XVI SIMPÓSIO DE ONCOBIOLOGIA
9 A 11 DE NOVEMBRO | 2022

LIVRO DE RESUMOS DO XVI SIMPÓSIO DE ONCOBIOLOGIA

Rio de Janeiro
2022

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REALIZAÇÃO

PROGRAMA DE ONCOBIOLOGIA

PATROCÍNIO

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Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro

APOIO

IBqM
Instituto de Bioquímica Médica Leopoldo de Meis

UFRJ

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Livro de Resumos

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Luiz Gustavo Dubois – ICB / UFRJ
Martin Hernan Bonamino – INCA
Marcelo Alex de Carvalho – IFRJ
Paulo Roberto Lira Lima – Programa de Oncobiologia
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Introdução

O Simpósio de oncobiologia está em sua décima terceira edição e será realizado no formato híbrido, on-line e presencial, no Centro de Ciências da Saúde da Universidade Federal do Rio de Janeiro, nos dias 09, 10 e 11 de novembro de 2022, na cidade do Rio de Janeiro.
O Simpósio cresce a cada ano e tem se estabelecido como um importante evento anual para o debate na temática da Biologia do Câncer. O simpósio tem o objetivo de integrar os grupos de pesquisa nesta área, além de trazer pesquisadores consagrados no âmbito nacional e internacional para oferecer uma perspectiva geral dos estudos que vem sendo desenvolvidos nos mais diversos aspectos da biologia tumoral.
Durante o evento temos as apresentações das conferências, das comunicações orais e dos trabalhos em pôsteres. Durante este tempo o simpósio promove um ambiente para debates sobre os avanços nas diversas áreas de estudo levando a um maior conhecimento e permitindo o avanço das nossas pesquisas. Gostaríamos de agradecer aos palestrantes convidados por aceitarem nosso convite e a todos os participantes por comparecerem ao evento.

Robson de Queiroz Monteiro
Coordenador do Programa de Oncobiologia

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SUMÁRIO

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09/11

ONLINE

09:50h – ABERTURA

10:00h – PALESTRA PLENA

Dra. Daniela Cerezo-Wallis (Centro Nacional de Investigaciones Oncológicas, Espanha & Yale University, EUA)
Tema: "Understanding innate immune cells in cancer: from their development to their implication in clinical responses"
Mediador: Dr. Robson Monteiro (IBqM/UFRJ)

11:00h – PALESTRA PLENA

Dr. Alvaro Monteiro (Moffitt Cancer Center, EUA)
Tema: "The BRCT domain: a modular platform for signal transduction in the DNA damage response"
Mediador: Dr. Marcelo Alex (IFRJ/ INCA)

12:00h – TRABALHOS SELECIONADOS IC/MsC/PhD

INICIAÇÃO CIENTÍFICA
Patrick Medeiros (INTO/UFRJ)
Orientadora: Dra. Amanda Cavalcanti (INTO)
Patient-derived tumor xenograft models as a platform for investigating multidrug resistance in osteosarcoma

MESTRADO
Marcos Magalhães (INCA)
Orientador: Dra. Paula Bernardo (INCA)
Promoter methylation is associated with miR-34b reduced expression and its restoration do not reverse cytarabine resistance phenotype in AML, in vitro

DOUTORADO
Gabriella Freitas (ICB-UFRJ)
Orientadora: Dra. Patrícia P. Garcez (ICB-UFRJ)
Glioblastoma microenvironment is remodelled by Zika virus infection in vitro and in vivo

Mediadora: Dra. Gabriela Nestal de Moraes (IBqM-UFRJ)

13:00h-14:30h
INTERVALO ALMOÇO

14:30h – TRABALHOS SELECIONADOS IC/MsC/PhD

INICIAÇÃO CIENTÍFICA
Afonso Arruda (UFF)
Orientador: Dr. Bruno Robbs (UFF)
Naphthoquinone-triazole-coumarin hybrids induce autophagy possibly by PKM2 inhibition in oral squamous cell carcinoma

MESTRADO
Renato Cardoso (ICB-USP)
Orientador: Dr. José Barbuto (ICB-USP)
Neuro-immune interactions in glioblastoma context: a new route?

DOUTORADO
Jhenifer Santos dos Reis (IBCCF-UFRJ)
Orientador: Dr. Leonardo Freire-de-Lima (IBCCF-UFRJ)
The participation of pp-GalNAc-T6 and O-linked glycans in multidrug resistance phenotype in tumor cells

Mediador: Dr. Martín Bonamino (Fiocruz/INCA)

15:30h – PALESTRA PLENA

Dra. Kandice Tanner (NIH/NCI, EUA)
Tema: "Microenvironment regulation of metastasis"
Mediador: Dr.Robson Monteiro (IBqM/UFRJ)

16:30h – PALESTRA PLENA
Dr. Martin Götte (Hospital Universitário Münster, Alemanha)
Tema: "Syndecans as pathogenesis factors and therapeutic targets in breast cancer"
Mediador: Dra. Juliana Motta (IBqM/UFRJ)

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PRESENCIAL

10:00h – PALESTRA PLENA
Dra. Kelly Magalhães (UnB, Brasil)
Tema: "Entendendo os mecanismos de agravamento ou atenuação do câncer pelos tecidos adiposos"
Mediador: Dr. Robson Monteiro (IBqM/UFRJ)

11:00h – TRABALHOS SELECIONADOS IC/MsC/PhD

INICIAÇÃO CIENTÍFICA
Amanda Gomes (INCA/UNIRIO)
Orientadora: Dra. Luciana Ferreira (INCA/UFF)
Osteopontin alters morphology and expression of differentiation-related genes in anaplastic thyroid cancer

MESTRADO
Ana Luiza Ferreira (ICB-UFRJ)
Orientadora: Katia Carneiro (ICB-UFRJ)
The molecular signature of exosome-related proteins is disrupted by histone deacetylase activity inhibition in the glioblastoma secretome: in silico and in vivo characterization

DOUTORADO
Carolinne Amorim (ICB-UFRJ)
Orientador: Dr. João Moraes (ICB-UFRJ)
Effect of extracellular vesicles derived from tumor-associated neutrophils on MDA-MB-231 cells

Mediador: Dr. Marcelo Alex Carvalho (IFRJ/INCA)

11:30h – SESSÃO DE PÔSTERES COFFEE BREAK

13:30h-14:30h – INTERVALO ALMOÇO

14:30h – PALESTRA PLENA
Dr. Thales Papagiannakopoulos (NYU, EUA)
Tema: "Uncovering Vulnerabilities in Genetic Subtypes of Lung Adenocarcinoma"
Mediador: Dr. Luiz Gustavo Dubois (ICB/UFRJ)

15:30h – SESSÃO DE PÔSTERES DRINKS & COFFEE BREAK

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ONLINE

10:00h – <u>PALESTRA PLENA</u>
Dra. Laurence Panicot-Dubois (Aix-Marseille Université, França)
Tema: “Platelet-educated cancer cells and tumor-educated platelets: an egg-and-chicken debate”
Mediador: Dr. Robson Monteiro (IBqM/UFRJ)

11:OOh – TRABALHOS SELECIONADOS PÓS-DOC

Marco Antonio Lacerda-Abreu (IBqM-UFRJ)
Hydrogen peroxide generation as an underlying response to high extracellular inorganic phosphate (Pi) in breast cancer cells

Caroline Pires Poubel (INCA)
Characterization of regulatory regions associated with FLT3 gene overexpression in acute leukemias

Mediador: Dr. Gustavo Dubois (ICB-UFRJ)

12:00h – ENCERRAMENTO DO EVENTO E PREMIAÇÃO

SESSÃO DE APRESENTAÇÃO DE TRABALHOS

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PRIMEIRA SESSÃO DE APRESENTAÇÃO DE TRABALHOS

10 DE NOVEMBRO

TARDE – 11:30 ÀS 13:30

SESSÃO DE TRABALHOS APRESENTADOS – MANHÃ

DATA: 10/11/2021

POSTER #	TITLE	TRACK	PRESENTER	PRESENTATION TYPE
155259	A miRNA-BASED PREDICTOR OF PROGNOSIS AND THERAPEUTIC RESPONSE FOR HIGH-GRADE SEROUS EPITHELIAL OVARIAN CANCER USING MACHINE LEARNING	Bioinformatics	Cristiane Esteves	MS
155042	EXPRESSION OF NUCLEAR XIAP REVEALS MOLECULAR SIGNATURES ASSOCIATED WITH MOST PROLIFERATIVE AND MIGRATORY FEATURES IN BREAST CANCER	Bioinformatics	Bruna Mendonça	DR
154976	IMPORTIN EXPRESSION AND INHIBITION IN BREAST CANCER: A POTENTIAL MODEL TO STUDY XIAP NUCLEAR LOCALIZATION	Cell Signaling	Carolina Ferreira	IC
155040	THE INFLUENCE OF PMA ON RESISTANCE TO MAPK PATHWAY INHIBITORS IN MELANOMA CELL LINES.	Cell Signaling	Gabriella Silva	IC
155207	INFLUENCE OF GALECTIN-3 ON DNA DAMAGE RELATED GENES EXPRESSION IN BREAST CANCER CELL LINES	Cell Signaling	Hugo Caramalho	IC
155263	β-CATENIN DESTABILIZATION OVERCOMES 5-FU RESISTANCE AND SYNERGISTICALLY IMPAIRS COLORECTAL CANCER GROWTH	Cell Signaling	Luiz Fernando Silva Oliveira	PD

154928	EXPRESSION EVALUATION OF HER-2 AND β1 INTEGRIN IN HER-2+ BREAST CANCER CELLS LINES AFTER TREATMENT WITH TRASTUZUMAB	Cellular Biology	Ana Luiza Sant'Anna	IC
155267	IMPLANTATION OF A MURINE MELANOMA ORTHOTOPIC METASTASIS MODEL	Cellular Biology	Cássio Hernandes Gumes da Silva	IC
155232	EVALUATION OF THE FREQUENCY OF ACTIVATED T CELLS IN THE PERIPHERAL BLOOD OF PATIENTS WITH HEAD AND NECK SQUAMOUS CELL CARCINOMA.	Cellular Biology	Daniela Medeiros	IC
155244	THE ROLE OF ACTIVE AND REACTIVE ENTERIC GLIAL CELLS TO COLORECTAL TUMOR CELLS	Cellular Biology	Gabriele Jardim	IC
155243	DEVELOPMENT OF A INTESTINAL MINI-ORGAN PLATFORM FOR TESTING FIRST-LINE PHARMACEUTICALS COMBINED WITH WNT/β-CATENIN PATHWAY INHIBITOR CHEMICAL COMPOUNDS	Cellular Biology	Julia França	IC
155261	THE ROLE OF LAMININ AND PTEN IN THE NEUROGENIC POTENTIAL OF ENTERIC GLIA.	Cellular Biology	Juliana Carvalho	IC
155121	ANTI-CD19 CAR-T CELLS OVEREXPRESSING THE PROTEIN PHF19 HAVE ALTERED MEMORY AND EXHAUSTION PHENOTYPES	Cellular Biology	Karina Hajdu	ES/AP
155256	STUDY OF THE ROLE OF CX43 ON ENTERIC GLIAL CELLS IN INFLAMMATION OF INTESTINAL EPITHELIAL CELLS	Cellular Biology	Yohana Fernandes	ES/AP

155239	CAR-T CELL FUNCTION EVALUATION IN LONG-TERM KILLING ASSAYS	Cellular Biology	Clara Andrade	MS
155196	GENERATION OF 19BBZ CAR-T CELLS IN TCR KNOCKOUT T CELLS	Cellular Biology	Eduardo Correia	MS
155158	ANTI-HER2 PIGGYBAC TRANSPOSON-BASED CAR T CELLS PRODUCTION: COMPARISON OF TWO DIFFERENT SCFV CLONES	Cellular Biology	EMMANUEL ARTHUR ARAGAO	MS
155264	INHIBITION OF THE WNT/β-CATENIN PATHWAY ENHANCE 5-FU EFFECTS IN MICE MODEL OF COLORECTAL CANCER	Cellular Biology	Hyvalker Allan Correia do Amaral	MS
155018	INFLAMMASOME ACTIVATION BY NEUTROPHIL EXTRACELLULAR TRAPS (NETs) IN HUMAN BREAST CANCER MODELS	Cellular Biology	ALEXANDER SILVA	DR
155122	THE ROLE OF TRANSCRIPTION FACTOR SOX2 IN THE MECHANISMS OF EPITHELIAL-MESENCHYMAL TRANSITION AND APOPTOSIS IN EXPERIMENTAL MODELS OF MELANOMA	Cellular Biology	Bárbara Carvalho	DR
155254	EXPLORING CAR-T CELL IMMUNOTHERAPY IN PRECLINICAL AND CLINICAL STUDIES THROUGH MATHEMATICAL MODELS	Cellular Biology	Emanuelle Arantes Paixão	DR
154753	A gaze at CDK9 isoforms in cell cycle and DNA damage response	Cellular Biology	Vanessa Fernandes	PD

155119	ANTINEOPLASTIC POTENTIAL OF Jatropha multifida L.	Drugs and/or Natural Products Therapy	Aryane Cazumbá	IC
155246	Development of bioactive-associated nanosystems and investigation of the antitumor effect on lung carcinoma cells	Drugs and/or Natural Products Therapy	Camila Nogueira da Silva Batista	IC
155236	CYTOTOXIC EFFECT OF ISOLATED COMPOUND FROM EREMANTHUS CROTONOIDES AGAINST CANCER CELL LINES	Drugs and/or Natural Products Therapy	Guilherme Freiman Wermelinger	IC
155262	EFFECT OF PIPERINE ON MUTANT p53 (R248Q) AGGREGATION IN NON-SOLID TUMORS	Drugs and/or Natural Products Therapy	Igor da Silva de Araujo	IC
155138	PRODUCTION AND EVALUATION OF THE ACTIVITY OF ESCHERICHIA COLI TYPE 2 L-ASPARAGINASE	Drugs and/or Natural Products Therapy	Anna Catharinna da Costa Novaes	MS
155153	INFLUENCE OF GREEN TEA (CAMELLIA SINENSIS) EXTRACT ON PERIPHERAL BLOOD CELLS STIMULATED BY CONDITIONED MEDIUM FROM BREAST TUMOR CELL LINES	Drugs and/or Natural Products Therapy	Emmanuele Andrade	MS
155233	DEVELOPMENT OF NEW COMBINATION STRATEGIES OF CHEMOTHERAPY WITH SELECTIVE CYCLIN-DEPENDENT KINASES (CDK) 4/6 INHIBITORS IN COLORECTAL CANCER	Drugs and/or Natural Products Therapy	Alana Souza	DR

155118	PCA3 SILENCING SENSITIZES PROSTATE CANCER CELLS TO DOCETAXEL	Drugs and/or Natural Products Therapy	Ana Emília Goulart Lemos	DR
154993	COTREATMENT OF CURCUMIN AND MELPHALAN INDUCES CELL DEATH IN MDA-MB-231 BREAST CANCER CELLS	Drugs and/or Natural Products Therapy	Daniel Galinis Vieira Lima	DR
155178	THE USE OF METFORMIN AS ADJUVANT TO RESTORE THE REDUCTION OF CISPLATIN CYTOTOXICITY IN THE PRESENCE OF ALLANTOIN	Drugs and/or Natural Products Therapy	Grazielle Paz	DR
155150	ANTITUMOR POTENTIAL OF OLIVE LEAF EXTRACT (Olea europaea) AFTER SIMULATED DIGESTION ON HEPATOCELLULAR CARCINOMA 3D MODEL	Drugs and/or Natural Products Therapy	Heloisa Pessoa	DR
155208	GENERATION AND OPTIMIZATION OF anti-CD19 CAR-T CELL FOR LEUKEMIA IMMUNOTHERAPY	Drugs and/or Natural Products Therapy	Leonardo Silva	PD
155205	THE IMPACT OF THE COVID-19 PANDEMIC ON BREAST CANCER SCREENING AND DIAGNOSIS IN BRAZIL	Education and Science communication	Giovanna Martins de Mello Alves	IC
155255	GENES ASSOCIATED WITH LEUKEMIA AND DOWN SYNDROME: SYSTEMATIC REVIEW STUDY	Genomics, Proteomics and Metabolomics	Alyssa Pires Marinho	IC
155200	GLYCOPHENOTYPE ALTERATIONS IN THE 4T1-LUC2 CELL LINE AFTER CHRONIC NICOTINE TREATMENT.	Metabolism and Glycobiology	Ariely Costa dos Santos	IC

155038	METABOLIC UPTAKE OF DIETARY N-GLYCOLYLNEURAMINIC ACID PROMOTE COLON CANCER GROWTH BY IMMUNE AND NON-IMMUNE MECHANISMS	Metabolism and Glycobiology	Ana Luiza dos Santos Lopes	DR
155210	THE EFFECT OF THE INCORPORATION OF EXOGENOUS SUGAR NEU5GC ON EGFR SIGNALING PATHWAY IN COLON CARCINOGENESIS	Metabolism and Glycobiology	Amanda Carlos Paulino	MS
155266	INTERPLAY BETWEEN PRC2/EZH2 COMPLEX AND LONG NON-CODING RNAS IN THYROID CANCER	Molecular Biology	Amanda Silva	IC
155095	INFLUENCE OF POLYMORPHISM IN THE GENES ENCODING LEPTIN AND ITS RECEPTOR IN THE DEVELOPMENT OF ENDOMETRIOSIS IN BRAZILIAN WOMEN	Molecular Biology	Isabelle Alves	ES/AP
154952	ASSOCIATION OF THE EPSTEIN-BARR ONCOVIRUS PHYLOGEOGRAPHY WITH ANCESTRALITY: A PERSPECTIVE OF MATERNAL INHERITANCE	Molecular Biology	Marcella Larrate da Silva	ES/AP
154889	Interleukin-8 polymorphism associated with low risk of endometriosis-related pelvic pain	Molecular Biology	Matheus Pereira de Mello	ES/AP
154962	THE ROLE OF DNMT3A AND TDT ON THE PROMOTION OF FLT3 AND NPM1 MUTATIONS IN ACUTE MYELOID LEUKEMIA	Molecular Biology	ANDRESA DAMASCENO	MS
154931	THERMOSENSITIVITY OF MISSENSE VARIANTS IN THE PALB2 WD40 DOMAIN	Molecular Biology	Daniel Soares Chrispim	MS

155022	SECRETED OSTEOPONTIN-A MEDIATE ANAPLASIC THYROID CARCINOMA PROGRESSION FEATURES BY αVβ3 INTEGRIN RECEPTOR	Molecular Biology	Daniella Mattos	MS
154953	ONCOGENIC ACTIVATION OF FLT3 IN PATIENTS WITH ACUTE MYELOID LEUKEMIA	Molecular Biology	Heloysa de Fátima Araujo Bouzada	MS
154808	IMPACT OF BRAFV600 MUTATIONS IN THE CLINICAL AND HISTOPATHOLOGICAL CHARACTERISTICS OF ACRAL MELANOMA AND ITS DERIVED EXPERIMENTAL MODELS	Molecular Biology	João Pedro Simões	MS
155230	MOLECULAR PROFILE OF T-CELL ACUTE LYMPHOBLASTIC LEUKAEMIA WITH CRLF2 OVEREXPRESSION	Molecular Biology	Ana Luiza Tardem Maciel	DR
154956	UNDERSTANDING THE PALB2 PROMOTER REGION AND ITS EXPRESSION REGULATION THROUGHOUT THE CELL CYCLE	Molecular Biology	Anna Beatriz Ribeiro Elias	DR
155247	AGGREGATION PROFILE OF THE p53 TUMOR SUPPRESSOR PROTEIN R337H MUTANT, CHARACTERISTIC OF A HEREDITARY CANCER SYNDROME (LI-FRAUMENI SYNDROME - LFS)	Molecular Biology	Gileno dos Santos de Sousa	DR
155017	A MECANISTIC ROLE FOR ATM/ATR-MEDIATED BRCA1 PHOSPHORYLATION IN THE DNA DAMAGE RESPONSE	Molecular Biology	Thiago Gomes	PD
154477	DEVELOPMENT OF CELLULAR LINEAGE DERIVED FROM A PATIENT WITH ALL PORTING TRANSLOCATION 8;22 (q24;q11) and DUP (1) (q21;q32).	Molecular Biology	Louise Deterling	PQ/DC

SEGUNDA SESSÃO DE APRESENTAÇÃO DE TRABALHOS

10 DE NOVEMBRO

TARDE – 15:30 ÀS 17:00

SESSÃO DE TRABALHOS APRESENTADOS – TARDE

DATA: 10/11/2021

POSTER #	TITLE	TRACK	PRESENTER	PRESENTATION TYPE
155006	Resistance to Hormone Therapy and Tumor Recurrence in Breast Carcinoma Patients: Possible Relation with the IL6/STAT3 Signalling Pathway	Cell Signaling	Maria Paula Mota Pereira dos Santos	IC
155215	CROSSTALK BETWEEN EPIDERMAL GROWTH FACTOR RECECPTOR (EGFR) AND PROSTAGLANDIN E2 (PGE2): A POSSIBLE LINK BETWEEN CERVICAL CANCER AND INFLAMMATION?	Cell Signaling	Vitória Ramos de Azevedo	IC
155191	CLOTRIMAZOLE IMPACT ON MACROPHAGE M2 POLARIZATION	Cell Signaling	José Xavier do Nascimento Júnior	MS
154961	AKT-MEDIATED REGULATION OF FOXK2 TRANSCRIPTION FACTOR: MOLECULAR MECHANISMS AND POTENTIAL ROLE IN BREAST CANCER DRUG RESISTANCE	Cell Signaling	Luciana da Torre Carneiro	DR
155235	MODULATION OF THE WNT/β-CATENIN PATHWAY AND ITS EFFECTS IN COLORECTAL CANCER CELL LINES	Cell Signaling	Marielly Mangelli	DR
155106	QUANTIFICATION OF NAÏVE AND MEMORY T LYMPHOCYTES IN THE PERIPHERAL BLOOD OF PATIENTS WITH SQUAMOUS CELL CARCINOMA OF THE HEAD AND NECK	Cellular Biology	Larissa Wermelinger	IC

155204	PRO-TUMORIGENIC EFFECTS OF NEUTROPHIL EXTRACELLULAR TRAPS (NETS) IN CASKI CERVICAL CANCER CELLS	Cellular Biology	Louise Jean Vidal dos Santos Silva	IC
154903	ROLE OF SULFATED FUCANS FROM SEA URCHIN ON PRIMARY TUMOR GROWTH	Cellular Biology	Mariana Gabry	IC
155221	INCREASED MIGRATORY CAPACITY OF HER2+ BREAST CANCER TUMOR CELLS AFTER TREATMENT WITH TRASTUZUMAB	Cellular Biology	Matheus Ferreira de Barros	IC
154937	TUMOR PROGRESSION STUDY: EVALUATION OF PREMETASTATIC LUNG NICHE FORMATION AND TUMOR CELL-PLATELET CONTACT	Cellular Biology	Nathália Dantas Nascimento de Oliva	IC
154910	P53 ON THE APPEARANCE OF CANCER STEM CELLS AND EXTRACELLULAR MATRIX CHANGES IN MICE COLORECTAL TUMORS	Cellular Biology	Pedro Henrique Sales Barbosa	IC
155209	ANTITUMOR POTENTIAL OF VITAMIN K SYNTHETIC DERIVATIVES ON WILD-TYPE p53 MCF-7 CELL LINE	Cellular Biology	Raissa Eduardo dos Santos	IC
155043	Study of tumor growth pattern and histopathological characteristics of patient–derived acral melanoma xenografts	Cellular Biology	Rebecca Martins Cadimo do Nascimento	IC

155019	AUTOLOGOUS BONE MARROW TRANSPLANTATION - RELATION BETWEEN CD34+ CELLS AND COLONY FORMING UNITS FOR QUALITY ANALYSIS IN CRYOPRESERVED APHERESIS PRODUCTS	Cellular Biology	Tatiely Silva dos Santos	IC
155253	NEUTROPHIL EXTRACELLULAR TRAPS (NETS) DRIVE THE SECRETION OF EXTRACELLULAR VESICLES (EVs) IN HUMAN BREAST CANCER CELLS	Cellular Biology	Manoela Bastos	MS
155186	APE1 REDOX INHIBITOR EXERTS ANTITUMOR EFFECT ON 3D BREAST CANCER CELL CULTURES IN NORMOXIC AND HYPOXIC MICROENVIRONMENT	Cellular Biology	Mariana Moreno de Sousa Rodrigues	MS
155023	INFLUENCE OF BCG (BACILLUS CALMETTE-GUÉRIN) ON MATURATION OF HUMAN DENDRITIC CELLS DIFFERENTIATED FROM MONOCYTES	Cellular Biology	Thamiris Lima	MS
154914	LABH INHIBITS PLATELET-TUMOR CELL INTERACTION INDEPENDENTLY OF ITS ANTICOAGULANT EFFECT	Cellular Biology	Carlos Fernandes	DR
155126	NEUTROPHIL EXTRACELLULAR TRAPS (NETS) DRIVE A CHEMORESISTANT PHENOTYPE IN HUMAN BREAST CANCER CELLS THROUGH PI3K/AKT/NF-kB PATHWAY	Cellular Biology	Juliana Lima de Souza	DR
155223	IMPROVEMENT OF CAR-T CELL THERAPY WITH ULTRA-FAST PROTOCOL AND IL-15 MEMBRANE BOUND ADDITION	Cellular Biology	Luiza Abdo	DR

155242	CHARACTERIZATION OF p53 AMYLOID AGGREGATES IN HEPATOCELLULAR CARCINOMA CELL LINES AND THEIR MODULATION BY PRIMA-1	Cellular Biology	Mariana Muniz da Paz	DR
155214	LONG-TERM RESISTANCE TO 5-FLUOROURACIL ACTIVATES DISTINCT CELLULAR EVENTS UNDERLYING DRUG RESISTANCE ON COLON CANCER CELLS	Cellular Biology	Annie Cristhine Moraes Sousa Squiavinato	PD
155257	EVALUATION OF THE ANTITUMOR EFFECT OF CURCUMINOIDS IN HUMAN LUNG CARCINOMA AND ADENOCARCINOMA CELL LINES	Drugs and/or Natural Products Therapy	Lorrane de Souza Chaves	IC
155116	EVALUATION OF ANTITUMOR POTENTIAL OF NOVEL CHALCONES AGAINST ORAL SQUAMOUS CELL CARCINOMA: IN VITRO AND IN SILICO ANALISYS	Drugs and/or Natural Products Therapy	Lucas Rubini Dias	IC
155000	ANTICANCER PROPERTIES OF A LOW ANTICOAGULANT BOVINE HEPARIN	Drugs and/or Natural Products Therapy	Marcos Roberto de Oliveira	IC
154934	EVALUATION OF THE TOXIC EFFECT OF PIPERINE AND DERIVATIVES IN HUMAN GASTRIC CARCINOMA CELL LINES	Drugs and/or Natural Products Therapy	Stefani Ingrid Martins Nascimento	IC
155241	SCREENING OF BIOACTIVE COMPOUNDS FROM FOODS IN A MURINE MODEL OF BREAST CANCER LINE 67NR	Drugs and/or Natural Products Therapy	Stêphanie Rocha Vieira Elexias	IC
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NAPHTHOQUINONE-TRIAZOLE-COUMARIN HYBRIDS INDUCES AUTOPHAGY POSSIBLY BY PKM2 INHIBITION IN ORAL SQUAMOUS CELL CARCINOMA

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INTRODUCTION AND OBJECTIVES: Mouth cancer is a malignant tumor of the oral cavity, very common in Brazil, the most common being squamous cell carcinoma (OSCC). Pyruvate kinase protein M2 (PKM2) is overexpressed in the worst prognosis of the disease. Inhibition of this protein in tumor cells might induce cell death by autophagy through energy privation. Autophagy refers to the process of degradation and recycling of cell components. 9 naphthoquinone-triazole-coumarin hybrids were used for cytotoxicity tests: CUME1-9. This work aims to characterize new selective molecules with antitumor activity OSCC, determine it´s acute toxicity *in vivo* and it´s molecular mechanism and death induction pathway. **MATERIAL AND METHODS:** The MTT assay was used to evaluate the viability of Fibroblasts, SCC4, SCC9 and SCC25 treated with the substances in order to calculate the IC50. The selectivity index (SI) was calculated by the ratio between the IC50 in fibroblasts and in SCC9. Stability was determined through cell viability using MTT assays. Apoptotic cell death phenotype was analyzed by flow cytometry. In autophagy, SCC9 cells were stability transduced with LC3-GFP plasmid and puncta formation was measured by fluorescent microscopy. The OSIRIS and ADMET-SAR platforms were used in the *in silico* evaluation to determine their pharmacological properties and molecular docking was performed with candidate binding targets. Acute toxicity assay was performed in C57BL6 mouse using 50, 62,5 or 75mg/Kg concentrations (CEUA: 982). Hemolysis assay in human blood was also performed, in order to establish whether cytotoxic activity is related to direct damage to the cell membrane or not. The selectivity index of CUME6 was evaluated in the lines B16F10, HEP2G and HT29. We performed Real Time in normal cells and in different tumor cells compared to the expression of the PKM1 and PKM2 genes, where significant differences were found. **RESULTS AND CONCLUSION:** The IC and SI values were used to select the most promising and stable substance at 37 ºC: CUME6. The IC50 in SCC9, SCC4, SCC25 and fibroblasts, in µM, were 28.21, 75.23, 68.83 and 100.7, respectively with

an SI > 2. CUME6 was selective in other tumor cells (B16F10 and HEP2G) with SI > 2. CUME6 didn't induce any apoptotic phenotype such as DNA fragmentation, phosphatidyl-serine exposure, or caspase-3 activation, but induced a phenotype of cell death by autophagy, forming vesicles and accumulating LC3 in punctas. It was evaluated *in silico* as a drug with good solubility (LOGS=-6.91), meeting 3 of the 4 criteria of the lipinski rule of 5. The number of violations for RIDGE 6 is 1, while for doxorubicin 3 and carboplatin 0. Molecular dockin showed that it's possibly capable of binding with hight affinity to PKM2 which might justify the autophagy cell death phenotype. CUME6 was not hemolytic and shown a LC50 of proximally 60mg/Kg in mice acute toxicity assay. Further trials should be carried out to determine other aspects of CUME6.

Keywords: carcinoma, triazole, synthesis, chemotherapy, oral cancer.

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OSTEOPONTIN ALTERS MORPHOLOGY AND EXPRESSION OF DIFFERENTIATION-RELATED GENES IN ANAPLASTIC THYROID CANCER CELLS

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INTRODUCTION AND OBJECTIVES: Anaplastic thyroid carcinoma (ATC) constitutes the minority of thyroid cancer cases. However, it contributes to most cancer-related deaths in this gland. ATC is characterized by its aggressive behavior, rapid tumor progression and poor prognosis. In addition, its cells present a high degree of cellular dedifferentiation, considered to be one of the hallmarks of the oncogenic process. This cellular dedifferentiation results in the negative regulation of thyroid functional genes, such as the sodium and iodide symporter, making it impossible to apply efficient therapies used in differentiated thyroid carcinomas, such as radioiodine therapy. In this regard, studies have been conducted aiming to regain the functionality of thyroid genes through the regulation of intracellular pathways and differentially expressed genes. Among the genes that show aberrant expression in thyroid carcinomas is osteopontin (OPN), a protein recognized as widely related to the progression of several tumor types and acquisition of poor prognostic features. However, the role of this protein in the anaplastic carcinoma pathophysiology remains unknown. Thus, this study aims to evaluate the role of OPN in dedifferentiation of ATC-derived cells. **MATERIAL AND METHODS:** OPN expression in an ATC-derived cell line was silenced by RNA interference, resulting in two distinct cell populations, named as OPN silenced cells (8505c-siOPN) and the control ones (8505c-siCN). Cell morphology, viability, and migratory and invasive capacities of both cells were analyzed. The expression levels of thyroid differentiation, mesenchymal and cancer stem cell (CSCs) markers were analyzed by RT-qPCR. **RESULTS AND CONCLUSION**: In cell morphological analysis, we found an increased cell circularity index in 8505c-siOPN cells, as well as a decrease in the amount of membrane projections, when compared to control cells. There were no significant differences in cell viability rates when comparing 8505c-siOPN and control cells, although apoptotic cell death index was slightly higher in the OPN-silenced cells. 8505c-siOPN cells also presented less invasion and migration capacity and increased expression of differentiation markers. Otherwise, the mesenchymal markers were increased in the OPN-silenced cells, while between the CSC markers, only NANOG was increased in the OPN-silenced cells. In summary, our study suggests that OPN silencing induces a more characteristically epithelial cell morphology, decreased migratory and invasion capacity, as well a trend toward increased expression of functional thyroid genes. Thus, our data suggest that inhibition of OPN expression is able to modulate some features of ATC dedifferentiation and poor prognosis, opening new perspectives for the development of redifferentiation therapies.

Keywords: Osteopontin, Thyroid Cancer, Dedifferentiation.
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PATIENT-DERIVED TUMOR XENOGRAFT MODELS AS A PLATFORM FOR INVESTIGATING MULTIDRUG RESISTANCE IN OSTEOSARCOMA

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INTRODUCTION AND OBJECTIVES: Osteosarcoma (OS) is the most common malignant bone neoplasm in children and young people. The survival of patients with metastatic disease is still low and the percentage of "poor responders" is between 47% to 71%. One of the mechanisms that may be associated with the poor response is the multidrug resistance (MDR) phenotype, and the expression of ABC superfamily transmembrane proteins. The difficulty of relevant clinical trials for a disease considered orphan from a commercial point of view is evident. Patient-derived xenograft (PDX) model is reproducible for several types of sarcomas and, in addition to recapitulating the phenotypic characteristics of the primary OS. This study aims to validate the OS PDX model, which has already been established by our group, as a tool for evaluating MDR by mapping the expression profile of ABC transporters. **MATERIAL AND METHODS:** The study was approved by human (CAAE: 69859417.20000.5273) and animal (014/2022) ethics committees. Tumor tissue samples were collected at the time of biopsy or surgical resection avoiding necrotic areas. After sectioning, fragments were stored in RNA later® and maintained in culture medium at 4°C until implantation. Fragments were implanted in the subcutaneous tissue of NOD scid gamma (NSG™) mice. Tumor growth was monitored once a week and when the tumor size reached the approximate volume of 1500 mm^3, it was transplanted to the next generation (P2) and processed for histological analysis and RNA extraction. **RESULTS AND CONCLUSION:** Over a period of four months, samples were collected from six patients, average age of 31 years, with histopathological diagnosis of osteosarcoma. Two samples were collected at biopsy and four at resection/disarticulation surgeries. We observed tumor growth in four out of six samples (66% engraftment rate). Two samples reached the second passage (P1) and none reached P2 after four months. The mean growth period of the samples that achieved P1 was 122.8 ± 17.6 days with a mean volume of 958.1 ± 638.1 mm^3. Also, our preliminary results confirmed the histological consistency with the corresponding parental osteosarcoma. Therefore, we consider the PDX a suitable model for preclinical tests and a promising tool to investigate and address several unanswered issues in clinical oncology.

Keywords: Osteosarcoma, Patient derived xenograft, Multidrug resistance.
Supported by: FAPERJ, Ministério da Saúde.

THE MOLECULAR SIGNATURE OF EXOSOME-RELATED PROTEINS IS DISRUPTED BY HISTONE DEACETYLASE ACTIVITY INHIBITION IN THE GLIOBLASTOMA SECRETOME: *IN SILICO* AND *IN VIVO* CHARACTERIZATION

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INTRODUCTION AND OBJECTIVES: Glioblastoma (GBM) is the most common and aggressive neoplasia of the Central Nervous System. We have previously shown that Histone Deacetylase (HDAC) activity inhibition disrupts the molecular signature of the GBM secretome, which is involved in shaping a favorable microenvironment for tumor growth and behavior. In this study, we focused on analyzing proteins associated with Extracellular Vesicles (EVs) characterized in the GBM secretome. EVs are nanostructures released by cells which are involved in intercellular communication and can be classified into different types, based on their sizes, as microvesicles and exosomes. Since exosomes are associated with tumor cell migration and metastasis, we aimed to explore the abundance of exosomes related proteins in the GBM secretome upon HDAC inhibition. **MATERIAL AND METHODS:** U87MG cells were treated with HDAC inhibitor Trichostatin 100nM (TSA;iHDAC) or DMSO for 72 hours when the supernatant was collected. Proteins were determined using quantitative NANOLC-MS/MS followed by *in silico* analysis using Uniprot, Vesiclepedia, and other bioinformatic tools. This strategy allowed us to identify the EVs-related proteins from both groups as well as to demonstrate pathways related to those proteins. In order to validate the *in silico* data, U87MG were treated with iHDAc for 72 hours when the supernatant was collected. We purified the EVs by ultracentrifugation method and perform further analysis, as Nanoparticle tracking and Western blotting aiming to distinguish the different populations of EVs. Furthermore, our *in silico* data identified RAB14 e RAB7A proteins exclusively present in the secretome of the treated group. Hence, we characterize RAB7A and RAB14 in U87MG and KNS42 cell lines of high and low gliomas, respectively. **RESULTS AND CONCLUSION**: Our *in silico* study demonstrated that both groups presented proteins most related to microvesicles than to exosomes. Interestingly, more pathways related to “Signal Transduction” were observed only in iHDAC group, including the AMPK signaling pathway which contains the RAB14 protein. Predicted PPI demonstrated significant interactions between RAB14/RAB7A in the treated group. Quantitative PCR demonstrated no significant differences in the expression of RABs genes after treatment with iHDAC in both cell lines which indicates that acetylation may not be involved in the control of those gene expressions. Immunocytochemistry showed that RAB7A is dispersed in the cytoplasm, while RAB14 is predominantly located in the perinuclear region. Those results support the hypothesis that the presence of RAB proteins in the secretome of the treated GBM cells might be due to a greater protein secretion without upregulation of the gene expression. Our findings indicate that HDAC activity is necessary for the proper pattern of GBM secretion and is mainly related to EVs proteins, which may impact the microenvironment and intercellular communication.

Keywords: Exosomes, Glioblastoma, Secretome.
Supported by: FAPERJ and CAPES

PROMOTER METHYLATION IS ASSOCIATED WITH miR-34b REDUCED EXPRESSION AND ITS RESTORATION DO NOT REVERSE CYTARABINE RESISTANCE PHENOTYPE IN AML, *IN VITRO*

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INTRODUCTION AND OBJECTIVES: Acute myeloid leukemia (AML) is a hematological neoplasm highly heterogeneous. The standard chemotherapy treatment is based on the association of one anthracycline with cytarabine (ara-C). However, patients' response to chemotherapy is reduced due to treatment resistance phenomenon, which contributes to AML poor overall survival. Deregulation of microRNAs expression is associated with resistance, tumor progression and relapse. miR-34b inhibits the translation of factors involved with proliferation and apoptosis regulation, being considered a tumor suppressor microRNA. miR-34b exogenous expression sensitizes solid tumor's cells to chemo- and radiotherapy. However, there is no literature report evaluating miR-34b potential to reverse chemoresistance or to potentialize synthetic compounds effects in AML. In this context, besides the study of chemoresistance, our group also performs *in vitro* testing of synthetic compounds' antitumoral effects. LQB-223 is a synthetic compound that has antitumor effect on different tumor cell lines, including AML. Therefore, the study aim is to evaluate miR-34b expression levels in AML cell lines with different resistance phenotypes along with the effect of miR-34b overexpression in treatment response in the chemoresistant cell line, HL-60R. **MATERIAL AND METHODS:** U937, HL-60 and HL-60R (ara-C chemoresistant cell line developed by our group) cell lines were selected to develop the study. Methylation specific PCR (MSP) was used to evaluate the methylation status of *miR-34b/c* promoter in the selected cell lines. Cell lines were treated with the synthetic compound LQB-223 to analyze miR-34b expression by quantitative PCR (qRT-PCR). Furthermore, miR-34b was overexpressed in HL-60R cell line by transfection of miRNA mimics with Lipofectamine reagent. The effect of miR-34b transient overexpression associated with ara-C or LQB-223 was evaluate by trypan blue exclusion and MTT assays, and the following flow cytometry assays annexin V, cell cycle distribution and DNA fragmentation. **RESULTS AND CONCLUSION**: Our data demonstrated that AML cell lines U937, HL-60 and HL-60R express low levels of miR-34b and *miR-34b/c* promoter methylated. Treatment with LQB-223 does not modulate miR-34b expression in AML cell lines. We performed cytotoxicity assays to evaluate the combined effect of miR-34b exogenous expression with ara-C or

LQB-223 treatments in HL-60R cell line. Our results demonstrated that there was no reduction of cell viability, no changes in cell cycle distribution and no induction of cell death by the overexpression of miR-34b alone or in combination with ara-C and LQB-223 treatments. In conclusion, the AML cell lines have low miR-34b expression due to methylation in the miR-34b/c gene promoter. Furthermore, miR-34b does not participate in LQB-223 mechanism of action. Finally, miR34b restoration did not reverse cytarabine resistance or potentialize LQB-223 antitumoral effect.

Keywords: Acute myeloid leukemia, microRNA-34b, chemoresistance, LQB-223.

