The Notch pathway drives the ability of the bone marrow niche to promote RNA editing in multiple myeloma

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Introduction and aims

Multiple myeloma (MM) is the second most frequently diagnosed hematological malignancy, and despite all the therapeutic advances it remains incurable due to the development of drug resistance. Recently, RNA editing has emerged as one of the important mechanisms that determines expression variability and therefore may be involved in the development of resistance to standard therapy. This process is mediated by adenosine deaminase acting on RNA (ADAR) enzymes that convert adenosines to inosines (A-->I editing) in double-stranded RNA (dsRNA) substrates. We hypothesize that ADAR1 activation in MM cells may be promoted by the normal cells of the bone marrow (BM) niche through the release of pro-tumor factor controlled by the oncogenic Notch pathway. Indeed, Notch is known to be hyperactivated in myeloma and it is crucial for the pathologic crosstalk between tumor cells and the surrounding BM microenvironment.

Aim of this work was to investigate how the Notch pathway contributes to the ability of the BM microenvironment stromal cells on their ability to boost RNA editing and drug resistance in MM, in order to provide the rationale for a Notch-directed therapy that may allow to inhibit the progression of this disease.

Methods

The MM cell line H929 were was co-cultured with the BM stromal cell (BMSC) line HS5. The use of GFP-positive HS5 cells (GFP+ HS5) allowed us to discriminate the two cells types in flow cytometry, while for molecular analysis, of H929 cells were carried out after immunomagnetic separation (CD138+ cells selection kit). Notch signaling was inhibited in HS5 using anti-Notch1 specific siRNAs (HS5 -N1KD), lentiviral vectors or 50μM DAPT. The effect of the co-culture on ADAR1 expression in MM cells was analyzed by qRT-PCR and Western blot. Intracellular levels of IL6 were analyzed using BD FACSVerse™ Flow Cytometry System. RNA editing levels of ADAR1 target genes in H929 cells were determined by RESSqPCR assay and Sanger sequencing using the Abi Prism 310.

Results and Conclusions

To test if stromal cells were able to promote ADAR1 activity in MM cells in a Notch-directed manner, we set up a co-culture system composed by the MM cell line H929 and the BMSC line HS5. H929 were co-cultured alone or on a layer of HS5 where Notch1 was inhibited (HS5-N1KD). Results showed that HS5 cells were able to boost the expression of one of the form of ADAR1, ADAR1-p150, in H929; interestingly, this effect is Notch-dependent, since ADAR1-p150 expression decreases when MM cells are cultured with HS5-N1KD. The next step was to investigate the outcome of ADAR1-p150 upregulation. It was correlated with an increase in the RNA editing activity in the cancer-related genes GLI1, APOBEC3D and AZIN1. We demonstrate that MM cells cultured with HS5 showed an increased level in the edited-form of the ADAR-

targeted transcripts such as GLI1, AZIN1 and APOBEC3D. Interestingly, this effect is lost when Notch1 is inhibited in BMSCs.

Our results showed that one of the key mediator of this process is IL6. Indeed, the Notch signaling positively regulates IL6 production in BMSCs, while IL6 silencing causes a decrease in the ability of these cells to boos ADAR1 activity in tumor cells.

These evidences offer new interesting details on the mechanisms that underline the ability of the bone marrow niche to promote tumor progression.