Lorena P. Burton, MD[1], Gang Deng, PhD[2], Cristian D. Yanes, BS[1], Jaydutt V. Vadgama, PhD[3], Michael E. Jung, PhD[2], Richard J. Pietras, PhD,MD[4], Diana C. Marquez-Garban, MD[1].
[1]Division of Hematology-Oncology, UCLA Department of Medicine, Los Angeles, CA, USA, [2]UCLA Department of Chemistry and Biochemistry, Los Angeles, CA, USA, [3]Division of Cancer Research and Training, Charles Drew University and UCLA Department of Medicine, Los Angeles, CA, USA, [4]Division of Hematology-Oncology, UCLA Department of Medicine and Division of Cancer Research and Training, Charles Drew University, Los Angeles, CA, USA.

Pancreatic ductal adenocarcinoma (PDCA) is a leading cause of cancer death in the US. Patients diagnosed with PDCA generally present with advanced disease with poor prognosis and limited treatment options. African American patients have higher incidence and mortality of PDCA than Caucasian American or any other ethnic group. Different factors have been attributed to contribute to this health disparity, among them higher incidence of Diabetes Mellitus type 2. To address the need for new therapeutic approaches, we note epidemiologic reports that patients with diabetes mellitus-type 2 treated with the biguanide drug metformin, but not other antidiabetic drugs, have a reduced risk of PDCA and an increased survival rate among those with PDCA. The main physiologic effect of metformin is to lower blood glucose and reduce hyperinsulinemia associated with insulin resistance. In the cell, metformin stimulates AMP-activated protein kinase (AMPK) that in turn inhibits mTORC1 which integrates signals from an array of intracellular pathways to regulate cell growth. Recent clinical trials describe modest antiproliferative effects from use of neoadjuvant metformin, but no significant clinical benefit occurred when metformin was dosed at glycemic control levels in patients with advanced cancers. These findings suggest that development of more potent anticancer analogues of metformin may help to boost clinical benefit and patient survival. Hence, we have designed new biguanide analogues of metformin, and screening of these compounds in preclinical PDCA models show that selected analogues are more efficacious in blocking tumor progression than parental metformin at lower doses. Using proliferation assays *in vitro*, PDCA cells (Panc 1, MIA Paca-2) were treated 72-hrs with metformin or analogues, and greater dose-dependent inhibition of PDCA cell proliferation was found with analogues as compared to metformin ($P<0.001$). Further, apoptosis was also markedly induced by metformin analogues as compared to parental metformin ($P<0.01$). Antitumor effects of metformin are attributed in part to activation LKB1-AMPK pathways and downstream blockade of mTOR signaling, which is often increased in PDCA cells. Using PDCA cells treated *in vitro* with analogues for 24-hrs, we find that analogues induce AMPK phosphorylation and suppression of mTOR signaling, thus blocking protein synthesis and tumor proliferation. With an *in vivo* PANC 1 xenograft model in nude mice, lead metformin analogues given by oral gavage daily significantly inhibited tumor progression over 28-days as compared to appropriate controls ($P<0.0001$). Our findings show that selected metformin analogues have potent anticancer activity in preclinical PDCA models and may have promise as new targeted therapeutics for patients afflicted with this deadly disease. [Funded by NIH/NCI R21CA176337 and NIH/NCI U54 CA143930]

Tumor Biology

HORMONE ACTIONS IN TUMOR BIOLOGY: FROM NEW MECHANISMS TO THERAPY

Nuclear ErbB-2-Induced Transcriptome Drives Triple Negative Breast Cancer Growth

Maria F. Chervo, PhD[1], Micaela Parra, MSc[2], Nicolas Bellora, PhD[2], Ezequiel Petrillo, PhD[3], Santiago Madera, MSc[1], Agustina Roldan Deamicis, MSc[1], Kohzoh Mitsuya, PhD[4], Violeta A. Chiauzzi, MSc[1], Cecilia J. Proietti, PhD[1], Roxana Schillaci, PhD[1], Tim H-M Huang, PhD[4], Rosalia I. Cordo Russo, PhD[1], Patricia V. Elizalde, PhD[1].
[1]Instituto de Biologia y Medicina Experimental IBYME-CONICET, Buenos Aires, Argentina, [2]Instituto Andino-Patagonico de Tecnologias Biologicas y Geoambientales (IPATEC), CONICET-UNComahue, Bariloche, Argentina, [3]Universidad de Buenos Aires (UBA), Facultad de Ciencias Exactas y Naturales, Departamento de Fisiologia, Biologia Molecular y Celular and CONICET-UBA, Instituto de Fisiologia, Biologia Molecular y Neurociencias (IFIBYNE), Buenos Aires, Argentina, [4]Department of Molecular Medicine, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA.