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NEURO-IMMUNE INTERACTIONS IN GLIOBLASTOMA CONTEXT: A NEW ROUTE?

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INTRODUCTION AND OBJECTIVES: Glioblastoma (GBM) is a grade IV glioma with the highest frequency and lethality among primary brain cancers in adults and the best treatment provides a median survival of 15 months. Unraveling the complex biology of GBM could open routes for new and needed therapies for this disease. Therefore, it is necessary to investigate not only the biology of the tumor itself, but also its microenvironment and the initiation and maintenance of the immune response against the tumor. The study of the α7 subunit of nicotinic acetylcholine receptors (α7nAChR) has the potential to meet this need. Since the literature provides evidence that supports the hypothesis that α7nAChR may be involved with pro-tumor and immunosuppressive phenomena. **MATERIAL AND METHOD** Monocyte-derived dendritic cells (mo-DCs) were stimulated with TNF-α and subsequently exposed to acetylcholine (ACh) 1, 25 or 100μM, Methyllycaconitine (MLA) - specific competitive antagonist of α7nAChR - 25μM, ACh+MLA, 100μM ACh + 25 or 50μM MLA and vehicle, the expression of molecules C80, CD83, CD86, CD40, CCR7 and PD-L1 were analyzed in a flow cytometer. Flow cytometry also used for analyzed the endocytic capacity and the expression of CD206 by immature mo-DCs. The mitochondrial viability of human GBM cells (U87MG) exposed to the same treatments was evaluated by MTT. **RESULTS AND CONCLUSION** Only ACh treatments were able to produce a trend (*p=0.0625) of reduction in the expression of co-stimulatory molecules CD80 [25 μM] and CD83 and [100 μM], CD40 [1 μM] and the co-inhibitor molecule CD274 [1 and 25μM]. The ratio between the expression of the CD274 molecule by the expression of co-stimulatory molecules was qualitatively higher in an ACh-mediated and “concentration-dependent” manner. The groups treated with MLA, simultaneously or not with ACh, presented ratio values between CD274 and co-stimulatory molecules very close to each other and especially for the CD274/CD40 ratio close to of vehicle value. Qualitatively the treatment of immature mo-DCs with ACh reduced the endocytic activity [1 and 25 μM], but without altering the expression of CD206. On the other hand, the group treated with MLA presented an expression index of CD206 approximately 32% lower in relation to the control index. The GBM cells exposed to 10μM of ACh + 20μM of MLA (1:2) exhibited the greatest reduction in viability compared to the control group, approximately 37%. Using bioinformatics web tools, expression of α7nAChR in GBM tumor cells is not a predictor of survival. Despite this, the α7nAChR in GBM cells positively correlates with the expression of some molecules known to be involved in tumor genesis, progression and/or recurrence, for example: TGFβI (p=1e-09,R=0.46), HIF-1α (p=1e-05,R=0.33), VEGFR (p=2.5e-04,R=0.28), PIK3CB (p=0,028,R=0.17) and PDGFR (p=0,042,R=0.16). Expanding these results could open the way for the exploration of α7nAChR as a therapeutic target adjuvant to immunotherapy or other treatment approaches for GBM.

Keywords: Glioblastoma, Acetylcholine, Dendritic Cells.
Supported by: CAPES

EFFECT OF EXTRACELLULAR VESICLES DERIVED FROM TUMOR-ASSOCIATED NEUTROPHILS ON MDA-MB-231 CELLS

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INTRODUCTION AND OBJECTIVES: Breast cancer is the second most common type of cancer in the world and the first most common among women. It is well established the relationship between cancer and inflammation, and neutrophil infiltration has been described in tumors since 1863. Immune system cells, including neutrophils, are recruited by the tumor microenvironment as a site of chronic inflammation and begin to favor tumor growth. The neutrophils present in the tumor site are called tumor-associated neutrophils (TAN) and can present 2 phenotypes: N1 (antitumor) or N2 (pro-tumor). As an *in vitro* polarization strategy for TAN, we use extracellular vesicles (EVs) derived from breast tumor cells (MDA-MB-231). Our aim was to investigate the role of EVs derived from MDA-MB-231 in the polarization of neutrophils to N2 phenotype and the effect of EVs derived from TAN in MDA-MB-231. **MATERIAL AND METHODS**: Neutrophils were isolated in a ficoll gradient from peripheral blood of healthy donors (CAAE 38257914.7.0000.5259) and were treated with MDA-EVs or MCF10 (non-tumoral cells)-EVs for different times at 37°C and 5% CO_2. We investigated MDA-EVs effects on neutrophils: chemotactic capacity (1h), production of neutrophilic extracellular traps (NET, evaluation of extracellular DNA after 3h), apoptosis protection (morphology and Annexin+/PI- for 20h), ROS production (probes DCF-DA and DAF-DA after 3h), cytokines secretion (ELISA, evaluation after 3h), as well as the specific labeling for CD95 (N1) and CD184 (N2) (flow cytometry after 3h). The tumor cells viability treated with polarized neutrophil was observed by MTT, pro-caspase-3 expression (blotting after 24h) and JC-1 probe was used for to investigate the mitochondrial potential integrity. The TAN-EVs was collected after 2h of polarization and mitochondrial ROS of tumor cells was measured (probe mitoSOX for 80 min) and the invasion capacity of MDA-MB-231 was observed by transwell-assay for 48 h. **RESULTS AND CONCLUSION:** We observed that MDA-EVs showed chemotactic capacity. Neutrophils treated with MDA-EVs produced more extracellular DNA than MCF10-EVs, suggesting NET production. Neutrophils treated with MDA-EVs had their half-life increased, and they were able to produce intracellular ROS. We also observed that neutrophils treated with MDA-EVs had an increase in the secretion of IL-8 and VEGF. Corroborating these data, we observed that MDA-EVs induced an increase in the N2 marker CD184. Neutrophils treated with MDA-EVs were able to increase viability and mitochondrial potential integrity of tumor cell. Furthermore, TAN-EVs (N2) are able to increase the mitochondrial ROS and invasion capacity of tumor cells. As we observed that TAN-EVs presented in their membrane L-Selectin, we used fucoidan as strategy to decrease the effect of TAN-EVs. Altogether, our results show that MDA-EVs can induce an N2-like

phenotype and TAN-EVs are able to increase the invasion capacity of tumor cells in a L-selectin dependent manner.

Keywords: extracellular vesicles; breast cancer; tumor-associated neutrophils

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GLIOBLASTOMA MICROENVIRONMENT IS REMODELLED BY ZIKA VIRUS INFECTION IN VITRO AND IN VIVO

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INTRODUCTION AND OBJECTIVES: Glioblastoma (GBM) is a glial primary brain tumor with a high incidence and the highest degree of malignancy. The gold standard therapy is based on surgical removal associated with radiotherapy and chemotherapy, and is highly ineffective, presenting an approximately one-year survival rate and a high level of recurrence. GBM is a tumor with a heterogeneous microenvironment (TME) and this TME secretes molecules that favor tumor progression, and the different cells present there respond differently to the treatment. Thus, TME contributes to the low effectiveness of GBM treatment, so TME has become a target of interest for new therapies. In this context, a new alternative is the use of oncolytic viruses to infect and kill tumor cells without causing damage to healthy cells. Recently, the oncolytic potential of Zika virus (ZIKV) in GBM was demonstrated by prolonged animal survival and reduction in tumor mass. Here we aim to investigate the effects of ZIKV infection in GBM cells on the crosstalk of tumor and non-tumor cells, particularly on endothelial cells and microglia. **MATERIAL AND METHODS:** Cell viability was measured with cell titer blue; cell proliferation was assessed using BrdU and cell death was analyzed through cleaved caspase labeling. GBM conditioned medium (CM) was collected two days post. Endothelial cells were treated with CM of ZIKV-infected GBM cells and migration was examined through scratch assay, the angiogenesis was measured using matrigel assay. Microglia were exposed to CM and the expression of cytokines was assessed through qPCR. Swiss mice were injected with human GBM cells and after tumor establishment, they were intratumorally infected with ZIKV.**RESULTS AND CONCLUSION**: We observed that ZIKV infects GBM cells leading to reduced cell viability and proliferation with consequent cell death by apoptosis. When endothelial cells are exposed to infected CM, they present a reduction in their migratory and proliferative capacity, in addition to a reduction in their angiogenic capacity. Another TME population affected by GBM infection is microglia. We found that when microglia cells are exposed to ZIKV-infected GBM cells CM they acquire an anti-tumoral profile, showing an increase of anti-tumoral cytokines and reduced pro-tumoral cytokines indicating that infected GBM CM reprograms microglia. In vivo results show that when immuno-competent mice injected with GBM are intratumorally infected with ZIKV, they exhibit a reduction in tumor angiogenesis, an increase in microglia recruitment to the tumor site, and a greater survival rate. Furthermore, we show that the CM from infected GBM can modulate TME indicating that ZIKV can help in GBM treatment. Altogether these results suggest that ZIKV infection in GBM cells alters the intercellular communication with non-tumor cells of the TME, specifically endothelial cells and microglia, promoting an antitumor phenotype in these cells.

Keywords: Glioblastoma, ZIKV, Oncolytic viruses, Tumor microenvironment, Microglia
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THE PARTICIPATION OF PP-GALNAC-T6 AND O-LINKED GLYCANS IN MULTIDRUG RESISTANCE PHENOTYPE IN TUMOR CELLS

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INTRODUCTION AND OBJECTIVE: Cancer cells show, when compared to normal cells, alterations in the glycoprotein glycosylation pattern due to changes in the expression and activity of enzymes involved in the biosynthesis of cellular glycoconjugates. Multidrug resistance (MDR) phenotype is one of the main obstacles to cancer chemotherapy. Recent studies indicate that the emergence of the resistance phenotype is accompanied by changes in the activity and/or expression of certain glycosyltransferases. Polypeptide-*N*-acetylgalactosaminyltransferase 6 (pp-GalNAc-T6), the enzyme responsible for the synthesis of the *O*-glycosylated oncofetal fibronectin (onf FN), has been described as a tumor glycobiomarker, and its expression has been correlated with poor prognosis. However, its participation in the development of resistance has never been investigated in cancer. Therefore, was established as a general objective, to analyze whether pp-GalNAc-T6, through the biosynthesis of onf FN and other *O*-linked glycans, could participate in the emergence and/ or maintenance of the drug resistance phenotype in human breast adenocarcinoma cells. **MATERIAL AND METHODS:** First, through the MTT assay, were selected sublethal doses of the chemotherapeutic drug doxorubicin (DOX) in MCF-7 cells (5, 10, 20 and 40 nM), that were used to induce chemoresistance. Subsequently, protein expression of ABC transporters and apoptotic proteins were analyzed by Western Blot (WB) and the MTT assay was repeated to analyze the IC_{50} of DOX, vincristine (VIN) and cisplatin (CIS). Then, the glycophenotype of parental and DOX-treated cells were compared through cell cytometry. Furthermore, were analyzed protein expression of pp-GalNAc-T6 and onf FN and its participation in the MDR phenotype by the downregulation of pp-GalNAc-T6 using the siRNA technique. **RESULTS AND CONCLUSIONS:** Results showed increased expression of both ATP-binding cassette (ABC) transporters (ABCG2 and ABCC1) and anti-apoptotic proteins (Bcl-2 and Bcl-xL) in DOX-treated cells. These cells were also resistant to VIN and CIS, indicating the emergence of the MDR phenotype. In addition, the acquisition of the MDR phenotype was accompanied by increased expression of Tn antigen, which is biossintetized by pp-GalNAc-T6, of onf FN, an unusual isoform of FN found in cancer cells and embryonic tissues, as well as pp-GalNAc-T6, suggesting that atypical *O*-linked glycans and/ or the glycosyltransferase could play an important role in this process. To analyze this hypothesis, pp-GalNAc-T6 was silenced, which promoted an increase in Bax and Bad and a reduction in Bcl-2 and Bcl-xL protein levels, as well as a reduction in IC_{50} for the analyzed chemotherapeutics. The results obtained so far demonstrate, for the first time, the increased expression of onf-FN and pp-GalNAc-T6 in chemoresistant cancer cells, suggesting that the altered expression of glycosyltransferases and their products might be used for therapeutic purposes in cancer treatment.

Key words: Cancer, Multidrug resistance, Glycophenotype Change, pp-GalNAc-T6, Oncofetal Fibronectin

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CHARACTERIZATION OF REGULATORY REGIONS ASSOCIATED WITH *FLT3* GENE OVEREXPRESSION IN ACUTE LEUKEMIAS

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INTRODUCTION AND OBJECTIVES: *FLT3* overexpression (*FLT3*-high) is a recurrent alteration in acute leukemias (ALs), which can lead to constitutive activation of its tyrosine kinase receptor by a ligand-independent mechanism. This results in increased cell proliferation, reduced apoptosis and inhibition of cell differentiation. Over time, many studies have associated *FLT3*-high with the presence of *FLT3* activating mutations (*FLT3*-mut), which is considered the main cause of its transcriptional dysregulation. However, many *FLT3*-high patients do not harbor these mutations, suggesting that there are other unknown mechanisms responsible for this gene overexpression. With the advent of omics approaches combined with advanced strategies developed by bioinformatics, it has been possible to identify and characterize the role of regulatory elements (e.g. enhancers) in the dysregulation of several proto-oncogenes expressions. Given this scenario, we hypothesized that enhancers might act as the activating mechanism accounting for *FLT3*-high in ALs. Therefore, this study aims to identify and characterize regulatory regions associated with *FLT3*-high, as well as to track potential genetic alterations present in these regions. **MATERIAL AND METHODS:** We have identified transcriptionally active regions using Chromatin Immunoprecipitation Sequencing (ChIP-Seq) data for H3K27ac marker in AL cell lines. We have also analyzed RNA-seq data from patients diagnosed with AML, B-ALL and T-ALL deposited in the Therapeutically Applicable Research to Generate Effective Treatments (TARGET) database to identify enhancer RNAs (eRNAs). The differential activity of transcribed enhancers was performed using the DESeq2 method, comparing *FLT3*-high and *FLT3*-mut with the *FLT3*-low expression group. We also evaluated single nucleotide variants (SNVs), small insertions/deletions (indels) and copy number alterations (CNAs) in these regions using Whole Genome/Exome Sequencing (WGS/WES) and SNP array data from AL samples. **RESULTS AND CONCLUSION:** We identified 385 active regulatory regions in patients with *FLT3*-high in the context of AML, 1,551 in B-ALL and 726 in T-ALL. Most of these regions (75.8%, 73.2% and 76.5%, respectively) have motif enrichment for the PU.1 transcription factor, encoded by the *SPI1* gene, an important regulator of *FLT3* positive transcription. In both AML and B-ALL cases, we identified high activity of an eRNA close to the *FLT3* region. In the context of B-ALL and T-ALL, we observed high activity of an eRNA located on chromosome 6, in which 20% of B-ALL patients have amplification. Our data suggest the participation of these enhancers/eRNAs in the oncogenic activation of the *FLT3* gene. However, additional studies will be needed to further elucidate these interactions.

Keywords: 1. Acute leukemia; 2. *FLT3*; 3. Overexpression; 4. Enhancers; 5. eRNA
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HYDROGEN PEROXIDE GENERATION AS AN UNDERLYING RESPONSE TO HIGH EXTRACELLULAR INORGANIC PHOSPHATE (PI) IN BREAST CANCER CELLS

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INTRODUCTION AND OBJECTIVES: According to the growth rate hypothesis (GRH), tumour cells have high inorganic phosphate (Pi) demands due to accelerated proliferation. Compared to healthy individuals, cancer patients present with a nearly 2.5-fold higher Pi serum concentration. In this work, we show that an increasing concentration of Pi had the opposite effect on Pi-transporters only in MDA-MB-231 when compared to other breast cell lines: MCF-7 or MCF10-A (non-tumoural breast cell line). **MATERIAL AND METHODS:** Breast cell lines maintained in IMDM or DMEN-F12 medium are exposed to 1 mM Pi, as stated by the manufacturer. Different concentrations of NaH_2PO_4 were added to the regular medium 1 h or 24 h before the assays. MCF-10A, MCF-7 and MDA-MB-231 cells (5×10^4 cells per well) were incubated at 37 °C, 5% CO_2 atmosphere, for 1 h in a reaction mixture (0.5 mL) containing 116 mM NaCl or choline chloride, 0.1 mM KH_2PO_4 or 1 mM KH_2PO_4 (depending on the type of Pi transporter), 5.4 mM KCl, 5.5 mM glucose, 50 mM HEPES (pH 7.2), 0.8 mM $MgCl_2$ and 2.5 µCi/nmol ^{32}Pi. **RESULTS AND CONCLUSION**: Here, we show for the first time that high extracellular Pi concentration mediates ROS production in TNBC (MDA-MB-231). After a short-time exposure (1 h), Pi hyperpolarizes the mitochondrial membrane, increases mitochondrial ROS generation, impairs oxygen (O2) consumption and increases PKC activity. However, after 24 h Pi-exposure, the source of H2O2 seems to shift from mitochondria to an NADPH oxidase enzyme (NOX), through activation of PKC by H_2O_2. Exogenous-added H_2O_2 modulated Pi-transporters the same way as extracellular high Pi, which could be reversed by the addition of the antioxidant N-acetylcysteine (NAC). NAC was also able to abolish Pi-induced Epithelial-mesenchymal transition (EMT), migration and adhesion of MDA-MB-231. We believe that Pi transporters support part of the energy required for the metastatic processes stimulated by Pi and trigger Pi-induced H2O2 production as a signalling response to promote cell migration and adhesion.

Keywords: Pi-induced H2O2 production, breast cancer cells, H+-dependent Pi transport, Na+-dependent Pi transport, cell migration.
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GENES ASSOCIATED WITH LEUKEMIA AND DOWN SYNDROME: SYSTEMATIC REVIEW STUDY

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INTRODUCTION AND OBJECTIVES: Among the genetic alterations, Down syndrome (DS) is the most frequent chromosomal anomaly which can occur in three distinct forms: meiotic nondisjunction, Robertsonian translocation, or mosaicism. The greatest risk factor for this alteration is the advanced age of the parents, especially the maternal. Down's syndrome brings a set of physical characteristics and clinical alterations, such as vision, hearing and thyroid problems, otitis, convulsion, congenital heart disease, in addition to leukemia, which can be acute myeloid (AML) or lymphocytic leukemia (ALL). Leukemia is a form of malignant blood cancer that results from the mutation of white blood cells with a 20% mortality rate in children. Children with DS have a 500x more chance to develop AML, and 20x for ALL than children without the syndrome. However, there is little evidence about this relationship. Therefore, the objective of the present work was to identify and analyze the main genes involved in the development of leukemia in children with DS, highlighting their relevance and biological effects. **MATERIAL AND METHODS:** The present work consists of a systematic review of articles published in the last ten years (2012) on the genes involved in the main forms of leukemia observed in children with DS. Scientific articles in Portuguese and English found in the Pubmed and SciELO search platforms were selected, using the following descriptors: Down syndrome and (“childhood leukemia” or “infantile leukemia” or “infant leukemia” or “children leukemia”) and (“gene expression” or “genetics expression”). **RESULTS AND CONCLUSION**: As preliminary results, eight hundred and twelve articles were found, remaining two hundred after the exclusion of duplicates. Of these two hundred, after exclusion criteria, 12 articles were included in this review, which 5 were cell culture (41.7%), 4 case-control studies (33.3%), 2 cohort and case-control (16.7%) and 1 cohort and cell culture (8.3%). Of the articles included, twenty-one genes had increased expression (*GATA1, GATA1s, CRLF2, RUNX1, MYB, CCND2, SKI, HMGN1, PAX5, DYRK1A, LRRC32, USP36, LDLR, RFLNB, MPL, IL3RA, IKZF1, ERG, GATA2, VWF* and *WT1*), and eleven had reduced expression (*IRF4, NOTCH1, FLT3, ETS1, MEF2C, HES1, EBF1, IGH, PAX5, DYRK1A* and *WT1*). It is worth noting that the expression of some genes is increased or decreased depending on the type of leukemia. The highlighting of genes pathways, level of expression and functions can be to elucidate gene targets to a better understanding of the genetic factors affecting risk to development leukemia in DS children. Together, these results may be help in the diagnosis, prognosis and treatment individualized of these diseases.

Keywords: Down Syndrome. Childhood Leukemia. Genetics.
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INTERPLAY BETWEEN PRC2/EZH2 COMPLEX AND LONG NON-CODING RNAS IN THYROID CANCER

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INTRODUCTION AND OBJECTIVES: Thyroid cancer is the most common endocrine malignancy, comprising different types of tumors, such as anaplastic thyroid carcinoma (ATC) the rarest type but most lethal. Different genetic and epigenetic alterations are associated with the etiology of ATC, especially those in the MAPK pathway. Long non-coding RNAs are a broad class of non-coding RNAs (>200nt in length) that regulate genes' expression and interact with epigenetic modifiers, such as the EZH2/PRC2 complex. EZH2 catalyzes the trimethylation of histone H3 lysine's 27 residue, resulting in chromatin compaction and silencing of specific genes. In thyroid cancer, an increase in EZH2 is observed in ATC but the impact of the EZH2 and lncRNAs interaction on thyroid cancer's progression remains unclear. Therefore, we investigated the role of epigenetic regulation in ATC biology and analyze the relationship between lncRNAs and the PRC2/EZH2 complex. **MATERIAL AND METHODS:** To compare the expression of different lncRNAs in the two types of thyroid tumors, we used five different cell lines of thyroid carcinoma (TPC1, BCPAP, KTC2, SW1736, and 8305C). We profiled the expression of 90 lncRNAs using the Human and Mouse LncProfiles qPCR Array Kit (System Biosciences). For that, we used 1ug of total RNA from each cell line to generate the cDNA, according to the manufacturer's instructions. The gene expression was analyzed by qPCR using SYBR Green and the expression was normalized using 5 endogenous controls. We used TPC1 gene expression as a control to normalize the expression among cell lines. To identify lncRNAs interacting with EZH2 in ATC, we performed an RNA-IP using the SW1736 cell line. The cell lysate was incubated with anti-EZH2 antibodies. Then, we incubated the sample with the magnetic beads and after several washes on a magnetic rack, the total RNA was extracted from the beads with the trizol reagent. The cDNA was transcribed with random primers and the validation of the RNA-IP (lncRNAs interacting with EZH2) was performed by qPCR with specific primers for the lncRNAs (H19, HOTAIR, MALAT1, MEG3, and XIST). **RESULTS AND CONCLUSION**: Our gene expression analysis identified 36 lncRNAs with differential expression among cell lines. Of all the 90 lncRNAs tested, in this study, we identified differential expression in four of these lncRNAs known to interact with the EZH2/PRC2 complex in other tissues (H19, HOTAIR, TUG1, and XIST). These findings suggest heterogeneity in the expression of lncRNAs within the same tumor histotype. Our EZH2-associated RNA immunoprecipitation data show that HOTAIR, MALAT1, and XIST lncRNAs interact with EZH2 in anaplastic thyroid carcinoma. Thus, additional experiments focusing on these lncRNAs may improve the understanding of the role and functions of such lncRNAs in association with EZH2 and how this regulation affects tumor aggressiveness.

Keywords: thyroid, epigenetics, molecular biology, chromatin modifiers, RNA biology.
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EXPRESSION EVALUATION OF HER-2 AND β1 INTEGRIN IN HER-2+ BREAST CANCER CELLS LINES AFTER TREATMENT WITH TRASTUZUMAB

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INTRODUCTION AND OBJECTIVES: Breast cancer is characterized as a high mortality tumor and high heterogeneity. To define an optimal and most effective treatment, breast carcinoma is molecularly subdivided as luminal, HER-2 positive and triple negative. The positive HER-2 subtype is based on an overexpression of the *human epidermal growth factor receptor* (HER-2). This subtype represents more than 20% of the breast cancer cases, being associated with a worse prognosis. HER-2 has been studied as a potential target for more effective gene therapies. One of the most efficient therapeutic routes is the drug Trastuzumab, since it is a monoclonal antibody to HER-2 and has a cytotoxic action on breast cancer cells. Despite this, about 70% of patients with a HER-2 positive tumor prove to be resistant to treatment. One of the mechanisms responsible for the resistance could be the overexpression of β1 integrin, which helps in the progression of the tumor through pathways that induce angiogenesis and cell proliferation. Therefore, our group focuses on investigating the integrin action inhibition by Recombinant Disintegrins, originally obtained from snake venoms, in order to reverse the scenario of resistance to Trastuzumab. **MATERIAL AND METHODS:** For this study, Western Blotting and Immunofluorescence experiments were performed. In order to evaluate the expression of HER-2 and β1 integrin in breast cancer cell lines treated with trastuzumab and the cellular localization of HER-2, E-cadherin and β1 Integrin. **RESULTS AND CONCLUSION:** The resistant cell line HCC-1954 shows a higher expression of β1 integrin than the cell line BT-474, which is sensitive to treatment by trastuzumab. BT-474 showed two bands corresponding to two isoforms of β1 integrin. Furthermore, HCC-1954 cells showed a higher expression of β1 integrin after treatment. Even being a cell line with strong migration capacity, the immunofluorescence assay demonstrated a high expression of E-cadherin in HCC-1954 cells, which could be related with the plasticity of the epithelial-mesenchymal transition.

Keywords: cellular biology, oncobiology, breast cancer.
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GLYCOPHENOTYPC CHANGES IN THE 4T1-LUC2 CELL LINE AFTER NICOTINE RESISTANCE INDUCTION

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INTRODUCTION AND OBJECTIVES: Smoking is a public health problem and a preventable cause of death, as tobacco contains substances with high carcinogenic potential, which can induce breast cancer, for example, and according to the National Cancer Institute, is the leading cause of death by cancer in the female population in almost all regions of Brazil. One of the greatest difficulties in the treatment of cancer is resistance to chemotherapeutics, so studies like this are extremely relevant to assess possible cellular changes and understand whether they are linked to cellular resistance. The present study aims to evaluate changes in the glycophenotypes of murine mammary gland adenocarcinoma cells (4T1-LUC2) induced by nicotine, one of the components of tobacco, in order to search for possible markers related to more invasive behaviors that are attributed to tobacco resistance. **MATERIAL AND METHODS:** Flow cytometry assay was performed to compare the glycophenotype between control and chronically nicotine treated cells, using the following FITC-conjugated lectins (and their specificities): PNA (Gal-GalNac), MAA (alpha 2- 3 linked), SNA (sialic acid linked to terminal galactose), AF, Con-A (alpha-mannose, alpha-D-glucose and D-fructose), HPA (alpha-N-acetylgalactosamine residues), PHA-E (terminal residues of galactose, N-acetylglucosamine and mannose), PHA-L (N-acetylglucosamine beta 1-2) and VVL (terminal N-acetylgalactosamine). The western blot assay was performed using antibodies against bax, bcl-2, oncofetal fibronectin and total fibronectin, aiming to verify changes in the expression of these proteins that can regulate resistance processes. In addition, the MTT assay was also performed to obtain the IC50 of nicotine in the cells studied. **RESULTS AND CONCLUSION:** The flow cytometry assay shows an increase in SNA and MAA binding of 40% and 15% respectively, along with a 25% decrease in PNA binding. The other lectins did not show significant changes. These findings suggest an increase in sugars that are specific in the cellular chemoresistance process. The western blot assay shows that bcl-2 expression is increased 3-fold in cells treated chronically with nicotine. The results showed that there were alterations in the glycophenotype of these cells and an increase in nicotine-induced antiapoptotic proteins, suggesting that the 4T1-LUC2 lineage cells may have developed a resistant phenotype.

Keywords: Chemoresistance, Nicotine, Cancer.
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ANTINEOPLASTIC POTENTIAL OF *Jatropha multifida L*.

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INTRODUCTION AND OBJECTIVE: *Jatropha multifida Linnaeus*, popularly known as "flor-de-coral", "flor-de-sangue", "merthiolate" is a plant species of the genus *Euphorbia*, whose chemotaxonomic characteristic is the production of latex. Euphorbiaceae contains about 290 genera and approximately 7.500 species, dispersed across continents, mainly in tropical regions. It is popularly used to treat various conditions, such as fever, pain, infection, wounds, ulcers and various infectious skin diseases; It is used by African folklore to treat bacterial infections. Preliminary academic reports demonstrated that this species has biological therapeutic potential. The objective is to investigate in the official literature the antineoplastic therapeutic biological potential of the species *Jatropha multifida L*. **MATERIAL AND METHOD:** Search using the descriptors "*Jatropha mutifida*", and "*Jatropha multifida* AND cancer" for each of the cited databases: PubMed, OPAS, Web of Science, BVS and Google. Reading of the abstracts was carried out, and, later, a selection of those that best suited the proposed objective was made. **RESULTS AND CONCLUSION:** Using descriptor "*Jatropha multifida*", 42 articles were found in the PubMed database, no articles were found in the OPAS database, 59 articles in the Web of Science database, 41 articles in the BVS database. The studies proved the various biological activities of medicinal interest of the species, including its antineoplastic effect, which was studied and evidenced in five articles, published in the years 2009, 2011, 2014, 2018 and 2019. The articles about antineoplastic activity were the same as those found in PubMed, Web of Science and BVS databases. They showed *in vitro* cytotoxic activity for cell lines of solid malignant neoplasms such as breast cancer, lung carcinoma, uterine cancer, liver cancer, mouse neuroblastoma; and against liquid malignant neoplasm cell lines, such as acute monocytic leukemia and acute promyelocytic leukemia. After searching for the articles found, it can be said that *Jatropha multifida L*. has potential antineoplastic activity. After receiving botanical certification of a specimen from Rio de Janeiro, assays will be carried out to elucidate cytotoxic activity and its possible mechanism of action.

Keywords: *Jatropha multifida*, cytotoxicity, cancer lineages.

There's no support, neither conflict of interest.

DEVELOPMENT OF BIOACTIVE ASSOCIATED NANOSYSTEMS AND INVESTIGATION OF THE ANTITUMORAL EFFECT ON LUNG CARCINOMA CELLS

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INTRODUCTION AND OBJECTIVES: Chronic diseases, such as cancer, affect the population worldwide, and often do not have an adequate treatment or even a cure, and continuous research is needed to improve this current picture. Bioactive groups have shown promise for this purpose, one of them being pentacyclic triterpenes, which belong to a family of terpenes and have several applications in pharmacological areas. However, the low solubility of these compounds is a limiting factor for their pharmacological application, since it influences bioavailability and pharmacokinetic mechanisms. In this work we intend to extract from plants and incorporate these triterpenes in nanosystems to facilitate their delivery into physiological systems and obtain a growth inhibiting activity, since existing studies show their ability to induce apoptosis and model the tumor environment, combining these features with their anti-inflammatory power. **MATERIAL AND METHODS:** For the extraction of triterpenes, two species of plants were used, Licania tomentosa and Maytenus robusta, where they were dried and macerated for an extraction with acetone and methanol in a 1:1 ratio of the material by the soxhlet method. After this period the solution was filtered and reduced in a rotary evaporator. After the initial volume reduction HCl will be added to the solution for the precipitation of the extracted compounds, which will be characterized next. For the polymeric encapsulation an alginate solution was prepared, where the triterpene extract will be added. This material will be dripped into a calcium chloride solution, which will be agitated by a sonicator during the process, to promote the breakage of the alginate solution drops. **RESULTS AND CONCLUSION:** For the main results we will be evaluating the potential of extraction of triterpenes from natural extracts, aiming to encapsulate the compounds in nanostructured polymeric systems, so that they are easily internalized by the cells. In this way we hope that the nano-systems will be able to direct the compounds into the tumor cells and, already inside them, release the compounds. After this step we will evaluate the antitumor potential of the

compounds, observing cytotoxic effects, from cell death, as well as the safe dosages of the compounds and nanosystems. With the progress of the project and the final results obtained, it is expected that a highly efficient, high quality extract will be obtained, which will be used in the next steps of the formulation development, such as the biological tests to prove the antitumor activity, cytotoxicity, and encapsulation efficiency in order to obtain an effective final drug.

Keywords: cancer, bioactive, nanosystem

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IMPORTIN EXPRESSION AND INHIBITION IN BREAST CANCER: A POTENTIAL MODEL TO STUDY XIAP NUCLEAR LOCALIZATION

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INTRODUCTION AND OBJECTIVES: Breast cancer is a neoplasm that has high incidence and mortality rates among women, mainly due to the development of resistance to treatment. Among the most well-known resistance mechanisms described, we have the evasion from apoptosis. XIAP (X-linked inhibitor of apoptosis protein) is an inhibitor of apoptosis (IAP) protein whose canonical functions are the direct inhibition of caspases and the ubiquitination of pro-apoptotic targets. XIAP is mainly expressed at the cytoplasm, but some studies have provided evidence on its expression at the nucleus. We have demonstrated in a previous work that nuclear expression of XIAP was associated with poor prognosis in a subgroup of patients with breast cancer. In addition, classical nucleocytoplasmic transport is often modified in cancer, which may result in altered subcellular localization of XIAP. In this context, the aim of the study is to investigate the expression and role of importins α and β1 in the mechanisms of nuclear translocation of proteins by using their respective inhibitors, ivermectin and INI-43, in breast cancer cells. **MATERIAL AND METHODS***:* To start this study**,** we analyzed gene and protein expression of α1, α2 and β1 importins in breast cancer cell lines by real time PCR and Western Blotting. Subsequently*,* we performed MTT and clonogenic assays with nuclear import inhibitors of α/β and β1 importins, Ivermectin and INI-43, respectively, in MCF-7 and MDA-MB-231 cell lines**.** Furthermore, we assessed cytotoxicity of inhibitors with lymphocytes isolated from peripheral blood of healthy individuals*.* In parallel, we are currently working on the generation of a doxorubicin resistant model (MCF-7 Dox^R) as a potential model to study aberrant subcellular localization of proteins, including XIAP**.** **RESULTS AND CONCLUSION:** We confirmed gene expression of the α1, α2 and β1 importins and verified differential protein expression in breast cancer cell lines exhibiting distinct drug resistance phenotypes. We also observed that MCF-7 and MDA-MB-231 cell lines were sensitive to importin inhibitors and could determine the subtoxic concentrations of the pharmacological inhibitors, that did not sensitize non-neoplastic cells. Furthermore, the MCF-7 Dox^R cell line showed overexpression of XIAP when compared to parental cells, indicating a potential cellular model for studying nuclear XIAP. In future experiments, the subtoxic concentrations of INI-43 and ivermectin will be used to investigate the potential modulation in XIAP subcellular localization in breast cancer models exhibiting endogenous and exogenous nuclear XIAP.

Keywords: XIAP protein; Breast cancer; Nuclear-cytoplasmic transport; Nuclear import; INI-43; Ivermectin.
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IMPLANTATION OF A MURINE MELANOMA ORTHOTOPIC METASTASIS MODEL

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INTRODUCTION AND OBJECTIVES: Melanoma is a type of skin cancer that has a high mortality rate when the patient has the disease in the metastasic stage. In the most commonly models used to study the mechanisms of melanoma metastasis in mice, the formation of the primary tumor occurs in a tissue distinct from the one in which the malignant neoplastic cell originated. This characteristic of those models means that the interactions that may occur between the stroma of the tissue of origin and the lineage of cancer cells used in those studies are disregarded, which, as already demonstrated, influences the behavior of tumor cells during lymphogenic and visceral metastases.
Thus, the general objective of the project is to implement an orthotopic metastasis model (in which the primary tumor develops in the tissue of origin of the B16F10 lineage) of murine melanoma in C57BL/6 mice, so that important characteristics of the disease can be defined about the evolution of the disease in mice throughout the metastasis process. In addition, mechanisms of interaction between B16F10 and lymph nodes, through which the lymphogenic and spontaneous metastasis that the model provides, will also be evaluated.
MATERIAL AND METHODS: The cells used in the experiments are from the B16F10 murine melanoma lineage that were transduced to express the Luciferin gene or the green fluorescent protein (GFP) gene. They are implanted intradermally on the dorsal side of the ear of mice and during the formation of the primary tumor they invade and are drained to the cervical lymph node that will act as a sentinel lymph node, initiating the process of lymphogenic metastasis. After the time required for lymph node invasion to occur, the mice are euthanized and samples from sentinel lymph nodes are collected to assess their invasion by transduced B16F10 cells.
RESULTS AND CONCLUSION: The expected results of this pilot study are, until then, to define the amount of time required for the cells to reach the lymph node and the intensity of this invasion that will happen during metastasis, and to find an adequate concentration of tumor cells to be injected into C57BL/6 mice, for the evolution of metastasis to occur at a rate consistent with the disease in humans, and to allow the analysis of the metastatic mechanisms involved. We also want to define which are other methods that can be used in the future with this model.

Keywords: Cancer, Metastasis, Melanoma.
Supported by: Conselho Nacional de Desenvolvimento Científico e Tecnológico, CNPq.

EVALUATION OF THE FREQUENCY OF ACTIVATED T CELLS IN THE PERIPHERAL BLOOD OF PATIENTS WITH HEAD AND NECK SQUAMOUS CELL CARCINOMA.

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INTRODUCTION AND OBJECTIVES: Head and neck cancers are diverse, common and serious. Data reveal that these neoplasms represent 4% of all types of cancer in Brazil and records show about 43,000 new cases annually. Surgery, radiotherapy and chemotherapy are the therapeutic weapons available to specialists. Recently, other types of treatment are being envisioned, based on immunology, in view of the relationship between cancer and inflammation. Activation of T cells and immune checkpoint molecules is important for the immune response to cancers. Evaluation of activation markers and immunological checkpoint molecules include human transmembrane C-type lectin protein (CD69) considered an early activation marker, interleukin-2 receptor alpha subunit (CD25) expressed on activated T and B cells, and the programmed death protein 1 (PD-1) expressed in activated T cells, may help to understand the immune response and help in the development of therapeutic strategies to enhance oncologic outcomes. To evaluate the frequency of activated CD4 and CD8 T cells in patients with head and neck squamous cell carcinoma. **MATERIAL AND METHODS:** A preliminary study of immunophenotyping assay was performed in peripheral blood, using flow cytometry to quantify activated CD4 and CD8 T cells. For this evaluation, the following antibodies CD3 Pacific Blue, CD4 Pacific Orange, CD8 PE, CD25 PerCP, CD69 APC and PD-1 FITC were used. 100,000 events were acquired for each patient sample and data analyzes were performed using the Infinicity program (Cytognos). The present study was approved by the Research Ethics Committee (CEP) under protocol number 3,967,267. **RESULTS AND CONCLUSION**: The results obtained in this preliminary study showed a mean frequency of the CD8/CD25+/CD69+/PD-1+ population (3.34%) higher than the CD4/CD25+/CD69+/PD-1+ population (1, 90%) within the lymphocyte region (CD3+). It is still necessary to increase the number of individuals in the study, but the results showed that the activated and reactive tumor CD8 population was increased, probably because it is the subpopulation of T cells with the greatest potential to mediate antitumor responses.

Keywords: head and neck squamous carcinoma, activated T lymphocytes, immunphenotyping.
Supported by: Secretaria Estadual de Saúde/RJ, FAPERJ.

THE ROLE OF ACTIVE AND REACTIVE ENTERIC GLIAL CELLS TO COLORECTAL TUMOR CELLS

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INTRODUCTION AND OBJECTIVES: In normal physiological conditions, the enteric glial cell (EGC) is essential to numerous functions, such as the regulation of the intestinal epithelial barrier (IEB). However, in a pathological condition, enteric glia become reactive and assume a pro-inflammatory role, expressing markers such as NFKB and overexpressing S100b. It has already been described that, in the colorectal cancer context, the glial cells act promoting the proliferation of tumor stem cells, stimulating the tumorigenesis, and expressing factors such as PGE2 and IL-1. Our project aims to comprehend how EGC becomes reactive when facing inflammatory and tumoral insults, and the implication of this reactive state to the IEB and colorectal cancer.
MATERIAL AND METHODS: We made culturing experiments of the colorectal tumor lineage HCT116 with or without conditioned medium of cells of the EGC lineage challenged with LPS or with the conditioned medium of HCT116 itself. We also performed a cell viability assay by MTT, subjecting HCT116 under different conditions involving treatment with conditioned medium of EGC, conditioned medium of LPS-treated EGC a and the HCT116-treated EGC and an immunocytochemistry assay under the same conditions as MTT.
RESULTS AND CONCLUSION: Our preliminary results indicate that, under conditions where the factors secreted by EGC were present, there was a similar cell proliferation compared to the control condition. But the LPS condition had a significant increase in tumor growth. This work will allow us to understand the role of EGC in its normal or reactive state in the maintenance of IEB and against colorectal tumors.

Keywords: Colorectal cancer, Enteric glia, Enteric nervous system.
Supported by: FAPERJ and CNPq.

THE INFLUENCE OF PMA ON RESISTANCE TO MAPK PATHWAY INHIBITORS IN MELANOMA CELL LINES.

