ImmunoTools *special* Award 2015

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THE EFFECT OF HYPOXIA ON MULTIPLE MYELOMA NICHE

Multiple myeloma (MM) is an incurable hematological tumor stemming from malignant plasma cells that accumulate in the bone marrow (BM). Despite recent advances in treatment, MM remains an incurable disease due to development of drug resistance which is critically enhanced by interactions of MM cells with the BM niche.

Indeed, it is well established that the BM microenvironment in MM is hypoxic, a condition that enhances neovascularization, increases glucose metabolism, and induces the expression of antiapoptotic proteins, finally promoting the progression of this disease. On the basis of the evidences described above, the aim of this project will be to characterize the effects of hypoxia on MM cells growth, proliferation and survival and their interaction wtih the BM niche. Furthermore we will evaluate the effect of hypoxia on cancer stem cells (CSCs) niche because it is known that MM stem cells (MMSCs) sustain MM maintenance and disease burden and are responsible of pharmacological resistance. As a matter of fact, MM relapse is most likely due to MMSCs surviving to chemotherapy.

The first set of experiments will be performed using MM cell lines (i.e. OPM2, U266) co-cultured on a layer of GFP^+-BMSC lines, HS5 or HS27. Hypoxia will be induced by incubating cells under hypoxic conditions (1% O_2). Variations in the expression of relevant genes and proteins in MM cells and BMSCs will be assessed and results obtained will be compared with cultures maintained in normoxic conditions (20% O_2). The effect of hypoxia will be analyzed both at gene expression (qRT-PCR) and protein levels using flow cytometry and ELISA assay:

- Flow cytometry analysis: first we will analyze apoptosis rate by using Annexin V and propidium iodide staining, then the cell cycle using propidium iodide staining and finally the expression of different proteins among which IL-6, VEGF, SDF-1a TNFa using a specific panel of antibodies.
- ELISA assay: we will evaluate the expression of cytokines i.e., IL-6, SDF-1a IL-8, TNFa, VEGF, MIP-1α and MIP-1β in the conditioned media (CM) of cell cultures.

As results we will expect that hypoxia is able to upregulate the expression of several pro-angiogenic factors if compared to normoxic controls.

The second part of experiments will be an ex-vivo validation using primary MM cells ($CD138^+$) isolated from patients' bone marrow aspirates as follows: $CD138^+$ MM cells will be immunomagnetically separated from BMSC using $CD138^+$ beads. After isolation the different cell populations will be characterized by flow cytometry using panels of antibody as follow:
- $CD138^+$ cells: $CD138^+$
- BMSC: $CD105^+/CD90^+/CD45^-/CD14^-$

Primary MM cells will be grown in medium supplemented with rh-IL6, rh-IGF-1 and rh-GM-CSF; we will set up co-culture using primary MM cells and primary BMSC under hypoxic or normoxic condition and we will evaluate the effect of hypoxia on the expression of different citokines. Primary MM cells will be sorted using anti-CD138 beads and then acquired by flow cytometry in order to analyse: apoptosis rate by using Annexin V and propidium iodide staining, the cell cycle using propidium iodide staining and the expression of different proteins among which IL-6, SDF-1a, VEGF, TNFa using a specific panel of antibodies. The results obtained by flow cytometry will be confirmed with ELISA assay on CM collected from primary co-colture in order to analyze the effect of hypoxia on the secretion of cytokines, e.g., rh IL-6, rh SDF-1a, eh IL-8, rh TNFa, rh VEGF, rh MIP-1α and rh MIP-1β.

The final part of this project will aim to investigate the effect of hypoxia on MMSC niche; up to now, results from several studies on MMSCs characterization provided discordant conclusions concerning the phenotype displayed by MM cells with clonogenic potential, possibly due to a high level of heterogeneity of MMSCs sub-populations. The MMSCs population is described with the following immunophenotype: $CD138^-CD20^+CD27^+$ and we will evaluate the contribution of hypoxia in maintaining the MMSCs analyzing the stemness markers by flow cytometry.

The final goal of this project is to provide a new insight into the MM biology in order to find a novel approach to interrupt the interaction between MM cells and the surrounding microenvironment.