ImmunoTools IT-Box-139 Award 2012

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Melanoma Cancer Stem Cell Aggressiveness and Immune Evasion upon microenvironment modification

Due to its resistance to current therapies, melanoma remains nowadays a significant cause of mortality. Mounting evidences suggest that cancer arise from a transformed stem cell, which is able to self-renew, differentiate into diverse progenies, and drive continuous growth (Nguyen LV. Nature 2012; 12:133-143). The presence of a small subpopulation of slowly dividing cancer stem cells might also explain why many cancers recur after treatment with irradiation or cytotoxic drugs, even when most of the cancer cells seem to be killed by the therapy. Indirect evidence supports the presence of stem-like cells in human melanoma. Indeed, melanomas show phenotypic heterogeneity both *in vivo* and *in vitro*, suggesting an origin from a cell with multilineage differentiation abilities and retaining their morphological and biological plasticity despite repeated cloning (Keith S.H. Pigment cell Melanoma Res. 2010; 23:746-759). Consequently, cancer stem cells (CSC) emerged as a potential therapeutic target for melanoma. Hence, one of the objectives of my PhD project is to deeply characterize the behavior of melanoma stem cells upon different microenvironment conditions using selected markers for the better understanding of tumor cells immune evasion mechanisms, interaction with immune cells, metastasis and angiogenesis formation.

For this study I have selected a human melanoma cell line from our collection (TVM-A12) that appeared to be a good candidate for the discrimination of stem-cell-like properties and for the studies on microenvironment dependent tumor alteration. The cell line was originally isolated in our laboratory from a human melanoma lesion. A previous study showed that TVM-A12 cells *in vitro* undergo a transition from adherent to a more malignant, non-adherent

phenotype when exposed to stress conditions by serum deprivation (Serafino et al. 2009). Hence, in continuation of this and other related findings of our lab, my study will focus on characterization of tumor related markers using this cell line.

Therefore, the main focus of this research investigation will be: A) to identify new melanoma cancer stem cell markers and characterize their specificity role with previously identified CSC markers; B) to find out the role of selected CD markers in melanoma associated metastasis and angiogenesis formation in different microenvironment conditions; C) to identify inter- and/or intra-cellular CD markers modulation by CSC to evade host antitumor immune cells and determine synergistic or additive role of regulatory T Cells and myeloid-derived suppressor cells through allogenic co-culturing.

Thus in chorus with the use of already recognized CD markers, cancer stem cells from TVM-A12 cell line will be analyzed using antibodies specific for CD13 and CD15 cell surface antigens, some findings indicated both might be used for identification of CSC (Christ B. Hepatology 2011; 53: 1388-90, Read TA. Cancer Cell 2009; 15:135-147). To determine the role of CD markers such as CD18, CD31, CD36, CD62p, CD105 and CD147 in metastasis and angiogenesis formation during malignancy, melanoma cancer stem cells and endothelial cells will be co-cultured or exposed to supernatant under different culture conditions and ultimately evaluate the role of those selected CD markers on cell migration as well as microvascular formation using the specific fluorochrome-conjugated antibodies (Yaojiong WU. Circulating Research 2006; 99: 315-22, Bendas G. Cell Bilogy 2012; 2012:1-10, Nishibaba R. Dermatology 2012; 39: 63-67). Furthermore, different conjugated antibody will be analyzed to functionally characterize the effect of allogeneic co-culturing of melanoma cancer stem cells, immune cells and immuno suppressive cells on the expression and modulation of certain markers associated with immune cell evasion (eg HLA-I, HLA-II, CD1a, CD27, CD33, CD38, CD44, CD95, Foxp3) and immune cell interaction (eg. CD3, CD4, CD5, CD8, CD45, CD58) using flow cytometry (Wallich R. Immunology 1998; 160: 2862-37, Malavasi F. Physiology Review 2008; 88:841-886, Kudo C. Patent Application Publication 2010; 18: 1-20, Lehner MG. Translational Medicinal 2011; 9:90).

Therefore, the chance to use these multiple type of ImmunoTools IT-Box 139 antibodies will help me to analyze all the above mentioned tumor related markers, which will not only further upgrade my PhD research project, but also, much more relevant, will have very important

implications in understanding the metastatic, angiogenetic and host immune evasion mechanisms to improve discovering of novel strategies for melanoma diagnosis and treatment.

The feasibility of this study is guaranteed by the long-term experience of our research group in the field cancer and our laboratory is well established with most advanced instrumentations such as cell culture and storing, flow cytometer, RealTime PCR, imaging, radioisotope rooms, biohazard P3 room, and an animal care facility.