

SIGNALING ALTERATIONS AND CYTOKINE PROFILES IN LATE METASTATIC COLORECTAL CANCER PROMOTE AN ANTI-INFLAMMATORY PROFILE AND PROLIFERATION ENHANCEMENT.

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INTRODUCTION: Colorectal cancer (CRC) is the most abundant type of neoplasia in developed countries and the second cause of death among cancers. Metastasis is the final step of the malignant transformation. Chemokine-mediated inflammation and focal-adhesion and cell-signaling proteins play an important role in tumour biology by influencing tumour growth, invasion and metastasis.

EXPERIMENTAL PROCEDURES: We have used KM12C and KM12SM CRC cell lines, which only differ in their metastatic properties, to study the CRC metastasis process, or, alternatively, tissue samples from 4 CRC patients with metastasis in liver to identify differentially regulated cytokines and proteins using the human cytokine antibody microarrays (RayBiotech) or the Panorama Antibody Array (Sigma). Spot intensities were normalized and ratios calculated to identify proteins differentially regulated. Western blotting analysis with both KM12 cells and CRC tissue extracts was performed to verify the results.

RESULTS: Differentially-regulated proteins and cytokine profiles in CRC metastasis have been identified using 1.2 or 0.8 fold-change as threshold for significant alterations. In cells, we found a cytokine-profile associated to a pro-inflammatory response in KM12C low-metastatic cells whereas KM12SM highly-metastatic CRC cells expressed an anti-inflammatory profile and proteins altering cell cycle and focal adhesions responsible for a proliferation enhancement. Western blotting analysis corroborated the results and the activation of the signaling pathways induced by the differentially expressed cytokines and proteins. The *in vivo* results using CRC tissue indicated a different cytokine balance than KM12 cells.

CONCLUSIONS: We have demonstrated that CRC tumour epithelial cells play an important role in the recruitment and maturation of macrophages and other effectors cells by identifying differentially regulated proteins and cytokines released by KM12 cells and CRC tissue. This profile was found to be different from that of CRC primary and metastatic tumoral tissue, which might reflect the cross-talk between different components of the tumour microenvironment.