Buenos Aires Breast Cancer Symposium

BA-BCS2024

September 3 – 6, 2024

IFIBYNE AUDITORIUM

FCEN-UBA

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Federico Waisberg, M. D.
Instituto Fleming, Buenos Aires

Scientific Committee

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Program

SEPTEMBER 3

15:00-17:45 Registration

17:45-18:00 Welcome from organizers

18:00-19:00 **Opening Conference**

Chairs: Claudia Lanari, IBYME-CONICET, Buenos Aires, Argentina and Edith Kordon, IFIBYNE-UBA-CONICET, Buenos Aires, Argentina.

Robert Clarke, University of Manchester, Manchester, UK

The role of cytokines in regulating cancer stem cells in therapy-resistance and metastasis

19:00-20:00 Welcome Reception at IFIBYNE

SEPTEMBER 4

9:30-11:00 **Session 1: Luminal breast cancer**

Chairs: Caroline Lamb, IBYME-CONICET, Buenos Aires, Argentina.
María F. Rubio, IDIM-UBA-CONICET, Buenos Aires, Argentina.
Fernando Petracci, Instituto Alexander Fleming, Buenos Aires, Argentina.

09:30-10:00 **Todd Miller**
Medical College of Wisconsin Cancer Center, Milwaukee, USA.
Leveraging endocrine and metabolic vulnerabilities in ER+ breast cancer.

10:00-10:30 **Joseph Jerry**
University of Massachusetts, Amherst, USA.
Metabolism, Signaling and Breast Cancer Riskcells from donors differing in breast cancer risk: variation in barriers to immortalization and estrogen signaling.

10:30-10:45 **Abstract selected for oral presentation**:
RUNX2 expression correlates with tumor progression in luminal breast cancer patients. **Yamil D Mahmoud**, IBYME-CONICET.

10:45-11:00 Discussion

11:00-11:30 **Coffee Break**

11:30-13:00 **Session 2: New treatments for Luminal and Her2+ breast cancer**

Chairs: Natalia Rubinstein, iB3, FCEyN, UBA, Buenos Aires, Argentina and Vanina Medina, BIOMED-CONICET, UCA, Buenos Aires, Argentina

11:30-12:00 **Jennifer Richer**
University of Colorado, Aurora, USA.
AR action in ER+ breast cancer – preclinical and clinical trial updates

12:00-12:30 **Federico Waisberg**
Instituto Fleming, Buenos Aires
New treatments and biomarkers in hormone resistant (HR+) breast cancer.

12:30-12:45 **Abstract selected for oral presentation**:
Inhibition of Rac1 activity enhances Tastuzumab sensitivity in Her2-positive breast cancer cells through cell cycle deregulation, **Virginia Judith Wolos**, Instituto de Oncología Ángel Roffo.

12.45-13:00 Discussion

13:00-15:00 **Lunch and Poster Session 1**

14:40-15:00 Sponsor presentation: LOBOV Científica

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Chairs: Roxana Schillaci, IBYME-CONICET, Buenos Aires, Argentina and Maria José Rico, Facultad de Ciencias Médicas, UNR, Rosario, Argentina.

15:00-15:30 **Roger Chammas**
Center for Translational Research in Oncology, University of São Paulo, Brazil.
miRNAs as biomarkers for early onset Triple Negative Breast Cancers.

15:30-16:00 **Paolo Ceppi**
University of Southern Denmark, Odense, Denmark.
Propionate suppresses epithelial-to-mesenchymal transition and reduces the aggressiveness of lung carcinoma, can we extrapolate to breast cancer?

16:00-16:30 **Pablo Mandó**
CEMIC, Buenos Aires, Argentina.
New therapeutic options for TNBC.

16:30-16:45 **Abstract selected for oral presentation:**

Enhancing Paclitaxel efficacy in triple negative breast cancer through DKC1 inhibition with R1D2-10, **Roman Nicolas Vilarullo**, Universidad Nacional de Quilmes.

16:45-17:00 Discussion

17:00-17:30 Coffee break

17:30-19.00 **Round Table 1 (Spanish)**

Coordinadoras: Mariana Salatino, IBYME-CONICET, Buenos Aires, Argentina y María Roqué, IHEM-CONICET, Mendoza, Argentina.

Utilidad clínica de la búsqueda de marcadores circulantes en biopsia líquida, para el redireccionamiento y/o seguimiento de la carga tumoral en pacientes con cáncer de mama.

Adriana De Siervi IBYME-CONICET y Oncoliq, Buenos Aires, Argentina.
Javier Stigliano, Hospital Nacional Prof. A Posadas, Haedo, Argentina.
Manglio Rizzo, Departamento Oncología, Hospital Universitario Austral, Pilar, Argentina.
Julieta Pandolfi, Anatomía Patológica. Lab. Secuenciación. Hospital Italiano de Buenos Aires, Argentina.
Diego Marzese (participante virtual) Division of Surgical Oncology, Duke University School of Medicine (DUSM), Durham, USA.

SEPTEMBER 5

09:30-11:15 **Session 4: Cancer Stem Cells and Tumor Initiation**

Chairs: Albana Gattelli, IFIBYNE-UBA-CONICET, Buenos Aires, Argentina and Catalina Lodillinsky, Instituto Ángel Roffo, Buenos Aires, Argentina

09:30-10:00 **Fariba Behbod**
University of Kansas, Kansas City, USA.
Ductal carcinoma in situ: Transcriptional diversity and stemness.

10:00-10:30 **Jochen Maurer**
University Hospital RWTH, Aachen, Germany.
Cancer stem cells as disease models in research – opportunities and challenges.

10:30-11:00 **William Muller**
Rosalind and Morris Goodman Cancer Center, Montreal, Canada.
Oncogene-mediated regulation of tumor immune microenvironment in mouse models of human breast cancer

11:00-11:15 Discussion

11:15-11:45 **Coffee Break**

11:45-13:30 **Session 5: Breast Cancer Genomics and Transcriptomics**
Chairs: Ignacio Schor, IFIBYNE-UBA-CONICET, Buenos Aires, Argentina and Marianela Candolfi, INBIOMED, FMED, UBA, Buenos Aires, Argentina.

11:45-12:15 **Martin Abba**
Universidad Nacional de La Plata, Buenos Aires, Argentina
Old questions, new tools

12:15-12:45 **Pedram Razavi**
Memorial Sloan Kettering Cancer Center, New York City, USA.
Deciphering the genomic mechanisms of resistance to CDK4/6 inhibitors

12:45-13:00 **Gonzalo Gomez Abuin**
Hospital Alemán, Buenos Aires
Utility of next generation sequencing in breast cancer

13:00-13:15 **Abstract selected for oral presentation:**
Exploring the effects of non-coding somatic mutations in BAF complex deregulation and the malignant characteristics of triple-negative breast cancer, **Pedro J. Salaberry**, IFIBYNE-UBA-CONICET.

13:15-13:30 Discussion

13:30-15:00 Lunch and Poster Session 2

15:00-17:00 Session 6: immunotherapy and Fighting treatment resistance
Chairs: Georgina Coló, INIBIBB-UNS, Bahía Blanca, Argentina and Germán A. Gil, CIQUIBIC, UNC-CONICET), Córdoba, Argentina

15:00-15-30 **Adrian Lee**
University of Pittsburgh, Pittsburgh, USA.
Endocrine resistance in breast cancer metastasis.

15:30-16:00 **Dejan Juric**
Massachusetts General Hospital, Boston, USA.
Novel PI3Ki inhibitors including mutant-selective PI3K inhibitors

16:00-16:30 **María Vivanco**
CIC bioGUNE, Basque Research and Technology Alliance, Derio, Spain.
Breast Cancer Heterogeneity and resistance to hormone therapy

16:30-16:45 **Abstract selected for oral presentation**:

Preclinical and clinical role of MUC4/TNF axis in metastatic triple-negative breast cancer, **Florencia Mauro**, IBYME-CONICET.

16:45-17:00 Discussion

17:00-17:30 Coffee Break

17:30-19:00 **Round Table 2 (Spanish)**

Coordinadores: Mario Rossi Universidad Austral, Pilar, Argentina y Pablo Mandó, CEMIC, Buenos Aires, Argentina

Jerarquización en investigación dentro de la carrera de formación de los médicos, experiencias en CEMIC, Fleming y Austral.

Manglio Rizzo, Servicio de Oncología Hospital Austral, Pilar, Argentina.
Fernando Petracci, Instituto Fleming, Buenos Aires, Argentina.
Estrella Levy, Instituto Fleming, Buenos Aires, Argentina.

SEPTEMBER 6

09:30-11:15 **Session 7: Early detection and treatment**

Chairs: Giselle Peters, Instituto Ángel Roffo, Buenos Aires, Argentina and Leandro E. Mainetti, Facultad de Ciencias Médicas, UNR, Rosario, Argentina.

09:30-10:00 **Vanesa Gottifredi**
Instituto Leloir, Buenos Aires, Argentina.
Targeting the chromosomic instability induced by cancer therapy.

10.00-10:30 **Catherine Park**
University of California, San Francisco, USA.
Evolution of breast radiotherapy options for today's early stage patients.

10:30-10:45 **Adriana de Siervi**
Oncoliq, CABA-Argentina.
miRNAs for early cancer detection in liquid biopsy.

10:45-11:00 **Abstract selected for oral presentation**
SPARC: a potential biomarker of the transition from in situ to Invasive breast cancer, **Marianela Sciacca**, Instituto de Oncología Ángel Roffo, Buenos Aires, Argentina.

11:00-11:15 Discussion

11:15-11:45 Coffee Break

11:45-13:30 **Session 8: Understanding and modeling breast cancer subtypes**

Chairs: Paola Rojas, IBYME-CONICET, Buenos Aires, Argentina and María T. Branham, IHEM-CONICET, Mendoza, Argentina

11:45-12:15 **Steffi Oesterreich**
University of Pittsburgh, Pittsburgh, USA.
Invasive lobular breast cancer – what's different and why is this important?

12:15-12:45 **Alana Welm**
University of Utah, Salt Lake City, Utah, USA.
Using patient-derived models for drug discovery and functional precision oncology in advanced breast cancer

12:45-13:00 **Abstract selected for oral presentation:**
RET receptor integrates tumor cell-adipocyte communication to promote breast cancer. **Sabrina A. Vallone**, IFIBYNE-UBA-CONICET.

13:00-13:15 **Abstract selected for oral presentation**:
Progesterone promotes triple-negative breast cancer metastasis through RANKL-expressing Treg cells. **Tomas Dalotto**, IBYME-CONICET.

13:15-13:30 Discussion

13:30-15:00 Lunch and Poster Session 3

15:00-16:00 **Session 9: Improving life quality**

Chair: Mario Rossi, Universidad Austral, Pilar, Argentina and Virginia Novaro, IBYME-CONICET, Buenos Aires

15:15-15:45 **Syril Pettit**,
Health and Environmental Sciences Institute (HESI), Washington DC, USA.
Addressing Cancer Therapy Related Adverse Effects – An Opportunity to Improve Quality of Life and Outcome for Patients.

15:45-16:00 Discussion

16:00-17:00 **Closing Conference**

Chairs: Martin Abba, Universidad Nacional de La Plata, Buenos Aires, Argentina and Mariana Salatino, IBYME-CONICET, Buenos Aires, Argentina.

Katherine Hoadley
UNC Lineberger Comprehensive Cancer Center, Chapel Hill, USA.

Quantitative Medicine for Breast Cancer Patients.

17:00-17:15 Discussion and closing remarks by the organizers

17:15-17:30 Coffee break

17:30-19:00 **Round Table 3 (Spanish)**

Coordinadores: Virginia Novaro, IBYME-CONICET, Buenos Aires, Argentina y Federico Waisberg, Instituto A. Fleming, Buenos Aires, Argentina.

Navegación en el sistema de salud para pacientes con bajo y alto riesgo de cáncer de mama. Situación actual en la región Norte, Centro y Sur del país.

Alejandro Di Sibio coordinador del Programa Nacional de Control de Cáncer de Mama del Instituto Nacional del Cáncer, Buenos Aires, Argentina
Marcela Kober del Instituto Misionero del Cáncer, Posadas, Argentina.
Romina Yapur, Hospital Villa Regina de Río Negro y Centro Oncológico Integral, General Roca, Argentina.

Conferences

Opening

Conference

Robert Clarke
University of Manchester, Manchester, UK
The role of cytokines in regulating cancer stem cells in therapy-resistance and metastasis.

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UNLP, La Plata, Argentina.
Old questions, new omics tools.

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Propionate suppresses epithelial-to-mesenchymal transition and reduces the aggressiveness of lung carcinoma, can we extrapolate to breast cancer?

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Center for Translational Research in Oncology, University of São Paulo, Brazil.
Circulating miRNAs as biomarkers for early onset triple negative breast cancer patients.

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Targeting the chromosomic instability induced by cancer therapy.

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A panel of normal breast epithelial cells from donors differing in breast cancer risk: variation in barriers to immortalization and estrogen signaling.

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Massachusetts General Hospital, Boston, USA.
Novel PI3Ki inhibitors including mutant-selective PI3K inhibitors.

Adrian Lee
University of Pittsburgh, Pittsburgh, USA.
Endocrine resistance in breast cancer metastasis.

Paolo Mandó
CEMIC, Buenos Aires, Argentina.
Innovative Strategies in the Management of Triple-Negative Breast Cancer.

Jochen Maurer
University Hospital RWTH, Aachen, Germany.
Cancer stem cells as disease models in research – opportunities and challenges.

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Medical College of Wisconsin Cancer Center, Milwaukee, USA.
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University of California, San Francisco, USA.
The evolution of breast radiotherapy options for today's early stage patients.

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CIC bioGUNE, Basque Research and Technology Alliance, Derio, Spain.
Breast Cancer heterogeneity and resistance to hormone therapy.

Federico Waisberg
Instituto Fleming, Buenos Aires, Argentina.
New treatments and biomarkers in hormone resistant (HR+) breast cancer.

Alana Welm
University of Utah, Salt Lake City, USA.
Using patient-derived models for drug discovery and functional precision oncology in advanced breast cancer.

Closing

Conference

Katherine Hoadley
UNC Lineberger Comprehensive Cancer Center, Chapel Hill, USA.
Molecular Characteristics of Metastatic Breast Cancer.

Abstracts Selected for Oral Presentation

SESSION 1

RUNX2 expression correlates with tumor progression in luminal breast cancer patients

Yamil D Mahmoud[1], Silvia Vanzulli[1], María Sol Rodriguez[1], Eunice Spengler[2], Paula Martínez Vazquez[2], Javier Burruchaga[2], Caroline A Lamb[1], Isabel A Lüthy[1], Claudia Lanari[1], Cecilia Pérez Piñero[1]

1- Instituto de Biología y Medicina Experimental (IBYME), CONICET, Buenos Aires, Argentina, 2- Hospital Zonal de Agudos “Magdalena V de Martínez”, General Pacheco, Argentina.

Presenting Author:
Yamil Damian Mahmoud
PhD Fellow - IBYME-CONICET
Buenos Aires, Argentina
Email: *yamil.dmah@gmail.com*

Luminal breast cancer (BrCa) is the most common subtype diagnosed, with major challenges in understanding the development of endocrine resistance and subsequent disease progression. RUNX2, a transcription factor (TF) linked to aggressiveness in triple-negative BrCa, has an unclear role in luminal BrCa. Our previous studies showed a correlation between FGFR2 and RUNX2 in ER+ breast cancer patients. Our aim was to determine if RUNX2 expression is associated with BrCa progression and its potential use as a prognostic marker in luminal BrCa. An exploratory study was done using BrCa samples from the Hospital Zonal de Agudos ‘Magdalena V. de Martínez.’ RUNX2 expression was assessed by immunohistochemistry (IHC) in primary tumor samples from non-progressors (n=26) and progressors (n=18). Progressors had higher RUNX2+ tumor cells and intensity scores in nuclei and cytoplasm. RUNX2 was also observed in cancer-associated fibroblasts, with higher staining scores in progressors, though not statistically significant. Then, we analyzed RUNX2 mRNA expression and its inferred activity in TCGA-BrCa patients categorized by PAM50. RUNX2 activity was among the 25 most variable TF, differing from gene expression, and was higher in Luminal A and Normal-like subtypes. ESR1 activity positively correlated with RUNX2 gene expression, while the gene expression correlated negatively. High RUNX2 activity was associated with worse progression-free interval (PFI), while high mRNA levels were linked to worse overall survival. High RUNX2 activity scores correlated with lower PFI in Luminal B patients. Notably, 89% of Luminal A patients had high RUNX2 activity scores, compared to only 11% of Luminal B patients. Our findings align with IHC studies, highlighting RUNX2's significant role in BrCa progression and suggesting that RUNX2 expression could serve as a prognostic and a predictive marker of therapy outcomes.

SESSION 2

INHIBITION OF RAC1 ACTIVITY ENHANCES TRASTUZUMAB SENSITIVITY IN HER2-POSITIVE BREAST CANCER CELLS THROUGH CELL CYCLE DEREGULATION

Virginia Judith Wolos[1], Marianela Abrigo[1], Ezequiel Lacunza[2,3], Daniel Fernando Alonso[3,4], Georgina Alexandra Cardama[3,4], Gabriel León Fiszman[1,3]

1- Universidad de Buenos Aires, Instituto de Oncología "Ángel H. Roffo", Área Investigación, Departamento de Inmunobiología, Buenos Aires, Argentina, 2- Centro de Investigaciones Inmunológicas Básicas y Aplicadas (CINIBA), Facultad de Ciencias Médicas, Universidad Nacional de La Plata, La Plata, Argentina, 3- CONICET, Argentina, 4- Centro de Oncología Molecular y Traslacional (COMTra), Universidad Nacional de Quilmes, Quilmes, Argentina

Presenting Author:

Virginia Judith Wolos

PhD Fellow - Universidad de Buenos Aires, Instituto de Oncología "Ángel H. Roffo", Área Investigación, Departamento de Inmunobiología

Buenos Aires, Argentina

Email: *vjwolos@gmail.com*

HER2 signaling to Rac1 GTPase and Rac1 pathway deregulation have been associated to increased survival of HER2-overexpressing breast cancer cells and resistance to targeted therapy. In this scenario, we evaluated the effects of Rac1 inhibition on breast cancer cells treated with trastuzumab, a HER2-directed antibody widely used for patient treatment. First, we assessed the effect of trastuzumab and the novel Rac1 inhibitor 1A-116 on the viability of BT-474 and SK-BR-3 cell monolayers. Not only single drug treatment significantly decrease the viability of both cell lines ($p<0.05$), but also a synergistic interaction was found using Combenefit software ($p<0.05$). To further study this effect, we evaluated drug combinations in 3D cultures. Whereas the growth of BT-474 cell spheroids was inhibited by both trastuzumab and 1A-116 ($p<0.05$), drug combination induced a significant reduction in spheroid volume ($p<0.05$). Moreover, interesting results were seen using trastuzumab-resistant BT-474-R cells. Spheroid growth was diminished by trastuzumab and 1A-116 separately compared to control spheroids ($p<0.05$). However, a significant reduction in spheroid volume was found after treatment with trastuzumab in combination with 1A-116 ($p<0.05$). To address the cellular processes behind Rac1 inhibition, we analyzed proteomic profiling data. Through a mass spectrometry-based label-free quantification approach, BT-474 cells treated with trastuzumab and 1A-116 were compared with cells treated with trastuzumab alone. The deregulation of proteins governing G1/S transition appeared to be particularly relevant ($p<0.05$). Thus, we next studied cyclin D1 expression and the cell cycle. We found significantly lower levels of cyclin D1 in BT-474 cells treated with both drugs together ($p<0.05$), along with an upward trend in G0/G1 cell cycle arrest. These results push us forward to further investigate the potential benefits of targeting Rac1 pathway in breast cancer patients with HER2-overexpression.

SESSION 3

Enhancing Paclitaxel efficacy in triple negative breast cancer through DKC1 inhibition with R1D2-10

Roman Nicolas Vilarullo[1], María del Pilar Casco[1], Lara Balcone[1], Julian Maggio[1], Diego Mengual Gómez[1], Daniel Eduardo Gomez[1], Romina Gabriela Armando[1]
1- Centro de Oncología Molecular y Traslacional (COMTra) - Universidad Nacional de Quilmes.

Presenting Author:
Roman Nicolas Vilarullo
PhD Fellow - Centro de Oncología Molecular y Traslacional (COMTra) - Universidad Nacional de Quilmes
Buenos Aires, Argentina
Email: *vilarulloroman@gmail.com*

One of the most commonly drugs used for triple-negative breast cancer (TNBC) treatment is Paclitaxel (PTX), a chemotherapeutic agent. However, due to its non-selective mechanism, PTX causes numerous side effects, which limit its use and dosage. Dyskerin Pseudouridine Synthase 1 (DKC1) is involved in several cellular functions, including the proper assembly of the telomerase complex, RNP biosynthesis, and the regulation of specific RNAs and microRNAs. To develop new anti-cancer treatments, we previously used docking-based virtual screening to create R1D2-10, a novel DKC1 inhibitor. R1D2-10 inhibited cell proliferation and telomerase activity in breast cancer (BC) cell lines, resulting in telomere shortening, senescence, and apoptosis.

In this study, database analysis revealed that DKC1 is overexpressed in BC tissue compared to healthy tissue, correlating with lower survival rates. TNBC showed the highest DKC1 expression among BC subtypes, with even higher levels in patients treated with PTX, indicating DKC1 as a potential target for TNBC treatment. We investigated the combined effect of R1D2-10 and PTX in MDA MB 231 and MDA MB 468 TNBC cell lines. Our findings demonstrated a synergistic effect on cell proliferation inhibition at various concentrations (1-20 μM/nM for R1D2-10 and PTX, respectively). Flow cytometry analysis showed a significant increase in Sub-G1 and G2/M populations, indicating cell cycle arrest. Additionally, the combined treatment elevated apoptosis levels, evidenced by increased Caspase 3/7 activity. RT-qPCR analysis revealed higher expression of pro-apoptotic genes bax/bcl2 and the cell cycle inhibitor p21 compared to monotherapy.

These promising results support the development of a combined therapy with R1D2-10, aiming to reduce PTX doses and associated side effects. We are conducting further studies to confirm the clinical potential of R1D2-10 for TNBC treatment.

SESSION 5

EXPLORING THE EFFECTS OF NON-CODING SOMATIC MUTATIONS IN BAF COMPLEX DEREGULATION AND THE MALIGNANT CHARACTERISTICS OF TRIPLE-NEGATIVE BREAST CANCER

Pedro J. Salaberry[1,2], Marina Pinkasz[1], Camila D. Arcuschin[1], Martín Iungman[1], Ignacio E. Schor[1,2]
1- Instituto de Fisiología, Biología Molecular y Neurociencias (UBA-CONICET), Buenos Aires, Argentina, 2- Departamento de Fisiología, Biología Molecular y Celular, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina.

Presenting Author:
Pedro Javier Salaberry
PhD Fellow - IFIBYNE (UBA-CONICET)
Buenos Aires, Argentina
Email: *psalaberry@fbmc.fcen.uba.ar*

While whole-genome sequencing of tumor samples has revealed the high frequency of somatic mutations in non-coding regions, limited effort has been made to understand their oncogenic potential. To understand the impact of non-coding somatic mutations and their contribution to disease characterization, we looked for mutations affecting the promoters of genes related to the malignancy of triple-negative breast cancer (TNBC) cells.
We used gene expression data from isogenic TNBC cell lines with different metastatic abilities to identify associated changes in the activity of key regulators, resulting in 354 differentially active regulators (FDR < 0.001). With this set as a seed, we obtained a network of potential regulators of metastasis in TNBC and leveraged the presence of highly functional promoter mutations reported in TNBC patients to look for recurrently mutated subnetworks. Using a network propagation analysis, we identified the chromatin remodeler BAF (mSWI/SNF) as a protein complex overrepresented in regulatory mutations in TNBC.
We tested the transcriptional impact of regulatory mutations affecting BAF genes using single-cell reporter assays and confirmed significant effects for mutations in the promoters of SMARCA4, SMARCC2, SMARCD2 and ARID1A (p-value < 0.001, Wilcoxon test). Finally, we are assessing the influence of the transcriptional modulation of these candidates in the malignant characteristics of TNBC cells. As a first attempt, we have diminished SMARCA4 and ARID1A levels with shRNA-mediated knock-down, and observed a decrease in stemness features in MDA-MB-231 cells. We believe this work presents an effort to explore the potential use of regulatory mutations to understand cancer progression and identify key pathways driving malignant phenotypes.

SESSION 6

Preclinical and clinical role of MUC4/TNF axis in metastatic triple-negative breast cancer

Mauro, Florencia[1] ; Bruni, Sofia[1] ; Schey, Aldana[2] ; Badalini, Agustina[3] ; Dupont, Agustina[3,4] ; Inurrigarro, Gloria[3] ; Figurelli, Silvina[4] ; Barchuk, Sabrina[5] , Lopez Della Vecchia, Daniel[5] ; Cordo Russo, Rosalia[1] ; Gil Deza, Ernesto[6] ; Urtreger, Alejandro[2] ; Mercogliano, María Florencia[1] ; Schillaci, Roxana[1]

1 Instituto de Biología y Medicina Experimental (IBYME-CONICET), Buenos Aires, Argentina. 2 Instituto de Oncologia Angel H. Roffo, Buenos Aires, Argentina. 3 Servicio de Patología Sanatorio Mater Dei, Buenos Aires, Argentina. 4 Servicio de Patología, Hospital Juan A. Fernández, Buenos Aires, Argentina. 5 Servicio de Ginecología, Hospital Juan A. Fernández, Buenos Aires, Argentina. 6 Instituto Oncológico Henry Moore, Buenos Aires, Argentina.

Presenting Author:
Florencia Mauro
PhD Fellow - IBYME-CONICET
Buenos Aires, Argentina
Email: *flor.luciana@hotmail.com*

We have demonstrated that TNF induces trastuzumab resistance through mucin 4 (MUC4) upregulation and it is an independent biomarker of poor response in HER2+ breast cancer. Here, we evaluated the role of the TNF/MUC4 axis in preclinical models of TNBC and the clinical impact of MUC4 in TNBC patients.
Soluble and transmembrane TNF were blocked with etanercept (E), and the dominant negative protein INB03 (DN) was used to block soluble TNF. BT-549 and MDA-MB-231 TNBC cell lines treated with E or DN exhibited a decrease in MUC4 expression. Conditioned media of MDA-MB-231 and BT-549 cells treated with E or DN impaired the invasion of both cell lines ($p<0.01$). We performed an in vivo assay on female BALB/c mice using s.c. LMM3 tumor, and we demonstrated that DN in combination with PD-1 blocking antibody prevented the appearance of lung metastasis ($p<0.05$ vs. IgG, DN, and anti-PD-1 groups).
To explore how DN influences cell seeding, LMM3 cells pre-treated with DN or IgG were injected into the tail vein of mice. Animals injected with IgG-treated cells were treated with IgG, and the ones injected with DN-treated cells were treated with DN or DN+anti-PD-1. Mice treated with DN+anti-PD-1 showed less lung metastasis vs. IgG and DN-treated groups ($p<0.05$). In a survival assay using female BALB/c bearing 4T1 tumors, we treated animals with the standard-of-care for TNBC patients, nab-paclitaxel+anti-PD-1 antibody, alone or with DN. We observed that the combined treatment significantly increased survival ($p<0.05$).
In a cohort of 47 TNBC patients (stage I-III), we proved that MUC4 expression was inversely correlated with TILs ($p=0.0003$) and tumor PD-L1 expression ($p=0.0003$). MUC4 proved to be an independent predictor of poor overall survival ($p=0.003$) and was associated with a higher metastasis risk ($p=0.04$). We propose TNF as a novel target for the treatment of TNBC patients and MUC4 as a predictive biomarker to guide a combined treatment of TNF-blocking agents with immunotherapy.

SESSION 7

SPARC: a potential biomarker of the transition from in situ to Invasive breast cancer

Marianela Sciacca[1,2], María del Pilar Carballo[3], Ezequiel Lacunza[4], Lina Marino[3], Noelia Paola Cardozo[5], Naira Rodríguez Padilla[1], Martin Abba[4], Érica Rojas Bilbao[3], Pablo J Sáez[6], Ana María Eiján[1,2], Catalina Lodillinsky[1,2]

1- Departamento de Inmunología, Área de Investigación, Instituto de Oncología Ángel H. Roffo, Universidad de Buenos Aires, Buenos Aires, Argentina. 2- Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). 3- Departamento de Patología, Área Diagnóstica, Instituto de Oncología Ángel H. Roffo, Universidad de Buenos Aires, Buenos Aires, Argentina. 4- CINIBA, Facultad de Ciencias Médicas, Universidad Nacional de La Plata, La Plata, Argentina. 5- Departamento de Bioterio y Cáncer Experimental, Área de Investigación, Instituto de Oncología Ángel H. Roffo, Universidad de Buenos Aires, Buenos Aires, Argentina. 6- Cell Communication and Migration Laboratory, Institute of Biochemistry and Molecular Cell Biology, Center for Experimental Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany.

Presenting Author:
Marianela Sciacca
PhD Fellow - Angel H. Roffo Institute of Oncology
CABA, Argentina
Email: *marianelasciacca@gmail.com*

Ductal carcinoma in situ (DCIS) is a proliferation of cells confined in the breast ductal-lobular unit. Currently, DCIS could be a precursor of invasive ductal carcinoma (IDC), but predicting this transition remains challenging. Previously, we have shown that MT1-MMP was essential for early breast progression using an intraductal xenograft model. In this work, RNAseq analysis from MT1-MMPhigh cells from invasive tumors post-intraductal inoculation was compared against a set of human high-grade DCIS previously described. SPARC emerges as one of the candidate genes involved in early breast cancer progression.
By IHC, we observed an over-expression of SPARC in neoplastic cells compared to normal tissue from 2 different cohorts of patients with IDC and DCIS foci (PICBIM n=116 and Roffo n=58). SPARC expression was higher in IDC than DCIS foci ($p<0,0001$) and correlated with MT1-MMP levels. SPARC-positive tumors were higher in high-grade and TNBC IDC tumors ($p=0,02$). In a metastatic cohort of patients (n=57), lymph node metastases did not express SPARC.
In the murine cell LM38-LP, MT1-MMP mRNA level and degradative capacity were reduced in KO SPARC cells ($p<0,05$). STRING analysis showed a medium interaction score between SPARC and TGF-B1. LM38-LP cells were sensitive to TGF-B1 pathway regulation ($p<0,05$; $p<0,001$) and KO SPARC LM38-LP cells reduced pSMAD 2/3 ($p=0,003$). SB431542 (20ng/ml) decreased SPARC and MT1-MMP expression in LM38-LP and human MCF10DCIS.com cell lines ($p<0,001$). TGF-B1 (2ng/ml) increased the degradative capacity of LM38-LP whereas, SB431542 and Galunisertib (1µg/ml), reduced it ($p<0,001$; $p<0,05$). On migration assays, we observed that the inhibitory effect of SB431542 was lost in KO SPARC cells ($p<0,0001$). In vivo, Galunisertib (50mg/kg) reduced the proportion and tumor area of IDC ($p= 0,04$).
These results reveal that SPARC is involved in early tumor progression via the TGF-β1-dependent mechanism, suggesting TGFRI as a target for SPARC-positive patients.

