Study of contribution of signalling pathways Notch, Wnt, Shh and BMP in mesenchymal stem cell pro-haematopoietic activity.

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Stem cells are primal cells that retain the ability to renew themselves through cell division and can differentiate into a wide range of specialized cell types. The mechanisms which control balance between the self-renewal and differentiation are still badly known. They consist of a complex whole of interactions between stem cells at their various stages of differentiation, stromal cells which form their micro-environment and many cytokines endowed with activating or inhibiting activity. Mesenchymal stem cells (MSCs) are able to support ex vivo haematopoiesis by providing components of the matrix, cytokines and essential growth factors to proliferation and differentiation of haematopoietic cells. The aim of this work consists in determining the contribution of signalling pathways Notch, Wnt, Shh and BMP in the support of haematopoiesis by MSCs by using various inhibitors of these ways.

We tested the effect on MSCs of following molecules: anti-human Jagged 1 Antibody and γ40 secretase inhibitor II (Notch pathway), Recombinant Human BMPR-IA/Fc chimera (BMP pathway), cyclopamine KAAD (Shh pathway) and recombinant human Dkk-1 (Wnt pathway). We analysed the phenotype, the MSC ability to differentiate into adipocytes, osteoblasts and chondroblasts, the colony-forming capacity and cytokine secretion profile. Compared to cultured without inhibitors MSCs, the presence of inhibitors didn't influence the phenotype. Cultured with inhibitors MSCs retain the differentiation potential; however chondroblasts differentiation didn't carry out with cyclopamine KAAD and γ40 secretase inhibitor II. Moreover inhibition of BMP pathway enhances osteoblasts differentiation. We observed that the CFU-F number didn't significantly change in comparison with control without inhibitor; signalling pathways didn't play an important role in the colony-forming capacity of MSCs. We used the BD^{TM} CBA Flex Sets to quantitatively measure IL-6 and IL-8. Compared to control without inhibitor, we observed an increase of IL-8 secretion in presence with anti-human Jagged 1 Antibody and recombinant human Dkk-1. We also observed a decrease, weak but significant, of IL-6 secretion in presence with Recombinant Human BMPR-IA/Fc chimera. We performed the same experiment with Human Th1/Th2 Cytokine Kit to measure the secretion of IL-2, IL-4, IL-5, IL-10, TNF and IFN-γ. MSCs didn't secret these molecules in our conditions.

Long term cultures were done with MSCs harvested at P2, P4, P7 and P10 and CD34+ cells in presence of inhibitor. We analysed by flow cytometry the number of CD10+, CD11b+, CD19+, CD33+ and CD34+ cells after 5 weeks of culture.
In contact with P2 MSCs, the inhibition of BMP pathway increases the CD34+ cells amplification. In contrast, with P7 and P10 MSCs, a reverse effect was observed. This result suggests that BMP pathway play a role in MSCs pro-haematopoietic activity; she can be inhibitive (P2 MSCs) or activative (P7 and P10 MSCs) on CD34+ cells amplification.
In presence with recombinant human Dkk-1, we observed a decrease of CD34+ cells and an activation of lymphoid differentiated CD10+ cells amplification in contact with P7 MSCs. With other MSC preparations the variation was less important.
In presence with anti-human Jagged 1 Antibody, we observed a decrease of CD34+ cells amplification in contact with P4, P7 and P10 MSCs. We also observed a stimulation of lymphoid differentiated CD10+ cells amplification.

In vivo, NOD/SCID mice were transplanted with the expansion product of CD34+ cells co-cultured for one week with P2 MSCs and inhibitors. All mice transplanted had a significant percentage of human chimerism (> 0,5%). The treatment of co-culture with inhibitors of BMP, Shh and Wnt pathways didn't modify the repopulating activity. In contrast, inhibition of Notch pathway enhanced the repopulating activity in NOD/SCID mice. This result suggests that Notch pathway contribute in support of haematopoietic stem cells in contact with MSCs, and that this activity is essentially inhibitive.

In conclusion, this four signalling pathways play a role in pro-haematopoietic activity of MSCs, but their contribution is variable in function of haematopoietic lineage and expansion in vitro of MSCs. Our results indicate that Notch pathway has an activator effect on CD34+ cells amplification in vitro but inhibit the repopulating activity in vivo.