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Spotlight

The mechanism of bone repair: Stem cells in the periosteum dedicated to bridging a large gap

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Bone repair requires the mobilization of stem cells from outer periosteum and inner bone marrow. A study by Jeffery et al.[1] shows that periosteal stem cells are dedicated to repairing a large defect and regenerating both bone and marrow stroma.

Bone has an amazing capability for repair after injury to restore its structure and function. The process of bone repair is orchestrated by stem cells in bone, generally termed skeletal stem/progenitor cells (SSCs). These stem cells are mobilized from both the outer periosteum and the inner bone marrow in response to injury, differentiate into bone-making osteoblasts, and deposit mineralized matrix at the injury site. It has been recognized for many decades that different types of injuries are repaired by different mechanisms. Most small and mechanically stable fractures heal by intramembranous bone formation, the process by which the cells directly differentiate into bone-making osteoblasts. In contrast, large and unstable fractures heal by endochondral bone formation, the two-step process in which transient fibrocartilage is formed at the fracture site ("soft callus") that then turn into bone ("hard callus"). It remains largely unclear whether periosteal and bone marrow stromal cells (BMSCs) have distinct cell fates during bone repair.

The first insight into this important question came from a fate mapping study using a unicortical bone xenograft of LacZ reporter mice that was subsequently fractured.[2] This pioneering study demonstrated that cells in the periosteum become both chondrocytes and osteoblasts, while cells in the bone marrow predominantly become osteoblasts, sparking the interest in "periosteal stem cells." In subsequent years, several studies applied more sophisticated genetic fate-mapping/lineage-tracing approaches using cell-type-specific *cre* and inducible *creER* lines to analyze periosteal cell fates during bone repair. Among those identified were cells expressing alpha smooth muscle actin (*αSMA-creER*),[3] Prrx1 (*Prrx1-cre* and *Prrx1-creER*),[4] and cathepsin K (*Ctsk-cre*).[5] Coupled with transplantation experiments, these studies have collectively supported the concept that periosteal stem cells play important roles in bone repair due to their robust self-renewal and chondrogenic differentiation potential.

However, a major limitation of the prior research on periosteal stem cells is that these genetic markers often mark BMSCs as well. BMSCs marked by *Lepr-cre* represents a major source of osteoblasts in adult bone marrow.[6] LepR$^+$ cells also express CXCL12 (LepR$^+$CXCL12$^+$ cells) and are composed of pre-osteoblast-like and pre-adipocyte-like cells. A quiescent pre-adipocyte-like subset of CXCL12$^+$ cells can also participate in bone repair.[7] How periosteal and bone marrow SSCs contribute differentially to bone repair remains largely undefined due to overlapping expression patterns of genetic markers.

To address this critical gap in knowledge, Jeffery et al.[1] undertook a side-by-side fate mapping of several *cre* and *creER* lines/alleles that have been reported to mark periosteal and/or bone marrow SSCs. The authors tested as many as 11 lines under steady-state conditions and bone repair of adult bones. Many of the results reported here are worth close attention. The authors even acknowledge that *Lepr-cre*, which the authors previously used to mark adult bone marrow SSCs, also marked a sizable fraction of periosteal cells, therefore they could not use *Lepr-cre* to make a distinction of the two SSCs. The authors eventually identified *Gli1-creER* and *Adiponectin-cre* as reasonable lines to mark periosteal and bone marrow SSCs, respectively. The authors found that *Adiponectin-cre* marked almost the entire population of *Lepr-cre*-marked BMSCs, but not periosteal cells. Of note, BMSCs marked by *Adiponectin-cre*/*creER* have been previously reported as marrow adipogenic lineage precursors (MALPs).[8] In contrast, *Gli1-creER* marked 20% of periosteal cells both in inner cambium and outer fibrous layers but only 2% of BMSCs. From this, the authors concluded that *Gli1-creER* preferentially labels periosteal SSCs. Under steady-state conditions, adult cortical bone osteocytes arose primarily from BMSCs marked by *Adiponectin-cre*, but not from *Gli1-creER*-marked periosteal cells. Therefore, Gli1$^+$ periosteal cells have distinctive cell fates from those of Adipoq$^+$ BMSCs in normal conditions.

Subsequently, the authors applied two different modes of bone injuries—drill injury and bicortical fracture—to enlist periosteal cells and BMSCs. Drill injuries were primarily repaired by Adiponectin$^+$ BMSCs, while bicortical fractures were primarily repaired by Gli1$^+$ periosteal cells (Figure 1). Of note, Gli1$^+$ cells have been previously shown to produce chondrocytes and osteoblasts during fracture healing.[9] When Gli1$^+$ cells were selectively ablated by diphtheria toxin fragment

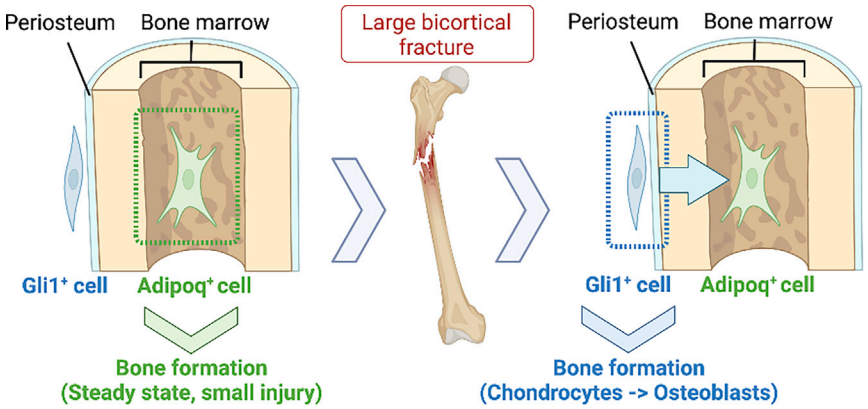

Figure 1. Gli1+ periosteal skeletal stem cells for repairing large bone fractures
Under steady-state conditions or small injuries, new bone is made by Adiponectin$^+$ cells in bone marrow. Gli1$^+$ periosteal cells are reserved for repairing extensive large bicortical fractures involving fibrocartilage and produce Adipoq$^+$ cells in bone marrow after healing. Created by BioRender.com.