SESSION 8

RET receptor integrates tumor cell-adipocyte communication to promote breast cancer.

Sabrina A. Vallone[1], Irene Ruiz-Garrido[2], Angela Lara[1], Ivana Nikolic[2], Alba C. Arcones[2], Marcos D. Palavecino[1], Martín García Solá[1], Gladys N. Hermida[3], Julian Naipauer[1], Guadalupe Sabio[2], Albana Gattelli[1]

1- Instituto de Fisiología, Biología Molecular y Neurociencias (IFIBYNE) (CONICET-UBA), Ciudad Universitaria C1428EGA CABA, Buenos Aires, Argentina., 2- Centro Nacional de Investigaciones Oncológicas (CNIO), Madrid, Spain., 3- Departamento de Biodiversidad y Biología Experimental (DBBE), Biología de Anfibios-Histología Animal, Facultad de Ciencias Exactas y Naturales (FCEN), Ciudad Universitaria C1428EGA CABA, Buenos Aires, Argentina..

Presenting Author:
Sabrina Vallone
PhD Fellow - IFIBYNE
Buenos Aires, Argentina
Email: *sabrivallone@gmail.com*

Adipocytes are the specialized cells for lipids storage and form the major cellular component of the breast organ microenvironment. The adipose tissue next to the breast tumor consists of modified adipocytes that share similarities with their precursors, known as pre-adipocytes. Understanding the interaction between both cell types will guide novel therapeutic strategies to treat breast tumors. Here, we identify RET receptor tyrosine kinase as a breast tumor-adipose tissue interplay regulator.

RET is overexpressed in 40% of breast tumors and high RET correlates with decreased survival in patients. RET-expressing tumors in our in vivo mouse models display abnormal adjacent adipose tissue. RNA-sequencing analysis revealed significant upregulation of genes involved in maintaining a pre-adipocyte phenotype, such as PDGF ligands, in RET-expressing glands compared to controls. Accordingly, in human breast tumor biopsies RET expression positively correlates with PDGFB. Mechanistically, we first have confirmed in vitro that PDGFB acts as a tumoral RET signaling downstream factor. Then, using co-culture systems of adipocyte cultures with RET-WT or CRISPR/Cas9-edited RET-KO tumor cells we pinpoint the effects of RET to keep PDGFR-positive pre-adipocytes phenotype. We show that the loss of RET on tumor cells reestablishes the adipocyte differentiation process. For that, we measured lipid incorporation and specific markers of adipocyte maturity. Importantly, we demonstrate that pre-adipocyte cells act as pro-tumoral in a RET-dependent manner. We found that co-cultures with pre-adipocytes enhance the proliferation of RET-WT cells. Furthermore, in vivo co-injection of pre-adipocytes and breast cancer cells promotes tumor growth in RET-WT tumors. Thus, the tumoral RET/PDGFB axis drives the harmful contribution of adipose tissue to breast cancer progression.

SESSION 8

Progesterone promotes triple-negative breast cancer metastasis through RANKL-expressing Treg cells

Tomas Dalotto[1], Perrotta Ramiro[1], Rosa Morales[1], Sabrina Gatto[1], Gabriel Rabinovich[1], Mariana Salatino[1]
1- IBYME.

Presenting Author:
Tomas DAlotto
Researcher - Glicomedicina-IBYME
Buenos Aires, Argentina
Email: *tomasdalotto@gmail.com*

Unresectable metastatic triple negative (TN) breast cancer is an aggressive disease with poor outcome and a short overall survival affecting about 15-20% of women diagnosed with breast cancer. Progestins can shape the immune response favoring a tumor-supportive rather than an anti-tumor immune response. In this sense, hormone replacement regimens and certain hormone-based contraceptives have been associated with an increased risk of aggressive breast cancer and recurrence. Here we studied in a mouse tumor model of TNBC (4T1) whether progestins can influence tumor progression by enhancing the suppressive activity of protumoral immune cells within the tumor microenvironment in a tumor cell-independent fashion. Progestin treatment using either MPA, Progesterone or levonorgestrel promoted an increase in the frequency of tumor-infiltrating effector Foxp3+ Tregs. Interestingly, lung metastatic burden was higher in progestin-treated mice, which was drastically reverted upon Treg cell depletion. Adoptive transfer of progestin-educated Tregs elicited an increase in the number of lung metastasis in Treg-depleted mice. Mechanistically, progestins activated progesterone membrane receptors on Tregs, enhancing their immunosuppressive activity and production of RANKL. We show that Tregs-derived RANKL acting directly on TN tumor cells stimulated epithelial to mesenchymal transition and a stem-like phenotype ultimately boosting their invasive/metastatic capacity. Finally, in vivo, blockade of RANKL with a monoclonal antibody impaired the metastatic potential of 4T1 tumors induced by progestins and progestin-educated Tregs. Our findings highlight the relevance of progestins in modulating antitumor immune response and harnessing immunosurveillance in the tumor microenvironment and describe a mechanism through which Tregs could directly promote a metastatic and aggressive phenotype on TNBC cells.

Poster Session 1

Biology of Her2/Neu and TNB cancers

Poster No. 01

Targeting RAC1: A Promising therapeutic approach for Triple-Negative Breast Cancer

Melisa B. Andersen[1], Juan Garona[2], Jesús S. Lemos[1], Paula L. Bucci[1], Daniel E. Gomez[1], Daniel F. Alonso[1], Georgina A. Cardama[1]

1- Centro de Oncología Molecular y Traslacional (COMTra), DCyT, Universidad Nacional de Quilmes, Bernal, Argentina, 2- Unidad de Investigaciones Biomédicas en Cáncer (IBioCAN), Centro de Medicina Traslacional (CEMET), Hospital de Alta Complejidad en Red S.A.M.I.C. El Cruce "Nestor Kirchner", Florencio Varela, Argentina..

Presenting Author:

Melisa B. Andersen

Undergraduate Student - Centro de Oncología Molecular y Traslacional (COMTra), DCyT, Universidad Nacional de Quilmes

Bernal, Argentina

Email: *melisabandersen@gmail.com*

RAC1 GTPase plays a key role in the regulation of multiple essential cellular processes. Aberrant activation of RAC1 is associated with tumorigenesis, invasion and metastasis in several tumor types, including breast cancer (BC). In triple-negative breast cancer (TNBC), RAC1 overexpression has been shown to lead to cancer metastasis and recurrence as well as to regulate chemosensitivity to cytotoxic agents by influencing DNA damage repair. 1-A116 is a small molecule previously developed by our team that inhibits RAC1 interaction with various GEFs, preventing its activation and showing interesting antitumor effects in several preclinical settings.

Since there is a pressing need to identify novel, efficacious therapies for TNBC due to its aggressive nature, high recurrence rates, and limited treatment options, we sought to evaluate 1A-116 RAC1 inhibitor as a potential novel treatment for TNBC. First, we performed a comprehensive analysis of RAC1 expression in BC using several bioinformatic platforms. RAC1 was upregulated in primary BC tissue in all stages compared with normal tissue and was also associated to shorter overall survival. Furthermore, strong correlations between RAC1 expression and signature gene subsets associated with migration, metastasis and immune evasion were found. These results highlight that RAC1 may have a relevant impact on BC clinical outcomes. We further evaluated the RAC1 inhibitor 1A-116 in a syngeneic murine TNBC model to assess its efficacy. Daily i.p treatment with 10 mg/kg 1A-116 significantly reduced tumor volume and similar results were observed when mice were treated once a week with 1 mg/kg i.v. Notably, 1A-116 treatment was well-tolerated in mice at the tested doses, with no observed toxicity, suggesting a favorable safety profile. These findings underscore the therapeutic potential of 1A-116 in targeting RAC1, a key target in TNBC, and support its further development in combination with chemoimmunotherapy to improve patient outcomes

Biology of Her2/Neu and TNB cancers

Poster No. 04

Norcantharidin: a promising natural compound for the treatment triple negative breast cancer.

Lizeth Aixa Ariza Bareño[1], Aldana M Schey[1], Andrés Bechis[1], Diego Javier Britez Neira[1], Luciana Cañonero[1], Laura B Todaro[1], Alejandro J Urtreger[1]
1- Instituto de Oncología "Ángel H. Roffo".

Presenting Author:
Lizeth Aixa Ariza Bareño
PhD Fellow - Instituto de Oncología "Ángel H. Roffo"
Ciudad Autónoma de Buenos Aires, Argentina
Email: *lisa19111@hotmail.com*

Triple negative breast cancer (TNBC) is an aggressive subtype characterized by the absence of estrogen and progesterone receptors, as well as HER2 overexpression. Due to the lack of specific targeted therapies, there is a clear imperative to explore new therapeutic strategies.
Norcantharidin (NCTD), a promising natural compound, has previously shown antitumor effects against lung and liver malignancies. However, its impact on TNBC remains unknown. Therefore, our work aimed to investigate the potential therapeutic implications of NCTD in TNBC.
Using human (HS578T, MDA-MB231) and murine (4T1) TNBC cell lines, we observed a significant antiproliferative effect of NCTD with IC50 values of 56 µM, 15 µM and 35 µM respectively, as determined by the MTS assay. Moreover, fluorescence microscopy (acridine orange/BrEt staining), flow cytometry (Annexin V/IP staining) and Western blot analysis (modulation of cleaved caspase 3, Parp and LC3-I/LC3II levels) revealed apoptosis and autophagy induction.
In vivo assays using BALB/c mice further supported our findings. Systemic administration of NCTD (2.5 and 3.75 mg/kg) significantly reduced both tumor size and local recurrence ($p< 0.001$ and $p<0.01$ respectively). This result is probably due not only to an effect on tumor mass, but also to an impact on cancer stem cells (CSC), since in vitro studies demonstrated that NCTD affects oncospheres formation ability.
In conclusion, our study highlights the significant antitumor activity of NCTD in TNBC, offering promising possibilities for its application as a therapeutic option. However, further research is necessary to optimize NCTD's efficacy, explore combination therapies, and fully elucidate the molecular mechanisms involved in its action.

Biology of Her2/Neu and TNB cancers

Poster No. 07

The Molecular Mechanism of Protein Phosphatase PP2A as a Potential Therapeutic Target to Counteract Metastasis in Breast Cancer

Gustavo Adolfo Barraza de la Torre[1], Joselina Magali Mondaca[1], Marina Ines Flamini[2], Angel Matias Sánchez[1]

1- Laboratorio de Transducción de Señales y Movimiento Celular, Instituto de Medicina y Biología Experimental de Cuyo (IMBECU), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Universidad Nacional de Cuyo, Mendoza, Argentina., 2- Laboratorio de Biología Tumoral, Instituto de Medicina y Biología Experimental de Cuyo (IMBECU), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Universidad Nacional de Cuyo, Mendoza, Argentina.

Presenting Author:

Gustavo Adolfo Barraza de laTorre

PhD Fellow - Laboraorio de transduccion de señales y movimiento celular, Instituo de Medicina y Biologia Experimental de Cuyo (IMBECU) - CCT-CONICET-Mendoza

Mendoza, Argentina

Email: *gbarraza@mendoza-conicet.gob.ar*

Breast cancer presents significant challenges due to its aggressive nature and the limited efficacy of current treatments. Our study focuses on PP2A, a key regulator in tumorigenesis. Phosphorylation at Tyr307 by CIP2A and SET critically inhibits the tumor suppressor function of PP2A and promotes metastasis. Therefore, our research aims to restore PP2A activity by targeting CIP2A and SET. First, we investigated the expression of PP2A, including its phosphorylated form (p-PP2A), in various breast cancer cell subtypes. Through extensive computational and experimental analysis, we identified high levels of p-PP2A in a triple-negative breast cancer cell line. In molecular docking and dynamics studies, Erlotinib interacts primarily hydrophobically with CIP2A, while FTY-720 binds to SET via hydrogen bonding and hydrophobic forces, stabilizing protein-drug complexes. Protein-protein docking simulations indicated that these drugs reduce the specific phosphorylation of PP2ATyr307 by CIP2A and SET, suggesting an indirect regulatory mechanism. In vitro experiments demonstrated the potent inhibitory effects of erlotinib and FTY-720 on CIP2A and SET, preventing PP2A/Tyr307 phosphorylation and restoring tumor suppressive function. In addition, these drugs disrupted key processes of cell migration and invasion, thereby counteracting metastasis. Our hypothesis proposes that targeting CIP2A and SET with Erlotinib and FTY-720 could effectively attenuate breast cancer metastasis by restoring PP2A activity. In conclusion, our research highlights the potential of Erlotinib and FTY-720 as promising therapeutic agents for breast cancer, particularly TNBC, by modulating PP2A regulatory pathways to suppress metastasis. Further clinical validation is critical to substantiate their therapeutic efficacy

Biology of Her2/Neu and TNB cancers

Poster No. 10

Metronomic chemotherapy in TNBC MDA-MB231 cells exposed to nicotine. Nitric Oxide Synthase participation.

Nicolás Britos[1], Abigail Vasquez[1], Yamila Sanchez[1], Mariano Barros[2], Noam Tanel[2], Alejandro Español[1]

1- Laboratory of Tumoral Immunopharmacology, Center of Pharmacological and Botanical Studies (CEFYBO) - CONICET - UBA. CABA. Buenos Aires. Argentina, 2- Chemistry and Biotechnology Area, ORT High School. CABA . Buenos Aires. Argentina.

Presenting Author:

Nicolás Britos

Undergraduate Student - Laboratory of Tumoral Immunopharmacology, Center of Pharmacological and Botanical Studies (CEFYBO) - CONICET - UBA,
Ciudad Autonoma de Buenos Aires, Argentina
Email: *bnicolasm@hotmail.com*

Triple negative breast cancer (TNBC) is the subtype with the worst prognosis. In conventional therapy, high doses of paclitaxel (PX) are used, generating several adverse effects. To avoid them, metronomic therapy (MT) arises based on the administration of lower doses with short drug-free intervals. Previously we demonstrated that a metronomic combination of the muscarinic agonist carbachol (Carb) with low doses of PX exerts an antitumor effect. Breast tumor tissue also expresses nicotinic receptors (NR), and nicotine (NIC) has been associated with resistance to conventional oncological treatment. This work aims to evaluate the signaling pathways involved in the effect of MT in the absence or presence of NIC in TNBC MDA-MB231 cells. By MTT assays we determined that NIC at a concentration like that of the smoking patients´ plasma (10-7M) increased cell viability (basal:100+/-12.7%; NIC:147.8+/-13.1%). Treatment with MT (PX10-8M+Carb10-11M) decreased cell viability (MT:67.6+/-1.8%) and the presence of NIC did not reduce the effectiveness of the treatment (MT+NIC:76.4+/-4.7%). The effect of MT was due to a mechanism dependent of PLC, PKC, Ras, MEK and NF-κB, since its selective inhibitors reduced the effect (105.1+/-9.8%; 93.5+/-10.5%; 91.8±12.3%; 90.9± 10.1% and 90.0+/-11.0% respectively). These values did not vary significantly when cells were treated in the presence of NIC. Since the mediators involved in the effect of TM are associated with the activation of nitric oxide synthase (NOS), using selective inhibitors we determined the participation of the NOS2 and 3 isoforms, which produce an increase of 114% in nitric oxide (NO) level that would be at least partially responsible for the increased levels of apoptosis. These results indicate that in TNBC MDA-MB231 cells, TM exerts its proapoptotic antitumor effect by increasing the levels of NO produced by the NOS2 and 3 isoforms in the presence or absence of NIC, highlighting the benefits of TM in these conditions

Biology of Her2/Neu and TNB cancers

Poster No. 13

INVOLVEMENT OF RUNX1 IN TUMOR HETEROGENEITY ON TNBC CELL LINES

Facundo Luis Couto[1], Sofía María Sosa[1,2], Natalia Brenda Fernández[1,2], Lucía Escobar[1], Natalia Rubinstein[1,2]
1- Instituto de Biociencias, Biotecnología y Biología Traslacional (iB3), FBMC-FCEN-UBA, 2- CONICET.

Presenting Author:
Facundo Luis Couto
PhD Fellow - iB3-FCEyN-UBA
CABA, Argentina
Email: *couto.facundo@gmail.com*

Triple negative breast cancer (TNBC) is an aggressive breast cancer subtype for which no effective targeted therapies are available. Growing evidence suggests that cancer cells with stem-like properties (CSC) may repopulate the tumor. One of the approaches that are getting great attention in treating the disease is to modulate the immune system of the patient. In TNBC, the expression of transcription factor RUNX1 correlates with poor prognosis. We identified that RUNX1 is relevant in tumor aggressiveness in TNBC cell models, for the regulation of oncogenes, cell migration and drug resistance. On the other hand, the transcription factor KLF4 is required to generate CSCs in TNBC. In addition, PD-L1 plays a crucial role in TNBC as it facilitates tumor cells to evade the immune response. Both KLF4 and PD-L1 have been described as RUNX1 target genes in other tumors. Our goal was to investigate the regulation of KLF4 and PD-L1 by RUNX1 in TNBC cell lines. To consider intratumor heterogeneity we used two cell culture models: attached and forced suspension (CSC-like). To inhibit RUNX1 transcriptional activity we used the commercial inhibitor AI-10-104. We found that when RUNX1 activity is inhibited in attached MDA-MB-231 and -468 cell lines KLF4 levels are increased, in a dose- and time-dependent manner ($p<0.05$). Growing MDA-MB-468 in forced-suspension significantly increased KLF4 and RUNX1 levels. Interestingly, under forced suspension conditions, KLF4 levels were notably reduced when RUNX1 was inhibited. Finally, by flow cytometry, we found that PD-L1 is strongly downregulated in the attached MDA-MB-231 treated with AI-10-104. Taken together, these results suggest that RUNX1 may act antagonistically in tumor intra heterogeneity and may contribute to immune evasion of TNBC. The characterization of this dual gene regulation within the intratumoral cell variation is crucial for the development of future therapeutic strategies.

Biology of Her2/Neu and TNB cancers

Poster No. 16

Insights into Androgen Receptor-Mediated Regulation of Acyl-CoA Synthetase 4 in Breast Cancer

Melina Andrea Dattilo[1,2], Paula Fernanda Lopez[1], Jesica Giselle Prada[1], Yanina Benzo[1,2], Ulises Daniel Orlando[1], Cristina Paz[1,2], Ernesto Jorge Podestá[1,2], Paula Mariana Maloberti[1,2]
1- Instituto de Investigaciones Biomédicas (INBIOMED), CONICET, Universidad de Buenos Aires, Paraguay 2155 (C1121ABG), Buenos Aires, Argentina, 2- Departamento de Bioquímica Humana, Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina.

Presenting Author:
Melina Andrea Dattilo
Researcher - INBIOMED (UBA-CONICET)
CABA, Argentina
Email: *mdattilo@fmed.uba.ar*

Acyl-CoA synthetase 4 (ACSL4) is involved in arachidonic acid metabolism and is linked to prostate and breast cancer aggressiveness. In prostate cancer, androgen receptor (AR) inhibition leads to ACSL4 overexpression, suggesting that this enzyme has an important role in hormone-resistant tumor. AR is also being studied in breast cancer (BC), potentially serving as a therapeutic target in AR+ BC. In triple-negative breast cancer (TNBC) AR expression has recently emerged as an important factor. TNBC AR+ is a potential target for AR antagonists, currently under clinical trials. However,70-80% of TNBC lack AR expression (QNBC), resulting in highly aggressive tumors with poor survival and resistance to chemotherapy. This study aims to explore androgenic regulation of ACSL4 in BC cells, evaluating AR and ACSL4 as therapeutic targets. AR-positive cells MCF-7 showed low ACSL4 levels while MDA-MB-231, a QNBC model, exhibited high ACSL4 expression. In this work, an AR consensus site was identified in the ACSL4 promoter. We observed that AR overexpression in MDA-MB-231 reduced ACSL4 promoter activity and mRNA levels. In MCF-7 and MDA-MB-231 cells transiently transfected with AR, dihydrotestosterone treatment decreased ACSL4 promoter activity and mRNA levels, whereas ACSL4 promoter activity was increased by AR antagonist bicalutamide (CDX). In MCF-7 cells AR stable silencing increased ACSL4 mRNA levels and ACSL4 promoter activity was increased by site-directed mutagenesis of the AR element. This regulation seems to be reciprocal, as the ACSL4 inhibitor PRGL493 increased AR mRNA levels in MDA-MB-231 cells. Combining submaximal doses of ACSL4 inhibitors (PRGL493 and Rosiglitazone) with CDX, MDA-MB-231 cell proliferation was synergistically decreased. All these findings indicate that AR reduces ACSL4 expression, and this effect is reciprocal. Our results also suggest the importance of evaluating in BC the effectiveness of therapies that combine ACSL4 and AR as therapeutic targets.

Biology of Her2/Neu and TNB cancers

Poster No. 19

Thyroid hormones modulate breast cancer metastasis formation by regulating the immune subset distribution in the lungs

Gonzalo Gonzalez[1], Maria Mercedes Debernardi[1], Johanna Abigail Diaz Albuja[1], Florencia Menay[1], Maria Alejandra Paulazo[1], Cinthia Rosemblit[1], Florencia Cayrol[1], Graciela Alicia Cremaschi[1], Helena Andrea Sterle[1]

1- Instituto de Investigaciones Biomédicas (BIOMED) – UCA – CONICET.

Presenting Author:

Gonzalo Gonzalez

PhD Fellow - Laboratorio de neuroinmunomodulación y oncología molecular

Puerto Madero, Argentina

Email: *gonzalezgonzalo4e@gmail.com*

Although both thyroid disease and breast cancer (BC) are more prevalent in women, the relationship between thyroid status and BC remains unclear. Previously, we demonstrated that hyperthyroid mice inoculated with 4T1 BC cells exhibit a higher tumor growth rate compared to euthyroid mice, whereas hypothyroid mice display a slower tumor growth rate but an increased number of lung metastases.

This study aimed to evaluate the mechanisms by which thyroid status modulates metastasis formation in BC. First, we treated 4T1 cells in vitro with triiodothyronine (T3) and thyroxine (T4) and assessed their migration using a wound-healing assay. The results indicated no direct effects of thyroid hormones on cell migration.

To further investigate the effects of thyroid status on BC metastasis formation in vivo, Balb/c mice were treated with T4 for 4 weeks (hyperthyroid, hyper), propylthiouracil (PTU) for 2 weeks (hypothyroid, hypo), or PTU for 2 weeks followed by daily injections of T3 for the last six days (reverted mice). The animals were then inoculated with 4T1 cells either orthotopically or intravenously (i.v).

Primary tumors from hypo mice showed increased levels of CXCL-16, CCL-5, and CCL-2 chemokines, which are involved in tumor cell migration. Additionally, hypo mice showed an increased proportion of myeloid-derived suppressor cells (MDSCs) in the lungs, which reverted to euthyroid levels upon T3 treatment. In contrast, hyper mice exhibited an increased proportion of cytotoxic T lymphocytes and B lymphocytes in the lungs. Hyper mice i.v. inoculated with 4T1 cells showed increased levels of NK cells, while hypo mice displayed higher levels of MDSCs. Furthermore, higher activity of matrix metalloproteinase-2 was detected in the lungs of hypo mice.

Our results suggest that thyroid status does not directly affect BC cell migration but significantly influences the antitumor immune response in the lungs, thereby affecting the metastatic potential of tumor cells.

Biology of Her2/Neu and TNB cancers

Poster No. 22

Triple-Negative Breast Cancer: effects of Tamoxifen in the lysosomal pathway

Laura Pereyra[1], Lorena Carvelli[1,3], Laura Vargas Roig[2,4], Miguel Sosa[1,3,4]
1- Instituto de Histología y Embriología- CONICET, 2- Instituto de Medicina y Biología Experimental- CONICET, 3- Facultad de Ciencias Exactas y Naturales- UNCuyo, 4- Facultad de Medicina- UNCuyo

Presenting Author:
Laura Lucía Pereyra
PhD Fellow - Instituto de Histología y Embriología de Mendoza (IHEM)- CONICET
Mendoza, Argentina
Email: *lauraluciapereyra@gmail.com*

Triple-negative breast cancer (TNBC) is a common cancer in women, accounting for 10–15% of all breast cancers. It's characterized by a poor prognosis and metastatic patterns and is associated with distant recurrence and a high risk of death. TNBC is an attractive research focus for oncology, because no therapeutic target has been found to date.
Cells of some tumors have increased the lysosomal biogenesis as response to its altered metabolism. These events affect lysosomal integrity and/or functionality, where increased levels of lysosomal proteases are observed. Tamoxifen (TAM) treatment of estrogen receptor (ER)-positive breast cancer reduces mortality by modulating hormone dependance of those tumours. However, TAM exerts effects by mechanisms other than interaction with ERs.
Breast cancer cells lines MDA-MB-231 (ER-negative, RP-negative and HER-2 negative) and MCF-7 (ER-positive, PR-positive and HER-2 negative) were incubated with 2 and 10 μM of TAM for 8 and 24 h, in presence or absence of 17-β-estradiol. After the treatments, we used LysotrackerTM Red DND-99 which provides fluorescence detection for live-cell staining of labeling acidic organelles such as lysosomes. We obtained the images using fluorescense mycroscopy and analize them using the software ImageJ. TAM tended to decrease the acidic compartments (AC) after 8 h in both cell lines, but the number of AC was recovered in the MDA-MB-231 cells at 24 h. This effect is more notorious to higher concentrations of TAM (10 μM) in triple negative cell line. This pronounced effect in the triple-negative cells might be influenced by differences in genomic expression, although further research is needed to confirm that. No differences were found in AC in presence of 17-β-estradiol alone in both cell lines. This indicates that TAM could act as lysosomotropic drug in breast cancer cells, independently of ER pathway.

Biology of Her2/Neu and TNB cancers

Poster No. 25

HISTAMINE MODULATES INTRATUMOR MICROBIOTA IN 4T1 TRIPLE NEGATIVE BREAST CANCER

Magdalena Pezzoni[1], Melisa B Nicoud[2], Sergio Nemirovsky[3], Ignacio A Ospital[2], Andrea Monti Hughes[1], Vanina A Medina[2]

1- Dpto Radiología, Centro Atómico Constituyentes, Comisión Nacional de Energía Atómica (CNEA), 2- Laboratory of Tumor Biology and Inflammation, Institute for Biomedical Research (BIOMED), School of Medical Sciences, Pontifical Catholic University of Argentina (UCA), National Scientific and Technical Research Council (CONICET), Buenos Aires 1107, Argentina., 3- Instituto de Química Biológica, FCEN, IQUIBICEN, CONICET-UBA, Argentina, 4- Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)

Presenting Author:
Magdalena Pezzoni
Researcher - Lab. Radiomicrobiologia.
CABA, Argentina
Email: *maguisur@outlook.com*

We have previously demonstrated that histamine produces a complex regulation of breast cancer (BC) immunobiology. Increasing evidence suggests that tumor microbiome impacts both the progression of BC as well as treatment's efficacy. In this study, we aimed to characterize the intratumor microbiota of the translationally relevant murine 4T1 BC model and to reveal whether it can be modified by histamine treatment.
We developed a syngeneic model by orthotopic inoculation of 4T1 cells in Balb/c mice, which were treated daily with histamine (s.c. 10 mg/kg) or left untreated. After 3 weeks, tumors were obtained, and DNA was extracted to analyze the bacterial composition by sequencing the hypervariable regions V3-V4 of 16S rRNA gene. Bioinformatics analysis was performed with QUIME 2 (v 2022.2.1).
Histamine reduced tumor growth and vascularization while increasing tumor apoptosis. The taxonomic analysis showed a predominance of phylum Proteobacteria (57.5 % of the overall reads sequenced), Firmicutes (13.3 %), and Actinobacteria (10.4 %) in untreated mice, which was like those reported in human BC. Bacteroidetes (2.5%) and Cyanobacteria (1.5%) were detected to a lesser extent. Two relevant genera found were Pseudomonas (Proteobacteria; 6.4 %) and Streptococcus (Firmicutes; 3.5 %). Preliminary studies showed that histamine produced changes in the distribution of order-level phylotypes, significantly decreasing Proteobacteria (48.2%), Actinobacteria (3.8%), Cyanobacteria (0.3%) while increasing Firmicutes/Bacteroidetes (F/B) ratio (7.4 vs. 5.6).
We conclude that histamine favorably modified the intratumor microbiota by reducing phyla associated with breast cancer risk and improving F/B. It also reduces Pseudomonas and Streptococcus genera both related to tumor development. However, the causal relationship between the microbiota and histamine-induced proliferation inhibition needs to be directly tested in future experiments.

Biology of Her2/Neu and TNB cancers

Poster No. 28

Metronomic chemotherapy combining paclitaxel and a muscarinic agonist for triple negative breast cancer treatment

Yamila Sanchez[1], Nicolás Britos[1], Lucía Vazquez[1], Laura Sapere[1], Alejandro Español[1]
1- Laboratory of Tumoral Immunopharmacology, Center of Pharmacological and Botanical Studies (CEFYBO) - CONICET - UBA, Buenos Aires, Argentina.

Presenting Author:
Yamila Sanchez
Researcher - Laboratory of Tumoral Immunopharmacology, Center of Pharmacological and Botanical Studies (CEFYBO) - CONICET - UBA, Buenos Aires, Argentina
CABA, Argentina
Email: *yamila18.sanchez@gmail.com*

Breast cancer is the most common type of cancer in women worldwide and triple negative breast cancer (TNBC) subtype is the most aggressive. The most used chemotherapy drug is paclitaxel (PX), which, when used at high doses, generates numerous adverse effects. To avoid it, metronomic chemotherapy (MC) arises as an alternative treatment schedule involving much lower drug doses administered at shorter intervals, reducing adverse effects and increasing antitumor effectiveness. Another approach to improve the efficiency of antitumor treatment is the use of selective targets present only in tumor cells. We demonstrate that muscarinic receptors are expressed in breast tumor cells but absent in non-tumor breast cells, making them a potential therapeutic target. Here we evaluated the effectiveness of a MC combining low doses of PX (10-8M) and the muscarinic agonist carbachol (10-11M) in human TNBC MDA-MB231 cells. By MTT assays we demonstrated that MC was as effective as conventional chemotherapy (CC) with PX (10-6M) in reducing cell viability in vitro (control: 100.0±10.2%; MC: 25.0±8.1%; CC: 11.9±12.1%). Furthermore, the residual cells after treatment with MC were more sensitive to a new cycle with PX evaluated by IC50 (control: 7.6x10-5 M; MC: 9.3x10-9 M; CC: 5.8x10-6 M). Since this effect may occur by the modulation of the drug transporter ABCG2 expression, we determined by Western blot (Wb) assays that MC reduced its expression (control: 100±9%; MC: 18±6%). Since angiogenesis is necessary for tumor growth, by Wb we evaluated VEGF-A levels and determined that MC significantly reduced it (control: 100±25%; MC: 50±23%) and reduced angiogenesis in vivo (No. vessels/cm2 skin) (normal skin: 3.0±0.1; cells alone: 4.0±0.1; MC: 2.9±0.2; CC: 4.3±0.2). Our results demonstrate that changing a conventional chemotherapy administration scheme by a metronomic one is a plausible alternative for TNBC treatment.

