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 2006, Nagy 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 humans, 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 NSC 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 these cells using several markers. Indeed, based on our previous results obtained from mouse cultures, NCSC were Nestin, Sox5, and P75NTR positive, while MSC were nestin and Sox5,2,3 negative and expressed a low level of P75NTR.

Surprisingly, we could not confirm those observations on human cell cultures, as all cells were nestin-positive and would express a low level of P75NTR. As Sox5,2,3 could not be considered as specific marker for NCSC, we performed several RT-PCR tests: it was pointed out that in order to obtain a correct differentiate and positive control for the analysis of our bone marrow samples. These two types of cells, their differentiation potential, sphere forming ability and performing RT-PCR and microwaves we were 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: CD305, CD17 and CD18 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.

Differentiation potential of human BMSC

We applied diverse protocols to induce human BMSC differentiation into adipocytes, osteocytes and chondrocytes. Figure 2 illustrates their multipotency. 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 MSC give rise to melanocytes and Schwann cells.

Control tissues

As the comparison between mouse and human is not so 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 cell 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, 0.25 ± 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 sphere’s characterization using immunofluorescence and RT-PCR (figure 4 and 5).

NCSC specific markers

NCSC specific markers expression was studied using RT-PCR and immunohistochemestries. 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 NCSC*) except for P75NTR expression. In immunohistochemistry (figure 6) Nestin and Tuj1 are expressed by all cells first passages Sox5,2,3 is negative and P75NTR is only express by spheres from BMSC and NCSC from skin. According to this results and based on these 4 markers we can hypothesized that BMSC, as in mouse, is made of MSC and also NCSC.

Perspectives

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

1) Functional characterization using differentiation potential
   - Differentiation into adipocytes, osteocytes, chondrocytes, melanocytes and Schwann cells
   - Nestin, Sox1,2,3, P75NTR, NCSC markers expression

2) RNA seq.
   - BMSC and NCSC are better characterized in vivo for their expression of specific markers.

3) Engraftment into chick embryos.
   - Development of BMSC, AT and NCSC into neural tissue (motor neurons)
   - Differentiation in 7 to 10 days after injection

It can be inferred that the isolated cells BMSC are closer to BMSC from AT than NCSC from skin.