A (DTA) expression or when canonical Wnt signaling was inactivated in Gli1$^+$ cells, fracture healing was delayed. Gli1$^+$ periosteal cells also regenerated bone and bone marrow stroma after healing of non-stabilized fractures. This is reminiscent of cells marked by *Hoxa11-creER* in the developing zeugopod, which give rise to LepR$^+$ cells in adult bone marrow.[10] These findings led the authors to conclude that different bone injuries are repaired by different types of SSCs.

The Jeffery study is insightful and opens a new chapter in skeletal stem cell biology by challenging the way we view the function of SSCs. However, many important questions remain to be answered by future studies. The first question is what is the identity of periosteal cells that contribute to adult cortical bone osteocytes under steady-state conditions. It is possible that another population of Gli1-negative periosteal SSCs are destined to become osteoblasts under steady-state conditions. Gli1$^+$ cells are likely to be not the sole type of periosteal SSCs. The second question is why Gl1$^+$ periosteal SSCs are normally quiescent and reserved for repairing a large and unstable defect. It is interesting to speculate that Gli1$^+$ SSCs represent reserve stem cells. The third question is what happens if the function of one SSC population is lost. It is possible that these two types of SSCs are interdependent. To answer these questions, even more sophisticated genetic approaches to simultaneously mark two different cell types (by *cre* and *dre,* for example) will be needed. Understanding the specific function of each subclass of SSC is fundamentally important to advance the field to decipher the mechanism of bone repair.

DECLARATION OF INTERESTS

The author declares no competing interests.

REFERENCES

1. Jeffery, E.C., Mann, T.L.A., Pool, J.A., Zhao, Z., and Morrison, S.J. (2022). Bone marrow and periosteal stem/progenitor cells make distinct contributions to bone maintenance and repair. Cell Stem Cell.
2. Colnot, C. (2009). Skeletal cell fate decisions within periosteum and bone marrow during bone regeneration. *J Bone Miner Res*. Feb *24*, 274–282. https://doi.org/10.1359/jbmr.081003.
3. Matthews, B.G., Grcevic, D., Wang, L., Hagiwara, Y., Roguljic, H., Joshi, P., Shin, D.G., Adams, D.J., and Kalajzic, I. (2014). Analysis of αSMA-labeled progenitor cell commitment identifies notch signaling as an important pathway in fracture healing. J. Bone Miner. Res. *29*, 1283–1294. https://doi.org/10.1002/jbmr.2140.
4. Duchamp de Lageneste, O., Julien, A., Abou-Khalil, R., Frangi, G., Carvalho, C., Cagnard, N., Cordier, C., Conway, S.J., and Colnot, C. (2018). Periosteum contains skeletal stem cells with high bone regenerative potential controlled by Periostin. *Nat Commun*. 02 *9*, 773. https://doi.org/10.1038/s41467-018-03124-z.
5. Debnath, S., Yallowitz, A.R., McCormick, J., Lalani, S., Zhang, T., Xu, R., Li, N., Liu, Y., Yang, Y.S., Eiseman, M., et al. (2018). Discovery of a periosteal stem cell mediating intramembranous bone formation. *Nature*. Oct *562*, 133–139. https://doi.org/10.1038/s41586-018-0554-8.
6. Zhou, B., Yue, R., Murphy, M., Peyer, J.G., and Morrison, S. (2014). Leptin-receptor-expressing mesenchymal stromal cells represent the main source of bone formed by adult bone marrow. Cell Stem Cell *15*, 154–168. https://doi.org/10.1016/j.stem.2014.06.008.
7. Matsushita, Y., Nagata, M., Kozloff, K.M., Welch, J.D., Mizuhashi, K., Tokavanich, N., Hallett, S.A., Link, D.C., Nagasawa, T., Ono, W., and Ono, N. (2020). A Wnt-mediated transformation of the bone marrow stromal cell identity orchestrates skeletal regeneration. *Nat Commun*. Jan *11*, 332. https://doi.org/10.1038/s41467-019-14029-w.
8. Zhong, L., Yao, L., Tower, R.J., Wei, Y., Miao, Z., Park, J., Shrestha, R., Wang, L., Yu, W., Holdreith, N., et al. (2020). Single cell transcriptomics identifies a unique adipose lineage cell population that regulates bone marrow environment. *Elife*. Apr *9*, e54695. https://doi.org/10.7554/eLife.54695.
9. Shi, Y., He, G., Lee, W.C., McKenzie, J.A., Silva, M.J., and Long, F. (2017). Gli1 identifies osteogenic progenitors for bone formation and fracture repair. Nat. Commun. *12*. 8:2043. https://doi.org/10.1038/s41467-017-02171-2.
10. Pineault, K.M., Song, J.Y., Kozloff, K.M., Lucas, D., and Wellik, D.M. (2019). Hox11 expressing regional skeletal stem cells are progenitors for osteoblasts, chondrocytes and adipocytes throughout life. *Nat Commun*. Jul *10*, 3168. https://doi.org/10.1038/s41467-019-11100-4.