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Abstract/Resumen: Toxicity from and resistance to ionizing radiation therapy constitutes a major obstacle to curative treatments for non-small cell lung cancer (NSCLC). Regimens for radiation therapy are often limited by toxicity to normal tissues and the development of resistance. Thus, strategies to reduce the total amount of ionizing radiation (IR) used are required. IR results in a wide variety of chromosomal DNA damage including DSB. Epigenetics refers to a set of mechanisms that regulate chromatin accessibility and therefore DNA-Based process such as DNA repair. Particularly, Jumonji (JmjC) histone lysine demethylases (KDM) play roles in DNA repair pathways. Our aim is to study if pharmacological inhibiton of JmjC could be used as a targeted therapy to radiosensitize NSCLC. Liquid colony formation assay was performed to determine IC50 of JIB-04, a JmjC pan-inhibitor, and radioresponse curves in Human NSCLCs cell lines (H1299, A549, HCC95 and HCC1719) and immortalized non-cancerous human bronchial epithelial cells (HBEC3KT and HBEC30KT). For in vivo experiments NSCLC cells were injected subcutaneously (H1299 and A549) into the right posterior leg of female athymic nude mice. Mice were treated for a total of 12 doses EOD with JIB-04 (50 mg/kg/day) by oral gavage or with vehicle; radiation was administered 4 hours after treatment. Tumor growth delay, survival and the dose enhancement factor (DEF) were then determined. Pharmacological inhibition of JmjC KDM using JIB-04 resulted in a strong sensitization of radio-resistant NSCLC (H1299, A549, HCC95) ($p<0.001$) but not radio-sensitive NSCLC (HCC1719) to radiation. In addition, we found that JIB-04 does not radiosensitize normal cells (HBEC3KT and HBEC30KT). In vivo, treatment with JIB-04 plus IR inhibit tumor growth compared with control mice and either treatment alone ($p<0.001$, DEF>6). Even more, mice treated with JIB-04 and IR survived significantly longer than mice treated with either agent alone or with vehicle even after the end of treatment. In conclusion, our study suggests that the epigenetic inhibitor JIB-04 could help to overcome radioresistance both in vitro and in vivo.

0855 - EVALUATION OF CIRCULATING LYMPHOCYTES SUBPOPULATIONS DURING THE GROWTH OF M-406 TRIPLE NEGATIVE MURINE MAMMARY TUMOR

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Abstract/Resumen: Inbred mice models provide an interesting tool for identifying factors that control susceptibility to breast cancer. M-406 mammary adenocarcinoma appeared in an inbred CBi mouse. CBi- mice were artificially selected from CBi. Cells of the immune system play an important role in tumor development. In order to determine their participation on tumor growth in genetically different hosts, CBi, CBi- and F1 reciprocal hybrids (F1A: CBi x CBi- and F1B: CBi- x CBi) were s.c. challenged with M-406, tumors were measured, and blood samples were taken on days 0, 7 and 14 in CBi and F1 and on days 0, 5, 8 and 12 in CBi- mice. Circulating CD4+, CD8+, Treg and Th17 cells were quantified (flow cytometry). Tumors grew exponentially in 100% of CBi (susceptible) and F1 female and male mice. However, in CBi- (resistant) after a short period of growth, reaching the maximum size on day 8 (female) and 12 (male), 100 % of tumors were rejected. CBi, F1A and F1B mice, did not differ in tumor volume doubling time (TVDT) for both sexes, while in CBi-, TVDT in males was higher than in females ($p<0.05$). We determined the ratio CD8+/Treg in CBi males: day 0 > day 14; ($P<0.05$); CBi females: day 0 day 12 ($p<0.01$) without differences in CBi- females; F1A males and females: day 7 > day 14 ($p<0.0001$; $p<0.001$, respectively); F1B: without differences between days or sexes. Conclusions: 1) The susceptible phenotype is dominant over the resistant. 2) CD4+ and Th17 lymphocytes could not explain tumor growth/rejection behavior in genetically different hosts. 3) CBi males and females utilize different antitumor immune mechanisms leading to tumor escape and growth, without modifying tumor growth rate. 4) The decrease in CD8+/Tregs ratio in CBi- males could be partly responsible for the observed delay in tumor growth. 5) The similar values in CD8+/Tregs ratios for F1A and F1B (males and females) could explain, in part, the absence of differences in tumor growth rate.