Biology of Her2/Neu and TNB cancers

Poster No. 31

Targeting ID4 to Reprogram Triple-Negative Breast Cancer for Endocrine Therapy Sensitivity

Carla Toro[1], Sebastian Real[1], Sergio Laurito[1], Maria Teresita Branham[1]
1- IHEM-CCT-CONICET.

Presenting Author:
Carla Daiana Toro
Undergraduate Student - IHEM
Mendoza, Argentina
Email: *carlatoro.lab@gmail.com*

Background: Triple-negative breast cancer (TNBC) is an aggressive subtype of breast cancer characterized by the absence of estrogen receptor, progesterone receptor, and HER2/neu expression, making it unresponsive to hormonal and HER2-targeted therapies. ID4, an inhibitor of differentiation protein, plays a crucial role in the reprogramming of mammary cells by regulating the transcription of lineage-specific genes. This study investigates ID4's potential to reprogram TN tumors into luminal tumors, making them sensitive to endocrine treatments. Methods: In-silico analyses evaluated ID4 expression in human TN tumors using public datasets. In vitro experiments on MDA-MB231 ER- breast cancer cell lines involved silencing ID4 using CRISPR-Cas9 or inhibiting it with AGX51. Gene and protein expression were analyzed via RT-qPCR and Western blot. Phenotypic changes were assessed using colony formation, migration, and wound healing assays. Cells were treated with Tamoxifen to evaluate endocrine response. Results: In-silico analysis stratifying ID4 expression in TN tumors into “high” and “low” groups revealed distinct luminal and basal gene expression patterns. The low ID4 group had increased luminal ($p<0.05$) and decreased basal signatures ($p<0.01$). Gene set enrichment analysis indicated enrichment of “estrogen response late and early” hallmarks. In vitro experiments confirmed a significant increase in ER and GATA3 expression and a decrease in EGFR expression ($p<0.05$) upon ID4 silencing, which also significantly reduced cell proliferation, migration, colony formation ($p<0.05$), and tumor size in an in vivo model ($p<0.01$). Tamoxifen treatment significantly decreased proliferation and migration in silenced cells ($p<0.05$). Treatment with AGX51 significantly reduced tumor size in an in vivo model ($p<0.01$). Conclusions: Our results highlight ID4’s influence on estrogen pathways and TNBC traits, suggesting ID4 targeting as a new treatment approach.

Biology of Her2/Neu and TNB cancers

Poster No. 34

Recovery of specific miRNAs in heparinized plasma obtained from HER2+ breast cancer patients who underwent a neoadjuvant therapy protocol.

Micaela Vivanco[1], Florencia Cascardo[1], Ayelén Pesse Viglietti[2], María Belén Bordignon[2], Estrella Levy[2], Virginia Novaro[1]
1- .Instituto de Biología y Medicina Experimental IBYME-CONICET, 2- Centro de investigaciones Oncológicas de la Fundación Cáncer (CIO-FUCA.

Presenting Author:
Griselda Micaela Vivanco
PhD Fellow - Instituto de biología y medicina experimental
Capital Federal, Argentina
Email: *mica.vivanco.ibyme@gmail.com*

The PI3K/AKT pathway is a critical downstream signal from HER2 receptor, and its alterations are potential biomarkers for predicting the effectiveness of HER2-targeted therapy. Understanding the regulation of this pathway can contribute to breast cancer management.

The recent increase in the clinical use of PI3K/AKT inhibitors for breast cancer requires characterizing patient populations at both clinical and molecular levels to provide more appropriate and personalized treatments. In previous studies using immunohistochemistry, we observed the activation state of PI3K/AKT pathway in HER2+ and triple-negative breast cancer patients compared to the luminal subtype.

Since biomarkers from liquid biopsies offer the advantage of non-invasively clinical monitoring, we aimed to identify plasma circulating miRNAs associated with the PI3K/AKT pathway specifically in HER2+ patients who undergo neoadjuvant treatment.

Biobanks allow the evaluation of a larger number of samples; however, most plasma samples are usually obtained with heparin, which has an inhibitory effect on RNA and DNA polymerase. Thus, we recognized the need to develop a protocol to counteract heparin interference. To address this, we evaluated different options to improve miRNAs extraction protocols using carriers, such as glycogen and tRNA, and heparin inhibitors, like LiCl and protamine sulfate.
We applied these optimized protocols to samples from five HER2+ breast cancer patients who received trastuzumab plus pertuzumab combined with chemotherapy before surgery. We verified the effectiveness of our protocols by amplifying miR16-5p, -155-5p, -195-5p, -21-5p, and -126-5p using RT-qPCR in pre- and post-therapy heparinized plasma samples. We are now validating this set of miRNAs in groups of anti-HER2 therapy responding and non-responding patients. This approach could be highly beneficial for monitoring and predicting responses to specific antitumor treatments.

Biology of luminal breast cancer

Poster No. 37

Doxorubicin-loaded sulfonated polyvinyl alcohol microspheres inhibit tumor growth in a murine model of breast cancer.

Luisa Ambrosio[1], Claudia Lanari[1], Maria Gisela Veron[2], Paola Rojas [1]
1- Instituto de Biología y Medicina Experimental (IBYME), CONICET, Buenos Aires, Argentina., 2- Centro Atómico Bariloche, CONICET, Río Negro, Argentina..

Presenting Author:
Luisa Ambrosio
Postdoc Fellow - Instituto de Biología y Medicina Experimental (IBYME)
buenos aires, Argentina
Email: *luisa.ambrosio.15@gmail.com*

Despite that new therapeutic strategies are currently being developed to treat the different tumor types; chemotherapy continues to be widely used for advanced breast cancer treatment. On the flip side, this therapy is often nonspecific and induces significant side effects. Thus, the development of drug delivery methods such as liposomes aimed to increase tumor-specific effects, increasing therapeutic efficacy and sustained and controlled release of the therapeutic drug reducing systemic side effects has gained relevance.
Microspheres can be administered locally either for drug delivery or they can also be used as embolic agents. At the Atomic Center Bariloche, sulfonated polyvinyl alcohol microspheres (SPVA; 35 µm) were designed and loaded with doxorubicin (Doxo-SPVA). To test the effect of these doxorubicin-loaded microspheres in an in vivo scenario we selected the luminal C4-HI tumor model which grows in BALB/c mice and was previously used to compare the effect of free doxorubicin vs doxorubicin pegylated liposomes. Fourteen days after subcutaneous tumor transplantation, female mice were treated with Doxo-SPVA (2 mg, locally), free doxorubicin (40 µg, locally), empty SPVA (2mg, locally), or remained untreated (n=5-7/group). Mice were treated once a week for 3 weeks. Tumors were measured with a caliper twice a week. One week after the third dose was administered tumors were excised and weighed. The tumors treated with Doxo-SPVA microspheres were significantly smaller than untreated mice ($p<0.05$). The therapeutic effect was like that obtained with the systemic dose proving the efficacy of the local administration. Future experiments will focus on studying whether the slow and continuous release of doxorubicin from the Doxo-SPVA microspheres produces an improved therapeutic effect in the long term compared to free or pegylated liposome doxorubicin.

Biology of luminal breast cancer

Poster No. 40

IMPACT OF PROMOTER POLYMORPHISM (rs251864) ON ZFP36/TTP TUMOR SUPPRESSOR EXPRESSION IN LUMINAL BREAST CANCER CELLS

Emilia Soledad Bogni[1], Angela Lara Montero[1], Pedro Salaberry[1], Jonathan Lamarre[3], Edith Kordon[1,2]

1- Instituto de Fisiología, Biología Molecular y Neurociencias (IFIBYNE), Universidad de Buenos Aires-Consejo Nacional de Investigaciones Científicas y Técnicas (IFIBYNE-UBA-CONICET), Ciudad Autónoma de Buenos Aires (CABA) 1428, Argentina., 2- Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Autónoma de Buenos Aires (CABA) 1428, Argentina., 3- Department of Biomedical Sciences, University of Guelph, Guelph, Canada ON N1G., 4-

Presenting Author:
Emilia Soledad Bogni
PhD Fellow - Instituto de Fisiología, Biología Molecular y Neurociencias (IFIBYNE)
Buenos Aires, Argentina
Email: *bogniemilia@gmail.com*

Tristetraprolin (TTP), a protein encoded by the ZFP36 gene, specifically induces the degradation of several inflammatory cytokines, oncogenes, and angiogenic factor mRNAs. Various authors have linked breast cancer (BC) aggressiveness to TTP expression deficiency. Furthermore, a single nucleotide polymorphism (SNP) in ZFP36 promoter region has been reported (rs251864) in which the AG or GG genotype is associated with worse prognosis compared to the AA genotype in Caucasian breast cancer patients in the USA. Here, in Argentina, we have found that the frequency of the allele G is approximately 50% each in a cohort of 54 BC patients. Interestingly, we have also found that the luminal BC cell lines MCF-7 and T47D have AA and GG genotypes at rs251864, respectively. Functionally, MCF-7 cells exhibit a rapid induction of ZFP36 RNA expression upon serum stimulation at 30 minutes and 1 hour. In contrast, T47D cells demonstrate a blunted response throughout the same time course. We then proceeded to clone out 640 bp including the polymorphic promoter region of each of these cell lines and to generate alternative reporter plasmids (pGL3-LUC). Luciferase assays using these constructs have also shown that the AA genotype exhibits serum-induced activation, while the GG genotype remains largely unresponsive. Additionally, we have identified a CpG island in the ZFP36 promoter, which co-localizes with the SNP and is a conserved regulatory element involved in serum-induced TTP expression. Therefore, we proceeded to treat T47D cells with the DNA methyltransferase (DNMT) inhibitor 5-Azacytidine for 48 hours before serum induction. Our results show that blocking DNMT activity sensitized T47D (GG) cells to serum treatment showing a similar response to MCF-7 (AA) cells. In summary, our data indicates that BC patients carrying the GG genotype at rs251864 might have a worse prognosis due to genetic and epigenetic events that are reducing ZFP36 transcription efficiency in tumor cells.

Biology of luminal breast cancer

Poster No. 43

ANDROGEN RECEPTORS AND WNT PATHWAY AS THERAPEUTIC TARGETS IN ENDOCRINE RESISTANT BREAST CANCER MODELS

Marcela Coianis[1], Virginia Figueroa[1], Sebastian Giulianelli[2], Claudia Lanari[1], Caroline Lamb[1]
1- Instituto de biología y medicina experimental (IBYME), 2- Instituto de Biología de Organismos Marinos (IBIOMAR)-CENPAT.

Presenting Author:
Marcela Coianis
PhD Fellow - Instituto de biología y medicina experimental
Buenos Aires, Capital Federal., Argentina
Email: *marcelacoianis@hotmail.com*

Endocrine resistance is still a major clinical problem in the treatment of breast cancer. Evidence suggests that dysregulation of growth factor signaling pathways contributes to endocrine resistance. Fibroblast growth factor 2 (FGF2) consists of a secreted form and several nuclear high molecular weight variants (HMW-FGF2). We previously showed that hormone-resistant tumors express higher HMW-FGF2 levels than endocrine-responsive variants and that HMW-FGF2-overexpression, in endocrine-responsive cells, induced tumor progression. Here, we aimed to explore the mechanisms underlying HMW-FGF2-induced hormone resistance. We show that HMW-FGF2-overexpression in T47D cell lines, induced hormone resistance, a dysregulation of the WNT signaling pathway (RNA-seq) and a decrease in estrogen and progesterone receptors, along with an increase in androgen receptor (AR) expression. We used endocrine-resistant cell lines expressing elevated HMW-FGF2 levels and their endocrine-responsive counterparts to target the AR and/or WNT pathways. Enzalutamide (ENZA; AR antagonist) was inhibited while dihydrotestosterone (DHT; AR agonist) increased cell proliferation only in resistant cell lines. Moreover, ICG-001 (WNT inhibitor) alone or combined with DHT reduced cell proliferation. In vivo, ENZA and LGK-974 (WNT inhibitor) inhibited T47D-HMW-FGF2 tumor growth, and the combined treatment induced a greater inhibition together with a reduction in the number of lung metastasis. To assess if there was a direct effect of WNT pathway activation on AR regulation we performed ChIP assays on TCF/LEF sites within the AR promoter. ICG-001 reduced the recruitment of β-catenin in the AR promoter. Our results suggest that, in endocrine-resistant cell lines with increased HMW-FGF2, an upregulated WNT pathway may modulate AR expression which, in turn, may guide tumor growth. In conclusion, targeting the WNT and/or AR pathway may be a promising therapy for endocrine-resistant breast carcinomas.

Biology of luminal breast cancer

Poster No. 46

Influence of aging on RET-mediated mammary tumor features and incidence

Clara de los Santos[1], Sabrina A. Vallone[1], Roberto P. Meiss[2], Edith C. Kordon[1], Albana Gattelli[1]
1- Instituto de Fisiología, Biología Molecular y Neurociencias (IFIBYNE) (CONICET-UBA), Ciudad Universitaria C1428EGA CABA, Buenos Aires, Argentina., 2- Academia Nacional de Medicina de Buenos Aires, Argentina..

Presenting Author:
Clara de los Santos
Undergraduate Student - IFIBYNE
Ciudad Autonoma de Buenos Aires, Argentina
Email: *claradelossa@gmail.com*

Most cancers arise in individuals over 60 and, as the global population ages, cancer is becoming a significant public health problem. Yet, the contribution of aging to oncogenic signals is largely ignored, with most preclinical studies designed in 2-month-old mice rather than older mice reflecting an age appropriate to the disease being modeled.
For several years, our lab has been studying the oncogenic function of RET receptor tyrosine kinase in breast cancer. RET is overexpressed in 40% of breast tumors with respect to normal tissue and high RET correlates with decreased survival. Using a doxycycline (DOX)-induced transgenic mouse system (RET/MTB), we previously demonstrated that RET expression in the mammary epithelium-induced estrogen receptor (ER) positive tumors, representing the human luminal subtype. Here, we aim to address the impact of aged microenvironment in the development of neoplastic lesions induced by RET. Firstly, we analyze mammary gland tissue from aged-virgin female mice. In addition to the reported morphological changes (histological analysis), we observe that aged glands (10 to 12-month-old, none cycling) express endogenous RET protein which is generally absent in young counterparts (2 to 4-month-old). Interestingly, phosphorylation (p) pattern of RET as well as ER, is differential: aged mammary gland displays high levels of the pY1062RET fully glycosylated isoform and a significant increase in pS118ER (Western blot) with respect to young mammary tissue. Then, we chronically DOX-induced RET overexpression in RET/MTB aged- vs. young-female groups. Surprisingly, we found that tumor incidence is reduced in older females (20%, 1/5) with respect to younger females (62,5%, 5/8), suggesting an aged-related protective role against RET oncogene. Signaling pathways activated by RET were analyzed in both epithelial tumor cells and adjacent mammary tissue microenvironment.

Biology of luminal breast cancer

Poster No.49

UNVEILING THE ROLE OF KLK4 AND KLK12 IN THE BREAST CANCER

Ingrid Pamela Ehrenfeld[1,5*], Maria Francisca Pavicic[1], Claudia Torres[1,5], Pia Bascur[1,5], Adriana Stuardo[1,5], Fabiola Sanchez[2,5], Alejandro Rojas[3,5], Tobias Dreyer[4], Victor Magdolen[4], Carlos Dario Figueroa[1,5], Larissa Turones[1,5]

1- Laboratory of Cellular Pathology, Institute of Anatomy, Histology and Pathology, Faculty of Medicine, Universidad Austral de Chile., 2- Laboratory of Immunology, Faculty of Medicine, Universidad Austral de Chile., 3- Institute of Medicine, Faculty of Medicine, Universidad Austral de Chile, 4- Technical University of Munich, Germany. 5- Center for Interdisciplinary Studies on the Nervous System (CISNe).

Presenting Author:
INGRID PAMELA EHRENFELD SLATER
Researcher - UNIVERSIDAD AUSTRAL DE CHILE
VALDIVIA, CHILE
Email: *ingridehrenfeld@uach.cl*

Kallikrein-related peptidases (KLKs), a family of 15 serine protease members, have been consolidated as key factors in establishing several diseases, like breast cancer (BC). BC is one of the most prevalent cancers in women worldwide. Considering the lack of investigation of the role of KLKs in BC, we decided to evaluate whether KLK4 and KLK12 impact cell survival and angiogenesis. In a conditioned medium (CM) of BC cells (MCF7 cell line) stimulated with KLK4 and KLK12 (2 and 10 ng/ml x 24 h) we found using Slot blot that these KLKs: a) increase the release of insulin growth factor (IGF) and its associated binding proteins (IGFBP3 and 7), b) modify the macrophage response by paracrine BC cells signaling through the increase of the release of the colony-stimulating factor (M-CSF), c) promote a reciprocal secretion of both KLKs in an autocrine signal pathway, even more pronounced under hypoxia (1% O2 x 24 h), and d) in particular KLK12 stimulation (10 ng/ml) increased the secretion of angiogenic factors like vascular endothelial growth factor and platelet-derived growth factor A/B (PDGFA/B). KLK12 (10 ng/ml x 24 h) also increased vascular endothelial growth factor (VEGF) secretion by BC and endothelial cells (EA.hy926) in CM. It also increased the levels of PDGF-β/α and VEGF-2 receptors in these cells evaluated by western blotting. Using a commercial adhesion assay, we demonstrate that KLK4 and KLK12 (10 ng/ml) increase the adherence of BC cells to components of the extracellular matrix, such as type 4 collagen and fibrinogen. These results emphasize these proteases, KLK4 and KLK12, as positive modulators of cancer progression due to activation of crucial survival and angiogenic pathways added to its potential to modulate the adhesion and tumor environment that can contribute to metastasis. Therefore, KLK4 and KLK12 may be important targets for developing new therapies and diagnostic tools.

Biology of luminal breast cancer

Poster No.52

FUNCTIONAL ANALYSIS OF GLUCOCORTICOID AND PROGESTERONE RECEPTOR CROSSTALK

Maximiliano Gutierrez[1], Norma Roxana Carina Alves[2], Adali Pecci[1], María Florencia Ogara[1]
1- Instituto de Fisiología Biología Molecular y Neurociencias (IFIBYNE). Universidad de Buenos Aires. CONICET. 2- UMYMFOR. Universidad de Buenos Aires. CONICET., 3- Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires.

Presenting Author:
Maximiliano Gutierrez
PhD Fellow - Instituto de Fisiología Biología Molecular y Neurociencias (IFIBYNE). Universidad de Buenos Aires. CONICET.
Ciudad Autónoma de Buenos Aires, Argentina
Email: *maxiguti1@gmail.com*

The glucocorticoid and progesterone receptors (GR and PR, respectively) are closely related members of the steroid receptor family of transcription factors. Despite they share similar structural and functional properties, as their DNA sequence recognition motif, the cognate hormones display very distinct physiological responses and even in tissues expressing both receptors they exert opposite biological actions in proliferation, differentiation and cell death. Results from our group demonstrated an antagonistic effect of activated GR on PR-dependent features in mammary epithelial cells. To evaluate whether GR activation could affect PR function, we analyzed the expression of several progestin target genes in MCF-7L cells which express both PR and GR. RT-qPCRs of selected genes show that GR activation by Dexamethasone (DEX) [10 nM] inhibited the R5020 [10 nM]-dependent induction of STAT5A, SNAI1A and EGFR, wherein potentiated R5020-mediated GREB1 and ELF5 expression induction. These results were confirmed by siRNA-mediated GR knockdown, where the progestin-dependent expression of those genes was restored. Moreover, cell cycle analyses performed in cells treated for 18 h with R5020 show that the percentage of cells accumulated in S phase was significantly higher compared to untreated cells (13.7±0.7% vs 10.2±0.3%). DEX alone did not affect S phase accumulation (10.3±0.6%) but inhibited R5020-mediated action (10.9±0.9%). To assess whether the presence of GR affects proliferation, survival and cell migration induced by progestin, clonogenic and wound healing assays were performed in MCF-7L cells. Clonogenic assay shows that treatment with R5020+DEX decreases the proportion of colonies by half compared to R5020 alone. In the same way, wound closure decreased by 20% when treated with both ligands compared to R5020 alone. These results seem to indicate that activated GR modulates PR-dependent cell proliferation and migration in mammary tumor epithelial cells.

Biology of luminal breast cancer

Poster No.55

Combination of GnRH Agonist/Antagonist with Protein Kinase Inhibitors Counteracts Growth and Metastasis in Breast Cancer

Joselina Mondaca[1], Juan Manuel Fernandez Muñoz[1], Fiorella Vanderhoeven[2], Gustavo Adolfo Barraza[1], Marina Inés Flamini[2], Angel Matias Sanchez[1]
1- Laboratorio de Transducción de Señales y Movimiento Celular., 2- Laboratorio de Biología Tumoral.

Presenting Author:
Joselina Mondaca
Postdoc Fellow - Instituto de Medicina y Biología Experimental de Cuyo (IMBECU), CCT-CONICET-Mendoza
Mendoza, Argentina
Email: *joselinammondaca@gmail.com*

Breast cancer (BC) is the most frequent malignant neoplasm in women, with metastases being the cause of 98% of deaths. Hormone-dependent BC represents approximately 80% of diagnosed cases and most frequently occurs in postmenopausal women, who often present elevated levels of gonadotropins. Currently, molecules directed against the gonadotropin-releasing hormone receptor (GnRHR) have been designed, such as the agonist leuprorelin (LEU) and the antagonist degarelix (DEGA) for the treatment of certain hormone-dependent tumors. These therapies aim to disrupt the hormonal environment that supports tumor growth. By reducing the levels of gonadotropins, these treatments can slow down or even halt the progression of hormone-sensitive BC. Tumor progression depends on the ability to invade and metastasize to distant sites, cell migration being essential in this process. Key proteins in these processes include the proto-oncogene tyrosine-protein kinase Src and focal adhesion kinase (FAK), which play critical roles in signaling and modulating metastatic pathways. In this work, we combined GnRH analogs with Src and FAK inhibitors to counteract tumor progression. By in silico analysis, we analyzed by miniarrays the effect of LH (5 and 50 mUI/ml) on genes involved in tumor development and progression. To compare the action of GnRHR agonist and antagonist, we explored the molecular interactions between GnRHR and LEU or DEGA. Through orthotopic tumor growth assay, we determined that DEGA decreases tumor growth while LEU has the opposite effect. PP2 and FAKi were found to reduce tumor volume and interestingly, combining DEGA plus PP2 or FAKi enhanced the inhibitory effect, increasing mice survival. Our findings reveal how gonadotropins regulate genes involved in tumorigenic processes. Despite the complexity of the LH signaling in BC, the approach based on GnRH antagonists administered in combination with PP2 or FAKi may be an effective strategy for treating BC patients.

Biology of luminal breast cancer

Poster No. 58

TGFβ signaling pathway participates in Heregulin induced luminal breast cancer cell migration

Mercedes Montani*[1], Angela Lara Montero*[1], Agustin Gonzalez[2], Pedro Salaberry[1], Julieta Aisemberg[2], Eva Wertheimer[2], Roxana Schillaci[3], Andrea De Laurentiis[1], Omar Coso[1,4], Edith Kordon[1,5]

1- Institute of Physiology, Molecular Biology, and Neurosciences (IFIBYNE-CONICET), University of Buenos Aires, CABA, Argentina, 2- Center of Pharmacological and Botanical Studies (CEFYBO-CONICET), University of Buenos Aires, CABA, Argentina, 3- Institute of Biology and Experimental Medicine (IBYME-CONICET). CABA, Argentina, 4- Department of Physiology, Molecular and Cellular Biology. 5- Department of Biological Chemistry (DQB), Faculty of Exact and Natural Sciences,University of Buenos Aires, CABA, Argentina.

Presenting Author:
Mercedes Agustina Montani
PhD Fellow - Instituto de Fisiología, Biología Molecular y Neurociencias (IFIBYNE)
CABA, Argentina
Email: *ma.montani@gmail.com*

results indicated that Heregulin (HRG) increased cell migration through ErbB3/P-Rex/Rac1 signaling activation in luminal breast cancer (BC) cells, and that this pathway also induced TGFβ2 expression, a molecule associated with migratory processes. Therefore, our goal was to establish the role of the TGFβ canonical pathway on Hrg-induced BC cell migration. To that end, we carried out experiments using specific pharmacological inhibitors and transient gene silencing to block mediators of the involved pathways. Analysis of mRNA and protein levels, as well as wound-healing assays in luminal BC cells, were performed.
Our results show that HRG induced not only TGFβ2, but also TGFβ1, TGFBR1, TGFBR2, SMAD3, and SMAD4 expression, and preliminary results indicate that Rac1 could be involved in these pathways. Supporting these data, we found positive correlations between HRG and TGFβ1, TGFβ2, TGFβ3, and TGFBR3 protein content in proteomic data sets from BC samples. In addition, HRG treatment induced phosphorylation and nuclear translocation of SMAD3, but this activation was blocked by adding either ErbB2 or TGFBR1 inhibitors. Furthermore, blocking TGFBR1 also inhibited HRG-induced cell migration. Importantly, after HRG treatment, SMAD3 phosphorylation was not affected by P-REX1 silencing and Rac1 activation increased when TGFBR1 was inhibited. This suggests that the HRG-induced TGFβ pathway does not contribute but competes with the activation of P-REX1/Rac1 signaling. Our results indicate that induction of TGFB ligands and SMAD-dependent pathway activation is required for HRG-induced migration in BC luminal cells. We are currently analyzing the impact of clinically relevant pharmacological ErbBs inhibitors to investigate the most effective way to reduce the combined effects triggered by HRG on BC cell migration and invasiveness. We believe our findings may contribute to the search for new combination therapies, which may lead to more successful treatments for luminal BC.

Biology of luminal breast cancer

Poster No. 61

Seeking the Truth Behind the Myth: Nuclear Role for Argonaute-1 as an Estrogen-Dependent Enhancer Coactivator

Ezequiel Nazer[1], Daphné Rageot[2], Juan Elgassi[1], Ezequiel Abraham[1], Alberto Kornblihtt[1]
1- IFIBYNE, 2- University of Paris.

Presenting Author:
Ezequiel Nazer
Researcher - IFIBYNE
Buenos Aires, Argentina
Email: *nazere@gmail.com*

Breast cancer is the leading cause of cancer-related death in women. Positive outcomes are largely due to the success of treatments targeting hormone receptors in hormone receptor-positive disease. However, a significant fraction of cases develops resistance and progress to metastatic disease. At the molecular level, disruptions of cell type-specific enhancers and their associated transcriptional programs play key roles in tumorigenesis. In breast cancer, many frequently mutated genes encode epigenetic regulators. Therefore, a deeper understanding of the underlying molecular mechanisms is required to uncover novel therapeutic targets and develop new drugs for treating estrogen receptor (ER)-positive breast cancers.

Recently, we have identified a non-canonical role for Argonaute1 (AGO1) as a coactivator of estrogen-induced enhancers. ChIP-seq analysis showed that AGO1 colocalizes with a vast majority of these enhancers in MCF7 cells (an ER+ breast cancer cell line) in an estrogen-dependent manner. Co-immunoprecipitation assays revealed that AGO1 interacts with ERα and RNA Pol II upon estrogen (estradiol, E2) treatment. Furthermore, AGO1 modulates the recruitment of ERα to induced enhancers, activates enhancer transcription, stabilizes induced enhancer-promoter interactions, and activates target promoters in response to E2. Overall, these results suggest that AGO1 plays a critical role in the activation of estrogen-induced enhancers.

Thus, it is tempting to hypothesize that AGO1 is a key determinant of estrogen-induced enhancers with potential as a "druggable" target to improve the treatment of ER+ breast cancer.

Biology of luminal breast cancer

Poster No. 64

Metabolic remodeling and the impact of the adipose microenvironment in breast cancer

Priscila Ayelén Pagnotta[1,2], María Luján Crosbie[3], Natalia Santiso[3], Anabela Ursino[3], Celeste Frascarolli[3], Alicia Amato[3], Rubén Dreszman[4], Juan Carlos Calvo[1], Judith Toneatto[1]
1- Institute of Biology and Experimental Medicine (IBYME), 2- Department of Biological Chemistry, Faculty of Exact and Natural Sciences, University of Buenos Aires, 3- Breast Surgery Section, Gynecology Staff, Churruca-Visca Police Medical Centre, 4- Microsurgery Clinic

Presenting Author:
Priscila Ayelén Pagnotta
PhD Fellow - Institute of Biology and Experimental Medicine (IBYME)
Buenos Aires, Argentina
Email: *priscila.pagnotta@gmail.com*

Breast cancer (BC) cells exhibit metabolic heterogeneity with different profiles influenced by their microenvironment. Reprogramming supports cancer growth and survival within this complex microenvironment. Our goal was to analyze the metabolic changes in the adipose microenvironment and their impact on BC reprogramming. We used adipose tissue (AT) explants from patients with BC (immediate tumor adjacency -ADJ- and >2 cm distance -DIST-), alongside normal controls (Normal). Additionally, we evaluated the effects of ADJ conditioned media (ADJ-CM) on changes in glucose metabolism in BC cells (MCF7 and T47D). ADJ explants increased MCT1 and decreased Glut4, LDHA and GAPDH protein expression levels compared to Normal explants. A subgroup of patients with high MCT1 also presented increased browning markers. ADJ-CM had a differential effect on MCF7 and T47D lines, decreasing MCT1 protein expression in MCF7 cells but increasing it in T47D cells. In T47D cells, ADJ-CM also downregulated MCT4 expression, while both ADJ-CM and Normal-CM induced an increase (or tendency to increase) in Glut4 transporters. A minority subpopulation with high glucose uptake was identified in T47D cells through cytometry assays. This subpopulation and overall glucose uptake tended to decrease after ADJ-CM treatment. Furthermore, changes in lactate transporter expression were contrasted with lactate efflux assay.
In sum, metabolic remodeling occurs in both tumor cells and the adipose stroma in BC. Peritumoral adipocytes could be decreasing glucose uptake, glycolysis and intracellular lactate synthesis, contrasting with the potential import of lactate for energy purposes or signaling the browning process. Soluble factors released by the tumor adipose microenvironment could induce a metabolic switch towards lactate utilization, thereby reducing glucose uptake and the glycolytic tumor population.

