

# Human Adult Bone Marrow and Adipose Tissue Harbor Stem Cells with Neural Crest Characteristics.

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## Introduction.

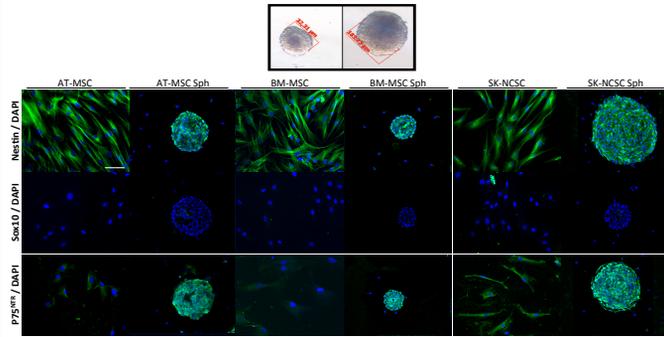
Adult neural crest stem cells (NCSC) are of extraordinary high plasticity and promising candidates for a use in regenerative medicine. Several NCSC locations have been described in rodent. However, very few information is available concerning their correspondence in human tissues. The main objective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

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  $\beta$ -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 those 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 same markers as mouse NCSC.

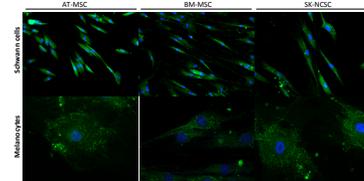
In adult mice bone marrow, MSC were weakly positive for Nestin and P75<sup>NTR</sup> but negative for Sox10 while, NCSC were Nestin, P75<sup>NTR</sup> and Sox10-positive. Moreover, we demonstrated that only NCSC had the ability to grow in suspension as spheres.

In this study, we showed that MSC from bone marrow, adipose tissue and NCSC from dermis were able to grow as spheres and were all Nestin-positives, but Sox10 negative. P75<sup>NTR</sup> was the only differentially expressed marker, but we could not link this expression to NCSC origin.



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

Differentiation potential experiments were performed on the three cells types to characterize their ability to differentiate into Schwann cells and Melanocytes (neural crest derived cells). After 5 to 8 weeks differentiation protocols, we obtained Schwann cells stained with anti-S100 $\beta$  antibody (some of them are also positive for MBP and P0) 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.



## 2. Transcriptomic characterization & proteomic validation.

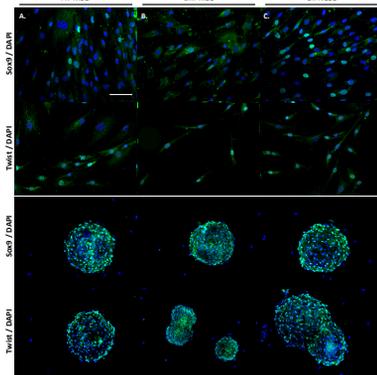
After a first characterization step by immunofluorescence we focused on neural crest and stem cell genes expression by our three different cell populations in adherent and sphere culture condition.

The three cells populations seem to present many similarities and adherent VS sphere comparison do not reveal big differences. Interestingly, we detected the expression of neural crest markers like *Slug*, *Sox9* and *Twist*.

According to these observation we decided to validate *Sox9* and *Twist* expression at protein level. For the first time we observed that in adherent culture condition **NOT ALL** cells are expressing these proteins whereas in spheres more than 80% of cells are!

This is for us a good information concerning sphere forming ability, indeed as expected spheres would really be an enriched population of neural crest cells. Moreover, *Sox9* and *Twist* should be good discriminant markers for neural crest identity.

	AT-MSC	BM-MSC	SK-NCSC	AT-MSC Sph	BM-MSC Sph	SK-NCSC Sph
AP2	-	+	-	+	+	+
Ascl1	+	-	-	+	+	+
Bmi3A	+	+	+	-	-	-
FoxD3	-	-	-	-	-	-
Msi1	-	-	+	-	-	+
Ngn1	-	-	-	-	-	-
Pax3	+	+	+	+	+	+
Pax6	+	+	+	+	+	+
Slug	+	+	+	+	+	+
Snail1	-	-	-	-	-	-
Sox2	+	+	+	+	+	+
Sox9	+	+	+	+	+	+
Twist	+	+	+	+	+	+

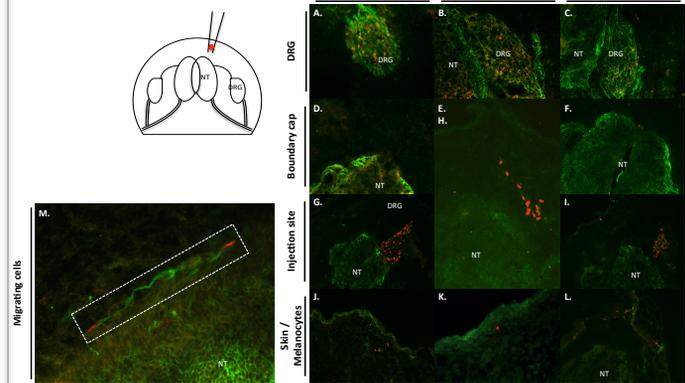


## 4. Injection into chick embryos.

The last step to confirm NCC 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-lumbar region of HHSt18 chick embryos, they were able to migrate and follow 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).

On the left, pictures represent longitudinal sections and are illustrating migrating cells. On the right, pictures represent transversal sections of interest territories.

Human cells were labelled using anti-human nestin antibody in red and chicken DRG and neural tube with anti- $\beta$ -3-tubulin antibody in green.



## Conclusion.

