Abstract/Resumen: Acute myeloid leukemia (AML) is a heterogeneous clonal disorder in which early hematopoietic cells fail to differentiate and do not undergo programmed cell death nor apoptosis. We previously showed that increments in intracellular cAMP levels play an important role in leukemic cell proliferation and differentiation. Histamine, through H2 receptor (H2R), stimulates cAMP formation and up-regulates MRP4 expression, responsible for cAMP efflux. The aim of this study was to evaluate the effect of combining treatment with H2R agonists, histamine (HDC) or amthamine (A), together with a MRP4 inhibitor (ceefourin1) or non-specific MRP4 inhibitors (probenecid and MK571) upon proliferation and differentiation of AML cell models. U937, HL60 and KG1a cell proliferation after 72 h treatment with 100μM HDC or 10 μM A and different concentrations of MRP4 inhibitors was assessed by cell count. H2R stimulation enhanced the concentration-dependent anti-proliferative effect of MRP4 inhibitors. Next, CD88 expression was evaluated by Western blot, as an AML terminal differentiation marker. HDC and A in combination with MK571 or probenecid augmented CD88 expression compared to single treatments. In accordance, c-Myc expression was down-regulated to a greater extent in the combined treatment. However, neither ceefourin1 nor its combined treatments induced CD88 expression, but induced apoptotic markers (caspase-3 and PARP activation). To evaluate the effect of ceefourin1 on leukemia cell proliferation in vivo, Swiss nu/nu mice were subcutaneously injected with U937 cells. Mice were treated with ceefourin1 for 3 weeks (i.p., 10 mg/kg daily), xenografts were measured periodically and their morphology was assessed by H&E staining. Although tumor growth was not affected, a significant increase in the apoptotic index was observed in mice treated with ceefourin1. Taken together, our results contribute to the rational basis of a polypharmacological approach in AML using H2R ligands and MRP4 inhibitors.

0513 - CD4, CD8 AND TREG LYMPHOCYTES IN THE TUMOR MICROENVIRONMENT OF M-406 MURINE TRIPLE NEGATIVE MAMMARY TUMOR GROWING IN HOSTS WITH DIFFERENT SUSCEPTIBILITIES

María Celeste CAPITANI | Antonela DEL GIÚDICE | Matías Ezequiel FUSINI | Leandro E. MAINETTI | Olga Graciela SCHAROVSKY | María Jose RICO | Viviana Rosa ROZADOS

INSTITUTO DE GENÉTICA EXPERIMENTAL. FACULTAD DE CIENCIAS MÉDICAS. UNIVERSIDAD NACIONAL DE ROSARIO

Abstract/Resumen: The tumor microenvironment contributes to several aspects of carcinogenesis and, therefore, offers promising targets for cancer therapy. CBi- mice was artificially selected for body conformation from CBi mice. The M-406 mammary adenocarcinoma appeared spontaneously in an inbred CBi female mouse and it is maintained in vivo in mice of the same line. Previously, we observed that when inbred CBi- mice were s.c. challenged with M-406 they showed 100 % takes and 100 % regressions (resistance). In contrast, the tumor grew exponentially (susceptibility) in CBi mice and in F1 hybrids (CBi x CBi- and CBi- x CBi). Our aim was to determine the participation of CD4, CD8 and Treg lymphocytes in tumors growing in hosts with different susceptibilities. Mice of the three genotypes were s.c. challenged with M-406 and when growing tumors reached the maximum size ethically allowed, they were sacrificed, tumor samples were obtained, fixed and included in paraffin. Also, tumors being rejected (CBi-) were excised at 12-17 days. CD4, CD8 and Treg cells were quantified by immunohistochemistry with antibodies for CD4, CD8 and Foxp3 in 30 fields of 1,000X. The number of CD4+ and CD8+ cells were higher and the number of Treg lower in CBi- than in CBi and F1, being CBi- > F1 for CD4 ($p<0.05$) and CD8 ($p<0.01$), and CBi- < F1 for Tregs ($p<0.001$). No significant differences were found for CBi vs. CBi- or F1 for the three types of cells tested. Also, the CD8/Treg ratio was higher in CBi- compared to CBi ($p<0.05$) and F1 ($p<0.001$). CD4/Treg ratio was higher in CBi- than in F1 ($p<0.001$). These results allow us to conclude that: 1) The decrease of Tregs and increase of CD8 and CD4 in CBi- tumors would allow the antitumor immune response to proceed and explain, at least partially, the rejection of M-406; 2) Higher number of intratumor Tregs would play a role in the susceptibility to M-406 shown by CBi and F1 mice; 3) The higher ratio CD8/Treg increased the differences among susceptible and resistant mice.

