THE UPREGULATION OF EPDR1 IS RELATED TO TUMOUR INVASIVENESS IN A COHORT OF LOCALISED COLORECTAL CANCER PATIENTS

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Introduction Colorectal cancer (CRC) represents a relevant public health problem. Despite new therapeutic advances, prognosis of patients diagnosed with advanced disease is still poor. The identification of new markers involved in the mechanisms of invasiveness represents a priority in order to better understand cancer development and generate new therapeutic targets. We describe here the possible role of EPDR1, a gene not yet well characterised, which encodes a protein related to ependymins, a family of piscine transmembrane proteins involved in cell adhesion.

To evaluate the role of EPDR1, a translational investigation was planned to explore the consequences of the upregulation of EPDR1 in cell models and in a well-balanced cohort of patients diagnosed with localised CRC.

Material and methods Expression of EPDR1 was determined by RT-qPCR in several CRC cell lines and in paired samples derived from CRC patients. The effects of silencing the gene on cell proliferation, invasion, migration and adhesion was studied in vitro.

147 patients diagnosed with localised disease, belonging to different stages, were prospectively selected in a single institution, according to different clinicopathological features such as stage, grading and MSI. All medical histories and the pathological reports were reviewed. All patients signed an informed consent at time of diagnosis. RNA was extracted form paraffin embedding samples of each primary tumour.

The statistical system R was used for all analyses.

Results and discussions Knockdown of the gene results in a decrease of cell proliferation, adhesion to collagen-coated plates, invasion and migration, while it was possible to observe an increase in necrosis among CRC cell lines. 140 patients were finally selected to perform the analysis, 7 were excluded because of the presence of secondary malignancy.

EPDR1 is more expressed in tumour than normal tissue among patients diagnosed with CRC. Interestingly, EPDR1 expression is directly related to T parameter, being higher among patients diagnosed with T3 and T4 CRC, independently from nodal involvement.

Conclusion EPDR1 seems to be a new marker of tumour invasiveness in CRC patients and its detection could predict tumour infiltration.

PO-183 IDENTIFICATION OF DIFFERENTIALLY HYPMETHYLATED GENES ASSOCIATED TO METASTASIS BEHAVIOUR IN COLORECTAL CANCER

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Introduction Colorectal cancer (CRC) is an important public health problem worldwide. In Chile, CRC is the fourth most frequent cancer and its incidence is rising. Sporadic CRC results from the accumulation of both acquired genetic and epigenetic changes that transform normal glandular epithelium into invasive adenocarcinoma. DNA methylation has an important role in colon carcinogenesis and also has been found involved in metastasis pathways of CRC. However, there are no studies that analysed whole genome in the search of specific methylated genes in metastasis of CRC, despite the next-generation sequencing platforms and methylation arrays available. Therefore, in this project it is proposed to find novel methylation markers of metastasis by comparing primary tumours and their corresponding lymph node metastasis, using a next-generation sequencing platform. The aim of this study was to identify differentially methylated genes associated with metastasis tumour behaviour in colorectal cancer.

Material and methods Five paired FFPE samples of CRC primary tumour and its corresponding lymph node metastasis...
were analysed with genome-wide Methyl-Seq bisulfite sequencing. Five differently hypomethylated genes were selected using bioinformatic tools. Bioinformatic analysis was realised comparing colorectal primary tumour vs lymph node metastasis using methylKit tool.

Results and discussions A total of 196 genes were detected as differentially methylated in their promoter region, 94 of which were hypomethylated on lymph node metastasis group. CS, RNF130, HERC6, ZNF717 and RNF216-IT1 genes presented differences over 50% in their methylation status, compared with CRC primary tumours group. According to their ontology, these genes are involved in regulation of tricarboxylic acid cycle, transcription, carbohydrate metabolic process and protein polyubiquitination.

Conclusion CS, RNF130, HERC6, ZNF717 and RNF216-IT1 genes were found hypomethylated in colorectal metastasis compared to primary tumour. These genes could be implied in metastasis behaviour in colorectal cancer, but further studies are necessary to evaluate their functions.

