ImmunoTools *special* Award 2015

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PTX3-derived FGF2 trap for the treatment of Multiple Myeloma

Despite advances in systemic and supportive therapies, multiple myeloma (MM) remains incurable because of chemotherapeutic resistance. Although high-dose chemotherapy with stem cell support has extended progression free and overall survival significantly, few, if any, patients are cured, indicating that novel biologically-based treatment approaches are urgently required.

The biological and clinical behavior of MM cells are not only determined by their genetic background, but also by the bone marrow (BM) microenvironment. The bone marrow milieu is represented by a heterogeneous cell population as well as extracellular matrix (ECM) proteins and secreted growth factors. The interactions of MM cells with the BM microenvironment activates a pleiotropic cascade of proliferative/antiapoptotic signaling pathways. These molecular events are triggered either directly, via cell adhesion molecule-mediated interactions of MM cells with BM stromal cells (BMSCs) or the ECM, or indirectly, triggered by growth factors released by BMSCs and/or MM cells. Importantly, this growth factor circuit between MM cells and BMSCs promotes MM cell growth, survival, and migration, contributing to MM progression and resistance to drug treatment. Among these growth factors, FGF2 and IL-6 appear to play a pivotal role. MM cells express and secrete FGF2, a potent angiogenic cytokine that contributes to the increased angiogenic potential of BM plasma cells in progressive MM. Stimulation of BMSCs with FGF2 induces a time- and dose-dependent increase in IL-6 secretion; conversely, stimulation with IL-6 enhances FGF2 expression and secretion by MM cell lines, as well as MM patient cells. In addition to paracrine production by cells in the BM microenvironment, IL-6 is also produced in an autocrine manner by the MM cells themselves. These data point to an IL-6/FGF2-mediated paracrine/autocrine interaction between MM and BMSCs triggering not only neovascularization, but also MM cell growth and survival. Thus, FGF2 represents a promising target for therapeutic strategy in MM. In keeping with the pivotal role of the FGF system in MM, a recurrent chromosomal translocation

identified in primary MM patients is the t(4;14) translocation associated with upregulation of FGF receptor 3 (FGFR3). Agents able to hamper FGF signaling are therefore of interest as a novel approach for the treatment of MM, in particular for those patients carrying the t(4;14). However, the development of drugs targeting the FGF pathway has been difficult and tyrosine kinase FGFR inhibitors have shown toxicity in clinical trials, possibly due to their broad, non-selective action.

The soluble pattern recognition receptor Pentraxin 3 (PTX3) is a member of the long pentraxin subfamily. Studies from our laboratory have shown that PTX3 has an unique N-terminal amino acid domain that binds FGF2 with high affinity and selectivity. This prevents FGF2 binding to cognate receptors (FGFRs) thus hampering the biological activity of FGF2.

Starting from the structure of PTX3 (340 KDa) we have recently identified the PTX3-derived pentapeptide ARPCA as the minimal sequence endowed with the FGF-neutralizing properties of PTX3. Molecular modeling based on ARPCA structure and library fishing have allowed the identification of a promising novel druggable orally active low molecular weight FGF-trap named NSC12 (480 Da).

Goal of the present project is the characterization of the antitumor activity of NSC12 in MM. This compound is anticipated to affect both tumor parenchyma and stroma, thus hampering alongside tumor growth and vascularization. At present, no pharmacophore drugs with FGF-trap properties are available in the clinics. NSC12 will represent the first low molecular weight FGF-trap druggable molecule to be used for anti-FGF therapy in MM.

This project is characterized by a “bedside to bench and back to bedside” approach with the final goal to identify new drugs to be used in the clinics for MM treatment with significant social benefits due to an increase of life expectancy and wellness of MM patients.

The ImmunoTools selected products would be of great benefit to this project as they would be used to: 1) evaluate the cytotoxic effects of NSC12 on MM cell lines by Annexin-V/Propidium Iodide double staining and flow cytometric analysis; 2) control the phenotype of endothelial cells, fibroblasts, and plasma cells isolated with specific magnetic microbeads from the bone marrow of MM patients; 3) stimulate human MM cells with FGF2 and/or IL-6; 4) Evaluate by ELISA IL-6 secretion upon treatment with NSC12; 5) evaluate by cytofluorimetric analysis the percentage of $CD138^{+}/CD19^{-}$ tumor cells in the peripheral blood of C57BL/6 mice transplanted with syngeneic murine Vk*MYC MM cells.

ImmunoTools *special* AWARD for **Arianna Giacomini** includes 24 reagents

FITC - conjugated anti-human CD19, CD105, Control-IgG1, Annexin V,

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

PerCP - conjugated anti-human CD45, Control-IgG1,

APC - conjugated anti-human CD31, Control-IgG1,

human IL-6 ELISA-set for 96 wells, (3 reagents),

recombinant human cytokines: rh FGF-b / FGF-2, rh IL-6,

FITC - conjugated anti-mouse CD19, isotype control IgG2b,

PE - conjugated anti-mouse CD34, isotype control IgG2b,

APC - conjugated anti-mouse CD45, isotype control IgG2b

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