ImmunoTools *special* Award 2014

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DELIVERY OF THERAPEUTICAL AGENTS INTO MELANOMA BY TGFβ-SWITCH-DEPENDENT HOMING OF MESENCHYMAL STEM CELLS (MSC) AND ENDOTHELIAL PROGENITOR CELLS (EPC)

Transforming Growth Factor-beta (TGFβ) plays a crucial role in progression and metastatic diffusion of many tumors by changing from tumour suppressor of premalignant stages to prooncogene at later stages of the disease **(1).** TGFb-dependent acquisition malignancy-related properties (named "TGFβ-switch") is connoted by loss of TGFβ-dependent growth inhibition, apoptosis and genomic stability, and by increased expression/activation of TGFβ. Tumor cells themselves may over-produce active TGFβ, as well as proteases that, besides activating TGFβ, also degrade extracellular matrix (ECM), with a consequent release of stored TGFβ. All these changes result in a micro-environment that promotes tumor growth, epithelial-mesenchymal transition (EMT) and modulation of a set of pro-metastatic genes. Morover accumulating evidences demonstrate that mesenchymal stem cells (MSCs) can be involved in tumor growth. MSCs favour both tumor growth and angiogenesis by providing a stromal support and by secreting angiogenic factors **(2,3)** that recruit endothelial precursor cells (EPCs) in tumor mass, promoting vascularization by incorporation in vessels and/or by supporting tumor angiogenesis **(4)**

Both EPCs and MSCs are selectively recruited within the tumor mass. In consideration of the EPCs and MSCs intra-tumor homing capabilities and of the expression of TGFβ receptor on their surface, that could force homing following interaction with tumor-produced TGFβ, these cells might be used in anti-cancer therapy as cellular vehicle for delivering molecules able to inhibit cancer cells invasion and angiogenesis in a tumor undergoing TGFβ over-secretion. Among molecules to be induced within "commandos cells" our previous data indicate as better candidates the following molecules: anti-TGFβ peptides and metalloprotease 12 (MMP12).

Therefore, the aim of this project is to deliver into tumors EPC and/or MSC populations bearing the adeno-associated virus (AAV) vectors encoding the metalloprotease 12 (MMP12), a protease with anti-angiogenic and anti-invasion activity. As tumor model I will utilize human melanoma cell lines.

I will isolate EPCs from cord blood and I will derive MSCs from bone marrow aspirate of volunteers according to standard procedure. Cells will be characterized by the expression of selected markers: CD34, CD133, absence of CD45 and appearance of endothelial-specific markers (KDR, CD144, CD141, CD105, VWF, CD31). The expression of the selected

markers will be monitored by flow cytometry analysis, RT-PCR and immunocytochemistry. MSC will be analyzed for the expression of several surface antigens as described **[5]:** CD45, CD14, CD44, CD166, CD90, CD73, HLA-DP, -DQ, -DR, HLA-ABC, CD105, CD27, APC.

I will be very pleased to use ImmunoTools cytokines such as: rh SDF-1, rh RANTES, rh IL6, rh IL10, rh BMP-2, rh BMP-7, to investigate their role on tumor microenviromental, cell recruitment, tumor growth and angiogenesis and anti-human antibodies for stromal cells characterization such as CD45, CD105, CD54, CD36, HLA-ABC, HLA-DP, HLA-DR, CD34, and Annexin V to evaluate the apoptosis induced by TGFbeta inibitors or TNFalfa.

References

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ImmunoTools *special* AWARD for **Alessio Biagioni** includes 25 reagents

FITC - conjugated anti-human CD27, CD36, CD45, CD54, CD55, CD105, HLA-ABC, HLA-DP, HLA-DR, Control-IgG1, Annexin V,

PE - conjugated anti-human CD34, CD44, Control-IgG1,

recombinant human cytokines: rh BMP-2, rh BMP-7, rh GRO-alpha, rh IFNgamma, rh IL-1alpha / IL-1F1, rh IL-6, rh IL-10, rh RANTES / CCL5, rh SDF-1α / CXCL12a, rh SDF-1β /CXCL12b, rh TNFα DETAILS more AWARDS