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Introduction and objectives: The mitogen-activated protein kinase (MAPK) pathway constitutes one of the signalling pathways commonly altered in cutaneous melanoma, as it is hyperactivated by oncogenes. The most common mutation in this pathway occurs in *BRAF*, causing uncontrolled cell cycle progression and neoplastic proliferation. Therefore, targeting BRAF is widely used as a therapeutic strategy against *BRAF*-mutant melanoma. However, despite the positive results, therapeutic resistance still presents a challenge. Phorbol 13-acetate-12 myristate (PMA) is a molecule analogous to diacylglycerol (DAG), an intracellular secondary messenger that activates protein kinase C (PKC), a protein whose different isoforms were shown to be related to melanoma prognosis. PMA has been also shown to activate the MAPK pathway. Therefore, the aim of this project is to study the effect of PMA in sensitivity to MAPK pathway inhibitors in melanoma cell lines. **Materials and methods**: 888mel cells were cultured in 96-well plates (3,000/well) and 24 hours later treated with 8 different doses of the BRAF inhibitor PLX4720 or the MEK1/2 inhibitor Trametinib in the presence and absence of 10nM of PMA. Cell viability was assessed after 3 days with CellTiter Blue (Promega) using the Spectramax iD3 plate reader at 560-600nm. Values were normalized considering the negative control (DMSO) as 100% viability and the positive control (Phenylarsine oxide) as 0% viable cells. Dose-response curves were generated in GraphPad Prism 9.0 from the average of the 3 experimental replicates and IC50 values were calculated from each condition. To analyse the role of PMA in PKC and MAPK activation, western blotting was performed with lysates from 888mel cells treated with 10nM PMA +/- 0.8μM of PLX4720 for 8 hours and 6 days. Detection of total and phosphorylated ERK and PKC were analysed. Vinculin and GAPDH were used as loading controls. **Results and Conclusion**: Dose-response assays showed that the presence of PMA significantly decreased sensitivity of 888mel cells to PLX4720 and Trametinib. Western blot analysis demonstrated that 8 hours of exposure to PMA increased phosphorylation of ERK and PKC, indicating activation of both proteins. We also observed that treatment with PLX4720 alone inhibited the MAPK pathway. However, in the presence of PMA, there was an increase in phosphorylation of

ERK and PKC, demonstrating that PMA can reverse the effects of PLX4720.

Keywords: The mitogen-activated protein kinases (MAPK), Phorbol 13-acetate-12 myristate (PMA), PLX4720 and Trametinib.

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THE IMPACT OF THE COVID-19 PANDEMIC ON BREAST CANCER SCREENING AND DIAGNOSIS IN BRAZIL

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INTRODUCTION AND OBJECTIVES: Breast cancer is the most commonly diagnosed neoplasm worldwide with a high rate of incidence and mortality and, therefore, it is an important global health problem. In Brazil, 66,280 new cases of breast tumor in women are expected for each year in the 2020-2022 biennium. The COVID-19 pandemic has presented significant challenges and implications for the screening and diagnosis of diseases. The reduced access to health care caused by the COVID-19 pandemic setting closures may result in delays in diagnosis and treatment that may impact the cancer incidence, disease staging and mortality. Thus, the aim of this study was to systematically review and describe the literature on the short-term effects of the COVID-19 pandemic on breast cancer screening and diagnosis in women from Brazil. **MATERIAL AND METHODS:** A literature research was performed to identify publications that studied the impacts of the COVID-19 pandemic on breast cancer screening and diagnosis in Brazil. Eligible studies were identified using two international databases (PubMed and SciELO) between the years 2020 and 2022. The search terms were: (breast cancer AND diagnosis AND COVID AND Brazil) OR (breast cancer AND screening AND COVID AND Brazil) OR (breast cancer AND screening AND diagnosis AND pandemic AND Brazil) OR (cancer de mama AND pandemia AND Brasil). **RESULTS AND CONCLUSION**: Eighty-two publications were identified in the databases, but only 8 articles were eligible. A total of 13.256.148 women between 2019 and 2021 were included and ages ranged from 25 to 69 years old, but most were between 50 and 60 years old. Two studies compared the percentage of mammogram exams performed in the 27 federative units of Brazil between 2019 and 2020 with a total reduction of 42% in the performance of mammograms. Five studies analyzed only women from the state of São Paulo and 1 study performed a snowball sampling, identifying 80.2% of delays or non-performance to mammograms in women with age to undergo the exam. The articles presented data for screening mammography and/or diagnostic mammography with risk classification and/or biopsy. We observed that the number of performed exams was reduced in about 35% to 83.4% during the pandemic, compared to the previous year. Three studies observed that 79.7-88.9% of confirmed cases of breast cancer during the pandemic were symptomatic. In addition, 2 studies described the impacts of the pandemic on breast cancer diagnosis: there was a reduction of 11.8% to 48.7% in the number of confirmed cases and probably four thousand cases were not detected in Brazil during the pandemic. In conclusion, we observed that the COVID-19 pandemic negatively impacted the screening and diagnosis of breast cancer in Brazil, compared to the year 2019.

Keywords: Breast neoplasm, COVID-19 pandemic, Detection of cancer, Diagnosis of cancer.
Supported by: FAPERJ e CNPq.

CYTOTOXIC EFFECT OF ISOLATED COMPOUND FROM *EREMANTHUS CROTONOIDES* AGAINST CANCER CELL LINES

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INTRODUCTION AND OBJECTIVE: Cancer is one of the main health problems in the world, according to The World Health Organization, in 2016, it was responsible for 71% of deaths from non-communicable diseases, standing out for its high incidence and low survival, despite advances in diagnosis and treatment. Therefore, the development of new drugs is extremely relevant. *Eremanthus crotonoides* are plants of the Asteraceae family, and the genus Eremanthus, they are endemic in Brazil. The genus Eremanthus are usually found in *cerrado* regions, but *E. crotonoides* can also be found in restinga forest. The aim of this study was to perform the phytochemical analysis of *Eremanthus crotonoides* leaves, evaluating the antitumor effect induced by the ethanol extract of the leaves and Centraterin, substance isolated from it, in cancer cell lines. **MATERIAL AND METHODS:** The crude ethanolic extract was prepared from *Eremanthus crotonoides* leaves. Then, the crude ethanolic extract of the leaves was partitioned in n-hexane, dichloromethane, ethyl acetate and n-butanol fractions. Of the dichloromethane fraction the Centraterin was isolated by thin layer chromatography. Cell viability and selective index determination assays were performed by MTT. The IC_{50} was calculated by a non-linear regression curve in the GraphPad Prism 5 program. For morphological analysis, cells of the HT-29 lineage were treated with Centraterin ($2xIC_{50}$) and a video was made in time lapse format. The determination of the formation of reactive oxygen species was performed using the Ros-Glo™ H_2O_2 Assay (Promega). Time-lapse video were performed to determine cell death morphology. Cell death pathway and cell cycle alterations were evaluated by flow cytometry. Pharmacological proprieties of Centraterin was determined by SwissADME analyses and “Lipinski Rule of 5”. **RESULTS AND CONCLUSION:** Viability assays showed that *E. crotonoides* ethanol extract was highly cytotoxic in tumor cells of the SCC9 lineage (IC50 11.37µg/ml) and in normal cells, primary cultured human tongue fibroblasts (IC50 12.40µg/ml). Centraterin also obtained high levels of cytotoxicity, showing against SCC9 (IC_{50} 1.703µM), HT-29 (IC_{50} 0.50µM), 4T1 (IC_{50} 0.74µM), B16F10 (IC_{50} 0.54µM) and HeLa (IC_{50} 1.30µM), and against normal cells, primary cultured human tongue fibroblasts (IC_{50} 1.04µM). Demonstrating that Centraterin is more than twice chemically active in tumor cells of the HT-29 lineage when compared to normal cells (Selective Index > 2). The morphological analysis indicated that Centraterin acts by a necrotic pathway of cell death that was not reversed by necrostatin (inhibitor of necroptosis). This phenotype is corroborated by flow cytometry analysis were is no effector caspases activation of DNA fragmentation. A high ROS (reactive oxygen species) production might explain this phenotype. Centraterine shown a good pharmacological profile were future experiments will determine its usefulness for cancer treatment.

Keywords: Câncer; Eremanthus crotonoides; Centraterin; Cytotoxicity;
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INFLUENCE OF GALECTIN-3 ON DNA DAMAGE RELATED GENES EXPRESSION IN BREAST CANCER CELL LINES

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INTRODUCTION AND OBJECTIVES: Cancer is among the leading causes of death worldwide. This disease can affect many tissues of the human body, breast cancer being the most frequent and important disease affecting women today. Its development and response to therapeutic approaches undergo molecular mechanisms related to DNA damage repair (DDR). In this context, our group has been studying the role and influence of galectin-3 protein *(*GAL3*)* in response to DNA damage inducing agents, such as chemotherapeutic drugs and ionizing radiation. The expression levels of GAL3 varies in several tumor types compared to healthy tissues and higher levels of *GAL3* were associated with worse prognosis in colorectal , breast and ovarian cancer, among others. Additionally, our group showed that the absence of *GAL3* can influence the expression of genes related to angiogenic and inflammatory processes, as well as increase resistance of different DNA damage-inducing agents, which may suggest that changes in this protein expression can also affect gene expression in the DDR pathway. This work aims to analyze the gene expression of selected DDR targets based on *in silico* database screening using GAL3 silenced breast cancer cell lines. **MATERIALS AND METHODS:** The expression of 134 genes encoding DDR-related proteins was evaluated using the *in silico* tool GEPIA2. Genes with statistically different expression between tumor and normal samples were selected to be further analyzed using reverse-transcriptase PCR and real-time PCR in breast cancer cell lines MCF7 and MDA-MB231 stably silenced for *LGALS3* or the unrelated control Scrambled (SCRB). **RESULTS AND CONCLUSION:** GEPIA database analysis returned 43 differentially expressed genes in breast cancer samples, of which *EXO1, WRN, LIG4, CTIP, UBE2N, TP53BP1, RAD51D, POL KAPPA, MDC1, BLM, H2AFX, RAD51, RMI1 and RMI2* have been tested to date. Mutations and expression changes in these genes were reported to be involved with angiogenesis and tumor progression of several cancer types. Differences between MCF7 and MDA-MB231 cells lacking *GAL3* expression and their respective controls were observed for *EXO1, WRN, LIG4, CTIP, UBE2N, TP53BP1, RAD51D, RMI2, POL KAPPA, MDC1 and BLM* genes. The data presented in this work may contribute to the elucidation of the mechanisms underlying *GAL3* roles, and its expression effects, in tumorigenesis and progression of breast cancer patients.

Keywords: Galectin-3, DNA damage, Breast Cancer

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EFFECT OF PIPERINE ON MUTANT p53 (R248Q) AGGREGATION IN NON-SOLID TUMORS

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INTRODUCTION AND OBJECTIVES: p53 plays an important role in tumor suppression, either by inducing cell cycle arrest and repairing cellular DNA damage, or by promoting cell apoptosis. However, several types of cancer are characterized by a high incidence of *TP53* gene mutation, which in turn is capable of altering p53 conformation. This can trigger p53 intracellular aggregation, causing loss of p53 normal functions, or even generating a gain in p53 oncogenic functions. Piperine, a bioactive compound present in black pepper, presents an important chemopreventive and chemotherapeutic action in *in vitro* and *in vivo* studies. However, the mechanisms of action of this compound have not been fully elucidated, especially regarding the possible involvement of p53 (wild-type or mutant form). Thus, the aim of this work is to investigate the effect of piperine on cells that do not express p53, in comparison with cells that express wild-type or mutant p53 (R248Q). **MATERIAL AND METHODS:** HL60 (acute promyelocytic leukemia cell line that does not express p53), REH (acute lymphoblastic leukemia cell line expressing wild-type p53), and Namalwa (lymphoma cell line expressing mutant p53 R248Q) were used in the experiments. Initially, mitochondrial metabolism was evaluated by the MTT reduction method. Then, immunocytochemistry experiments were performed under a fluorescence microscope. Subsequently, a p53 Seprion-ELISA analysis was performed. Finally, analysis were carried out by fluorescence spectroscopy. **RESULTS AND CONCLUSION:** MTT reduction assay demonstrated that piperine promoted a dose- and time-dependent effect on HL60, REH, and Namalwa cell lines. However, it had a greater cytotoxic effect on HL60 cells and a lesser effect on Namalwa cells, suggesting a possible p53-independent mechanism. The immunocytochemistry experiments indicated the presence of amyloid-like aggregates in HL60, REH and Namalwa cell lines. However these aggregates only co-localized with p53 in Namalwa cells. suggesting the existence of amyloid-like p53 aggregates in this lymphoma cell line, whereas piperine has shown to increase these p53 aggregates. Then, by p53-seprion-ELISA, we verified that the cell extract of Namalwa had the highest amount of p53 in the amyloid fraction, even when compared to the positive control (MDA-MB-231). In addition, piperine further increased these p53 aggregates in Namalwa cells. Finally, the data obtained by fluorescence spectroscopy suggest that piperine was able to induce the formation of mutant p53 (R248Q) aggregates *in vitro*. Together, our results suggest that Namalwa cells could be more resistant to piperine action, possibly due to the increased aggregation of mutant p53 (R248Q). Thus, the investigation of the involvement of p53 in the action of compounds, such as piperine, may be important to elucidate the mechanisms of action in cancer cells.

Keywords: Piperine, p53 and Cancer.

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LONCHOCARPIN ENHANCES 5-FU CHEMOTHERAPY EFFECTS AND IMPAIRS COLORECTAL CANCER ONCOSPHERES GROWTH

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INTRODUCTION AND OBJECTIVE: The Wnt/β-catenin signaling pathway plays a central role in regulating the levels of the β-catenin protein and is associated with events such as the establishment of embryonic axes, controlling cell proliferation, adhesion, and differentiation, as well as stem-cells maintaining in adult tissues. However, uncontrolled activation of the pathway results in many cancers such as colorectal cancer (CRC). The chemotherapy drug 5-Fluorouracil (5-FU) is widely used in CRC treatments but has limited activity in many cases, and this resistance and tumor recurrence may be associated with many factors such as intra-tumor heterogeneity, tumor microenvironment (TME), and the existence of cancer stem cells (CSCs), which is impossible to solve with the standard approaches currently available in clinical practice. In this sense, we sought to develop a platform of three-dimensional cell structures such as oncospheres and organoids, to test the effects of the combination of the chemotherapy drug 5-FU with Wnt/β-catenin inhibitor such as the chalcone lonchocarpin. **MATERIAL AND METHODS:** We used the CRC cell lines HCT116 and DLD-1, which harbor a mutation in β-catenin and APC, respectively, to develop a three-dimensional cell culture by oncospheres through a hanging drop. To analyze the effects of 5-FU and lonchocarpin in 3D cell culture, the oncospheres were treated with 10μM 5-FU and 20 μM lonchocarpin alone or combined 1:1 for 48 hours. The control group corresponds to the volume corresponding to the vehicle of each drug, DMSO, and saline. **RESULTS AND CONCLUSION**: We observed that the combined treatment with 5-FU and lonchocarpin altered the CRC spherical morphology and decreased the size of the spheres compared to the control group. However, the combined treatment of 5-FU and lonchocarpin was more efficient compared to either 5-FU or lonchocarpin alone. Our data support that the inhibition of the Wnt/β-catenin signaling pathway can enhance 5-FU effects in 3D cancer models.

Keywords: Wnt signaling; 5-FU; colorectal cancer

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THE ROLE OF LAMININ AND PTEN IN THE NEUROGENIC POTENTIAL OF ENTERIC GLIA.

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Introduction and Objective: The enteric nervous system (ENS) constitutes the intrinsic innervation of the digestive system. Enteric glial cells (EGCs) and neurons that composite the ENS form the myenteric and submucosal plexus. EGCs have important functions, such as regulating the intestinal epithelial barrier, acting on communication between neurons, as well as acting as a progenitor cell in certain situations. The EGC differentiation into neurons is well known in vitro and some situations of injury in vivo. However, signaling pathways involved in this process are still not known. Laminin is an extracellular matrix protein present in the muscular layers of the intestinal wall, and the EGCs are found in the midst of this laminin network. PTEN is a phosphatase that inhibits PI3K/AKT signaling. Deletion of PTEN in the ENS has already been shown to result in enteric ganglion hyperplasia. In ex vivo myenteric plexus culture, PTEN inhibition increases EGC proliferation and differentiation into neurons.

In our previous work, Veríssimo et al (2019), we showed that, when culturing EGCs most of the cells after 3 days consisted of GFAP-positive EGCs and only a minority were double positive for GFAP and βIIItubulin. After seven days, most had dual expression of GFAP and βIIItubulin, which indicates that the EGCs were funder transdifferentiation into neurons. A process that has its rate reduced when cells are cultured on polylaminin, suggesting that this can inhibits the neuronal differentiation of EGCs from adult mice. The objective of this work is to investigate the differentiation potential of EGC in vitro, the role of laminin in this process, and the relationship with PTEN activity.

Materials and Methods: EGC cultures from Swiss or C57BL/6 mouse on fibronectin or polylaminin substrate. The cells will be evaluated by immunofluorescence and western-blotting, for the expression of glial (GFAP and S100β) and neuronal (βIIItubulin and HUC/D) markers, as well as the presence of PTEN, active or inactive form, and also AKT (pathway effector). The proliferation rate will also be evaluated.

Results and Conclusions: Our initial data indicate an increase in proliferation in the presence of fetal bovine serum, which also interferes with cell morphology. The percentage of cells doubly labeled for GFAP and βIIItubulin is being evaluated. In a next step we will have the analysis for PTEN, phospho-PTEN. We believe that the PI3k/AKT pathway may be involved through PTEN in activating the neurogenic potential of enteric glia.

Keywords: Enteric glia, Neurogeneses, Laminin.

GLICOPHENOTYPIC ANALYSIS OF HUMAN LUNG ADENOCARCINOMA CELL LINE TREATED CHRONICALLY WITH NICOTINE

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INTRODUCTION AND OBJECTIVES: Smoking is a risk factor for the development of several types of cancers due to the presence of carcinogens in cigarette smoke. It has already been demonstrated that nicotine, the main addictive component of cigarettes, has a fundamental role in cancer initiation and progression since it promotes survival, proliferation, resistance to therapy and activation of epithelial-mesenchymal transition (EMT) program. The epithelial-mesenchymal transition occurs by the loss of the epithelial phenotype of cancer cells and acquisition of mesenchymal characteristics - such as increased invasion capacity and motility - which confers to cancer the ability to establish metastasis. In addition, studies have already demonstrated the potential of nicotine in inducing multiple drug resistance (MDR), a phenotype that is the main obstacle in cancer treatment. Furthemore, it is observed that cancer cells have a differentiated glycosylation pattern when compared to normal cells, and this glicophenotypic alteration is capable of inducing metastasis and drug resistance. The literature has already described the potential of nicotine in modulating the intracellular phenotype of glycosylation, but its action on extracellular glycosylation has never been investigated. Thus, the general objective of this work is to evaluate the impact of chronic treatment with nicotine on the extracellular glycophenotype of lung cancer tumor cells and how this affects tumor progression. **MATERIAL AND METHODS:** First, through the MTT assay, we found the concentrations of IC-20 and IC-50 of the A549 lung adenocarcinoma cell line, which were 3mM and 12mM, respectively. Thereafter, the cells were submitted to chronic treatment with nicotine for 1 month. Subsequently, we analyzed the acquisition of resistance not only to nicotine but also to the chemotherapeutic agents cisplatin and doxorubicin in parental and nicotine-treated cells through the MTT assay. In addition, using flow cytometry, with the help of lectins, we compared the glycophenotype of nicotine-treated and parental cells. **RESULTS AND CONCLUSION:** Glycophenotypic analysis revealed reduced expression of the epitope Galβ1-3GalNAc, monitored by *Peanut agglutinin lectin* (PNA), as well as bisected *N*-linked glycans, which were investigated with the lectin *Phaseolus vulgaris erythroagglutinin* (PHA-E). Regarding the *O*-linked glycans, it was observed an increase in the expression of Tn antigen, which was evidenced by the *Helix pomatia* lectin (HPA), suggesting that these glycans, in the cells treated with nicotine, were truncated. Taking together these preliminary data indicate that there is accumulation of truncated *O*-linked glycans and reduced expression of sialoglycans, as well as tri or tetra-antennary and bisected *N*-glycans. New experiments are underway in our laboratory to better understand the impact of these changes on glycosylation in cells chronically exposed to the carcinogen nicotine.

Keywords: Cancer, nicotine, glycosylation, epithelial-mesenchymal transition, multiple drug resistance

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QUANTIFICATION OF NAÏVE AND MEMORY T LYMPHOCYTES IN THE PERIPHERAL BLOOD OF PATIENTS WITH SQUAMOUS CELL CARCINOMA OF THE HEAD AND NECK

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INTRODUCTION AND OBJECTIVES: Head and neck cancer (HNC) is a frequent tumor arising from multiple anatomical subsites that corresponds to a group of aggressive and genetically complex primary neoplasms. Worldwide, head and neck cancer is responsible for approximately 930,000 new cases and more than 460,000 deaths annually, with a high prevalence in Southeast Asia, Brazil and Central Europe. In view of the low survival rate and current treatments, it is essential to deepen the studies that can point to biomarkers for better management, decision making of new therapeutic modalities/target therapy and monitoring/prognosis with greater precision, leading to improve the therapeutic success. This study aimed to quantify naïve T lymphocytes, which participate in the immune response against activation by pathogens or other stimuli such as tumor cells, and memory T cells that are part of an important mechanism for long-term protection, as the generation and Persistence of memory T lymphocytes are vital events of antitumor immunity, given their ability to persist for prolonged periods, as well as being activated and migrating rapidly. To determine the frequency of naïve and memory T lymphocytes in the peripheral blood of patients with head and neck squamous cell carcinoma.
MATERIAL AND METHODS: A preliminary study of immunophenotyping assay was performed in peripheral blood, using flow cytometry to quantify naïve and memory T lymphocytes using antibodies conjugated to fluorochromes: CD3 Pacific Blue, CD4 Pacific Orange, CD8 PE, CD45RA PerCP, CD62L APC, CD27 FITC, CCR7 PECy7. 100,000 events were acquired for each patient sample and data analyzes were performed using the Infinicity program (Cytognos). The present study was approved by the Research Ethics Committee (CEP) under protocol number 3,967,267.
RESULTS AND CONCLUSION: In a preliminary study, a mean frequency of the population of naïve CD4 T lymphocytes (2.52%) and CD8 naïve T lymphocytes (2.35%) was observed in 13 patients. The mean frequency of central memory CD4 T lymphocytes expressing CD45RO+, CD27+ and CCR7+ was 3.31% and 5.91% for central memory CD8 T lymphocytes expressing CD45RO+, CD27+ and CCR7+, in 19 patients. In relation to effector memory CD4 T lymphocytes with CD45RO+, CD27-/+ and CCR7- phenotype the mean was 8.21% while the mean of effector memory CD8 T lymphocytes with CD45RO+, CD27-/+ and CCR7- phenotype was 7 .96% in 19 patients. It is important to increase the number of individuals in the study, in order to understand and try to relate possible changes in these subpopulations of the immune system with the clinical status/evolution of the patients and also to determine if any subpopulation could become a biomarker associated with prognosis.

Keywords: head and neck squamous carcinoma, naïve and memory T lymphocytes, immunphenotyping.
Supported by: Secretaria Estadual de Saúde/RJ, FAPERJ.

EVALUATION OF THE ANTITUMOR EFFECT OF CURCUMINOIDS IN HUMAN LUNG CARCINOMA AND ADENOCARCINOMA CELL LINES

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INTRODUCTION AND OBJECTIVES: Since the 1960s, changes have been observed in the profile of diseases that affect human populations. Infectious and parasitic diseases ceased to be the main cause of death and were replaced by diseases of the circulatory system and cancers. The progressive rise in the incidence and mortality from chronic degenerative diseases is the main cause of population aging, a result of the intense urbanization process and the actions of health promotion and recovery. In this scenario, cancers have become an increasingly relevant public health problem, particularly in the last two decades. Among the different types, lung cancers stand out as the deadliest. The high costs of treatments, added to the adverse effects resulting from the available therapies, point to the need to seek new therapeutic alternatives that present reduced side effects, in addition to lower costs for the patient. It has been well accepted that over 80% of the antitumor drugs developed in the last thirty years come from natural products or from semisynthetic/synthetic derivatives thereof. This finding validates the strategy of searching for new therapeutic alternatives among compounds of natural origin. Extensive studies suggest that curcumin, the active ingredient found in the phytotherapy *Curcuma longa*, interferes with multiple cell signaling pathways, providing support for its potential role in modulating cancer development and progression. However, despite its potential, the use of curcumin *in vivo* faces pharmacokinetic (PK) limitations that make its therapeutic application difficult. To circumvent these PK limitations, in this work, a series of molecules were planned through the strategy of molecular simplification, which, applied to the structure of curcumin, generated thirteen molecules. **MATERIAL AND METHODS:** To evaluate the *in vitro* cytotoxic effects of curcumin and its synthetic analogs on human adenocarcinoma cell lines (A549 and H460), the MTT assay was carried out. **RESULTS AND CONCLUSION:** Some of the molecular simplification derivatives proved to be more active than the natural diaryleptanoid. Among the most active simplified analogs, the derivatives in which a six-membered ring was introduced stand out. This structural feature implements a conformational restriction to the planned molecules. In addition, no toxic effect was observed on human leucocytes obtained from buffy coat bags donated by healthy donors. New experiments are

going on in our laboratory to determine the possible action mechanisms responsible for such effects on transformed cells, but thus far, it is plausible to especulate that such molecules might be used as a prototype for the design of anticancer drugs, since they presented no cytotoxic effect on healthy human cells.

Keywords: Lung Cancer, Curcumin, Medicinal Chemistry.
Supported by: Fundação do Câncer, FAPERJ, CNPq and CAPES

PRO-TUMORIGENIC EFFECTS OF NEUTROPHIL EXTRACELLULAR TRAPS (NETs) IN CASKI CERVICAL CANCER CELLS

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INTRODUCTION AND OBJECTIVES: Cervical cancer is the fourth most common cancer among women worldwide. In spite of great advances in available treatments, a great number of patients still face cancer recurrence, especially in the advanced stages of the disease. The infiltration of neutrophils in tumors has been associated with worse prognosis, and evidence shows that neutrophil extracellular traps (NETs) can stimulate tumor growth, aggressiveness and metastasis. Data published by our group show that NETs promote a pro-metastatic phenotype in breast cancer cells; however, little is known about the influence of NETs on cervical cancer progression and chemoresistance. In the present study, we investigated the pro-tumorigenic effects of isolated NETs on the cervical cancer cell line CASKI, especially in regard to the acquisition of resistance to apoptosis. **MATERIALS AND METHODS**: In this study, we employed the cervical cancer cell line, CASKI, which is derived from a metastatic site in the small intestine and displays an aggressive phenotype. Neutrophils isolated from the blood of healthy donors were stimulated with PMA to produce NETs which were further isolated using an established protocol. To evaluate cytotoxicity through MTT assay, the cervical cancer cells were treated with isolated NETs for 24 h. Following this, samples were generated for real-time PCR, and the expression of Bcl-2, Bcl-xL, Bax and BIM was analyzed. Finally, treatment with cisplatin was performed for 48 h. **RESULTS AND CONCLUSION**: *In vitro* analysis showed that NETs were not cytotoxic to the cell line CASKI. NETs treatment significantly downregulated the mRNA expression of BIM, a proapoptotic protein, but also downregulated the expression of the antiapoptotic protein Bcl-xL. Western blot experiments to fully corroborate these findings are currently underway. Moreover, MTT assay results showed that NETs are able to protect CASKI cells against cisplatin treatment. In summary, we conclude that NETs modulate the expression of apoptosis-related proteins and induce resistance to cisplatin treatment in CASKI cells. Considering the data obtained so far, we intend to perform functional assays like the colony forming assay and apoptosis analysis by flow cytometry to further investigate the ability of NETs to protect cervical cancer cells from the cytotoxic effects of the chemotherapeutic drugs cisplatin and paclitaxel. Additionally, we plan to evaluate the effects of NETs treatment on a primary cervical cancer cell line, C33A.

Keywords: cervical cancer, NETs, apoptosis, chemoresistance.
Supported by: CAPES, CNPq, Fundação do Câncer and FAPERJ.

EVALUATION OF ANTITUMOR POTENTIAL OF NOVEL CHALCONES AGAINST ORAL SQUAMOUS CELL CARCINOMA: *IN VITRO* AND *IN SILICO* ANALISYS

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INTRODUCTION AND OBJECTIVES: Oral Squamous Cell Carcinoma (OSCC) have high incidence and low survival rates turning novel treatment desired. Chalcones have antitumor properties where some might interact with murine double minute 2 (MDM2) that is a protein involved in apoptosis resistance when overexpressed, acting as a p53 regulator. Our objective is to characterize new chalcones that may provide a better therapeutic outcome for OSCC patients. **MATERIAL AND METHODS:** A total of 7 novel chalcones were synthesized and characterized by *in silico* analysis using SwissADME, for QSAR evaluation of pharmacokinetics and druglikeness. Doxorubicin and Nutlin-3A were also submitted. We performed *in vitro* experiments (MTT cell viability assay) using OSCC SCC9 cell line. Normal tissue fibroblasts were used to verify selectivity *in vitro*. Literature was reviewed for potential targets that could account for the observed biological effects. Docking was performed using AutoDock4 on MDM2 (PDB ID: 4HG7). Based on AutoDock4 scoring function, the most favorable complex was submitted to a 100 ns molecular dynamics simulation in GROMACS using the AMBER99SB forcefield. A simulation of the complex MDM2-nutlin was performed as control. **RESULTS AND CONCLUSION:** SwissADME showed that all chalcones has no violations of the “Lipinski Rule of 5” and might have excellent GI absorption while controls doxorubicin has 3 violations of the rules and bad GI absorption; and nutlin-3A an already established inhibitor of Mdm2, violated 1 Lipinski rule. The new Chalcones have high cytotoxic potential towards SCC9 cells in special Ch3 (IC50 = 3.8μM); Ch1 and Ch6 IC50 ~ 9μM and Ch2 and Ch7 > 10 μM. Ch3 was shown to be highly selective towards cancer cells compared to normal fibroblasts (S.I.=6.5). Ch4 and Ch5 were not soluble. Ch3 shown a binding energy (-9.18 kcal/mol) close of Nutlin-3a (-9.52 kcal/mol). All other docked chalcones had values above -9 kcal/mol which correlates with the determined IC50. RMSD and cluster analyses from molecular dynamics confirmed a high stability of the control complex of MDM2 with Nutlin-3a and with some fluctuation between interaction dynamics Ch3, where the complex did not dissociate and some clusters reveal significant interaction of the chalcone with the MDM2 hydrophobic pocket. In conclusion, Ch3 showed promising *in vitro* anti-cancer potential. More *in silico* analysis are needed to better describe the possible interaction it can form with Mdm2, or other targets that may explain the experimental evidence.

Keywords: chalcones, molecular docking, molecular dynamics

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ANTICANCER PROPERTIES OF A LOW ANTICOAGULANT BOVINE HEPARIN

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INTRODUCTION AND OBJECTIVES: Evidence has emerged that heparin biological effects go beyond their typical anticoagulant and antithrombotic activities. For instance, anti-inflammatory, antiviral, and antimetastatic effects have been suggested by several groups. However, due to the high hemorrhagic potential of porcine heparin (gold-standard for clinical use), it is a challenge employing this polysaccharide for alternative therapeutic purposes. On the other hand, bovine heparin presents heterogeneous structure and about 55% of porcine heparin anticoagulant potential. Recently, our laboratory purified a fraction from bovine intestinal heparin that presents even a lower anticoagulant activity (about 15% of porcine heparin), named LABH. Therefore, our aim in this study was to test the LABH antitumoral and antimetastatic activities *in vivo*. **MATERIAL AND METHODS:** For this, we used two experimental models: (1) the Lewis lung carcinoma cells (LLC cell line) injected subcutaneously in C57BL/6 mice followed by daily treatment with different heparin preparations; and (2) the melanoma cells (B16F10 cell line) injected directly into the tail vein after a single dose of heparin treatment. The heparin doses used were 2, 4, 8 and 20 mg/Kg and animals were euthanized after 28 (1) or 21 (2) days for primary tumor and lungs removal. **RESULTS AND CONCLUSION**: (1) Data showed that the tumor growth was delayed at the first 2 weeks in groups treated with both porcine heparin and LABH. However, at the 28th day, despite a tendency of tumor area and weight reduction in heparin-treated animals compared to controls, no statistical difference was noted. (2) In the metastasis experiments, we observed that both porcine heparin and LABH reduced, in a dose dependent manner, the number of lung metastatic foci. Moreover, heparin treatment increased the number of lungs completely free of metastasis. Our results suggest that, although losing anticoagulant activity, LABH maintains the antitumoral and antimetastatic potential described for porcine heparin with the benefit of presenting very low bleeding side effect.

Keywords: heparin, cancer, metastasis, therapy.
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IDENTIFICATION OF METABOLITES IN BREAST CANCER CELLS AFTER PIPERINE TREATMENT

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INTRODUCTION AND OBJECTIVE: Breast cancer is a common disease in women all over the world. In addition to genetic factors, lifestyle is strongly linked to the development of cancer, which is why an inadequate diet may promote tumor progression. Although modifications in food habits can be useful, discoveries based on food and nutrition can lead to new therapies and chemoprevention derived from bioactive compounds. Piperine (1-Piperoylpiperidine) is an alkaloid from black pepper (*Piper nigrum* L.), characterized by its antioxidant and anti-inflammatory potential. Recently, studies have investigated the effectiveness of natural compounds by analyzing the impact on proliferation and survival of cancer cells. However, studies investigating the effect of natural compounds on the metabolism of breast cancer cells are still limited. The aim of this study was to evaluate the metabolic profile of MDA-MB-231 cells after treatment with the natural alkaloid piperine.

MATERIAL AND METHODS: The viability of MDA-MB-231 cells was evaluated by MTT assay. The phases of the cell cycle were analyzed in flow cytometry after labeling with RNAse-PI. The metabolic profile of the cells was evaluated by ^{1}H-NMR spectroscopy.

RESULTS AND CONCLUSION: Piperine had a cytotoxic effect in MDA-MB-231 cells having an IC_{50} of 280 µM for 24 hours. The piperine was able to increase 4.91-times the number of cells in sub-G0/G1 phase of cell cycle after 48 hours of treatment. The metabolites identified from the ^{1}H-NMR spectrum included fatty acid (formate), carbohydrates (acetate, ethanol, lactate), nucleotide sugar (UDP-glucose) and amino acids (alanine, glutamate, valine, isoleucine, threonine and methionine). Piperine increase 2.64, 2.03, 1.40, 3.26, 1.71, 1.66, 1.21, 1.99, 1.89, 1.95 and 1.24-times the metabolites levels of formate, acetate, ethanol, lactate, UDP-glucose, alanine, glutamate, valine, isoleucine, threonine and methionine, respectively. In summary, our results demonstrated the *in vitro* anti-breast cancer effect of piperine, and the metabolic differences between untreated and piperine-treated MDA-MB-231 cells.

Keywords: MDA-MB-231, Piperine and Metabolic Profile.

Supported by: FAPERJ, Fundação do Câncer, CNPq and CAPES.

RESISTANCE TO HORMONE THERAPY AND TUMOR RECURRENCE IN BREAST CARCINOMA PATIENTS: POSSIBLE RELATION TO THE SIGNALLING PATHWAY IL6/STAT3

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INTRODUCTION AND OBJECTIVES: Breast carcinoma is one of the most common diseases in women, being responsible for numerous deaths annually. The IDC (Invasive Ductal Carcinoma) is classified into 4 subtypes based on the expression of Estrogen receptors (ER) and Human Epidermal growth factor receptor 2(HER 2): Luminal; Luminal – HER2; HER2; Basal Like. This classification has predictive value regarding response to treatment and prognosis, where these biomarkers are pharmacological targets. The resistance to hormone therapy and tumor recurrence in breast carcinoma has been associated with the activation of the IL6/STAT3 signalling pathway. Through the induction of metastasis, this pathway has been playing an important role in tumor progression, where your activation is related to a poor prognosis in breast carcinoma patients. Therefore, we evaluated the expression of activated STAT3 in tumor samples of patients diagnosed with subtype luminal A Breast Carcinoma and its relation with the response to treatment with tamoxifen. **MATERIAL AND METHODS:** 84 samples obtained from biopsy and surgery of CDI ER+HER2- patients were analyzed. Samples of healthy mammary tissue were obtained from mammoplasty and used as negative control. The evaluation of the expression of activated STAT3 was performed by Immunohistochemistry. The primary antibodies applied in this experiment were obtained from the company Abcam: anti – STAT 3 [EPR23968-52]. The positive control used in the Immunohistochemistry technique was established by the fabricant. **RESULTS AND CONCLUSION:** Preliminary results indicated the presence of activated STAT3 in 84 samples. This result suggested that the signalling pathway has been activated. We hypothesize that the activation of STAT3 could be correlated with patients poor prognosis, tumor recurrence and hormone therapy resistance. Thus, we will evaluate their clinicopathological features in an attempt to correlate our results with it.

Keywords: Breast carcinoma, Luminal, STAT 3, proliferation.

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GM2/GM3 CONTROLS THE ORGANIZATIONAL STATUS OF CD82/MET MICRODOMAINS IN THE BLADDER CANCER CELL LINE

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INTRODUCTION AND OBJECTIVES: Bladder cancer ranks 9th and 13th among the cancers with the highest incidence and mortality in the world, respectively. According to the National Cancer Institute (INCA), there are an estimated 10,640 new cases of bladder cancer between 2020-2022 in Brazil. One of the many factors that contribute to tumor malignancy and metastasis is the modulation of cell adhesion that induces cell activation, motility and growth. The modulation of cell adhesion can occur by grouping gangliosides, amphiphilic glycophingolipids, found in cell membranes. These are often associated with signal transducing proteins, growth receptors, integrins, G proteins, and tetraspanins, forming cell surface domains that contribute to cell phenotype. We previously described the formation of a GM2/GM3 heterodimer, which interacts with CD82 tetraspanin, controlling epithelial cell motility, inhibiting tyrosine kinase signaling induced by the integrin-hepatocyte growth factor (cMet). The aim of this project is to understand how gangliosides control the organization of CD82 and cMet in the bladder cancer cell line. **MATERIALS AND METHODS:** Tritium labeling was performed to verify the ability of GM2 and GM3 to form complexes in aqueous solution. To study the molecular basis of the GM2 and GM3 interaction, molecular modeling and simulations of the gangliosides in methanol and water were performed. Bladder cancer cell line (YTS1) and a normal bladder cell line (HCV29) were utilized. Cell line YTS1/CD82+ was obtained by stably transfecting the human CD82 gene in a pcDNA3 vector into YTS1 cells. To study the impact of ganglioside expression on the organization of the main components of the glycosynaptic domain, cells were pre-treated with D-threo-1-phenyl-2-palmitoylamino-3-pyrrolidino-1-propanol, an inhibitor of glucosylceramide synthase. Cells were than harvested, pelleted, resuspended in Brij 98 lysis buffer, and the lysate was centrifuged. The supernatant was subjected to sucrose density gradient ultracentrifugation to separate low density membrane fractions. Fractions were analyzed using western blot. **RESULTS AND CONCLUSIONS:** We observed that the GM3/GM2 complex alters the location of CD82 tetraspanin on the cell surface, thus increasing the phosphorylation of Src2 kinase. These results raise the possibility that microdomain organization dictates cell phenotype, suggesting that malignancy may result from microdomain data disorganization.

Keywords: glycosphingolipids, cancer, microdomains, CD82, cMet, tetraspanin, gangliosides

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ROLE OF SULFATED FUCANS FROM SEA URCHIN ON PRIMARY TUMOR GROWTH

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INTRODUCTION AND OBJECTIVE: Sulfated fucans (SF) are marine polysaccharides mainly constituted of sulfated α-L-fucopyranose varying in sulfation pattern. These natural polysaccharides have a high molecular weight (>100 kDa) and can be found in algae and marine invertebrates. The SF of marine invertebrates possess a regular, linear and repetitive structure. In sea urchins, these sulfated polysaccharides occur in the jelly coat that surrounds the egg and show great structural similarity to heparin. Heparin is frequentlyused in cancer-associated thrombosis treatment, but is limited by its relevant side effects. Besides, some recent studies with heparin report an antitumor effect that may be related to different mechanisms of action, the main one being the ability to inhibit P-selectin. Considering cancer is an important public health matter, showing constant increasing incidence ratios, the search for new drugs to improve cancer treatment is critical. Thus, considering the structural similarity between SF and heparin, we aim to verify the potential antitumor role of SF from *Arbacia lixula* sea urchin using *in vitro* and *in vivo* experimental models. **MATERIAL AND METHOD**: Sulfated polysaccharides extracted from the egg jelly coat of female *A. lixula* sea urchin were purified by anion exchange chromatography on DEAE-cellulose. In order to investigate the direct antitumor properties of SF, mouseB16F10 melanoma cells line ($1x10^4$ cells/well), maintained in DMEM-high glucose medium, suplemented with 10% fetal bovine serum (FBS), were seeded for 24, 48 and 72 hours in 96-well plates in the presence of SF or porcine heparin (both 1, 10, 50, 100, 150 and 200µg/mL) and the culture medium suplemented with 5%FBS. To access tumor and normal cells' toxicity, B16F10 and African Green Monkey Kidney cell line (Vero) were seeded overnight ($1x10^4$ cells/well) in 96-well plates and incubated with SF (1, 10, 50, 100,150 and 200µg/mL) for 24 hours. All analysis were quantified by the MTT method. In the *invivo* model, B16F10 cells ($5x10^5$ cells/animal) were subcutaneously inoculated into theright flank of ketamine/xylazin anesthetized C57/BL6 mice. Animals were daily treated with intraperitoneal injection of 100 µL of sterile PBS or polysaccharides (4 mg/kg). Tumor volume (H) was accessed by using a caliper for measuring the two major diameters (d1 and d2) every two days after they were palpable, throughout 15 days of experiment by the formula: H x L2 x 0.5, where H = highest measurement obtained and L = lowest measurement obtained. **RESULTS AND CONCLUSION**: SF extracted from the egg jelly coat of *A. lixula,* but not heparin, inhibited the proliferation of B16F10 tumour cells. SF is cytotoxic for melanoma tumor cells, but not for normal cells Vero. In the preliminary *in vivo* assay, SF, but not heparin, showed a trend towards a decreasing local tumor growth. These results suggest that SF from *A. lixula* could be a potential candidate for anti-tumor drug research.

