ImmunoTools *special* Award 2015

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Notch pathway drives osteoclastogenesis in multiple myeloma

Multiple myeloma (MM) is a plasma cells (PCs) disorder characterized by the accumulation of malignant PCs in the bone marrow (BM) and it accounts alone for about 10% of all hematologic cancer. 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.

MM is associated with organ damages including hypercalcaemia, renal dysfunction, anaemia, and bone disease, which is mediated by the dysregulation of the ratio between osteoblasts and osteoclasts (OCLs) in favour of the second cells type. Bone lesions are observed in up to 80% of patients. It is known that MM cells may both directly contribute to increase the level of the receptor activator of NF-κB ligand (RANKL) and other pro-osteogenic chemokines and may induce BM-stromal cells to increase the production of osteogenic factors that promote OCLs formation. OCLs support MM cell survival, proliferation and drug resistance, and promote TGF-β release from the bone matrix, which plays a role antagonizing patient's anti-tumor immune responses.

We have demonstrated that MM can drive osteoclastogenesis by contemporaneously activating Notch signaling on tumor cells and osteoclasts through the overexpression of Notch ligands belonging to the Jagged family. Active Notch signaling in MM cells induces the secretion of RANKL, which can be further boosted in the presence of stromal cells.

The evidences that the osteoclastogenic potential of MM cell depends on Notch activity indicate that Notch signaling may represent clinically relevant target to prevent myeloma bone disease.

This project is aimed to provide, by *ex vivo* experiments, the proof of principle that a Notch-directed therapeutic approach may be effective to inhibit MM osteoclastogenesis.

To this, based on reported data of a positive correlation between levels of circulating TNF-α and bone disease in MM patients, we will screen sera of MM patients to detect level of TNF-α using an ELISA. From patients with high levels of TNF-α respect to healthy donor we will isolate:
- $CD14^+$ monocytes (precursors of osteoclasts) from blood;
- $CD138^+$ MM cells from bone marrow aspirate;
- Bone marrow stromal cells (BMSC) from bone marrow aspirate.

$CD14^+$ monocytes will be isolated using immunomagnetical beads;$CD138^+$ 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:
- $CD14^+$ monocytes: $CD3^-/CD19^-/CD14^+$
- $CD138^+$ cells: $CD138^+$
- BMSC: $CD105^+/CD90^+/CD45^-/CD14^-$

Primary MM cells will be grown in medium supplemented with h-IL6, h-IGF-1 and h-GM-CSF; primary monocytes will be grown in medium containing h-MCSF.

We have previously shown that conditioned medium (CM) from co-culture system of BMSC and $CD138^+$ cells can enhance OCLs differentiation if compared to single culture either of BMSC or $CD138^+$. Therefore, to evaluate the osteoclastogenic potential of patients MM cell, CM obtained from co-culture of primary BMSC and $CD138^+$ cells will be used to induce OCLs differentiation of primary $CD14^+$ monocytes. In addition, we will use the murine monocyte/macrophage cell line Raw 264.7 which is able to generate OCLs if stimulated with CM.

The effect of Notch signalling on osteoclastogenic potential of MM cell will be studied by an inhibitory approach.

As we previously reported in vitro, Notch pathway withdrawal reduces the ability of MM cells to promote ostoclastogenesis. To confirm this data ex-vivo, we will block Notch signalling in $CD138^+$ cells using the, γ-secretase inhibitor, DAPT, both in single and BMSC co-culture systems.

Previously, we have reported that Notch pathway withdrawal reduced the ability of MM cells to promote osteoclastogenesis *in vitro*, thus we expect that CM from DAPT-treated primary cultures will develop lower osteoclastogenic potential than untreated control.

Finally we will analyse by ELISA the pool of cytokines involved in OCLs maturation produced by patients MM cells among which: h-RANKL, h-IL6, h-IGF1 and h-VEGF. The effect of Notch on release of these cytokines will be detected in CM of primary MM cells treated with DAPT. We expect an increased production of these molecules in MM-BMSC co-cultures rather than single culture that could be reduced by Notch signalling blockade using DAPT.

This work may provide the rational for an effective Notch-directed approach to contrast MM patients relapse and bone disease, improving the standard treatments, providing a valuable option for patients with advanced disease.