0865 - ADRENERGIC RECEPTORS IN BREAST CANCER: DIFFERENTIAL EFFECTS OF ALPHA 2A AND 2C-ADRENERGIC RECEPTOR EXPRESSION ON TAMOXIFEN SENSITIVITY IN STABLY TRANSFECTED LUMINAL MCF-7 CELLS.

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Abstract/Resumen: Breast cancer is the most frequently diagnosed and leading cause of cancer death among women worldwide. Epinephrine and norepinephrine, released during stress, bind to 9 different adrenoceptors. Our group has already described (SAIC 2015, poster 660) by in silico analysis in a great database that patients with high expression of Alpha2A-adrenoceptors (A2A-AR) have better disease-free survival than those with lower expression, mainly in luminal tamoxifen-treated ones. Contrarily, a high expression of Alpha2C-AR was associated with worse outcome in luminal B but not in luminal A patients. The aim of the present work was to study the sensibility of tamoxifen on A2A or A2C-AR-overexpressing cells. The human luminal breast cancer MCF-7 cells were stably transfected with A2A or A2C-AR or the empty vector. The expression of A2-AR and Estrogen Receptor Alpha (ER) was measured by RT-qPCR, the sensitivity to tamoxifen by tritiated thymidine incorporation and ER, progesterone receptor and pERK relative to ERK, by Western Blot. They were all performed in the absence of adrenergic stimulation because catecholamines released during stress bind to all receptors and no specific ligand for individual A2-AR exists yet. We successfully over expressed alpha2A and alpha2C on MCF-7 cells: 65 (A2A) and 28 % (A2C) increase compared with empty vector (pCDNA, $p<0.05$ and $p<0.01$, respectively). When analyzing the sensitivity to tamoxifen treatment, the A2A cells exhibited an EC50 of $2.867e^{-}10$ vs. 4.250 e-10 of pCDNA, $p<0.01$; while A2C of $1.202e^{-}9$, $p<0.001$.This was accompanied by a decrease in both cases of ER levels measured by RT-qPCR, $p<0.05$ and WB. A2A cells also showed diminished cell proliferation ($p<0.01$) in the absence of any stimulation when compared with pCDNA and A2C. We suggest that the increase of tamoxifen sensitivity in A2A cells could be due to the combined effect of inhibiting ER expression and cell proliferation.

0871 - 4-METHYLUMBELLIFERONE INDUCES SENESCENCE, INHIBITS MIGRATION AND MODULATES CD44 AS WELL AS RHAMM IN HUMAN GLIOBLASTOMA CELL LINES

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Abstract/Resumen: 4-methylumbelliferone (4MU) is a non-toxic coumarin derivative used as inhibitor of hyaluronan (HA) synthesis, but there are reports about independent effects of this inhibition. Currently, this drug is being studied on several neoplasms. Nevertheless, little is known about its effects on glioblastoma (GBM), the most frequent and malignant primary tumor of the central nervous system. HA is strongly involved in tumor progression, favoring cell proliferation and migration through its main receptors, CD44 and RHAMM, both associated with poor prognosis. GBM shows higher levels of HA than normal brain tissue. Given that current therapy for this tumor is ineffective and highly toxic, new drugs are needed for GBM treatment. Our hypothesis is that 4MU is a potential new drug for GBM therapy. Therefore, the aim of this work was to evaluate the effects of 4MU on cell proliferation, migration, senescence induction, expression of CD44 and RHAMM, and the receptors involved in HA-induced migration on LN229 and U251 human GBM cell lines. Cell proliferation was evaluated by BrdU incorporation assay, migration by the wound healing assay, senescence by SA-ß-gal assay and expression of receptors by Western blot (WB) and immunofluorescence (IF). We found that 4MU inhibited cell proliferation and migration in a dose-dependent manner in both cell lines ($p<0.05$). These effects were not prevented by the co-treatment with HA. Besides, 4MU increased the percentage of SA-ß-gal+ cells in a dose-dependent manner in U251 cell line, but in LN229 cells only at the higher dose ($p<0.05$). Furthermore, 4MU modulates the expression of RHAMM and CD44 ($p<0.05$). Regarding the implication of CD44 and RHAMM in HA-induced migration, we evaluated this process using blocking antibodies which prevented the effect of HA ($p<0.05$). In conclusion, we demonstrated that 4MU inhibited all studied processes involved in GBM malignancy, thus being a promising therapy for GBM.

0883 - IMIQUIMOD TREATMENT OF TRANSFORMED CELLS: NF-KB AND TLR-7/8 SIGNALLING INDEPENDENT DEATH.

