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EDITORES RESPONSABLES

Claudia Pérez Leirós
Pablo Baldi
Alberto Crottogini

The aim of this study was to determine the somatic and germline mutation spectrum in high-risk BRCA1/2 mutation-negative HBOC patients from Argentina.
Tumor and matched normal samples were collected from eleven patients. The DNA was extracted from peripheral blood and tumoral tissue. Library preparation was performed using the GeneRead (Qiagen) workflow for cancer predisposition panel (141 genes) .Clonal amplification and sequencing was performed on the Ion PGM platform (Thermofisher).
We could identify somatic variants in 5 of 11 samples analyzed due to low DNA quality of 6 of the samples. After quality and functional filtering we found 278 somatic variants on Tumor suppressor genes (16.8%) and Oncogenes (5.72%). The most frequent somatic altered genes were: POLE, SMARCA4, ATR. Regarding sequence ontology: 60,5% were nonsense, 33% missense, 3% stopgain and 1,8% splice site. Mutational signature have been also analyzed showing the presence of signatures 19 and 30 mainly.
The developed paired tumor/normal workflow allowed us to identified tumor exclusive mutations. This approach helps to understand the genomic biology of HBOC tumors. Our data shows that NGS based gene panel sequencing is an tool for identify germline and somatic variants. However, we still have to improve the methodology to increase the efficiency of the workflow that is dependent of preanalytic parameters.

536. (572) **ANALYSIS OF TELOMERE LENGTH IN WOMEN WITH POLYCYSTIC OVARY SYNDROME**

Andrea Millan, Mariela Velazquez, Giselle Adriana Abruzzese, Silvina Cocucci, Andea Molli, Mabel Graffigna, Gustavo Frechtel, Alicia Beatriz Motta, Gloria Cerrone
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Polycystic ovary syndrome (PCOS) is the most common endocrine disorder affecting women in their reproductive age. We aimed to determine the absolute LT (LTa) in women with PCOS in comparison with healthy controls and their association with metabolic variables and the presence of hyperandrogenism.
We analyzed 86 control women and 130 PCOS patients (16-46 years of age). Measurements of biochemical, clinical, anthropometric and hormonal variables were made. Biochemical hyperandrogenism (HA) presence was determined (total testosterone levels higher than 0.9ng/mL). LTa determination was performed on genomic DNA from peripheral blood leukocytes by Real Time PCR absolute quantitative method. The relationship kpb of telomeric sequences and copies of the single copy gene RPLPO (radio T/S) was determined. Statistical analysis were carried out by one-way ANOVA and linear regression. PCOS patients have a higher weight, body mass index (BMI), greater waist circumference (WC), higher levels of triglycerides (TG), and fasting plasma glucose as compared to controls. An inverse relationship was observed between LTa and age (p=0.004). PCOS patients presented increased LTa as compared to controls (p=0.001, adjusted for age: p=0.005). Moreover, we found higher levels of LTa in PCOS-HA women as compared to PCOS-NHA and Controls (p=0.004). In PCOS patients, we found an association between higher LTa and lower BMI (p=0.040), lower WC (p = 0.004), lower TG levels (p = 0.049), lower DPB (p=0.001) and higher c-HDL (p=0.004).
In conclusion, LTa presents an inverse relationship with age in the studied population. The significantly increased LTa in PCOS patients (as compared to controls) could be a consequence of the presence of different metabolic, but mainly, hormonal components. A lower LTa was associated with presence of metabolic syndrome components, while biochemical HA was associated with higher LTa. Our results contribute to knowledge about the role of LT in the pathophysiology of PCOS.

