In Vivo Identification and Regulating Niche of Mesenchymal Stem Cells

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Purposes

• Techniques for studying stem cells in vivo.
  ---Label retaining analysis
  ---Lineage tracing analysis
  ---Inducible genetic system (CreERT, tetO)
  ---Reporter strain (LacZ reporter, GFP reporter)

• Fundamental concepts for stem cell study
  niche, self-maintenance, quiescence, transit amplifying cells, multipotential, hedgehog signaling,

• How to design experiments and how to publish on high level journals.
• Part 1. The neurovascular bundle provides a niche for incisor MSCs
• Part 2. The suture provides a niche for MSCs of craniofacial bones.
Definition of MSCs

• “First, MSC must be plastic-adherent when maintained in standard culture conditions. “
• “Second, MSC must express CD105, CD73 and CD90, and lack expression of CD45, CD34, CD14 or CD11b, CD79alpha or CD19 and HLA-DR surface molecules. “
• “Third, MSC must differentiate to osteoblasts, adipocytes and chondroblasts in vitro.”

Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 2006 8(4) 315-317
Fundamental questions for MSCs

• What are the in vivo identity and physiological functions of MSCs?

• What is the regulating niche for the MSCs?
Hypothesis

• MSCs are regulated by various types of niches.
• Gli1 is a marker for MSCs of various mesenchymal organs.
• Classical MSC markers do not define the most primitive MSC population in vivo.
Part 1. The neurovascular bundle provides a niche for incisor MSCs

08/2011—10/2013
Mouse incisor is an excellent model for studying MSC

All mesenchymal cells in incisor turn over within a month
H2BGFP label retaining analysis based on the slow cycling property of stem cells

- **Wnt1-Cre; tTA\textsuperscript{flox/+}; tetO-H2BGFP**

```
W
Cre-mediated
Tissue-specific

T
Dox regulation
Tet-Off

H
Label retaining
reporter

CNC

Chase

Label retaining
cells

Dox +

1mo

Dox free

Dox(+)  chasing

WTH
mouse

E0.5  E8.5  NB  1month  2 month, harvest
```
Label retaining cells of the incisor mesenchyme surround the neurovascular bundle (NVB)

Wnt1-Cre; tTA flox; tetO H2BGFP
H2BGFP based label retaining analysis

αSMA — artery
CD31 — pan-vasculature
β3-tubulin — nerve
H2BGFP — label retaining cells
LRCs are negative for classical MSC markers
Gli1+ cells within the incisor are localized surrounding the NVB centered on the arteries and nerves

*Gli1-LacZ* mouse incisor
Gli1-LacZ mouse incisor

Incisor cross sections
**Gli1-CreERT;ZsGreen$$^{\text{flox}}$$ lineage tracing analysis**

- **24 hours**
- **1 week**
- **2 weeks**
- **4 weeks**

**Gli1-CE**

**ZsGreen**
Gli1+ cells are quiescent

- Gli1-LacZ; Wnt1-Cre; tTA^{flox}; tetO-H2BGFP chased for 1 month, then double staining with betaGal+H2BGFP

Almost all Gli1+ cells are LRCs, but not all LRCs are Gli1+
The nerve provides Shh
Denervation significantly reduces Gli1 activity
Denervation disrupts incisor mesenchyme homeostasis

One month after denervation
The neurovascular bundle centered around the nerves and arteries provides a niche for dental mesenchymal stem cells.
“ALL MSCs are Pericytes”
Majority of Gli1+ cells do not express classical MSC markers.

NG2+ cells express classical MSC markers and they are pericytes.
Incisor MSCs are typical MSCs in vitro
Gli1+ cells give rise to nearly the entire MSC population on the culture dish.
Molars lack Gli1+ cells but have NG2+ cells that contribute to repair.
Part 1. Summary

- Incisor MSCs are peri-arteriole cells regulated by accompanying sensory nerves.

- Pericytes labeled with classical MSC markers are an MSC sub-population derived from more primitive Gli1+ cells and function mainly in injury repair but not homeostasis.

- Mouse molars do not contain Gli1+ MSC population, but only NG2+ pericytes population.
Summary

• MSCs are regulated by various types of niches.
• Gli1 is a universal marker for MSCs of various mesenchymal organs.
• Classical MSC markers do not define the most primitive MSC population in vivo.
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Thank you