Triple negative breast cancer (TNBC) refers to tumors that do not express clinically significant levels of estrogen and progesterone receptors, and lack membrane overexpression or gene amplification of ErbB-2 tyrosine kinase receptor. Transcriptome and proteome heterogeneity of TNBC poses a major challenge to precision medicine. Gene expression analyses have categorized TNBC into distinct molecular subtypes. Up to 78% of clinical TNBCs belong to the basal-like (BL) subtype. Here we found ErbB-2 in an unanticipated scenario: the nucleus of TNBC (NErbB-2). Our study on ErbB-2 alternative splicing, using a PCR-sequencing approach combined with RNA interference, revealed that BL TNBC cells express the canonical ErbB-2 (WTErbB-2), encoded by transcript 1, and the non-canonical isoform c, encoded by alternative transcript 3 (T3). The latter was not previously reported in normal or malignant cells. To characterize the isoform c we designed siRNAs targeting T3 (T3 siRNAs), which silenced up to 93% of said isoform. Transfection of T3 siRNAs into BL cells expressing only isoform c or both isoform c and WTErbB-2 was sufficient to decrease cell proliferation. Intratumoral injections of T3 siRNAs into mice bearing BL TN tumors also blocked *in vivo* growth. To explore whether isoform c growth-promoting effect is due to its functions as a transcriptional regulator, we performed RNA-seq in BL cells expressing only this isoform. We identified a set of genes differentially regulated in BL cells where we evicted isoform c from the nucleus, as compared to control cells. In the up-regulated group, we found enrichment of pro-apoptotic and tumor suppressor genes and in the down-regulated one, genes involved in proliferation and stemness. We used gene set enrichment analysis (GSEA) to identify the biological processes associated with these isoform c-regulated genes. We found a pronounced enrichment of gene sets related to apoptosis, activation of DNA damage pathways and cell cycle arrest in response to eviction of nuclear isoform c. GSEA

also revealed negative regulation of gene sets involved in cell motility, cellular differentiation and growth pathways in BL cells lacking nuclear isoform c expression. These results suggest that NErbB-2 function modulates tumor growth and promotes a metastatic phenotype in TNBC. Furthermore, our clinical findings identified NErbB-2 as an independent predictor of shorter OS
(HR 2.54; 95% CI 1.22-5.28; P = 0.013), DFS (HR 2.91; 95% CI 1.44-5.87; P = 0.003), and DMFS (HR 2.59; 95% CI 1.20-5.60; P = 0.015) in 99 TN primary tumors. Our discoveries challenge the present scenario of drug development for personalized BC medicine that focuses on wild-type proteins, which conserve the canonical domains and are located in their classical cellular compartments, highlighting the potential of NErbB-2 isoforms as novel therapeutic targets and clinical biomarkers in TNBC.

Tumor Biology

HORMONE ACTIONS IN TUMOR BIOLOGY: FROM NEW MECHANISMS TO THERAPY

Osteoblast-Induced Prostate Cancer Cell Migration and Invasion Is Mediated Through TGF-β1/SMAD2 Signal Pathway and Blocked by 17α-Estradiol

Jian Shi, MD, Lian Zhao, MD, Brittany Duncan, BS, Jie Su, PhD, Jale Manzo, BS, He Liu, MD, Yuan-Shan Zhu, MD, PhD.
Weill Cornell Medical College, New York, NY, USA.