INCREASED MIGRATORY CAPACITY OF HER2+ BREAST CANCER TUMOR CELLS AFTER TREATMENT WITH TRASTUZUMAB

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INTRODUCTION AND OBJECTIVES: Breast cancer (BC) is the most common type of cancer amongst women and was responsible for approximately 680,000 deaths worldwide in 2020. Among the subtypes of BC, those that are HER2 positive stand out, characterized by the overexpression of HER2 receptors, which are related to a worse prognosis, greater tumor aggressiveness and lethality. In this sense, trastuzumab, a drug widely used in the treatment of this BC subtype, which consists of humanized monoclonal anti-HER2 antibodies, aims to block the functioning of these receptors. However, the large majority of patients have resistance or acquire it throughout immunotherapeutic treatment, and may even have relapses and metastatic tumors. Previous studies highlight the role of integrins in the resistance to antineoplastic therapies. Integrins are membrane proteins that interact with the extracellular matrix, activating signaling cascades that promote survival, proliferation and cellular migration. Our group has already shown the overexpression of integrins in tumor cells resistant to treatment with trastuzumab, suggesting a possible compensatory pathway of survival. Nowadays, our goal is to study the role of integrins in the migration of these resistant cells, through their modulation with inhibitors (recombinant disintegrins). **MATERIALS AND METHODS:** To analyze cell migration, Wound Healing (WH) assay was performed for the HCC1954 (resistant to trastuzumab) and BT474 (as control) cell lines after treatment. Gap quantifications were done with the software ImageJ. **RESULTS AND CONCLUSION:** WH assays showed that these cells, that are resistant to trastuzumab (HCC1954), acquire greater migratory competence after treatment when compared to control. *In vivo*, this could result in an increased metastatic tumor capacity. In addition, initial results suggest that disintegrins reduced the migration of these resistant cells, pointing to a possible participation of integrins in the gain of migratory capacity. Thus, it is essential to understand the relationship between the use of trastuzumab in the therapy of BC HER2 positive and the gain of migratory capacity of these tumor cells, in order to minimize the risk of metastasis, which in the case of BC, commonly occurs in the brain, lungs, bones and liver; fundamental organs for the maintenance of life.

Keywords: Breast Cancer, HER2, trastuzumab, migration, integrins, metastasis, wound healing.
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STUDY OF RELATION BETWEEN MIR-210 AND P53 IN CANCER

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INTRODUCTION AND OBJECTIVES: Cancer is one of the major public health problems in Brazil. p53 pathway is a central pathway in response to DNA damage and its deregulation is associated with tumorigenesis in more than 50% of tumor' types. In addition, microRNAs are small non-coding RNAs that regulate gene expression posttranscriptionally by inhibiting translation of target mRNAs. miR-210 expression deregulation has been associated with cancer treatment resistance. Our group demonstrated that the inhibition of miR-210 associated with radiotherapy reduces cellular viability in glioblastoma cell lines with wildtype *TP53*. Thus, this work aims to evaluate whether there is a correlation between the expression of miR-210 and *TP53* mutational *status* in different solid tumors, *in vitro* and *in silico*. **MATERIAL AND METHODS:** miR-210 expression was compared between tumor and non-tumor tissues, *TP53* wildtype and mutated samples, and evaluated in disease staging and in nodal metastasis cases, *in silico*. Cell lines from breast, lung, esophagus cancer and glioblastoma with different *TP53* mutational *status* were used to evaluate miR-210 expression by RT-qPCR and p53 protein levels by Western blotting. **RESULTS AND CONCLUSION**: *In silico* analysis revealed a higher expression of miR-210 in tumor tissues when compared to non-tumor tissues in breast, prostate, esophageal, lung and cervical squamous cell carcinoma. Corroborating this data, samples derived from different disease stages and nodal metastasis showed higher expression of miR-210 compared to non-tumor tissue. Additionally, we observed a higher expression of miR-210 in samples with mutated *TP53* compared to samples with wild-type *TP53* in breast cancer, prostate and lung adenocarcinoma. Only in prostate cancer we observed a poor overall survival in cases with high expression of miR-210. There was a higher expression of miR-210 in breast cancer and glioblastoma cell lines with *TP53* mutation, corroborating the data of *in silico* analysis. However, there was a lower expression of miR-210 in lung cancer cell lines with *TP53* mutation when compared to cell lines with wild type *TP53*. The esophageal cancer cell line with a thermosensitive mutation in *TP53* did not show a significant difference in microRNA expression neither in p53 protein levels when cultivated at 32°C or at 37°C. Therefore, miR-210 is overexpressed in breast, prostate, esophageal, lung and cervical squamous cell carcinoma, however high expression is associated with worse survival only in prostate cancer. miR-210 appears to be associated with the presence of a *TP53* mutation in breast and lung cancer *in silico* and in glioblastoma *in vitro*. In lung cancer cell lines, the inverse relation was observed for miR-210 expression and *TP53* mutation.

Keywords: Cancer, p53, miR-210.
Supported by: FAPERJ, Ministério da Saúde.

TUMOR PROGRESSION STUDY: EVALUATION OF PREMETASTATIC LUNG NICHE FORMATION AND TUMOR CELL-PLATELET CONTACT

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INTRODUCTION AND OBJECTIVE: The metastatic cascade is composed by multiple steps and tumor cells need to acquire specific phenotype to be able to form secondary tumors. Recent studies pointed to the fact that tumor cells start to prepare premetastatic niches in distant organs before their own arrival and it is crucial for metastasis success. In one of the metastatic steps, circulating tumor cells should be capable of surviving in the bloodstream, and for this, they bind to platelets forming aggregates that protect them from shear stress and NK cell attack. In this context, heparins, a known anticoagulant drug, may be used to disrupt tumor cell-platelet interaction. Our study aims (1) to characterize the lung premetastatic niche formation, and (2) to evaluate the ability of different heparins in blocking platelet interaction to tumor cells. **MATERIAL AND METHOD:** For this, (1) colon carcinoma cells (MC38) were injected subcutaneously into C57BL/6 mice. In some groups we performed a second injection of MC38 cells, but this time into the tail vein when the primary tumors were visually noted. After 4 weeks, lungs were collected for analysis. As a first step, tissue architecture, extracellular matrix (ECM) changes and cellular organization were analyzed by histology. In another attempt, (2) melanoma cells (B16F10-GFP) were cultured in plates and platelets were put in contact in the presence of porcine heparin (HPI), bovine heparin (HBI), low anticoagulant bovine heparin (LABH), low molecular weight heparin (LMWH) or chondroitin-4-sulfated (C4S). This interaction was analyzed by confocal microscopy. **RESULTS AND CONCLUSION:** (1) H&E images showed that the group restricted to the primary tumor showed lung tissue reorganization and visible changes in cell density and ECM. The 2-week metastasis-induced group did not present large visible metastases, but changes in ECM and cellular organization could be observed. In the 4-week metastasis-induced group, a large metastasis with an extensive area of ECM deposition was seen. Finally, the group with primary tumor and 2-week metastasis showed extensive ECM reorganization, areas of higher cell density, unpreserved alveolar structures and possible micrometastases. (2) The data on B16F10-GFP-platelets interaction *in vitro* revealed that, although LABH is capable of blocking this contact, HPI and HBI were more efficient. Both LMWH and C4S did not interfere with this interaction. Finally, our preliminary results on premetastatic sites demonstrate that is possible to track tissue alterations and we might interfere with tumor cell survival in the circulation using heparins as antimetastatic drugs.

Keywords: premetastatic niches, circulating tumor cells, extracellular matrix, heparin

Financial support: CNPq and FAPERJ

P53 ON THE APPEARANCE OF CANCER STEM CELLS AND EXTRACELLULAR MATRIX CHANGES IN MICE COLORECTAL TUMORS

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INTRODUCTION AND OBJECTIVES: Colorectal cancer is the 2nd most common type of cancer in both women and men in Brazil, except for non-melanoma skin cancers. TP53 is a tumor suppressor gene that encodes P53, responsible for mechanisms such as senescence, DNA repair, and programmed cell death in response to DNA damage. This gene is mutated in about 60% of all colorectal cancer cases. Recently, while searching for better anticancer therapies, many studies have found associations of TP53 mutations with the dynamics of the appearance and resistance of tumor stem cells. In intestinal tissue, both adult and cancer stem cells have been identified and tracked through the leucine-rich repeat-containing Gprotein coupled receptor 5 (LGR5) biomarker. Moreover, the tumor's microenvironment, such as the extracellular matrix, seems to be influenced by different p53 status. One of the extracellular matrix compounds known as Laminin appears to promote a metastatic phenotype to cancer cells, contributing to tumor malignancy. Therefore, we aimed to investigate new roles of TP53 in the appearance of LGR5 positive CRC cells and modifications in laminin deposition. **MATERIAL AND METHODS**: CEUA`s approved number was 085/15. Induction of inflammation-associated colorectal tumors, using the AOM/DSS protocol, in wild-type (WT), heterozygous (HET), and knockout (KO) mice for TP53. Azoxymethane (AOM) was used as a carcinogen and Dextran sodium sulfate (DSS) as a pro-inflammatory agent. Mice were monitored by clinical signs and their tumor development was investigated by colonoscopy. At the end of the protocol, mice were euthanized and had their colon removed, the large intestines were processed to investigate histopathological morphology, LGR5 expression, and immunofluorescence of Laminin; tumor fragments were also removed for Western-Blot analysis. **RESULTS AND CONCLUSION**: Results showed a higher mortality coefficient among KO individuals, compared to HET and WT mice; KO animals also had worse clinical signs of severity such as acute weight loss (more than 10% in 3 days or less), adoption of antalgic posture and dysentery. KO mice presented macroscopic tumors in 100% of cases, averaging 15 ± 9 per animal, followed by WT (74%, 6 ± 6 tumors) and HET (56%, 6 ± 7 tumors). WB analysis of either basal or tumoral LGR5 expression showed no statistical difference among WT, HET and KO animals. However, KO tumors showed increased levels of LGR5 compared to basal expression; this pattern does not repeat in WT animals. Besides that, immunofluorescence analysis shows that extracellular matrix appears to be enriched in laminin in tumoral and peri-tumoral zones.

Keywords: Colorectal Cancer, Cancer Stem Cells, p53, Laminin

Supported by: FAPERJ, CNPq, PIBIC, PRONON-Ministério da Saúde.

GLYCOPHENOTYPIC ANALYSIS OF HUMAN GLIOBLASTOMA CELL LINES CULTIVATED IN TWO-DIMENSIONAL (2D) AND THREE-DIMENSIONAL (3D) MODELS

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INTRODUCTION AND OBJECTIVE: Cancer is a group composed of more than 100 diseases that originate from a disturbance in the proliferation or apoptosis capacity of cells in a tissue. More specifically, glioblastoma (GBM) is a type of glioma that originates from glial cells in the central nervous system (CNS) and corresponds to 58% of all gliomas and 48% of all malignant tumors in the CNS. GBM has a high relapse rate due to its resistance to the current treatment protocol with temozolomide (TMZ) and the impossibility of surgical removing the tumor with a clean margen due to the localization in the CNS. Cell cultures are central to the study and the development of new drugs and treatment for cancer, being invaluable for basic research and for direct application. However, they have limitations, being hard to answer more complex problems and to take broad conclusions, though it has a lot of space for development. More recently, three dimensional (3D) cell cultures models have emerged as a novel and improved technique, theoretically being more close to *in vivo* and natural systems. 3D culture methods have rapidly evolved and are now one of the most promising experimental approaches in the biological sciences. Still, despite the importance of glycobiology in the field of cancer, little is known about the possible differences in these models, compared to the monolayer cultures. Studies in this area can give insights on the factors that enable cells to interact and organize, possibly show different patterns in a single sphere and more. Thus, our objective is to implement the 3D culture in our laboratory and compare the glicophenotype of 2D and 3D GBM cultures to evaluate if there is any difference in the expression pattern of glycoconjugates and how it may impact the cell biology. **MATERIAL AND METHODS:** Two glioblastoma cell lines were used, A172 and GBM-95. Spheroids of both cells were formed with 30.000 cells each and photographs were taken in four time points (3, 7, 14 and 21 days). Western blot was performed to evaluate the production of extracellular matrix components. A lectin assay was conducted with a panel of 8 fluorescent lectins and the analysis was conducted at the cytometer. **RESULTS AND CONCLUSION:** In order to analyze the glycophenotype of cell cultured in both 2D and 3D models, flow cytometry analysis by using FITC-lectins with distinct glycan-structure specificities were conducted, and some tendencies were observed, such as the lower expression of sialic acid (SA) in cell glycoconjugates carried by cells cultured in 3D model. New experiments are going on in our lab to investigate how the low expression of sialoglycans might impact the formation and maintenance of the spheres.

KEY WORDS**:** Câncer, Glycobiology, 3D culture, Spheroid

Supported by: CNPq, CAPES, FAPERJ, Fundação do Câncer

ANTITUMOR POTENTIAL OF VITAMIN K SYNTHETIC DERIVATIVES ON WILD-TYPE p53 MCF-7 CELL LINE

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INTRODUCTION AND OBJECTIVES: p53 is a phosphoprotein composed of 44 kDa and 393 aminoacids; it is encoded by the *TP53* gene that is located on the short arm of chromosome 17. When active, p53 adopts a tetrameric conformation and it is regulated mostly by the MDM2 protein. Known as the "guardian of the human genome", p53, under stress conditions can promote cell cycle arrest in the G1/S phase, the induction of p21, among other defense mechanisms, allowing DNA repair. If these mechanisms fail, p53 triggers the apoptotic cascade signals and the cells can go into apoptosis, preventing the error from spreading. There are a small number of studies on the role of vitamin K on the activation of wild-type p53, however, there is evidence that vitamin K2 is able to induce apoptosis via p53 in hepatocarcinoma cell lines expressing wild-type p53 (Smmc-7721). Knowing that vitamin K2 induces apoptosis by activating wild-type p53, our aim is to test a generation of 12 new menadione derivatives with methyl-1,2,3-triazole substitutions in breast cancer cell lines expressing wild-type p53. **MATERIAL AND METHODS:** To clarify the mechanism of p53 activation in breast cancer cell lines expressing wild-type p53, we screened the vitamin K-derived compounds through the MTT reduction assay on MCF-7 (wild-type p53) and MDA-MB-231 (mutant p53) cell lines in at 24, 48 and 72h and selected those with better effects on MCF-7. Concentration-response curves of the selected compounds were obtained and their IC_{50} values were calculated. Using the non-tumor cell line MCF10A, we obtained the selectivity index for each selected compound. **RESULTS AND CONCLUSION:** On the screening assay, using the compounds at 10 µM, we observed that after 72 h, six compounds (RCT-02, RCT-04, RCT-07, RCT-11 and RCT-12) were more effective in decreasing the cell viability of MCF-7 cells when compared to MDA-MB-231 cells. The compounds that decreased the viability of MCF-7 cells by 50% or more, with less effect on MDA-MB-231 cells, were chosen to continue this study. Subsequently, with the concentration-response curve, we were able to calculate the IC_{50} values of these compounds and also the selectivity index and we observed that these compounds are more selective for tumor cell lines compared to the non-tumor cell line. With these results, we intend to evaluate the capacity of these compounds to modulate wild-type p53 and also to elucidate the molecular components involved in the process.

Keywords: p53, Apoptosis, Vitamin K.

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STUDY OF TUMOR GROWTH PATTERN AND HISTOPATHOLOGICAL CHARACTERISTICS OF PATIENT–DERIVED ACRAL MELANOMA XENOGRAFTS

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INTRODUCTION AND OBJECTIVES: Recent scientific advances have contributed to a better understanding of the biology of melanoma and the establishment of new therapeutic strategies. However, these advances have not contemplated acral melanoma (AM), a subtype with worse prognosis which is relatively more common non-European descent populations, such as those in Latin America, and for which experimental models are still lacking. Patient-derived xenograft (PDX) mouse models are a high-fidelity tool for Oncology research and have high predictive power for preclinical studies. These models accurately represent the histological and genetic characteristics and recapitulate the microenvironment of human tumors during in vivo passaging. Here, we report the characterization of PDX models of different phases of AM progression with respect to the histopathological characteristics and the dynamics of tumor growth during three serial passages in vivo. **MATERIAL AND METHODS:** Tumor fragments derived from the first generation (X1) of the PDX model AM025a, a local recurrence on the heel, and its respective lymph node metastasis, AM025b, were used to establish a new passage (X2) in immunocompromised (NSG) mice. These tumors were enzymatically digested and the obtained cells were subcutaneously implanted in NSG mice (X3, $2x10^6$ cells/mice). The same procedure was performed for two more serial passages (X4 and X5). Tumor growth was measured 3 times a week. For histology analysis, paraffin-embedded tumor tissues from the PDX models (X1 to X5) and from the surgically-removed patient sample were analyzed by H&E staining and by immunohistochemistry for Ki-67, to evaluate cell proliferation, and human-specific Lamin A/C, to evaluate percentage of human cells. **RESULTS AND CONCLUSION**: Tumor growth analyses showed no consistent and significant differences across in vivo passaging. H&E staining demonstrated that AM-PDX maintained the morphological characteristics of the patient tumor. Importantly, an increase in Ki-67 staining especially in the late PDX passages (AM025a-X5; AM025b-X4) compared to the first ones (AM025a-X1 and X2; AM025b-X1) shows that in vivo passaging confers higher proliferative potential to the PDX models (p<0,01). Human-specific Lamin A/C staining showed preservation of the percentage of human cells during in vivo passaging (variation of ~1,2% between the X1-X5 AM025a passages and variation of ~0,6% between the X1-X5 AM025b passages). The results shown here are important for the characterization of acral melanoma PDX models and for their use in functional experiments in the future.

Keywords: Acral Melanoma. Patient Derived-Xenograft. PDX models.
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EVALUATION OF THE TOXIC EFFECT OF PIPERINE AND DERIVATIVES IN HUMAN GASTRIC CARCINOMA CELL LINES

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INTRODUCTION AND OBJECTIVE: Cancer is a multifactorial disease, which encompasses the evasion of growth suppressors, resistance to cell death, replicative mortality and in particular the activation of invasion and colonization of new sites. In Brazil, approximately 600,000 new cases were recorded in 2021, leading to 252,786 deaths. Among several types of cancer, this work highlights gastric cancer, which affects about 21,230 people worldwide. It is well known that current treatments are very invasive, being necessary to expand new studies related to identifying natural and / or synthetic molecules with antitumor properties. Over the last 20 years, several works have demonstrated that piperine, a natural alkaloid isolated from *Piper nigrum,* has antitumor properties. However, its antimetastatic effects are still poorly understood. In this study, we evaluated the cytotoxic effect of piperine and its derivatives on human gastric carcinoma cell lines (KATTO III and HGC-27). **MATERIAL AND METHODS:** The molecules used in this study were: (i) the Natural Alkaloid Piperine, (ii) Piperine Acid, (iii) Methoxy Piperine, (iv) Cinnamamide, (v) Hydrazide Piperine, and (vi) Hydrogenated Piperine. The cytotoxic effect of all molecules was monitored by MTT assay, which is a colorimetric test to assess cellular metabolic activity. NADH-dependent cellular oxidoreductase enzymes can, under defined conditions, reflect the number of viable cells present. MTT was crucial to determine the IC50 of Piperine and its derivatives for further studies. **RESULTS AND CONCLUSION:** Among the molecules tested, Piperinic Acid has no toxic effect on HGC-27. Regarding the KATO III cell line, Piperinic Acid and Hydrogenated Piperine did not present cytotoxic effects. New studies are going on in our lab to identify the molecules that have anti-epithelial-mesenchymal transition effect.

Keywords: Cancer, Piperine, Derivatives

Supported by: Fundação do Câncer, FAPERJ, CNPq and CAPES

SCREENING OF BIOACTIVE COMPOUNDS FROM FOODS IN A MURINE MODEL OF BREAST CANCER LINE 67NR

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INTRODUCTION AND OBJECTIVE: Breast cancer is the neoplastic disease that most affects the female population and is also largely responsible for deaths in this population. Cancer is a disease with multiple factors, and inadequate food is responsible for 35% of the mortality rate, with a direct relationship to the development of carcinogenesis, acting in the stages of initiation, promotion and propagation of tumors. However, there is also a great deal of evidence that proves an inverse relationship between regular consumption of fruits and vegetables and the risk of developing specific cancers. It is known that a balanced diet containing fruits and vegetables may be able to reduce the incidence of chronic non-communicable diseases like the breast cancer. Although modifications in food habits can be useful, discoveries based on food can lead to new therapies derived from bioactive compounds, due to its antioxidants effects. Recently, studies have investigated the effectiveness of natural compounds by analyzing the impact on proliferation and survival of cancer cells. The aim of this study was to examine the cytotoxicity potential of bioactivity compounds of foods like oxyresveratrol found in blackberry, resveratrol in grape, piperine in black pepper, ascorbic acid in citrus fruits, polydatin in grape juices, caffeic acid in coffee, chrysine in honey and perillic acid a derivative of perillic alcohol present in lavender and peppermint essential oils in breast cancer murine cells 67NR and RAW 264.7 macrophages line.

MATERIAL AND METHODS: Initially, a screening test was performed to select the most promising compounds for therapeutic targets. oxyresveratrol (ORV), resveratrol (RV), piperine (PIP), ascorbic acid (AA), polydatin (POL), perillic acid (PA), caffeic acid (CA) and chrysine (CR) were all investigated. Posteriorly, the viability of 67NR and RAW 264.7 cells was evaluated by the MTT assay.

RESULTS AND CONCLUSION: The screening test showed that ORV, PIP and RV had the best inhibition potential in 67NR cells, with a reduction of 73.55, 55.19 and 54.14 %, respectively, after 24 h treatment. After, the 67NR cells were treated with different concentrations of ORV, PIP and RV. Our results demonstrated citotoxicity after 24 and 48 h treatment, with IC_{50} of 118.40 and 57.71 for ORV, 143.62 and 69.80 for PIP and 39.60 and 32.21 for RV, respectively. Besides, our results showed that RAW 264.7 cells treated with ORV, PIP and RV for 24 and 48 h not respond to a significant cytotoxicity concentration. In summary, our results demonstrated the cytotoxicity of ORV, PIP and RV in breast cancer cell murine, which lead us to suggest them as promising candidates for *in vivo* assay.

Keywords: Breast Cancer; 67NR; Bioactive Compounds, Foods.

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RELATION BETWEEN CD34+ CELLS AND COLONY FORMING UNITS FOR QUALITY ANALYSIS IN CRYOPRESERVED APHERISIS PRODUCTS FOR AUTOLOGOUS BONE MARROW TRANSPLANTATION

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INTRODUCTION AND OBJECTIVE: Bone marrow transplantation (BMT) is one of the indications for the treatment of hematological diseases. The quantification of hematopoietic progenitor cells (PHC) by phenotype (CD34+/CD45+) or by cell culture (Clonogenic Assay evaluates Colony Formation Units-CFU) is used to determine the quality of leukapheresis products applied to BMT. The ratio between CD34+ and CFUs of granulocytes and macrophages (CFU-GM) shows great variations, but may express damage resulting from cryopreservation. The objective was to evaluate the loss of quality of these post cryo products using pre cryo RCD34/CFU as references. **MATERIAL AND METHODS**: In this study we included 399 clonogenic assay of 120 patients transplanted from 2013 to 2019 at the HUCFF Bone Marrow Transplantation Unit. Of these, 69 had multiple myeloma, 35 had Hodgkin's lymphoma, and 16 had non-Hodgkin's lymphoma. Therefore, 170 fresh samples and 170 thawed samples and 38 thawed samples were submitted to a new culture to confirm the result. To assess the amount of MHC by flow cytometry, samples were incubated with anti-CD45 and anti-CD34 antibodies and analyzed as indicated by ISHAGE/ISCT. The clonogenic assay was performed by culturing nucleated cells of the leukapheresis product in semi-solid culture containing Iscove's Medium, agar, fetal bovine serum and conditioned medium of the 5637 strain, as a source of GM-CSF. The plates were incubated for 12-14 days. Afterwards, the samples were fixed with formalin vapor until the colonies were counted. RCD34/CFU was determined by the ratio between the number of CD34+ cells and the number of CFU from apheresis. The cryopreservation method was automated and the products were kept in liquid N2. Statistical analysis of values was performed using the Mann-Whitney and t-paired tests. **RESULTS AND CONCLUSION**: The median of RCD34/CFU in fresh samples was 35 (5-2143) and in thawed samples it was 102 (2-19171). The loss of post-cryo proliferative capacity was significant $p<0.0001$ (Main-Whitney U). Post cryo samples that had losses outside of tolerance levels were repeated and showed similar results (RCD34/CFU with median 113 and post repeat 472). The loss of post-cryo proliferative capacity is very variable between patients. It is necessary to identify the associated risk factors to predict satisfactory collection for patients with greater risk of loss of post-cryo graft quality, a study of patients' clinical characteristics or cytological properties in these products may help in the interpretation of these results. The clonogenic assay in cryopreserved products reveals the loss of proliferative capacity of post-thawed progenitors and can add quality values, along with cell viability. We observed that a rate of about 25% of samples show significant hematopoietic loss in vitro. The results were confirmed by repetition and showed reproducibility.

Keywords: cancer, hematopoiesis, Clonogenic Assay, Colony Formation Units, hematopoietic progenitor cells, Bone marrow transplantation.
Supported by: CNPq and CAPES

CROSSTALK BETWEEN EPIDERMAL GROWTH FACTOR RECECPTOR (EGFR) AND PROSTAGLANDIN E2 (PGE2): A POSSIBLE LINK BETWEEN CERVICAL CANCER AND INFLAMMATION?

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INTRODUCTION AND OBJECTIVES: Cervical cancer is the third most common tumor in women in Brazil and the fourth leading cause of cancer death in women around the world. Chemotherapy and radiotherapy are widely used treatments for cervical tumors, however, in recent years survival rates have stabilized, highlighting the need for new therapeutic modalities to treat these patients. The epidermal growth factor receptor (EGFR) is a proto-oncogene highly expressed in cervical cancer. EGFR signaling has associated with different cellular functions such as cell migration, cell death, cell differentiation and angiogenesis. In addition, studies have shown that EGFR induces the expression of the inflammatory enzyme cyclooxygenase-2 (COX-2), whose product is prostaglandin E2 (PGE2) - an agonist for the EP receptors class. Cyclooxygenase enzymes have two isoforms: COX-1, encoded by a gene constitutively expressed in different tissues; and COX-2 is encoded by a gene, whose expression is induced under pathological conditions such as inflammation and cancer. Recent literature data point to a new mechanism of action of EP receptors and other GPCRs, based on EGFR transactivation. Thus, this work aimed to investigate the transactivation of the EGFR signaling pathway by PGE2 in vitro and the pro-tumor effects associated with it. **MATERIAL AND METHODS:** In this work, we used The Cancer Genome Atlas (TCGA) database to estimate the impact of EGFR, COX-1, COX-2 and mPGES-1 gene expression in the survival rate of patients with cervical tumors. PGE2 was used as an agonist of EP receptors, while panitumumab was used as a pharmacological inhibitor of EGFR activation in CASKI and HeLa cell lines. Cell migration assays were performed using the Boyden chamber. **RESULTS AND CONCLUSION**: RNAseq analysis of the TCGA database revealed that there is a positive correlation between EGFR expression and genes encoding COX-1, COX-2 and mPGES-1 in 306 cervical tumor samples. Patient survival analyzes showed that EGFR and COX-2 have a negative prognostic value, impacting on a worse overall survival in patients with tumor overexpression of this enzyme. In vitro analyses demonstrated that PGE2 activates the MAPKs (Mitogen-Activated Protein Kinases) pathway in cervical tumor cell lines by an EGFR-dependent mechanism. In the cell migration assay, we observed that PGE2 increases the migratory capacity of these cells, while the pharmacological inhibition of EGFR reverses this phenomenon. Therefore, a better understanding of the crosstalk between the EGFR pathway and the PGE2 biosynthesis pathway can help us understand the biology of cervical tumor cells and reveal new therapeutic targets.

Keywords: Cell Signaling, Cervical Cancer, Epidermal Growth Factor Receptor (EGFR), Cyclooxygenase-2 (COX-2).

Supported by: FAPERJ, CNPq and CAPES.

ESPECIALIZAÇÃO E APERFEIÇOAMENTO

INFLUENCE OF POLYMORPHISM IN THE GENES ENCODING LEPTIN AND ITS RECEPTOR IN THE DEVELOPMENT OF ENDOMETRIOSIS IN BRAZILIAN WOMEN

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INTRODUCTION AND OBJECTIVES: Endometriosis is a benign and heterogeneous gynecological disease, influenced by genetic and environmental factors. It is a pathology that negatively impacts the physical, mental, and social well-being of women due to the high presence of painful symptoms and infertility, in addition to being a risk factor for the development of gynecological cancers. In addition, the definitive diagnosis is invasive, and the pharmacological treatment is used only to alleviate the symptoms, being considered a relevant public health problem. Although its etiopathogenesis is not fully elucidated, it is known that the endocrine pathway may play an important role in the development of endometriosis, with emphasis on the leptin hormone together with its receptor LEPR which has already been recognized as an important mediator of immune regulation, angiogenesis, and inflammation. Single nucleotide polymorphisms (SNPs) involved in the regulation and biosynthesis of leptin (LEP) and its receptor (LEPR) are of great importance as possible genetic markers of the disease. Therefore, this study aims to evaluate the influence of *LEP –2548G>A* and *LEPR 109A>G* SNPs on the development of endometriosis and its clinical characteristics in Brazilian women. **MATERIAL AND METHODS:** This study consisted of 237 endometriosis cases and 226 controls recruited from two referral hospitals in Rio de Janeiro. Genotyping of *LEP –2548G>A* and *LEPR 109A>G* SNPs was performed by the real-time polymerase chain reaction (PCR) technique. A binary logistic regression was used to evaluate the associations between the studied SNPs and endometriosis and its characteristics, obtaining odds ratios (OR) and their respective 95% confidence intervals (95% CI). **RESULTS AND CONCLUSION:** Mean age (39 ± 8 *versus* 36 ± 7, respectively) and mean body mass index (28.8 ± 6.1 Kg/m^2 *versus* 26.5 ± 5.3 Kg/m^2, respectively) were significantly different between controls and cases. Endometriosis cases had a higher prevalence of all clinical symptoms of the disease, and a higher frequency of family history of endometriosis when compared to the control group (P<0.001).The variant allele frequency for *LEP -2548G>A* SNP was 32.7% and 28.5% for the cases and controls, respectively, and for *LEPR 109A>G* SNP was 17.9% and 17.9% for the cases and controls, respectively. No significant differences were found for both studied SNPs between cases and controls. However, this study observed that the *LEPR 109A>G* SNP was positively associated with chronic pelvic pain (OR = 1.75; 95% CI = 1.05-2.89) and dyspareunia (OR = 1.78; 95% IC = 1.01-3.12) among women with endometriosis. In conclusion, the *LEPR 109A>G* SNP presented an increased risk of developing chronic pelvic pain and dyspareunia in endometriosis, which may contribute to the understanding of the molecular mechanisms of the disease and to aid in the early diagnosisand exploration of therapeutic options for the painful symptoms present in women with endometriosis.

Keywords: Endometriosis. Polymorphisms. Leptin. Leptin receptor.
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ANTI-CD19 CAR-T CELLS OVEREXPRESSING THE PROTEIN PHF19 HAVE ALTERED MEMORY AND EXHAUSTION PHENOTYPES

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INTRODUCTION AND OBJECTIVES: CAR T cells are genetically modified to express a synthetic receptor that recognizes surface antigens. CAR-T therapy cost is largely impacted by using clinical-grade viral vectors. Transposon-based systems are more economic than viral vectors, being a good strategy for cost reduction. Another limitation is short-term persistence of T-cells in vivo, often restricted by acquisition of an exhausted phenotype. Recent work showed that Phf19, a member of the PRC2 complex, can induce formation of memory cells and avoid terminal differentiation of CD8+ lymphocytes. To address the phenotype and function of anti-CD19 CAR-T cells overexpressing Phf19 generated with transposon systems Sleeping Beauty (SB) and piggyBac (PB). **MATERIAL AND METHODS:** To generate CAR-T cells, we electroporated peripheral blood mononuclear cells from healthy donors with the SB or PB transposases along with the respective transposon plasmid. Cells were activated with anti-CD3/CD28 beads and expanded for 8 to 12 days. T cell phenotype was assessed by flow cytometry, and we evaluated lysis capacity through Calcein-AM assay. **RESULTS AND CONCLUSION:** Our results show that the PB system is more effective in providing CAR expression with the present construct containing Phf19 when compared to the SB system. We were able to obtain around 2 million CAR+ cells per well with transfection rates ranging between 20-30% 24h post-electroporation and about 10-15% on day 12 of expansion. We did not observe significant differences in lytic capacity with Phf19 overexpression in a co-culture with CD19+ leukemia cell line Nalm-6. Regarding T cell phenotype, Phf19 overexpression led to increased formation of central memory-like T cells marked by increased expression of CCR7, and a reduction of effector memory-like cells. Interestingly, we also observed increased levels of the inhibitory receptor PD-1 in Phf19+ CAR-T cells, particularly within the CD4+ compartment. These results suggest an ambiguous role for Phf19 in T lymphocytes that may differ between CD8+ and CD4+ cells and paves the way for assessment of the effector function of these cells in vivo. Particularly, we believe Phf19 indirectly upregulated both CCR7 and PD-1, which we intend to further explore.

Keywords: CAR-T cells, Immunotherapy, Exhaustion, Transposon systems, Gene therapy

Supported by: FAPERJ and CNPq

ASSOCIATION OF THE EPSTEIN-BARR ONCOVIRUS PHYLOGEOGRAPHY WITH ANCESTRALITY: A PERSPECTIVE OF MATERNAL INHERITANCE

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INTRODUCTION AND OBJECTIVES: The Epstein-Barr virus (EBV) is a saliva-borne human herpesvirus, present asymptomatically in more than 90% of the adult world population. However, it is causally associated with benign and malignant diseases, which may depend on ethnographic, socioeconomic and genetic factors, being relevant to discern viral factors that are geographically restricted from those associated with specific tumorigenic processes. One of the main hypotheses of viral transmission in developing countries is through early childhood contact between mother and child, thus, the present study aims to investigate the correlation of the phylogeographic origin of EBV circulating in Brazil with maternal ancestry in asymptomatic individuals, in order to contribute to the hypothesis of intrafamilial transmission. **MATERIAL AND METHODS**: We selected 45 asymptomatic EBV+ donors in saliva previously characterized for the *LMP1* oncogene and the type by *EBNA3C*. The genetic variability of mitochondrial DNA (mtDNA) was evaluated by sequencing by the Sanger method, through hypervariable regions I and II and the maternal ancestry of these individuals was attributed through the classification in haplogroups using mitochondrial public databases. Further, multigene phylogenetic reconstruction was performed by Maximum Likelihood method. **RESULTS AND CONCLUSION:** The characteristics of this sampling were obtained as follow, a median of 26 years of age and 93.3% had Brazilian origin with approximately 81% from the southeast macro-region. Mitochondrial ancestry was established for 43 individuals, where the main classification obtained was European (40%), followed by African (33%), Amerindian (18%), Asian (5%) and unclassified (4%). A multigenic phylogenetic tree suggested the monophyletic grouping of some unranked sequences, suggesting a possible limitation in the diversity of sequences from a public database. The EBV type of the studied population belong 77.8% to EBV type 1 and 22.2% type 2. The *LMP1* oncogene belong to 4 different clades: Mediterranean (51.4%), Raji (40.5%), B95-8 (5.4%) and China2 (2.7%). The Mediterranean variant has a strong association with European ancestry, while the variant related to the Raji lineage, with African ancestry (p=0.038). Our results correlate the phylogeographic origin of EBV with maternal ancestry, in this way, reinforce the hypothesis that EBV diversity is linked to human migration, bringing the perspective of its transmission possibly through maternal parental care. Furthermore, the clade related to Raji variant were related to individuals with African and Native American, strengthens the hypothesis that this variant is an EBV recombinant originating from these two populations.

Keywords: Epstein-Barr virus. Mitochondrial DNA. Human migrations.
Supported by: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and INCT para Controle do Câncer.

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INTERLEUKIN-8 POLYMORPHISM ASSOCIATED WITH LOW RISK OF ENDOMETRIOSIS-RELATED PELVIC PAIN

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INTRODUCTION AND OBJECTIVES: Endometriosis is a gynecological inflammatory disorder described as thepresence of ectopic endometrial glands and stroma outside the uterus, affecting10% of reproductive age women. Among the recurrent symptoms, the most prevalent in endometriosis aredysmenorrhea, chronic pelvic pain, dyspareunia and infertility. Although the disease's etiology is not entirely understood, there areevidence that genetic factors andinflammatory processes may contribute to endometriosis development, also,it may be a precursor of gynecological tumors, such as ovarian cancer. Inflammation is characterized by infiltration of inflammatorycells and tissue up-regulation ofcytokines such as interleukin (IL)-8. IL-8 is a chemokine known to be involved on endometriosis pathogenesis,which increase vascular permeability and production of angiogenic factors even asstimulates proliferation of endometrial cells. Some studies have shown that IL-8 was overexpressedin women with endometriosis cases compared to endometriosis free women, and thus, single nucleotide polymorphisms (SNPs) in this gene may affect its expression. The *IL-8* rs4073 *(-251) A>T*SNP is commonly associated with up-regulation of IL-8expression. The aim ofthestudy was to investigate the role of *IL-8* rs4073 *A>T*SNP inendometriosis' development and its related symptoms.**MATERIAL AND METHOD:**This case-control study included 207 women with endometriosis (cases) and 193 without the disease (controls)recruited in two reference hospitals from Brazil. Genotyping of the SNP was performed by the real time polymerase chain reaction using TaqMan validated assay. Associations were evaluated by a binary logistic regressionmodel, using odds ratios (OR) and 95% confidence intervals (CI). **RESULTS AND CONCLUSION:**The results showed thatcases were younger (36 ± 6.8 versus 39 ± 8.4), had a lowerbody mass index (26.5 ± 5.3 Kg/m^2 versus 35.7 ± 6.3 Kg/m^2), a high frequency of all painful endometriosis-related symptoms and infertility(48.8% versus 14.0%)than the controls (P<0.001). Minor allele frequencies were 42.3% for cases and 39.9% for controls. There were no significant associations between the *IL-8* rs4073SNP and the development or staging(controls versus DIE cases, and controls versus III/IV cases) of endometriosis. However, theSNP was associated with chronic pelvic pain among endometriosiscases (*AT+TT* model, OR=0.54; 95% CI=0.29-0.98). Therefore, the present study hypothesized that the*IL-8* rs4073 *A>T*SNP contributes to lower IL-8 expression and, consequently,decreases endometriosis-related pelvic pain in women with endometriosis. Finally, these data can contribute to the accurate diagnosis of the symptoms of thedisease,preventing its complications, and assisting in the individualized treatment ofwomen with endometriosis.

Keywords: Endometriosis, Interleukin, Polymorphism.

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STUDY OF THE ROLE OF CX43 ON ENTERIC GLIAL CELLS IN INFLAMMATION OF INTESTINAL EPITHELIAL CELLS

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INTRODUCTION AND OBJECTIVES: Enteric Glial Cells (EGC) and enteric neurons compose the Enteric Nervous System. Mucosal EGCs play an important role in controlling the integrity of the Intestinal Epithelial Barrier (IEB) by reducing the permeability and as a defense in inflammation. However, in some cases EGC becomes reactive, releasing pro-inflammatory cytokines and S100B protein, that in high concentrations contributes to the fragility of the IEB and intestinal inflammation. Communication between EGCs occurs through connexin43 (cx43) channels. In addition, some factors may be released by these Cx43 hemichannels. Ablation of Cx43 in EGC results in increased fluid in feces, which indicates a role of these hemichannels in IEB regulation. The objective of this work is to investigate in vitro the role of cx43 in the EGCs response and its consequences for colon epithelial cells, against inflammation promoted by lipopolysaccharide (LPS). **MATERIAL AND METHODS**: Interaction of the EGC lineage - CRL2690, with intestinal epithelial cells of the Caco-2 lineage, through co-culture experiments and culture of one cell type with the conditioned medium of the other cell type, with or without treatment with LPS, and cx43 inhibitor (43gap26). Caco-2 cells will be evaluated by immunocytochemistry and western blotting (WB) for the expression of the ZO-1 cell junction protein. EGCs will be evaluated for expression (immunocytochemistry and WB) of GFAP, S100B and Cx43, and release (ELISA) of S100B. Cell viability (MTT) and proliferation will be evaluated in both cell types. **RESULTS AND CONCLUSION:** Caco-2 showed no significant difference in cell viability when exposed for 24h to LPS, or to the conditioned media of EGC, LPS-treated EGC, or HCT116 conditioned media-treated EGC; EGC showed decreased cell viability about 10% when exposed to LPS and 43gap26 and expressed twice as much S100b in the presence of LPS. Treatment with LPS plus cx43 inhibitor in EGC seems not to change cx43 expression. Caco-2 treated with LPS shows discontinuity sites in ZO-1 expression zones. However, in co-culture with EGC, LPS does not cause these failures in the occlusion zones between Caco-2 cells, but failures regions are there if co-culture is treated with LPS and 43gap26. EGCs seems to protect IEB from inflammatory insults, which is mitigated when cx43 pathways are inhibited. These data suggest that factors secreted by cx43 hemichannels of EGC play an important role in this protection.

