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Human Endogenous Retroviruses (HERV) in colorectal cancer patients

Human Endogenous Retroviruses (HERV) are remnants of ancient exogenous retroviral infections of the humans, representing about 8% of the human genome. Although the majority of HERVs genes are highly defective with large deletions, stop codons and frameshift in the open reading frame (ORF), HERVs LTRs still retain their function, such as a promoter, enhancer and transcriptional factor binding site and potentially regulate their neighboring viral and cellular genes. HERVs do not replicate and produce infection, except for the human species-specific HERV-K. Despite this inactivation, many retroviral sequences contain intact ORFs that produce retroviral proteins. HERVs encoded proteins have been detected in a variety of human cancer, such as melanoma, testicular cancer, kidney cancer, breast cancer and prostate cancer. Several reports have shown the existence of a relationship between the HERVs expression and colorectal cancer, based on the mRNA expression profile of HERVs in normal and tumour tissues, but conclusive evidence is still lacking. We will evaluate the presence of HERV in peripheral blood mononuclear cells collected from colorectal cancer patients and healthy control, to clarify if HERV can be used as diagnostic biomarker for this type of cancer, using flow cytometry (anti-human CD4 APC, CD8 PE, CD19 APC and antibody direct against HERV stained in FITC) (IgG1-PE control will be included).

Extracellular vesicles (EVs) are part of an important mechanism of intercellular communication: EVs are composed of exosomes and microvesicles, secreted in plasma and they contain also RNA. EVs play important roles in many diseases, such as cancer: several studies have implicated EVs in driving the formation of a pre-metastatic tumour niche and are capable of stimulating tumour progression. It has been reported that, in vitro, tumor-derived microvesicles (EVs) are enriched in retrotransposon elements, such as LINE-1, Alu and in HERV-K. and there is no report regarding the presence of HERV sequences in EVs derived from clinical specimens. In order to clarify if HERV sequences are packaged and may be transferred to other cells and contribute to cellular genomic instability, for each colorectal cancer patient enrolled in our study, a tube of plasma and tumor tissue will

be obtain at surgery. A tube of plasma from healthy subject will be also collected to compare the results. First, the tumor tissue collected will be dissociated to a single cell suspension and cultured to obtain primary tumor cell line and characterized using traditional markers (anti-human CD44, CD24, CD133 FITC conjugated). The supernatant will be collected and EVs will be isolated by ultracentrifugation and will be stained with phycoerytrin (PE) conjugated antibodies specific to CD9, CD63, CD81, a typical marker of EVs, and anti HERV antibody FITC conjugated to determinate the HERV presence and the expression.

Furthermore, EVs isolated from the plasma of the patients will be analyzed by means of flow cytometry to discriminate if they derive from tumor or normal cells, using specific antibody for discriminating the colorectal cancer cell (anti-human CD147 stained APC and HERV antibody).

The last part of the project will be focused on the analysis of the capability of HERV gene products to act as tumor-associated antigens. It will be verificated whether the PBMCs collected from the patients in colture, after stimulation with the HERV antigen, are able to activate TH_1 and/or TH_2 responses. These data will be assessed by the human ELISA set for IFN-γ (TH_1), IL-6 (TH_2) and IL-10 (T-reg).