NOVEL APPROACHES TO THE CHARACTERIZATION OF METASTASIS IN COLORECTAL CANCER

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Colorectal cancer (CRC) remains as a major cause of mortality in the developed countries due to the absence of appropriate biomarkers for diagnosis. Progression to metastasis is the critical point in colorectal cancer survival. Metastasis of CRC is very poorly understood at the moment. In previous studies, the use of 2D-DIGE and antibody microarrays led to the identification of differentially-expressed proteins in primary CRC tumors as potential specific biomarkers of CRC. Both approaches were complementary and enabled us to identify a large collection of potential tumoral tissue biomarkers that is being currently investigated. These proteins included isoforms and post-translational modifications responsible for modifications in signalling pathways.

Recently, we have been working along two different lines for the search of colorectal cancer biomarkers related with metastasis: On the one hand, two colorectal cancer cell lines (KM12C and KM12SM), representing non-metastatic *versus* highly metastatic cells were compared to find and quantify the differences in protein expression at the cell surface level by using a SILAC approach. We were able to identify 291 membrane and membrane associated proteins from these two cell lines. A total of 66 proteins were differentially expressed more than 1.5-fold. Together with CEA and EGFR we identified an elevated number of cell receptors, CDs and cell adhesion molecules among the most deregulated proteins in metastatic cells. These proteins were further validated by using different techniques.

On the other hand, we have used high density protein microarrays comprising 8000 human proteins to identify autoantibody signatures in the sera of CRC patients. The screening was performed using sera from CRC patients in advanced stages, including metastasis. A total of 43 proteins were differentially recognized with a statistically significant value $p < 0.04$, 26 proteins showed higher prevalence in CRC sera and 17 showed lower prevalence. Furthermore, an ELISA assay was developed using these purified recombinant proteins for testing their discriminatory power in a different subset of human sera. The results confirmed the presence of a discriminatory autoantibody signature for CRC diagnosis and point out new individual markers of disease with a potential diagnostic capacity in metastasis.