Keywords: Enteric Glial Cells, Inflammation, Reactivity, Connexin43, Enteric Nervous System

Supported by: CNPq and Capes.

MESTRADO

THE EFFECT OF THE INCORPORATION OF EXOGENOUS SUGAR NEU5GC ON EGFR SIGNALING PATHWAY IN COLON CARCINOGENESIS

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INTRODUCTION AND OBJECTIVES: Sialic acids (sia) are a family of nine carbon-monosaccharides, present in the terminal portions of glycoconjugates, such as glycoproteins and glycolipids. They have an important role in extracellular signaling, mainly due to their electronegative nature, position and abundance on cell surface. N-acetylneuraminic acid (Neu5Ac) and N-glycolylneuraminic acid (Neu5Gc) are the main forms of sialic acids in mammals. Humans are deficient in Neu5Gc, since the enzyme CMAH, responsible for the hydroxylation of Neu5Ac into Neu5Gc, is inactive in humans. However, Neu5Gc can be detected in healthy and malignant human tissues, due to its metabolic incorporation from Neu5Gc rich foods, such as red meat. In Brazil, colorectal cancer (CRC) is the third most prevalent cancer and the second cause of death related with cancer in the world. Red meat consumption was previously associated with the progression of CRC. Previous data from our group shows that CRC cells lines incubated with Neu5Gc induced greater activation of Wnt pathway. From this we wondered if incorporation of Neu5Gc could alter other signaling pathways, such as the EGFR signaling pathway. EGFR is an important pathway in colon carcinogenesis and nearly 50% sporadic CRC cases show changes in this signaling pathway. **MATERIAL AND METHODS:** CRC cell lines were induced to incorporate Neu5Gc and stimulated with different concentrations of rEGF (EGF recombinant) to evaluate cell viability by MTT assay and the expression of proteins involved in EGFR signaling pathway by western blot. We also performed an immunoprecipitation assay of the EGFR to check for Neu5Gc incorporation at the receptor. **RESULTS AND CONCLUSION:** Ours results shows that CRC cells exposure to exogenous Neu5Gc led to it is incorporation on the EGFR. However, no changes in cell viability were observed between incubation Neu5Gc or Neu5Ac. Western blot analysis revealed an increase in p-AKT (activated AKT) in cells incubated with Neu5Gc, suggesting an increased cellular response to rEGF. Our results suggest that Neu5Gc incorporation can modulate the EGFR signaling pathway through increased activation of AKT and its targets, supporting the need of further studies regarding the importance of Neu5Gc intake to signaling pathways involved in CRC progression.

Keywords: Colorectal Cancer; Sialic acids; Neu5Gc; EGFR. Supported by:

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THE ROLE OF DNMT3A AND TDT ON THE PROMOTION OF FLT3 AND NPM1 MUTATIONS IN ACUTE MYELOID LEUKEMIA

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Acute myeloid leukemia (AML) is a malignant neoplasm, in which precursor myeloid lineage cells acquire mutations that block their differentiation and maturation programs. The etiology of the disease is associated with a highly heterogeneous genomic landscape, in which alterations in the *FLT3* and *NPM1* genes - identified in 25% and 33% of AML patients, respectively - stand out for their impact on the clinical prognosis. Recently, a study suggested that the enzyme terminal deoxynucleotidyl transferase (TdT) is responsible for the formation of small insertions or duplications within *FLT3* (*FLT3*-ITD) and *NPM1* (NPM1c) genes. These alterations are associated with other pre-existing mutations in cells, that affect the *DNMT3A* gene. As this DNA methyltransferase regulates gene expression, we investigate whether mutations in *DNMT3A* can contribute to the dysregulation of TdT expression and, thereby, facilitate the occurrence of mutations in *FLT3* and *NPM1* during the molecular pathogenesis of AML. Bone marrow and/or peripheral blood samples from patients diagnosed with AML were used to obtain DNA and RNA. The regions with the highest prevalence of insertions in *FLT3* (exon 14), *NPM1* (exon 12) and mutation in *DNMT3A* (exons 19 and 23) were amplified by PCR. Microinsertions in *NPM1* and *FLT3* were identified by fragment analysis and Sanger sequencing, while point mutations in *DNMT3A* were identified by Sanger sequencing. Our current series includes 66 patients, of which 61% are pediatric (0 - 18 years old). The proportion of female and male patients is similar (47% and 53%, respectively) and 15% had the promyelocytic leukemia (AML-M3). Mutations in *FLT3* and *NPM1* were identified in 15% and 18% of cases, respectively. Regarding the prognostic risk classification, 15% of the patients were classified as favorable risk, 84% as intermediate risk and 2% as adverse risk. The average size of the insertion in *FLT3* was 54 base pairs, while the microinsertions in *NPM1* were only 4 base pairs. The type A mutation (c.860_863dupTCTG) was the most frequently observed in *NPM1*, corresponding to 56% of the mutations in this gene. Currently, we are investigating mutations of the *DNMT3A* exon 19, and identified one sample with a point mutation at residue 735 (c.735TAC>TCC), which leads to a substitution of the amino acid residue tyrosine for serine.The frequency of *NPM1* and *FLT3* mutations verified here was similar to that reported in the literature. We also plan to investigate mutations in the exon 23 of *DNMT3A*. In addition, we have already standardized the quantification of *DNTT* gene expression levels by RT-qPCR.

Keywords: Acute Myeloid Leukemia, FLT3 mutations, NPM1 mutations.
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PRODUCTION AND EVALUATION OF THE ACTIVITY OF *ESCHERICHIA COLI* TYPE 2 L-ASPARAGINASE

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INTRODUCTION AND OBJECTIVES: The E. coli type II L-asparaginase enzyme (EcA2) is an amidohydrolase, which catalyzes the hydrolysis of L-asparagine into aspartate and ammonia. This enzyme is a key product in the clinical protocol for the treatment of Acute Lymphoblastic Leukemia (ALL) by depleting plasma asparagine, an essential amino acid for neoplastic cell growth. The therapeutic use of EcA2 is only possible upon importation from foreign manufactures with marketing authorization by ANVISA. Consequently, the availability of drugs containing L-asparaginase depends on the steps from production, transport logistics and import bureaucracy. This is very significant considering the 8-month shelf life for this biopharmaceutical, as well as the complexity of the production method, which poses constant risk for patient care. In addition, the risk of inactivation, development of side effects and even the need to interrupt the treatment, stimulate the emergence of new producers of this enzyme and the development of L-asparaginase variants with improved pharmacological properties. In this work we report the establishment of bulk production of EcA2 in bioreactor, aiming the scale-up of this productive method. **MATERIAL AND METHODS:** The gene of *E. coli* BL21 (DE3) strain EcA2 was cloned into bacterial plasmid used for homologous expression, followed by grown in fed-batch in a bench-scale bioreactor for expression of the recombinant EcA2 (rEcA2). Purification of rEcA2 was achieved by tangential flow filtration and chromatography. The enzymatic activity and conformation of the rEcA2 were evaluated in comparison with commercial EcA2. We also evaluated the cytotoxic activity of rEcA2 compared to commercial EcA2 on CCRF-CEM lymphoid leukemia cells. The steps in the production pipeline were monitored in regard to the content of L-asparaginase, metabolites and other impurities, by 1D ^{1}H-NMR and gel electrophoresis, in addition to the structural integrity of rEcA2, by NMR, circular dichroism and fluorescence spectroscopy. **RESULTS AND CONCLUSIONS:** Our process resulted in satisfactory production of rEcA2 in high yield and purity, with conformation and activity in close agreement with commercial EcA2. Critical steps of the production protocol were identified as well as fundamental quality assessment methods in order to increase the quality of the produced biopharmaceutical, especially in comparison to the commercially available formulations.

Keywords: L-asparaginase type II, Acute Lymphoblastic Leukemia, Bioreactor, 1D ^{1}H-NMR.

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CAR-T CELL FUNCTION EVALUATION IN LONG-TERM KILLING ASSAYS

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INTRODUCTION AND OBJECTIVE: One of the first steps in screening a new design of CAR-T cell is to measure the cancer cell-killing ability of an effector cell with a cytotoxicity assay. The most used tests for evaluating the capacity of cytotoxicity activity of CAR-T cells in vitro are the chromium (51Cr)-release assay and other tests like calcein fluorescent and bioluminescent assays (Kiesgen et al, 2021). However, these tests only evaluate the lysis for a short period (4h-24h). The benefit of a long assay is to understand how the cells are behaving in terms of kinetics, memory, and exhaustion marks which assays in 24 hours do not predict. Therefore, our objective is to standardize a long cytotoxicity assay using different tumor lineages and define whether our CAR construct has a cytotoxicity effect against the CD19+ cells using this method. **MATERIAL AND METHOD:** Nalm-6-GFP and RS4;11-GFP are tumor cell lineage of B-Cell Acute Lymphoblastic Leukemia that expresses CD19 and were used as target cells. As CAR-T effector cells we used human cells modified with the CAR-encoding pT4-19BBz Sleeping Beauty transposon vector. We used different ratios of CAR-T/tumor cells defined by the total number of T cells and incubated cells at 37ºC for killing evaluation at 0h, 24h, 48h, and 72h time points. To discriminate the living from death cells, we used Fixable Viability Dye - eFluor™ 780 before gating GFP+ target cells. T cells without CAR (mock) were used as a control in the same conditions as CAR-T cells. We evaluated the number of remaining living GFP+ cells to determine the proportion of killed cells. Tests were performed in duplicate. **RESULTS AND CONCLUSION:** We performed the tests on 3 different donors and we saw a decrease of alive cells in the first 24h to at least 60% in Nalm-6 and RS4;11 in all E:T ratios. In agreement with our previous results (Chicaybam et al., 2020), the lysis of Nalm-6 is lower than the observed for RS4;11 in each time point and ratio. The present data shows we have better results of lysis and the largest difference between CAR-T cells and mock at times later than 24h. CD19BBz positive cells promote at least 15% of leukemia killing even in the lower E:T ratio at 72h. Furthermore, we observed that only 1.7% of RS4;11 cells were alive at the highest ratio and 5.4% at the lowest ratio at 72h. Similar results were also obtained with the Nalm-6 cell line. Thus, we demonstrate that our 19BBz CAR-T cells are capable of killing tumor cells efficiently even in lower doses and this result may be important for in vivo tumor killing dynamics. In the next steps of this study, we will use a modified Nalm-6 cell line expressing low levels of CD19 and evaluate the kinetics using a calcein and caspase assay for flow cytometry and high throughput microscopy-based evaluations of killing dynamics.

Keywords: Immunotherapy, CAR-T, acute lymphoblastic leukemia
Supported by: FAPERJ, CNPq, PRONON, CAPES and Ministério da Saúde

A miRNA-BASED PREDICTOR OF PROGNOSIS AND THERAPEUTIC RESPONSE FOR HIGH-GRADE SEROUS EPITHELIAL OVARIAN CANCER USING MACHINE LEARNING.

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INTRODUCTION AND OBJECTIVES: Ovarian cancer (OvC) is one of the neoplasms with the highest incidence among women worldwide, with significantly increased mortality. The vast majority of patients are diagnosed in advanced stages of the disease since early stages present unspecific symptoms and inaccurate diagnoses. Currently, not many clinical biomarkers are available to aid in the diagnosis or predict the prognosis of HGSOC. The application of omics approaches on non-invasive samples, such as liquid biopsies, combined with artificial intelligence techniques shows promise, not only for identifying effective biomarkers for prognosis and diagnosis of OvC, but also for decision making. Therefore, our goal is to build a predictor of prognosis for patients diagnosed with ovarian cancer, as well as to identify new biomarkers. **MATERIAL AND METHODS:** Prognostic groups were defined based on the number of years of overall survival and their vital status: Patients with less than 3 years of survival and with Dead status were allocated as the group with poor prognosis and patients with ≥ 3 years were considered as a good prognosis. Based on the miRNA expression data from samples of patients diagnosed with ovarian cancer obtained from the database of The Cancer Genome Atlas (TCGA) project, we applied variable selection methods (FCBF, Cox Univariate Regression, and ElasticNet) to identify relevant predictors. Subsequently, samples were subject to a ten-fold split (outer cross-validation), wherein each 10% split was used as a held-out set, with the remaining 90% used as a training set. Random Forest (RF) classifiers were trained with hyperparameter optimization performed through a grid search with ten-fold internal cross-validation, leading to a total of ten separate AUC (Area Under Curve) values. The mean of these ten values was calculated as a summary AUC metric. **RESULTS AND CONCLUSION**: After applying the variable selection methods, 47 miRNAs were selected as important for the clinical outcome of patients with OvC. Regarding the performance of the models on the test data set, RF showed excellent metrics: 0.848 AUC (Area Under the Curve); 0.761 sensitivity; 0.771 precision, and with an accuracy of 0.763. Among the miRNAs with greater predictive potential, miRNA-205-5p was considered an excellent predictor by the RF model to classify patients with good or poor prognosis regarding survival. Published studies show that this miRNA has a mechanism of action dependent on tumor cell-derived exosomes and has the ability to regulate angiogenesis in the tumor, thus being a potential therapeutic target for OvC. Furthermore, independently evaluating the miRNAs indicated by the RF model as having the highest predictive potential, miRNA-143-3p showed an AUC of 0.700. Through in vitro experimental studies, authors have already demonstrated that this miRNA acts as a tumor suppressor in ovarian cancer cell lines.

Keywords: miRNA; machine learning; prognosis. liquid biopsies.
Supported by: Brazilian Ministry of Health / INCA-MS.

THERMOSENSITIVITY OF MISSENSE VARIANTS IN THE PALB2 WD40 DOMAIN

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INTRODUCTION AND OBJECTIVES: *PALB2* is a tumor suppressor gene that encodes for a homonymous protein that works as a scaffold connecting BRCA1 and BRCA2 proteins. The BRCA1-PALB2-BRCA2 complex plays an important role in genome integrity maintenance and in homologous recombination repair, a DNA repair pathway. The interaction between PALB2 and BRCA2 forms a stable heterodimer mediated by PALB2 WD40 domain and BRCA2 N-terminal region. The clinical impact of missense mutations (the substitution of a single amino acid residue) is particularly difficult to interpret, usually being classified as variants of uncertain significance (VUS). The pathogenicity of PALB2 missense variants can be explained by the destabilization of their interaction with BRCA1 or BRCA2. Interestingly, variants that impact on the structure of the WD40 domain of PALB2 may expose a nuclear export signal (NES) that routes the protein to the cytoplasm. The altered subcellular localization of PALB2 is related to the loss of protein functions such as interaction with BRCA2 and DNA repair. However, it is not clear whether reversal of this phenotype is able to rescue the role of PALB2 in DNA repair. In this work, our goal is to identify and characterize the thermosensitivity of PALB2 missense VUS located in the WD40 coding region. **MATERIAL AND METHODS:** We performed a literature curation to identify PALB2 VUS with cytoplasmic localization. Variants were generated by site-directed mutagenesis strategies using PrimeSTAR® Max DNA polymerase and specific primers with the mutation of interest; and later confirmed by automatic Sanger sequencing. The subcellular localization assay is performed in HEK293FT cells. Cells are cotransfected with constructs encoding histone H2B fused to the Cherry epitope (H2B-Cherry, nuclear localization control) and eGFP (control, empty vector) or eGFP-PALB2 (wild-type or variant). After 24 hours, cells are plated on glass coverslips (previously treated with 2% w/v gelatin solution in PBS) and kept at 37°C or 32°C for 12 hours. The slides are analyzed under a fluorescence microscope, subcellular localization is evaluated using ImageJ software (National Institute of Health, USA). Data will be grouped into three sets considering subcellular localization: (1) predominantly nuclear, (2) predominantly cytoplasmic or (3) equivalent nuclear and cytoplasmic localization. Protein profile will be evaluated by immunoblotting. **RESULTS AND CONCLUSION**: We selected 18 variants to be analyzed. Of these, 11 are already generated and confirmed by sequencing. We are currently performing the subcellular localization assay. We expect that the study of the thermosensitivity of PALB2 missense VUS located in the WD40 coding region will contribute as a molecular tool for the structural and functional characterization of the protein.

Keywords: cancer, mutations, *PALB2*, thermosensitivity.
Supported by: INCA, FAPERJ, Fundação do Câncer and CNPq.

SECRETED OSTEOPONTIN-A MEDIATE ANAPLASIC THYROID CARCINOMA PROGRESSION FEATURES BY αVβ3 INTEGRIN RECEPTOR

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INTRODUCTION AND OBJECTIVES: Thyroid cancer (TC) is the most frequent endocrine neoplasm worldwide, representing 3% of all estimated tumors. The anaplastic thyroid carcinoma (ATC) is one of the least frequent among the TC subtypes. ATC presents rapid tumor progression and high metastatic rates, reflecting a poor prognosis. Osteopontin (OPN) is a multifunctional protein able to activate tumor progression-related pathways in several tumor types, including TC. Previous studies from our group indicated that OPNa splicing variant is overexpressed inducing progression features in thyroid tumors, including in ATC cell lines. However, the molecular mechanisms by which OPNa promote these effects are still poorly understood. This study aimed to investigate whether integrin and/or CD44 receptors can mediate OPNa effects on distinct aspects related to tumor progression. **MATERIAL AND METHODS:** The expression of αvβ3 integrin and CD44 surface receptors was evaluated by flow cytometry in the representative ATC cell line, 8505c, which stably overexpress OPNa (8505c-OPNa cells). OPN levels secreted into the conditioned medium (CM) from 8505c-OPNa cells was quantified by ELISA assays. The wild-type 8505c cell line was tested for cell growth, migration and invasion rates in response to culturing in the presence of CM secreted by 8505c-OPNa cells. **RESULTS AND CONCLUSION:** The expression of αvβ3 integrin was detected on the 8505c-OPNa cell surface, while no CD44 expression was observed. However, αvβ3 integrin expression was not increased in 8505c-OPNa cells when compared to control cells. We also found that CM enriched with secreted OPNa stimulated cell growth, migration and invasion of 8505c wild type cells. Notably, the neutralization of the αvβ3 receptor using an anti- αvβ3 integrin specific antibody inhibited the invasive potential of these cells. Taken together, these findings support that OPNa secreted by the 8505c-OPNa ATC cell line may impact on key functional aspects of ATC progression and that cell invasion could be mediated by the αvβ3 integrin receptor.

Keywords: osteopontin, anaplastic thyroid tumor, splicing variants

Financial support: CNPQ, CAPES, FAPERJ, MS

GENERATION OF 19BBZ CAR-T CELLS IN TCR KNOCKOUT T CELLS

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INTRODUCTION AND OBJECTIVES: A promising treatment consists in using autologous T cells bearing chimeric antigen receptors (CARs), synthetic constructs designed to redirect lymphocyte specificity to tumor membrane antigens. Although this approach is showing good results, it still shows some limitations, such as the cost of production, quality and quantity of starting materials, and cell preparation time. To circumvent these limitations, allogenic CAR-T cells can be generated. To avoid graft versus host disease (GVHD), genes encoding the TCR can be knocked out and the CAR transgene be delivered through a viral approach, transposons, such as Sleeping Beauty (SB), or donor DNA for homologous recombination. The use of SB can optimize the production of CAR-T since it is cheaper and less laborious compared to viral assembly and delivery. The use of donor DNA can provide some improvements in the cells generated by knock-in (KI) of the transgene in specific genetic sites. The main objective of this work is the generation of allogenic 19BBz CAR-T cells KO for TCR via CRISPR and CAR+ via the SB system or CAR donor KI. **MATERIAL AND METHODS:** To achieve this goal, PBMCs were isolated by gravity density and, in some experiments, submitted to CD3 purification columns, electroporated in 2B or 4D nucleofector devices with CRISPR RNPs, SB and/or donor DNA. The cells were stained with monoclonal antibody panels to verify the KO of the TCR and evaluated by flow cytometry. **RESULTS AND CONCLUSION**: The editing system was optimized and tested in Jurkat cells, which showed, 21 days after the edition, 40% of KO of TCR and 41 days later 70% of KO, with better results in conditions with a reason of 1:3 (Cas9/gRNA). When using PBMC as starting material, the rate of CD3 negative cells was 38%, and after *in vitro* expansion the rate of KO in T cells gathering CD4+ and CD8+ was 20%. When the SB transposon carrying CAR was co-delivered with the RNP we achieved 15-20% of editing (CD3 negative cells). At last, we tested the editing system alone, co-delivered with SB or donor DNA (for KI). Remarkably, the system alone shows poor performance (5-9% editing), but when we used it along with SB the KO rate was 21-25%, with more than 20% of CAR+ in this population with higher CAR expression in the CD3 negative subpopulation. We used a non-coding donor DNA sequence, which impaired CAR expression to test the KI condition to compare it with KO rates. This condition with a donor DNA generated a stable population of 50-60% of CD3 negative cells throughout the expansion. With these results, we can conclude that the editing system works in the Jurkat cell line, total PBMCs, and CD3 purified population. Furthermore, it was possible to generate allogenic CAR-T cells with the

SB system, with the CD3 negative population showing some advantage in the expression of the CAR molecule. The non-coding KI sequence promoted higher KO rates, maintaining the CD3 negative population during the expansion period.

Keywords: CRISPR/CAS9, Immunotherapy, CAR-T
Supported by: FAPERJ, CNPq, PRONON, CAPES and Ministério da Saúde

ANTI-HER2 PIGGYBAC TRANSPOSON-BASED CAR T CELLS PRODUCTION: COMPARISON OF TWO DIFFERENT SCFV CLONES.

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INTRODUCTION AND OBJECTIVE: The PiggyBac (PB) system consists in a non-virus transposon/transposase gene delivering tool. Chimeric antigen receptors (CARs) are molecules capable of redirecting immune cells against a specific tumor antigen (Chicaybam et al, 2020); several studies elect the HER2 receptor as a good target due to its specific overexpression in different solid tumors. The aim of this work was to evaluate and compare the PB system-based transposition efficacy of two different anti-HER2 CARs (4D5 and FRP5) on primary peripheral blood mononuclear cells (PBMCs) isolated from healthy donors, besides the phenotype characterization and in vivo antitumor capacity. **MATERIAL AND METHOD:** The two clones of anti-HER2 CARs were synthetized and cloned into the PBCAG plasmid vector; for transposition, PMBCs were isolated by Ficoll gradient and electroporated with 20ug of the transposase and 10ug of the 4D5 or FRP5 carrying PB plasmid. After electroporation, the cells were cultured for up to 12 days and the receptor expression, memory and exhaustion phenotype were analyzed at different times by flow cytometry. For in vivo antitumor evaluation, 3x10^6 SK-OV-3 LUC+ per NSG mice were injected in the right flank and the animals were treated peritumorally with 1,3x10^6 CAR-T cells on day 41, when the tumor volume was approximately 500 mm3; and tumor volume and bioluminescence were monitored. **RESULTS AND CONCLUSION:** A constant expression of the two receptors was observed, reaching an average of 25% of cells expressing the CAR 12 days after transduction. On the eighth day of expansion, the phenotypes of central memory (CD45RO+ CCR7+) and effector memory (CD45RO+ CCR7-) were also evaluated, in addition to exhaustion-related markers (PD1, TIM3 and LAG3) in CAR-T CD4+ and CD8+ populations. In both cases we observed a similar phenotype in the evaluated anti-HER2 CAR receptor populations, evidencing a higher frequency of cells with central memory phenotype. For exhaustion-associated receptors, we observed a lower frequency of expression among CD8+ CAR-T cells expressing the FRP5 clone. For the CD4+ subset, we could observe an increase of the markers for either of the CAR constructs compared with untransduced cells. In the in vivo assay, we observed non-tumor-related death. While 4D5 exhibited decrease on tumor volume and bioluminescence, with on mouse reaching full regression, FRP5-based CAR wasn't able to control tumor burden. In summary, the two evaluated anti-HER2 CARs demonstrated consistent expression in PBMCs from different donors, with predominant central memory phenotype and low frequency of inhibitory receptor expression. At the chosen dose and tumor volume for treatment, the 4D5 seemed more prone to induce tumor burden control, maybe due to its increased affinity for the HER2 antigen. Additional in vivo experiments will be performed with lower total T cell numbers to avoid non-tumor-related deaths and confirm the antitumor capacity from both CARs.

Keywords: CAR-T Cells; Her2; Solid Tumor
Supported by: CNPq, FAPERJ, PRONON, Ministério da Saúde.

INFLUENCE OF GREEN TEA (*CAMELLIA SINENSIS*) EXTRACT ON PERIPHERAL BLOOD CELLS STIMULATED BY CONDITIONED MEDIUM FROM BREAST TUMOR CELL LINES

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INTRODUCTION AND OBJECTIVES: The most common cancer among women is breast cancer, which despite advances in diagnosis and treatment, is the leading cause of death from the disease in several countries around the world, including Brazil. Recent studies have shown that breast cancer consists not only of tumor cells but also of significant changes in what we call the tumor microenvironment, which has been considered a potential therapeutic target. An important factor in reducing the risk of cancer is diet. Numerous epidemiological pieces of evidence demonstrate an inverse relationship between the consumption of plant-based foods and the incidence of the disease. The bioactive compounds naturally present in these foods are responsible for the benefits they provide to human health. Camellia sinensis is the raw material for green tea, a beverage rich in phenolic compounds - especially catechins - that have anticancer potential. Our group previously tested the effects of green tea extract (GTE) on breast tumor cells, with an impact on reducing migration, cell viability, and modulation of the tumor suppressor protein p53. However, the investigation of these processes in cells of the immune system, such as in peripheral blood mononuclear cell (PBMC) cultures, still needs to be explored. To this end, the aim of this work was to investigate the influence of GTE on the modulation of peripheral blood mononuclear cells when exposed to the conditioned medium of two breast cancer cell lines, simulating in vitro the tumor microenvironment. **MATERIAL AND METHODS**: PBMC cells were collected from healthy individuals and exposed to a conditioned medium of MCF-7 and MDA-MB-231 cells in the presence and absence of different concentrations of GTE. Analyses to assess cell viability, lymphocyte activation, and cell death were performed to elucidate the effects of ECV in modulating cells of the immune system. **RESULTS AND CONCLUSION**: Preliminary results indicate that the conditioned medium of both tumor lines, in the absence of ECV, causes exacerbated lymphocyte multiplication, while in the presence of ECV the immune response appears to be more controlled. The concentrations of ECV studied were not shown to be toxic to PBMC cells. Further assays are in progress to better characterize the effects of ECV on cells of the immune system.

Keywords: PBMC, Breast cancer, Catechins, Tumor microenvironment.
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ONCOGENIC ACTIVATION OF *FLT3* IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

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INTRODUCTION AND OBJECTIVE: Acute myeloid leukemia (AML) has great genetic heterogeneity and treatment response is still poor for the majority of patients. Mutations in the *FLT3* (*fms-like tyrosine kinase 3*) gene are often observed. This gene encodes a tyrosine kinase receptor that is very important in hematopoiesis, since its activation regulates cellular processes such as transcription, proliferation and apoptosis. Two types of mutations are the most common: internal tandem duplication in the juxtamembrane domain (*FLT3*-ITD) and point mutations in residues D835/I836 of the tyrosine kinase domain (*FLT3*-TKD). As a consequence of constitutive activation of the FLT3 receptor, there is a high stimulus for proliferation of leukemic clones. In addition, overexpression of *FLT3* is associated with a worse prognosis in patients with AML, which may occur regardless of the presence of activating mutations. Because there is still little clarification on this subject, we aim to determine the levels of *FLT3* gene expression associated with its state of oncogenic activation in AML patients. **MATERIAL AND METHODS:** Samples from patients diagnosed with AML were referred from different centers: INCA-RJ, Prontobaby-RJ, IMIP-PE, IPPMG-UFRJ, Hemorio and Amaral Carvalho Hospital-SP. Furthermore, the main genetic alterations associated with prognostic risk as defined by the European LeukemiaNet (ELN) are under investigation: mutations in *FLT3* (ITD and TKD), *NPM1* and *WT1*. The search for *FLT3-ITD* and *NPM1* mutations are being performed by fragment analysis, while the determination of *FLT3-TKD* and *WT1* mutations are being performed by Sanger sequencing. Finally, to verify the association between the expression of *FLT3* and markers of this pathway activation, transcript (RNA-seq) and protein (RPPA) expression data were obtained from the CCLE database. Subsequently, a differential expression analysis between two groups of AML cell lines (*FLT3* mutation vs. low expression of *FLT3*) was performed. **RESULTS AND CONCLUSION**: At the moment, we included 63 samples, with most patients being male (51%) and with a median age of 12 years (range: 0-79 years); the FAB M3 subtype represents 13% of cases. Regarding gene mutations, 14% of patients have alterations in the *NPM1* gene, 13% in *FLT3-ITD*, 1.3% in *FLT3-TKD*, and no patient showed alteration in *WT1*. Considering the stratification of prognostic risk defined by the ELN, 8 patients were classified with favorable prognosis, 6 with an intermediate and 2 with an adverse prognosis. In regard to in silico approaches, three genes stand out differentially expressed between the two cell lineage groups (mutation in *FLT3* vs. low expression of *FLT3*): *BCL2, STAT5* and *NDRG1*. They showed expression proportional to that observed for *FLT3* (R=0.62, p=0.00016; R=0.62, p=0.00016; R=0.7, p=7.7e-06, respectively). Furthermore, their transcriptional levels were directly proportional to their protein levels (R=0.19,

p=6.2e-12; R=0.9, p=1.5e-12; R=0.61, p=0.00018, respectively). Currently, we plan to use these markers to better understand the mechanisms of oncogenic activation of *FLT3* in AML, which is essential for improving the prognostic risk stratification of these patients as well as for assisting the development of new therapies for this disease.

Keywords: Acute myeloid leukemia, *FLT3*, mutation, gene expression.
Supported by: FAPERJ, CNPq, CAPES, Alexander von Humboldt Foundation, Swiss Bridge Foundation.

INHIBITION OF THE WNT/β-CATENIN PATHWAY ENHANCE 5-FU EFFECTS IN MICE MODEL OF COLORECTAL CANCER

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INTRODUCTION AND OBJECTIVES: The Wnt/β-catenin signaling pathway plays a central role in the establishment of the embryonic axes and the regulation of homeostasis in adult tissue. However, the upregulation of this pathway is associated with cell abnormal proliferation and differentiation in many diseases. The Wnt/β-catenin pathway is upregulated in a wide variety of cancers, such as colorectal cancer (CRC), in which Wnt components are altered in around 90% of the cases, especially the tumor suppressor gene APC. The chemotherapy drug 5-Fluorouracil (5-FU) remains the first-line treatment for CRC, but despite its initial reduction of the tumor mass, tumor recurrence after treatments remains the main barrier to an effective outcome for the patients. Recent studies have shown that the chemotherapy treatment with 5-FU promotes the stemness of CRC cells through p53-mediated Wnt/β-catenin pathway activation, which could relate to tumor recurrence after the treatment. Therefore, we hypothesized that the use of Wnt/β-catenin signaling pathway inhibitors could increase the chemotherapy treatment response and impair tumor recurrence. Thus, we sought to test the effects of the combination of 5-FU with the chalcone lonchocarpin, an inhibitor of the Wnt/β-catenin pathway, in a mouse model of Wnt/β-catenin-associated CRC. **MATERIAL AND METHODS:** We used the APC-CPC Cmah -/- mice (APC knockout) as a model, which spontaneously develops intestinal tumors around the third month of life. The mice were treated with drug vehicle (control); 5-FU i.p (25 mg/kg) every 48 hours; lonchocarpin orally (5 mg/kg) daily and a combination of both drugs. The mice were treated for 21 days and after euthanasia, the organs (intestines, livers, and kidneys) were collected for further histopathological examination as well as biochemical and molecular characterization by immunohistochemistry, western blotting, and q-RT PCR for the Wnt/β-catenin pathway target genes, cell proliferation, and pluripotency markers. **RESULTS AND CONCLUSION:** No significant body weight loss or deaths were registered during the experimental period in treated mice. Macroscopically, we could see a drastic reduction in tumor number and size in mice from the combined treatment group compared to the control and the mice from both 5-FU or lonchocarpin monotherapy groups. We also evaluated macroscopical aspects of the livers and kidneys of all groups, and no toxicity was observed. The next steps are the biochemical and molecular characterization, as well as the evaluation of cell proliferation and cell death induction. So far, our data support the hypothesis that targeting the Wnt/β-catenin signaling pathway can improve 5-FU effects in CRC treatments.

Keywords: Wnt/β-catenin, 5-Fluorouracil, Colorectal cancer.
Supported by: FAPERJ and CNPq

IMPACT OF *BRAF*^{V600} MUTATIONS IN THE CLINICAL AND HISTOPATHOLOGICAL CHARACTERISTICS OF ACRAL MELANOMA AND ITS DERIVED EXPERIMENTAL MODELS

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INTRODUCTION AND OBJECTIVES: Despite its low incidence, melanoma is the deadliest type of skin cancer. One of its subtypes, acral melanoma (AM), develops in glabrous skin of the palms, soles, and nail apparatus. Its incidence is relatively higher in populations originated from Africa, Asia and Latin America. Regardless of advances in the understanding of the genetics of melanomas, most studies explore target-specific therapeutic approaches for non-acral cutaneous melanomas, which are more prevalent in populations of European ancestry. This occurs, for example, with BRAF inhibitors such as Dabrafenib (DAB), since the frequency of patients carrying *BRAF*^{V600} mutations is, on average, 60% higher in cutaneous melanomas than in AM. Histopathological features are crucial for AM staging and prognosis and consequently, for the clinical care of the patient. However, it is still not clear whether these features are associated with *BRAF*^{V600} mutations in AM. It is also not clear if response to BRAF inhibitors in AM is similar to that observed for cutaneous melanomas. Therefore, the aim of this study is to evaluate the correlation between histopathological parameters and the presence of *BRAF*^{V600} mutations in AM cases and derived PDX models, and analyze how BRAF-mutated PDX respond to DAB. **MATERIAL AND METHODS:** A collection of 28 PDX was established from 77 AM samples from 61 patients treated at the Brazilian National Cancer Institute (INCA). Clinical and histopathological data were collected from medical records. The presence of *BRAF*^{V600} mutations was analyzed by Sanger or whole exome sequencing from patient samples and PDX models. One *BRAF*^{V600E}-mutated PDX was selected for analyzing the effect of DAB in tumor growth *in vivo*. For that, AM058-X1 cells were expanded and inoculated in 10 immunocompromised (NSG) mice ($1x10^6$ cells/animal). When the tumors reach 100 mm^3, mice will be divided into control and treated groups. DAB (30 mg/kg) will be administered 6x/week by oral gavage and tumor volume will be monitored 2x/week. **RESULTS AND CONCLUSION:** Preliminary histopathological analysis was performed in 42 patient samples from primary tumors and 21 from metastatic lesions. Analyses of the primary tumors show that most were classified as clinical stage III (62%), with Breslow Depth > 4mm (83%) and IV/V Clark level (97%). There was also evidence of microsatellitosis (10%) and vascular (28%) or perineural (20%) invasion in primary tumors. Median mitotic index (mitoses/mm^3) was 7 in primary and 6 in metastatic tumors. Regarding tumor cell morphology, both primary and metastatic tumors were predominantly epithelioid. Sequencing results from the cases already analyzed show that 23% of the AM samples (n=5) and of the PDXs (n=2) have the *BRAF*^{V600E} mutation. The *BRAF*^{V600E}-mutated PDX is currently being evaluated *in vivo* for DAB sensitivity.

Keywords: Acral melanoma, BRAF^{V600}, Dabrafenib.

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CLOTRIMAZOLE IMPACT ON MACROPHAGE M2 POLARIZATION

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INTRODUCTION AND OBJECTIVES: Macrophages are cells of the innate immune system, present in all tissues where function as sentinels. They are extremely specialized cells capable of detecting and responding to pathogens invasion, tissue damage and maintaining homeostasis. Due to their plasticity, these cells are extremely responsive to microenvironment stimuli and can change their phenotype and functions. In addition to their important role in tissue homeostasis, macrophages are also involved in several pathologies. Macrophage activation is conventionally defined in a classically activated state (M1) in which these cells assume pro-inflammatory characteristics (stimulated by LPS and IFNγ) and the alternative activation state (M2) with opposite characteristics assuming an anti-inflammatory profile (activated by IL-4). Tumor-associated macrophages (TAM) are macrophages present in the tumor microenvironment with M2-like characteristics due to the immunomodulatory profile. Its tumorigenic role is highlighted in the literature, where it is described for playing important roles in tumor progression, migration, metastasis, angiogenesis and immunomodulation. Investigate the mechanisms of M2 polarization is essential to understand tumor biology and its microenvironment, generating the possibility of new therapeutic strategies. The objective of this work is to evaluate the effect of clotrimazole, an important PI3K pathway inhibitor, on M2 polarization. **MATERIAL AND METHODS:** Murine macrophage cell line J774 and bone marrow-derived macrophages were polarized with IL-4 and then treated with 5μM clotrimazole for 24h. Subsequently, immunofluorescence, Western blotting and qPCR assays were performed to evaluate the effects on the phenotype, modulated pathways and the gene expression of M2 markers. **RESULTS AND CONCLUSION**: After treatment with CTZ, a modulation in the PI3K pathway was observed, characterized by the decrease in the phosphorylation of the downstream effectors. Furthermore, a modulation on M2 activation phenotype was observed when these macrophages are treated with clotrimazole, observed by immunofluorescence and Western blotting of classic M2 activation markers. In addition, the effects of CTZ on the MAPK and NFkB pathways were analyzed, which corroborate with our hypothesis that there is a modulation in these pathways that are linked to phenotypic changes in these cells. These results support the hypothesis that CTZ modulates M2 polarization, inhibiting important pathways for M2 phenotype. These data confirm previous data and support our hypothesis that CTZ can modify the macrophage phenotype and directly impact its tumorigenic effects in the tumor microenvironment.

Keywords: Macrophages, Polarization, Clotrimazole
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GLYCOLIPIDS FROM *SARGASSUM FILIPENDULA* AS A NATURAL SOURCE OF INHIBITION OF ABCC1 TRANSPORTERS IN CANCER CELLS

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INTRODUCTION AND OBJECTIVES: Cancer is one of the leading causes of death worldwide. Despite advances in chemotherapy, tumor diversity, side effects, and Multidrug Drug Resistance (MDR) make it increasingly difficult to treat this disease. MDR is characterized by ABC transporter overexpression, which expel drugs used in chemotherapy, being the main obstacle to their treatment efficiency. Several products of natural or synthetic origin are potential ABC inhibitors and reverse MDR phenotype when co-administered with drugs. Marine macroalgae species of the genus *Sargassum* present a high content of glycolipids, which exhibit different biological activities. Therefore, the purpose of this research is to evaluate the glycolipids from *S. filipendula* as an inhibitor/modulator of the main transporters ABC involved to resistance (ABCB1, ABCC1 and ABCG2) in cancer cells.

MATERIAL AND METHOD: Glycolipids were obtained from the Laboratory of Functional Compounds of the University of Antioquia, characterized as sulfoquinovosyldiacylglycerols (SQDG). 7 glycolipids, named according to the retention time in the column in the separation process, were evaluated at concentrations of 25 to 200 µg/mL. Lucena-1 cells are resistant to vincristine sulfate and were used as model due to overexpression of ABC transporters. ABC-mediated efflux assays were divided into two 30-minute stages: substrate accumulation and efflux in the absence or presence of glycolipids. The dyes Rhodamine 123, CFDA and Mitoxantrone were used as fluorescent substrate for transport of the ABCB1, ABCC1 and ABCG2 proteins, respectively. The cytotoxicity assay of the glycolipids was performed by Propidium Iodide labeling, a DNA intercalating dye. The dye fluorescence intensity was measured by flow cytometer.