537. (112) **MOLECULAR ANALYSIS OF AN ARGENTINE DYSTROPHINOPATHY COHORT: DIAGNOSTIC ALGORITHM, GENETIC ASSESSMENT AND DMD GENE CHARACTERIZATION**

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Introduction: Dystrophinopathies are X-linked recessive diseases caused by mutations in DMD gene. Hitherto there is no effective treatment for these pathologies, which enhances the importance of performing genetic assessment in order to detect mutation carriers and prevent diseased newborns. However, two mutation-specific gene therapies were recently approved: Exon 51 Skipping (Eteplirsen) and Premature Stop Codon Read-through (Ataluren). Therefore, accurate detection and characterization of the causing mutation is essential to allow genetic counseling, patient follow-up and determine the suitable gene therapy.
Materials and Methods: We have analyzed 200 boys with clinical diagnosis of Dystrophinopathy, 12 symptomatic women, 240 females at-risk of being carriers and 15 prenatal diagnoses. A diagnostic algorithm was designed for each case, implementing MLPA, PCR, Whole Exome Sequencing, Sanger Sequencing, STRs segregation analysis and HUMARA assay.
Results: The selected strategy allowed disease confirmation in 71.7% (152/212) of the affected boys and symptomatic females. 12 were candidates for Eteplirsen, while 22 were suitable for Ataluren. On the other hand, we were able to establish as carriers 72/255 women/fetuses, while could exclude from being carriers/affected 143/255. As for gene characterization, we could establish an association between the most frequent deletion/duplication intron breakpoints and the abundance of STR loci and, we have detected 3 haplotypes blocks within the SNPs identified by the Exome technique.
Conclusions: In the present work, we have characterized a Dystrophinopathy argentine population and contributed to the understanding of the genetic/molecular basis of these pathologies. This study was supported by PTC Therapeutics and University of Buenos Aires, Argentina.

ONCOLOGÍA / ONCOLOGY 7

538. (599) **hTERT EXPRESSION IS REGULATED BY THE ACTIVATION OF HSF1**

Cecilia Maricel Lotufo[1], Nadia Romina Zgajnar[1], Mario Galigniana[1, 2]
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Cancer cells achieve proliferative immortality by upregulating telomerase. hTERT is the catalytic subunit with reverse-transcriptase activity, which forms complexes with a template functional RNA, Hsp90, p23, and other accessory proteins. Recently, we demonstrated that two Hsp90-binding immunophilins, FKBP51 and FKBP52, are overexpressed in cancer cells and associated to hTERT. FKBP51 is also an antiapoptotic factor that undergoes nuclear-mitochondrial trafficking and binds to the hTERT•Hsp90 nuclear heterocomplex in a peptidylprolyl-isomerase (PPIase)-independent manner enhancing telomerase enzymatic. This effect is PPIase-dependent. hTERT nuclear localization is favored by FKBP52 via the cytoplasmic Hsp90•FKBP52•dynein retrotransport machinery, and because FKBP52 anchors hTERT to nucleoskeleton structures. In this study we analyzed the regulation of hTERT expression and subcellular relocalization. The disruption of hTERT heterocomplex with radicicol (Hsp90 inhibitor) or by overexpression of Hsp90-interacting TPR peptide, delocalizes nuclear hTERT to the cytoplasm. This Hsp90-free hTERT is degraded via proteasome unless it is targeted to mitochondria, where it seems to complement the antiapoptotic effects of FKBP51. Oxidative stimuli (H2O2, arsenite, BSO, tert-butyl-hydroperoxide, etc.) also disengage hTERT from nuclear structures favoring its nuclear export. Importantly, oxidative stress increases hTERT expression. Because high ionic strength, high glucose, heat-shock, etc. also show similar effect, we postulat-

ed that the HSF1 activation could be involved. This was confirmed due to the lack of hTERT induction in HSF1-KO cells compared to wild-type cells, and by the high basal expression of hTERT due to the mere overexpression of HSF1, even in the absence of stimuli. It is concluded that overall expression level of hTERT depends on HSF1 activation, whereas its subcellular localization is commanded by Hsp90.