Biology of luminal breast cancer

Poster No. 67

Elucidating the role of FGFR2 activation and RUNX2 expression in the progression of luminal breast cancer

María Sol Rodríguez[1], Silvia Vanzulli[1], Sebastián Giulianelli[2], John Bushweller[3], Caroline Lamb[1], Isabel Lüthy[1], Claudia Lanari[1], Cecilia Pérez Piñero[1]
1- Instituto de Biología y Medicina Experimental (IBYME-CONICET), 2- Instituto de Biología de Organismos Marinos)IBIOMAR-CCT CENPAT-CONICET), 3- Department of Molecular Physiology and Biological Physics, 4-

Presenting Author:
Maria Sol Rodriguez
PhD Fellow - Instituto de Biología y Medicina Experimental (IBYME-CONICET)
CABA, Argentina
Email: *msolrodriguez91@gmail.com*

Luminal breast cancer (BrCa) is the most common subtype diagnosed in patients, and the major challenge is to understand the mechanisms related to the development of endocrine resistance and disease progression. RUNX2 is a transcription factor associated with tumor aggressiveness in triple-negative BrCa. Its role in luminal tumors is still unclear. Our previous studies showed that FGF2 promotes BrCa cell proliferation through FGFR2 and ligand-independent hormone receptor activation. We also observed higher RUNX2 expression in hormone-independent (HI) mouse mammary carcinomas.
We aimed to evaluate FGFR2 and RUNX2 interaction in luminal BrCa models. IBR1, MCF7, and T47D cells were stably transfected with a constitutively active FGFR2 (R2CA), a RUNX2 expression plasmid, or an empty vector. R2CA and RUNX2 overexpression caused increased cell proliferation, HI growth in vivo, and metastasis development. Conversely, silencing FGFR2 or RUNX2 reduced these parameters. Interestingly, RUNX2 rescued the FGFR2-silenced phenotype, generating a more aggressive tumor phenotype compared to Control, shFGFR2- and RUNX2-overexpressing tumors. Additionally, RUNX2 overexpressing tumors generated an endocrine and FGFR inhibitor-resistant phenotype in vivo. RUNX inhibitors (AI-14-91 or CADD522: CAD) reduced cell proliferation in vitro in all the cell lines. In vivo, CAD only reduced the growth of T47D tumors with endogenous RUNX2 expression (Control vs CAD treated group: 58% of tumor size-reduction; $p<0.0001$), suggesting the need to generate more efficacious inhibitors for in vivo use.
In summary, our results indicate a complex interaction between FGFR2 and RUNX2 in regulating tumor progression and aggressiveness in luminal BrCa and suggest that RUNX2 expression could serve as a prognostic and predictive marker of therapy outcomes. The therapeutic effect of RUNX2 inhibitors in combination with other therapies deserves further investigation.

Biology of luminal breast cancer

Poster No. 70

Invasive mammary carcinomas with different progesterone receptor isoform ratios: metastatic vs. proliferative ability, which is worse?

Leo Saldain[1], Andrés Elia[1], Gabriela Pataccini[1], Martín Abba[2], Claudia Lanari[1], Paola Rojas[1]
1- Instituto de Biología y Medicina Experimental (IBYME), 2- Universidad de La Plata.

Presenting Author:
Leo Saldain
PhD Fellow - Instituto de Biología y Medicina Experimental (IBYME)
Buenos Aires, Argentina
Email: *leosaldain@gmail.com*

There is a controversy regarding the prognosis of the progesterone receptor (PR) isoform ratio in luminal breast cancer. We suggested that excess in isoform B over isoform A of PR (PRB-H tumors) is associated with the luminal B subtype. Thus, patients with PRB-H tumors would have lower survival compared with PRA-H patients. This study aimed to compare the metastatic and proliferative patterns of a PRA-H murine antiprogestin-sensitive tumor (C7-2-HI) with its antiprogestin-resistant PRB-H variant (C7-2-HIR) and to search candidates to decipher their biological behavior. Two approaches were performed. To test early metastases, tumors were inoculated subcutaneously in BALB/c mice and euthanized after 42 days (n=7-8/group). Lungs were histologically examined. A higher number of foci were generated by the PRA-H tumor compared to the PRB-H counterpart (Mann-Whitney, $p<0.01$), regardless that the proliferation rate of the former was almost twice lower than the latter (linear regression, $p<0.01$). In the late metastases setting surgery was performed in both groups (n=5-8/group) at similar tumor sizes, and lungs were examined after 90 days. The PRA-H model generated several lung foci, and in the PRB-H model, there were either none or few huge foci ($p<0.05$). A similar trend was observed when tumors were excised at the same time points. RNA-Seq studies showed up-regulation in motility and migration genes and a down-regulation in the G2 phase genes in the PRA-H tumor (log fold change (LFC) > 1 and FDR < 0.05) compared to the PRB-H variant. These results confirm that when PRA-H tumors progress to a PRB-H phenotype there is an increase in the proliferative state in detriment of the metastatic ability. Depending on the metastatic spread before surgery, PRA-H tumors may have an increased number of foci than PRB-H tumors, but they might grow very slowly. On the contrary, the PRB-H metastasis may grow so fast that may affect normal organ function in a shorter time.

Biology of luminal breast cancer

Poster No. 73

Effect of CDK2 inhibitors on cell proliferation in an experimental human breast cancer model.

Pedro Joaquín Villagra Delgado[1], Lucía Vottero[1], Claudia Lanaria[1], Victoria Fabris[1]
1- INSTITUTO DE BIOLOGÍA Y MEDICINA EXPERIMENTAL.

Presenting Author:
Pedro Joaquín Villagra Delgado
Undergraduate Student - INSTITUTO DE BIOLOGÍA Y MEDICINA EXPERIMENTAL
EGT, Argentina
Email: *pjoaquinvilla@gmail.com*

Seventy percent of breast cancer express hormonal receptors and are treated with endocrine therapy, which targets mainly the estrogen receptor (ER) pathway. Progesterone receptor (PR) has also been explored as a therapeutic target and antiprogestins have been proposed to treat luminal breast carcinomas expressing higher levels of isoform A of PR (PRA) than isoform B (PRB). Since in luminal carcinomas the cyclin D1/RB/E2F cell cycle pathway is highly activated, CDK4/6 inhibitors are used to treat metastatic ER+/HER2- breast cancer in combination with endocrine therapy. However, resistant tumor cells may bypass the cell cycle inhibition through the activation of CDK2. Thus, CDK2 inhibitors in combination with endocrine therapy and/or CDK4/6 inhibitors may be an option to treat these tumors. Our study aims to evaluate the effect of two CDK2 inhibitors, Roscovitine (Rosco) and Dinaciclib in T47D cells which express both PR isoforms, and in the T47D variants that only express isoform PRA (T47D-YA) or PRB (T47D-YB), alone or in combination with the antiprogestin mifepristone. The three cell lines were similarly inhibited by Rosco with an IC50 of 12 μM. Rosco (5 and 10 μM) inhibited serum and hormone-induced T47D cell proliferation, with a decrease in ki-67 expression and RB phosphorylation ($p<0.01$). The combined treatment of Rosco (2 μM) and mifepristone (10 nM) was more efficient to inhibit cell proliferation only in T47D-YA cells ($p<0.05$). Dinaciclib (10 nM) inhibited the proliferation of T47D-YA and -YB cells (25% and 38% of inhibition respectively, $p<0.01$). The combined studies are in progress. We conclude that Roscovitine and Dinaciclib would be efficient to treat luminal breast carcinomas and the combination of mifepristone with Roscovitine may be a therapeutic option in tumors expressing higher levels of PRA than PRB.

Poster Session 2

Breast Cancer Genomics and Transcriptomics

Poster No. 02

Adrenergic receptor expression from public breast cancer databases

Celine Almeida Gouvêa[1], María Sol Rodríguez[1], Cecilia Pérez Piñero[1], Isabel Alicia Lüthy[1]
1- Instituto de Biología y Medicina Experimental – CONICET.

Presenting Author:
Celine Almeida Gouvêa
PhD Fellow - Instituto de Biología y Medicina Experimental – CONICET
Buenos Aires, Argentina
Email: *gouveaceline@gmail.com*

Breast cancer is the most diagnosed female malignancy globally (23% of all cancers in females) with 15% mortality by this disease, as informed by GLOBOCAN for 2022. Our group has characterized the expression of α2-adrenergic receptors in breast cancer models. We have described that α2B (ADRA2B) and α2C (ADRA2C) are expressed in most of the cell lines and their stimulation is associated with cell proliferation and tumor growth while the stimulation of β2 (ADRB2) has the opposite effect. We have previously investigated GEO data for adrenergic receptor expression and its relationship with disease-free survival (DFS). The present investigation aimed to analyze TCGA and METABRIC databases to assess this relation. We found that ADRA2A expression was higher in luminal A, while ADRA2B was higher in basal-like in both databases. ADRB2 was lowest in the basal-like BrCa subtype. Then, we performed Kaplan Meier analysis for the data. Data from METABRIC patients revealed that high expression of ADRA2A was associated with significantly better DFS in luminal A and B, claudin-low and normal subtypes. TCGA data showed that only luminal A ADRA2A had this effect. ADRB2 high expression was associated with better DFS in luminal B and claudin-low tumors, while in TCGA only in the whole cohort, but lost significance when analyzing by subtypes (as previously seen in GEO). On the contrary, in METABRIC, ADRA2B high expression had a significantly lower DFS in luminal in general, HER2, claudin-low and normal. In TCGA the significantly lower DFS was in luminal A and basal. The previous study in GEO showed no effect of this receptor on DSF. For ADRA2C, a high expression was associated with lesser DFS in luminal B and claudin-low in METABRIC and none in TCGA. These results, with our previous on GEO ones, show that the results obtained interrogating different databases can vary, probably due to different ancestry, highlighting the importance of investigate several ones with different ancestry.

Breast Cancer Genomics and Transcriptomics

Poster No. 08

Transcriptomic profile of primary luminal breast carcinomas with imbalanced progesterone receptor isoforms expression

Andres Elia[1], Leo Saldain[1], Joaquin Merlo[1], Paula Martínez Vazquez[2], Javier Burruchaga[2], Eunice Spengler[2], Paola Rojas[1], Claudia Lanari[1]
1- Instituto de Biología y Medicina Experimental, 2- Hospital Magdalena V de Martinez.

Presenting Author:
Andres Elia
Postdoc Fellow - IBYME
La plata, Argentina
Email: *elia.andresm@gmail.com*

Luminal breast carcinomas usually show imbalanced levels of progesterone receptor (PR) isoforms A (PRA) and B (PRB). The clinical impact of the PRA/PRB ratio remains poorly understood. We have previously demonstrated that tumors with higher levels of PRB than PRA (PRB-H) expressed higher Ki67 and HER2, and lower PR levels than those with higher levels of PRA than PRB (PRA-H). In agreement, transcriptomic studies matched the PRB-H pattern with the luminal B subtype and the PRA-H pattern with the luminal A subtype.
Since, this cohort included samples with different histological types, or HER2 expression, this study aimed to find differential signatures among PRA-H and PRB-H tumors in a homogeneous cohort of samples and select candidates to identify PRA-H tumors. The relevance relies in that only these tumors would respond to an antiprogestin therapy.
Patients are routinely accrued at the Hospital Magdalena V de Martinez, Argentina. Surgery samples are frozen or fixed. Western blots categorize tumors as PRA-H or PRB-H. A total of 18 primary IC-NST tumors (11 PRA-H and 8 PRB-H) passed the control quality to perform a Poly(A) RNA sequencing study.
Among 14,771 genes, 129 were related to the PR isoform imbalance. Various followed the same trend observed previously such as CRISP3 or KRT16. Gene set enrichment analysis showed an up modulation of pathways related to proliferation, such as PI3K/AKT/mTOR and KRAS in PRB-H tumors (Hallmark gene set, NES > 1.7, $p < 0.05$) and a downmodulation of substrate adhesion and cell junction assembly biological processes in PRA-H tumors, suggesting a more invasive profile (GO biological process, NES < -1.6, $p < 0.05$).
Finally, we selected a set of 20-40 genes that recapitulate the biological differences observed between both groups that may be used to design a diagnostic panel to predict which patients would respond to an antiprogestin treatment

Breast Cancer Genomics and Transcriptomics

Poster No. 11

Expression and Subcellular Localization of TP73 Isoforms as Prognostic Factors in Breast Cancer Molecular Subtypes

Laura C Gomez[1,2], Mayra L Sottile[3], Analía L Redondo[1,3], Laura M Vargas-Roig[1,2,3]
1- Laboratorio de Biología Tumoral, IMBECU-CCT CONICET Mendoza, 2- Facultad de Ciencias Médicas, Universidad de Mendoza., 3- Facultad de Ciencias Médicas, Universidad Nacional de Cuyo.

Presenting Author:
Laura Gomez
Researcher - Laboratorio Biología Tumoral . IMBECU-CCT CONICET Mendoza
Mendoza, Argentina
Email: *glconstanza@gmail.com*

Breast cancer (BC) is a prevalent disease in our country. BC are classified into molecular subtypes based on gene expression variations: normal breast-like, luminal A, luminal B, HER2, and basal-like. We previously reported significant differences in TP73 methylation among these subtypes. Luminal A tumors often show unmethylated TP73, while all basal-like tumors exhibit methylated TP73. The p73 protein is structurally and functionally like the p53 tumor protein. However, the TP73 gene produces multiple isoforms that have antagonistic properties.
Here, we analyzed the expression differences of TP73 exons in the TCGA breast cancer RNASeq dataset using EdgeR tools. This allowed us to identify the prevalent isoforms expressed in each BC subtype. Additionally, we examined the expression and subcellular localization of the TA-p73 and ΔNp73 isoforms in 137 invasive breast tumors and 5 BC cell lines using immunohistochemistry and immunofluorescence techniques. Our results revealed that TP73 is overexpressed in all BC subtypes. Surprisingly, this up-regulation depends mostly on the over-expression of exons corresponding to the TAp73-specific domains. Notably, significant downregulation of the 4th exon was observed in all subtypes, suggesting a differential gene promoter usage favoring TAp73 isoform expression. Furthermore, we found that the TA-p73 isoform predominantly localized in the nucleus of luminal BC tumors, while basal-like and ErbB2+ tumors exhibited cytoplasmic expression. In contrast, the ΔNp73 isoform was mainly localized in the cytoplasm of all breast tumor subtypes. Additionally, the TA-p73 isoform was mostly nuclear in luminal BC cell lines, whereas it was primarily cytoplasmic in basal-like cell lines. These findings provide valuable insights into the expression patterns and subcellular localization of TP73 isoforms in different BC subtypes, highlighting the complex biology of TP73 and its potential implications for prognosis and treatment.

Breast Cancer Genomics and Transcriptomics

Poster No. 14

WARNING! Your cell line is just a model. A study of COL1A1 alternative promoters in breast cancer cells.

Martin Iungman[1,2], Pedro J. Salaberry[1,2], Ignacio E. Schor[1,2]
1- Instituto de Fisiología, Biología Molecular y Neurociencias (UBA-CONICET), Buenos Aires, Argentina. 2- Departamento de Fisiología, Biología Molecular y Celular, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina.

Presenting Author:
Martin Iungman
PhD Fellow - IFIBYNE
Capital Federal, Argentina
Email: *martin.iungman@gmail.com*

Although tumor DNA sequencing uncovers frequent mutations in non-coding areas, their role in cancer development remains poorly understood. To address this gap, we investigated the impact of these mutations in regulatory regions of genes linked to the aggressive behavior of triple-negative breast cancer cells. During our exploration, COL1A1 stood up as a strong candidate, with a putative mutation in an unannotated intronic alternative promoter. COL1A1 gene codes for type I collagen, a constituent of the extracellular matrix and is a canonical target of the TGFb pathway, with a predominantly main protein-coding isoform consisting of 51 exons and only one annotated promoter. However, CAGE data from breast cancer (BC) cell lines showed the predominant usage of an alternative transcription start site, located close to the 3' end of the first intron, coinciding with a reported enhancer. This potential transcript was confirmed by RT-PCR from extracted RNA of multiple BC cell lines. We could further validate these findings by looking at RNA splicing patterns across various cell lines with RNA-seq. To account for potential artifacts arising from culturing cells in a flat dish (2D), which may not fully represent real tumors, we compared these findings to data from MDA-MB-231 cells grown in a 3D culture system and observed the same results. However, when we analyzed RNA-seq data from eight BC patients, all of them categorically used the canonical promoter. We conclude that the misregulation of COL1A1 promoter usage is probably inherent to a cell line artifact and not inherent to BC tumor biology. Since this could be the case for several "cancer-specific isoforms", we are undertaking a systematic comparison between RNA isoform usage in BC cell lines and patients' samples. BC cell lines are great models to explore cancer biology, although they might present many anomalies with uncertain causes hence the extrapolation of results to clinical patients must be made with caution.

Breast Cancer Genomics and Transcriptomics

Poster No. 17

ANALYSIS OF TRISTETRAPOLIN (TTP) BIOLOGICAL ROLE AND TRANSCRIPTIONAL REGULATION IN BREAST CANCER CELLS

Angela Lara Montero[1], Pedro Salaberry[1], Micaela Stedile[1], Karina Cicinelli[1], Noemi Jordanovski[2], Eliana Querol[2], Florencia Costa[2], Mary Carrasco[2], Daniela Maltagliatti[2], Verónica Sanchotena[2], Laura Leguina[2], Silvia Vornetti[2], Nicasio Cuneo[2], Eva Wertheimer[3], Claudia Arias[2], Gabriela Acosta[2], Diego Flaks[2], Edith Kordon[1,4]

1 Institute of Physiology, Molecular Biology, and Neurosciences (IFIBYNE-CONICET), University of Buenos Aires, CABA, Argentina, 2 Marie Curie Oncology Hospital, CABA, Argentina, 3 Center of Pharmacological and Botanical Studies (CEFYBO-CONICET), University of Buenos Aires, CABA, Argentina. 4 Department of Biological Chemistry (DQB), Faculty of Exact and Natural Sciences. (FCEN), University of Buenos Aires, CABA, Argentina.

Presenting Author:
Angela Lara Montero
Postdoc Fellow - Instituto de Fisiología, Biología Molecular y Neurociencias (IFIBYNE)
CABA, Argentina
Email: *angelalaramt@gmail.com*

Tristetraprolin (TTP) is an anti-inflammatory and tumor suppressor protein that induces the degradation of specific mRNAs. We and others have reported that TTP is downregulated in invasive breast cancer (BC) compared with normal tissue. To better understand the role of this protein in BC development, we carried out an integrative analysis using data from The Cancer Genome Atlas, the Molecular Taxonomy of Breast Cancer International Consortium, the Clinical Proteomic Tumor Analysis Consortium, and samples from Hospital Curie BC patients. Using this information, we found that BCs display lower TTP expression than adjacent normal tissues. In addition, TTP levels were lower when the clinical stage of BC patients was higher, and reduced TTP expression was associated with poor prognosis in pre-menopausal women. Besides, we have determined a negative correlation between TTP and MKI67 expression in BC and their adjacent tissue, but not in normal breast samples. Finally, we found negative correlations between TTP levels and Ki-67 protein, tumor size as well as Cyclin D1 and TNFα mRNAs. These observations support the role of TTP as a tumor suppressor and anti-inflammatory protein in BC.
Surprisingly, we not only found a positive correlation between TTP and the inflammatory cytokine IL6 mRNAs in all BC molecular subtypes but also in normal tissue. Moreover, TTP expression correlated positively with phosphorylated STAT3 (a transcription factor commonly activated by IL6) levels. Therefore, we postulated that IL6 induces TTP expression through STAT3 activation in mammary cells. Our results show that this cytokine induces TTP expression in luminal breast cancer cells in culture in a time-dependent manner. We propose that this signaling pathway is part of inflammation normal regulation, which remains active in breast cancer cells. We postulate that our results may support the reported paradoxical activities of IL6 and STAT3 in normal and tumor mammary cells.

Breast Cancer Genomics and Transcriptomics

Poster No. 20

RNA-binding proteins in cancer: exploratory analysis of the neurodegenerative disease-related protein TDP-43

Lionel Muller Igaz[1], Lara Robalo[2], Maria Caçador[2], Mafalda Dias[2], Caroline Lamb[3], Luisa Alejandra Helguero[2]

1- IFIBIO Houssay, Grupo de Neurociencia de Sistemas, Facultad de Medicina, Universidad de Buenos Aires -CONICET, Buenos Aires, Argentina., 2- Department of Medical Sciences and Institute of Biomedicine – iBiMED, University of Aveiro., 3- Instituto de Biología y Medicina Experimental (IBYME), CONICET, Argentina.

Presenting Author:
Lionel Muller Igaz
Researcher - IFIBIO Houssay (CONICET-University of Buenos Aires)
Buenos AIres, Argentina
Email: *lmuller@fmed.uba.ar*

RNA-binding proteins (RBPs) play central roles in regulating posttranscriptional gene expression, and their abnormal expression or function amplify the effects of cancer-driver genes, accelerates tumor progression and promote aggressiveness. TDP-43 is a RBP that, amongst other functions, participates in mRNA metabolism. It is a key player in neurodegenerative diseases like frontotemporal dementia and amyotrophic lateral sclerosis. Although recent evidence suggests a potential role in other pathogenic processes, the involvement of this protein in cancer is still unclear. In this work, we aimed to i) investigate the differential expression of TDP-43 in different cancer types, and ii) analyze the levels and localization of TDP-43 in breast cancer (BC) cell lines in vitro (MCF7, MDA-MB-231) using different TDP-43 antibodies. For the first purpose, we interrogated the CPTAC public dataset (breast, glioblastoma, colon and lung) using cBioportal to characterize mutations in the TARDBP gene and stratify samples according to the 25% and 75% quartiles of TDP-43 expression to identify the overrepresented biological pathways. Interestingly, RBPs and RNA metabolism were the top pathways present in the 75% quartile. Secondly, we used different antibodies raised against the N-terminal (N-t), C-t or internal regions of TDP-43, to investigate the localization of TDP-43 in cultured BC cell lines. Remarkably, although most cells studied so far (in culture, or in rodent and human tissue) had a predominantly nuclear TDP-43 localization, we show evidence of abundant cytoplasmic expression in BC cell lines under basal culture conditions. Moreover, since TDP-43 cytoplasmic mislocalization and aggregation are considered as pathological features in human neurological diseases, we are expanding our analysis to determine TDP-43 levels, localization and aggregation in BC samples. In summary, we provide evidence for a potential role of TDP-43 in BC that deserves further investigation.

Breast Cancer Genomics and Transcriptomics

Poster No. 23

The Liver X Receptor interferes with Estrogen Receptor-dependent genomic regulation in MCF7 cells

Evelyn Olszanowski[1], María Florencia Ogara[1], Agustina Lafuente[1], A. Silvina Nacht[2], Belén Benitez[1,3], Santiago Andrés Rodríguez-Seguí[1,4], Diego Presman[1], Guillermo P. Vicent[5], Adali Pecci[1,6]

1- CONICET-Universidad de Buenos Aires, Instituto de Fisiología, Biología Molecular y Neurociencias (IFIBYNE), C1428EHA, Buenos Aires, Argentina. 2- Gene Regulation, Stem Cells and Cancer Program, Center for Genomic Regulation (CRG), The Barcelona Institute of Science and Technology, Barcelona 08003, Spain. 3- CONICET-Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales, Instituto de Química Biológica de la Facultad de Ciencias Exactas y Naturales (IQUIBICEN), C1428EHA, Buenos Aires, Argentina. 4- Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales, Departamento de Fisiología, Biología Molecular y Celular, C1428EHA, Buenos Aires, Argentina. 5- Molecular Biology Institute of Barcelona, Consejo Superior de Investigaciones Científicas (IBMB-CSIC), Barcelona, 08028, Spain. 6- Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales, Departamento de Química Biológica, C1428EHA, Buenos Aires, Argentina.

Presenting Author:
Evelyn Olszanowski
PhD Fellow - IFIBYNE
CABA, Argentina
Email: *eve.olszanowski@gmail.com*

Liver X Receptors (LXRs) belong to the nuclear receptor's superfamily of ligand-activated transcription factors, whose endogenous agonists are oxysterols. They play a key role in the regulation of cholesterol homeostasis, induce the de novo synthesis of triacylglycerides, and counteract pro-inflammatory effects. LXRs are also known to compromise cell proliferation in several cancer models. However, their role in breast cancer (BC) has not been depth studied. Here, we have examined the potential involvement of LXRs in BC cells with special emphasis on their possible crosstalk with the Estrogen Receptor alpha (ERα). We performed colony formation (CFA) and propidium iodide staining assays in MCF-7 cells treated with or without the ERα agonist, Estradiol (E2) and the LXR synthetic agonist, GW3965. Our results showed that GW3965 impaired the cell proliferation capacity induced by E2. To understand the functional pathways involved in these effects, we performed bulk RNA-seq experiments. The differentially expressed genes between E2 and E2+GW3965 conditions revealed several genes whose expression was affected by GW3965, which are widely enriched in terms associated to DNA replication, cell cycle checkpoint signaling and transition between the cell cycle stages (padj<0.05, DESeq2 and clusterProfiler) including genes such as E2F1, E2F7, PCNA, BRCA1, CCNE2, CDK2, CDC7, MKI67, EEIG1 and the MCM family. In addition, we studied the dynamics of the nuclear organization of the Liver X Receptor in LXR-GFP transfected MCF-7 live cells using super-resolution microscopy (Airyscan). We found that the combined treatment (E2+GW3965) led to a decrease in the number of nuclear condensates (foci) of LXR, compared to the GW3965 treatment alone. These results combined show that the LXR interferes with Estrogen Receptor-dependent genomic regulation in MCF7 cells, contributing to the idea of an interaction between these two receptors.

Breast Cancer Genomics and Transcriptomics

Poster No. 26

UNCOVERING GENE EXPRESSION REGULATION OF R-SPONDIN3, AN ONCOGENE INVOLVED IN BREAST CANCER PROGRESSION

Ana Laura Ortiz[1], Carla María Felcher[1], Pedro Javier Salaberry[1], Marcos Palavecino[1], Edith Claudia Kordon[1,2]

1- Instituto de Fisiología, Biología Molecular y Neurociencias-CONICET-UBA, Argentina, 2- Departamento de Química Biológica (DQB), Facultad de Ciencias Exactas y Naturales. (FCEN), Universidad de Buenos Aires (UBA), Argentina.

Presenting Author:
Ana Laura Ortiz
PhD Fellow - Instituto de Fisiología, Biología Molecular y Neurociencias
Ciudad Autónoma de Buenos Aires, Argentina
Email: *aniortiz97@gmail.com*

We have determined that R-spondin3 (RSPO3), a secreted protein that potentiates Wnt signaling pathway, is a key modulator of tumor progression and stem cell behavior in triple-negative (TN) breast cancer (BC) cells. The highest RSPO3 mRNA expression levels have been detected in TN cells and TNBC tumors compared with the other BC subtypes. However, immunohistochemical analysis showed that 70% of tumor samples of patients are positive for RSPO3, regardless of its molecular classification.
Pharmacological blockade of RUNX-CBFβ activity inhibited RSPO3 expression in the TN cell line MDA-MB-231. In these cells, it has been determined that RUNX1 binds to its DNA motif at the end of RSPO3 first intron. Our in silico analyses suggest that this site constitutes a relevant putative regulatory region of the human RSPO3 gene (named RE4), as indicated by combined bioinformatic studies of publicly available data from ChIP-seq and ATAC-seq, as well as transcription factor (TF) binding motives in the human genome. Besides, not only RUNX1, but also STAT3, and the SWI/SNF chromatin remodeling complex bind to RE4, while the presence of estrogen receptor (ER) was detected attached to the promoter region of RSPO3 in luminal BC cells. Therefore, our goal is to analyze the regulation of RSPO3 gene expression in a subset of human BC cell lines, which includes both luminal and TN phenotypes. Our preliminary results indicate that RUNX-CBFβ regulates RSPO3 in various TNBC cell lines, while in luminal cells, expression of this oncogene is induced by estradiol treatment. We are currently analyzing the possible interaction between RUNX1 and ER in luminal cells and the putative involvement of the SWI/SNF complex in RSPO3 expression regulation. In summary, we propose that differential regulatory mechanisms would be responsible for ER+ and ER- high RSPO3 protein levels found in BC patient samples.

Breast Cancer Genomics and Transcriptomics

Poster No. 29

GENOMIC PROFILE OF GENETIC VARIANTS ASSOCIATED WITH HEREDITARY BREAST/ OVARIAN CANCER: EXPERIENCE FROM THE ANALYSIS OF MULTIGENE PANEL TESTING IN ARGENTINA

Mayra Lis Sottile[1,2], Analia Lourdes Redondo[1,2], Laura Constanza Gómez[1], Cecilia Montes[3], Alejandra Mampel[2,4], Laura Maria Vargas-Roig[1,2]

1- IMBECU, CCT Mendoza, CONICET, UNCuyo, Argentina, 2- Facultad de Ciencias Médicas, UNCuyo, Mendoza, Argentina, 3- Instituto Modelo de Ginecología & Obstetricia, Córdoba, Argentina, 4- Centro Oncológico de Integración Regional (COIR), Mendoza, Argentina.

Presenting Author:
Analia Lourdes Redondo
Researcher - IMBECU CCT Mendoza
Mendoza, Argentina
Email: *analia_redondo@yahoo.com.ar*

Breast cancer (BC) is a disease with high incidence in Argentina and the leading cause of cancer death in women in our country. Hereditary BC and ovarian cancer (HBOC) are mainly caused by deleterious germline mutations in BRCA1 or BRCA2 genes. However, a number of these cancers are due to germline mutations in other susceptibility genes with low frequency or reduced penetrance. We aimed to characterize the frequency of pathogenic (P) and likely pathogenic (LP) variants in HBOC susceptibility genes in the Argentine population. 354 women from Mendoza and Cordoba with early-onset BC/OC or a family history of cancer were included. Patients were tested using NGS panel containing at least 14 high- and moderate-penetrance HBOC genes. P and LP variants were identified in 17.2% (61/354) of cases. Fifty-one of these carriers presented a diagnosis of BC (3 with bilateral BC), 5 of OC, and 5 were healthy women. The mean age of cancer diagnosis was 41.5 years. Thirty (8.5%) patients carry germline P/LP variants in BRCA1/2 and 31 (8.7%) in other HBOC susceptibility genes. A total of 66 P/LP variants were detected: 30/66 (45.4%) in BRCA1/2, 10 in PALB2 (15.2%), 5 in CHEK2 (7.6%), 3 in TP53 (4.5%), 3 in ATM (4.5%), 2 (3%) in RAD51C, MLH1, MUTYH, MITF and PMS2, and 5 in other less frequent genes. Four (0.85%) patients were double heterozygote carriers of germline P/LP variants. The co-occurrence of P/LP variants was identified in the following genes: BRCA2+BARD1, TP53+CDKN2A, MLH1+MITF and B RCA2+CHEK2. In addition, a novel LP variant was detected in BRCA2: c.1744del (p.Thr582LeufsTer2), a frameshift mutation that introduces a premature stop codon in the protein. Our findings would indicate that multigene panel testing gives more accurate information on cancer risk and thus would allow the implementation of more appropriate surveillance strategies in germline P/LP variants carriers.

Breast Cancer Genomics and Transcriptomics

Poster No. 32

Differential Response to All-Trans Retinoic Acid in Breast Cancer Cells: Effects on Metastatic Pathways and Gene Expression

Fiorella Vanderhoeven[1], Joselina Mondaca[2], Analía Redondo[1], Angel Matias Sanchez[2], Marina Ines Flamini[1]

1- Laboratorio de Biología Tumoral. Instituto de Medicina y Biología Experimental de Cuyo (IMBECU). UNCuyo-CONICETLaboratorio de Biología Tumoral. Instituto de Medicina y Biología Experimental de Cuyo (IMBECU). UNCuyo-CONICET, 2- Laboratorio de Transducción de Señales y Movimiento Celular. Instituto de Medicina y Biología Experimental de Cuyo (IMBECU). UNCuyo-CONICET..

