For the *in vitro* study, we obtained ACM for the high-fat environment and H_2O_2 for the ROS effect. They were used in the XTT cytotoxicity assay. ACM co-culture with the hepatocytic AML12 cell line increased the sensitivity of ROS-related cell cytotoxicity, which was related to ferroptosis by Erastin in the high-fat/high-ROS cell environment. However, these results can be suppressed by UAMC3203, a ferroptosis inhibitor. As for the animal model experiments, the mice had higher weight, Alanine Aminotransferase (ALT) levels, and developed tumors. Their liver section showed lipid accumulation, histological fibrosis, and iron accumulation in the WD and WD+CCl_4 groups, which corresponds to the IRP1 and IRP2 upregulated in both groups by qPCR result.

Conclusion

In conclusion, we have established the effect of ferroptosis on hepatic steatosis *in vivo* and *in vitro* in nonalcoholic steatosis hepatitis (NASH). These results may enable the development of precise health prevention strategies by inhibiting ferroptosis to prevent the occurrence of liver diseases including hepatic steatosis and HCC.

EACR23-0908
Semaphorin 4A-expressing bone marrow-derived myeloid cells promote tumor progression.

S. Brundu[1,2], G. Sardo[1,2], Y. Qiu[1,2], G. Franzolin[1,2], C.F. Cojocaru[1,2], F. Maione[3,4], E. Giraudo[1,2]
[1]Candiolo Cancer Institute - IRCCS, Laboratory of Tumor Microenvironment, Candiolo, Italy
[2]University of Torino, Department of Science and Drug Technology, Torino, Italy
[3]Candiolo Cancer Institute – IRCCS, Laboratory of Cancer Epigenetics, Candiolo, Italy
[4]University of Torino, Department of Oncology, Torino, Italy

Introduction

Semaphorins (Semas) and Plexins are a family of proteins first identified as axonal guidance molecules and recently discovered as regulator the tumor microenvironment and cancer growth. Accumulating evidences indicate that several Semas critically involved in the immune response and regulate tumor progression. We previously described that Sema4A, overexpressed in myeloid cells under inflammatory conditions, exerted a pro-angiogenic effect. Despite its established role in regulating immune cell functions, until now Sema4A role in tumor-associated inflammation and cancer progression is still controversial and poorly investigated.

Material and Methods

We used two mouse: i) an orthotopic mouse model of pancreatic ductal adenocarcinoma (PDAC); ii) a transgenic mouse model of spontaneous multistep tumorigenesis of HPV16-induced cervical cancer (HPV16/E2). Moreover, by means of lentiviral shRNA technology we efficiently silenced Sema4A in bone marrow (BM)-derived myeloid cells in tumor bearing PDAC or HPV16/E2 mice to study the role of Sema4A during tumor progression.

Results and Discussions

We observed increased levels of Sema4A and its receptors in PDAC and HPV16/E2 compared with normal tissues. Interestingly we noticed that Sema4A was mainly expressed by myeloid cells and particularly by pro-tumoral M2-like macrophages. In addition, Sema4A was expressed in wild type BM myeloid cells and up-regulated in PDAC or HPV16-derived BM. Stemming from these findings, we efficiently silenced Sema4A in BM cells in tumor bearing from the two models and we demonstrated that the lack of Sema4A expression significantly hampered tumor growth e metastasis spreading compared with controls.
Notably, we observed that Sema4A silencing induced a shift from M2 toward M1 anti-tumor phenotype, to decrease regulatory Tregs and to enhance the recruitment of $CD8^+$ T cells, along with induction of tumor vessel normalization in both PDAC and HPV16/E2 models. Finally, we observed that Sema4A promotes proliferation and migration of PDAC and that the depletion of PlexinB1 in these cells, reverted these effects.

Conclusion

We demonstrated that Sema4A express by BM myeloid cells contributes to tumor progression by acting on tumor-associated macrophages and T cells in PDAC and cervical cancer, and that its receptor PlexinB1 may be mainly involved in Sema4A-induced tumor growth and invasiveness. In conclusion, Sema4A representing a novel predictive biomarker and a new potential therapeutic target to inhibit the progression of pancreatic and cervical cancers.

EACR23-0923
Role of the NADPH oxidase NOX4 in the liver tumor microenvironment

R. Espinosa Sotelo[1], I. Peñuelas-Haro[1], M. Herranz-Itúrbide[1], D. Caballero-Díaz[1], E. Crosas-Molist[1], E. Bertran[1], I. Fabregat[1]
[1]Bellvitge Biomedical Research Institute IDIBELL, TGF-β and Cancer Group. Oncobell Program, L'Hospitalet de Llobregat- Barcelona, Spain

Introduction

The progression of hepatocellular carcinoma (HCC) is known to be a complex process involving different mutations in hepatocytes, caused by a continuous inflammatory environment where Reactive Oxygen Species (ROS) likely contribute to an oxidative stress and its consequences. The NADPH oxidase NOX4, downstream from the Transforming Growth Factor (TGF)-β pathway, has been proposed as relevant regulator of liver tumor cell proliferation and invasion, playing a tumor suppressor function. However, whether NOX4 expression (either in the tumor cell or in the stroma cells) influences liver tumor microenvironment, regulating fibroblast activation or inflammation, remains unidentified.

Material and Methods

Wild Type (WT) or NOX4-/- mice (14 days-old) were treated either with PBS or Diethylnitrosamine (DEN), as a model of induced experimental hepatocarcinogenesis. Tissues (tumor and non-liver tumor areas) were gathered at 9 months and 11 months of treatment. Additionally, in order to know whether the expression of NOX4 is relevant for the observed effects, either in the tumor cells or in the immune cells, we have started doing in vitro experiments, 3D co-cultures of activated macrophages and HCC spheroids, where the expression of NOX4 will be modulated through silencing NOX4 with shRNA.

Results and Discussions