Prostate cancer (PCa) is curable if it is diagnosed and treated in localized and regional stage. However, PCa outcome is poor once it has distant metastasis. Approximately 70% to 100% of PCa deaths have bone metastasis, which may be associated with a specific bone microenvironment. In this study, we investigated the effect and molecular mechanism of osteoblast cells on stimulation of PCa cell migration and invasion and examined the effectiveness of 17α-estradiol on blocking osteoblast-induced PCa cell migration and invasion using in vitro cell analysis. PCa cells (PC3, LNCaP and DU145), osteoblast hFOB, kidney CV-1, breast tumor MCF-7 and liver cancer Huh-7 cells (ATCC) were cultured in RMPI-1640 or DMEM media supplemented with or without fetal bovine serum (FBS) at 37 °C in a 5% CO_2-humidified incubator. hFOB condition media (HCM) without FBS were collected at different times of hFOB cell culture. Transwell and wound-healing experiments were used to determine PCa cell migration and invasion. Cell migration and invasion in PC3, DU-145 and LNCaP PCa cells were markedly promoted by co-culturing hFOB osteoblast cells or HCM, but not by cells or condition media originated from kidney (CV-1), liver (Huh-7) and breast (MCF-7). Compared to other non-osteoblast cell conditioned media, HCM had much higher levels of several cytokines and chemokines including tumor growth factor (TGF) β1. Both HCM and TGF-β1 produced a dose- and time-dependent induction of PCa cell migration and invasion as well as SMAD2 phosphorylation without altering cell proliferation. These HCM and TGF-β1 effects were inhibited by a specific TGFβ receptor inhibitor, LY2157299, as well as by 17α-estradiol in a dose-dependent manner. Most intriguing, 17α-estradiol significantly inhibited the HCM and TGF-β1-induced PCa cell migration and invasion at very low nanomolar concentrations, presumably mediated through estrogen receptor β. These findings suggest that TGF-β1 is a major factor in mediating hFOB cell stimulation of PCa cell migration and invasion, and 17α-estradiol is a potential agent to block PCa cell bone metastasis, probably through inhibition of TGF-β1/SMAD2 signal pathway.

Tumor Biology

HORMONE ACTIONS IN TUMOR BIOLOGY: FROM NEW MECHANISMS TO THERAPY

Preclinical Evidence of the Efficacy of Lewis Y Car T Cells in Patient-Derived Models of Prostate Cancer

Gail Petuna Risbridger, PhD[1], Laura Helen Porter, BSc(hons)[1], Joe Zhu, PhD[2], David Byrne, Nil[2], Natalie Lister, PhD[3], Arun Azad, MD, PhD[2], Michael Hofman, MD[2], Ian Vela, MD, PhD[4], Melbourne Urological Research Alliance (MURAL), Nil[1], Renea A. Taylor, PhD[3], Paul Neeson, PhD[2], Phil Darcy, PhD[2], Joseph Trapani, MD, PhD[2].
[1]Monash University, Clayton, Victoria, Australia, [2]Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia, [3]Monash University, Clayton, Australia, [4]Queensland University of Technology, Brisbane, Queensland, Australia.

Chimeric antigen receptor T (CAR T) cell therapy is an adoptive immunotherapy that has led to new treatments for lymphoma, leukemia, and other blood cancers; however, its efficacy for prostate cancer remains unproven. Here we report pre-clinical evidence of the efficacy of CAR T cell therapy against the Lewis Y antigen (LeY) using patient-derived models of prostate cancer. To assess the expression of LeY on prostate tumours, we performed immunohistochemistry on a cohort of 41 patient-derived xenografts (PDXs). Cytoplasmic and membrane expression were separately assessed and quantified, for each patient. Overall, 61% (25/41) of PDXs were positive for membrane LeY expression, of which 18 PDXs had greater than 50% membrane-positive cells, and considered most suitable to detection and stable binding by anti-LeY CAR T's. To determine the *in vitro* sensitivity to CAR T cytotoxicity, we selected 4 PDXs with high and 2 PDXs with low LeY expression using 3 androgen receptor (AR)-positive adenocarcinomas and 3 AR-negative tumors expressing neuroendocrine markers. Next we established organoids for *in vitro* co-culture assays where organoids were co-incubated with an equal number of anti-LeY+ CAR T cells or Empty vector control CAR T cells (Ev CAR T). Using time-lapse microscopy we reported destruction of organoids by LeY+ CAR T cells as indicated by their morphological collapse and uptake of propidium iodide from the culture medium; control Ev CAR T cells produced no cytotoxicity. Over the 48h assay, the level of target cell death of the LeY+ organoids was correlated to the intensity LeY surface expression. Target cell death mediated by the CAR T cells required perforin and granzyme B, as potent and highly specific small molecule inhibitors of perforin (SN34960) and granzyme B (C20) applied alone or in combination greatly decreased PI uptake, indicating organoid survival. Neither inhibitor adversely affected CAR T cell viability as measured by PI and Annexin V staining. This demonstrated canonical activation of granule exocytosis pathway by the CAR T cells, leading to organoid cell death. To assess CAR T cell efficacy *in vivo*, we selected one PDX