RESEARCH HIGHLIGHT

Shaping the sinuses: a novel *Krt14*$^+$*Ctsk*$^+$ cell lineage driving regenerative bone formation

Seoyeon Bok[1] and Matthew B. Greenblatt[1,2]✉

Cell Research (2022) 32:791–792; https://doi.org/10.1038/s41422-022-00694-y

The specific skeletal cell types involved in bone formation around the sinus remain unclear. In a recent paper published in *Cell Research*, Weng et al. identify a novel lineage with mixed epithelial and osteoblast features that mediates bone formation in regenerative procedures involving the maxillary sinus.

The past few years have seen a revolution in defining the cell types forming the skeleton. The field has moved from defining cells based on morphology towards a combination of using cre-based genetic markers to label specific lineages and cell surface markers to define discrete cell types within those lineages. This work has led not only to increasingly precise definitions of skeletal stem cells,[1–3] but moreover to an understanding that bone is not formed by a single stem/progenitor cell, but rather through the combined actions of several distinct lineages of stem and progenitor cell types, each with distinct anatomic location and physiologic function.[2,4–6]

Weng et al.[7] have identified a lineage of cells marked by both the epithelial marker keratin 14 (*Krt14*) and a marker associated with periosteal skeletal stem cells,[5] cathepsin K (*Ctsk*) that reside in the Schneiderian membrane, an epithelial layer lining the maxillary sinus. The investigators were interested in the cellular basis of bone formation in maxillary sinus floor lifting (MSFL, also termed maxillary sinus floor augmentation), a procedure used to augment the thickness of the posterior maxillary bone to facilitate integration of dental implants.[8] In this procedure, elevation of the Schneiderian membrane off of the underlying maxillary bone triggers new bone formation. After developing a mouse model of this procedure, single-cell RNA-sequencing was used to characterize the cell types formed in response, revealing the presence of a cell population expressing an unusual combination of osteoblast lineage and epithelial markers, *Krt14*$^+$*Ctsk*$^+$ lineage cells. Genetic deletion of these cells using diphtheria toxin-triggered killing of *Ctsk*-expressing cells impaired mineralization in the MSFL model, identifying this lineage as a source of the osteoblasts mediating bone formation in this setting (Fig. 1).

Not only do the *Krt14*$^+$*Ctsk*$^+$ lineage cells identified here display shared markers with a recently described *CTSK*-lineage stem cells present in the outer periosteal layer covering long bones, but these two populations also apparently share conserved functional characteristics. Both populations appear to mediate intramembranous bone formation, perhaps indicating that *CTSK* expression is a convergent feature of populations mediating intramembranous bone formation. Additionally, the ability of *Krt14*$^+$*Ctsk*$^+$ lineage cells to undergo osteoblast differentiation and facilitate new bone formation after being lifted off their underlying bone substrate in MSFL has interesting parallels to the biology of the periosteal membrane covering the outer surface of long bones, where lifting of the periosteum off of the bone surface triggers similar bone formation. Clinically, this is best known through the radiographic observance of so-called "Codman triangle", a triangular area of newly formed subperiosteal bone created in response to a tumor protruding through the bone into the surrounding tissue and thereby lifting the periosteum off of the underlying bone.[9] The similarity of this phenomenon to the MSFL procedure argues that both take advantage of a similar osteogenic reaction triggered by loss of bone adherence and invites determination of an underlying shared mechanism. The existence of human disorders such as Pyle's disease characterized by defects in the outer cortex of long bones indicating defective periosteal bone formation together with sinus defects[10] provides tantalizing phenotypic links between bone formation at these sites and suggests a human relevance for the parallels between *CTSK*-lineage periosteal stem cells in long bones and *Krt14*$^+$*Ctsk*$^+$ lineage cells. However, further study of the cellular basis for these phenotypes is needed.

These findings point to several important areas for subsequent investigation. First, the *Krt14*$^+$*Ctsk*$^+$ lineage cells studied here are defined primarily in terms of lineage markers driven by *Krt14* and *Ctsk* gene regulatory elements. Thus, the cells under study here represent a lineage of cells, and the distinct cell types comprising that lineage have yet to be fully determined, though the single-cell RNA-sequencing performed provides initial insights into this question. Additionally, much remains to be learned about the physiologic importance of these *Krt14*$^+$*Ctsk*$^+$ lineage cells, including their role in the developmental formation and subsequent maintenance of the bone surrounding the sinuses.