Presenting Author:
Fiorella Vanderhoeven
PhD Fellow - Laboratorio de Biología Tumoral- IMBECU-CCT-CONICET-MENDOZA
Mendoza, Argentina
Email: *fiiore.vander@gmail.com*

Breast cancer (BC) is women's most frequent malignant neoplasia and has a high mortality rate. All-trans retinoic acid (RA), a vitamin A-derived pleiotropic signaling molecule, regulates critical genetic programs and shows promise for treating various neoplasias, including BC. However, its use in solid tumors is limited. Previous studies have shown that sensitivity to RA varies among BC cells. We aimed to determine RA's effects on metastatic processes and characterize the expression profiles of target genes using the Gene Expression Omnibus (GEO) public repository and assess its impact on cell viability in different BC cell lines. We performed Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), and Differential Gene Expression (DGE) analysis, using the GSE103426 database. In-vitro MTT assays and RT-q-PCR experiments were conducted to evaluate the effect of RA on cell viability. Our GO analysis revealed that RA regulates biological processes such as cell migration, motility, epithelial cell differentiation, epithelial-mesenchymal transition, and apoptosis in MDA-MB-231 cells. KEGG analysis indicated that RA downregulates pathways involving differentiation, inflammation, proliferation, angiogenesis, and inhibitory pathways of apoptosis, invasion, migration, and metastasis. Through DGE, we demonstrated that RA modulated the expression of SRC, PTK2, VIM, and CTTN, validated by RT-q-PCR. We confirmed the absence of retinoic acid receptors (RARs) and the reduced ability of RA to inhibit MDA-MB-231 cell viability, indicating resistance to RA. Conversely, the viability of T-47D cells decreased at lower RA doses. In conclusion, despite the absence of RARs, RA affects and modulates mRNA levels in RA-resistant cells, downregulating pathways involved in metastatic processes. These results suggest RA could be significant as a potential therapy for metastatic breast cancers lacking specific treatment options.

Cancer Stem Cells, Primary Tumor Initiation and Early Metastasis

Poster No. 35

ANALYSIS OF GPC3 EFFECT ON CANCER STEM CELL POPULATIONS

Lizeth Ariza Bareño*[1], Maia Jazmin Martinez Gomez*[1], Diego Britez Neira[1], Andrés Bechis[1], Magali Delgado Pastore[1], Ana Clara Lugones[1], Laura Todaro[1], Alejandro Urtreger[1], María Giselle Peters[1]

1- Research Area. Institute of Oncology "Angel H. Roffo".

*equal contribution

Presenting Author:

Lizeth Ariza Bareño

PhD Fellow - Research Area. Institute of Oncology "Angel H. Roffo"

Buenos Aires, Argentina

Email: *lisa19111@hotmail.com*

Cancer stem cells (CSCs) are involved in cancer relapse and metastasis. These cells possess the ability to self-renew and differentiate into non-tumorigenic cell progeny. Additionally, the epithelial-mesenchymal transition (EMT) may either enhance or decrease the CSC population in a cell type-specific manner.

We have demonstrated that GPC3 expression reverses the EMT undergone by breast cancer cells and modulates several signaling pathways, including Wnt/β-Catenin, which is involved in CSC regulation. Given the relevance of CSCs in metastasis and the roles of EMT and GPC3 in this process, we aimed to investigate the relationship between GPC3 and breast CSCs.

We evaluated the mammosphere-forming ability of breast cancer cells with genetically modified GPC3 expression. We found that MDA-MB231-vector control cells form small clusters, whereas MDA-MB231-GPC3 cells developed large concentrically arranged mammospheres that were more numerous and had an average diameter about 50% larger than those of MDA-MB231-vector ones. On the other hand, the GPC3 silencing in MCF-7 cells did not affect morphology or size but resulted in a 3 to 4-fold reduction in mammosphere formation compared to control MCF-7 sh scramble cells.

Cancer Stem Cells, Primary Tumor Initiation and Early Metastasis

Poster No.38

The galectin-1-glycan axis in tumour progression and metastasis in breast cancer.

Magalí Berton[1], Ramiro Perrotta[1], Tomás Dalotto-Moreno[1], Yamil D. Mahmoud[1], Sabrina Gatto[1], Rosa Morales[1], Gabriel A. Rabinovich[1], Mariana Salatino[1]
1- Institute of Biology and Experimental Medicine (IBYME), CONICET.

Presenting Author:
Magalí Berton
PhD Fellow - Institute of Biology and Experimental Medicine (IBYME), CONICET
Buenos Aires, Argentina
Email: *magaliberton13@gmail.com*

Galectin-1 (Gal1), a glycan-binding protein, plays a key role in the creation of an immunosuppressed tumour microenvironment (TME) acting as a negative regulator of the immune response, fostering tumour immune escape, and stimulating an invasive, stem-like phenotype, enhancing dissemination in cancer. Our group focuses on the TME in breast cancer, which has the highest incidence amongst women worldwide. To further study the role of Gal1 in mammary tumour progression and metastasis, we developed a transgenic model using MMTV-PyMT mice WT or KO for lgals1-/- (PYMT-KO). Using this experimental model, we observed that PyMT-KO mice present an increased latency in tumour burden and a longer tumour-free survival time, with a lower number of transformed ducts and lung metastasis, alongside a reduced mammary gland branching morphogenesis (whole mount).
In hopes of understanding the role that Gal1 plays in mammary glands, we performed cell sorting based on surface markers EPCAM and CD49f to categorize the isolated mammary cells from WT and KO mice into four main populations: luminal, basal, stromal, and mammary stem cells (MaSC). We observed that lgals1-/- KO mice present a decreased percentage of luminal and MaSC cells in comparison to the Gal1 sufficient strain, which presents an enriched population of basal cells (spectral FACS). Each population's purity was evaluated by Real Time PCR using specific markers and we notoriously point out a reduction in the expression of progesterone receptor in the mammary gland and in the luminal population in the lgals1-/- KO mice. By scRNAseq (in silico analysis) and qPCR, we found that Gal1 is synthesized by basal cell lineages and MaSC cells, which may in turn promote branching morphogenesis. Our findings highlight the relevance of Gal1 in regulating normal mammary gland morphogenesis and assert its critical role in metastatic spreading in breast cancer.

Cancer Stem Cells, Primary Tumor Initiation and Early Metastasis

Poster No. 41

Lapatinib treatment induces cytotoxic effects on different tumor cell lines and decreasesf Stemness in triple-negative breast cancer cell lines.

Diego Javier Britez Neira[1], Andrés Bechis[1], Luciana Cañonero[1], Lizeth Aixa Ariza Bareño[1], Aldana Schey[1], Alejandro Jorge Urtreger[1], Laura Beatriz Todaro[1]
1- Instituto de Oncología "Ángel H. Roffo".

Presenting Author:
Diego Javier Britez Neira
PhD Fellow - Instituto de Oncología "Ángel H. Roffo"
Ciudad de Buenos Aires, Argentina
Email: *djbritezneira@gmail.com*

The human epidermal growth factor receptor (HER) family comprises tyrosine kinase receptors that play a critical role in breast and gastric cancers. Lapatinib, a dual tyrosine kinase inhibitor (TKI) that targets HER1 and HER2 by binding to the ATP-binding site of their intracellular domains. Currently, this inhibitor is indicated in combination with capecitabine in advanced HER2-positive breast cancer after progression following standard treatment (anthracyclines, taxanes and trastuzumab) and in postmenopausal patients in whom hormonal therapy is indicated.
Previously, our group had shown that HER2 was expressed and active (phosphorylated form) in the CSC subpopulation of several triple-negative and HER 2-negative mammary cancer cell lines. To study the implications of Lapatinib therapy in other breast tumor subtypes and its effect on CSCs, we proposed to study the effect of HER inhibitor therapy in different human and murine tumor cell models.
In this study, we observed decreased viability in different breast cancer models including human (HCC70, HS578T) and murine (4T1, LM38-LP) triple-negative, estrogen-positive (MCF7) and HER2-positive (BT-474) cell lines upon lapatinib treatment. Similar effects were also found in the human hepatocellular carcinoma (Hep G2, HUH7) and prostate cancer (PC3) cell lines.
Moreover, in different triple-negative breast cancer lines, a significant decrease in oncospheres (culture enriched in CSC) formation and size was also observed.
Finally, to elucidate the underlying mechanisms, we compared the effects of lapatinib with Trastuzumab (monoclonal antibody against HER2) and Cetuximab (monoclonal antibody against EGFR). Lapatinib significantly affected cell viability in several cell lines and Cetuximab showed similar effects. However, trastuzumab showed no effect.
Our findings, along with previous results, highlight the potential repositioning of lapatinib beyond HER2-positive breast cancer treatment.

Cancer Stem Cells, Primary Tumor Initiation and Early Metastasis

Poster No.44

The agrotoxicant chlorpyrifos increases CSC subpopulation and regulates the expression of CSC markers and molecular targets involved in resistance to antiestrogen therapy

Marianela Lasagna[1,2], Lucía Enriquez[1], Daniel Zappia[3], Mariana Mardirosian[1,2], Gabriela Martín[1], Noelia Miret[2,4], Andrea Randi[4], Mariel Núñez[2], Claudia Cocca[1,2]

1- Instituto de Química y Fisicoquímica Biológicas “Prof. Alejandro C. Paladini” (IQUIFIB) UBA-CONICET, Buenos Aires, Argentina., 2- Laboratorio de Radioisótopos, Cátedra de Física, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina., 3- Instituto de Investigaciones Farmacológicas (ININFA), UBA-CONICET, Buenos Aires, Argentina., 4- Laboratorio de Efectos Biológicos de Contaminantes Ambientales, Departamento de Bioquímica Humana, Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina.

Presenting Author:

Marianela Lasagna

Postdoc Fellow - Instituto de Química y Fisicoquímica Biológicas “Prof. Alejandro C. Paladini” (IQUIFIB) UBA-CONICET, Buenos Aires, Argentina

Buenos Aires, Argentina

Email: *marianelalasagna86@gmail.com*

The organophosphorus chlorpyrifos (CPF) is currently classified as an Endocrine Disruptor (EDs). EDs have been associated with resistance to endocrine therapy in breast cancer and loss of Estrogen Receptor α (ERα) expression is critical in this process. It is postulated that histone deacetylase 1 (HDAC1) interacts with ERα and suppresses ERα transcriptional activity. Additionally, Cancer Stem Cells (CSC) show a self-renewal capacity and differentiation potential that contribute to tumor progression and therapy resistance. CSC are characterized by the expression of stem cell markers such as OCT4, SOX2, Nanog, ALDH1A1 and CD44+/CD24-. In this study, we investigated whether CPF can induce mechanisms associated with resistance to antiestrogen therapy. Our experiments were performed using MCF-7 cell line. We analyzed if CPF (0.05 and 50 μM) induces CSC proliferation by mammosphere assay and/or CSC markes (CD44, CD24, Oct4 and Nanog) ERα, HDAC1 and the co-repressor SMRT expression in monolayer cells and mammospheres by RT-qPCR. CPF at 0.05 μM increases the subpopulation of CSC derived from MCF-7 cells ($p<0.05$), decreases ERα and HDAC1, and increases SMRT mRNA expression ($p<0.05$) in monolayer-grown cells. CPF 50 μM decreases SMRT ($p<0.05$) and CD24 ($p<0.01$) mRNA levels in these conditions. In mammospheres, both CPF concentrations induce a decreased ERS1 ($p<0.001$) and HDAC1 ($p<0.001$) expression, while only CPF 0.05 μM decreased SMRT ($p<0.01$) levels. We observed that CPF 0.05 upregulates CD44 ($p<0.01$) and Oct4 ($p<0.001$) and downregulates CD24 ($p<0.01$) expression. CPF 50 μM enhances the expression of Oct4 ($p<0.01$) and Nanog ($p<0.05$). Our study demonstrates that CPF can increase CSC subpopulation and can modulate the expression of CSC markers and molecular targets involved in antiestrogen therapy resistance. Therefore, CPF exposure could affect the outcome of breast cancer patients treated with endocrine therapy.

Cancer Stem Cells, Primary Tumor Initiation and Early Metastasis

Poster No. 47

RUNX1 TRANSCRIPTIONAL ACTIVITY FAVORS A FINGERPRINT OF DRUG RESISTANCE IN TNBC CELL LINES

SOFIA MARIA SOSA[1], FACUNDO COUTO[1], NATALIA FERNANDEZ[1], NATALIA RUBINSTEIN[1]
1- Instituto de Biociencias, Biotecnología y Biología Traslacional (iB3); Departamento de Fisiología, Biología Molecular y Celular (FBMC), Facultad de Ciencias Exactas y Naturales (FCEN), Universidad de Buenos Aires (UBA)..

Presenting Author:
SOFIA MARIA SOSA
PhD Fellow - Instituto iB3
CABA, Argentina
Email: *sofiamariasosa@gmail.com*

Triple-negative breast cancer (TNBC) is associated with epithelial-mesenchymal transition (EMT) and an enrichment in cancer stem cells (CSC) which are both involved in tumor chemoresistance and metastasis. Our group has shown that RUNX1 is implicated in the aggressiveness of this breast cancer subtype by promoting cell migration and regulating tumor gene expression, and in chemoresistance of TNBC-androgen responder cell lines. However, the mechanisms involved are still undetermined. Moreover, RUNX1 protein expression in TNBC correlates with poor patient prognosis. We aimed to evaluate RUNX1 relevance during drug treatment in human TNBC. Here we show that using a RUNX1 transcriptional activity commercial inhibitor (AI-10-104) in TNBC MDA-MB-231 and -468 cell lines there is a significant decrease in cell viability and migration and a significant increase in apoptosis. Interestingly, in a forced suspension cell model (which promotes a CSC phenotype), RUNX1 expression is significantly increased compared to the attached cell population, and the inhibition of RUNX1 transcriptional activity decreases OCT4, ABCC1 and ALDH1 gene expression in the forced-suspended subpopulation. Moreover, RUNX1 inhibition in the MDA-MB-231 cell line prevents mammosphere formation capacity. RUNX1 mRNA is significantly upregulated in doxorubicin (Doxo)- and paclitaxel (Px)-treated cell lines. Also, its protein expression is upregulated in PX resistant cell lines. We determined that loss of RUNX1 transcriptional activity significantly enhances Doxo and Px toxicity in TNBC cell lines by reducing viability and enhancing apoptosis. Also, mammospheres already established and then treated simultaneously with Doxo and AI-10-104 show a significant number reduction compared with Doxo alone. In addition, PX-resistant MDA-MB-468 cell line treated with AI-10-104 recovers Px sensitivity. Therefore, our data strongly suggests that RUNX1 may be involved in the generation of TNBC chemoresistant cells.

Early detection and treatment

Poster No. 50

Anticancer activity of novel copper (II) compounds with an acylhydrazone ligands against 2D and 3D human breast cancer models.

Lucía M. Balsa[1], Olivia Espindola Moreno[1], Lucía Santa Maria de la Parra[1], Fagner da Silva Moura[2], Nicolás A. Rey[2], Ignacio E. León[1]

1- CEQUINOR (UNLP, CCT-CONICET La Plata, asociado a CIC), La Plata, Argentina, 2- Department of Chemistry, Pontifical Catholic University of Rio de Janeiro, RJ, Brazil.

Presenting Author:
Lucía Mariana Balsa
Postdoc Fellow - CEQUINOR (CONICET-UNLP)
La Plata, Argentina
Email: *luciambalsa@gmail.com*

Breast cancer is the most common cancer in women. Triple Negative Breast Cancer (TNBC) is the greatest invasive class, with a poor prognosis due to side effects exerted by chemotherapy and the low effectiveness of novel treatments. In this sense, copper compounds have shown to be potentially effective as antitumor agents, attracting increasing interest as alternatives to usually employed platinum-derived drugs.
This work aims to evaluate the antitumoral activity of a series of copper (II) compounds in 2D and 3D cancer models: two monomeric complexes derived from thiophene hydrazide (1) and methoxyphenyl hydrazide (2), and a dimeric complex derived from furane hydrazide (3).
The cytotoxic activity in the 2D model was tested against two breast cancer cell lines MDA-MB-231 (TNBC) and MCF7. The complexes significantly reduced cell viability in both cell lines (MDA-MB-231 IC50: 1 1.65μM, 2 1.56μM, 3 1.30μM; MCF7 1 1.67μM, 2 1.70μM).
Antitumoral activity of 1 and 2 was tested in MCF7 3D models. Complexes diminished the cell viability of spheroids (IC50: 1 2.64μM; 2 2.23μM), affecting the spherical shape. Moreover, pretreatment of cells with 1 and 2 lead to a decrease in mammosphere formation.
Further analysis demonstrated that the complexes conveyed cells to apoptosis and decreased the cancer stem cell population. Moreover, 1 and 2 showed a great genotoxicity, and inhibition of proteasomal activity.
Finally, label-free quantitative proteomics was used to identify the molecular mechanisms of 1 and 2 in TNBC cells. Both complexes increased proteins involved in ER stress and UPR, as well as down-regulated proteins related to DNA replication and repair, in addition to GOF-mutant p53. Moreover, we found a novel and interesting effect for a copper metallodrug, the downregulation of proteins related to lipid synthesis and metabolism.
Taken together, these results suggest that these complexes are good potential candidates to evaluate on breast in vivo assays.

Early detection and treatment

Poster No. 53

Transcriptional factor STAT3 as a potential target for nanoparticle-delivered therapeutics, towards CD44+ breast cancer cells

Ariadna Birocco[1], Agustín Blachman[1], Lucía Quiroz[1], Juan Manuel Lázaro Martinez[2], Sergio Moya[3], Graciela Calabrese[1]

1- Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Departamento de Ciencias Biológicas, Cátedra de Biología Celular y Molecular, Ciudad Autónoma de Buenos Aires, Argentina, 2- Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica. Cátedra de Química Orgánica II, Departamento de Química Orgánica. Instituto de Química y Metabolismo del Fármaco (IQUIMEFA) UBA-CONICET. Ciudad Autónoma de Buenos Aires, Argentina., 3- Center for Cooperative Research in Biomaterials (CIC biomaGUNE), Basque Research and Technology Alliance (BRTA), Donostia-San Sebastian, Spain, 4-

Presenting Author:

Ariadna Birocco

PhD Fellow - Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica
Ciudad autónoma de Buenos Aires, Argentina
Email: *birocco.ariadna@gmail.com*

Despite advances, metastasis continues to be the main reason for therapeutic failure in breast cancer (BC), making it crucial to develop new strategies that target this process. BC cells responsible for tumor dissemination have an invasive and proliferative phenotype; with a deregulation of the STAT3 pathway. These cells also overexpress CD44, a hyaluronic acid receptor that can bind many extracellular matrix components, such as Dermatan Sulfate (DS). Our research group reported nanoparticles (NPs) based on chitosan and DS loaded with the IRW. This delivery platform selectively interacts and is internalized through the CD44 receptor and modulates the pharmacological stress tolerance to 5-fluoracil in colorectal cancer. The present work describes the study of new NPs designed to deliver anti STAT3 compounds towards BC cells: (1) DS/chitosan NPs, loaded with Flubendazole (Flu-NPs), an antihelminthic repurposed as an anti-STAT3 antitumoral agent, and (2) NPs loaded with siRNA (siRNA-NPs). Both formulations were synthesized by ionotropic gelation and displayed a similar hydrodynamic diameter (Flu-NPs: 199±48 nm and siRNA.NPs: 202±28 nm). PEC characterization confirmed that both nanoformulations can encapsulate Flu (100 µM) and scramble siRNA (1 µM). Uptake was studied by confocal microscopy and flow cytometry, using two human BC cell lines with different CD44 expression, MDA-MB-231 and MCF-7. In both analyses, there was a positive correlation between NPs uptake and CD44 expression. Regarding its effects, wound healing assays and proliferative assays were conducted to study migration and proliferation. Flu-NPs show a significantly higher inhibition of cell viability and cell migration in MDA-MB-231 cultures, compared to the CD44-low expressing MCF-7 cells. These results support the use of both NPs as a strategy for delivering active compounds targeting STAT3, to inhibit cellular dissemination in BC.

Early detection and treatment

Poster No.56

MIR-28 IMPAIRS PROLIFERATION AND METASTATIC CHARACTERISTICS OF TRIPLE NEGATIVE BREAST CANCER CELLS

Athina Carducci Sartorio[1], Juana Moro[1], Agustina Grinpelc[1], Karen Daniela Graña[1], Leandro Vera-Sanchez[1], Flavia Piccioni[2], Fiorella Campo Verde Arbocco[3], Adriana De Siervi[1], Paola De Luca[1]
1- Instituto de Biología y Medicina Experimental (IBYME-CONICET), 2- Laboratorio de Inmunobiología del cáncer - Instituto de Investigaciones en Medicina Traslacional (IIMT) - Universidad Austral - CONICET, 3- Laboratorio de Hormonas y Biología del Cáncer - Laboratorio de Endocrinología de la Reproducción y Lactancia, IMBECU CONICET. Universidad de Mendoza, Facultad de Ciencias Médicas, 4-

Presenting Author:
Athina Carducci Sartorio
Undergraduate Student - Laboratorio de Oncología Molecular y Nuevos Blancos Terapéuticos, Instituto de Biología y Medicina Experimental (IBYME-CONICET)
Buenos Aires, Argentina
Email: *athina.sartorio@gmail.com*

Breast cancer (BC) is the most frequent tumor and the leading cause of cancer death in women worldwide. Triple-negative breast cancer (TNBC) is the histologic subtype with the worst prognosis and fewest therapeutic options. MiRNAs are small non-coding RNAs that regulate gene expression. Aberrant expression of miRNAs in body tissues are linked to pathologies like BC. Using bioinformatic approaches, we identified miRNAs whose expression is altered in BC tissue and correlates with patient survival, such as miR-28-3p and miR-28-5p, which are decreased in BC tissue compared to adjacent normal tissue. We aimed to investigate the effect of miR-28-3p/5p in TNBC. The hypothesis is that miR-28-3p/5p has tumor suppressor functions in TNBC.
We determined miR-28 expression levels in PAM50 basal-like BC tumors and normal mammary tissue (NT) from TCGA BRCA and GTEx project data sets. We found that miR-28-5p was significantly increased in basal-like BC tissue compared to NT. Analysis using UCSC Xena showed that miR-28-3p/5p expression is increased in basal-like BC compared to the other PAM50 BC subtypes. Expression of miR-28-3p correlates with higher disease-free interval and progression-free interval of basal-like BC patients.
To investigate the effect of mir-28 in TNBC, we generated stable-transfected 4T1 cells with an expression vector from this miRNA or control (pSGIPX). We evaluated the effect of the miRNAs in proliferation, clonogenicity, adhesion and migration through in vitro assays. We found that miR-28-3p/5p decreased cell viability, migration and adhesion, and clonogenic assay demonstrated that they decreased both the number and size of clonogenic foci.
Our results suggest that miR-28-3p/5p could have potential therapeutic implications limiting TNBC cells' ability to proliferate and spread by impairing the proliferation, clonogenic capability, adhesion and migration of TNBC cells.

Early detection and treatment

Poster No. 59

Exploring Racemic 5,5-(aryl-alkyl) substituted thiadiazolines as Promising Agents for Treatments of Breast Cancer

Tatiana Jazmin Goldberg[1], Aldana Maria Sólimo[1,2], Gabriel Jasinski[4], Albertina Moglioni[3,2], Mariana Callero[1,2]

1- Universidad de Buenos Aires. Instituto de Oncología Angel H. Roffo. Area de Investigaciones, 2- Consejo Nacional de Investigación Cientificas y Técnicas, 3- Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Instituto de la Química y Metabolismo del Fármaco., 4- Universidad de Buenos Aires, Facultad de Farmacia y Bioquíca. Cátedra de Química Medicinal.

Presenting Author:

Tatiana Jazmin Goldberg

Undergraduate Student - Universidad de Buenos Aires. Instituto de Oncología Angel H. Roffo. Area de Investigaciones Buenos Aires, Argentina

Email: *tatianajazmingoldberg@gmail.com*

Thiadiazolines (TDZs) are pentagonal heterocyclic compounds derived from thiosemicarbazones (TSCs). TDZs have exhibited promising biological effects as chemotherapeutic drugs, leading to the possibility that they could also prove efficacious against different forms of cancer. This is supported by the anti-tumor properties of various TSCs, their chemical precursors.

To explore the anti-tumor effects of four TDZs (B20, B21, B23, and B24) on breast cancer, experiments were conducted using human and murine breast cancer cell lines. The cytotoxicity of the compounds was assessed through MTS assay to determine IC50 values after 48 hours of treatment. It was observed that hormone-dependent cell lines were more responsive to B20 and B23 (both substituted with chlorine in positions 3 and 4 of the aromatic ring) compared to B21 and B24, while triple negative cell lines showed higher IC50 values for all the TDZs (LM05 Mix B20: 3.34uM, B21: 44uM, B23: 8.2uM, B24: 17.2uM; MCF-7 B20: 25uM, B21: 270uM, B23: 33.2uM, B24: 40uM; 4T1 B20: 380uM, B21: undefined, B23: 74.6uM, B24: 58.5uM, LM3 B20: 54.9uM, B21: undefined, B23: 41.8uM, B24: 64.4uM and MDA-MB-231 B20: 176.7uM, B21: undefined, B23: 141.8uM, B24: 52.3uM).

Furthermore, through the Ethidium Bromide and Acridine Orange staining technique, we found that the cytotoxicity of B20 and B23 on hormone-dependent cell lines was due to apoptosis. Additionally, the ability of LM05 Mix and MCF-7 cells to form colonies was reduced under the treatment of B20 and B23 compared to control cells (LM05 Mix B20: 36±6% and B23: 50,4±0.2%, MCF7: B20: 33±10% and B23: 43±12%, $p<0,05$). Moreover, the cell cycle analysis revealed that treatment with B20 and B23 led to a G2 phase cell cycle arrest in sensitive cells.

Overall, these findings suggest that B20 and B23 have promising anti-tumor effects on hormone-dependent breast cancer cells and warrant further investigation into their mechanism of action as potential therapies for breast cancer.

Early detection and treatment

Poster No. 62

Impact of let-7b-5p on triple negative breast cancer growth and progression.

Agustina Grinpelc[1], Juana Moro[1], Erick Merizalde[1], Athina Carducci Sartorio[1], Leandro Vera Sanchez[1], Karen Daniela Graña[1], Georgina Daniela Scalise[1], Flavia Piccioni[2], Fiorella Campo Verde Arbocco[3], Adriana De Siervi[1], Paola De Luca[1]

1- Instituto de Biología y Medicina Experimental (IBYME-CONICET)., 2- Laboratorio de Inmunobiología del cáncer - Instituto de Investigaciones en Medicina Traslacional (IIMT) - Universidad Austral - CONICET. 3. Laboratorio de Hormonas y Biología del Cáncer - Laboratorio de Endocrinología de la Reproducción y Lactancia, IMBECU CONICET. Universidad de Mendoza, Facultad de Ciencias Médicas.

Presenting Author:
Agustina Grinpelc
Researcher - Instituto de Biología y Medicina Experimental (IBYME-CONICET).
Buenos Aires, Argentina
Email: *agus.grin19@gmail.com*

Breast cancer (BC) is a leading cause of cancer-related deaths among women, with triple-negative breast cancer (TNBC) being particularly aggressive and challenging to treat.

MiRNAs are small non-coding RNAs that regulate gene expression. Their aberrant expression is linked to pathologies such as BC. Previously, we found that let-7b-5p was diminished in BC tissue compared to adjacent normal tissue using bioinformatic approaches. This work aimed to investigate the effect of let-7b-5p in TNBC. We hypothesize that let-7b-5p acts as a tumor suppressor in TNBC.

We analyzed let-7b-5p expression levels in PAM50 basal-like BC tumors and normal mammary tissue (NT) from TCGA BRCA and GTEx project data sets. We found that let-7b-5p was significantly diminished in PAM50 basal-like BC tissue compared to NT. We also found that its expression is decreased in basal-like BC compared to the other PAM50 BC molecular subtypes using UCSC Xena tool.
Then, we evaluated let-7b-5p effects in the TNBC cells, MDA-MB-231 and 4T1, using stable transfections with expression vectors or transient transfections with a let-7b-5p mimic or negative control (NC), respectively.
Overexpression of let-7b-5p reduced the clonogenic capability of MDA-MB-231 cells. On the other hand, let-7b-5p increased the viability under serum deprivation conditions and cell adhesion of 4T1 cells. Moreover, let-7b-5p reduced the migration of both, 4T1 and MDA-MB-231 cells.
Finally, the effect of treatment with a single dose of PEI nanoparticles containing a let-7b-5p mimic or NC was analyzed in female Balb/c mice with 4T1 orthotopic tumors. Tumor size was decreased on days 11 and 12 after treatment with the let-7b-5p mimic compared to NC.

These findings constitute the initial step for developing therapies for TNBC based on a specific let-7b-5p miRNA mimic. Further research is needed to understand the mechanisms by which let-7b-5p influences TNBC and evaluate its effect on metastasis.

Early detection and treatment

Poster No. 65

Design of a platform to rank chemotherapeutic drugs with a multidimensional approach

Florencia Hernandez[1], Lilen Ivonne Caimi[1], Ginette Moyano[1], Vanesa Gottifredi[1], Nicolás Luis Calzetta[1]
1- Instituto de Investigaciones Bioquímicas de Buenos Aires.

Presenting Author:
Florencia Hernandez
Undergraduate Student - Fundación Instituto Leloir
Buenos Aires, Argentina
Email: *fhernandez@leloir.org.ar*

Cancer is the leading cause of death worldwide. As a result, over 100 chemotherapy drugs have been approved to cure or control the disease, while there are still thousands more candidates undergoing clinical trials. One of the challenges currently faced by cancer researchers is the need to rank these drugs based on their safety and efficacy, as the only available variables. We propose to design, generate and test a novel platform that compares drugs using a multidimensional pipeline. Such an approach may provide substantial improvement the traditional unidimensional approach based on cancer cell-killing potential. By applying automated image capture by fluorescence microscopy, our platform is designed to simultaneously- and comparatively evaluate cytotoxic capacity, impact on the cell cycle profile, incorporation of genomic instability features and levels of DNA damage accumulation. A drug that (1) has high cytotoxic efficacy (2) causes massive DNA damage, and (3) causes cell death without leading to cell cycle arrest will be categorized as best candidate by our platform. We have succeeded in fine-tuning this platform using the DNA dye, DAPI, and the antibody against histone γH2AX as a DNA damage marker. Furthermore, we also adapted our setting to perform a novel imaging analysis called QIBC (quantitative imaging-based cytometry), which reveals the stage of the cell cycle at which a chemotherapeutic drug induces DNA damage. Such settings will therefore produce information related to the mode of action of the drug candidates and use that information to rank them. We initiated the development of this platform using the osteosarcoma cell line, U2OS, exposed to a checkpoint kinase 1 inhibitor (CHK1i) in our experimental setting that we have used extensively in the past. The pipeline will then be used to compare a list of drugs that is currently being evaluated in clinical trials to test the ranking power of our platform.