539. (624) **PREDICTIVE BIOMARKERS OF RESPONSE TO TREATMENT WITH BACILLUS CALMETTE-GUERIN (BCG) IN PATIENTS WITH SUPERFICIAL BLADDER CARCINOMA**

Maria Teresa Pombo[2], Mariana Aris[1], Gustavo Villoldo[2], Pablo Mando[1], Camean Juan[2], Adrian Burioni[2], Mora Amat[2], Walter Astorino[2], Alberto Villaronga[2], María Marcela Barrio[1]
[1]*Fundación Cáncer FUCA* , [2]*Instituto Médico Alexander Fleming*

Intravesical administration of live attenuated Bacillus Calmette-Guerin (BCG) is the main therapy for intermediate/high grade non-muscle invasive bladder cancer (NMIBC). However, the response rate is only 60%, with a 5-year recurrence rate of 30-40%. In addition, for those patients (pts) with tumors staged as T1 or Cis (carcinoma in situ) that do not respond to BCG, the risk of progression to muscle-invasive disease could reach 50%. Intravesical BCG acts as a local immunomodulator, inducing a massive response of inflammatory cells (Th1 polarization) and ultimately the generation of a cytotoxic response that eliminates the tumor. Our hypothesis is that pts with a pre-existing tumor microenvironment of Th2-polarized lymphocytes and eosinophils would be susceptible to polarization towards Th1 after administration of BCG and respond to therapy. Instead, pts that already have a Th1-polarized tumor microenvironment, would not respond to BCG, probably because the tumor has already developed escape mechanisms to the Th1 response. In the search for a biomarker score to predict BCG response, pre-treatment biopsies of NMIBC pts (n=26), we evaluated by immunohistochemistry the polarization of the tumor microenvironment, quantifying the density and degranulation of eosinophils and T-bet+ (Th1), GATA-3+ (Th2) lymphocytes, all at maximal specific immune population focus. All pts received a 6-week induction plus a 3-week maintenance intravesical instillations of 120mg BCG, Danish strain SSI. Non-responders were defined as any recurrence after BCG treatment. A Th2 score was defined combining lymphocyte polarization GATA3+/Tbet+ (G/T) plus eosinophils density plus eosinophils degranulation. We observed a modest tendency towards the higher Th2 score, with response to BCG (no-recurrence) (Fisher´s exact test, p=0.23).G/T top quartile (>38) is clearly associated to BCG response, although near statistical significance (p=0.063). Given these preliminary results, a prospective study will be initiated to evaluate the score as a predictive biomarker of clinical response to BCG for NMIBC pts.

540. (709) **ROLE OF CAMP EFFLUX MEDIATED BY MRP4 IN PANCREATIC CANCER CHEMORESISTANCE**

Nicolás Di Siervi, Ana Sahores, Agustin Yaneff, Natalia Gomez, Angela Rodríguez-González, Carina Shayo, Carlos Davio
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Pancreatic ductal adenocarcinoma (PDAC) ranks among the most lethal of human malignancies. This is due to several factors: lack of early diagnosis, extensive local tumor invasion, early systemic dissemination, and extremely poor response to chemotherapy. Thus, there is an urgent need to improve the therapeutic of PDAC. Previous results from our laboratory indicate that cAMP efflux mediated by MRP4 is critical in PDAC cell proliferation, migration, tumorigenicity, and tumor growth rate. Therefore, the inhibition of MRP4 should be considered an alternative strategy for pancreatic cancer treatment, either alone or combined with chemotherapeutic agents. In this study, we hypothesized that the efflux of cAMP by MRP4 could be responsible of an adaptive advantage, critical in the development of chemoresistance. We treated BxPC-3 human pancreatic cancer cells with clinically used chemotherapeutic drugs which are not substrates of MRP4 (10μM gemcitabine, 50μM 5-fluorouracil, or 5μM paclitaxel; 24 h). Western blot analysis demonstrated a significant increase in MRP4 protein levels in all treated cells ($p<0.01$). Chronic (8 months) treatment with crescent doses of gemcitabine reduced sensitivity to this agent, with a significant shift in IC50 ($P<0.01$) and a concomitant increment of MRP4 levels. Moreover, the addition of cAMP to BxPC-3 cells (100μM) activated proliferative (pERK/ERK) and survival (pAKT/AKT) pathways, which are key in the adaptation to chemotherapy. Also, incubation with cAMP and not its metabolites, adenosine or 5'AMP (50μM), was able to induce a transient increase in Ca+2 intracellular levels, suggesting a direct effect of extracellular cAMP on tumor cells. Collectively, our results indicate that exposure to chemotherapeutic agents induces MRP4 expression, augmenting cAMP efflux, which in turn may act as an autocrine factor on neoplasic cells and as a paracrine factor in the tumor microenvironment. Inhibiting MRP4-cAMP transport may represent a novel therapeutic strategy to prevent or delay PDAC chemoresistance.