RESULTS AND CONCLUSION: 6 of 7 glycolipids inhibited substrate efflux mediated by ABCB1 and ABCC1. The glycolipid 1 showed no inhibitory effect on ABC transporters. Glycolipids 2, 3, and 4 showed higher inhibitory capacity than glycolipids 5, 6, and 7 for the membrane transporters ABCC1 and ABCB1; increasing their inhibition in a dose-dependent way. Glycolipids 2, 3, and 4 inhibited above 80% and about 50% of cells with ABCC1 and ABCB1 activity, respectively, suggesting a greater affinity for the ABCC1 transporter. Furthermore, none compounds exhibited an inhibitory capacity for ABCG2 transporter. None of these metabolites showed a cytotoxic effect at the concentrations tested at 24 h. The difference between the glycolipids is due to the structural diversity of the carbon chains composing the fatty acids, as well as the degree of unsaturation of the molecule. These differences could affect the binding of these glycolipids to transporter sites and,

consequently, their efflux. The results suggest that SQDGs, being anionic organic compounds, could be competitive inhibitors and, possibly a source of phytotherapeutic agents capable of reversing the MDR phenotype in cancer cells.

Keywords: ABC transporters, *Sargassum filipendula*, glycolipids, cancer.

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CLOTRIMAZOLE IS EFFECTIVE AND SAFE IN THE TREATMENT OF ENDOMETRIOSIS

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INTRODUCTION AND OBJECTIVES: Endometriosis is a gynecological disease characterized by the displacement of endometrial tissue out of the uterine cavity. Although considered a benign disease, endometriosis frequently presents characteristics of malignancy and presents an increased risk of developing epithelial ovarian cancer. Several factors contribute to the development of the disease such as chronic inflammation, hormonal changes, and genetic factors. During inflammation, reactive oxygen species (ROS) are released, a product of the activation of macrophages in the peritoneal cavity, being a fundamental factor in the adhesion of endometrial tissue and damage at the cellular level. The endometriosis current clinical treatment is based on the use of hormones (combined analogues of androgen, progestin, antiprogestin and gonadotropin-releasing hormone), but these drugs action only in relieving symptoms and has several side effects. In addition, the surgery (laparoscopy) is an invasive treatment and presents disease recurrence of 2 to 5 years later. Clotrimazole (CTZ) is an antimycotic drug used in clinical medicine for the treatment of Candida Albicans and has already shown reduction of ROS production, endometriotic cell growth and disease development. This study investigated the therapeutic effects of CTZ on inducible nitric oxide synthase (iNOS), modulation of the antioxidant defense system and oxidative stress biomarkers in an experimental model of endometriosis. **MATERIAL AND METHODS:** Animals were treated in accordance with protocols approved by the CEUA from UEZO (009/2019). Eighteen female Wistar rats were randomized into two groups after autologous endometrial implantation and treated with CTZ (200 mg/kg) or vehicle via esophageal gavage for 15 consecutive days. Macroscopic, histological, and immunohistochemical analyses of iNOS were performed. Levels of superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST), glutathione (GSH), lipid peroxidation (LPO) and protein carbonylation (PTC) were measured in brain and liver. **RESULTS AND CONCLUSION**: At the end of the treatment, the growth, maintenance, and implant size of the endometriotic lesions and iNOS immunoreactivity were significantly lower in the CTZ group compared to the control. Liver levels of LPO, PTC, and SOD were reduced, and GST activity were increased. However, there was no change in SOD, GSH and GST levels in brain. These results suggest that CTZ interferes with reactive nitrogen species (RNS) production by downregulating iNOS expression and, therefore, enhances the antioxidant system, promoting atrophy and regression of endometriotic lesions, without adverse effects upon the brain and/or liver. CTZ is a promising drug for the treatment of endometriosis due to its effectiveness and without adverse effects.

Keywords: Endometriosis, Clotrimazole, Oxidative Stress
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NEUTROPHIL EXTRACELLULAR TRAPS (NETS) DRIVE THE SECRETION OF EXTRACELLULAR VESICLES (EVs) IN HUMAN BREAST CANCER CELLS

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INTRODUCTION AND OBJECTIVES: Extracellular vesicles (EVs) are particles naturally released from cells, delimited by a lipid bilayer, and not subjected to replication. Virtually all cells are capable of secreting EVs, including cancer cells. EVs' release has been linked to metastasis. Also, EVs have a complex role in cellular communication affecting the stroma and immune cells, such as neutrophils. Our group has shown that neutrophils extracellular traps (NETs) induce a change in MCF7 cells into a more aggressive phenotype through the epithelial-mesenchymal transition (EMT). Little is known about the role of NETs in the vesiculation of cancer cells. Thus, this study aims to analyze NETs' ability to stimulate EVs secretion from human MCF7 breast cancer cells. **MATERIAL AND METHODS:** We used the breast cancer cell line MCF7, described as luminal A, epithelial-like, and less aggressive. Neutrophils isolated from the blood of healthy donors were stimulated with Phorbol 12-myristate 13-acetate (PMA) to produce NETs which were further isolated using an established protocol. Tumor cells were treated with isolated NETs for 24h and EVs were further isolated from the conditioned media using an established protocol. Isolated EVs were submitted to Zetaview particle analysis, protein quantification and microscopy evaluation. For *in silico* analysis, we used the online platform GEPIA2 to evaluate the correlation between neutrophil states and NETs gene-signatures and EV generation-related genes (Rabs) in The Cancer Genome Atlas (TCGA-BRCA) cohort. **RESULTS AND CONCLUSION:** Our results suggest that although EVs size remains similar with or without NET stimulus, treatment with NETs induce an increase in the secretion of EVs by MCF7 cells. Transmission electron microscopy confirmed that NETs enhance the MCF7 vesiculation. We found a positive correlation between vesiculation genes (Rabs) and neutrophil states / NETs gene signatures, with emphasis on Rab27A, Rab27B, RAB9a and Rab14. Rab27A showed a higher correlation (>0,35) with neutrophil states / NETs gene signatures compared with other Rabs. Real-time PCR showed that NETs upregulated the mRNA expression of Rab27B in MCF7 cells. Overall, the protumor effect of NETs may derive, at least in part, from their ability to enhance the release of EVs from tumor cells.

KEYWORDS: Breast cancer, neutrophil extracellular traps (NETs), extracellular vesicles (EVs), Rab GTPases.

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APE1 REDOX INHIBITOR EXERTS ANTITUMOR EFFECT ON 3D BREAST CANCER CELL CULTURES IN NORMOXIC AND HYPOXIC MICROENVIRONMENT

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INTRODUCTION AND OBJECTIVES: Breast cancer tumor hypoxia is associated with aggressiveness, worse prognosis, and treatment resistance. Therefore the search for molecular targets is essential to increase the success of anticancer therapies. In this scenario, the bifunctional AP-endonuclease 1 (APE1) protein acts as an endonuclease in the base excision DNA repair pathway and activates transcription factors through its redox domain, both functions are involved in tumor promotion and progression. Although APE1 inhibitors are already in clinical trials and APE1 protein has emerged as a potential target for cancer treatment, no studies consider the breast cancer hypoxia microenvironment and 3D cell cultures/spheroids. Therefore, we aimed to analyze the antitumor effect of the APE1 inhibitor, APX2009, in breast cancer 3D cell cultures in a hypoxic microenvironment. **MATERIAL AND METHODS:** The MDA-MB-231 and MCF-7 breast cancer cell lines were cultured in low adherence plates in DMEM/F12 medium supplemented with EGF and B27 to form 3D/spheroids cell cultures. For the 3D viability assay, APX2009 inhibitor treatments were carried out for 72 hours after five days of cell culture. For spheroid formation assay, APX2009 inhibitor was added at the time of the cell seeding under normoxia or hypoxia. The hypoxic microenvironment was carried out for 24 hours in 1% of oxygen in the hypoxia chamber. In both assays, 3D/spheroid cell viability was assessed using CellTiter-Glo 3D reagent. **RESULTS AND CONCLUSION**: The 100 µM of APX2009 decreases the viability of the MDA-MB-231 3D cell cultures, while for MCF-7 3D cell cultures, viability was also reduced from 50 µM. Thus, these results suggested that the MCF-7 cell line is more sensitive to the APE1 redox domain inhibitor than MDA-MB-231, in the 3D cell cultures, which mimics the tumor. In the spheroid formation assay, the 50 µM of APX2009, in normoxia, decreased the viability of MDA-MB-231 cells, whereas, in hypoxia, the same data were reached by using 20 µM. For the MCF-7, the cell viability was also reduced in normoxia and hypoxia from 20 µM. Additionally, APX2009 inhibits tumorigenesis in hypoxia blocking spheroid formation, which encourages further studies on APX2009 as an antitumor promise.

Keywords: Breast cancer spheroids, Hypoxia, APE1
Supported by: FAPERJ, CNPq, and CAPES

EFFECTS OF THE STEROID OUABAIN ON THE LYMPHOCYTE POPULATION IN A SUBCUTANEOUS MODEL OF MURINE MELANOMA (B16F10)

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INTRODUCTION AND OBJECTIVES: Ouabain (OUA) is a steroid initially used to treat heart failure and such effects are due to its potential as a specific inhibitor of Na+/K+-ATPase. OUA has been described as an endogenous substance, therefore, it has a role in the modulation of the immune system and acts as a modulator of inflammatory responses. Melanoma is a tumor derived from epidermal melanocytes, considered the most lethal skin cancer, with high mortality rates. The aim of, this study was investigate the *in vivo* effects of OUA on the immune response of C57BL/6 mice with subcutaneous melanoma. **MATERIAL AND METHODS:** C57BL/6 mice were injected intraperitoneally for three consecutive days with 200 ul of DMEM medium in the control (CTR) and melanoma (MEL) groups or with 0.56mg/Kg of OUA in the Ouabain (OUA) and melanoma plus. Ouabain groups (MEL+OUA). On the 4th day, half of the animals from the CTR and OUA groups were euthanized for internal control of the experiment and the MEL and MEL+OUA groups were inoculated subcutaneously on the flank with melanoma (B16F10). On the 21st day, all other animals were euthanized to remove the spleen, mesenteric and inguinal lymph nodes. The cells were stained with different antibodies and flow cytometry analysis was performed. **RESULTS AND CONCLUSION**: Our preliminar results show that, on the 4th day, the treatment with OUA decreases the percentage and absolut number of B cells in the spleen, confirming the previous results of our group. In inguinal lymph node, ouabain decreases the number absolut number of B lymphocytes besides CD4+ and CD8+ T lymphocytes. On the 21st day there was a decrease in percentage of CD4+ T cells in the MEL+OUA group when compared to the control group in inguinal lymph node. On the other hand, there was an increase in the absolut number of B cells in the MEL+OUA group when compared to the other groups. These results point to a possible effect of ouabain on the recruitment of B lymphocytes to the tumor draining lymph node.

Keywords: Ouabain, immunoregulation, B cells, T cells.

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CYTOTOXIC AND ANTIOXIDANT POTENTIAL FROM LEAVE EXTRACT OF *Begonia luxurians Scheidw.* AGAINST BREAST CANCER CELL LINES

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INTRODUCTION AND OBJECTIVE: Breast cancer is the most common cancer in women. Worldwide, with approximately 2.3 million estimated cases in 2020 and the most frequent cause of cancer death in this population. Chemotherapy has been the most important treatment option for breast cancer but this therapeutic is not completely effective due side effects and toxicity of drugs. Evidences shows that natural products isolated from plants extracts, have wide application prospects in cancer prevention and treatment. *Begonia luxurians Scheidw.* is a specie of genus Begonia (*Begoniaceae*) endemic in Brazil and distributed in the Southeast region, mostly in Espírito Santo, Minas Gerais, and Rio de Janeiro states. Previous studies reported biological activities of secondary metabolites from plants of the genus Begonia including anticancer potential. The aim of this study was to analyse the cytotoxic effect and antioxidant potential of leaves extract of *Begonia luxurians Scheidw.* in different breast cancer cell lines. **MATERIAL AND METHODS:** The viability of MDA-MB-231, MCF-7, 4T1 and murine macrophage cells RAW 264.7 cells strains were evaluated by MTT method. The phases of the cell cycle were analyzed in flow cytometry after labeling with RNAse-propidium iodide (PI). The characterization of cell death was investigated by labeling with PI and Annexin-V. The antioxidant capacity was evaluated by the methods of TEAC (*Trolox Equivalent Antioxidant Capacity*) and FRAP (*Ferric-Reducing Ability of Power*) and by the production of reactive oxygen species (ROS) by flow cytometry after labeling with the DCFDA. **RESULTS AND CONCLUSION**: Our results demonstrated that the crude extract from leaves of *Begonia luxurians Scheidw.,* in different concentrations (25, 50, 75, 100 and 200 µg/mL), after 24 hours showed cytotoxicity in MDA-MB-231 and 4T1 cells with IC_{50} = 111.4 ± 21.6 and 114.5 ± 15.52 µg/mL, respectively, and showed no cytotoxicity in MCF-7 and RAW 264.7 cells. The *Begonia luxurians Scheidw* extract increase 5.78-fold the sub-G0/G1 phases of cell cycle after 24 hours of treatment in MDA-MB-231 and increase the percentage of PI positive cells and annexin-V/PI positive cells by 13,51 and 7-fold, respectively, in relation to untreated control which characterizes late apoptotic and necrotic cells. Furthermore, we found that *Begonia* extract demonstrated values for TEAC and FRAP of 10.57 ± 0.18 µM Trolox/mL and 70.27 ± 4.24 µM Fe_2SO_4/mL, respectively and decrease the ROS production 20.98 and 24.13-fold in MDA-MB-231 and 4T1 cells, respectively. In conclusion, our results demonstrated the cytotoxic and antioxidant potential from leaves

extract of *Begonia luxurians Scheidw.* on different breast cancer cell lines. These results leads us to suggest that leaves from *Begonia luxurians Scheidw* can be an important source of new anti-cancer molecules development.

Keywords: *Begonia luxurians Scheidw.*, Breast cancer, Natural products.
Supported by: FAPERJ, CNPq, Fundação do Câncer and CAPES

HAPLOTYPES OF THE EPSTEIN-BARR VIRUS REVEALS A RECOMBINANT VARIANT WITH TRANSFORMATION POTENTIAL CIRCULATING IN BRAZIL

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INTRODUCTION AND OBJECTIVE: The Epstein-Barr virus (EBV) is a γ-herpesvirus that asymptomatically infects >90% of the adult population worldwide. However, EBV is etiologically associated with the development of neoplasms. EBV own a dsDNA genome of ~170kb and is divided into EBV1 and EBV2 with distinct biological properties. The carcinogenic mechanisms mediated by EBV are not completely understood, and viral oncogenic variants may act directly on these mechanisms. Here, we describe the diversity of the *BZLF1* viral gene related to the lytic cycle transactivator Zta and its haplotypic relationship with other viral targets, to understand the evolution and adaptation of strains with a high transformative potential present in our region. **MATERIALS AND METHODS:** This study included 70 EBV+ samples, consisting of malignant cases (ML, 36%) and asymptomatic carriers (AC, 64%) previously characterized for the Zp promoter, *LMP1* oncogene and type by *EBNA3C* gene. The classification of the coding region of the *BZLF1* gene and the relationship of haplotypes was performed by Bayesian phylogenetic analysis, while DNA motifs inducing recombination (DMIR) were quantified using the find motifs tool. **RESULTS AND CONCLUSION:** Phylogenetic analysis of *BZLF1* indicated the presence of two main clades with high support (PP>0.85), where our sample had a prevalence of 27% for the clade BZLF1-A and 73% for the clade BZLF1-B. Through polymorphism analysis, the BZLF1-A clade proved to be the most diverse. In the haplotypic analysis with the viral Type and the Zp promoter, the clade BZLF1-A mainly clustered the haplotype with high replicative capacity Type 1+V3 (p<0.001). When analyzed *BZLF1* clade with the Raji/Argentine clade of viral oncogene *LMP1*, that clustered mainly Brazilian lymphomas, this haplotype was shown to be associated with the BZLF1-A clade (p=0.020). Then, the multigene analysis between *BZLF1* and *LMP1* was able to stratify the different haplotypes determined in our sample together with references of worldwide geographic origin. Highlighting the haplotype BZLF1-A+Raji/Argentine+Type1+V3 that demonstrated a possible recent origin, corroborating the hypothesis that this variant originated in the late evolutionary history of EBV. Finally, when evaluated the presence of recombination-inducing motifs, BZLF1-A+Raji/Argentine haplotype showed to possess a greater number of the motifs (p<0.001), strengthening the hypothesis of the recombinant potential of this variant. Although no clinical outcome was directly associated with a specific clade or haplotype, interestingly, the ML group had a greater amount of recombination motifs than the AC (p=0.011). Our results highlight a great genetic diversity of EBV circulating in Brazil, drawing attention to a possible recombinant variant present in neoplasms and associated with haplotypes with functional gain. This work call attention to the importance of monitoring and evaluating genetics determinants of EBV-mediated oncogenicity.

Keywords: Epstein-Barr virus, Lymphoma, Oncovirus, EBV Diversity.
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NEW TRISUBSTITUTED IMIDAZOLES AS EGFR-TYROSINE KINASE INHIBITORS: DESIGN, SYNTHESIS AND PHARMACOLOGICAL ACTIVITY

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INTRODUCTION AND OBJECTIVES: Lung cancer is a serious health problem, 85% of them are represented by the non-small cell lung cancer (NSCLC). In recent years, molecular targeting has achieved great progress in therapeutic treatment of cancer and plays a crucial role in the current clinical treatment of NSCLC. Among them, EGFR-targeted therapies are considered very promising. EGFR is a cell membrane receptor that plays a key role in cancer development and progression. Ligand-activated EGFR-dependent signaling is involved in cell proliferation, apoptosis, angiogenesis, invasion, and metastasis. However, due the high genomic instability of NSCLC, tumors lead to the rapid development of resistance against the classical EGFR tyrosine kinase inhibitors (TKIs). The main goal of the project is to contribute to the discovery of new antitumor prototypes, acting by the inhibition of the kinase domain of EGFR. We described here the synthesis and pharmacological activity by phenotypic assays of four new trisubstituted imidazoles derivatives, designed as new EGFR TKIs. **MATERIAL AND METHODS:** The new compounds were synthetized, and the relative purity was determined by HPLC. The cytotoxic profile was evaluated by the MTT assay and antiproliferative capacity by SRB assay, using three different cell lines of lung cancer: PC9 (EGFR L858R), H1975 (EGFR L858R + T790M) and H292 (EGFRwt). Erlotinib (first-generation inhibitor), WZ-4002 and Osimertinib (both third-generation inhibitor) were used as a control. **RESULTS AND CONCLUSION:** First, screening was performed against the PC9, H1975 and H292 cell lines, using the target compounds at 10 μM. Compounds were inactive on H292. Therefore, the cytotoxic potency, the CC_{50} and the antiproliferative assay were determined against PC9 and H1975. As expected, erlotinib was less potent (CC_{50} = 0.14 μM and 12.14 μM in PC9 and H1975, respectively) than both osimertinib (CC_{50} (PC9) = 0.01 μM and 0.22 μM) and WZ-4002 (CC_{50} = 0.07 μM and 0.23 μM). The synthesized compounds were equipotent to erlotinib in PC9 cells. However, against H1975 they displayed great cytotoxic activity with CC_{50} of 1.28 μM; 1.35 μM, 2.14 μM and 2.26 μM. Results of cell proliferation assay corroborate the results of cytotoxic activity. The comparative cytotoxic and antiproliferative activity of those compounds with the standard drugs are now being investigated.

Keywords: NSCLC, EGFR, tyrosine kinase inhibitors, trisubstituted imidazoles.
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CAPSAICIN-INDUCED METABOLIC ALTERATIONS IN BREAST TUMOR CELL LINES

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INTRODUCTION AND OBJECTIVES: Breast cancer is a public health problem that affected about 88,492 Brazilian women in 2020. The current oncological treatments compromise the well-being of patients and do not always contribute to increasing life expectancy. In this spectrum, phytochemicals have been described as a potential alternative treatment. Capsaicin is a component of hot red peppers and chili peppers. Several studies have reported that compound exhibited pro-apoptotic, anti-metastatic, and anti-angiogenic effects in cancer cell lines. Previous data have shown a significant correlation between capsaicin and downregulation of hexokinase-2 expression. It is well knowing that hexokinase presents important role of glycolysis, meanwhile, the interaction of this pathway with capsaicin remains unknown. In time course and dosage studies capsaicin show induces increase reactive oxygen species and intracellular calcium levels promoting apoptosis. Therefore, causes decreased of mitochondrial membrane potential in cancer cells. In the present study, the effect of capsaicin on breast tumor growth, enzymatic activities of glycolytic and oxidative metabolism as well as its cytotoxicity will be investigated.**MATERIAL AND METHODS:** The analyses will be performed on invasive tumor (MDA-MB-231), non-invasive tumor (MCF-7) and non-tumor (MCF-10A) cell lines. These cells will be subjected to capsaicin treatment at varying concentrations and times. Viability and proliferation will be measured by MTT, Trypan blue, and cell migration assay. The quantification of lactate dehydrogenase activity will also be quantified. This assay is also an indicator of apoptosis and cell necrosis. Mitochondrial function will be assessed by high-resolution respirometry (OROBOROS). The cytotoxicity of capsaicin will be measured by comparing the effects on tumor and non-tumor cells. To evaluate the oxidative metabolism of glucose, the enzymatic activities of hexokinase and citrate synthase will be performed. **RESULTS AND CONCLUSION**: This research work looks into contribute to a greater understanding of cancer metabolism and critical analysis of capsaicin effects in the glycolytic pathway in breast tumor cell lines, elucidating its possible mechanisms of action. In this spectrum, we also wish to study the cytotoxicity of capsaicin through comparing tumor and non-tumor cells.

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PARTICIPATION OD ECTO-5'NUCLEOTIDASE(CD73) IN THE GENERATION OF EXTRACELLULAR INORGANIC PHOSPHATE: POSSIBLE INVOLVEMENT IN METASTATIC PROCESS IN BREAST CÂNCER

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INTRODUCTION AND OBJECTIVES: Inorganic phosphate (Pi) is one of the most essential nutrients for the maintenance of cell life. Pi is essential for various biochemical reactions, such as kinase/phosphatase signaling, ATP formation, lipid, carbohydrate and nucleic acid biosynthesis. Recent studies have shown that Pi in the breast cancer microenvironment is significantly elevated (2 mM) when compared to normal tissues (1 mM). CD39 ecto-nucleotidases (Ecto-ATPases, which hydrolyses ATP to ADP and AMP) and CD73 (ecto-5'nucleotidase, which hydrolyzes AMP to adenosine and Pi) are very important for the generation of extracellular Pi through its cooperative action. In this context, it was also observed that increased levels of CD73 in tumor tissue is associated with a poor clinical prognosis. However, the mechanism by which CD73 acts remains to be better elucidated. Therefore, this work aims to biochemically characterize CD73, and understand if there is a correlation between its tumor effect and Pi production. **MATERIAL AND METODS:** In order to measure the ecto-phosphohydrolasic activity, the released Pi was measured using the Fiske-Subbarow methodology. **RESULTS AND CONCLUSION:** In this work, a linearity of enzymatic activity up to 40 minutes was demonstrated in MDA-MB-231 cells. Its predominant 5'nucleotidase activity is ecto-5'nucleotidase, it was also observed that the predominant ecto-phosphatase activity is ecto-5'nucleotide and it has great affinity with the substrates 5'AMP, 5'CMP and 5'UMP. These cells had Km= 1.80 ± 0.08 mM 5'AMP and showed to have greater activity at alkaline pH, in addition to being insensitive to levamisole (a specific alkaline phosphatase inhibitor). In order to comparatively evaluate the ecto-5'nucleotidase activity we used as a models three different breast cancer cell lines: MCF10-A (non-tumor breast cancer cell line), MCF-7 (luminal A breast cancer cell line), and MDA-MB-231 (triple-negative breast cancer cell line). Thus, it was observed a high activity of the enzyme in the MDA-MB-231, there was no significant activity in MCF-7 and a low activity in MCF-10A. Despite, the difference in ecto-5'nucleotidase activity between MDA-MB-231 and MCF-10A, both have a high level of expression of the ecto-enzyme, suggesting that its expression level is not a good parameter for the potential metastatic, since the high expression of ecto-5'nucleotidase does not mean that there is a high enzymatic activity. Furthermore, the results presented in this work indicate that the ecto-enzyme that has the predominant activity in phosphate generation is ecto-5'nucleotidase, suggesting that this ecto-enzyme can be a therapeutic target, since by inhibiting it, there will be a decrease in the concentration of Pi in the tumor microenvironment.

Keywords: Breast cancer, ecto-5'nucleotidase

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EFFECTS OF GREEN TEA EXTRACT (Camellia Sinensis) COMBINED WITH DOXORUBICIN ON HEPATOCELLULAR CARCINOMA CELL LINES

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INTRODUCTION AND OBJECTIVES: Cancer is the second leading cause of death worldwide and has emerged as a major public health problem. Primary liver cancer, mostly hepatocellular carcinoma (HCC), presents late diagnosis, which results in limited therapeutic alternatives. In the last years new strategies based on the use of dietary chemopreventive agents for cancer management have been developed. Derived from one of the most popular and consumed beverages in the world, green tea extract (GTE) is associated with a reduced cancer risk development. This effect can be promoted by the mixture of bioactive compounds present in food matrix, especially phenolic compounds. Previous studies have described the use of GTE as an anticancer agent in different tumor cell lines, as well as its association with doxorubicin (DOX), a chemotherapeutic anthracycline. The association between GTE and DOX plays a role in prevention of cardiotoxicity in Wistar rats, usually caused by DOX, as well as the inhibition of the mensechymal epithelial transition process (MET) in vitro. However, more investigation is needed to clarify the mechanisms of action involved. This study aims to elucidate the antitumor role of GTE, DOX, and the association between them, in HCC cell lines HepG2 (p53 wild-type) and Hep3B (p53 null). **MATERIAL AND METHODS:** GTE was obtained as previously described by our group. Cells were exposed to GTE at concentrations of 31.2 µg, 62.4 µg, 125 µg, 250 µg, 500 µg, 750 µg and 1000 µg. Cell viability was assessed using the resazurin reduction assay. Data were examined using analysis of variance (ANOVA) and significance or difference between groups will be established using Tukey's test. **RESULTS AND CONCLUSION**: Cell viability preliminary assays revealed that GTE reduced HepG2 viability in a time- and dose-dependent manner. Cell morphological changes observed by optical microscopy also suggests a cytotoxic effect of GTE. These findings corroborate previous studies by our group in breast cancer cell lines. Ongoing experiments with GTE + DOX will provide insights into the action of this association and support its anticarcinogenic potential on hepatocellular carcinoma

Keywords: green tea; doxorubicin; cancer.
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INFLUÊNCIA DO BCG (BACILLUS CALMETTE-GUÉRIN) NA MATURAÇÃO DE CÉLULAS DENDRÍTICAS HUMANAS DIFERENCIADAS A PARTIR DE MONÓCITOS

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INTRODUCTION AND OBJECTIVES:It is currently known that immunotherapy with dendritic cells (DCs) can promote an antitumor response in patients with multiple myeloma. These cells fused with tumor cells further enhance this response. Preliminary results show a significant degree of response; however, understanding this response and its improvement is justified. In this study, the addition of BCG at the maturation stage of DCs represents a new model not yet explored that can improve the protocol of immunotherapy for DCS.**MATERIAL AND METHODS**: DCs were obtained from the isolation of monocytes from the blood of 6 healthy donors. After the isolation step, monocytes were cultured for 5 days with RPMI-1640 medium supplemented with 10% fetal bovine serum, IL-4 and GM-CSF for differentiation into immature DCs. For terminal maturation (on the 5th day) TNF-α and IFN-α (DC-TNF) or dead BCG (DC-BCG) were added in a 1:1 ratio. On the 6th day, DC-TNF and DC-BCG were co-cultured with autologous and allogeneic lymphocytes previously stained with CFSE fluorochrome, this culture lasted 5 days. To phenotypically characterize the DCs, after their terminal maturation, the following markers were used: CD86, HLA-DR, CD1A, PD1, CD40, CD83 and CD80. The strain used was the freeze-dried Moreau-RJ and the bacillus was killed by gamma radiation. **RESULTS AND CONCLUSION:** DC-TNF and DC-BCG cultures showed high rates of cells expressing HLA-DR: 99% and 98%; CD1A: 77% and 85% and CD86: 99% and 98%, respectively. Cellular expression rate of CD80 on DC-TNF: 53%, and on DC-BCG: 47%. The rate of cells expressing CD40: on DC-TNF: 30%, on DC-BCG: 20%, the marker PD-1 on DC-TNF: 18%, on DC-BCG: 15%, and CD83 on DC-TNF:36% and in DC-BCG:32%. However, the percentage values of the expression of these markers were not significantly different. MFI levels (mean fluorescence intensity) in HLA-DR were high in DC-TNF and DC-BCG: 9,189 and 7,575, respectively. CD86 expression levels were also high, with DC-TNF: 11,981 and DC-BCG: 11,232. CD1A MFI levels were high for both DC-TNF and DC-BCG: 3,957 and 4,721, respectively. DC-TNF and DC-BCG had MFI for PD-1: 772 and 1293; and CD40: 1354 and 883, respectively. MFI levels for CD80 were always low, DC-TNF:843 and DC-BCG:721. And MFI levels for CD83 were DC-TNF:2,196 and DC-BCG: 4,181. There was a significant difference in CD80 and HLA-DR MFI levels ($p<0.05$). In lymphocyte proliferation, we observed that there is greater stimulation by DC-BCG, both in autologous and allogeneic proliferation. DC-BCG showed higher autologous lymphocyte proliferation: 11%(2-24%) and allogeneic 21%(12-35%), while DC-TNF cells: autologous 5% (2-19%), and allogeneic 16 %(9-32%). The use of dead BCG promotes the maturation of DCs at levels equivalent to TNF. But BCG potentiates lymphocyte proliferation at a higher rate than TNF. To interpret the results obtained, a study of the production of cytokines in quantity and quality will be the subsequent objective study.

Keywords: dendritic cell, BCG, cancer, immunotherapy
Supported by: CNPq and CAPEs

DOUTORADO

DEVELOPMENT OF NEW COMBINATION STRATEGIES OF CHEMOTHERAPY WITH SELECTIVE CYCLIN-DEPENDENT KINASES (CDK) 4/6 INHIBITORS IN COLORECTAL CANCER

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INTRODUCTION AND OBJECTIVES: Colorectal cancer (CRC) is the second most common type of cancer in Brazil in both women and men, excluding non-melanoma skin cancer. Also, only 10-20% of IV-stage patients are eligible for curative treatment, and about 65% of CRC patients present relapse disease. Cell cycle dysregulation is one of the main characteristics of cancer, providing sustained proliferative signaling. Therefore, some new therapies, which target cell cycle enzymes, have emerged in the last decade, including the cyclin-dependent kinase (CDK) 4/6 inhibitors, Palbociclib and Abemaciclib. These inhibitors have been used to treat advanced breast cancer that expresses Retinoblastoma (RB), the primary target of the CDK 4/6. Similar to breast cancer, CRC has modifications in the RB pathway that include amplifying CCNE1, CDK4, and CDK6, as well as the loss of CDKN2A. Therefore, CRC is a potential candidate for CDK4/6 inhibitor therapy. This work aims to evaluate the efficacy of new CDK4/6i agents as monotherapy or with traditional chemotherapy in CRC cells. The use of these new inhibitors can optimize treatment regimens regarding how to combine these drugs with others and what resistances may arise**. MATERIAL AND METHODS**: CRC cell lines (HCT116) were cultured in DMEM/F12 medium supplemented with 10% fetal bovine serum and incubated at 37ºC and 5% CO2. Dose-response curve analysis of 48 hours of treatment with first-line chemotherapy drugs, SN-38 (the active metabolite of irinotecan), oxaliplatin (OXA), and 5-fluorouracil (5-FU), was performed to determine doses that resulted in different cell viabilities (EC10, EC30, EC50) to be combined with CDK4/6i Palbociclib (PALBO) and Abemaciclib (ABE) low (EC20) and high (EC50) doses in the high-content screening platform using the Live/Dead kit. Moreover, a TUNEL reaction kit was performed to identify apoptosis cells in response to mono and combined therapy. RESULTS AND **CONCLUSION**: Cells viability with first-line chemotherapies (EC10, EC30, and EC50) and CDK4/6i (EC20 and EC50) were determined in the HCT116 colorectal cell line after 48 hours of treatment. Therefore, cells were treated with low concentration and high concentration combined therapies. Some combinations were more effective in killing cancer cells than monotherapy, presenting a synergic effect: OXA 0.6uM + ABE 300nM, OXA 0.6uM + ABE 35nM, OXA 0.6uM + PALBO 400nM, SN38 1.2nM + PALBO 400nM. Furthermore, TUNEL preliminary tests showed increased apoptotic cells when combined OXA + ABE therapy in comparison to monotherapy.

Keywords: Cyclin-Dependent Kinases (CDK) Inhibitors, Colorectal Cancer, High Content Screening, Chemotherapy.
Supported by: CNPq, CAPES, FAPERJ, Ministério da Saúde (PRONON).

INFLAMMASOME ACTIVATION BY NEUTROPHIL EXTRACELLULAR TRAPS (NETs) IN HUMAN BREAST CANCER MODELS

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INTRODUCTION AND OBJECTIVES: Components of the immune system, such as Neutrophil Extracellular Traps (NETs) and inflammasomes, may favor the development and progression of cancer. A correlation between these two mechanisms, where the activation of one depends on the other, has already been shown in diseases such as lupus erythematosus, diabetes and cancer. However, the role of the inflammasome in tumor progression when activated by NETs has not been observed in the context of breast cancer. Here we evaluated whether isolated NETs could upregulate elements of the inflammasome pathway in human breast carcinoma cell lines. **MATERIAL AND METHODS:** Human breast cancer cell lines MDA-MB-231 and MCF7 were stimulated with NETs isolated of neutrophils from the blood of healthy donors and stimulated with PMA. The breast cancer cell lines were starved in a serum-free medium and further treated with isolated NETs for 16 hours. In some cases, samples were treated with MCC950 and Anakinra, inhibitors of the NLRP3 inflammasome and IL-1 receptor, respectively. The expression of the inflammasome genes was analyzed by rt-PCR. ELISA was used to assess the release of IL-1β. RNA-seq data from breast cancer patients deposited in The Cancer Genome Atlas (TCGA) database were assessed **RESULTS AND CONCLUSION:** An increase in the gene expression of NLRP3, Caspase1 and IL-1β was observed in tumor cell lines when stimulated with NETs, as compared to cells without stimulation. The release of IL-1β by tumor cells stimulated by NETs was also observed. Treatment with Anakinra, decreased the gene expression of IL-1β, G-CSF and IL-8. TCGA analysis showed a positive correlation between breast cancer subtypes and inflammasome gene expression.

Keywords: NETs; Inflammasome; Cancer

Supported by: CNPq

PCA3 SILENCING SENSITIZES PROSTATE CANCER CELLS TO DOCETAXEL

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INTRODUCTION AND OBJECTIVES: Prostate cancer (PCa) is an androgen dependent disease and chemotherapy with docetaxel (DXT) represents the standard first line treatment in patients with castration resistant PCa. Efforts are needed to improve DXT therapeutic efficacy, since drug resistance is the main limitation. Prostate cancer antigen 3 (*PCA3*) is an overexpressed prostate long non-coding RNA (lncRNA) that modulates PCa cell survival through modulating AR signaling, besides controlling the expression of key cancer-related genes. However, the contribution of this lncRNA to chemoresistance requires further investigation. This study aims to investigate whether *PCA3* knockdown sensitizes PCa cells to DXT. **MATERIAL AND METHODS:** The DXT concentration that reduces cell viability by 50% (IC_{50}) was determined in human prostate carcinoma cell line LNCaP by MTT assay. LNCaP cells were treated with DXT, in combination or not with *PCA3* specific silencing using specific siRNA molecules. Cell viability was evaluated by MTT and trypan blue staining exclusion assays. LNCaP cell morphology analysis was performed by phase contrast microscopy. Flow cytometry analysis was conducted to assess apoptosis index and cell cycle. **RESULTS AND CONCLUSION**: LNCaP cells were sensitive to DXT in a dose-dependent manner, with an IC_{50} of 11,8nM. In response to *PCA3* silencing in combination with DXT treatment, cell viability rates were reduced 14,9% ($p \geq 0,05$). The number of viable cells also reduced 20,6% ($p < 0,05$) in relation to cells transfected with siRNA control sequence in combination with DXT treatment. In addition, apoptotic cell death rates were slightly increased 2,0%, while there was an 8,0% increase of cells in G1 phase of cell cycle and a 2,8% decrease of cells in G2 phase ($p \geq 0,05$). Moreover, in response to *PCA3* silencing and DXT combined treatment, morphological analysis evidenced a reduction on LNCaP cell clusters and density, as well as a 33,3% significant reduction ($p < 0,05$) in the number of cells adhered to the substrate in relation to control transfected cells in DXT presence. In conclusion, our findings provide early evidence that *PCA3* can sensitizes PCa cell response to DXT by modulating cell viability, apoptotic index, and cell cycle. These data suggest that *PCA3,* besides controlling cell survival, can also modulate PCa resistance to DXT.

Keywords: *PCA3*, docetaxel, chemoresistance, cell survival.
Supported by: Programa de Oncobiologia, Proppi-UFF, FAPERJ and CAPES

METABOLIC UPTAKE OF DIETARY *N*-GLYCOLYLNEURAMINIC ACID PROMOTE COLON CANCER GROWTH BY IMMUNE AND NON-IMMUNE MECHANISMS.

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INTRODUCTION AND OBJECTIVES: Every living cell is covered with an extense layer of glycoconjugates, including glycoproteins and glycolipids. The majority of these glycans present a terminal sialic acid. While most mammals synthesize both N-acetylneuraminic sialic acid (Neu5Ac) and N-glycolylneuraminic sialic acid (Neu5Gc), humans do not synthesize Neu5Gc due to a mutation in the Cmah gene, which encodes the only enzyme capable of hydroxylating Neu5Ac into Neu5Gc. Despite this, Neu5Gc can be found in healthy human tissues and, at higher levels, in carcinomas such as colorectal cancer (CRC). Ingestion of red meat is the only known source of Neu5Gc incorporation in humans and data shows an association of its intake with CRC risk. Here, we investigate if the metabolic incorporation of Neu5Gc by CRC cells could affect signaling pathways relevant to its progression **MATERIAL AND METHODS:** HCT 116 cells were fed with 2mM of Neu5Ac or Neu5Gc and stimulated with 2nM Wnt3a. APC-CPC Cmah-/- mice were fed either with a Neu5Gc-rich diet or a Neu5Gc-free diet. In addition, the mice were subjected to an immunization process for Neu5Gc or Neu5Ac **RESULTS AND CONCLUSION:** HCT-116, human CRC cells, when fed with diferente concentrations of Neu5Gc presented an increased Wnt3a binding to the cell surface, suggesting that Neu5Gc could influence Wnt/Fzd interaction. The increased response of Neu5Gc-fed cells to Wnt3a resulted in increased activation of the Wnt signaling pathway, as seen in luciferase reporter assay, augmented expression of Axin2 and SP5, and higher cell proliferation rates. Using a human-like Cmah-/- mice model, presenting a mutation in the Wnt pathway, that spontaneously develops CRC (APC-CPC Cmah-/- mice) we found a higher number of polyps, that were also bigger in size, in mice fed with a Neu5Gc-rich diet. These results demonstrate that metabolic uptake of Neu5Gc can promote CRC growth both in vitro and in vivo. The incorporation of Neu5Gc is associated with the induction of xenosialitis, an inflammatory process provoked by circulating anti-Neu5Gc. Here, our animal model was submitted to induction of xenosialitis by the immunization with Chimp red cell membrane ghosts, we found that these animals presented an increased number of polyps and tumor load when fed a Neu5Gc-rich diet. In these mice, we also observed higher mRNA expression of

regulatory genes, such as IL-10. On another hand, we observed lower mRNA expression of TGF-β and IL 22. Pro-inflammatory genes such as TNFα, IL-12, and IL-6 did not increase neither decrease. Our data suggest that dietary ingestion of Neu5Gc can promote CRC growth by immune and non-immune mechanisms likely contributing to the human-specific risk of CRC associated with red meat consumption.

