ImmunoTools *special* Award 2016

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Molecular targeting of Notch signaling as a novel therapeutic strategy for multiple myeloma treatment

Multiple myeloma (MM) is still incurable plasma cell malignancy due to the localization of MM cells in the bone marrow (BM), which promotes tumor survival, osteolysis and drug resistance.

The oncogenic Notch signaling plays a crucial role in MM progression. The Notch family includes 4 transmembrane receptors (Notch1-4) activated upon binding to 5 ligands (Jag1 and 2, Dll1,3 and 4). The overexpression of Notch ligands, Jag1 and 2 leads to aberrant Notch activation in MM resulting in tumor growth and stimulating MM cells to establish pathological interactions with BM. The evidence of our group and literature data showed that these effects can be interfered by knocking-down Jag1 and 2 in MM cells. This lays foundation for a development of a new therapeutic strategy in MM aiming to disrupt Notch-Jag interaction.

The goal of this project is to provide a rationale to target Notch-Jag interaction in MM by an unprecedented approach based on small molecules. This approach has several advantages, i.e. small molecules can be easy delivered, are metabolically stable and orally active. Additionally, targeting only Notch signaling members dysregulated in MM promises to avoid the intestinal toxicity caused by currently used pan-Notch inhibitors directed to all Notch isoforms.

This project will rely on 100 small molecules able to disrupt Notch-Jag complex already selected *in silico* by a multistep approach based on protein-protein docking and virtual high-throughput screening (HTS). Selected compounds will be subjected to *in vitro* HTS using a Notch-responsive reporter and proliferation assays on MM cell lines. After screening, biologically active hit compounds will be undergone to *in silico* leading compound refinement and characterization in order to identify lead candidates with improved pharmacological properties. Up to 4 lead compounds with the best pharmacokinetic profile will be undergone to a thorough biological characterization on a panel of MM cell lines and primary MM cells from treated or relapsed/refractory MM patients.

The biological analyses will include the outcome of Notch signaling inhibition upon compounds treatment on key features of myeloma cell biology and on an interaction

of MM cells with the surrounding BM stromal cells (BMSCs). To study the pathological interaction, we will get advantage of co-culture system of MM and BMSC line, GFP^+ - HS5.

To this, changes in *proliferation, cell cycle and apoptosis* in single and co-culture systems upon compounds treatment will be measured by flow cytometry using volumetric counting, propidium iodide staining and AnnexinV/PI staining, respectively. The effect of small molecules on BMSC-induced drug resistance to currently used anti-MM drugs will be assessed by apoptosis assay in single and co-culture systems. Moreover, variations in the expression and secretion of factors inducing MM cell proliferation, apoptosis, drug resistance and osteoclast maturation, i.e. IL-6, SDF1α, IGF-1, VEGF, TNF-α, bFGF, OPG/RANKL ratio, MIP-1α, MIP-1β will be assessed by ELISA and flow cytometry using a panel of antibody.

Ex-vivo validation of the promising compounds will be performed using primary MM cells isolated from patients' BM aspirates using $CD138^+$beads. MM cell population will be characterized by flow cytometry using CD138 antibody whereas BMSC fraction will be evaluated using CD105, CD90, CD45 and CD14 antibodies. Primary MM cells will be grown in medium supplemented with rh-IL6, rh-IGF-1 and rh-GM-CSF either alone or co-cultured on a layer of BMSC fraction. The effect of compounds on the biological features of primary MM cells and on the expression of different factors will be performed as mentioned above.

We suppose that a detailed functional and molecular characterization of the lead compounds will result in one or two promising compounds for future *in vivo* validation on a murine models in order to provide the basis for an effective Notch-targeted approach in future clinical trials.