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 HSC activity. 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?

Characterization of BMSC cultures

❖ MSC cell surface markers

According to the guidelines (Dominici *et al.*, 2007) more than 95% of cells are expressing surface molecules: CD105, CD73 and C90 whereas less than 2% are expressing hematopoietic markers like CD45, CD34, CD14, CD19 and HLA-DR.
FACS results presented in *figure 1* allowed us to confirm stromal and non hematopoietic identity for our populations.

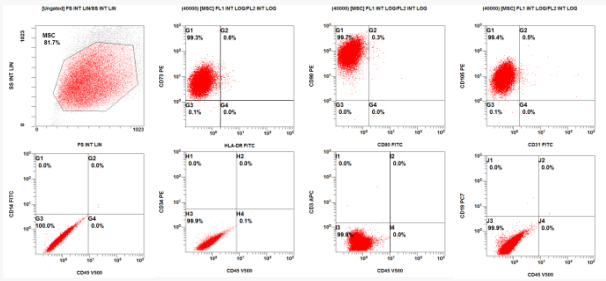

Fig. 1. FACS experiment in BMSC at low passage.
> 95% of cells are expressing CD105, CD73 and CD90
< 2% of cells are expressing hematopoietic factors: CD45, CD34, CD14, CD19 and HLA-DR

❖ Differentiation potential of human BMSC

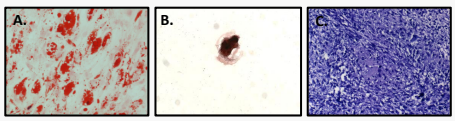

Fig. 2. Differentiation experiments in BMSC population.
A. Adipocytes (Oil Red-O)
B. Osteocytes (Alizarin Red)
C. Chondrocytes (Toluidine Blue)

We applied diverse protocols to induce human BMSC differentiation into adipocytes, osteocytes and chondrocytes. *Figure 2* illustrates their multipotentiality. Moreover, we performed melanocyte and Schwann cells differentiation from our populations. Indeed, it is known that MSC and NCSC can differentiate into adipocytes, osteocytes and chondrocytes but only NCSC can give rise to melanocytes and Schwann cells.

❖ **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 presents this property.
Indeed, **0,05 ± 0,03%** of human cells are able to grow as spheres (with a diameter between 30 to 300 µm) (*figure 3*).
We thus performed spheres characterization using immunofluorescence and RT-PCR (*figure 4 and 5*).

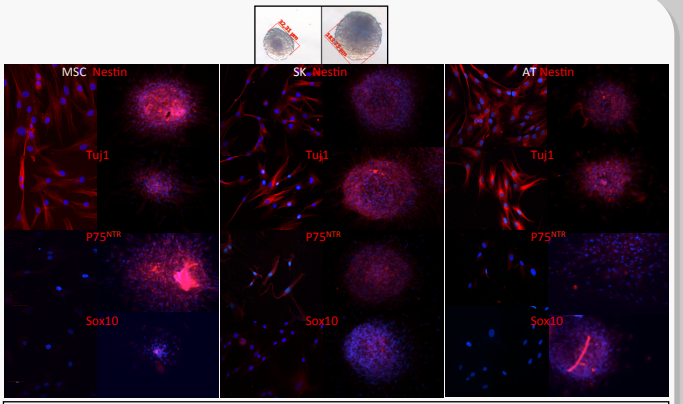

Fig. 3 and 4. Spheres present a diameter range between 30 and 300µm.
SK represents NCSC from skin and AT MSC from adipose tissue. Due to this picture you can conclude that:
*- **In adherence, MSC are closer to MSC from AT***
*- **In sphere population, MSC are closer to NCSC from SK than to MSC from AT (P75NTR stainning)***

❖ NCSC specific markers

The expression of NCSC specific markers was studied using RT-PCR and immunohistochemistry. In RT-PCR (*figure 5*) 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* expression.
Based on immunohistochemistry results, (*figure 4*) 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.

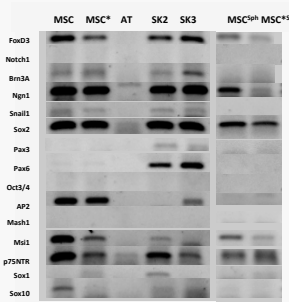

Fig. 5 RT-PCR for NCSC markers using MSC (MSC and MSC), MSC from adipose tissue (AT), NCSC from skin (SK2 and SK3) and spheres from MSC (MSC^{sph} and MSC*^{sph}).*

❖ 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 6*).

Fig. 6. Differentiation potential of NCSC from skin, MSC from adipose tissue and stromal cells into Schwann cells.

Perspectives

In order to deeper characterize BMSC population and the hypothesized sub-populations (MSC and NCSC) two main experiments could be performed:

1) **RNA seq**
   - RNA seq will be perform in our 3 populations (BMSC, AT and SK) and also in spheres from these populations in order to identify NCSC specific markers in human
2) **Engraftment into chick embryo**
   - Engraftment of 200 – 250 cells into 7-11 somites embryo (about 30h)
   - Sacrifice between 1 to 4 days after injection
   - Identification using human cell marker

Concerning hematopoietic support: protein array in order to study hematopoietic secreted factors and also co-culture will soon be carried out.