Keywords: Colorectal cancer; Neu5Gc; Wnt signaling pathways and anti-Neu5Gc antibodies
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MOLECULAR PROFILE OF T-CELL ACUTE LYMPHOBLASTIC LEUKAEMIA WITH *CRLF2* OVEREXPRESSION

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INTRODUCTION AND OBJECTIVES: *CRLF2* overexpression is a potential biomarker of poor prognosis in T-cell acute lymphoblastic leukaemia (T-ALL) and also a candidate for targeted therapy. Recently, we demonstrated that *CRLF2* overexpression (*CRLF2*-high) associates with mutations leading to the NOTCH1 intracellular protein stabilisation, however the mechanisms underlying *CRLF2* deregulation in T-ALL remains unknown. Therefore, we aimed to evaluate the molecular profile of patients with *CRLF2*-high and delineate the mechanisms that contribute to this gene dysregulation. **MATERIAL AND METHODS:** We analysed data from 264 patients (*Therapeutically Applicable Research to Generate Effective Treatments* - TARGET) and 8 T-ALL cell lines (Cancer Cell Line Encyclopedia and Gene Expression Omnibus). Information of gene expression, mutations, copy number changes and gene fusions generated by RNA sequencing (RNA-seq), whole exome sequencing and microarrays were assessed to categorise the patients into *CRLF2* subgroups and to delineate the molecular landscape of these leukaemias. We also analysed RNA-seq data from previous studies that investigated the Polycomb Repressive Complex 2 (PRC2) loss in LOUCY and JURKAT cell lines through BET proteins inhibition and *EZH2* silencing, respectively. The *CRLF2* region was investigated by chromatin immunoprecipitation (ChIP) followed by sequencing performed in T-ALL cell lines for H3K27ac, H3K4me^1 and H3K4me^3. Finally, we performed a quantitative ChIP (qChIP) assay to evaluate MEF2C binding in *CRLF2* regulatory regions. **RESULTS AND CONCLUSION:** Initially, we observed an enrichment of early T-cell precursor ALL (ETP-ALL) samples in the *CRLF2*-high subgroup (23.08% vs 4.02%, p=7.579e-06) and these molecular entities showed similarities in their transcriptional profile. Likewise, *JAK3*, *RUNX1* and *EZH2* mutations and *TCF7* deletions were associated with higher *CRLF2* levels (p=0.0063, p=0.0009, p=0.0170 and p=0.0102, respectively). *CRLF2* expression in LOUCY, an ETP-ALL cell line with *CRLF2*-high, was restored after BET inhibitors treatment (p=0.0095). However, in JURKAT, the *EZH2* silencing did not upregulate *CRLF2* expression. We observed active enhancer regions in LOUCY and identified 104 transcription factors (TFs) that recognize these regions. We filtered those TFs according to their association with ETP-ALL and the *CRLF2*-high subgroup and further investigated the *MEF2C* gene as a potential regulator of this dysregulation in T-ALL. Subsequently, through qChIP assay we demonstrated that MEF2C binds

CRLF2 enhancer region. In the present study, we demonstrated the association of *CRLF2* overexpression with the ETP-ALL subtype and with some molecular alterations. Furthermore, the PRC2 activity loss seems to be a mechanism underlying *CRLF2* upregulation in ETP-ALL. Although, in other subtypes, PRC2 loss alone is unable to account for this gene dysregulation, suggesting that MEF2C activation may be a crucial trigger for this molecular event.

Keywords: 1. T-cell acute lymphoblastic leukaemia; 2. *CRLF2*; 3. *MEF2C*; 4. ETP-ALL; 5. gene expression.

Supported by: Ministério da Saúde, FAPERJ, CNPq, Fundação do Câncer and CAPES.

UNDERSTANDING THE *PALB2* PROMOTER REGION AND ITS EXPRESSION REGULATION THROUGHOUT THE CELL CYCLE

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INTRODUCTION AND OBJECTIVES: Germline mutations in the *PALB2* tumor suppressor gene are associated with breast and pancreatic cancer predisposition. *PALB2* encodes for a homonymous protein with a pivotal role in genomic integrity maintenance through the homologous recombination (HR) DNA repair pathway. The protein works as a scaffold protein, connecting BRCA1 and BRCA2 to promote the recruitment of the RAD51 recombinase to DNA-damaged sites. Despite the growing interest in PALB2, both the promoter region of gene, and its expression regulation are not fully understood. The present study seeks to characterize the *PALB2* promoter *locus*, defining its minimal promoter region, and also analyze expression regulation. **MATERIAL AND METHODS:** The Genome Browser platform was used to track down *in silico* H3K27Ac signal (euchromatin marker) upstream *PALB2* gene and, therefore, identify the putative *PALB2* promoter region. The selected region was generated through PCR using BJ cells genomic DNA as a template; different deletion fragments were further generated to map the minimal region with promoter activity. The activity was assessed through a luciferase reporter expression assay in HEK293FT cells. **RESULTS AND CONCLUSION:** We identified a sequence enclosing a 1001 nucleotide upstream of *PALB2* as the putative gene promoter region (named *1001*). We demonstrated that 1001 has a promoter activity by its ability to activate the *luciferase* transcription (the reporter gene). To better map these putative *PALB2* promoter region, we generated different fragments and identified a minimal regulatory region (1001Δ366) that exhibits similar levels of transcription activation observed for *1001*. We also demonstrated that the segment *1001* and smaller different fragments showed an increase in transcriptional activity upon DNA damage induced by treatment with ionizing radiation (IR) when compared to non-IR cells. Further, we also observed an increase in *PALB2* mRNA and protein levels after IR (10Gy) damage and PARP inhibition (Olaparib 2,5uM – 10uM). Interestingly, we demonstrated that the levels of *PALB2* mRNA and PALB2 protein varied throughout the cell cycle, showing an increase during the S and G2/M phases, in which HR is active. In summary, our data identified a minimal regulatory region of *PALB2* and suggested that it is responsive to different DNA damage agents, particularly during the S and G2/M phases of the cell cycle.

Key-words: DNA damage response, PALB2, promoter region

Supported by: INCA, FAPERJ, Fundação do Câncer, CNPq

THE ROLE OF TRANSCRIPTION FACTOR SOX2 IN THE MECHANISMS OF EPITHELIAL-MESENCHYMAL TRANSITION AND APOPTOSIS IN EXPERIMENTAL MODELS OF MELANOMA

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INTRODUCTION AND OBJECTIVES: Melanoma is an aggressive skin cancer due to its high invasive and migratory capacity, and its neoplastic progression can be associated with processes that are hallmarks of cancer: resistance to apoptosis and epithelial-mesenchymal transition (EMT). Other primary markers involved in cell differentiation and usually up or downregulated during carcinogenesis are the genes that encode transcription factors, such as SOX family proteins, including SOX2. SOX2 is associated with an undifferentiated cell phenotype and anti-apoptotic properties related to tumorigenesis promotion. Thus, the objective of the present work was to investigate the involvement of the SOX2 protein in the apoptosis and the epithelial-mesenchymal transition regulation through an in vitro model using A375 and SK-MEL-28 melanoma cell lines and in vivo model using canine spontaneous melanoma samples. **MATERIAL AND METHODS:** Melanoma cell lines (A375 and SK-MEL-28) were seeded and transfected with the pEF1/Myc-His/SOX2+ vector for SOX2 protein overexpression. SOX2 overexpression was confirmed 24 hours after the transfection protocol through the identification and quantification of the protein by immunofluorescence, and transfected cells' viability was evaluated by MTT assay. In spontaneous canine melanoma, we evaluated histopathological parameters, and immunohistochemistry (IHQ) was performed to correlate and identify markers of proliferation (Ki67), apoptosis (Caspase-3), EMT with E-cadherin (epithelial cells marker), and N-cadherin (mesenchymal cells marker), and the transcription factor protein SOX2. **RESULTS AND CONCLUSION**: Results showed that A375 and SK-MEL-28 cells transfected with the pEF1/Myc-His/SOX2+ vector significantly increased SOX2 protein expression, with a consequent decrease in cell viability in 44% of A375 cells and 48% of SK-MEL-28 cells when compared to their controls. In the canine spontaneous melanomas model, it was observed by IHQ that positive cases for SOX2 were associated with the presence of neoplastic emboli and with a higher apoptotic index. Additionally, there was a positive correlation between increased expression of Ki67 and Caspase-3, Caspase-3 and E-cadherin, cytoplasmic SOX2, and nuclear SOX2 staining. When evaluated separately, cutaneous melanomas showed a lower proliferative index, a positive correlation between Ki67 and SOX2 cytoplasmic staining and Caspase-3 and E-cadherin, as well as a negative correlation

between Caspase-3 and membrane N-cadherin. Thus, it was observed that SOX2 overexpression in human melanoma cell lines decreased the viability and metabolism of neoplastic cells; on the other hand, in the canine spontaneous melanoma model, SOX2 protein expression was more evident in tumors that contained worse prognostic features. In the canine melanoma model, we also observed that EMT and apoptosis are more present in melanomas with more controlled tumor progression.

Keywords: SOX2, Apoptosis, EMT, Melanoma, Ki-67.
Supported by: FAPEMIG, CNPq and CAPES

EXPRESSION OF NUCLEAR XIAP REVEALS MOLECULAR SIGNATURES ASSOCIATED WITH MOST PROLIFERATIVE AND MIGRATORY FEATURES IN BREAST CANCER

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INTRODUCTION AND OBJECTIVE: X-linked inhibitor of apoptosis protein (XIAP) is an inhibitor of apoptosis protein aberrantly expressed in cancer. XIAP performs its canonical antiapoptotic functions at the cytoplasm, but growing evidence implicate an unexplored role at the nucleus. Our group has demonstrated that nuclear XIAP is associated with unfavorable outcome in breast cancer patients as well as increased in vitro cell growth and chemoresistance. Here, we aimed to explore transcriptomic signatures associated with XIAP nuclear oncogenic functions in breast cancer. **MATERIAL AND METHOD:** MCF-7 human breast cancer-derived cells overexpressing XIAP variants: pEBB (empty vector), $XIAP^{wildtype}$, $XIAP^{H467A}$ (cytoplasmic location) (lack of ubiquitin ligase activity; cytoplasmic location), $XIAP^{\Delta RING}$ (RING deletion; nuclear location) and $XIAP^{NLS}$ (Nuclear Localization Signal insertion; nuclear location) were compared by RNA microarray considering a ≥ 2-fold-change as criteria to define the differentially expressed genes (DEGs). Pathway analysis and related processes were obtained through gene enrichment analysis using MetaCoreTM and gene ontology (GO) dataset. The Venn diagram was produced using InteractiVenn. The STRING® database was used to construct a protein network. Interest genes were validated by qRT-PCR. **RESULTS AND CONCLUSION:** $XIAP^{NLS}$ overexpression induced a global repression in gene expression in comparison to $XIAP^{wild\ type}$ and $XIAP^{\Delta RING}$. The most representative DEGs were involved in the regulation of cellular processes such as localization, transport, cell proliferation, migration, motility and protein phosphorylation. Following validation, *MMP13*, *IGFPB6* and *TFPI2* turned out to be the most differentially expressed in $XIAP^{NLS}$ in relation to $XIAP^{\Delta RING,}$ genes closely linked to cell proliferation and migration. Interestingly, *MMP13* expression was found increased, and *IGFBP6* and *TFPI2* reduced in invasive breast cancer versus normal tissues. Notably, *TFPI2* was also associated with favorable clinical outcome. Functional assays with candidate genes and XIAP nuclear interactomes are currently being performed and will help understand nuclear XIAP role in breast cancer aggressiveness.

Keywords: Breast cancer; Nuclear XIAP; Transcriptomic signatures.
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LABH INHIBITS PLATELET-TUMOR CELL INTERACTION INDEPENDENTLY OF ITS ANTICOAGULANT EFFECT

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INTRODUCTION AND OBJECTIVES: At present, the heparin, mainly obtained from porcine intestinal mucosa (HPI), is commonly used in the treatment of cancer-associated thromboembolism and several clinical studies indicate that it prolongs patient survival. However, the risk of bleeding associated to this therapy has limited its use as a potential anticancer drug. Recently, we isolated a fraction from bovine heparin (HBI) with very low anticoagulant activity, named LABH (low anticoagulant bovine heparin). The LABH displays an interesting antimetastatic activity in an *in vivo* hematogenous metastasis assay. Thus, our aim in this work was to investigate the LABH mechanism of action as an antimetastatic agent *in vitro*. **MATERIAL AND METHODS:** For this, isolated platelets from healthy volunteers were incubated for 30 minutes with MV3 cells (human melanoma cell line) on adhesion/interaction assay or tested for 15 minutes on MV3 cell-induced platelet aggregation in the presence or absence of heparins (HPI, HBI and LABH). We also evaluated the heparin interference on the interaction of U937 cells (human lymphoma cell line) to immobilized P-selectin, a key molecule in tumor cell-platelet crosstalk; the thrombin activity in supernatants of MV3-platelets interaction; and MV3-induced procoagulant activity using platelet-poor plasma. **RESULTS AND CONCLUSION**: All three heparins tested were efficient in blocking MV3-platelet adhesion and aggregation in a concentration-response manner, but LABH required higher concentrations than HPI. When analyzing the direct binding of U937 cells to P-selectin, we could observe that HPI and HBI inhibited at the same level (~ 60% of inhibition with 100 µg/mL), while LABH was less effective (~ 45% of inhibition with 100 µg/mL). Moreover, the LABH did not prevent the MV3 cell-induced procoagulant activity. Finally, we could not detect thrombin activity within the supernatants of MV3-platelets interaction. Collectively our results suggest the LABH as a promising drug for cancer patients because it significantly reduced lung metastasis and platelet-tumor cell adhesion by coagulation-independent mechanisms. As perspectives for this work we intend to investigate other potential biological activities of LABH that may affect tumor progression, for instance angiogenesis and tumor cell-endothelium interaction.

Keywords: LABH, antimetastatic activity, platelets, p-selectin.
Supported by: FAPERJ and CNPq.

COTREATMENT OF CURCUMIN AND MELPHALAN INDUCES CELL DEATH IN MDA-MB-231 BREAST CANCER CELLS

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INTRODUCTION AND OBJECTIVE: In women, breast cancer is the leading cause of cancer death having an estimated 2.26 million cases in 2020. Chemotherapy is usually associated to long-term toxicity affecting overall survival. Several studies have shown that curcumin shows therapeutic potential against various cancers. However, no studies have yet investigated the combined use of curcumin with chemotherapeutic agents such as melphalan. Hence, in the present study we assessed the anticancer effects of cotreating MDA-MB-231 breast cancer cells with curcumin and melphalan. **MATERIAL AND METHODS:** The cell viability assay was performed using MTT. The cell cycle phases were analyzed in flow cytometry after labeling with RNAse-PI. The analysis of apoptosis and necrosis were conducted with annexin V-FITC/PI by flow cytometry. The proteins involved in apoptosis were analyzed by Western Blot and by Immunocytochemistry. **RESULTS AND CONCLUSION**: Curcumin and melphalan had a cytotoxic effect in MDA-MB-231 cells having an IC_{50} of 23.29 µM and 18.62 µM for curcumin and 187.90 µM and 37.78 µM for melphalan for 24 and 48 hours, respectively. Cotreatment with 11.65 µM of curcumin and 93.95 µM of melphalan for 24 h decreased cell viability by 28.64%. Cotreatment with curcumin + melphalan for 24 and 48 h reduced the G0/G1 phase population by 1.33 and 1.56-fold, respectively. The cotreatment with curcumin + melphalan for 24 h increased annexin-V/PI-positive cells ratio by 5.55-fold, indicating apoptotic cell death. Lastly, cotreatment with curcumin + melphalan for 48 h increased annexin-V/PI-positive cells ratio by 2.33-fold, indicating late apoptosis. Our Western blotting results demonstrate that PARP, capase-7, caspase-9 and p21 levels were increased by 2.44, 2.88, 1.68 and 1.32-fold, respectively, after cotreatment of curcumin and melphalan. In addition, immunocytochemistry results showed that curcumin + melphalan increased caspase-3, caspase-7 and caspase-9 by 2.03, 1.78, and 1.67-fold, respectively. Overall, our results evidence the *in vitro* anti-breast cancer effect by co-treatment of curcumin and melphalan, which may suggest an important source of new molecules to support anticancer therapy.

Keywords: Breast cancer, Curcumin, and Melphalan.

Supported by: Fundação do Câncer, FAPERJ, CNPq and CAPES

EXPLORING CAR-T CELL IMMUNOTHERAPY IN PRECLINICAL AND CLINICAL STUDIES THROUGH MATHEMATICAL MODELS

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INTRODUCTION AND OBJECTIVES: CAR-T cell immunotherapy consists of the genetic modification of T lymphocytes aiming to increase their ability to detect and kill tumor cells that express specific antigens. Currently, several CAR-T cell designs and combinations with other therapies have been studied in preclinical trials. The FDA-approved CAR-T therapies have shown expressive complete response rates, but many patients still suffer a relapse and/or become resistant to therapy within the first few months or years. The barriers that prevent the effective success of the therapy are not completely understood but are related to patient-specific and product heterogeneities, among other issues. The CAR-T cell dynamics is marked by a multiphasic behavior with great variability in duration and characteristics. There are still several challenges to be faced and in this context, the mathematical modeling may represent a useful tool to better understand the mechanisms and improve the effectiveness of the therapy. Thus, our objective is to develop mathematical models that help in the planning and in the analysis of preclinical and clinical experiments. Specifically, we aim at identifying key parameters associated with patient-specific responses to CAR-T immunotherapy. **MATERIAL AND METHODS:** We developed a mathematical model that describes CAR-T immunotherapy against hematological tumors in immunodeficient mice, considering populations of effector and memory CAR-T cells and tumor cells. An *in silico* platform, called CAR**T***math*, was developed, allowing researchers from different areas to reproduce the obtained results and explore new tests with the model. The model was then extended to represent the dynamics in patients by including the multiphasic dynamics of CAR-T cells through phenotypic differentiation. The CAR-T cells were divided into functional (distributed and effector), memory, and exhausted phenotypes and we considered patient and infused product heterogeneities and antigen-dependent expansion. The model is tested against different hematological malignancies and therapy outcomes. **RESULTS AND CONCLUSION**: The model developed for preclinical scenarios was able to reproduce several cases reported in the literature, with different CAR receptors and tumor targets. *In silico* experiments yield insights on immune checkpoint inhibitors, dosing strategies, and uncertainties impacting treatment outcomes. In patients, a wide variety of dynamic behaviors were represented and potential markers of response to therapy were obtained. Specifically, the joint assessment of the area under the curve with the corresponding fraction of non-exhausted CAR-T cells was considered the most promising for outcome classification. The models developed have allowed the exploration of several questions regarding therapy, generating promising insights into underlying mechanisms. We hope that future developments can further contribute to the development of preclinical and clinical studies.

Keywords: Hematological cancers, CAR**T***math*, Multiphasic dynamics, Patient and CAR-T heterogeneities. Antigen-dependent expansion.

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AGGREGATION PROFILE OF THE p53 TUMOR SUPPRESSOR PROTEIN R337H MUTANT, CHARACTERISTIC OF A HEREDITARY CANCER SYNDROME (LI-FRAUMENI SYNDROME - LFS)

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INTRODUCTION AND OBJECTIVES: Gene mutations in somatic cells are responsible for most cases of cancer. Nonetheless, germline cell mutations, described as hereditary mutations in which mutated genes are transferred from parents to children, also promote an increase in the predisposition to the development of certain types of cancers. LFS is inherited in an autosomal dominant pattern cancer predisposition syndrome associated with a mutation in the TP53 gene, capable of causing cancer in the first years of life and multiple tumors over a patient's lifetime. The most common mutations in the TP53 gene are the missense mutations which are found most frequently in the central DNA-binding domain (DBD) of the protein, called hotspot mutations. In addition to those hotspot mutations, the predominantly germline mutation R337H is located in the oligomeric domain (OD) and has been reported in individuals diagnosed with LFS in Brazil, prevalently in the South and Southeast regions of the country. Mutations in the p53 DBD are known to promote loss of protein function due to reduced conformational stability, favoring a greater tendency to aggregate formation that contributes to p53 accumulation in tumors, the effects of gain-of-function, loss of function, and tumor progression. However, the impact of the mutations in OD and how they might affect p53 protein folding is still poorly understood in the literature. Our main goal is to evaluate the aggregational behavior of the mutant p53 R337H to better understand LFS disease. **MATERIAL AND METHODS:** The full-length (FL) p53 protein bearing or not the R337H mutation was heterologously purified by two affinity column followed by gel filtration. After that we started *in vitro* experiments using spectroscopic techniques such as fluorescence, light scattering, and circular dichroism. Molecular biology techniques also were used to investigate the aggregates characteristics. **RESULTS AND CONCLUSION**: Aggregation behavior was assessed using a light scattering aggregation kinetics, comparing p53 FL wild-type and p53 FL R337H, showing that at acidic pH, p53 FL R337H considerably increases aggregation when compared with wild-type. Dynamic light scattering experiments also demonstrated variations in the hydrodynamic radius of the aggregates with the pH variation. Thioflavin T (ThT) fluorescence measurement, a well know probe to monitor in vitro amyloid fibril formation, was also performed, and the amyloid aggregates formation was confirmed for the R337H mutant. We also used the dot-blot assay to confirm the presence of amyloid oligomers and fibrils using the anti-oligomer antibody (A11) and the anti-fiber antibody (OC) respectively. The data emphasize that a microenvironment that undergoes pH changes can promote p53 R337H aggregation and since it is known that tumor cells are usually more acidic than normal cells our findings can suggest a way for LFS tumor development and a possible target for future treatments.

Keywords: p53 R337H, Li-Fraumeni syndrome, aggregation.
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THE USE OF METFORMIN AS ADJUVANT TO RESTORE THE REDUCTION OF CISPLATIN CYTOTOXICITY IN THE PRESENCE OF ALLANTOIN

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INTRODUCTION AND OBJECTIVES: The tumor lysis syndrome (TLS) is a metabolic disorder frequently associated with hyperuricemia. To treat TLS, rasburicase has been used, producing allantoin. Recently, our group showed that high levels of allantoin causes impairment of cisplatin cytotoxicity in K562 cell line. Due the importance of the maintenance of the concomitant use of cisplatin and rasburicase, our group evaluated and showed in a recent study the capacity of metformin in recover cisplatin cytotoxicity in the presence of allantoin in K562 cells. This study aims to understand how metformin restores cisplatin cytotoxicity.**MATERIAL AND METHODS:** For this study, the K562 cell line was maintained in RPMI 1640 medium supplemented with 10% of fetal bovine serum and 0.5% of penicilin-streptomicin at 37ºC and 5% of CO_2. During the experiments, the cells were incubated in RPMI 1640 low glucose medium (0.5mM) for 2h and then treated with metformin (0.1 to 3mM), cisplatin (16.5 to 33µM), allantoin (100 and 200µg/ml), rotenone (0.125 to 2µM) and their combination for 48h. Cell viability, cell cycle, morphology analysis and NMR assays were performed. **RESULTS AND CONCLUSION**: Our results showed: (1) the synergism between metformin and cisplatin; (2) the recovery of cisplatin cytotoxicity by metformin at morphology level; (3) the alterations of cisplatin effect in cell cycle by allantoin; (4) the absence of metformin-cisplatin interaction; (5) the interaction between metformin-allantoin; (6) the synergism between rotenone and cisplatin; (8) the capacity of rotenone to restore cisplatin cytotoxicity in the presence of allantoin at mitochondrial and cell cycle level, such as done by metformin. This study showed that metformin recovers cisplatin cytotoxicity in the presence of allantoin through its action on mitochondria and in an independent way.

Keywords: metformin, cisplatin, allantoin.
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ANTITUMOR POTENTIAL OF OLIVE LEAF EXTRACT (*Olea europaea*) AFTER SIMULATED DIGESTION ON HEPATOCELLULAR CARCINOMA 3D MODEL

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INTRODUCTION AND OBJECTIVES: The olive leaf is a source of bioactive compounds, especially polyphenols, that exhibit antioxidant activity and antitumor potential on different experimental models. Most studies regarding the biological effects of polyphenols are conducted by the administration of isolated compounds, however the use of Olive Leaf Extract (OLE) allows to evaluate the anticancer potential of complex plant matrix. Additionally, the investigation of OLE effects after in vitro digestion allows to explore its potential considering consumption. This study aims to investigate the role of OLE after simulated in vitro digestion on cell viability of Hep-G2 Hepatocellular carcinoma (HCC), cultivated in a two-dimensional (2D) and three-dimensional model (3D). **MATERIAL AND METHODS:** The OLE (*Olea europaea*, cultivar Coratina) was obtained in the Puglia region (South Italy) and will be subjected to in vitro digestion. The digested fraction will be characterized. HepG2 cells spheroids will be exposed to different concentrations of OLE and OLE digested fraction during 24 h-48 h. After that, the cell viability (Alamar Blue®), induction of apoptosis (Annexin V-FITC) and the expression of proteins involved (Immunocytochemistry and Western blotting analysis) will be evaluated. **RESULTS AND CONCLUSION:** Preliminary results showed that the spheroids were spontaneously formed (4 days). The morphology and size of the spheroids were modified by exposition to OLE. The future perspectives are to demonstrate the effect of the OLE and its digested fraction into two cell culture models in addition to investigating the molecular pathways involved.

Keywords: Olive leaves. Cancer. 3D culture.
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NEUTROPHIL EXTRACELLULAR TRAPS (NETS) DRIVE A CHEMORESISTANT PHENOTYPE IN HUMAN BREAST CANCER CELLS THROUGH PI3K/AKT/NF-kB PATHWAY

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INTRODUCTION AND OBJECTIVES: Neutrophil Extracellular Traps (NETs) have been associated with various aspects of tumor progression, such as primary growth, aggressiveness, and metastasis. Our group has recently demonstrated that NETs promote a pro-metastatic phenotype in nonaggressive breast cancer cell lines through the epithelial-mesenchymal transition (EMT) process. This has been linked to resistance to chemotherapeutic agents, but little has been reported regarding the role of NETs in driving these events. Thus, this study aims to analyze NETs' ability to modulate an antiapoptotic phenotype in human breast cancer cells. **MATERIAL AND METHODS:** We used three breast cancer cell lines in this study: MCF7 and T47D (described as epithelial, less aggressive and sensitive to chemotherapeutic agents) and MDA-MB231 (described as undifferentiated, highly aggressive and resistant to chemotherapeutic agents). Neutrophils separated from the blood of healthy donors were stimulated with Phorbol 12-myristate 13-acetate PMA to produce NETs which were further isolated using an established protocol. The cell lines were treated with isolated NETs for 24 or 48 h. In some cases, prior to the treatment with NETs, cells were treated for 1h with either PI3K (LY294002), AKT (MK-2206) or NF-kB (BAY117085) inhibitors. Samples were collected for real-time PCR and Western Blot, and the expression of Bcl-2 and Bax was analyzed. A clonogenic assay, MTT assay and Western Blot for effector components of the apoptotic pathway were performed to evaluate the functional impacts of NETs on cellular response to doxorubicin. **RESULTS AND CONCLUSION:** *In vitro* analysis showed that NETs were not cytotoxic for none of the cell lines. NETs treatment upregulated the mRNA expression of Bcl-2, an antiapoptotic protein, in all cell lines, while downregulating the expression of Bax, a proapoptotic protein. Colony formation was improved in cells treated with NETs as compared to untreated cells. Notably, NETs were able to protect cells against doxorubicin treatment. Treatment with PI3K, AKT and NF-kB inhibitors prevented the protective effects of NETs towards doxorubicin, as evidenced by MTT assay. In sum, we conclude that exposure of breast cancer cell lines to NETs confer a chemoresistant phenotype, functionally impacting responses to doxorubicin, via PI3K/AKT/NF-kB pathway. **KEYWORDS:** Breast cancer, neutrophil extracellular traps (NETs), apoptosis, chemoresistance. **SUPPORTED BY:** CAPES, CNPq, Fundação do Câncer and FAPERJ.

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AKT-MEDIATED REGULATION OF FOXK2 TRANSCRIPTION FACTOR: MOLECULAR MECHANISMS AND POTENTIAL ROLE IN BREAST CANCER DRUG RESISTANCE

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INTRODUCTION AND OBJECTIVES: FOXK2 is a transcription factor which modulates drug sensitivity in breast cancer cells. In contrast, drug-resistant cell lines exhibit constitutively high FOXK2 protein levels, suggesting that post-translational modifications might impair its functions. An *in silico* analysis revealed AKT oncogenic kinase as the main putative regulator of FOXK2 by phosphorylation. Then, the objective of this project is to evaluate the effect of AKT modulation in the regulation of FOXK2 protein levels and the impact of the AKT-FOXK2 axis on drug resistance in breast cancer. **MATERIALS AND METHODS:** The MTT and clonogenic assays were performed to assess drug-induced cytotoxicity in MCF-7 (chemosensitive) and MDA-MB-231 (chemoresistant) cell lines. AKT1 and GSK3 knockdown was done by RNA interference. LY294002 and MK-2206 were used for pharmacological inhibition of AKT. Overexpression of FOXK2 and constitutively active AKT was performed by transient transfections. The NE-PER kit was used for cytoplasmic and nuclear extractions. Protein levels in whole and fractioned lysates were examined by Western Blotting following electrophoresis in SDS-PAGE and Phos-tag gels. RNA levels were examined by real-time PCR (SYBR Green Master Mix method). **RESULTS AND CONCLUSION**: Inhibition of AKT, but not GSK3β, resulted in a reduction in endogenous and exogenous FOXK2 protein levels, particularly in the lower mobility bands. Interestingly, FOXK2 RNA levels are increased in AKT-inhibited cells, suggesting that modulation of FOXK2 protein levels is possibly dependent on post-translational modifications. Differently from MCF-7, FOXK2 and phosphorylated AKT are predominantly found in the nucleus of MDA-MB-231 cells. Also, FOXK2 subcellular localization is not modulated following AKT inhibition in MDA-MB-231 cells. Experiments involving lambda-phosphatase treatment and Phos-tag gels suggest that FOXK2 mobility is altered in AKT-modulated cell models. Notably, an *in silico* analysis identified higher levels of phosphorylated FOXK2 at different sites in breast tumor samples. Finally, inhibition and overexpression of AKT impact drug response, which is associated with modulation of FOXK2 protein bands. In summary, our results suggest that AKT can regulate FOXK2 protein levels in drug-resistant breast cancer cells. Future investigations will determine whether AKT directly phosphorylates FOXK2, as well as identify the crucial sites for AKT-FOXK2 interaction and biological functions.

Keywords: Breast cancer drug resistance, FOXK2 transcription factor, AKT oncogenic kinase.

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IMPROVEMENT OF CAR-T CELL THERAPY WITH ULTRA-FAST PROTOCOL AND IL-15 MEMBRANE BOUND ADDITION

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INTRODUCTION AND OBJECTIVES: Despite the advancement of new technologies for immunotherapy, gene therapy is far from democratized. Among them is CAR-T cell therapy, which has a great response in B-cell tumor patients but is very expensive. Basically, this therapy occurs with leukapheresis to remove the cells, taken to specialized laboratories, activated to proliferate, genetically modified with viral vectors to insert CAR, expanded for about 15 days, and returned to the hospital to treat the patient. This logistics can last about 1 month. In this project, we propose an ultra-fast protocol to decrease the time, cost, and complexities of CAR-T cell therapy. We use a transposon-based non-viral vector called Sleeping Beauty (SB) or PiggyBac (PB) that allows us to not activate the cell before gene insertion and consequently, the non-obligation to expand these cells. We insert CAR into T cells and in less than 24h we use these cells to treat grafted mice with leukemia. This protocol is called Point-of-care (POC) approach. **MATERIAL AND METHODS:** Mononuclear cells were isolated by Ficoll and electroporated using the Nucleofector IIb with SB plasmids encoding 19BBz CAR and SB100x transposase or PB plasmids encoding 19BBz or 19BBz-mbIL-15 with PBase transposase. For *in vivo* model, NSG mice were injected iv. $5x10^6$ RS4;11 or 10^5 Nalm-6 and after 3 days were treated with recently electroporated CAR-T cells. for the expanded cell protocol, we activated with anti-CD3/CD28 beads for 8-12 days. **RESULTS AND CONCLUSION**: Both mice models (RS4;11 and Nalm-6) were treated with our protocol to produce CAR-T cells showed improved survival when compared to mice treated with mock electroporated cells and decreased tumor burden in blood and spleen was observed. Head-to-head comparison of 19BBz cells used in POC approach or expanded for 8-12 days *in vitro* showed similar antitumor activity *in vivo* against RS4;11 cells, leading to equivalent improvements in mice survival. After that, we added an IL-15 membrane bound receptor (mbIL-15) to CAR to improve cell persistence and animal survival and we used PB vector to insert the transgene. We noticed that the tumor burden evaluated by bioluminescence and the survival of animals that had 19BBz-mbIL15 was better when compared to only 19BBz (dose of $3x10^5$). We can conclude that our proposed Point-of-care approach to CAR cell therapy can be explored as an alternative with less cost, time, and complexities. Furthermore, mIL15 added to CAR appears to bring benefits in fighting tumor in animal model.

Keywords: CAR-T cells, Point-of-care, Immunotherapy, Sleeping Beauty, PiggyBac

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CHARACTERIZATION OF p53 AMYLOID AGGREGATES IN HEPATOCELLULAR CARCINOMA CELL LINES AND THEIR MODULATION BY PRIMA-1

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INTRODUCTION AND OBJECTIVES: Mutations in p53, a tumor suppressor protein, are present in more than 50% of cases of cancer. In hepatocellular carcinoma (HCC), the third leading cause of cancer death worldwide, p53 mutations are the most frequent mutations that favor tumor development. These mutations promote wild-type p53 (WTp53) loss of function and oncogenic gains-of-function, which vary according to the type of mutation. Also, amyloid aggregation and a prion-like behavior of mutant p53 (*mutp53)* are observed. Overall, available therapies are not only limited, but also the presence of *mutp53* contributes to tumor progression and therapeutic resistance. Based on this scenario, it is still necessary to search for new pharmacological targets, such as *mutp53*. A promising chemotherapy agent, PRIMA-1 (P1) is a small molecule capable of recovering the functional conformation of p53 and restoring its transcriptional activity. Thus, the objective of this work is to investigate the aggregation of *mutp53* in the HCC model, for the characterization of a new pharmacological target using P1. MATERIAL AND METHODS: initially, mutants Y220C and R249S were analyzed through different aggregation prediction algorithms (AGGRESCAN, Tango, Waltz, and ZipperDB). HCC cell lines, Hep3B (p53 null), HepG2 (WTp53), Huh-7 (p53 Y220C), and PLC/PRF/5 (p53 R249S), and the effects of P1 were evaluated using 2D and 3D cell culture models by western blotting, dot-blot, confocal fluorescence microscopy, MTT assay, immunoprecipitation, Annexin /PI assay, migration, clonogenic assay, and RT-qPCR. RESULTS AND CONCLUSION: Y220C and R249S showed the same propensity to aggregate as WTp53, and a higher level of p53 and amyloid oligomers were observed in cells expressing these mutants when compared to WT and p53 null cell lines. In addition, the amyloid oligomers of Huh-7 and PLC/PRF/5, found mostly in the cytoplasm, colocalized and immunoprecipitated with p53, corroborating that p53 is in the amyloid state. To analyze their prion-like behavior, total cell lysates from *mutp53* cell lines induced the aggregation of WTp53C, which may suggest the ability of *mutp53* to convert WTp53 into an aggregated form. A greater reduction in the viability of *mutp53* cell lines was observed under P1 treatment and this effect remained after its removal. p53 levels and p53 amyloid oligomers were reduced as well. p53 gene targets were activated after P1 treatment, which indicates p53 reactivation. Also, P1 prevented the formation and disrupted 3D cells while reducing their viability through apoptosis induction. Cell migration and colony formation were inhibited. Through the associated treatment of P1 and cisplatin, the chemosensitization of Huh-7 cells was 6 times better compared to chemotherapy alone. Taken together, these results demonstrate that p53 amyloid aggregation is a potential pharmacological target for HCC, and P1 can be a new candidate for combination therapy with cisplatin.

Keywords: Hepatocellular carcinoma, p53, Mutant p53, Amyloid aggregation, PRIMA-1
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REGULATORY ROLE OF THE PIGMENT C-PHICOCYANIN IN A MURINE MELANOMA MODEL

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INTRODUCTION AND OBJECTIVES: Melanoma has a high lethality rate and one of the characteristics of this disease is the decrease in T lymphocytes in the tumor environment. In this scenario, C-FC, a photosynthetic pigment, appears as a possible treatment, since it has antitumor activity acting on different cellular pathways. Thus, we evaluated the action of C-FC on murine melanoma and its possible immunomodulatory effect. **MATERIAL AND METHODS:** Cell viability of B16F10 and melan-a cell lines was analyzed by trypan blue after exposure to C-FC (100, 200, 400 µg/mL) at 24-72h. C57BL/6 mice were used for *in vivo* experiments and for tumor induction (Mel) $1x10^6$ cells of the B16F10 line were injected. The animals in the C-FC and C-FC+MEL groups received dorsal subcutaneous injections of C-FC (80 or 525 mg/Kg) for 3 days. Control (CTR) and Mel animals received sterile water. After euthanasia, the organs of interest were removed, the cells were stained with anti-CD4+ and anti-CD8+ antibodies and analyzed by flow cytometry. **RESULTS AND CONCLUSION**: Cell viability showed that all C-FC concentrations tested decreased the number of viable cells 72h after exposure, with a cytotoxic effect to 400 µg/mL at 48 and 72h. Treatment with C-FC did not alter the viability of the melan-a line, demonstrating its specificity for tumor line. We then evaluated the action of C-FC in the *in vivo* model at a dose of 80 mg/kg. Tumor-bearing animals treated with C-FC (Mel+C-FC) had a reduction in the % of CD8+ T lymphocytes in the spleen compared to the CTR and C-FC. In the thymus, there was a decrease in the absolute n° of total lymphocytes and in the absolute n° of CD4+/CD8+ T lymphocytes in the Mel and Mel+ C-FC in relation to the C-FC. The Mel+C-FC group also showed a reduction in the % of CD4+ T cells in relation to C-FC. The C-FC was not able to reverse the immunosuppressive effects of the tumor, in addition to significantly reducing the population of T lymphocytes in the thymus and spleen. Thus, we tested an overdose (525 mg/Kg) to analyze the toxicity of C-FC and its ability to modulate lymphoid organs in the absence of the tumor. The treated animals showed a decrease in the absolute n° of total lymphocytes and absolute n° and % of CD4+ and CD8+ T lymphocytes in the spleen. However, in the inguinal lymph node there was an increase in the absolute n° of total lymphocytes and in the absolute n° of CD4+ and CD8+ T lymphocytes. We assume that C-FC overdose induce the recruitment of T lymphocytes from the spleen to peripheral lymph nodes. We conclude that C-FC exerts different effects depending on the concentration: in the tumor-free animals only the highest concentration modulated the spleen lymphoid organs and inguinal lymph node. C-FC at a lower dose does not reverse the immunosuppressive effect of melanoma, and it is interesting to investigate the action of

overdose on the tumor, as this dose is capable of recruiting T lymphocytes to the lymph nodes draining the injection.

Keywords: Phycobiliprotein, Skin cancer, T cells, Immunoregulation.
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MODULATION OF THE WNT/β-CATENIN PATHWAY AND ITS EFFECTS IN COLORECTAL CANCER CELL LINES

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INTRODUCTION AND OBJECTIVES: The Wnt/β-catenin signaling pathway has been studied over the last years, and is well known that it plays an essential role in embryo development, cell fate decision, stem cell self-renewal, and tissue homeostasis. However, mutations in its key components are related to many cancer types such as colorectal cancer (CRC). The most common mutation is found in the gene APC, which promotes a constitutive activation of the Wnt/β-catenin pathway, disrupting tissue homeostasis and benefits cancer cells promotes tumor cell growth. The chemotherapy drug oxaliplatin (L-OHP) is one of the most used drugs in advanced CRC treatment. However, information about L-OHP and DL79FA effects on the Wnt/β-catenin pathway are still lacking. For this reason, we aimed to evaluate the effect of both L-OHP and DL79FA as inhibitors of the Wnt/β-catenin pathway and investigate the mechanisms and impacts on tumor cell growth. **MATERIAL AND METHODS:** In this study, we employed the colorectal cancer cell lines RKO, DLD-1, HCT 116, and SW480. The cells were treated with L-OHP and DL79FA, for 24 hours and then we characterized the activity of the Wnt/β-catenin signaling pathway through gene reporter assay and western blotting. We also evaluated cell viability, proliferation, and cell cycle dynamics. **RESULTS AND CONCLUSION:** So far, our results have demonstrated that both DL79FA and L-OHP inhibit the Wnt/β-catenin signaling pathway in a concentration-dependent manner in a specific gene reporter assay, with an IC50 of 11.73 μM and 1.5 μM, respectively. Subsequently, we demonstrated that total β-catenin levels were decreased in RKO cells treated with BIO, a GSK3 inhibitor, and cotreated with L-OHP and DL79FA. In addition, both drugs decreased cell viability as well as tumor growth in all cell lines. The next step will be to demonstrate whether the combination of DL79FA and L-OHP synergistically impairs tumor cell growth.