Early detection and treatment

Poster No.68

Use of ascitic fluid from patients to evaluate the mechanisms of action of PARP inhibitors

Ginette Moyano[1], Candelaria Mares Ahlers[1], Florencia Menay[2], Glenda Ernst[3], Ernesto Korbenfeld[3], Brenda Iglesias[4], Kevin Madauss[5], Helena Sterle[2], Florencia Cayrol[2], Graciela Cremaschi[2] , Sabrina Florencia Mansilla[1], Vanesa Gottifredi[1]

1- Fundación Instituto Leloir, Instituto de Investigaciones Bioquímicas de Buenos Aires, CONICET, 2- Instituto de Investigaciones Biomédicas UCA, CONICET, 3- 3Hospital Británico, Buenos Aires, Argentina, 4- GlaxoSmithKline, Oncology R&D, London, United Kingdom. 5 GlaxoSmithKline, Global Health R&D, Upper Providence, PA, United States

Presenting Author:
Ginette Moyano
PhD Fellow - Fundacion Instituto Leloir
CABA, Argentina
Email: *gmoyano@leloir.org.ar*

High-grade serous ovarian carcinoma (HGSOC) is the most common subtype of epithelial ovarian cancers (EOC), accounting for 70% of them. Approximately 50% of HGSOCs are associated with abnormalities in the homologous recombination (HR) pathway, such as mutations in breast cancer genes 1 and 2 (BRCA1 and BRCA2), resulting in defective repair of the DNA which is known as “homologous recombination deficiency”.
Furthermore, advanced stages of ovarian cancer are frequently associated with the accumulation of ascitic fluid in the patient's abdomen, known as ascites, which is made up of cellular and acellular components. This provides a direct source of patient tumor cells.
Once surgically removed, ascitic fluid has no value from a clinical perspective but provides a source of tumor cells directly from patients, which can be used to validate results obtained from commercial cell line assays. Likewise, they are useful to evaluate their sensitivity to different drugs, in our case, the poly ADP-ribose polymerase (PARPi) inhibitors Olaparib and Niraparib, which are a therapeutic strategy used to generate selective lethality of HR-deficient tumor cells.
We have established a work protocol generating primary cultures from tumor patient cells derived from ascitic fluid. Compared to other cancer types, primary cultures of ovarian cancer cells are relatively easy to obtain and establish in vitro due to their high viability, strong surface adhesion, and rapid cell division. Using serial trypsinizations we were able to select the tumor cells and eliminate the rest of the cell populations. We performed in vitro assays on already established primary cultures, which allowed us to characterize key markers for this type of tumor, such as HR, BRCA, p53, and CK7 status. We are currently evaluating the effect of different PARPi, looking for possible correlations between sensitivity to these drugs and the aforementioned markers.

Early detection and treatment

Poster No.71

Evaluation of Histamine H3 and H4 Receptors as Prognostic Biomarkers in Triple-Negative Breast Cancer

Melisa Nicoud[1], Daniela Speisky[2], Mónica Táquez Delgado[1], Juan Luis Uriburu[2], Vanina Medina[1]
1- Laboratory of Tumor Biology and Inflammation, Institute for Biomedical Research (BIOMED), School of Medical Sciences, Pontifical Catholic University of Argentina (UCA), National Scientific and Technical Research Council (CONICET), Buenos Aires, Argentina, 2- British Hospital, Buenos Aires, Argentina.

Presenting Author:
Melisa Nicoud
Postdoc Fellow - Institute for Biomedical Research (BIOMED)
Buenos Aires, Argentina
Email: *mbnicoud@gmail.com*

Triple-negative breast cancer (TNBC) is an aggressive breast cancer subtype, accounting for 10-15% of all breast cancer cases. There are neither universally accepted prognostic markers, nor molecular targets related to TNBC. Therefore, the identification of biomarkers for personalized therapies is an unmet medical need. We have previously characterized the histamine H3 (H3R) and H4 (H4R) receptors in TNBC experimental models, demonstrating critical roles in tumor progression.
In this study, we aimed to evaluate the expression of H3R and H4R in TNBC samples and the association with clinicopathological parameters. Fifty female patients with TNBC who underwent breast surgery at the British Hospital of Buenos Aires, Argentina, were retrospectively studied using archived paraffin-embedded tumor tissue specimens. Immunohistochemical analysis showed a higher H3R expression in neoplastic cells in comparison to normal ducts of the histopathologically normal peritumoral breast tissue (Mann Whitney test, P = 0.0177). A high level of H3R was associated with poor overall survival (OS) in TNBC patients (Gehan-Breslow-Wilcoxon test: $\chi 2=4.162$, P = 0.0413). Conversely, a low H4R expression was observed in aggressive tumors while its high expression correlated with improved overall and relapse-free survival (RFS) (RFS: P = 0.016; OS: P = 0.019). In addition, both receptors were expressed in murine 4T1 and human MDA-MB-231 TNBC cell lines, and treatment with H4R agonists or H3R antagonists demonstrated significant antiproliferative and pro-apoptotic effects. We conclude that both receptors might represent promising prognostic biomarkers in TNBC associated with tumor aggressiveness and patient survival. Targeting these receptors alone or in combination with current therapies may enhance TNBC treatment strategies.

Early detection and treatment

Poster No. 74

The displacement of translesion DNA synthesis polymerases from the replisome sensitize tumor cells to genotoxins

Yiovana Verónica Okraine[1], María Belén de la Vega[1], Sofia Venerus Arbilla[1], Florencia Hernandez[1], Sabrina Florencia Mansilla[1], Vanesa Gottifredi[1]
1- Fundación Instituto Leloir-IIBBA-CONICET.

Presenting Author:
Yiovana Verónica Okraine
PhD Fellow - Fundación Instituto Leloir-IIBBA-CONICET
CABA, Argentina
Email: *vero_okraine@yahoo.com*

The proliferating nuclear antigen (PCNA) is a crucial component of the DNA replication machinery. Replicative DNA polymerases bind to PCNA to efficiently achieve duplication of the genomic material. PCNA also promotes auxiliary DNA replication events such as Translesion DNA Synthesis (TLS), a DNA replication transaction that involves specialized polymerases (S-Pols) to replicate damaged DNA. Because cancer cells do not stop DNA replication cycles, even in the presence of DNA damage, it has been proposed that TLS is a good target for cancer therapy. However, inhibiting TLS is challenging because TLS Polymerases compensate for each other. Indeed, specific inhibitors of TLS polymerases do not block the TLS process globally. An alternative possibility to inhibit TLS is to block the interaction of all TLS polymerases with PCNA at once. We have previously shown that the stable expression of the cyclin kinase inhibitor, p21, does so through PCNA binding. Here we tested if a small peptide comprising the PCNA binding region of p21 achieves TLS polymerase displacement from PCNA. Our results indicate that a short peptide comprising the C-terminal PCNA interacting region of p21 displaces all S-pols from PCNA and sensitizes cells to different treatments including cisplatin, chk1 inhibitors and Olaparib. Such observation was recapitulated when using a small peptide corresponding to the PCNA binding region of the S-Pol eta. These results suggest that small peptides with high affinity for PCNA capable of displacing PCNA partners such as S-Pols are versatile tools that can be used to sensitize tumour cells to different genotoxins.

Early detection and treatment

Poster No. 76

Antiproliferative activity and mechanism of action of novel potent anticancer metallocompound CuHL1 on human TNBC cells

Lucía Santa Maria de la Parra[1], Lucía Mariana Balsa[1], Olivia Espindola Moreno[1], Nazia Nayeem[2,3], Maria Contel[2,3], Ignacio Esteban León[1,4]

1- CEQUINOR (UNLP, CCT-CONICET La Plata, Asociado a CIC), Departamento de Química, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, La Plata 1900, Argentina, 2- Brooklyn College Cancer Center BCCC-CURE, Brooklyn College, The City University of New York, 2900 Bedford Avenue, Brooklyn, New York 11210 (USA), 3- Department of Chemistry, Brooklyn College, The City University of New York, 2900 Bedford Avenue, Brooklyn, New York 11210 (USA), 4- Cátedra de Fisiopatología, Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, La Plata 1900, Argentina

Presenting Author:

Lucía Santa Maria de la Parra

PhD Fellow - CEQUINOR (UNLP, CCT-CONICET La Plata, Asociado a CIC)

La Plata, Argentina

Email: *luciasantamaria@quimica.unlp.edu.ar*

Breast cancer is the most common cancer among women, contributing to 32.1% of cases in Argentina (Globocan2020). Triple-negative breast cancer (TNBC) is an aggressive subtype of breast cancer characterized by high invasiveness, high metastatic potential, propensity for relapse, and poor prognosis. Consequently, developing innovative TNBC therapeutic strategies has become essential for clinical practice.

Here we demonstrated the anticancer potency of our most recently synthesized and reported Cu(II) complex derived from acylhydrazone, [Cu(N−N−Fur)(NO3)(H2O)] (CuHL1), on a panel of human TNBC cell lines each distinctive features (MDA-MB-231, MDA-MB-157, MDA-MB-468 and HCC1806). CuHL1 presented a highly cytotoxic effect on all cells tested, showing IC50 values between 1.5 and 2.7 μM at 24 h of treatment. Further analyses were carried out on MDA-MB-231 cells to reveal the mechanism of action of the complex. CuHL1 produced an increment of reactive oxygen species from 1 μM when tested after 3 h of incubation. This complex also induced apoptosis cell death, as can be seen by an augment of early apoptotic cells at 1 μM and an increment of late apoptotic cells at 1 and 2.5 μM after 24 h treatment. Finally, proteomic analysis was performed through label-free quantification using the Orbitrap LC-MS/MS (Thermo ScientificTM, Waltham, MA, USA). Among the 34 differentially expressed proteins, 19 were up- and 15 down-regulated by the treatment with 1 μM of CuHL1. Interestingly, BCAR3 (Breast cancer anti-estrogen resistance 3) was downregulated and it was reported previously that high BCAR3 mRNA expression, specifically in TNBC, correlates with poorer outcomes and chemoresistance effects in patients. Despite it would be necessary to carry out other proteomic assays to validate our results, CuHL1 is positioned as a promising candidate for potential anti-TNBC therapies and would be attractive to further test this complex on in vivo studies.

Early detection and treatment

Poster No.78

CYTOTOXIC ACTION OF SHIGA TOXIN IN TRIPLE-NEGATIVE BREAST CANCER: POTENTIAL THERAPEUTIC USE AND INVOLVEMENT OF TDP-43

Alipio Pinto[1], Noelia Victoria Miret[2], Ana Belen Ramos Aloi[1], Florencia Vassallu[3], Lionel Muller Igaz[3], Andrea Silvana Randi[2], Jorge Goldstein[1]

1- Universidad de Buenos Aires - CONICET. Instituto de Fisiología y Biofísica Bernardo Houssay (IFIBIO Houssay), Facultad de Medicina, Departamento de Ciencias Fisiológicas. Laboratorio de Neurofisiopatología. Buenos Aires, Argentina., 2- Universidad de Buenos Aires, Facultad de Medicina, Departamento de Bioquímica Humana, Laboratorio de Efectos Biológicos de Contaminantes Ambientales, Buenos Aires, Argentina., 3- Universidad de Buenos Aires - CONICET. Instituto de Fisiología y Biofísica Bernardo Houssay (IFIBIO Houssay), Facultad de Medicina, Departamento de Ciencias Fisiológicas. Grupo de Neurociencia de Sistemas. Buenos Aires, Argentina., 4-

Presenting Author:

Alipio Vasconcelos Esteves Pinto

Researcher - Universidad de Buenos Aires - CONICET. Instituto de Fisiología y Biofísica Bernardo Houssay (IFIBIO Houssay), Facultad de Medicina, Departamento de Ciencias Fisiológicas. Laboratorio de Neurofisiopatología. Buenos Aires, Argentina.

Buenos Aires, Argentina

Email: *pintoalipio@gmail.com*

Shiga toxin (Stx) causes hemolytic uremic syndrome. The cytotoxic effect of Stx is mediated by its receptor, globotriaosylceramide (Gb3), which has a limited expression profile in human cells but is overexpressed in many neoplastic cells, including breast cancer. Triple-negative breast cancer (TNBC) is the most aggressive and difficult to treat. On the other hand, TDP-43 is a key protein in the pathogenesis of several neurodegenerative diseases, and both TDP-43 aggregation and abnormal cytoplasmic mislocalization are critical features leading to neuronal degeneration. However, little is known about its role in other diseases, such as cancer. In this work we explore a) the potential of Stx as a novel cytotoxic agent in the human TNBC cell line MDA-MB-231, and b) the changes in TDP-43 elicited by these toxins. TNBC cells were treated with Stx1, Stx2, or anti-Gb3 antibodies, while the non-tumorigenic mammary epithelial cell line NMuMG and VERO cells were used as negative and positive controls for Gb3 expression, respectively. Gb3 expression and Stx uptake were observed in MDA-MB-231 and VERO cells. MTT assay results showed that 10 ng/ml of Stx1, Stx2, and anti-Gb3 significantly reduced TNBC cell viability by 50%, 40%, and 10% respectively after 48 hours. Moreover, 10 ng/ml of these toxins significantly increased the number of cells with karyorrhexis and autophagy. In addition, these treatments reduced the percentage of mitotic cells, BrdU incorporation and cell migration rate. Pre-incubation with PPMP (a Gb3 synthesis inhibitor) significantly reversed all observed effects. Remarkably, Stx significantly increased both total TDP-43 expression and cytoplasmic mislocalization, while both parameters were significantly decreased when cells were also treated with PPMP. In summary, MDA-MB-231 cells are susceptible to Stx and anti-Gb3, suggesting that Stx could be used as an antineoplastic agent in TNBC. We also propose that TDP-43 may play a role in Stx2-mediated cytotoxicity.

Poster Session 3

Fighting treatment resistance. Immunotherapy

Poster No. 03

A novel strategy to kill FANC/BRCA-deficient tumors: depleting cellular glutathione.

Ariel Abramovici Blasco[1], Sebastián Omar Siri[1], María Candelaria Mares Ahlers[1], Yiovana Verónica Okraine[1], Vanesa Gottifredi[1]
1- IIBBA - CONICET.

Presenting Author:
Ariel Isaac Abramovici Blasco
PhD Fellow - Fundación Instituto Leloir / IIBBA (CONICET)
CABA, Argentina
Email: *aabramovici@leloir.org.ar*

Introduction: Formaldehyde (FA) is a ubiquitous, very reactive aldehyde that damages proteins and nucleotides. As a result, cells rely on two systems to deal with its effects: a detoxification pathway dependent on glutathione (GSH) and, in case the FA reaches the DNA, the ability to repair the damage caused involving the Fanconi/BRCA pathway.

Objective: To evaluate whether the pharmacological depletion of GSH selectively kills FANC/BRCA-deficient cells.

Materials: A variety of cellular models were selected, each of them consisting of a pair of cell lines: the wild-type and one deficient in one of the major FANC/BRCA proteins, such as BRCA1 (FANCS), BRCA2 (FANCD1) and FANCD2. Six-day survival assays were conducted; in addition to immunofluorescences of phosphorylated H2AX histone (γH2AX) and total micronuclei count as indirect indicators of replication stress and genomic instability, respectively.

Results: While some FANC/BRCA-deficient cell lines demonstrated a higher sensitivity to GSH-depleting agents (L-BSO and Erastin) relative to their proficient counterparts, this was not a universal phenomenon. For example, the ovarian-derived PEO1/4 cell lines exhibited a clear synthetically lethal effect that was absent in the DLD1 and U2OS pairs. Interestingly, that the synthetically lethal effect of L-BSO on PEO1 cells was not preceded by a differential induction of γH2AX.

Conclusions: Our preliminary results indicate that targeting GSH metabolism may be a promising therapeutic approach in some FANC/BRCA-deficient tumors. However, further validation is necessary to confirm and expand our findings. For instance, we hypothesize that cancerous cells being affected by GSH depletion in a tissue-dependent manner could at least partially explain the discordant results observed. Furthermore, we believe that differences on the relative importance of each FANC/BRCA factor could also account for at least some of the differences seen.

Fighting treatment resistance. Immunotherapy

Poster No. 06

Soluble TNF blockade boosts the antitumoral effects of trastuzumab deruxtecan in HER2 positive breast cancer model

Sofia Bruni[1], Florencia Luciana Mauro[1], Agustina Dupont[2], Maria Florencia Mercogliano[1], Roxana Schillaci[1]

1- Instituto de Biología y Medicina Experimental, Buenos Aires, Argentina, 2- Hospital Juan A. Fernández, Buenos Aires Argentina.

Presenting Author:

Sofia Bruni

PhD Fellow - Instituto de Biologia y Medicina Experimental (IBYME-CONICET)
Buenos Aires, Argentina
Email: *sofibruni@hotmail.com*

DESTINY-Breast03 demonstrated that the overall survival of HER2+ metastatic breast cancer (BC) patients treated with trastuzumab-deruxtecan (T-DXd) was 52.6% Therefore, more efforts are needed to improve these results. We have shown that mucin 4 (MUC4) expression, induced by soluble TNF (sTNF), is an independent biomarker of poor response to trastuzumab in HER2+ BC. We have also demonstrated that blocking sTNF with INB03 (DN) decreases MUC4 expression and overcomes trastuzumab resistance in preclinical models.

We assessed whether blocking sTNF with DN enhances the antitumor activity and immune response associated with T-DXd. Nude mice bearing the trastuzumab-resistant human breast tumor JIMT-1 (HER2+MUC4+), were treated with IgG 5 mg/kg, T-DXd 5, 2.5 or 1.25 mg/kg, DN 10 mg/kg, or the combinations (n=6-8). IgG and T-DXd were administered i.v. on days 0, 7, and 14. DN was administered i.p. twice a week for 3 weeks. We analyzed the number of mitoses by HE staining and tumor-infiltrating leukocytes by flow cytometry.

The dose-response curves showed tumor growth inhibition of 83% (T-DXd 5 mg/kg), 61% (T-DXd 2.5 mg/kg), and 37% (T-DXd 1.25 mg/kg), versus IgG ($P<0.0001$, 0.001 and 0.05, respectively). Adding DN enhanced this inhibition, increasing it to 98%, 81% and 73%, ($P<0.0001$ in all cases) respectively. T-DXd 1.25 mg/kg + DN achieved an antitumor effect similar to T-DXd 5 mg/kg alone. Also, it reduced the number of mitoses and promoted an effective antitumor immune response mediated by macrophages and NK cells, compared to T-DXd 1.25 mg/kg alone.

We conclude that adding DN could reduce the optimal dose of T-DXd without compromising antitumor efficacy. As sTNF and MUC4 expression have shown to be relevant players in the response to T-DXd, we propose that patients with HER2+MUC4+ BC, or those who have progressed on T-DXd treatment could benefit from adding sTNF-blocking agents to enhance T-DXd antitumor effect.

Fighting treatment resistance. Immunotherapy

Poster No.09

Differential expression of regulatory T cells marker genes from peripheral blood of breast cancer patients

Romina Canzoneri[1], Valentina Guai[1], Martina Calderone[1], Aldo Cretón[1], Marina Teresita Isla Larrain[1]

1- Centro de Investigaciones Inmunológicas Básicas y Aplicadas (CINIBA), FCM, CICPBA-UNLP.

Presenting Author:

Romina Canzoneri

Researcher - Centro de Investigaciones Inmunológicas Básicas y Aplicadas, FCM, UNLP

La Plata, Argentina

Email: *canzonerir@hotmail.com*

Introduction: Breast cancer (BC) is the main cause of death from cancer in women in Argentina. Therefore, immunological studies of the mechanisms involved in this pathology are relevant.
Objectives: To evaluate the expression of Treg cell marker genes in peripheral blood (PB) from breast cancer patients and women free of disease and characterize the tumor samples.

Materials and methods: PB and tumor samples from 41 BC patients and PB from 10 women free of disease (controls) were evaluated. Total RNA and cDNA were obtained from lymphocytes. FOXP3, CTLA-4, TNFR-2, and LAG-3 Treg markers gene expression was evaluated by PCR and qPCR. Clinical and histopathological data of BC patients were also registered. IDO, Foxp3, CD4, CD8, and CD45R0 protein expression were studied in tumor samples by immunohistochemistry.

Results: In BC patients, 76% (31/41) of samples expressed FOXP3, while 100% (10/10) of control samples expressed this transcription factor. Besides, CTLA-4, TNFR-2, and LAG-3 expression was evaluated by PCR in selected samples from 22 BC patients and in controls. Pathological samples presented a differential gene expression profile in contrast to control samples. All the genes were expressed in BC samples, while the control samples only expressed FOXP3 and LAG-3. Subsequently, the expression levels of the genes commonly expressed, both in BC and control samples, were evaluated by qPCR. The expression levels of FOXP3 and LAG-3 were significantly higher in the BC samples compared to the controls ($p<0.01$).

By IHC, 40% of samples showed Foxp3 expression, while 38% expressed Foxp3 in Tumor-infiltrating lymphocytes (TILs). Eighty-eight percent of samples expressed CD45R0+ TILs; 76%, CD8+ and 93%, CD4+ TILs. IDO was expressed in 54% of tumor samples.

Conclusions: These results show the relevance of the study of immunological markers in PB liquid biopsies and tumor samples to evaluate the immunological status of BC patients with potential therapeutic implications.

Fighting treatment resistance. Immunotherapy

Poster No.12

Relevance of the correct activation of key mitotic proteins for the survival of cells harboring deficient expression of the BRCA1 and BRCA2 tumor suppressors

María Candelaria Mares Ahlers[1], Sebastián Omar Siri[1], Ariel Isaac Abramovici[1], Ginette Moyano[1], Lilen Ivone Caimi[1], Vanesa Gottifredi[1]
1- Fundación Instituto Leloir.

Presenting Author:
María Candelaria Mares Ahlers
PhD Fellow - Fundación Instituto Leloir-Laboratorio de ciclo celular y estabilidad genómica
CABA, Argentina
Email: *cmares@leloir.org.ar*

Hereditary breast and ovarian cancers are autosomal dominant diseases frequently caused by mutations in the breast cancer susceptibility genes BRCA1 and BRCA2 (BRCA1/2). BRCA1/2 are DNA repair genes, and their protein products regulate homologous recombination (HR). While deficient HR is a trigger for tumorigenesis, it also represents an Achilles heel that can be targeted during cancer treatments. In such a notion, BRCA1/2-deficient cancer cells are highly sensitive to poly-ADP-ribose polymerase inhibitors (PARPi) due to the trapping of PARP on DNA. The persistence of those adducts augments double-strand break formation, which is selectively toxic in BRCA1/2-deficient cells without affecting the normal cells of patients. Such a phenomenon of increased lethality due to the genetic context is defined as "synthetic lethality" (SL). However, probably following the high genomic instability that these drugs propitiate, resistance to PARPi has been repeatedly reported in the clinic, so other therapeutic alternatives are necessary. Previous data obtained by our team indicate that a deficiency in BRCA1/2 could generate an exacerbated dependency on regulators of the M phase. Based on these, this project aimed to identify molecular targets with already characterized pharmacological inhibitors, which may directly or indirectly modulate the M phase, and which also induce SL in BRCA1/2 deficient contexts to identify alternative conditions to PARPi. Five of the nine mitotic inhibitors evaluated caused SL in only one of the two genetic contexts (BRCA2 and not BRCA1). This could suggest a mechanism of action different from the one of PARPi. Supporting this hypothesis, some of these mitotic inhibitors did not show signs of replicative stress or genomic instability. In conclusion, our results contribute to the identification of possible novel pharmacological targets that could provide therapeutic alternatives for the treatment of tumors with BRCA2 deficiency.

Fighting treatment resistance. Immunotherapy

Poster No. 15

LINS01 HISTAMINE H3 RECEPTOR ANTAGONISTS OVERCOME PLACLITAXEL CHEMORESISTANCE IN 4T1 TRIPLE NEGATIVE BREAST CANCER

Ignacio Ospital[1], Melisa Nicoud[1], Paolo Lauretta[1], Agueda Velazco[1], João Fernandes[2], Vanina Medina[1]

1- Laboratory of Tumor Biology and Inflammation, Institute for Biomedical Research (BIOMED), School of Medical Sciences, Pontifical Catholic University of Argentina (UCA), National Scientific and Technical Research Council (CONICET), Buenos Aires, Argentina, 2- Departamento de Ciências Farmacêuticas, Universidade Federal de São Paulo (UNIFESP), Diadema-SP, Brazil.

Presenting Author:
Ignacio Ospital
PhD Fellow - Institute for Biomedical Research (BIOMED)
Buenos Aires, Argentina
Email: *ignaciospital@gmail.com*

Triple-negative breast cancer (TNBC) is an aggressive and difficult to treat subtype of breast cancer. Cancer mortality is due to disseminated disease that has become resistant to multiple therapeutic modalities. Nonspecific cytotoxic agent paclitaxel (PTX) is a standard of care, although its treatment is associated with severe side effects and the acquisition of chemoresistance.
We have recently demonstrated the expression of histamine H3 receptor (H3R) in TNBC samples and the antitumoral efficacy and safety of the LINS01 series of H3R antagonists in TNBC experimental models. The aim of this work was to evaluate whether LINS01022 and LINS01023 compounds could potentiate PTX therapy in 4T1 TNBC cells and in PTX-resistant 4T1 cells (4T1 R).
4T1 cells were exposed to increasing concentrations of PTX until resistance was acquired. The expression of H3R in 4T1 R was confirmed through immunocytochemistry. Both LINS01022 and LINS01023 compounds produced a dose-dependent inhibition of clonogenic proliferation in 4T1 R, demonstrating low IC50 values (3.2 and 0.9 μM, respectively), exhibiting an even more potent antiproliferative effect than the one observed in the respective 4T1 parental cells. Both LINS01022 and LINS01023 potentiated PTX-induced reduction of cell proliferation and viability in both 4T1 R and 4T1 parental cells.
Multidrug resistance-1 (MDR1) acts as a chemotherapeutic drug efflux pump that is involved in chemoresistance of tumor cells. An increased expression of MDR1 was observed in 4T1 R cells. The efflux modulating effects of LINS01 compounds were investigated using the rhodamine 123 accumulation assay.
Interestingly, LINS01 compounds showed a potent MDR1 efflux pump inhibitory action in 4T1 R cells, which contributes to overcoming PTX resistance. We conclude that H3R antagonists LINS01 might be potential therapeutic candidates against TNBC with the capacity to reverse PTX resistance.

Fighting treatment resistance. Immunotherapy

Poster No.18

Extracellular vesicles derived from TNF-α conditioned macrophages promote endocrine resistance in breast tumor cells

Maria Celeste Rodriguez-Baili[1], German Alejandro Gil[1]
1- CIQUIBIC-CONICET.

Presenting Author:
Maria Celeste Rodriguez- Baili
PhD Fellow - CIQUIBIC-CONICET. Química Biológica Ranwel Caputto, FCQ.UNC.
Cordoba, Argentina
Email: *celeste.rodriguez@unc.edu.ar*

Breast cancer is one of the leading tumors diagnosed worldwide and the primary cause of cancer-related death in women. Nearly 70% of diagnosed breast tumors are estrogen receptor-positive (ER+), becoming the receptor a key therapeutic target. Despite the efficacy of hormonal therapies, some patients develop resistance, either initially or during treatment. Understanding tumor progression and resistance mechanisms requires studying the tumor microenvironment, where tumor cells interact closely with endothelial cells, cytokines, soluble factors, fibroblasts, and immune cells, particularly macrophages, which are highly influential in tumor behavior. Cells communicate through direct contact, endocrine and paracrine soluble factors, and extracellular vesicles (EVs). These lipid bilayer vesicles facilitate the transfer of their cargo to recipient cells, inducing phenotypic changes. Here, we isolated EVs derived from TNF-α-conditioned macrophages and investigated their effects on MCF-7 ER+ breast tumor cells. Our findings indicated that these vesicles enhance proliferation, migration, induced epithelial-mesenchymal transition and a tumor stem cell phenotype. Furthermore, we identified a link between these EVs and endocrine resistance, demonstrating that vesicles not only increased cell proliferation in the presence of Tamoxifen, an ER modulator, but also transferred endocrine resistance to previously sensitive cells.

Fighting treatment resistance. Immunotherapy

Poster No. 21

Mifepristone treatment reverts T cell exclusion and immunotherapy resistance programs in hormone-dependent breast luminal tumors

Mariana Salatino[1], Joaquin Merlo[1], Magalí Bertón[1], Tomas Dalotto-Moreno[1], Ramiro Perrotta[1], Andres Elia[1], Karina Mariño[1], Claudia Lanari[1], Gabriel Rabinovich[1]
1- Institute of Biology and Experimental Medicine, CONICET.

Presenting Author:
Mariana Salatino
Researcher - LAB OF GLYCOMEDICINE -IBYME-CONICET
CABA, Argentina
Email: *MARIANSALATINO@GMAIL.COM*

Endocrine context is a critical factor that may modulate immune function in breast cancer and, accordingly, should be considered during immunotherapy regimens. Given the immunosuppressive and tolerogenic activities of the sexual hormone progesterone (Pg) and its roles in promoting breast cancer initiation, we aim to evaluate the effect of the antagonist of the progesterone receptor Mifepristone (MFP) as an endocrine therapy to alter the immune profile and to activate the antitumor immune response. We evaluated the MFP-profiled breast cancer immune landscape in a mouse model of luminal tumours and in human tumour samples derived from the MIPRA clinical trial, where breast cancer patients harboring HR+ tumours were treated with MFP before surgery. Using RNA-seq and flow cytometry, we interrogated the tumour immune infiltration profile, including several global gene expression signatures associated with T-cell exclusion and Immune Checkpoint Inhibitors (ICI) resistance. We observed that treatment with MFP in luminal breast tumours restrains the progestin-mediated tolerogenic infiltrate composed of Tregs and exhausted CD8 and M2 macrophages. Importantly, in tumours, MFP downregulates the suppressive pathway of IDO and Galectin-9, which in turn contributes to the persistence of a population of CD8 T cells that highly produce granzymes and express low TIM3 and PD-1, exhibiting a reinvigorating phenotype. More importantly, we showed that in both mouse models and human tumours, treatment with MFP reverts transcription programs associated with T cell exclusion and ICI resistance and significantly up-regulates programs associated with PD-1/PD-L1 response. Our work provides additional bases for a new therapeutic modality based on the use of antiprogestin endocrine therapy with MFP toward sensitizing luminal breast tumours to ICI treatment.

Fighting treatment resistance. Immunotherapy

Poster No. 24-A

4HER project: Impact of HER Family Co-Amplifications on Trastuzumab Efficacy and Clinical Outcomes in HER2-Positive Breast Cancer

Carla Torres Orellana[1], Sergio Laurito[1], Pablo Mandó[2], María Roqué[1], Sebastián Real[1]
1- Instituto de Histología y Embriologia de Mendoza (IHEM), CONICET-UNCuyo, Mendoza, Argentina, 2- Centro de Educación Médica e Investigaciones Clínicas "Norberto Quirno" (CEMIC), CABA, Argentina.