541. (610) **CEEFOURIN-1: THERAPEUTIC POTENTIAL OF MULTIDRUG RESISTANCE PROTEIN 4 (MRP4) PHARMACOLOGICAL INHIBITION IN ACUTE MYELOID LEUKEMIA AND PANCREATIC DUCTAL ADENOCARCINOMA**

Angela Rodríguez-González, Agustin Yaneff, Nicolás Di Siervi, Natalia Gomez, Antonela Diaz Nebreda, Carlos Davio, Carina Shayo, Ana Sahores
Instituto de Biología y Medicina Experimental (IBYME - CONICET)

Multidrug resistance-associated protein 4 (MRP4) transports anionic compounds and the dysregulation of its expression has been historically associated with drug resistance in several pathological conditions, including cancer. Thus, this protein is a potential therapeutic target in some types of neoplasias. Ceefourin-1, a specific inhibitor of MRP4, has recently been developed. Taking in consideration that MRP4 is the principal transporter of cAMP and that the balance between intra- and extracellular levels of this cyclic nucleotide is crucial in acute myeloid leukemia (AML) and in pancreatic ductal adenocarcinoma (PDAC), ceefourin-1 seems to be a promising compound for cancer therapy. The aim of this study was to assess the efficacy and mechanism of action of ceefourin-1 as an anticancer drug in AML and PDAC models. We evaluated the effect of ceefourin-1 on cAMP extrusion in AML (U937; HL-60) and PDAC (Panc1; BxPC3) cell lines through concentration response curves in a radio-binding assay. Both systems revealed a significant decrease in cAMP efflux in basal and stimulated (25μM forskolin) conditions. Ceefourin-1 inhibition of MRP4 activity was confirmed by measuring intracellular cAMP levels by FRET using Epac-SH187 as a cAMP molecular sensor in HEK293T cells. Treatment of leukemic and pancreatic cancer cells with different concentrations of ceefourin-1 showed that viability is affected only at the highest concentration (100μM; 200μM). MRP4 inhibition with ceefourin-1 and a non-specific MRP4 inhibitor (MK-571) has a concentration-dependent anti-proliferative effect ($p<0.01$) in these cell lines. Finally, acute toxicity was evaluated in Balb/c mice treated with ceefourin-1 for two weeks (sc; 2 and 10 mg/kg /3 times a week). No significant toxic effects were observed, except for mild leukocytosis only with the highest dose. These results show that ceefourin-1 represents a promising selective MRP4 inhibitor for AML and PDAC and leads us to propose future experiments to test its efficacy in vivo.

542. (617) **DEREGULATION OF NON-CODING RNAS IS ASSOCIATED WITH CLINICAL OUTCOME OF CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA**

Mercedes Abbate[1, 2], Javier Nahuel Brandani[1, 2], Geraldine Gueron[1, 2], Elba S. Vazquez[1, 2], Javier Cotignola[1, 2]
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