Keywords: Wnt/ β-catenin pathway, Oxaliplatin, DL79FA.
Supported by: CNPq, FAPERJ,CAPES

EUTERPE OLERACEA (AÇAÍ) EXTRACT HAS PROTECTIVE EFFECTS IN EXPERIMENTAL BREAST CANCER TREATED WITH THE FAC-D PROTOCOL

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INTRODUCTION AND OBJECTIVES: Breast cancer is the most common cancer and the leading cause of cancer death among women worldwide, with the exception of non-melanoma skin tumors. In addition, it has been recognized as one of the main public health problems in the world. In Brazil, the most common chemotherapy regimen is the FAC-D protocol (5-fluorouracil, doxorubicin, and cyclophosphamide, with docetaxel or paclitaxel), which produces several adverse effects. It has already been shown that açaí, a fruit native to Brazil, has anti-inflammatory, antioxidant, anticancer and cardioprotective effects. Thus, the aim of this study was to evaluate the use of açaí with the FAC-D protocol in an *in vivo* breast cancer model. **MATERIAL AND METHOD:** Approved by CEUA UEZO (nº 008/2014), breast cancer was induced in 40 female Wistar rats (8 weeks old, 150 g) by subcutaneous injection of 25mg/kg of 7,12-dimethylbenzanthracene (DMBA) in the mammary gland. Treatment with açaí (200 mg/kg) or saline solution was started after 60 days, via gastric tube for 45 consecutive days. The FAC-D protocol was started after 90 days of induction by intraperitoneal injection for 3 cycles with an interval of 7 days each. After treatment, blood (leucogram, glycemia and biochemistry), tumor (macroscopic and histological), heart (macroscopic, histological and immunohistochemical), liver and kidneys (macroscopic and histological) were collected for analysis. **RESULTS AND CONCLUSION:** Histological analysis showed invasive carcinoma in both groups, however, the saline group presented more inflammatory clusters. No differences were observed based on body weight, blood glucose, AST, ALT, creatinine, urea and on macroscopic and histological analysis of liver and kidneys in both groups. However, açaí treatment decreased CK and CKMB levels and increased the number of neutrophils and monocytes. The histopathology of the heart showed normal myocardium histology in the treatment with açaí, while the saline group presented greater toxicity with loss of cardiac tissue architecture. Picrosirius red analysis revealed higher staining in heart samples from the açaí group, while H2AX immunohistochemistry revealed lower immunostaining in the açaí group. In conclusion, açaí berry was shown as a promising adjuvant treatment in breast cancer.

Keywords: Açaí, breast cancer, treatment.

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ANTITUMOR ACTIVITY OF NAPHTHOQUINONES MANNICH BASES AGAINST ORAL SQUAMOUS CELLS CARCINOMA

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INTRODUCTION AND OBJECTIVES: Oral squamous cell carcinoma (SCC) is currently considered a public health problem in Brazil. Carboplatin and Cisplatin are the drugs most used in chemotherapy for this type of cancer, however, they have many side effects, such as nephrotoxicity and acquired resistance. Thus, the search for more effective chemotherapy drugs is justified. The present study aims to show the synthesis of eighteen synthetic substances from fused naphthoquinones based on Mannich adduct and testing them on OSCC models. **MATERIAL AND METHODS:** Compounds synthesis occurred through the fusion of lawsone to a Mannich reaction (MB1-18). Cytotoxicity and selectivity were evaluated by MTT assays in OSCC (SCC4, SCC9, SCC25) and fibroblast. The following assays were also carried out: hemolysis assays; clonogenic assay; *in vivo* acute toxicity (CEUA: 982); *in silico* prediction; molecular docking; caspase inhibitor assay; necroptosis inhibition; autophagy inhibition; flow cytometry and cell cycle assays. **RESULTS AND CONCLUSION**: Among the eighteen 1,4-naphthoquinones tested, only the methyl benzyl (4-chlorophenyl) (3-hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl) methyl) carbamate (MB10) demonstrated a selectivity index higher than 2 (SI: IC_{50} on normal cells / IC_{50} on tumor cells) considering the 3 strains of OSCC (SCC9; SCC4 and SCC25) and normal mouth cells (human fibroblasts). The most selective substance (MB10) reduced the formation of clones in SCC9 via clonogenic assay. The tests for reactive oxygen species (ROS) demonstrated that the substance MB10 does not produce ROS in SCC9. *In vivo* tests showed that the LD_{50} of MB10 was around 150 mg/kg and signs of toxicity were observed in the lungs and kidneys in higher concentrations. *In silico* predictions of MB10 indicated indicated that their chemical and biological characteristics by lipinski's rule of 5 exhibit better drug profile than carboplatin and doxorubicin. The computational target fishing strategy used indicated that the MB10 could exert its anticancer action by inhibiting topoisomerase I and IIβ and hPKM2. Autophagy cell death followed by latte apoptosis was observed by the presence of autophagosomes and their inhibition through fluorescence microscopy, which is related to the Docking assay molecular structure suggesting that inhibition of hPKM2 leads to energy deprivation and consequently autophagy. The stress generated by the autophagic process leads to late apoptosis, active caspases 3/7 intense DNA fragmentation,

phosphatidyl serine exposure and activation of effector caspases at late times were observed. For the cell cycle analysis, it was shown that the substance does not cause an arrest in the cell cycle. Furthermore, inhibition of autophagy by 3MA partially inhibited cell death that was further inhibited by co-treatment with pan-caspase inhibitor (ZVAD). The results indicated that 1 of the 18 Mannich bases synthetic naphthoquinone compounds has a good profile for the treatment of OSCC.

Key words: Oral Cancer, Naphthoquinones, Mannich aducts.

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HEPARIN ANTIMETASTATIC EFFECTS IN AN EXPERIMENTAL MODEL OF BREAST CANCER-INDUCED METASTASIS

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INTRODUCTION AND OBJECTIVE: Cancer is the second leading cause of death in the world. Studies suggest that cancer patients are more prone to develop thrombosis and much poorer prognosis. For this reason, it is common to associate in clinical practice cancer treatment with antithrombotic prophylaxis. It allowed the realization of cohort studies whose results showed a strong relationship between the use of heparin and increased survival of cancer patients, including a reduction in the number of metastases. Heparin is a glycosaminoglycan used as an anticoagulant drug. However, studies project other pharmacological applications for this molecule since it presents anti-inflammatory, antiviral and antimetastatic potential. Porcine intestinal heparin (HPI) is the most used clinically, however, due to its potent anticoagulant effect, HPI often leads to hemostatic imbalance which is a risk for most patients. Data from our laboratory showed that the heparin extracted from bovine intestine (HBI) and its low anticoagulant bovine heparin fraction (LABH) are less sulfated and present about 50% and 10% of HPI anticoagulant activity respectively. The objective of this work is to investigate and compare the performance of these distinct heparins (HPI, HBI and LABH) in preventing the occurrence of metastases, for this making use of an experimental model of murine mammary carcinoma (4T1). **MATERIAL AND METHODS:** Firstly, we tested the cancer-induced metastasis model using different number of 4T1 cells injected directly into the tail vein of BALB/c mice. Then, the antimetastatic activity of heparins was evaluated through their injection in different concentrations immediately before tumor cell application. After 28 days, lungs were collected for macro and microscopical analyses using Indian ink and histological tools. **RESULTS AND CONCLUSION:** Our results indicated that the injection of 5.10^4 cells per animal produced the best scenario for further experiments, as the assay with 10^5 resulted in a greater variation of metastatic foci number. When analyzing different doses of HPI, we could see that the 4 mg/kg dose proved to be sufficient and capable of promoting a reduction in metastatic foci as we did not find a significant difference when we treated with 16 mg/kg. Next, we performed a comparison between HPI, HBI and LABH treatments, and analyzing the macroscopic lung foci we observed a similar reduction of metastatic nodules with all heparins tested. In addition, H&E images of different animal tissues revealed micrometastasis also in the liver. Finally, bovine heparins (HBI and LABH) that present greater clinical safety regarding bleeding side effect, seem to be good alternatives for antimetastatic treatment of breast cancer. As perspectives, we will extend our evaluation to a spontaneous metastasis model.

Keywords: antimetastatic activity, heparin, 4T1, breast cancer.

Funding source: CNPq and FAPERJ.

EVALUATION OF GALECTIN-3 IN DNA DAMAGE REPAIR MEDIATED BY POLY-ADP- RIBOSYLATION: INVESTIGATION OF SENSITIVITY FACTORS TO PARP1 INHIBITOR TREATMENT

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INTRODUCTION AND OBJECTIVE: Galectin is a family of proteins that recognize and bind β-galactosides. Among them, galectin-3 stands out as the only member of the chimeric galectin subfamily. It is known that tumor cells are under stress conditions, such as hypoxia and nutrient deprivation, and that galectin-3 levels are increased, promoting the proliferation and survival of these cells. Our group identified poly ADP-ribose polymerase 1 (PARP1) as a novel protein-interacting partner of galectin-3. PARP1 promotes the addition of ADP-ribose polymers to proteins involved in DNA damage repair processes. Studies show that PARP1 is compromised both in repairing the single stranded DNA and in double strand break of DNA. The purpose of this work is to analyze the performance of galectin-3 in the repair steps to DNA damage dependent on PARP1. **MATERIAL AND METHOD:** Using the shRNA stable silencing technique, the MDA-MB231 and MCF-7 cells were silenced for the LGALS3 gene and an unrelated gene used as a negative control (scrambled, SCRB). The sensitivity profile of MDA-MB231 and MCF-7 silenced cells treated for 48h with cisplatin, irradiation or pharmacological inhibitor of PARP1 (PJ34) was evaluated by colorimetric assays with tetrazolium salts (MTT). The level of PARP1 protein in MDA-MB231 and MCF-7 silenced cells was evaluated by immunoblotting. The gama-H2AX focus in MDA-MB231 cells treated with irradiation for different periods of time was evaluated by immunofluorescence. **RESULTS AND CONCLUSION:** After 48 hours of cisplatin and PJ34 treatment, MDA-MB231 shGAL3 cells are more resistant to treatment than MDA-MB231 shSCRB cells. However, MCF-7 shGAL3 cells are more sensitive to treatment compared to MCF-7 shSCRB cells. When cells were treated only with PJ34, we observed that MCF-7 shGAL3 cells are more sensitive to the treatment compared to MCF-7 cells shSCRB. Nonetheless, MDA-MB231 shGAL3 cells are more resistant to PJ34 treatment than MDA-MB231 shSCRB cells. The level of PARP1 protein in MDA-MB231 shSCRB was higher than in MDA-MB231 shGAL3. Although, the MCF-7 shSBRB shows lower PARP1 protein level than MCF-7shGAL3. After irradiation, the ɣ-H2AX level was higher in MDA-MB231 shSCRB than in MDA-MB231 shGAL3. Thus, LGALS3 expression may be a relevant factor in the effect of PARP1 inhibition treatment in combination with Cisplatin. In addition, the PARP1 protein level may have a role in PJ34 and Cisplatin breast cancer cells sensitive. In MDA-MB231 shGAL3 cells, the low level of ɣ-H2AX shows a delay in DNA damage repair.

Keywords: Galenctin-3, PARP1, DNA Damage Repair.
Supported by: FAPERJ, CNPq and CAPES

TOB2 INDUCES CELL GROWTH AND CELL DEATH INHIBITION IN CANCER CELLS AND PRESENT A POTENTIAL BIOMARKER VALUE IN DIFFERENT TUMORS

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INTRODUCTION AND OBJECTIVES: TOB2 (transducer of ERBB2, 2) belongs to BTG/Tob antiproliferative family involved in mRNA deadenylation and cell cycle arrest. Although several members of its family are considered a tumor suppressor, TOB2 role in cellular growth and cell death is poor explored. Moreover, TOB2 value as diagnosis, prognosis and response to therapy biomarker in tumors is unknown. Our aim is to investigate the role of TOB2 modulation in cell growth and cell death in tumor and tumorigenic cells *in vitro* and to evaluate *in silico* the impact of TOB2 expression in overall survival (OS), its value to differentiate normal and tumor from different tissues and its value to predict chemotherapy response in breast cancer. **MATERIAL AND METHOD:** MDA-MB-231, H460, U251-MG and HEK293T cell lines were used in the study. Crystal violet, Trypan blue and clonogenic assays were performed after TOB2 overexpression or inhibition by specific siRNA for 24h. Cell cycle profile and cell death (Anexin V) were assessed after TOB2 overexpression for 24, 48 and 72h using flow cytometry. Western blot was used to confirm TOB2 modulation. PCR array was applied to investigate cancer pathways altered after TOB2 overexpression for 24h in U251-MG cells. Web-based tools were used to collect data from OS, tissue gene expression, gene methylation and response to therapy. Biomarker potential was evaluated by ROC curve and AUC value. AUC values above 0.6 (positive or negative) were considered potencial biomarker. **RESULTS AND CONCLUSION**: TOB2 overexpression in MDA-MB-231, H460, U251-MG and HEK293T cells increased clonogenic potential and crystal violet cells stanning. Confirming these data, TOB2 inhibition showed opposite effect. However, trypan blue assay showed no difference after TOB2 overexpression in all cells evaluated. TOB2 overexpression in all cell lines induced no cell cycle arrest and no impact in cell death, except in U251-MG cells, where TOB2 overexpression induced reduction in Anexin V cells. PCR array initial data demonstrated expression increase of *CASP9, TGFBR2, CDC42, CDKN1A, ITGB1, SOS1, CDKN1B, FGF2, WNT1, MAPK8, APC, BCAR1, CASP8, CDH1, BAX, COL1A1, GSK3B, PTEN, AKT1, AKT2, BRAF and ITGB3* after TOB2 overexpression. *In silico* analysis demonstrated that higher TOB2 expression is associated with better OS rates in rectum adenocarcinoma, breast and uterus cancers. Also, TOB2 could predict treatment response in breast cancer. Conversely, TOB2 expression is associated with a pro-tumor role in acute leukemias and in prostate, pancreatic and esophageal cancer. Additionally, TOB2 is associated with worse OS in pancreatic cancer. Finally, TOB2 may be associated with tumorigenesis in low grade gliomas, but not in glioblastoma, the most aggressive glioma subtype. Our findings suggest a pro-tumor role of TOB2 in

tumor cells and a potential value as biomarker in the diagnosis, prognosis and response to therapy.

Keywords: TOB2, pro-tumor role, cell growth, biomarker.

Supported by: Fundação do Câncer, FAPERJ and CAPES

ANTITUMOR ACTIVITY AND CELL DEATH PATHWAY OF SYNTHETIC MOLECULE BASED ON THE GENUS PIPER IN ORAL SQUAMOUS CELLS CARCINOMA (OSCC)

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INTRODUCTION AND OBJECTIVES: In traditional medicine, many plants, such as those of the Piper genus, are used in the treatment of oral squamous cell carcinoma (OSCC). Thus, isolated substances derived from these plants are often used as a base molecule for the synthesis of analogues, which may also have significant antitumor effects. The objective of this work is to evaluate whether synthetic molecules based on a substance isolated from Piper have antitumor activity in SCC9 cells of OSCC and great selectivity, for the production of a possible treatment with fewer side effects than platinum-based drugs commonly used in the clinic. **MATERIAL AND METHODS:** A collection of esters and amides was prepared from 3,4,5-trimethoxybenzoic acid. Fourteen esters (RHE1, RHE2, RHE3, RHE4, RHE5, RHE6, RHE7, RHE8, RHE9, RHE10, RHE11, RHE14, RHE15 and RHE16) and eight amides (RHA1, RHA2, RHA3, RHA4, RHA5, RHA6, RHA7 and RH8) were obtained. From this, the viability assay (MTT) was performed on SCC9 and normal gingival fibroblasts, in which substances with greater activity and selectivity were selected to continue in assays to determine the type of cell death. **RESULTS AND CONCLUSION:** Substances RHE5, RHE6, RHE10, RHE11 and RHE15 were the most active and selective in SCC9, with RHE11 (IC50= 68.69; SI= 53.12) being selected to continue the tests, due to its greater activity and selectivity. In addition to cell viability, it was observed that RHE11 did not show hemolytic activity in human erythrocytes. Video time-lapse microscopy of SCC9 treated with RHE11 demonstrated that these cells exhibit a morphology indicative of cell death by apoptosis. From this, flow cytometry was performed, in which an increase in annexin V+PI labeling, positive labeling of a single PI, active caspase-3/7 and an increase in sub-G0 were observed. All these results showed that RHE11 showed antiproliferative activity and selectivity, indicating a possible cell death by apoptosis process in SCC9.

Keywords: Cancer, Cytotoxicity, Oral Cancer, Synthetic Molecules, Apoptosis.
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PÓS-DOC

LONG-TERM RESISTANCE TO 5-FLUOROURACIL ACTIVATES DISTINCT CELLULAR EVENTS UNDERLYING DRUG RESISTANCE ON COLON CANCER CELLS

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INTRODUCTION AND OBJECTIVES: Drug-resistant cell line models are crucial for understanding resistance mechanisms and developing new therapeutics. However, little is published about these cell lines, including the methodology and development process. The present work aimed at the establishment and characterization of a long-term drug resistance model in an effort towards exploring the cellular mechanisms underlying drug resistance. **MATERIAL AND METHODS:** The colon cancer cell line HCT-116 was treated with intermittent, repeated, and increasing 5-fluorouracil (5-FU) doses followed by cell recovery in a drug-free medium. To assess the acquired resistance, cell viability, thymidylate synthase (TS), and efflux pump levels were measured. The cell cycle distribution and proliferation were determined using flow cytometry and clonogenic assays. In addition, apoptosis was evaluated by flow cytometry and caspase 3/7 detection, while autophagy was determined by LC3B detection. Moreover, the activation of epithelial-mesenchymal transition (EMT) was evaluated by mRNA and protein measurement using epithelial and mesenchymal markers, as well as by migration and invasion transwell assays. Analysis of drug resistance and mesenchymal gene signatures their correlation, and prognostic values were performed using the GEPIA database. **RESULTS AND CONCLUSION**: The 5-FU-treated cells (HCT-116 5FUR) using a prolonged treatment protocol were significantly more resistant than parental cells. Likewise, cell viability and IC50 values were also observed to increase in HCT-116 5FUR cells when treated with increasing doses of oxaliplatin, indicating a cross-resistance mechanism to other cytotoxic agents. Moreover, HCT-116 5FUR cells exhibited metabolic and molecular changes, as evidenced by increased TS levels and upregulated mRNA levels of *ABCB1*. HCT-116 5FUR cells were able to overcome S phase arrest and evade apoptosis, as well as activate autophagy, as indicated by increased LC3B levels. Cells treated with low and high doses displayed EMT features, as observed by decreased E-cadherin and claudin-3 levels, increased vimentin protein levels, and increased *SLUG, ZEB2,* and *TWIST1* mRNA levels. Furthermore, HCT-116 5FUR cells displayed enhanced migration and invasion capabilities. Also, resistant cells developed cell heterogeneity and acquired a significant mesenchymal-like population, indicated by quantification of the morphometric parameters as circularity index. Interestingly, we found that the 5-FU drug-resistance gene signature is positively associated with the mesenchymal signature in colorectal cancer (CRC) samples, and that *ABCB1* and *ZEB2* co-expressed at high levels could predict poor outcomes in CRC patients. Overall, the 5-FU long-term drug-resistance model established here induced various cellular events, and highlighted the importance of further efforts to identify promising targets involved in more than one cellular event to successfully overcome drug resistance.

Keywords: drug resistance; 5-fluorouracil; apoptosis; autophagy; epithelial-mesenchymal transition; colorectal cancer.
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IDENTIFICATION AND FUNCTIONAL CHARACTERIZATION OF INTERACTION PARTNERS OF THE TUMOR SUPPRESSOR PALB2

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INTRODUCTION AND OBJECTIVES: The DNA damage response (DDR) proper function maintenance is fundamental for tumor suppression activity. DNA double-strand breaks (DSB) pose the greatest threat to DNA stability and the homologous recombination repair (HR) pathway is the most accurate DSB repair mechanism. Following DSB, RAD51 recombinase is recruited to DNA damage sites in HR repair, promoted by the BRCA1-PALB2-BRCA2 complex. PALB2 (*PArtner and Localizer of BRCA2*) interacts with BRCA1 at DNA damage sites and functions as a linker between BRCA1 and BRCA2. BRCA1-PALB2-BRCA2 complex formation is crucial for the appropriate HR function. Mutations in the genes encoding for these proteins are associated with a high risk of developing breast cancer and other tumor types. Besides being associated with hereditary breast cancer, the *PALB2* gene, also known as *FANCN*, is also associated with pancreatic cancer and Fanconi Anemia syndrome. Little is known about PALB2 protein complexes formation and, since its identification, few studies have explored its protein interaction partners. In view of PALB2 relevance for HR repair and the need for better understanding its functions, this work aims to characterize PALB2 WD40 domain new interaction partners by conducting a yeast two-hybrid (Y2H) screening. **MATERIAL AND METHODS:** We have conducted a Y2H screening using PALB2 C-terminal (WD40 domain) segment as a bait and a universal human cDNA library (Clontech, USA) as prey. Isolated clones were sequenced and the results analyzed by sequence alignment using BLAST. Interactions identified will be validated by GST-pull-down assays using human cell lines. Functional characterization of the validated PALB2 heterodimers will be performed by co-immunoprecipitation and co-immunofluorescence microscopy assays. Other assays to better assess the identified targets' role in the BRCA1-PALB2-BRCA2 complex formation and function will be conducted. **RESULTS AND CONCLUSION**: We have identified 7 new PALB2 WD40 interactions. We highlight the newly identified partners COPS5 and SNAPIN. We identified two independent clones coding for COPS5. Both COPS5 and SNAPIN were also detected in an unpublished Y2H screening conducted by our group using PALB2 full-length as a bait, strongly suggesting they could be bonafide PALB2 functional partners. COPS5 is part of the COP9 signalosome (CSN) deneddylase complex. Neddylation/deneddylation, similar to ubiquitination and sumoylation, is involved in different cell signaling processes, including DDR. SNAPIN is associated with organelles protein docking and has a potential role in cell cycle regulation. We expect that the resulting data from this project will validate and characterize new interacting partners of PALB2, contributing to the better understanding of the cellular functions of this important tumor suppressor.

Keywords: DNA damage repair, PALB2, homologous recombination, yeast two-hybrid.

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GENERATION AND OPTIMIZATION OF anti-CD19 CAR-T CELL FOR LEUKEMIA IMMUNOTHERAPY

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INTRODUCTION AND OBJECTIVES: CAR-T-cell immunotherapy has achieved high response rates in treatment-refractory patients with B-cell malignancies. This therapy involves the genetic modification of isolated T cells frompatient to express an anti-CD19 CAR (Chimeric Antigen Receptor), expanded *in vitro* and then re-infused into the patient. CAR-T-cell therapy has shown considerable advance in recent years, being approved by regulatory agencies in US, Europe and recently in Brazil. Most current methods for CAR-T-cell generation use viral vectors for T-cell genetic modification and can take up to 15 days to generate the infusion product. To adapt this protocol to our local reality, we are developing simple and less costly manufacturing protocols to meet the increasing demand for this therapy. The main goal of this work is generate a anti-CD19 CAR-T cells with a short *in vitro* expansion protocol based on the non-viral Sleeping Beauty (SB) transposon-based vector system. **MATERIAL AND METHODS:** The 19BBz CAR sequence was provided by Dr Dario Campana (Memphis, TN) and was cloned in the transposon vector pT4/HB (provided from Wolfgang Uckert (Addgene plasmid#108352). PBMCs were collected from healthy donors after signed board–approved informed consent. Mononuclear cells were isolated by density gradient centrifugation with Ficoll-Hypaque-1077 and electroporated with plasmids enconding 19BBz CAR and the SB100x transposase. The expansion of CAR-T cells was performed using G-REX culture wells for 8 and 12 days. For the xenograft mouse model, eight- to twelve–week-old female NOD-SCID IL2R gamma null (NSG) mice were injected on the tail vein with Nalm-6 Luc-GFP cells and treated 2 days later with 2.5 $x10^5$, 5 $x10^5$ CAR-T and 3 $x10^6$ cells or control cells expanded but not expressing the CARs. For *in vivo* imaging, mice were injected i.p. with 75 mg/kg d-luciferin and tumor burden was verified by bioluminescence using IVIS Lumina XR. For cell analysis, cells or organs were processed and analyzed by flow cytometry. **RESULTS AND CONCLUSION**: Using the protocol described herein we generate from 9 $x10^7$ total PBMCs a mean of 1.34 $x10^7$ CAR-T cells after 8 days of expansion. CAR-T cells generated showed cytotoxic effect against CD19+ leukemia cells *in vitro.* Furthermore, CAR-T cells treatment improved overall survival rates (40% at 2,5 $x10^5$ dose after 37 days, 77% at 5 $x10^5$ dose after 86 days and over 60% at 3 $x10^6$ after 85 days) of NSG mice inoculated with 1x 10^5 Nalm-6 B-cell leukemia, leading to a significative reduction in the tumor burden**.** Finally, infused CAR-T cells persisted for up to 50 days, showing that they are capable of long-term persistence and antitumor response. The current protocol can generate a cellular product campatible with regulatory requeriments and performance of a phase zero clinical assay.

Keywords: Leukemia, CAR-T, Immunotherapy, CD19, Transposon
Supported by: INCA, Inova-FIOCRUZ, FAPERJ, Ministério da Saúde - PRONON and CNPq

β-CATENIN DESTABILIZATION OVERCOMES 5-FU RESISTANCE AND SYNERGISTICALLY IMPAIRS COLORECTAL CANCER GROWTH

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INTRODUCTION AND OBJECTIVES: Colorectal cancer (CRC) remains the second leading cause of cancer-related deaths worldwide. Despite advances regarding 5-fluorouracil (5-FU)-based therapies for CRC, the 5-year survival rate is less than 15%. It has been demonstrated the Wnt/β-catenin signaling pathway is critical for CRC chemoresistance besides its well-known role in CRC development and progression. In this sense, targeting the Wnt/β-catenin signaling could be promising in overcoming CRC progression and resistance. Thus, we targeted the Wnt/β-catenin pathway and demonstrated that the chalcone Lonchocarpin, a potent β-catenin inhibitor, can enhance 5-FU effects and impairs colorectal cancer growth. **MATERIAL AND METHODS:** In this study, we used several colorectal cancer cell lines with different degrees of malignancy and Wnt signaling statuses, such as RKO (carcinoma in situ), HCT 116 (adenocarcinoma), and DLD-1 (adenocarcinoma) cells, which harboring mutations in β-catenin and APC, rendering the Wnt pathway constitutively active. Cells were treated with different concentrations of 5-FU and lonchocarpin, both individually and in combination, for 48 hours. We then assessed Wnt/β-catenin signaling activity, as well as tumor cell proliferation, growth, viability, and survival. **RESULTS AND CONCLUSION:** First, we showed that 5-FU activated the Wnt/β-catenin signaling pathway and enhanced β-catenin levels while lonchocarpin inhibits the gene reporter activity in a concentration-dependent manner. Next, we showed that low concentrations of lonchocarpin can synergistically enhance the effects of 5-FU on cell growth and viability. Interestingly, the combined treatment also significantly reduced cell proliferation and impaired growth of colorectal oncospheres. Finally, we show that the combined treatment of 5-FU and lonchocarpin is more effective in suppressing the long-term survival of colorectal cancer cells compared to 5-FU alone. Together, our data support the hypothesis that targeting the Wnt/β-catenin can enhance the effects of the chemotherapeutic 5-FU and can improve the efficiency of CRC therapies.

Keywords: Wnt signaling; 5-fluorouracil; chemoresistance
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A MECANISTIC ROLE FOR ATM/ATR-MEDIATED BRCA1 PHOSPHORYLATION IN THE DNA DAMAGE RESPONSE

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INTRODUCTION AND OBJECTIVES: DNA Damage Response (DDR) is dysregulated in several diseases, especially in cancer. BRCA1 tumor suppressor protein plays a key role in the homologous recombination (HR)-mediated DDR axis. Mechanistically, BRCA1 orchestrates two crucial steps of HR. First, BRCA1 promotes the DNA end resection process through its interaction with CtIP. Then, BRCA1 recruits PALB2 to the DNA-damaged sites through a strikingly fine-tuned direct interaction mediated by their coiled-coil domains. This interaction was shown to be regulated in a cell cycle and DNA damage-dependent manner by post-translational modifications in PALB2, such as ATM/ATR and CDKs-mediated phosphorylation, but the mechanisms underlying these associations are not fully understood. Interestingly, since the 1990s is known that ATM/ATR and CDKs-mediated BRCA1 phosphorylation is pivotal to HR repair and genomic maintenance, however it is not clear their mechanistic role. As some of these modifications occur near to PALB2-interacting region, we sought to evaluate the putative role of BRCA1 phosphorylation in modulating PALB2 association. **MATERIAL AND METHODS:** BRCA1/PALB2 interaction was evaluated by a GST pulldown approach in HEK293FT cells. BRCA1 and PALB2 phosphorylation sites were mutated by site-directed mutagenesis in which serine residues were replaced by alanine or glutamic acid (mimicking a phosphodeficient or a phosphomimetic mutant, respectively). **RESULTS AND CONCLUSION:** Here, we uncover a mechanistic role for ATM/ATR-mediated BRCA1 phosphorylation. Our data indicate that the region harboring the ATM/ATR and CDKs phosphorylation sites is necessary to fully promotes PALB2 binding. However, its global dephosphorylation or CDKs phosphorylation does not affect BRCA1-PALB2 association. Interestingly, a BRCA1 phosphodeficient mutant of specific ATM/ATR sites disrupts PALB2 interaction with no effect on BRCA1-CtIP association. Further analyses demonstrate that this phenotype is independent of PALB2 phosphorylation, previously shown to modulates PALB2-BRCA1 interaction after DNA damage. Finally, a global dephosphorylation compromises BRCA1-PALB2 interaction, but remarkably, a BRCA1 phosphomimetic mutant is sufficient to sustain PALB2 interaction. Taken together, these results identify that ATM/ATR-mediated BRCA1 phosphorylation modulates its interaction with PALB2 in the DDR and, therefore, has implications in genomic integrity maintenance and cancer knowledge.

Key words: DNA Damage Response; Homologous Recombination; BRCA1.

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LONG NON-CODING RNAs CONTRIBUTING FOR *CRLF2* OVEREXPRESSION IN ACUTE LYMPHOBLASTIC LEUKAEMIA

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INTRODUCTION AND OBJECTIVE: *CRLF2* overexpression (*CRLF2*-high) has been associated with unfavourable prognosis in B-cell acute lymphoblastic leukaemias (B-ALL). Occurrence of *CRLF2* rearrangements (*CRLF2*-r) and *CRLF2* F232C mutations account for only half of the cases with this gene overexpression. Long non-coding RNAs (lncRNAs) play a role in the development and progression of leukaemia and can interfere in the transcriptional regulation of protein-coding genes. In this scenario, we hypothesise that the dysregulation of lncRNAs might be a potential mechanism underlying *CRLF2*-high in B-ALL patients. **MATERIAL AND METHODS:** We included 126 B-ALL cases from the Therapeutically Applicable Research to Generate Effective Treatments (TARGET) cohort and delineated their molecular profile based on Whole Genome Sequencing (WGS) and RNA sequencing (RNA-seq) data. Differentially expressed (DE) lncRNAs in *CRLF2*-high patients were identified using DESeq2. Lncpath was used to obtain functional pathways influenced by the DE lncRNAs. The lncRNA and mRNA pairs experimentally validated were obtained from the LncRNA2Target database, and the interactions were tested by correlation analysis using RNA-seq TARGET data. Chromatin accessibility landscape was evaluated by Chromatin Immunoprecipitation Sequencing (ChIP-Seq) using H3K27ac and H3K4me1 marks, comparing B-ALL cell lines with *CRLF2*-high and low, to identify putative enhancers that could be linked to *CRLF2* regulation. Peak regions and transcription factor (TF) motifs were visualised in IGV software. All analyses used the GRCh37 genome as reference. **RESULTS AND CONCLUSION**: Were identified 293 up- and 70 down-regulated lncRNAs in *CRLF2*-high context. Among them, we identified five potential interactions lncRNA-target previously reported and also positively correlated in B-ALL context: *RPL34-AS1-MIR3663*, *LINC00161-MIR21*, *LINC00161-MIR590*, *PWRN1-MIR21* and *PART1-MIR149*. Interestingly, *RPL34-AS1* and *MIR21* were also correlated with *CRLF2*, showing a high expression in *CRLF2*-high patients (p=0.036 and p=0.004, respectively). Additionally, *MIR3663*, a known target of *RPL34-AS1*, was exclusively expressed in *CRLF2*-high patients. The characterisation of *RPL34-AS1* region revealed enhancer peaks in all B-ALL cell lines evaluated, independently of *CRLF2* status. Furthermore, we observed that this region is enriched with TFs motifs in common with *CRLF2* promoter, including *FOXM1*, *PML*,

TBP and *YY1* sites. Additionally, we assessed the expression profile of these, but only *YY1* was positively correlated with *CRLF2* expression (p=0.050). Considering the functional role of ribonucleoproteins in gene regulation, we will validate the potential regulatory interactions between *RPL34-AS1* and *CRLF2* using small interfering RNA (siRNA) assays. Our findings indicate a likely mechanistic role for these lncRNAs interactions on leukemogenesis which could unravel novel biomarkers, and clarify how the expression of *CRLF2* is regulated in those leukaemias.

Keywords: lncRNAs, *CRLF2*, gene expression, acute lymphoblastic leukaemia.

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APOPTOSIS MECHANISMS IN THE ANTITUMOR ACTION OF LQB-461 IN JURKAT'S LEUKEMIC LINEAGE MICROARRAY

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INTRODUCTION AND OBJECTIVES: Leukemias stand out for being the main type of childhood câncer and current treatments have strong side effects, also present a high rate of development of resistance to drugs used in chemotherapy. Several studies seek to develop new drugs that have multiple pharmacological properties, ensuring better therapeutic efficacy and fewer side effects[1]. We develop a study with novel synthetic molecules, analogous to LQB-278 (IC_{50} = 6,7μM), already described for its *in vitro* antileukemic action[2]. The cytotoxicity of analogues was previously evaluated on tumor cell lines (Jurkat, K562, PC3, A549 and MCF-7) and non-tumor cells (NIH-3T3), and LQB-461 stood out, presenting greater antileukemic action compared to its derivative (IC_{50} = 2,8μM), without toxicity in non-tumor cells (IC_{50} > 10 μM), showing to induce in Jurkat cells a cytostatic effect at a concentration of 3μM, and death by apoptosis pathway at 5μM [1]. Thus, our objective in the present work was to study the mechanism of death indution by apoptosis, investigating possible genes involved and target proteins in the action of LQB-461 in Jurkat cells *in vitro*. **MATERIAL AND METHODS:** Jurkat cells (1x10^5/ ml) were incubated 72h at 37 °C and 5% CO_2 in RPMI medium-1640. RNA was extracted from cell and the microarray was performed using gene chip with the Human Exon 1.0 ST array. For qPCR, complementary DNA synthesis was carried and reactions were performed according to Fast SYBR Green Master Mix. Apoptosis was determined using Annexin-V-FITC apoptosis kit and cells was concomitant labeled with the APC-conjugated anti-human CD95 (Fas) antibody and analyzed by flow cytometer. LC-3 protein was analized by western blot, the lysates were loaded by electrophoresis, transferred to nitrocellulose membranes and labeled with anti-LC-3 antibodie. **RESULTS AND CONCLUSION**: a microarray assay was carried out, which changes in GDE expression confirmed the importance of the intrinsic apoptosis pathway in the action of LQB-461 at a concentration of 5μM. Through real-time PCR, we validated the increased expression of *CDKN1A* and *BAX* genes, important mediators of the intrinsic pathway. Through the extrinsic pathway of apoptosis, we found also an increase in the expression of Fas receptor by flow cytometry, showing the presence of a population more sensitive and another more resistant to death. In addition, considering the importance of autophagy in cellular resistance, it was demonstrated by western blotting that LQB-461 promoted a significant decrease in the expression of the LC-3 protein, an autophagic marker, at the concentration that induces apoptosis (5μM). Our studies have shown that this synthetic molecule, LQB-461, at a concentration of 5μM, has promising results by activating the mechanism of death by intrinsic and extrinsic apoptosis pathway in Jurkat lymphocytic leukemia cells, in addition to inhibiting autophagy, which may be related to the overlapping of cellular resistance mechanisms.

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Keywords: microarray, apoptosis, leucemia, synthetic molecules.
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GAZE AT CDK9 ISOFORMS IN CELL CYCLE AND DNA DAMAGE RESPONSE

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INTRODUCTION AND OBJECTIVES. The DNA damage response (DDR) comprises an interwoven network that assures genomic integrity and constitutes a crucial barrier to cancer development and progression. The cell cycle checkpoint and DNA repair pathways play essential roles in the DDR because they allow a cell cycle-dependent and lesion-specific response. In a previous study, we identified CDK9 as a putative interactor of the DDR-related proteins BRCA1, BARD1, and PTIP. CDK9 acts in transcription elongation and presents two isoforms: $CDK9_{42k}$ and $CDK9_{55k}$, which differ only by additional 117 amino acid residues at the N-terminal region of 55k. Previously, we demonstrated that BRCA1 recruitment to DNA damaged sites, and consequently the homologous recombination (HR) repair, depend on $CDK9_{42k}$. However, the literature suggests a possible role for $CDK9_{55k}$ in the non-homologous end joining (NHEJ) repair. Here we investigate CDK9 regulation through cell cycle and explore functional differences between $CDK9_{42k}$ and $CDK9_{55k,}$ focusing on HR and NHEJ pathways. **MATERIAL AND METHODS.** We characterized CDK9 levels (both isoforms) throughout cell cycle, by real-time PCR and immunoblotting in synchronized MCF7 and hTERT-BJ cell lines. The impact of CDK9 isoforms overexpression (OE) on cell cycle dynamics and cell survival was evaluated in MCF7 cells using ionizing radiation (IR) and chemotherapeutic agents. To better understand the role of CDK9 in DNA repair, we evaluated the protein levels and subcellular localization of each isoform after IR treatment along with 53BP1 ionizing radiation-induced foci formation (IRIF) in MCF7 cells OE $CDK9_{42k}$ or $CDK9_{55k}$. Subsequently, we also assessed the HR and NHEJ proficiency in MCF7 cells OE $CDK9_{42k}$ or $CDK9_{55k}$. **RESULTS AND CONCLUSION:** Our results demonstrate that $CDK9_{55k}$ levels (mRNA and protein) oscillate throughout the cell cycle, presenting a marked increase in G1/S transition, while $CDK9_{42k}$ levels show no changes. Cell cycle dynamics and cell survival evaluation reinforced this correlation once $CDK9_{55k}$ OE induces cell accumulation in G1 phase and confers resistance to chemotherapy treatment compared to control or cells OE $CDK9_{42k}$. Further, we demonstrate that $CDK9_{55k}$ protein levels are increased upon IR treatment and only $CDK9_{42k}$ presents IRIF. Although $CDK9_{55k}$ do not form IRIF, cells OE $CDK9_{55k}$ show an increase in 53BP1 IRIF and NHEJ efficiency, in contrast to cells OE $CDK9_{42k}$, that present no alterations in 53BP1 IRIF and an increase in HR efficiency. Taken together, our data suggest that CDK9 plays a dual role in DDR, possibly coordinating HR and NHEJ pathways through its two isoforms and that $CDK9_{55k}$ levels are cell cycle regulated and response to DNA damage.

Keywords: CDK9, DNA damage, 53BP1, BRCA1, cell cycle.
Supported by: Fundação do Câncer, FAPERJ, CNPq and MS

PESQUISADOR PROFESSOR

DEVELOPMENT OF CELLULAR LINEAGE DERIVED FROM A PATIENT WITH ALL PORTING TRANSLOCATION 8;22 (q24;q11) and DUP (1) (q21;q32).

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INTRODUCTION AND OBJECTIVES: During a prospective study of acute lymphoblastic leukemias in childhood, several bone marrow cell samples from patients were placed in culture. One of these samples was able to create an immortal cell line. This work was carried out through experimental descriptive research type Case Study, where several techniques were used, including cariotypic analyses during and after establishment of the lineage, which could identify chromosomal aberrations and the possible implications in the creation of the lineage. The Lymphoblastid Cell Line B called 199 presented t (8;22)(q32;q11) and dup (1)(q21;q32). Our results showed that the cell line 199 originates from FAB L3 b lymphoblastide cell. Karyotypic studies revealed that the cell line 199 carries the karyotype of 53 XY, +X, dup (1)(q21;q32), -4, +6, +7, t (8;22)(q24;q11), +8, +11, +15, +16, +20 deriving therefore from one of the clones originally present in the patient's bone marrow. **MATERIAL AND METHODS:** During the beginning of the study, several different clones were found. Most of them present ing several chromosomal aberrations: t (1;6)(q21;q23-q26); t (1;11)(q21;p15), t (1;12)(q21;q24); t (1;14)(q21;q32) and t (1;22)(q21;q11). However, these clones did not last long in culture. After the culture was established, cariotypic studies were performed and RNA was extracted from the sample and submitted to RT-PCR reactions to verify the expression of some genes involved with cell immortalization and to identify possible expression alterations. The primers were designed using the sequences already existing in several works already published. **RESULTS AND CONCLUSION:** It is believed that clone t (8;22) and dup (1), because it presents duplication of chromosome 1 in regions q 21 to q 32, where immortalization genes, such as the CLK-2 gene, were of crucial importance for the creation of the lineage. Other possible explanations are the fact that the presence of translocation t (8;22) also allowed a high expression of genes of genetic transcription factors, such as: Ikaros, NF-EM5, NFAT-1, c-myc, GATA-1 and 2. All involved in hematopoietic development. The c-MYC gene acts directly on cell proliferation, however other samples driving translocation with c-MYC have not been able to become immortal. This type of translocation is estimated to occur in 2% of all Leukemias and Type B lymphomas, including Burkitt Lymphomas. Probably the duplication of chromosome 1 in the regions q21 and q32 allowed the creation of the lineage, because in this region it presents genes that control not only cell proliferation, but are also responsible for regulating telomere size, a very important structure in cell aging. In other studies it was also possible to observe that the duplication of chromosome 1 in regions q21 to q32 are extremely important in the formation of strains being this the main event of transformation and generation of the cell lineage 199.

Keywords: Leukemias, Lineages and Translocation.