Presenting Author:
Carla Torres Orellana
PhD Fellow - IHEM, CONICET-UNCuyo
Mendoza, Argentina
Email: *carlaorellana40@gmail.com*

INTRODUCTION: Trastuzumab (TZM) resistance remains a primary cause of mortality in HER2-positive breast cancer (BC) patients. We hypothesize that this resistance may be partially due to synergistic interactions among members of the HER oncogene family, compensating for inhibition when one member is targeted. Previously, we developed an MLPA-based tool to assess CNV of the four HER oncogenes. Our current objective is to investigate the association between co-amplification of HERs and resistance to TZM.
OBJECTIVE: To analyze the correlation between CNV/expression of the four HER oncogenes and clinical variables in HER2-positive BC patients using both clinical and computational approaches.
RESULTS: Clinical study: DNA was extracted from FFPE tissue of 49 HER2-positive patients. Using our MLPA X026 probemix (MCR Holland), we confirmed 42/49 tumors as HER2-positive. Co-amplification of HER2 with at least one other HER oncogene was observed in 12/42 samples (29%). Among 42 patients, 6 (14.3%) experienced disease recurrence; notably, 1/6 was HER2-negative (refractory to TZM), while all the remaining 5 patients exhibited HERs co-amplification ($p=0.001$). Significant correlations were found between HER2/EGFR and HER2/HER3 co-amplification and recurrence ($p<0.001$ and 0.03).
In silico study: Analysis of TCGA data from 67 HER2-positive BC patients (PAM50RNAseq) revealed that high co-expression of HER2/HER3 significantly correlated with decreased overall survival (<0.0001). Differential gene expression analysis indicated downregulation of tumor microenvironment (TME)-related genes in these tumors. This altered TME affects immune cell populations such as NK, macrophages, and CD8 cells, along with reduced INF and cytokine signatures ($p<0.05$).
CONCLUSION: Co-amplifications of HER2 with HER3 reduce OS in HER2-positive BC patients, potentially influencing the tumor microenvironment and antitumor response. Further studies are needed to elucidate how HERs “work together for the family".

Fighting treatment resistance. Immunotherapy

Poster No. 24-B

Impact of Thyroid Hormones on Chemotherapy Efficacy in Triple-Negative Breast Cancer Cells via Integrin αvβ3

Johanna Díaz Albuja[1], Celeste Díaz Flaqué[1], Florencia Menay[1], Gonzalo Gonzalez[1], Mercedes Debernardi1, Alejandra Paulazo[1], Florencia Cayrol[1], Cinthia Rosemblit[1], Helena Sterle[1], Graciela Cremaschi[1]

Instituto de Investigaciones Biomédicas IOMED UCA-CONICET

Presenting Autor:

Johanna Abigail Díaz Albuja

Laboratorio de Neuroinmunomodulación y Oncología Molecular (Dra. Cremaschi). Instituto de Investigaciones Biomédicas BIOMED UCA-CONICET

diazalbuja@gmail.com

Chemotherapy resistance is a leading cause of treatment failure in breast cancer, often due to mechanisms like the overexpression of multidrug transporters that enhance drug efflux and reduce efficacy. Recent studies suggest that thyroid hormones (THs) may influence tumor processes through integrin αvβ3, their membrane receptor. However, the exact role of THs in modulating chemotherapy response and the underlying mechanisms are still unclear. The aim of this study was to investigate the impact of physiologic concentrations of THs on chemotherapy response in MDA-MB-231 triple negative breast cancer (TNBC) cells in vitro. Our results demonstrate that THs activate integrin αvβ3-dependent signaling pathways, including PI3K-AKT and MAPK, in these cells. Notably, cell viability assays showed that THs attenuate the efficacy of the chemotherapeutic agents Doxorubicin (DOX) and Paclitaxel (PTX). This effect was inhibited by cilengitide, an αvβ3 integrin inhibitor, thus indicating that THs' modulation of chemotherapy response is mediated by integrin αvβ3. Subsequently, we investigated whether THs could activate mechanisms associated with chemotherapy resistance and found that THs increase the expression and activity of ABC-type transporters. Additionally, the treatment of this cell line with increasing doses of DOX led to the development of resistant clones that were not only resistant to DOX but also to PTX, which was associated with increased expression of multidrug transporters. Finally, we also found that THs could further enhance the expression of these proteins, including MDR1 and BCRP, in DOX-resistant cells. In conclusion, our study shows that THs significantly influence chemotherapy response in TNBC by activating integrin αvβ3 signaling pathways and promoting resistance mechanisms, which could have implications for improving therapeutic strategies in TNBC.

Fighting treatment resistance. Immunotherapy

Poster No. 24-C

4-methylumbelliferone sensitizes breast cancer spheroids to chemotherapeutic treatment with epirubicin: new perspectives for pharmacological repositioning

Daiana L. Vitale[1], Paolo Rosales[1], Antonella Icardi[1], Candela Morán[1], Ina Sevic1, Laura Alaniz[1]

Laboratorio de Microambiente Tumoral - Centro de Investigaciones Básicas y Aplicadas (CIBA) - Universidad Nacional del Noroeste de la Provincia de Buenos Aires – Centro de Investigaciones y Transferencia del Noroeste de la Provincia de Buenos Aires (CITNOBA) – UNNOBA-UNSAdA-CONICET – Argentina.Laboratorio de Microambiente Tumoral - Centro de Investigaciones Básicas y Aplicadas (CIBA) - Universidad Nacional del Noroeste de la Provincia de Buenos Aires – Centro de Investigaciones y Transferencia del Noroeste de la Provincia de Buenos Aires (CITNOBA) – UNNOBA-UNSAdA-CONICET – Argentina.

Presenting Autor:

Daiana L. Vitale

Universidad Nacional del Noroeste de la Provincia de Buenos Aires

vitaledai@gmail.com

Tumor extracellular matrix (TECM) influence drug resistance due to an imbalance in the synthesis and degradation of its components, including hyaluronan (HA). HA accumulates in the TECM, impeding drug distribution and inducing pro-tumoral signals. UDP-glucuronic acid (UDP-GlcA) together with N-acetyl-glucosamine, are involved in HA synthesis. UDP-GlcA plays a key role in the elimination of chemotherapeutic drugs as epirubicin (EPI). On the other hand, candidate molecules for drug repositioning include 4-methylumbelliferone (4MU), an orally approved dietary supplement derived from coumarins. 4MU specifically inhibits HA synthesis by binding to UDP-GlcA and depleting the cellular pool required for HA synthesis. The aim was to propose a treatment with EPI + 4MU in two breast cancer models to reduce EPI elimination and affect HA synthesis. Spheroids of MDA-MB-231 and MCF-7 cells were established and after 5 days they were treated with EPI, 4MU, or EPI + 4MU for 3 days. EPI + 4MU treatment reduced tumor cell viability (MTS) and increased early apoptosis (flow cytometry) compared to control conditions. This effect was associated with a higher intracellular accumulation of EPI (flow cytometry), a decrease in spheroids size (microscopy) and the downregulation of efflux pumps involved in EPI resistance (RT-qPCR). Besides, EPI + 4MU treatment decreased the expression of the major HA synthase HAS2 and showed a tendency to increase hyaluronidases HYAL1 and HYAL2 (RT-qPCR). Moreover, this effect was shown when HA accumulation on the cell surface of tumor cells obtained from treated spheroids was determined by flow cytometry. In conclusion, 4MU promotes remodeling of the breast cancer ECM, affecting HA metabolism. Indeed, the combination of 4MU with EPI reduced EPI inactivation and elimination. Finally, combined treatment reduced the activation of cellular mechanisms involved in drug resistance, sensitizing tumor cells to EPI and enhancing the efficacy of antitumor therapy.

Metabolism, Signaling and Breast Cancer Risk

Poster No.27

Synergistic Antitumor Effects of Metformin and Alpha-Lipoic Acid on Breast Cancer Cell Viability

Vanina Alejandra Alamino[1,2,3], Leandro Eduardo Nieto[1], Victoria Leonhard[1,2], Karina Lilian Bierbrauer[1,2], Laura Raquel Comini[1,2], Claudia Gabriela Pellizas[2,3], Dante Miguel Beltramo[1], Roxana Valeria Alasino[1,2]

1- Centro de Excelencia en Productos y Procesos (CEPROCOR), Santa María de Punilla, Córdoba, Argentina, 2- Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). 3. Departamento de Bioquímica clínica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba (UNC).

Presenting Author:

Vanina Alejandra Alamino

Researcher - CEPROCOR (Centro de Excelencia en Productos y Procesos)- CONICET
Santa María de Punilla, Córdoba, Argentina
Email: *vanina.alamino@unc.edu.ar*

Breast cancer is the most frequently diagnosed cancer among women worldwide, underscoring the critical need for developing innovative strategies to control tumor growth. Current research emphasizes the significance of combination therapies in enhancing efficacy, reducing toxicity, and mitigating drug resistance. One particularly promising approach is drug repurposing, where clinically approved drugs are identified for new therapeutic applications. Aberrant metabolic activity, a hallmark of cancer, contributes significantly to cellular immortality and sustained proliferative activity. Thus, compounds that can modulate energy metabolism present a potent strategy for controlling tumor growth. Among such compounds, metformin (Met) and alpha-lipoic acid (α-LA) have well-documented effects on cellular metabolism and are widely used to treat various pathologies. In our study, we investigated the effects of Met and α-LA on the viability of the breast tumor cell line 4T1. The cells were treated with different concentrations of Met (1, 5, and 10 mM) and α-LA (0.05 and 0.1 mM), both individually and in combination. Cell viability was then assessed using methyl thiazolyl tetrazolium (MTT) assays. Our findings revealed a statistically significant decrease in cell viability when treated with both Met and α-LA simultaneously, compared to individual treatments. This indicates a strong synergistic effect, as confirmed by a positive synergy score for all four algorithms analyzed in SynergyFinder. These results suggest that the combination of Met and α-LA exerts a potent antitumor activity, highlighting a promising therapeutic avenue for breast cancer treatment. The implications of this study could open new doors for leveraging existing drugs in innovative ways to combat one of the most challenging diseases worldwide.

Metabolism, Signaling and Breast Cancer Risk

Poster No. 54

In vitro and in vivo effect of the combination of 2’-nitroflavone and safingol in breast cancer

Juan M. Anselmi Relats[1], Leonor P. Roguin[1], Nora M. Marder[1], Magalí C. Cercato[1], Julieta Marino[1], Viviana C. Blank[1]
1- Instituto de Química y Fisicoquímica Biológicas.

Presenting Author:
Juan Manuel Anselmi Relats
PhD Fellow - Instituto de Química y Fisicoquímica Biológicas
Buenos Aires, Argentina
Email: *jm.anselmirelats@gmail.com*

Sphingosine kinase-1 (SPHK1), the enzyme that catalyzes the synthesis of the pro-oncogenic molecule sphingosine-1-phosphate, is commonly upregulated in breast cancer cells and has been linked with poorer prognosis. Therefore, SPHK1 targeting drugs have been proposed for breast cancer treatment, with better antitumor results when they are combined with chemotherapy. Previously, we demonstrated that the synthetic flavonoid 2’-nitroflavone (2´NF) exerted a potent and selective antiproliferative effect in murine HER2-positive LM3 mammary tumor cells. As we found that these cells overexpress SPHK1, we explored the antitumor action of the combination of the SPHK inhibitor safingol with 2´NF. To quantitatively characterize the interaction between 2´NF and safingol, dose-effect curves were analyzed by Compusyn software. Results showed combination indexes indicative of synergism in cells incubated with 5 µM of 2´NF and 0.6 µM of safingol (0.72±0.06, 48h and 0.71±0.01, 72h). Similar results were obtained when human HER2-positive MDA-MB-453 breast cancer cells were treated with 2´NF and safingol (0,65 ± 0,08, 48 h and 0,73 ± 0,06, 72 h). To explore the in vivo effect of the combination, we employed a syngeneic LM3 breast cancer murine model. Mice were treated with either vehicle, 2’NF (0.7 mg/kg), safingol (0.5 mg/kg) or a combination of both drugs. 2’NF treatment reduced tumor volume by 38 % ($p<0.05$) and safingol had no significant effect compared to vehicle-treated mice. However, when both drugs were administered together, tumor volume was reduced by ~85% ($p<0.0001$). Moreover, western blot analysis of tumor lysates revealed that combined treatment increased PARP cleavage and Bax protein levels and decreased anti-apoptotic Bcl-xL and Bcl-2 protein levels ($p<0.05$). In summary, the therapeutic efficacy of the combined treatment, which employs low doses of each drug, makes this formulation an attractive potential treatment for HER2-positive breast cancer.

Metabolism, Signaling and Breast Cancer Risk

Poster No.33

Boosting Antitumor Action of a Copper(II)-Hydrazone Compound Using Functional Polymer Nanoparticles.

Tugce Boztepe[1,2], Aldana Sólimo[3,4], Tatiana Goldberg[3], Lucia Santa Maria de la Parra[1], German Islan[2], Mariana Callero[3,4], Ignacio León[1,5]

1- CEQUINOR (UNLP, CCT-CONICET La Plata, Asociado a CIC), Departamento de Química, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, La Plata 1900, Argentina, 2- Laboratorio de Nanobiomateriales, CINDEFI—Departamento de Química, Facultad de Ciencias Exactas, Universidad Nacional de La Plata-CONICET, La Plata B1900, Argentina, 3- Universidad de Buenos Aires, Instituto de Oncología Angel H.Roffo, Area Investigaciones, 4- Consejo Nacional de Investigaciones Científicas y Técnicas, CONICET. 5- Cátedra de Fisiopatología, Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, Argentina

Presenting Author:
TUGCE BOZTEPE
Postdoc Fellow - CEQUINOR-CINDEFI-CONICET
LA PLATA, Arjantin
Email: *tugceboztepe@gmail.com*

Copper-based complexes have received attention due to promising antitumor activity both in vitro and in vivo. Drug delivery systems have been designed to address the drawbacks of anticancer drugs, including dose-limiting toxicity, chemoresistance, and poor water solubility. Eudragit® is a polymer widely utilized in pharmaceutical formulations to enhance the stability, solubility, and controlled release of drugs. Previously, we have synthesized and reported a novel copper (II)-hydrazone complex ([Cu(N-N-Fur)(NO3)H2O], CuHL1) that demonstrated an anticancer effect on triple-negative breast cancer (TNBC) cells. Next, we aimed to evaluate the combination of CuHL1 with Eudragit® on this type of cell. First, the nanoparticles containing Eudragit® E100 and S100 loaded with CuHL1 (ES-CuHL1) were synthesized by nanoprecipitation technique followed by ultrasonication. The formulation showed an encapsulation efficiency higher than 90%, with nanoparticles ranging from 253 nm (ES) to 342 nm (ES-CuHL1). By MTS assay we found that the nanoformulation induced significantly higher cytotoxicity on MDA-MB-231, 4T1, and HS578T TNBC cells compared to free compound at 0.75 μM (measured as cell viability respect to control cells; 4T1: 67±10% ES-CuHL1 vs. 94±4% CuHL1; HS578: 84±5% vs. 109±6%, $p<0.05$) and at 2 μM on MDA-MB-231: 41±6% vs. 60±3%). Moreover, the treatment with ES-CuHL1 increased the number of apoptotic cells compared to free compound, in every cell line. Finally, by clonogenic assays we observed a significant decrease in cell clonogenic capacity with nanoformulation compared to free CuHL1, at 0.5 μM in MDA-MB-231 and 4T1 cells (Clonogenic capacity respect to its control, 4T1: 72±7% ES-CuHL1 vs. 93±8% CuHL1; MDA-MB-231: 1±0% vs. 87±6% vs $p<0.05$). Overall, the findings suggest that ES-CuHL1 nanoparticles could be a more potent anti-tumor option compared to free CuHL1 against TNBC. Further research is required to evaluate its potential as a treatment for this type of tumor.

Metabolism, Signaling and Breast Cancer Risk

Poster No.36

EXPOSURE TO ENDOCRINE DISRUPTOR IMIDACLOPRID ENHANCES CELL PROLIFERATION AND MIGRATION IN HER2 POSITIVE BREAST CANCER MODEL

Sol Bujan[1], Noelia Victoria Miret[1], Alejandro Nicola Candia[2], Carolina Pontillo[1], María Agustina Leguizamón[1], Florencia Chiappini[1], Marianela Candolfi[2], Andrea Randi[1]

1- Universidad de Buenos Aires, Facultad de Medicina, Depto de Bioquímica Humana, Laboratorio de Efectos Biológicos de Contaminantes Ambientales, 2- Universidad de Buenos Aires, Facultad de Medicina, Instituto de Investigaciones Biomédicas, Laboratorio de Inmunoterapia Antitumoral.

Presenting Author:

Sol Bujan

PhD Fellow - Universidad de Buenos Aires, Facultad de Medicina, Depto de Bioquímica Humana, Laboratorio de Efectos Biológicos de Contaminantes Ambientales

Ciudad Autónoma de Buenos Aires, Argentina

Email: *sol__bujan@hotmail.com*

Neonicotinoids are insecticides used worldwide for fruits and vegetables. Imidacloprid (IMI) is among the 10 most detected agrochemicals in studies in Argentina and Brazil, with a prolonged residual effect in the soil, accumulating due to its repeated application, and may affect human health. IMI increases the expression of aromatase and the secretion of estradiol in breast cancer cells, so exposure to IMI could collaborate with tumor development and progression, stimulating estrogen receptor (ER) pathways such as G protein-associated ER (GPER). Some endocrine disruptors activate the aryl hydrocarbon receptor (AhR), a transcription factor that modulates processes such as inflammation, proliferation and migration. The indoleamine 2,3-dioxygenase (IDO) produces kynurenine, which is involved in modulating the immunosuppressive microenvironment in tumors. Our objective was to examine whether exposure of HER2(+) LM3 breast cancer cells to IMI (0.01, 0.1, 1 and 10 μM) alters cell viability, proliferation and migration, as well as the activity of metalloprotease (MMP)9, and whether these effects are mediated by the AhR, IDO and GPER pathways using specific inhibitors. In addition, we studied its action on the expression protein of AhR, GPER and its downstream pathway ERK1/2. Our results showed that IMI (10 μM) increases cell viability at 48 h ($p<0.05$) (MTT assay) and proliferation (clonogenic assay) ($p<0.05$) through AhR and GPER pathways in LM3 cells. Low doses of IMI (0.01 and 0.1 μM) enhance cell migration (wound healing assay) ($p<0.01$) in a manner dependent on AhR, GPER and IDO. The activity of MMP-9 (gel zymography) shows a tendency to increase with IMI (1-10 μM). Furthermore, exposure to IMI enhances GPER and ERK1/2 expression by WB at 0.1 μM ($p<0.05$), showing a tendency to reduce AhR levels at 0.01 and 0.1 μM. All these findings suggest that exposure of populations to IMI could contribute to the development and progression of HER2-positive breast cancer.

Metabolism, Signaling and Breast Cancer Risk

Poster No.39

THYROXINE PROMOTE MCF-7 CELL VIABILITY VIA GENOMIC PATHWAYS

Rocío Cano[1], Leila Zyla[1], Matias Ferrando[1], Silvina Goméz[1], Virginia Pistone Creydt[1], Constanza López Fontana[1], Rubén Carón[1]
1- Instituto de Medicina y Biología Experimental de Cuyo, Universidad Nacional de Cuyo.

Presenting Author:
Rocio Cano
PhD Fellow - Instituto de Medicina y Biología Experimental de Cuyo (IMBECU)
Mendoza, Argentina
Email: *rociyasmin@gmail.com*

Hypothyroidism is considered a protective factor against breast cancer (BC). However, prolonged exposure to or excessive doses of thyroxine (T4) in thyroid replacement therapy may potentially elevate BC risk. The contribution of thyroid hormones (HT) to BC can be attributed to their genomic and non-genomic effects. Genomic mechanisms entail T4 binding to intranuclear thyroid receptors (TRα or TRβ1), which, in turn, regulate the transcription of numerous genes. Non-genomic mechanisms involve T4 binding to plasma membrane receptors, including αVβ3 integrins, as well as cytoplasmic TRβ1 or TRα. These interactions activate pathways such as ERK and AKT/PI3K, ultimately promoting cell proliferation. In this study, our objective was to investigate the biological impact of T4 on MCF-7 breast tumor cells and examine its potential genomic and non-genomic effects. To achieve this, we utilized a final concentration of 10-9 M of T4, administered both alone and in combination with a TR inhibitor (1-850, Cayman Chemical, MI, USA), in a culture medium of DMEM/F12 with phenol red and 1% charcoalized fetal serum (FBSc) for 24 h (genomic effects) and 20-minute (non-genomic effects). We assessed cell proliferation via MTT assays, and cell viability using Trypan Blue, and explored genomic and non-genomic pathways through western blot analysis. After 24 h of T4 treatment, we observed that T4 enhanced cell proliferation and increased MCF-7 cell viability. However, the addition of 1-850 reduced their viability and increased TRβ1 expression. Moreover, T4 suppresses apoptotic processes, as indicated by reduced expression of caspases (3, 7, and 8). Meanwhile, a 20-minute T4 treatment activated the pERK/ERK pathway. In conclusion, T4 primarily exerts its proliferative effects on hormone-sensitive mammary tumor cells through genomic pathways.

Metabolism, Signaling and Breast Cancer Risk

Poster No.42

GEF-H1 drives breast cancer cells to tumor formation and metastasis

Lucía Fernández Chávez[1], Karen Schweitzer[1], Exequiel Gonzalo Alonso[1], María Julia Ferronato[1], María Eugenia Fermento[1], Eliana Noelia Alonso[1], María Marta Facchinetti[1], Alejandro Carlos Curino[1], Georgina Pamela Coló[1]

1- Laboratorio de Biología del Cáncer, Instituto de Investigaciones Bioquímicas de Bahía Blanca (INIBIBB-UNS-CONICET). Departamento de Biología, Bioquímica y Farmacia, UNS, Bahía Blanca, Argentina.

Presenting Author:

Lucía Fernández Chávez

PhD Fellow - Laboratorio de la Biología del Cáncer, Instituto de Investigaciones
Bahía Blanca, Argentina

Email: *luciafchavez@hotmail.com*

Rho GTPases are involved in several biological and pathological processes, including gene transcription, cell polarity, migration and invasion. Rho guanine nucleotide exchange factor-H1 (GEF-H1) is the unique RhoA activator that binds to microtubules and its localization and activity is regulated in part by fibronectin-binding integrins. The aim of this work is to investigate the role of GEF-H1 in breast cancer progression. We report that the expression of GEF-H1 is higher in human breast cancer biopsies than in normal tissue. Furthermore, the expression of GEF-H1 is higher in breast cancer cell lines than in non-tumoral cell lines. We observed a significant reduction in the number of focal adhesions, stress fibers formation and altered downstream signaling in GEF-H1 knockout (KO) breast cancer cell lines, resulting in decreased cell proliferation, migration, adhesion and invasion. In addition, orthotopic implantation of GEF-H1 KO cells into mammary fat pads of Balb/c mice showed a significant delay in tumor formation and lung metastasis development compared to control breast cancer cells. These results suggest that activation of GEF-H1/RhoA mediates cytoskeletal remodeling and signaling pathways involved in cell proliferation, migration, and invasion of breast cancer cells. In vivo assays and human biopsy studies suggest that GEF-H1 expression indeed contributes to breast tumor progression and could be postulated as a potential biomarker.

Metabolism, Signaling and Breast Cancer Risk

Poster No.42

Imidacloprid as a risk factor for breast cancer development

Noelia V. Miret[1], M. Agustina Leguizamón[1], Alejandro Español[2], Sol Buján[1], Carolina Pontillo[1], Florencia Chiappini[1], Andrea Randi[1]

1- Universidad de Buenos Aires, Facultad de Medicina, Departamento de Bioquímica Humana, Laboratorio de Efectos Biológicos de Contaminantes Ambientales, Paraguay 2155, 5° piso, (CP 1121) Buenos Aires, Argentina, 2- Universidad de Buenos Aires, Facultad de Medicina, Centro de Estudios Farmacológicos y Botánicos (CEFYBO).

Presenting Author:
Noelia Victoria Miret
Researcher - Universidad de Buenos Aires, Facultad de Medicina, Departamento de Bioquímica Humana, Laboratorio de Efectos Biológicos de Contaminantes Ambientales
Ciudad Autónoma de Buenos Aires, Argentina
Email: *noeliamiret@hotmail.com.ar*

Imidacloprid (IMI) is a neonicotinoid insecticide widely used in agriculture, which binds to the nicotinic acetylcholine receptor (nAChR). Increasing evidence shows the potential risk to humans of IMI exposure and IMI has been postulated as an endocrine disruptor. For other neonicotinoids, their ability to bind and activate the G protein-coupled estrogen receptor (GPER) was probed. Given that nAChR and GPER activation are implicated in breast cancer, we hypothesize that IMI exposure produces alterations in the mammary gland that favor tumorigenesis.
Mammary epithelial cell line NMuMG was exposed to IMI (0.01-10 μM) or vehicle (DMSO) for 24 h. IMI does not alter cell viability (MTT assay) but promotes cell motility. IMI boosted cell migration at 1 and 10 μM (wound healing assay) and the activity of metalloprotease (MMP)2 at 10 μM and MMP9 at 0.1 and 10 μM (gel zymography). In addition, 10 μM IMI increased GPER and α7-nAChR protein levels after 24 h of treatment, as well as c-Src phosphorylation, a kinase downstream of GPER and α7-nAChR, after 1, 2 and 4 h (western blot). Next, cells were preincubated with specific inhibitors -1 μM G15 for GPER, 1 μM mecamylamine for nAChR, 1 μM methyllycaconitine citrate for α7-nAChR or 0.2 nM PP2 for c-Src- for 1 h and cell migration was assayed. Results showed IMI-promoted wound healing was blocked in the presence of each inhibitor. Finally, female pre-pubertal BALB-c mice were treated with IMI (0.01, 0.1 and 10 mg/kg/day) orally for 4 weeks and the whole mammary gland was mounted and hematoxylin-eosin-stained sections were examined. IMI (10 mg/kg/day) enhanced ductal hyperplasia and the number of terminal end buds (TEBs). IMI (0.1 mg/kg/day)-treatment induced ductal growth but reduced branch density.
In summary, IMI increases mammary epithelial cell motility through GPER and α7-nAChR and promotes preneoplastic lesions and TEBs retention. Our results support the hypothesis that IMI represents a risk factor for breast cancer.

Metabolism, Signaling and Breast Cancer Risk

Poster No.48

WNT5A IS INVOLVED IN THE PRO-TUMORIGENIC ROLE OF GPAT2 EXPRESSION IN MDA CELLS

Verónica V. Moscoso[1], Oriana Martiarena[1], Fiorella J. Ferremi[1], Elizabeth R. Cattaneo[1], Maria Gonzalez Baro[1], Mauro A. Montanaro[1]
1- INIBIOLP. Instituto de Investigaciones Bioquímicas de La Plata, CONICET – UNLP.

Presenting Author:
Verónica Victoria Moscoso
PhD Fellow - INIBIOLP. Instituto de Investigaciones Bioquímicas de La Plata, CONICET – UNLP
La Plata, Argentina
Email: *vmoscoso@med.unlp.edu.ar*

We have shown that human glycerol-3P-acyltransferase 2 (GPAT2) is overexpressed in various human cancer cell lines, including breast cancer MDA-MB-231 cells. We also showed that GPAT2 knockdown decreases cell proliferation, anchorage-independent growth, migration, and tumorigenicity, and increases staurosporine-induced apoptosis. GPAT2 features a significant positive correlation with the histological grade of human breast carcinomas. Additionally, it can modulate the expression levels of several non-coding RNAs. Transcriptomic analysis showed that the Wnt5A gene and the Wnt pathway are downregulated after GPAT2 silencing. Furthermore, we found that GPAT2 knockdown in MDA-MB-231 cells impacts the expression levels of several long non-coding RNAs (LncRNAs). Following the identification, selection, and annotation of differentially expressed LncRNAs in GPAT2-silenced cells versus control cells, we identified three LncRNAs (LINC1085, CTD2066L21.3, and NOVA1.AS1) predictive of overall survival in patients with breast cancer. Moreover, we identified specific miRNAs associated with the selected LncRNAs. Specifically, we found that hsa-mir-106a-5p, associated with NOVA1.AS1, shows a significant positive correlation with breast cancer patient survival and a strong negative correlation with the Wnt signaling pathway, thereby establishing this pathway as a candidate to explain the effect of GPAT2 expression on MDA cells. In this work, we knocked down Wnt5A in MDA cells using shRNA methodology. By performing cell proliferation and wound healing assays, we were able to reproduce the phenotype obtained by silencing GPAT2 in this same cell line. Importantly, the expression level of GPAT2 was not affected by Wnt5A silencing.

Metabolism, Signaling and Breast Cancer Risk

Poster No.51

Biological activity of two new copper(II)-neocuproine complexes and L-dipeptides (L-Ala-L-Phe) in two breast cancer cell lines.

Katherine Seneth Muñoz Garzon[1], Natalia Alvarez[2], Gianella Facchin[2], Delia B. Soria[1], Ana Laura Di Virgilio[1]

1- CEQUINOR (CONICET-UNLP) Bv120 N1465 e 60 y 64, La Plata, Argentina, 2- Área de Química Inorgánica, DEC, Facultad de Química Gral. Flores 2124, Montevideo..

Presenting Author:
KATHERINE SENETH MUÑOZ GARZON
Undergraduate Student - CEQUINOR-CONICET
LA PLATA, ARGENTINA
Email: *kseneth22@gmail.com*

Breast cancer has emerged as the most diagnosed cancer in Argentina, with an incidence of 21,631 new cases reported in 2022. Chemoprevention strategies aim to reduce the risk of developing invasive breast cancer, and chemotherapy remains a primary treatment for metastatic cases, particularly in triple-negative breast cancer (TNBC). However, metal-based drugs like cisplatin, commonly used in chemotherapy, exhibit significant side effects and the potential for resistance with prolonged use. Recent research has explored the combination of metal complexes with amino acids to enhance pharmacokinetic properties and reduce toxicity. In this context, we investigated the effects of two copper complexes with neocuproine [CuCl2(neo)] (1) and compared with a ternary complex with a dipeptide (alanine and phenylalanine) [Cu(ala-phe) (neo)]·4H2O (2) in two human breast cancer cell lines (MCF-7 and MDA-MB-231). Notably, both complexes demonstrated IC50 values below 3 μM and clonogenic inhibition in both cell lines. However, the mechanisms of action varied according to the cell line. In MCF-7, direct DNA damage was observed using the comet assay, while in the MDA-MB-231 cell line, reactive oxygen species (ROS) production occurred. Additionally, late apoptosis and necrosis were induced by complex 2 in MCF-7, and necrosis in MDA-MB-231 (preliminary data). Furthermore, exhibited migration inhibition in both cell lines. These promising results suggest that the ternary copper complex 2 warrants further evaluation as an alternative therapeutic approach for breast cancer treatment.

Metabolism, Signaling and Breast Cancer Risk

Poster No.54

IN VITRO EFFECT OF NEW HSP90 INHIBITORS ON BREAST CANCER CELLS

Iara Sofía Santa Cruz[1], Sol Ciucci[2], Alejandra Erlejman[2,3], Mario Galigniana[1,2], Gisela Mazaira[2,3]
1- IByME-CONICET, Buenos Aires, Argenitna, 2- epartamento de Química Biológica, FCEN, UBA. Buenos Aires, Argentina, 3- IQUIBICEN/CONICET. Buenos Aires, Argentina.

