In vitro and *in vivo* Characterization of Adult Bone Marrow Neural Crest Stem Cells

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Introduction

Adult stem cells, due to their self-renewal ability and *in vitro* multilineage differentiation properties present an attractive autologous source of material for cell therapy in neurological disorders.
In 2008, Nagoshi and collaborators demonstrated that there are neural crest-derived stem cells (NCSC) into bone marrow stroma. According to this information, it can be interesting to use these cells (NCSC) presenting neural origin instead of mesenchymal stem cells (MSC) in cell therapy protocols for neurological disorders.
In order to transpose these procedures in humans, the first step is to determine if in human, as in mouse, bone marrow stromal cells are a mixed population containing inter alia MSC and NCSC.

Many strategies were developed to identify MSC and NCSC population from human adult bone marrow. First of all the general population was cultivated and sphere-forming ability based cell culture was tested. Using both approaches, we characterized those cells using several markers. Indeed, based on our previous results obtained from mouse cultures, NCSC were Nestin, Sox10 and $P75^{NTR}$-positives, while MSC were nestin and Sox10-negatives and expressed a low level of $P75^{NTR}$.
Surprisingly, we could not confirm those observations on in human cells, as all cells were nestin-positive and would express a low level of $P75^{NTR}$. As Sox10 could not be considered as specific marker for NCSC, we performed several RT-PCR comparisons to determine specific human NCSC/MSC markers.
We also cultivated MSC from adipose tissue and NCSC from dermis in order to obtain respectively good negative and positive control for the analysis of our bone marrow samples. Using these two types of cells, their differentiation potential, sphere forming ability and performing RT-PCR and microarray we hope to be able to identify and characterize NCSC population into human bone marrow stromal cells.

Once these steps will be performed, a confirmation step will be carried out using chicken embryo engraftment in order to follow the migration and the fate of injected cells. This experiment should validate the presence of NCSC in human bone marrow.

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% of cells are expressing hematopoietic markers like CD45, CD34, CD14, CD19 and HLA-DR.
FACS results presented in *figure 1* allowed us to confirm stromal or mesenchymal identity for our populations.

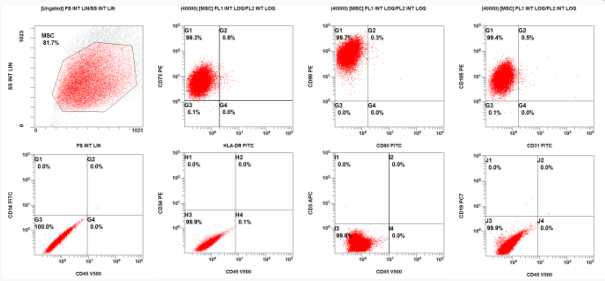
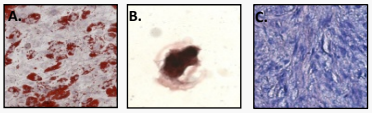


Fig. 1. FACS experiment in BMSC at low passage.
> 95% of cells are expressing CD105, CD73 and CD90
< 2% of cells are expressing hematopoietic factors: CD45, CD34, CD14, CD19 and HLA-DR

❖ Differentiation potential of human BMSC

Fig. 2. Differentiation experiments in BMSC population.
A. Adipocytes (Oil Red)
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 are performing melanocyte and Schwann cells differentiation into 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.

❖ Control tissues

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. 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 and dermis 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 between MSC and NCSC clonal population in mouse, only NCSC are able to grow as sphere in specific medium. We thus try to obtain sphere from human stromal population which 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 200 µm) (*figure 3*).
We thus carried out spheres' characterization using immunofluorescence and RT-PCR (*figure 4 and 5*).

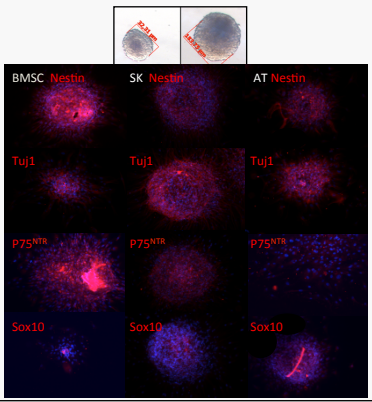


Fig. 3 and 4. NCSC spheres present a diameter range between 30 and 200µm.
They are positive for NCSC markers Nestin, Tuj1 and $p75^{NTR}$ but negative for Sox10.
SK represents NCSC from skin and AT MSC from adipose tissue. Due to this picture you can conclude that in sphere population BMSC are closer to NCSC from SK than to AT ($P75^{NTR}$ stainning).

❖ NCSC specific markers

NCC specific markers expression was studied using RT-PCR and immunohistochemistries. In RT-PCR (*figure 5*) you can observe that MSC from adipose tissue (AT) don't express NCSC specific markers whereas BMSC and NCSC from skin do. However, there are not significant differences between BMSC in adherence or in sphere (MSC and MSC^{sph}) except for *Pax6* expression.
In immunohistochemistry (*figure 6*) Nestin and Tuj1 are expressed by all cells since first passages Sox10 is negative and $P75^{NTR}$ is only express by spheres from BMSC and NCSC from skin. According to this results and based on this 4 markers we can hypothesized that BMSC, as in mouse, is made of MSC and also NCSC.

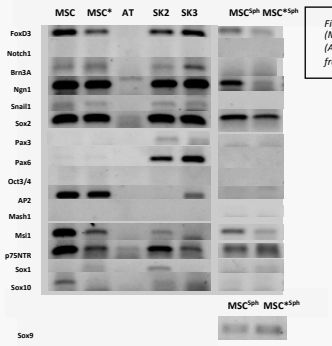


Fig. 5 RT-PCR for NCSC markers using BMSC (MSC and MSC), MSC from adipose tissue (AT), NCSC from skin (SK2 and SK3) and spheres from BMSC (MSC^{sph} and MSC^{*sph}).*

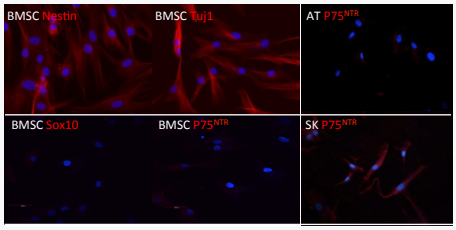


Fig. 6 Immunohistochemistry for BMSC using NCSC specific markers and also for AT and SK with $P75^{NTR}$. It can be notice that in adherent cells BMSC are closer to MSC from AT than SK.

Perspectives

In order to deeper characterize BMSC population and the hypothesize sub-populations (MSC and NCSC) three mains experiments could be performed :

1) **Functional characterization using differentiation potential**
   - Differentiation into melanocytes and Schwann cells
   - Usually, only NCSC are able to give rise to these to populations
2) **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
3) **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