ImmunoTools *special* Award 2014

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Role of Fibroblast Growth Factor-2 isoforms in human melanoma vascularization

Overexpression of growth and survival-promoting factors is an important hallmark of neoplastic cells and a major driving force for tumor progression and dissemination (1).

Expression of fibroblast growth factor 2 (FGF2) has been identified as an important characteristic of melanoma cells in contrast to normal melanocytes (2) and, being a strong mitogenic and angiogenic factor, FGF2 is up-regulated in melanoma patients and contributes to melanoma growth and progress (3).

FGF2 belongs to the family of Fibroblast growth factors (FGFs) and is a potent inducer involved in proliferation and differentiation of a wide variety of cells derived from mesoderm and neuroectoderm. FGF-2 is also involved in tumor neovascularization. It is produced in many cell types and tissues, and its pleiotropic biological roles can partly be explained by the different modes of action of this factor. The paracrine and autocrine action of FGF2 are mediated by its secretion and specific-receptor recognition (FGFR1-4), but FGF2 also exhibits an intracrine action, thereby allowing a direct effect on intracellular targets in the absence of secretion. The different modes of action of FGF-2 are the direct consequence of a process of alternative initiation of translation on the mRNA: the AUG-initiated form of 18 kDa is mostly cytosolic (low molecular weight FGF2, LMW), whereas the CUG-initiated forms of 22, 22.5, 24 and 34 kDa (high-molecular-weight FGF-2, HMWs) are predominantly localized in the nucleus because of a nuclear localized sequence (NLS) at N-terminal. The biologic roles of these HMWs FGF2 isoforms remain mostly unknown (4).

Cancer poor prognosis primarily results from metastases that are resistant to conventional therapies. To sustain tumor survival and metastasis is necessary a blood supply, but targeting angiogenesis process to inhibit tumor growth and metastasis formation have given disappointing results. Indeed, a novel mechanism of aggressive tumor perfusion, called “vasculogenic mimicry” (VM), was discovered, allowing *de novo* formation of perfusable, matrix-rich, vasculogenic-like networks by aggressive tumor cells themselves in 3 dimensional matrices *in vitro*, which parallels matrix-rich networks in aggressive tumors (5). Human aggressive melanoma is an example of VM-able cancer in which tumor cells were shown to coexpress endothelial and tumor markers and form channels, networks, and tubular structures, containing plasma and red blood cells, indicating a perfusion pathway for rapidly growing tumors, as well as an escape route for metastasis. Interestingly, these findings agree with very early reports by others suggesting the perfusion of melanoma tumors via non-endothelial-lined channels (6)**.**

The aim of this project is to investigate the role of different FGF2 isoforms in the tumor vascularization of human primary and metastatic melanoma and to study the collaboration between FGF2 expressing-melanoma and endothelial cells. We will use stable transfected melanoma cells which selectively overexpress LMW FGF2 and HMWs FGF2.

As HMWs FGF2 seem to be expressed only by aggressive melanomas, we would like to investigate if there could be a connection between the expression of these isoforms and the vascular mimicry process.

Tumor vascularization will be detected in all its phases. In order to do this, we will use: VE-cadherin, EphA2, VEGFR1, HIF1alpha, Twist1, Notch, Nodal antibodies to detect vasculogenic mimicry markers in melanoma cells; GRO-alpha, CTGF, CD154, SDF-1α, VEGF-A, IL-8 cytokines to investigate endothelial cell mediated melanoma chemotaxis and migration into blood vessels; Annexin V antibody, TRAIL and TNF-alpha cytokines to evaluate tumor cells survival in the circulatory system (anoìkis-resistance); matrix-metalloproteinases, urokinase plasminogen activator (uPA) activity and other proteolytic assays to investigate extravasation ability of melanoma cells; CD18, CD61, CD47, CD54, CD62P antibodies to study engraftment capacity of tumor cells to the second site.

If a selectively involvement of HMWs FGF2 in tumor vascularization will be confirmed, these isoforms could be considered another possible target to inhibit tumor growth and metastasis formation in human aggressive melanoma.

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