In vitro and *in vivo* Characterization of Adult Bone Marrow Neural Crest Stem Cells and their Implication in Hematopoietic Support

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Introduction

Human bone marrow is made of Hematopoietic Stem Cells (HSC) and stromal cells that are supporting hematopoiesis. Stroma compartment contains mesenchymal stem cells (MSC) and many other cell types constituting helpful microenvironment for hematopoiesis process. This helpful microenvironment is also called niche.

More specifically, Hematopoietic Stem Cell niches are defined as cellular and molecular microenvironments that regulate stem cell function together with stem cell autonomous mechanisms. Many different cell types have been characterized as contributors to the formation of HSC niches, however, several components remain unknown and contribution by as yet undefined cells have been speculated.

On the one hand, Méndez-Ferrer and collaborators strongly suggested that nestin-positive MSC are spatially associated with HSC and highly express several factors for HSC maintenance. In the last months, they also shown that these MSC are able to promote HSC proliferation.

On the other hand, according to Nagoshi *et al.*, and Wislet *et al.*, it has recently been demonstrated that, in mouse, nestin-positive MSC was a mixed population mainly composed of neural crest stem cells (NCSC).

In this context, it is tempting to ask if in human NCSC are also present in bone marrow stroma and if they are supporting HSC maintenance and proliferation by being part of HSC niches?

❖ **As the comparison between mouse and human is not as easy as we hoped we thought to use tissue control in order to better characterize our stromal population(s).**

In literature, it has been shown that there are MSC in adipose tissue and more interestingly that NCSC can be isolated from skin and more particularly form dermis or hair follicles.
Thanks to a collaboration with an aesthetic surgeon we obtained adipose tissue (AT) and dermis (SK) from abdominoplasties and we respectively isolated MSC and NCSC.

NCSC in human bone marrow ?

❖ Sphere-forming ability

In the lab, it has been shown that in mouse : between MSC and NCSC clonal population, only NCSC are able to grow as sphere in specific culture medium. We thus tried to obtain spheres from human stromal population since this characteristic is a good tool in order to isolate NCSC from MSC which don't present this property. Interestingly, a restricted number of human BMSC present this property.
Indeed, **0,05 ± 0,03%** of human cells are able to grow as spheres (with a diameter between 30 to 600 µm) (*figure 1*).
We thus performed spheres characterization using immunofluorescence and RT-PCR (*figure 2 and 3*).

❖ NCSC specific markers

The expression of NCSC specific markers was studied using RT-PCR and immunohistochemistry. In RT-PCR (*figure 3)* you can observe that MSC from adipose tissue (AT) don't express NCSC specific markers whereas MSC and NCSC from skin (SK) do. However, there are no difference between MSC in adherence or in sphere (MSC and MSC^{Sph}) except for *Pax6* and *Msi1* expression.
Based on immunohistochemistry results, (*figure 2)* Nestin and Tuj1 are expressed by all cells since first passages, Sox10 is negative and P75^{NTR} is only express by spheres from MSC and NCSC from skin. According to this results and based on this 4 markers we can hypothesized that MSC, as in mouse, is made of MSC and also NCSC.

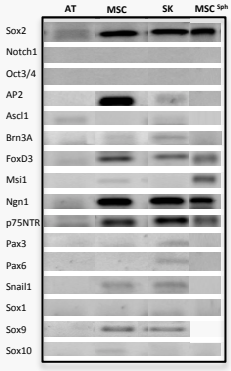

Fig. 3 RT-PCR for NCSC markers using bone marrow stromal cells (MSC), MSC from adipose tissue (AT), NCSC from skin (SK) and spheres from bone marrow stromal cells (MSC^{Sph}).