The combined epithelial and skeletal features of this lineage are also especially noteworthy for defying established cellular classifications. Accordingly, it will be of great interest to determine whether there is a dichotomy between epithelial and osteoblastic fates within this lineage and where in the differentiation hierarchy this fate decision occurs. The co-expression of epithelial and osteoblast lineage markers, which are normally mutually exclusive, also raises fundamental questions about the transcriptional basis for specifying these *Krt14*$^+$*Ctsk*$^+$ lineage cells and whether epithelial structures serve as sources for osteoblasts in other

[1]Department of Pathology and Laboratory Medicine, Weill Cornell Medicine, New York, NY, USA. [2]Research Division, Hospital for Special Surgery, New York, NY, USA.
✉email: mag3003@med.cornell.edu

Published online: 14 July 2022

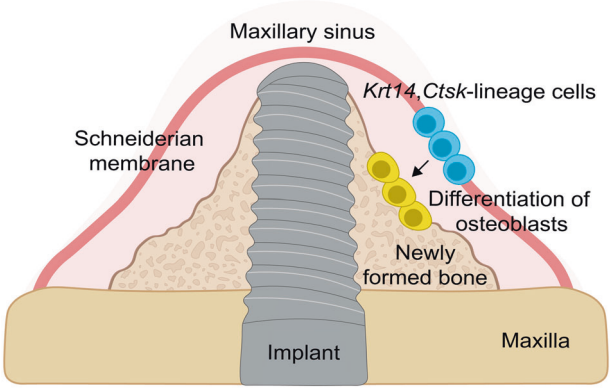

Fig. 1 *Krt14*$^{+}$*Ctsk*$^{+}$ lineage cells contribute to de novo bone formation in the MSFL model. Weng et al. identified a cell population expressing a combination of epithelial and osteoblast lineage markers, *Krt14*$^{+}$*Ctsk*$^{+}$ lineage cells, in the Schneiderian membrane. When MSFL is induced, this lineage differentiates into osteoblasts, which in turn mediates new bone formation. Krt14, keratin 14; Ctsk, cathepsin K; MSFL, maxillary sinus floor lifting.

contexts. In older literature, transplantation of transitional epithelium from bladder or other sites to an intramuscular site was associated with induction of osteogenesis; however, this finding should be revisited with modern tools to clarify whether the bone formed is truly graft derived or induced from local host cells.[11] Taken together, this work furthers the model that bone is formed by a diverse set of cells that are specialized for their local anatomic niche, specifically identifying a cell with features of both epithelial cells and *CTSK*-lineage periosteal stem cells mediating regenerative bone formation around the maxillary sinus.

REFERENCES

1. Chan, C. K. F. et al. *Cell* **160**, 285–298 (2015).
2. Mizuhashi, K. et al. *Nature* **563**, 254–258 (2018).
3. Chan, C. K. F. et al. *Cell* **175**, 43–56.e21 (2018).
4. Ambrosi, T. H. et al. *Elife* **10**, e66063 (2021).
5. Debnath, S. et al. *Nature* **562**, 133–139 (2018).
6. Shen, B. et al. *Nature* **591**, 438–444 (2021).
7. Weng, Y. et al. *Cell Res.* https://doi.org/10.1038/s41422-022-00687-x (2022).
8. Starch-Jensen, T. & Jensen, J. D. *J. Oral Maxillofac. Res.* **8**, e3 (2017).
9. McDonald, J. & DenOtter, T. D. In: StatPearls. Treasure Island: StatPearls Publishing (2022).
10. Kiper, P. O. S. et al. *N. Engl. J. Med.* **374**, 2553–2562 (2016).
11. Huggins, C. *Proc. Am. Philos. Soc.* **113**, 458–463 (1969).

ADDITIONAL INFORMATION

Correspondence and requests for materials should be addressed to Matthew B. Greenblatt.