Abstract Tesi Raffaella Belvedere

Annexin 1 (ANXA1) is a multifunctional protein of 37 kDa, and represents the first characterized member of the annexin superfamily, so called since their main property is to bind (i.e. to annex) cell membranes in Ca^{2+}-dependent manner. ANXA1 is over-expressed in tissues from patients affected by pancreatic carcinoma (PC), where the protein seems to be associated with the malignant transformation and the poor prognosis. In this PhD project, experiments were performed to understand the role of ANXA1 in human PC development with particular attention to migration and invasion processes. We observed in all the analyzed PC cell lines, a huge expression and a localization of ANXA1 mostly on the motility sub-structures. Interestingly, in MIA PaCa-2 cells we found also two cleaved forms of ANXA1 (33 and 3 kDa) that localize at cellular membranes and are secreted outside the cells, as confirmed by MS analysis. MIA PaCa-2 and PANC-1 cell lines express Formyl Peptide Receptors (FPRs) 1 and 2: the treatment of this cells with the ANXA1 mimetic peptide, Ac2-26, induced intracellular calcium release, consistent with nFPR activation, and significantly increased cell migration/invasion rate. ANXA1 effects on MIA PaCa-2 and PANC-1 migration and invasiveness were observed both by down-modulating its expression through siRNAs and by treatment with a blocking antibody. The importance of the secreted form of ANXA1 in cellular motility was confirmed when MIA PaCa-2 were compared with PANC-1 cells that lack both the cleaved and the externalized forms. Moreover, the treatment of PANC-1 cells with MIA PaCa-2 supernatants, significantly increased the migration rate of these cells. To better characterize the functional role of the protein in PC progression, ANXA1 Knock-Out (KO) clones from MIA PaCa-2 cells were obtained. The expression of several proteins was affected by the absence of ANXA1, particularly the cytoskeletal organization was negatively conditioned. In fact, MIA PaCa-2 ANXA1 KO lost their migratory and invasive capabilities, proliferated more rapidly and seemed to acquire a less aggressive phenotype. To confirm this aspect the MIA PaCa-2 wild type, PGS (the scrambled vector) and ANXA1 KO were implanted to create orthotopic xenograft *in vivo*. The PC mass of ANXA1 KO MIA PaCa-2 was not significantly smaller than the other experimental points, but the metastatization degree appeared particularly reduced as showed on livers of mice with MIA PaCa-2 wild type and PGS which showed a higher degree of metastatic lesions compared to MIA PaCa-2 ANXA1 KO.

This project provides new insights on the role of ANXA1 in PC progression. In *in vitro* models, the intracellular ANXA1 is involved in the maintenance of the cytoskeleton integrity. When secreted, the protein stimulates PC cells migration and invasion through FPR activation. This is confirmed by *in vivo* xenograft experiments where ANXA1 appears to stimulate the metastatization process.