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Abstract/Resumen: The immunotherapeutic agent imiquimod (IQ), an agonist of the Toll-like receptors (TLR) 7/8, has been reported to be effective in the treatment of several skin pathologies including melanoma and infantile haemangioma. In immune cells, the classic pathway for IQ signalling comprises TLR7 and NF-KB activation. Previously, we have demonstrated that IQ causes cell death, oxidative stress and loss of migratory ability in haemangioma and melanoma cells in vitro. In order to gain insight on IQ signalling mechanism in transformed cells, we studied TLR expression and the involvement of NF-kB in IQ-induced cell death. Murine melanoma B16F-1 and haemangioma H5V cells were treated with IQ (0, 5, 10 and 50 µg/mL) in the presence or absence of an NF-KB inhibitor (BAY 11-7082) during 24 hours. Cell viability was analysed by crystal violet staining and nuclear morphological changes were evaluated by a nuclear morphometric analysis (NMA) with ImageJ on Höescht 33258-stained nuclei. TLR-7/8 expression was assayed by RT-qPCR. Both H5V and B16F-1 cells suffered loss of viability (circa 50 %) at IQ 10 µg/mL but inhibition of NF-KB did not modify cell death levels. Likewise, NMA showed an increased number of small and regular nuclei (50-60 %, $p<0.05$) at IQ 10-50 µg/mL associated to apoptotic cells. The percentage was similar either with or without BAY 11-7082 treatment. In addition, after incubation with IQ+BAY, a slight tendency to the appearance of large regular nuclei, compatible with senescent cells, was detected in both cell lines accompanied by cytoplasmic vacuolization. With respect to TLR7 expression, low levels were obtained for H5V cells (0.13 ± 0.10) compared to ganglion and resulted undetectable for B16F1, as well as TLR8 expression in both cell lines. Consequently, these results suggest that IQ would be exerting its cytotoxic effect without involving NF-kB and TLR-7/8 signalling.

0893 - ASSESSMENT OF BACULOVIRAL VS ADENOVIRAL VECTORS FOR GENE DELIVERY IN EXPERIMENTAL BRAIN CANCER

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Abstract/Resumen: We aimed to compare the transduction efficiency and neuropathology of adenoviral (AdV) vs baculoviral (BV) vectors in order to develop therapeutic strategies for the treatment of brain cancer. Although AdVs can be produced in high titers and yield good transduction efficiency in the brain, the population exhibits pre-existing anti-AdV immunity, leading to transient transgene expression. BVs primarily infect insects at larval stage, but they also transduce cells from other species. Even though BVs are less stable than AdVs after long-term storage, their advantage is that pre-existing immunity against BVs has not been reported in humans. Our general hypothesis is that BVs may lead to more stable transgene expression than AdVs upon injection into naïve and neoplastic brain. We constructed AdV and BV encoding tdTomato under the control of the CMV promoter. Human and rat GB cell lines were incubated with different doses of AdV or BV for 48 h and transduction efficiency was assessed by microscopy. AdV (MOI 50-500) and BV (MOI 500-2000) transduced GB cell lines with similar efficiency. AdV ($\sim 10^7$ UFP) and BV ($\sim 10^6$ UFP) were injected by stereotactic surgery into orthotopic GL26 GB growing in the brain of C57Bl/6 mice and 5 d later, mice were perfused/fixed and brains were sectioned in cryostat. We detected comparable expression of tdTomato within tumors injected with either vector. AdV or BV were also injected into the striatum of naïve mice and 5 d later, brains were processed for immunohistochemistry to identify glial cells, showing that transduced brain cells were GFAP+. CD45 staining showed similar immune cell infiltration around BV and AdV injection sites and no signs of neurotoxicity were observed. Our findings indicate that both vectors transduce GB and glial cells with similar efficiency without evident neurotoxicity. Given that humans do not present pre-existing immunity against BVs, BV may constitute a valuable tool for delivery of therapeutic genes in the brain.

0896 - LOOKING FOR DRUG SYNERGY AGAINST CANCER THROUGH POLYAMINE METABOLISM IMPAIRMENT: INSIGHT THE METABOLIC EFFECT OF INDOMETHACIN OVER KRAS-MUTATED LUNG CANCER CELLS.

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Abstract/Resumen: Non-small cell lung cancer (NSCLC) is the most lethal and prevalent lung cancer type. Mutations in the