Role of intracellular and extracellular annexin A1 in migration and invasion of human pancreatic carcinoma cells

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BACKGROUND:
Annexin A1 (ANXA1), a 37 kDa multifunctional protein, is over-expressed in tissues from patients of pancreatic carcinoma (PC) where the protein seems to be associated with malignant transformation and poor prognosis.
METHODS:
The expression and localization of ANXA1 in MIA PaCa-2, PANC-1, BxPC-3 and CAPAN-2 cells were detected by Western Blotting and Immunofluorescence assay. Expression and activation of Formyl Peptide Receptors (FPRs) were shown through flow cytometry/PCR and FURA assay, respectively. To investigate the role of ANXA1 in PC cell migration and invasion, we performed in vitro wound-healing and matrigel invasion assays.
RESULTS:
In all the analyzed PC cell lines, a huge expression and a variable localization of ANXA1 in sub-cellular compartments were observed. We confirmed the less aggressive phenotype of BxPC-3 and CAPAN-2 compared with PANC-1 and MIA PaCa-2 cells, through the evaluation of Epithelial-Mesenchymal Transition (EMT) markers. Then, we tested MIA PaCa-2 and PANC-1 cell migration and invasiveness rate which was inhibited by specific ANXA1 siRNAs. Both the cell lines expressed FPR-1 and -2. Ac2-26, an ANXA1 mimetic peptide, induced intracellular calcium release, consistent with FPR activation, and significantly increased cell migration/invasion rate. Interestingly, in MIA PaCa-2 cells we found a cleaved form of ANXA1 (33 kDa) that localizes at cellular membranes and is secreted outside the cells, as confirmed by MS analysis. The importance of the secreted form of ANXA1 in cellular motility was confirmed by the administration of ANXA1 blocking antibody that inhibited migration and invasion rate in MIA PaCa-2 but not in PANC-1 cells that lack the 33 kDa ANXA1 form and show a lower degree of invasiveness. Finally, the treatment of PANC-1 cells with MIA PaCa-2 supernatants significantly increased the migration rate of these cells.
CONCLUSION:
This study provides new insights on the role of ANXA1 protein in PC progression. Our findings suggest that ANXA1 protein could regulate metastasis by favouring cell migration/invasion intracellularly, as cytoskeleton remodelling factor, and extracellularly like FPR ligand.