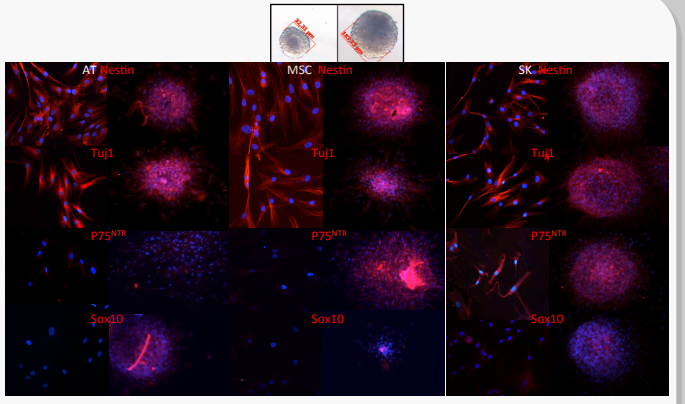

Fig. 1 and 2. Spheres present a diameter range between 30 and 300µm.
SK represents NCSC from skin and AT MSC from adipose tissue. Due to this picture you can conclude that:
*- **In adherence, MSC are closer to MSC from AT***
*- **In sphere population, MSC are closer to NCSC from SK than to MSC from AT (P75^{NTR} stainning)***

❖ Differentiation potential of NCSC

A third method used in order to characterize NCSC sub-population into bone marrow stroma was the study of differentiation potential into NCSC derived populations like Schwann cells. NCSC derived from skin AND stomal cells from bone marrow presented this ability whereas mesenchymal cells from adipose tissue not (*figure 4*).

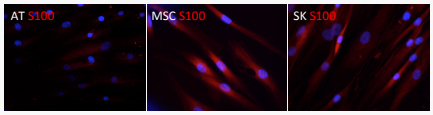

Fig. 4. Differentiation potential of NCSC from skin, MSC from adipose tissue and stromal cells into Schwann cells.

NCSC & MSC in hematopoiesis support

❖ MSC VS NCSC secretome study

In order to study NCSC and MSC implication in hematopoiesis we first of all performed a cytokine array.
We used 72h conditioned medium of our three cell types in adherence and sphere culture condition. After analysis these cytokines were selected (*figure 5*) as :

- The most secreted by adherent cells but not by spheres like Osteoprotegerin
- The most secreted by spheres but not by adherent cells like TIMP-2 or NAP-2
- The most secreted by only one cell type like IL-8 secreted by AT-MSC in both culture conditions but only by sphere for the other cell types.

These results are only preliminary results and are currently confirmed and quantified using ELISA test.

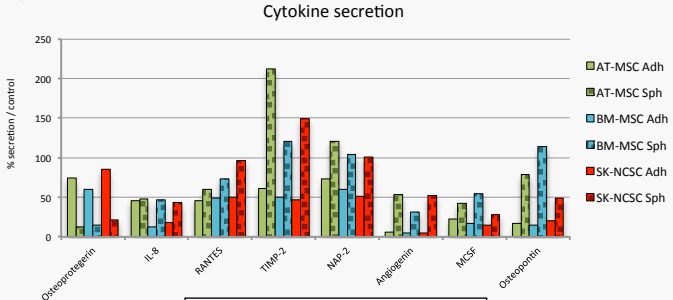

Fig 5. Selection of interesting cytokines after cytokine array.

❖ Co-culture development

A co-culture protocol is actually tested using MSC or NCSC and T-cells. We will analyse T-cell:

- Activation
- Proliferation
- Migration

We will then focus on HSC using similar techniques.
The aim of this part of the work is in the first time to explain co-culture results and observations thank to the cytokines identified in the cytokine array.

Perspectives

The last and final point in human bone marrow stromal cells characterization will be:

Engraftment into chick embryo (collaboration in Stockholm)

- Engraftment of 200 – 250 cells into 18HH stage embryo
- Sacrifice between 1 to 4 days after injection
- Identification of migrating cells using human cells marker

Concerning hematopoiesis support:

Preliminary results were just obtained and will be compare with co-culture observations.
We hope we will be able to link MSC or NCSC effects on T-cells or HSC with specific cytokines detected in the cytokine array.