Abstract

Pancreatic cancer (PC) is one of the most aggressive cancers in the world and it correlates to poor prognosis and high mortality due to late diagnosis, even if early diagnosed. Resectable PC patients have unfavorable outcome due to several factors like chemoresistance by tumor microenvironment (TME) and by tumor cells per se. Recent studies have focused on TME that plays a critical role in PC progression, highlighting the strong relationship between the microenvironment and metastasis. Several extracellular factors are involved in its development and metastasis. In PC, the protein Annexin A1 (ANXA1) appears overexpressed and may be identified as an oncogenic factor, also as component in tumor-deriving extracellular vesicles (EVs). Indeed, these microvesicles are known to nourish the TME. Our published data have highlighted that autocrine/paracrine activities of extracellular ANXA1 depend on its presence in EVs. The aim of the first and second year of my PhD project has been to investigate the paracrine effect of ANXA1 on cellular components of TME, mainly stromal cells like fibroblasts, endothelial ones to demonstrate how the ANXA1-EVs complex can stimulate this mechanism. EVs from Wild Type (WT) and ANXA1- Knock-Out (KO) MIA PaCa-2 cells, obtained by CRISPR/Cas9 genome editing system, have been purified from cell conditioned medium by differential centrifugation and then administrated on stromal cells.

Moreover, as oncogenic factor, ANXA1 needs to be inhibited, mainly by blocking its extracellular form, as a new model of cancer adjuvant therapy. Heparan sulfate (HS) is a glycosaminoglycan of the extracellular matrix known to bind growth factors and cytokines, generating a kind of reservoir in the extracellular environment. One of these molecules is represented by ANXA1 and previous study has shown that ANXA1 notably binds to sulfate glycans, mainly HS and heparin. In this regard, the second annual aim has been to investigate the interaction between HS and ANXA1 and how this glycosaminoglycan could influence ANXA1 oncogenic action. In this way, it would be possible to confirm the relevance of ANXA1 in PC progression as actor of the cross-talk among tumor cells and the microenvironment.

In order to amplify the knowledge about the role of ANXA1 on PC stroma, the aim of my third year has been to demonstrate that the complex ANXA1/EVs modulates the macrophage polarization further contributing to cancer progression. The WT and ANXA1 KO EVs have been administrated to THP-1 macrophages finding that ANXA1 is crucial for the acquisition of a pro-tumor M2 phenotype. The M2 macrophages activate endothelial cells and fibroblasts to induce angiogenesis and matrix degradation, respectively. Once shown in vitro the multifaceted role of ANXA1 in the intensification of PC-stroma cells cross-talk, we have also found a significantly increased presence of M2 macrophage in mice tumor and liver metastasis sections previously obtained by orthotopic xenografts with WT cells. Finally during the third year of the PhD program, I had the opportunity to work at “Institut national de la santé et de la recherche

médicale" (INSERM), Marseille (FR), where I deepened the role of ANXA1 in TME using tumor 3D model, both in monoculture and co-culture with cancer associated fibroblasts (CAFs) and monocytes.

Taken together, our data interestingly suggest the relevance of ANXA1 as potential diagnostic/prognostic and/or therapeutic PC marker. In this way, it would be possible to confirm the relevance of ANXA1 in PC progression as actor of the cross-talk among tumor cells and the microenvironment.