0522 - ACTIVITY OF INTESTINAL EFFLUX PUMPS IN M-406 MAMMARY ADENOCARCINOMA BEARING MICE WITH METABOLIC SYNDROME (MS) TREATED WITH CYCLOPHOSPHAMIDE (CY) METRONOMIC CHEMOTHERAPY (MC)

María Manuela BARRANCO (1) | Martín José HABIB(1) | Bianca DI CARLO(1) | Nicolás SIGAL(1) | María SYLVESTRE BEGNIS(1) | Felipe ZECCHINATI(2) | Antonela DEL GIÚDICE(3) | María Jose RICO(3) | Viviana Rosa ROZADOS(3) | Olga Graciela SCHAROVSKY(3) | Silvina Stella Maris VILLANUEVA(2) | Fabiana GARCÍA(1) | Leandro Ernesto MAINETTI(3)

LABORATORIO DE FISIOLOGÍA METABÓLICA, FACULTAD DE CIENCIAS MÉDICAS, UNIVERSIDAD NACIONAL DE ROSARIO (1); INSTITUTO DE FISIOLOGÍA EXPERIMENTAL (IFISE-CONICET). FAC. DE CS. BIOQUÍMICAS Y FARMACÉUTICAS. UNR (2); INSTITUTO DE GENÉTICA EXPERIMENTAL. FACULTAD DE CIENCIAS MÉDICAS. UNIVERSIDAD NACIONAL DE ROSARIO (3)

Abstract/Resumen: High fat diet (HFD) cause obesity and MS, which are associated with breast cancer progression. MC (chronic, low dose, with no extended rest periods, drug administration) is a promising treatment strategy with low toxicity. Some chemotherapeutic drugs are substrates of multidrug resistance-associated protein 2 (Mrp2) and P-glycoprotein (P-gp), efflux pumps that limit the intestinal absorption and are modified by alterations produced in MS. We previously demonstrated the induction of MS in CBi male mice. The aim was to assess the alteration produced by MS in Mrp2 and P-gp activity as intestinal biochemistry barrier and its influence on the therapeutic effect of Cy MC. CBi male mice (5 weeks n= 20/group), were fed with a chow diet (C) and a diet with 40 % calories of fat (HFD) throughout the experiment. At 16 weeks, the development of MS was confirmed by biochemical and morphological parameters. The activity of Mrp2 and P-gp was evaluated using the in vitro model of everted intestinal sacs. Once the MS features were settled, mice were challenged s.c. with M-406 (day 0); when the tumor was palpable, mice were distributed into 4 groups (n= 8/group): GI: C no treatment, GII:C+Cy (30 mg/kg/day in drinking water), GIII: HFD no treatment and GIV: HFD+Cy. Efflux of Mrp2 substrate DNP-SG decreased 64% in HFD respect to C ($p<0.05$); transport rate of rhodamine 123 by P-gp decreased 55 % in HFD vs. C ($p<0.05$). At the end of the experiment (day 22), the tumor volume was lower in GII vs GI and in GIV vs GIII ($p<0.0001$).The % inhibition of tumor growth in GII was greater than that of GIV ($p= 0.0524$). It was observed a 30% decrease in body weight in GIV, indicating toxicity. We conclude that the induction of MS impairs the intestinal activity of Mrp2 and P-gp and these modifications may produce a change in Cy absorption, leading to toxicity along with therapeutic effect; also, MC has lower antitumor effectiveness in animals with MS so that the drug schedule should be re-designed.

0536 - ANTITUMOR EFFECT OF RAC1 INHIBITION IN 2D, 3D AND IN VIVO GLIOBLASTOMA MODELS

Lucas VALDEZ CAPUCCINO | Julian MAGGIO | Pablo LORENZANO MENNA | Daniel Fernando ALONSO | Daniel E. GOMEZ | Georgina A. CARDAMA

LABORATORIO DE ONCOLOGÍA MOLECULAR, UNIVERSIDAD NACIONAL DE QUILMES

Abstract/Resumen: Glioblastoma Multiforme is the most common central nervous system cancer among adult patients with one of the worst prognoses. Despite current therapeutic