**Introduction**

Adult neural crest stem cells (NCSC) are of extraordinary high plasticity and promising candidates for a use in regenerative medicine. Several locations like skin, dental pulp or bone marrow have been described in rodent. However, very few information is available concerning their correspondence in human tissues. The main objective of this project was then to isolate and characterize NCSC from adult human bone marrow.

In adult mouse, neural crest stem cells (NCSC) were identified in many tissues like dental pulp, cornea, skin, adipose tissue or even bone marrow stroma. These cells were characterized by Nagoshi et al., in 2008 thanks to a double transgenic mouse expressing β-galactosidase under the Wnt1 promoter. In our laboratory, we characterized two clonal populations of NCSC and mesenchymal stem cells (MSC) isolated from a similar mice model: Wnt1-Cre / R26R-LacZ. From these two populations, we observed that NCSC clones presented different properties compared to MSC clones: the expression of NCSC specific markers and the ability to grow as sphere in specific culture medium. Based on these results, we focused on the characterization of NCSC in different human tissues like bone marrow stroma, adipose tissue and dermis.

### 1. Human NCSC do not express the same markers than mouse NCSC.

In adult mice bone marrow, MSC were weakly positive for nestin and P75<sup>NT</sup> but negative for Sox10 while, NCSC were Nestin, P75<sup>NT</sup> and Sox10-positive. Moreover, we demonstrated that only NCSC had the ability to grow in suspension as spheres. In this study, we showed that all cell types were able to grow as spheres and were all Nestin and β3-tubulin-positive, but Sox10 negative. P75<sup>NT</sup> was the only differentially expressed marker, but we could not link this expression to NCSC origin.

### 2. Transcriptomic characterization of NCSC and other stem cell gene expression.

After the first characterization step by immunofluorescence we focused on NCSC and stem cell genes expression by the different cell populations in adherent and sphere culture condition.

It seems that the three cells populations present many similarities excepted for Ascl1, Pax3 and Pox6 expressed only by MSC from adipose tissue and NCSC from dermis; they are respectively involved in neural differentiation and autonomous nervous system development, the specification of neural plate border and finally on CNS development.

Sphere culture condition does not induce the expression of specific markers. We just observed a slight expression for AP2 and P75<sup>NT</sup>.

### 3. Differentiation potential into neural crest derived cells

Differentiation potential tests were performed on the three cells types to characterize their ability to differentiate into Schwann cells and Melanocytes. After a 5 to 8 week differentiation protocols, we obtained Schwann cells stained here with anti-S100β antibody and melanocytes stained with anti-Trp1 antibody. It is important to notice that the three cells types in adherence and sphere culture conditions were able to differentiate into Schwann cells and melanocytes.

### 4. Injection into chick embryos

Finally the last step to confirm NCSC presence into human bone marrow and adipose tissue was the injection into chick embryos. Indeed, it has been shown by Ledouarin that when NCSC were injected into thoraco-lumar region of stage HH18 chick embryo, they were able to migrate and followed chick NCSC migration pathways. Thus, 72h after injection NCSC can be localized into peripheral neural crest targets: the dorsal root ganglion (DRG), in the area of the boundary cap, in the dermal layer of the skin (a few cells observed close to the surface of the embryo corresponding to melanocytes migration territory) and finally in fiber tract leaving DRG.

On the left, pictures represent longitudinal sections and are illustrating cells migration; on the right pictures represent transversal sections of the interest territories. Human cells were labelled using anti-human nuclei antibody in red and chicken DRG and neural tube are stained with anti-β3-tubulin antibody in green.

### Conclusions

- **Proteins**
- **Genes**
- **Diff Potential**
- **Chick Injections**
- **NCSC**