ANNEXIN A1 CONTRIBUTES TO PANCREATIC CANCER CELL PHENOTYPE, BEHAVIOUR AND METASTATIC POTENTIAL INDEPENDENTLY OF FORMYL PEPTIDE RECEPTOR PATHWAY

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Annexin A1 (ANXA1) is a Ca2+-binding protein over-expressed in pancreatic cancer (PC) tissues, where its expression was associated to the increase of metastatization degree, a minor cell differentiation and a minor time of survival of patients (Bai et al., 2004; Chen et al., 2012). To better define the role of ANXA1, we reported the extracellular protein mediates PC cell motility acting on Formyl Peptide Receptors (FPRs) (Belvedere et al., 2014). Next, we described other mechanisms by which intracellular ANXA1 could mediate PC progression. We obtained ANXA1 Knock-Out (KO) MIA PaCa-2 cells using the CRISPR/Cas9 genome editing technology, together with PGS, an empty vector we used as technical control. We selected this cell line for the mesenchymal-like phenotype and for the presence of secreted ANXA1 in the forms of 37 kDa (full length), 33 and 3kDa (cleaved peptides). Wild type (WT), PGS and ANXA1-KO MIA PaCa-2 cells were first analyzed by LC-MS/MS showing altered expression of 62 proteins (36 of them appeared up-regulated and 26 down-modulated). These proteins could be involved in several cell pathways as proliferation, trafficking, metabolism, cytoskeletal organization and others. Based on the previous data, we focused on the cytoskeletal dynamics. We validated, by other techniques as Western blotting, cytofluorimetric analysis and RT/PCR, the down-modulation of vimentin and lamin A/C, the up-regulation of CD44 and the exclusive expression of cytokeratin 8/18 in ANXA1-KO MIA PaCa-2, all markers whose disregulation lead to the acquisition of a less aggressive or more epithelial phenotype. In these ANXA1-KO clones, we further highlighted a strong disorganization of F-actin fibers, which are concentrated all around plasma membrane and not structured in bundles protruding structures towards the plasma membrane. As a result, ANXA1-KO MIA PaCa-2 strongly lost their migratory and invasive capabilities. Moreover, the absence of ANXA1 did not seem to influence the expression of FPRs, since we observed similar expression levels of FPR1 and 2 by cytofluorimetric assay, on WT, PGS and ANXA1-KO MIA PaCa-2 cells. Thus, we performed further migration and invasion assays in presence of fMLP (endogen FPRs ligand), Ac2-26 (mimetic ANXA1 peptide) and Boc-1 (receptor antagonist), both alone than in co-administration. The receptors appeared active since migratory and invasion rate increased in presence of agonists and decrease following Boc-1 effects. But ANXA1-KO MIA PaCa-2 cells, although showed a similar trend, did not acquire the same speed of WT and PGS MIA PaCa-2 in their basal condition. This result highlighted the capability of the intracellular ANXA1 to operate in the cytoskeletal orchestration with a mechanism that appeared independent of FPRs. The acquisition of a less aggressive phenotype has been further investigated in vivo. WT, PGS and ANXA1-KO MIA PaCa-2 cells were engrafted orthotopically in the pancreas of SCID mice. No differences were found about PC primary mass, conversely liver metastatization appeared particularly reduced in ANXA1-KO MIA PaCa-2 engrafted mice confirming a relevant role of ANXA1 in the PC metastatization process (Belvedere et al., 2016).

The investigation of the role of ANXA1 in PC progression led us to study possible disregulation of micro-RNA profile in our in vitro models. Indeed, miRNAs represent crucial multitasking factors promoting or suppressing tumour progression and it is reported that ANXA1 can regulate the expression of multiple miRNAs in several tumour system, including PC. In order to further explain the mechanisms of action of ANXA1, total miRNAs from WT, PGS and ANXA1-KO MIA PaCa-2 cells were extracted and purified through the smallRNA-Sequencing technique as reported in (Hashim et al., 2014). According to the statistic tests, in ANXA1-KO MIA PaCa-2 28 miRNAs appeared down-modulated and 19 were over-expressed. These differences have to be better defined and validated, also in vivo, to demonstrate a loop ANXA1-miRNAs, which could be an appealing contribute to the creation of new combined biomarkers panel for PC diagnosis and therapeutic strategies.

Bai et al. (2004). World J Gastroenterol. 10, 1466-70.

Chen et al. (2012). Asian Pac J Cancer Prev. 13, 4707-12.

Belvedere et al. (2014). BMC Cancer. 14, 961.

Belvedere et al. (2016). Sci Rep. 6, 29660.

Hashim et al. (2014). Oncotarget. 5, 9901-10.