Presenting Author:
Iara Sofía Santa Cruz
PhD Fellow - Lab. Receptores Nucelares, IByME - CONICET
Ciudad Autónoma de Buenos Aires, Argentina
Email: *iarasofiasantacruz@gmail.com*

Hsp90 maintains the active form of specific client proteins that already have a stable tertiary structure, including steroid receptors and various oncoproteins. Due to their high proteotoxic stress, cancer cells rely heavily on chaperones. Hsp90 inhibitors are the only chemotherapeutic agents known to strongly impact all hallmarks of cancer, making Hsp90 a promising target for cancer therapy. Numerous Hsp90 inhibitors are currently undergoing clinical and preclinical trials with varying results but have shown nephro- and hepatotoxicity. This study aimed to examine the biological actions of synthetic compounds designed via computational modelling for their potential inhibitory effect on Hsp90's intrinsic ATPase activity, which is crucial for its function. We evaluated these compounds on Hsp90 ATPase activity in vitro, as well as on cell viability and migration in breast cancer models. Additionally, their potential to inhibit glucocorticoid receptor (GR) nuclear translocation was tested. Geldanamycin (GA), a known Hsp90 inhibitor, served as a positive control. Pyrazoline-derivative compounds (C3, C6, and 4F) confirmed in silico predictions by effectively inhibiting Hsp90 ATPase activity. As anticipated, GA treatment inhibited nuclear import of the steroid receptor in normal cells and reduced cell viability and migration in both MDA-MB-231 and MCF7 cell lines. The synthetic drugs similarly inhibited cell viability and migration but did not affect GR nuclear import in normal cells. Interestingly, the compounds were observed to affect cytoskeletal stability, resulting in a more adherent cell phenotype. This lack of effect on steroid receptor inhibition suggests these drugs could have significant pharmacological benefits, avoiding certain side effects. The study provides new insights that could aid in the development of more effective and less toxic drugs.

Metabolism, Signaling and Breast Cancer Risk

Poster No. 57

Effect of neonicotinoid insecticide imidacloprid exposure on breast cancer cells

Chiara R. Santos[1], M. Agustina Leguizamón[1], Sol Buján[1], Carolina Pontillo[1], Florencia Chiappini[1], Marianela Lasagna[2], Claudia Cocca[2], Andrea Randi[1], Noelia V. Miret[1]

1- Universidad de Buenos Aires, Facultad de Medicina, Departamento de Bioquímica Humana, Laboratorio de Efectos Biológicos de Contaminantes Ambientales, Paraguay 2155, 5° piso, (CP 1121) Buenos Aires, Argentina, 2- Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Laboratorio de Radioisótopos, Junín 954, primer subsuelo, (CP 1113), Buenos Aires, Argentina.

Presenting Author:

Chiara Rita Santos

Medical/Resident - Universidad de Buenos Aires, Facultad de Medicina, Departamento de Bioquímica Humana, Laboratorio de Efectos Biológicos de Contaminantes Ambientales

Ciudad Autónoma de Buenos Aires, Argentina

Email: *santoschiaraa@gmail.com*

The incidence of breast cancer is increasing globally and exposure to endocrine disruptors (EDs) has become a potential risk factor for this disease. Different studies have linked breast cancer progression with pesticide exposure. Imidacloprid (IMI) is a neonicotinoid insecticide widely used and is postulated to act as an ED. Given that IMI modulates estradiol secretion in breast cancer cells, it has been suggested that it could promote this disease. Our objective was to evaluate the possible impact of IMI exposure on cell viability, proliferation, motility and estrogen receptor (ER)-α and G protein-coupled ER (GPER) protein expression in MCF-7 (ER+) and MDA-MB-231 (ER-) breast cancer cells. Cells were exposed to environmentally relevant concentrations of IMI (0.01-10 μM) for 24 h or vehicle (DMSO). Results showed that IMI does not alter cell viability (MTT assay) but produces an increase in cell proliferation only in MCF-7. The clonogenic assay showed an enhancement in the number of colonies at 0.01 μM, while the expression of the proliferation marker PCNA (western blot, WB) was increased at 0.1, 1 and 10 μM in MCF-7. No alterations were observed in the number of colonies of MDA-MB-231 after IMI treatment. On the other hand, WB results showed a reduction in the levels of ER-α at 0.01 μM and GPER at 10 μM in MCF-7. In addition, IMI decreased GPER expression in MDA-MB-231 at 0.01, 0.1 and 1 μM. Finally, metalloprotease-9 activity was increased in MCF-7 at all assayed doses (gel zymography), whereas cell migration was enhanced in MDA-MB-231 at 0.1 μM (wound healing assay). In conclusion, IMI exposure induces alterations in breast cancer cells that promote cell proliferation and motility, suggesting that it may be involved in breast cancer progression.

Metabolism, Signaling and Breast Cancer Risk

Poster No. 60

HEMEOXIGENASE-1 GENETIC VARIANTS EFFECTS ON BREAST CANCER PROGRESSION

Karen Schweitzer[1], Exequiel Gonzalo Alonso[1], Lucía Fernández Chávez[1], Georgina Pamela Coló[1], Eliana Noelia Alonso[1], María Julia Ferronato[1], Eugenia Fermento[1], Alejandro Carlos Curino[1], María Marta Facchinetti[1]

1- Instituto de Investigaciones Bioquímicas de Bahía Blanca.

Presenting Author:

Karen Schweitzer

PhD Fellow - Instituto de Investigaciones Bioquímicas de Bahía Blanca

Bahía Blanca, Argentina

Email: *karens96.ks@gmail.com*

Hemoxygenase-1 (HO-1) is a microsomal enzyme that catalyzes the degradation of the heme group (canonical role) and can be cleaved at its C-terminal end, followed by translocation to the nucleus, to perform functions at the transcriptional level (non-canonical role). Our laboratory has already demonstrated that HO-1 has antitumoral activity in breast cancer (BC) and that nuclear HO-1 is not enzymatically active. The aim of this work was to study the effect of genetic overexpression of HO-1 variants on cellular processes related to cancer progression, and the molecular mechanisms through which HO-1 modulates the cellular processes investigated. To accomplish this goal, we used the hormone-dependent BC cell line T47D and the triple-negative BC cell lines 4T1 and MDA-MB-231. These cell lines were stably transfected with plasmids overexpressing the HO-1 variants (full-length (FL-HO1), full-length without enzymatic activity (H25A-HO1) and truncated (T-HO1)). We observed significant differences in cell viability between wild-type (WT) cells and cells overexpressing HO-1 variants in the three cell lines evaluated ($p<0.05$, two-way ANOVA). We found that the WT cell line displays a higher rate of proliferation than those that overexpress FL in the three cell lines analyzed. However, the T-HO1 form behaves differently among cell lines, displaying more proliferative activity in 4T1 and MDA-MB-231 than in T47D cells. The H25A form appear to behave in the same way among cell lines, showing a similar phenotype to WT cells. These results indicate that the overexpression of HO-1 FL seems to display an anti-tumor role. The behavior of the H25A and the T-HO1 variants would indicate that the anti-tumor behavior is the result of both HO-1 enzymatic activity and its nuclear localization. In addition, cell line hormone-dependency would also contribute to the differential effects. Altogether, these results provide evidence of the canonical and non-canonical roles of HO-1 in BC.

Metabolism, Signaling and Breast Cancer Risk

Poster No. 63

CYTOTOXIC EFFECTS OF NOVEL NAPHTHOQUINONES ON MDA-MB-231 BREAST CANCER CELLS: INDUCTION OF OXIDATIVE STRESS AND APOPTOSIS

Hassen Nadir Sebik Vasquez[1], Lucas Nunes Vanazzi[1], Efrén Xavier Silva Moreta[1], Mariana Daniela Kovalovsky Barreiro[1], Alicia Juana Klecha[1], Horacio Eduardo Romeo[1], María Laura Barreiro Arcos[1]

1- Laboratory of Physiology and Experimental Microsurgery, Institute of Biomedical Research (BIOMED-CONICET), Catholic University of Argentina (UCA).

Presenting Author:

Hassen Nadir Sebik Vasquez

Undergraduate Student - Laboratory of Physiology and Experimental Microsurgery, Institute of Biomedical Research (BIOMED-CONICET), Catholic University of Argentina (UCA)

Buenos Aires, Argentina

Email: *hassensebik@uca.edu.ar*

INTRODUCTION: Breast cancer is one of the leading causes of cancer-related mortality in women worldwide. To develop more effective compounds against cancer and with fewer side effects than traditional chemotherapeutics, novel naphthoquinones (NfQs) with potential anticancer activity have been synthesized. OBJECTIVES: The aim of this study is to evaluate the action of the NfQ 3,4-dihydro-2,2-dimethyl-2H-naphtho[1,2-b]pyran-5,6-dione and its structural analogs NfQ1 (2-phenyl-3,4-dihydro-2H-benzo[h]chromene-5,6-dione) and NfQ2 (2-p-tolyl-3,4-dihydro-2H-benzo[h]chromene-5,6-dione) on the viability of MDA-MB-231 breast adenocarcinoma cell line, analyzing their ability to induce ROS production, mitochondrial dysfunction, and apoptosis. METHODOLOGY: MDA-MB-231 cells were incubated with NfQs (0 to 15 μM) for 24 h, and cytotoxic effects were quantified using the MTT assay. The CC50 was calculated from dose-response curves for each NfQ. Nuclear morphology was evaluated by fluorescence microscopy after staining cells with DAPI. Levels of ROS were assessed by flow cytometry using the probe DCFH-DA, and the production of O2- was evaluated using the NBT reduction technique. Mitochondrial membrane potential was assessed by flow cytometry using rhodamine-123. RESULTS: NfQs reduced the viability of MDA-MB-231 cells at concentrations above 0.5-1 μM after 24 h of culture, with similar CC50 values ($p<0.01$). Microscopic observation of DAPI-stained cells revealed condensed chromatin, membrane blebbing, and formation of apoptotic bodies, all events consistent with apoptosis. Incubation with NfQs increased levels of ROS and O2- compared to untreated cells ($p<0.01$). Additionally, NfQs induced alterations in mitochondrial membrane potential. CONCLUSIONS: NfQs exert cytotoxic effects on MDA-MB-231 cells by inducing oxidative stress and mitochondrial dysfunction, thereby promoting cellular apoptosis. These findings could contribute to improving current chemotherapy treatments.

Metabolism, Signaling and Breast Cancer Risk

Poster No. 66-A

Association between nutrition and tumor microenvironment in breast cancer

Erica Daniela Solla[1], Franco Fabián Roldán Gallardo[1], Cristina Alicia Maldonado[1], Amado Alfredo Quintar[1]

1- Universidad Nacional de Córdoba (UNC). Facultad de Ciencias Médicas. Centro de Microscopía Electrónica. Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). Instituto de Investigaciones en Ciencias de la Salud (INICSA, CONICET-UNC)..

Presenting Author:

Erica Daniela Solla

PhD Fellow - Centro de Microscopía Electrónica, INICSA - Facultad de Ciencias Médicas, Universidad Nacional de Córdoba

Cordoba, Argentina

Email: *erisolla@gmail.com*

Studies suggest an association between nutrition and tumor development. Dietary patterns with foods rich in carbohydrates (PBA) and saturated fatty acids (PCS), represented by fructose (F) and palmitic acid (PA), are associated with the risk of breast cancer, although the cellular mechanisms are not clear. The objective was to evaluate the effects of diet on interactions of the tumor microenvironment, between cancer-associated fibroblasts (CAFs) and breast tumor cells, mediated by extracellular vesicles (EVs). Balb/c mice fed 2 months with PBA, PCS, or PBA+PCS were implanted s.c. with LM3 breast tumor cells. Tumors from mice on the PBA+PCS diet showed a higher frequency of CAFs, collagen and ultrastructural signs of increased EV secretion in transmission electron microscopy (TEM). Immunohistochemistry with CD63 revealed more intense and diffuse staining in the cell membrane, suggesting greater release of exosomes. In vitro, the mammary CAFs F88 cell line was stimulated with F40mM, AP250uM, F+AP or their vehicles for 24h. EVs were isolated from supernatants by sequential ultracentrifugation, characterized by MET and labeled with CD63 using immunogold. F+AP increased the frequency of EVs of 20-30nm compared to controls or F and AP alone. MCF-7 tumor cells were stimulated for 24 h with F88 conditioned media treated with F, AP and F+AP and with F88 EVs treated equally. Conditioned media and F88 EVs treated with F+AP increased MCF-7 cell proliferation, determined by bromodeoxyuridine and cell count. This protumor action of EVs was inhibited by preincubating MCF-7 with genistein, suggesting clathrin-independent uptake. F88 EVs treated with F+AP decreased cell apoptosis, measured by annexin V flow cytometry in MCF-7 and MDA-MB-231 cells. These results indicate the pathogenic effect of dietary patterns rich in F and AP in the breast tumor microenvironment, through the release of pro-proliferative EVs,

Metabolism, Signaling and Breast Cancer Risk

Poster No. 66-B

Antitumoral effects of natural derivatives in human breast cancer cells

Cayado-Gutiérrez N [1], Cuello-Carrión FD [1], García CE [2], Martín IB [3], Garro HA [4], Pungitore CR [4], Fanelli MA [1].

1- Oncology Laboratory, Institute of Medicine and Experimental Biology of Cuyo (IMBECU), CCT-CONICET, Universidad Nacional de Cuyo, Mendoza, Argentina. 2- University Institute of Bio-Organic "Antonio González", Universidad de La Laguna, La Laguna, España. 3- Institute of Natural Products and Agrobiology, San Cristóbal de La Laguna, Santa Cruz de Tenerife, Islas Canarias, España. 4- Chemical Technology Research Institute (INTEQUI), CONICET. Department of Chemistry, Universidad Nacional de San Luis, San Luis, Argentina.

Presenting Autor:

Niubys de los Milagros Cayado Gutiérrez

Oncology laboratory. Institute of Medicine and Experimental Biology of Cuyo (IMBECU), CONICET.

ncayado@mendoza-conicet.gob.ar

Despite advances in breast cancer treatments, recurrence, and metastasis still occur. Phytochemicals and their derivatives are promising as alternative anticancer agents. Coumarin derivatives have demonstrated anticancer activity in various tumor cells. 3-isopropyl-4-methyl-5,7-dihydroxycoumarin (C13) is a new semi-synthetic coumarin with inhibitory activity against Taq DNA polymerase in vitro, making it an attractive target for cancer research. Additionally, chalcone derivatives combined with halogens and O-benzyl groups have shown significant antitumor effects in oral squamous cell carcinoma and colon cancer, respectively. However, the biological activity of novel chalcones modified by chlorine (JS23) and O-benzyl groups (X2) remains unknown. Searching for new antineoplastic alternatives, we studied the antitumor effects of three natural derivatives—C13, JS23, and X2—in human MCF-7 breast cancer cells. Cells were exposed to serum-free media containing these derivatives at concentrations ranging from 0 to 50 μM for 24, 48, and 72 h. The compounds were dissolved in dimethyl sulfoxide (DMSO), and cells treated with 1% DMSO for 48 h were used as negative controls. Cell viability was assessed by MTT assay. Ki-67 immunostaining, TUNEL, senescence-associated β-galactosidase, and wound healing assays were performed on C13-treated cells. C13 and X2 significantly inhibited cell viability with the same IC50 value (25 μM) in a dose- and time-dependent manner, although X2 reached this pattern at 30 μM. JS23 exhibited the strongest cytotoxic activity in a dose-dependent but time-independent manner (IC50=10.03 μM). C13-treated cells at IC50 concentration showed a Ki-67 proliferation index of 43.01%, an apoptotic mean value of 0.21, dose-dependent induction of senescence, and significant cell migration inhibition after 6 to 72 h of treatment in a dose-independent manner. These natural derivatives could represent promising alternatives to current breast cancer therapies.

Understanding and modeling breast cancer subtypes

Poster No. 69

Tumoral PD-L1-dependent regulation of TAM immunosuppresion during TNBC progression

Paula Anabella Aguirre[1], Lilian Fedra Castillo[13,1], Marcos Daniel Palavecino[2], Paula Macarena Gonzalez[1], Sabrina Aldana Vallone[2], Roberto Meiss[4], Santiago Rodriguez-Seguí[2], Omar Adrian Coso[2], Eva Wertheimer[5], Edith Claudia Kordon[2], Marina Simian[3], Emilse Andrea Errasti[6], Eugenio Antonio Carrera-Silva[7], Manuel De la Mata[2], Albana Gattelli[2], Juan Pablo Fededa[1]

1- Instituto de Investigaciones Biotecnológicas (UNSAM/CONICET), San Martin, PBA, Argentina, 2- Instituto de Fisiología, Biología Molecular y Neurociencias (UBA/CONICET), CABA, Argentina, 3- Instituto de Nanosistemas (UNSAM), San Martin, PBA, Argentina, 4- Academia Nacional de Medicina, CABA, Argentina.5Centro de Estudios Farmacológicos y Botánicos (UBA/CONICET), CABA, Argentina.6-Instituto de Farmacología, Facultad de Medicina (UBA), CABA, Argentina. 7-Instituto de Medicina Experimental (ANM/CONICET), CABA, Argentina.

Presenting Author:
Paula Aguirre
PhD Fellow - Instituto de Investigaciones Biotecnológicas (IIBio - UNSAM/CONICET)
San Martín - Buenos Aires, Argentina
Email: *paguirre@iib.unsam.edu.ar*

T-cell PD-1 engagement by tumoral PD-L1 is widely recognized as one of the main immunosuppressive mechanisms driving cytotoxic T-cell exhaustion. However, it is unclear how tumoral PD-L1 modulates immune evasion in non-T cell PD1+ immune populations, such as tumor-associated macrophages (TAMs).

To interrogate this, we generated a PD-L1 KO TNBC-like tumor model in the murine EO771 cell line using CRISPR/Cas9 editing, allowing us to profile the immune infiltrates of the tumoral microenvironment (TME) in vivo during tumoral progression.

Using flow cytometry (FC) to characterize the immune infiltrates of early vs late-stage WT tumors, we found a late-stage decrease in F480+ CD206+ populations, suggesting that M2 TAM polarization is inhibited during tumor development. Furthermore, analyzing PD-1 expression of immune infiltrates we observed an increase in PD1+ M2 TAMs at late-stage, suggesting that advanced tumors are more responsive to PD-L1.
In addition, examining PD-L1 KO vs WT tumors at early and late-stage we found that tumoral PD-L1 inhibits M2 TAM polarization exclusively at late stage. Using bone marrow-derived macrophages in tumoral cells & conditioned media co-culture experiments, we found that M2 TAM inhibition is a direct effect that involves tumor cell-to-macrophage contact. Interestingly, M2 TAMs from late-stage WT tumors showed increased MHCII+ expression, suggesting an improvement in antigen-presentation potential. Moreover, using FC to analyze GFP+ tumor cell phagocytosis, we found that tumor progression triggers phagocytosis exclusively in M2 TAMs. Comparing PD-L1 KO vs WT tumors, we observed that tumoral PD-L1 inhibits TAMs phagocytosis both in vivo and in vitro.
Altogether, these results suggest that M2 TAMs acquire anti-tumoral features during tumor progression and that tumoral PD-L1 dependent inhibition of M2 polarization plays a critical role in TAM immunosuppression during late stages of TNBC progression.

Understanding and modeling breast cancer subtypes

Poster No. 72

Characterization of Novel M46 Murine Breast Cancer Model

Noelia Paola Cardozo[1], Carolina Belén Iglesias[1], Carla Pulero[2], María de las Nieves Pelagatti[1], Marianela Vence[1], Fernanda Roca[2], Lina Marino[2], Erica Rojas Bibao[1], Yanina Verónica Langle[1]
1- Departamento de Bioterio y Cáncer Experimental - Área de Investigación - Instituto de Oncología Ángel H. Roffo, 2- Departamento de Anatomía-Patológica - Área de Diagnostico - Instituto de Oncología Ángel H. Roffo.

Presenting Author:
Noelia Paola Natalia Cardozo
Researcher - Departamento de Bioterio y Cáncer Experimental - Instituto de Oncología Ángel H. Roffo
Ciudad Autónoma de Buenos Aires, Argentina
Email: *cardozo.paola83@gmail.com*

Breast cancer (BC) is the second most common tumor worldwide and is the first in incidence in women. Nowadays, there are only a few pre-clinical syngeneic murine models for BC study.
M46 tumor spontaneously appeared in a BALB/c female of the Instituto Roffo Animal´s Facility. Tumor was maintained through subcutaneous (sc) transplantation and cryopreservation. The objective of this study was to characterize the novel M46 breast tumor to establish a new pre-clinical murine model. M46 tumor was transplanted with a trocar needle into the sc of females and males BALB/c mice. Tumor grew in all animals, being euthanized after 20 days of inoculation. Tumor volume reached 1029 ± 367.3mm3 in female and 1040 ± 367.4mm3 in male (mean ± SD). Tumor, lungs and liver were fixed to histological analysis and metastasis quantification. The histopathological diagnostic determined that M46 is a High Grade (GIII) invasive carcinoma, with a Nottingham Score of 8 (GH3, GN3, GM2). It has sections with atypical neoplastic proliferation consisting of sheets of epithelial cells with anisodiskaryosis and numerous atypical mitotic figures. M46 presents areas of necrosis and hemorrhage, with a few areas of associated stroma accompanied by mild inflammatory infiltrate. These pathological features are related with an aggressive tumor associated with the general disease that we observed in our mice. M46 tumor developed spontaneous lung metastasis in 100% of mice, with 11 (2-100) and 9 (2-18) metastasis per lung (median and range) in female and male respectively. The incidence of liver metastasis was 55% in female and 70% in male with a median of 1 per liver. There are no significant differences between females and males in the tumor size, tumor growth kinetics, lung metastasis and liver metastasis development. In conclusion, our findings establish M46 as a novel pre-clinical murine model of invasive and metastatic BC. This model holds promise as a valuable tool for further research into this disease

Understanding and modeling breast cancer subtypes

Poster No. 75

Participation of RUNX1 in mitochondrial dynamics and reprogramming in AR+ TNBC

Natalia Brenda Fernández[1,2], María Florencia Pignataro[1,2], Lucía Escobar[1], Facundo Luis Couto[1], Javier Santos[1,2], Natalia Rubinstein[1,2]

1- Instituto de Biociencias, Biotecnología y Biología traslacional (iB3); Departamenteo de Fisiología y Biología Molecular y Celular (FBMC); Facultad de Ciencias Exactas y Naturales (FCEyN); Universidad de Buenos Aires (UBA), 2- Consejo Nacional de Ciencia y Tecnología (CONICET).

Presenting Author:
Natalia Brenda Fernández
Researcher - iB3-FCEyN-UBA
Ciudad Autónoma de Buenos Aires, Argentina
Email: *natyfernandez24@gmail.com*

Triple negative breast cancer (TNBC) is one of the most aggressive cancer subtypes in which 35% of patients suffer tumor recurrence. Increasing evidence suggests that this high recurrence is due to the presence of tumor cells with stem cell-like characteristics (CSC). It has been reported that the acquisition of a CSC phenotype correlates with an increased expression of the androgen receptor (AR). We previously showed that AR regulates the expression of the transcription factor RUNX1 in AR+ TNBC cell lines and PDX. RUNX1 expression correlates with a poor prognosis in TNBC patients, and we reported that it participates in the CSC generation and chemoresistance in TNBC. Recent evidence indicates that CSC presents an imbalance towards the use of mitochondrial oxidative phosphorylation producing functional and morphological reprogramming. The AR and RUNX1 signaling pathways have been associated with mitophagy and mitochondrial function in some cancer models. Here we show, for the first time, that inhibition of RUNX transcriptional activity with the commercial inhibitor AI-10-104 leads to mitochondria reorganization and fragmentation. Moreover, MDA-MB-453 and SUM-159PT cells lines treated with AI-10-104 have an accumulation of the immature isoform of the mitochondrial protein frataxin (FXN) with the consequent decrease of the mature and functional isoform. FXN is a small protein involved in the biosynthesis of Fe-S clusters and their transfer to key cell metabolism proteins; alterations in FXN leads to neurodegenerative and metabolic disorders. On the other hand, we didn't find changes in the expression of the mitophagy genes BECLIN1 and ATG7, suggesting that it would not be involved at these temporal points. We hypothesize that the AR/RUNX signaling pathway participate in mitochondrial reprogramming favoring the survival of TNBC cells, and our aim is to characterize the role of AR and RUNX1 as potential new modulators of mitochondrial metabolism and dynamics in AR+ TNBC.

Understanding and modeling breast cancer subtypes

Poster No. 77

Subtyping breast cancer PDX: androgen and glucocorticoid receptors, EGFR and lineage markers

Gabriela Pataccini[1], Luisa Ambrosio[1], Paula Martínez Vázquez[2], Javier Burruchaga[2], Eunice Spengler[2], Claudia Lanari[1]

1- Instituto de Biología y Medicina Experimental (IBYME-CONICET), Buenos Aires, Argentina, 2- Hospital Magdalena V de Martínez, General Pacheco, Buenos Aires, Argentina.

Presenting Author:
Gabriela Pataccini
Postdoc Fellow - Instituto de biologia y medicina experimental (IBYME)
Buenos Aires, Argentina
Email: *gaby.pataccini@hotmail.com*

Patient-derived tumor xenografts (PDX) are reliable models generated when tumor tissue is transplanted from patients into immune-deficient mice. Breast cancer PDX usually retain ER, PR, or HER2 expression as their parental tumors. Other nuclear receptors such as androgen (AR) and glucocorticoid receptors (GR), are currently being explored as therapeutic targets. Our goal was to characterize twelve PDX developed in our laboratory regarding AR, GR, phospho-PR (p-PR), lineage markers (CK5/6 and CK8/18) and EGFR expression, used to sub-classify triple-negative (TN) breast cancers (TNBC). ER+ PDX (BC-AR685, BC-AR707, BC-AR767 and BC-AR781) were AR+, GR+, EGFR-, and expressed luminal markers (CK8/18+). Interestingly, they exhibited low or null PR levels (except for BC-AR685) but were stained positive for p-PR (except for BC-AR767). BC-AR767 expressed HER2, and BC-AR781 acquired HER2 expression. Only one out of the 12 PDX was ER-, PR- and HER2+ (BC-AR474). These PDX expressed AR, EGFR, CK8/18 and p-PR. PDX BC-AR485 originated from a TN lymph node recurrence during chemotherapy, although the primary tumor was ER+. This PDX showed an intense CK5/6 and GR staining, and mild EGFR and p-PR expression. The remaining TN PDX (BC-AR546, BC-AR553, BC-AR631, BC-AR687, BC-AR775 and BC-AR815) expressed CK8/18. BC-AR815 also showed intense CK5/6 staining, whereas BC-AR687 showed CK5/6+ foci. BC-AR815 was the only AR- TN PDX. Strong AR staining in CK8/18+ tumors suggest they belong to the luminal androgen receptor positive (LAR) subtype. GR was co-expressed with AR in 3 out of 6 TN PDX. EGFR and p-PR were highly expressed in BC-AR553 PDX. In general, PDX and parental tumors showed similar patterns. These PDX are excellent tools for studying tumor progression and testing the combination of AR and GR ligands with standard or novel therapies. p-PR expression in PR-negative PDX, underscores the role of the PR pathway even in tumors considered as PR negative.

Understanding and modeling breast cancer subtypes

Poster No. 79

LEFT-RIGHT EPIGENETIC AND BIOELECTRIC DIFFERENCES IN BREAST CANCER

Sebastián Real[1], Sergio Laurito[1], María Roqué[1]
1- Instituto de Histología y Embriología de Mendoza, CONICET-UNCuyo.

Presenting Author:
Sebastián Real
Researcher - IHEM, CONICET-UNCuyo
Mendoza, Argentina
Email: *tazreal@gmail.com*

In previous work, we identified a differential DNA methylation profile between left (L) and right (R) breast tumors. L tumors exhibited enhanced membrane depolarization, increased proliferation, and elevated stemness scores. Given the inherent asymmetry of L-R mammary glands, it is reasonable to expect bioelectric and epigenetic differences, both responsive to environmental modulation. One key question we are exploring now is whether bioelectricity and epigenetics form a dual circuit. In this work, we present our latest findings addressing this question. Through in-silico analyses, we found increased expression of DNA methylation writers in L tumors (Welch's unpaired t-test, p=0,004). To investigate potential global methylation differences between L-R tumors, we examined RRBS data from three paired L-R xenograft tumors. Additionally, we used R-TCGA BioLinks to analyze the complete methylation of 350,000 CpG sites. We also conducted MS-MLPA methylation analyses on cells cultured in L-R conditioned medium, targeting 20 CpG sites of imprinted regions. All approaches revealed no significant differences between L-R tumors (Wilcoxon test and paired T-test, p>0,05). We also examined methylation differences in specific ICH but found no strong correlations with laterality, leading us to conclude there are no enzymatic activity differences, despite the increased expression in L tumors. However, when MDA-MB231 cells were cultured in L-R conditioned medium, the bioelectric difference was abolished upon treatment with DNMT inhibitors. Specifically, the R-treated cells shifted from a hyperpolarized to a depolarized state, whereas the L-treated cells showed no response. These observations strongly suggest that bioelectric differences depend on DNA methylation writers, although not on their expression nor activity level. The underlying mechanisms are currently under investigation in our group, using animal models, cell culture models, and bioinformatics approaches.

Understanding and modeling breast cancer subtypes

Poster No. 80

Studying tumor microenvironment components in breast cancer: association between hyaluronan metabolism and DNA repair mechanisms

Candela Morán[1], Daiana Luján Vitale[1], Paolo Rosales[1], Antonella Icardi[1], Laura Alaniz[1], Ina Sevic[1]

1. Laboratorio de Microambiente Tumoral - Centro de Investigaciones Básicas y Aplicadas (CIBA) - Centro de Investigaciones y Transferencia del Noroeste de la Provincia de Buenos Aires (UNNOBA-UNSAdA-CONICET)

Presenting Autor:

Candela Morán Maidana

Centro de Investigaciones Básicas y Aplicadas (CIBA)

candemoran.cm43@gmail.com

In recent years, cancer had an increase in recurrences, due to its heterogeneity and molecular complexity. This could explain, in part, the failure in the efficacy of treatment for cancer patients. Tumor microenvironment (TME) consists of cells and macromolecules that surround a tumor cell. Its main non-cellular component is the extracellular matrix (ECM), a complex network of proteins and polysaccharides that is highly deregulated in tumor context. Among the ECM components that are altered in tumors are hyaluronan (HA) and molecules associated with its metabolism. On the other hand, TME alterations can influence the expression of tumor-associated genes, such as BRCA1 and BRCA2, mainly involved in DNA repair. The aim of this study was to analyze alterations in HA signaling (CD44), synthesis (HAS2) and degradation (HYAL1) in tumor tissue (TT) and non-tumor adjacent tissue (NAT) from breast cancer patients, and its association with the expression of BRCA1 and BRCA2 and with patients' clinical data. Additionally, we analyzed the expression of these genes in 3D spheroids of MDA-MB-231 and MCF-7 breast cancer cells after HA degradation (HYAL) or inhibition of its synthesis (4-MU), also evaluating the volume and area of the spheroids generated. mRNA expression in both patients and cell samples was evaluated by RT-qPCR. The levels of HA and BRCA1, BRCA2 and CD44 proteins from patients were analyzed by immunohistochemistry. Breast cancer patients showed lower levels of HAS2 (mRNA) and BRCA1 and BRCA2 (protein) in TT compared to NAT. Besides, we found a strong positive correlation between CD44 and HYAL1 mRNAs. In turn, a decrease in the size of the spheroids was observed when HA synthesis was inhibited. This work presents important evidence regarding the influence of ECM, particularly HA and molecules associated with its metabolism on DNA repair genes, which is crucial for identifying key markers to predict tumor progression and guide potential therapeutic approaches.