PO-184 ROLE OF UBQUITIN-CONJUGATING ENZYMES IN CHROMOSOME INSTABILITY AND BREAST CANCER METASTASIS

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Introduction Chromosome Instability (CIN) is a hallmark in cancer being aneuploid in found in most of the tumours. In addition, high levels of CIN in primary tumours predict poor outcome in several cancer types. During last years, it has been demonstrated the role of aneuploidy during primary tumour generation in some transgenic mouse models. However, the role and functional consequences of CIN and aneuploidy during the metastatic process has not been deeply explored.

Material and methods
- Previous screening from the group (Gawrzak et al. 2018) identified several candidates genes potentially relevant in breast cancer (BC) dormancy.
- WB and IF were performed for analysis of mitosis and study of chromosome segregation.
- Cells were labelled with GFP-Luciferease for tracking tumour cells in mice.
- CIN70 signature was obtained by analysing RNA expression data from paired primary and metastatic tissues from BC (Cejalvo et al. 2017).

Results and discussions Taking advantage from a previous loss of function screening approach in dormant breast cancer cells we have identified an Ubiquitin-conjugating enzyme (UBE) as a candidate gene to control metastasis in BC. UBE has a pivotal role during cell division by controlling the stability of key mitotic players. UBE abrogation prolongs the spindle assembly checkpoint in several breast cancer cell lines, thus delaying mitosis exit. Further analysis shows that UBE depletion impaired the normal segregation of chromosomes during cell division increasing aneuploidy rates. Interestingly, in vivo studies with different breast cancer cell lines show an increase in the metastatic abilities of UBE downregulated cells. Additionally, we compared a signature of chromosomal instability (CIN70) between paired primary and metastatic tissues form BC. CIN70 score is clearly increased in metastasis, emphasising the importance of aneuploidy for the acquisition of the traits required for cancer metastasis in BC.

Conclusion Overall, our results suggest that CIN generated by disturbed levels of UBE increases cell plasticity to facilitate metastatic growth in BC.

PO-185 TOWARDS DYNAMIC TARGETING OF TGF-β IN METASTATIC MELANOMA USING INTRAVITAL MICROSCOPY

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Introduction Melanoma patients diagnosed with liver metastasis have a poor prognosis. TGF-β inhibitors give mixed results in clinical trials with metastatic melanoma patients. To more specifically target TGF-β in liver metastasis melanoma patients, it is necessary to unravel the spatio-temporal function of the TGF-β pathway in hepatic colonisation of melanoma cells. TGF-β is a multifunctional cytokine that signals via TGF-β receptors and downstream SMAD effector proteins. SMAD proteins regulate transcription by binding, among others, to CAGA elements in the DNA. It can exert both pro- and anti-tumorigenic functions, depending on cellular context. It acts on tumour cells, as well as the tumour micro-environment and the immune system. In metastatic melanoma cells, TGF-β can stimulate invasion and metastasis. To unravel its exact role during the different processes of metastasis, we will investigate the spatio-temporal patterns of TGF-β signalling during metastatic colonisation using intravitral microscopy (IVM).

Material and methods Spatio-temporal patterns of TGF-β signalling will be studied in vitro and in vivo. For our in vivo studies, we are using an experimentally induced liver metastasis model, injecting highly aggressive B16F10 melanoma cells in immune competent C57BL/6 mice. By injecting these tumour cells in the mesentric vein, cells will be transported directly to the liver, the first capillary network the cells will encounter. An abdominal imaging window will be placed after cell injection to visualise the different steps of metastasis in real-time using IVM. We developed a rapid CAGA12-GFP-based transcriptional reporter, which expresses a fluorophore upon TGF-β receptor activation and SMAD binding. Upon the expression of this reporter in B16F10 cells, activation of the TGF-β pathway can be monitored over time. By genetic manipulation of B16F10 cells to express dominant negative or constitutively active TGF-β receptors, the role of TGF-β can be assessed for the different steps of metastasis.

Results and discussions We confirmed earlier reports showing that B16F10 cells show a transcriptional CAGA response upon TGF-β stimulation. Using our liver metastasis model, the injected B16F10 cells are able to perform the steps of metastasis and form liver metastasis within a short time frame. The use of the rapid TGF-β reporter during intravitral imaging will show the involvement of the TGF-β pathway in the different phases of metastasis.