response. We claim that signaling through Myd88 plays a key role in liver cancer development in mice. Adult C57BL/6 wild-type (WT) and Myd88-/- mice (23-25 g) were subject to a model of early liver cancer development. This was induced by administration of 2 i.p. doses of diethylnitrosamine (75 mg/kg bw) 2 weeks apart. One week after the last injection, mice received 20 mg/kg bw of 2-acetylaminofluorene by gastric probe 3 days a week for 3 weeks. All studies were performed before the initiation of treatment and showed no difference between genotypes. After cancer development, Myd88-/- mice showed lower body but higher liver weights than WT mice. We confirmed the complete absence of liver Myd88 protein expression by immunoblotting, as well as Myd88 mRNA expression, when evaluated by qPCR. Liver histology analysis showed scatter alterations on hepatocyte architecture, with accumulation of cytosolic lipid droplets (+23%) and increased inflammatory infiltration (+45%) in Myd88-/- mice compared to WT mice. Hepatic enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were slightly increased (+15%, and +12%, respectively) in plasma of Myd88-/- mice compared to WT mice, indicating a mild liver damage. Then, we evaluated proliferation and apoptosis by immunoblotting. We found that Myd88-/- mice presented with decreased protein expression of proliferation cell nuclear antigen (PCNA) (-35%), with a slight decrease in caspase-3 expression and no changes in Bax and cytochrome c expressions in total liver homogenates. These studies represent the first steps in the evaluation of the role of Myd88 in liver cancer development, and demonstrate that Myd88 is involved in prevention of chemical hepatocarcinogenesis; exposing, once again, the tight relationship between the immune system and the de development of cancer.

O2. Epidermal growth factor receptor (EGFR) activation induces the expression of multidrug resistance associated protein 4 (MRP4/ABCC4) in a pancreatic cancer human cell line

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Pancreatic ductal adenocarcinoma (PDAC) is the most frequent type of pancreatic cancer and has one of the worst prognosis. The poor overall survival is associated with the overexpression of epidermal growth factor receptor (EGFR), a known member of the ErbB family of receptor tyrosine kinases, and the multidrug resistance associated protein 4 (MRP4/ABCC4). Our previous results show that high levels of MRP4 are associated with an increase in tumor cell proliferation, metastatic invasion and up-regulation of EGFR protein levels in PDACs cell lines. The aim of our study was to evaluate the regulation of MRP4 by EGFR activation in a pancreatic cancer cell model. To accomplish our objective, we treated the pancreatic cancer cell line BxPC-3 with EGF (0.1ng/µl). EGFR activation was confirmed by ERK phosphorylation at 5, 10, 20, 30, and 40 min after EGF treatment. MRP4 protein expression was evaluated by western blot using whole cell extracts following incubation with EGF for 0, 24 and 48 h, using histone as loading control. MRP4 expression levels were also assessed 48 h after treatment with EGF alone or in combination with the EGFR inhibitor CL 387-785 (1µM). Our results confirm that EGFR is quickly activated upon incubation with EGF, as evidenced by a 4-fold increase in the pERK/total ERK ratio detected ($P<0.001$) at 5 min and normalized at 40 min. Maximal induction of MRP4 expression (86%, $P<0.001$) was observed in cells treated with EGF for 48 h. Furthermore, EGF-mediated MRP4 induction was abolished by co-treatment with CL387-785 ($P<0.05$), while its expression did not change by treatment with this EGFR inhibitor alone. These data demonstrate that EGFR activation produces increments in MRP4 protein levels in a PDAC cell line. In summary, it is possible that MRP4 and EGFR, both PDAC poor prognosis markers, are co-regulated by a positive feed-back which ultimately enhances their effect upon each other.

O3. Antiproliferative and apoptotic effects of combined treatment of interferon alpha-2b (IFN) and vitamin e (vit E) on SK HEP-1 cells

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Our group demonstrated that IFN is an effective antitumor agent in the prevention and treatment of hepatocellular carcinoma (HCC) given its apoptotic and antiproliferative effects. Besides, it was shown that vit E exerts inhibitory effects on liver cancer due to its apoptotic, antiangiogenic and antiproliferative activities. Previously, we observed that combined treatment of IFN with vit E significantly decreased cell viability, migration and invasion of human HCC cell line SK HEP-1, in comparison with single treatments.

The aim of the present work is to deepen the study of the effects of combining IFN and vit E, with particular focus on proliferation and apoptosis. Methods: SK-HEP 1 cells were treated with 10000 U/l IFN and 25 uM δ-Tocotrienol (an isomer of vit E). Treatments were performed for 72 h using single drugs (IFN-group and E-group) and their combination (IFN-E-group). Also, a control group treated with drugs vehicles was included. We performed: a) annexin V-FITC assay to determine total apoptosis by flow citometry, b) western blot studies to analyze the expression of PCNA (marker of proliferation), proapoptotic Bax and antiapoptotic Bcl-Xl proteins, and c) dichlorofluorescein assay to evaluate reactive oxygen species (ROS) production. Results: IFN-E-group showed a higher increase in total apoptosis (+520%*&), compared with monotherapies (IFN-group: +75%*; E-group: +90%*). Also, IFN-E-group showed a significant decrease in PCNA expression (-35%*&) together with an increase in Bax protein expression (+65 %*&) and a decrease in Bcl-Xl expression (-85%*&), compared with monotherapies (IFN-group: PCNA: -17%*, Bax: +23%*, Bcl-Xl: -55%*; E-group: PCNA: -20%*, Bax: +25%*, Bcl-Xl: -60%*). On the other hand, IFN-E-group showed a significant increase in ROS production (+480%*&) compared with monodrug therapies (IFN-group: +50%*, E-group: +150%*) (*p<0.05