Published in final edited form as:

Plast Reconstr Surg. 2019 September ; 144(3): 656–657. doi:10.1097/PRS.0000000000005914.

Discussion: Adipose-Derived Stem Cells and Ceiling Culture-Derived Preadipocytes Cultured from Subcutaneous Fat Tissue Differ in Their Epigenetic Characteristics and Osteogenic Potential

Mimi R. Borrelli, M.B.B.S., M.Sc., Michael T. Longaker, M.D., M.B.A., Derrick C. Wan, M.D.

Hagey Laboratory for Pediatric Regenerative Medicine, Department of Surgery, Division of Plastic Surgery, and the Stanford Institute for Stem Cell Biology and Regenerative Medicine, Stanford University School of Medicine.

Sasahara et al.¹ explore the heterogeneity in progenitor cells derived from adipose tissue in terms of osteogenic potential and epigenetic regulation. Adipose-tissue is a rich source of multipotent cells with powerful immunomodulatory, regeneration-promoting, antifibrotic, antiapoptotic, and proangiogenic properties. Recent work has revealed the existence of multiple subpopulations of progenitor cells in adipose tissue with distinct functional characteristics; adipose-derived stromal cells expressing bone morphogenetic protein receptor-1A have enhanced capacity for adipogenesis,² whereas CD248⁺ adipose-derived stromal cells have augmented angiogenic potential,³ and adipose-derived stromal cells negative for CD105 (endoglin) have enhanced osteogenic capacity.⁴ Increased expression of CD90 (Thy1) or bone morphogenetic protein receptor-1B has also been found to identify pro-osteogenic adipose-derived stromal cell subpopulations.^{5,6} In this article, the authors compared adipose-derived stromal cells to the less explored but more homogenous ceiling culture-derived preadipocyte population. Unlike adipose-derived stromal cells, which are obtained from the stromal vascular fraction of adipose tissue in the sediment of centrifuged collagenase-digested fat,⁷ ceiling culture-derived preadipocytes are the collection of mature adipocytes found floating after centrifugation that are subsequently propagated by ceiling culture.⁸ The ceiling culture-derived preadipocyte population has been reported to have greater adipogenic potential than adipose-derived stromal cells,⁹ but the osteogenic potential of ceiling culture-derived preadipocytes compared with adipose-derived stromal cells has not previously been explored. The authors show for the first time that the osteogenic capacity of ceiling culture-derived preadipocytes exceeds that of adipose-derived stromal cells.

Defining the identity and roles of these various progenitor subpopulations is extremely informative from a clinical perspective, as it can help in the development of cell-based therapies targeted toward specific outcomes. Reconstruction of the human skeleton is limited by the availability of autologous bone grafts, the risks associated with cadaveric or

Derrick C. Wan, M.D., Department of Surgery, Stanford University Medical Center, 257 Campus Drive, Stanford, Calif. 94305, dwan@stanford.edu, Twitter: @mimiborrelli.

Disclosure: None of the authors has a financial interest to declare in relation to the content of this Discussion or of the associated article.

xenogenic materials, and the robustness of prosthetic/synthetic alternatives. Consequently, deficiencies of bone following trauma or infection (or because of congenital absence) result in an enormous reconstructive challenge.¹⁰ Although it has long been known that stem and progenitor cells derived from adipose tissue have the potential to form bone, discovery of cell subpopulations with enhanced osteogenic potential can significantly help to address this clinical need.

The exact mechanisms responsible for guiding lineage-specific differences within multipotent cells remain unknown. The authors uniquely compare the epigenetic landscapes of adipose-derived stromal cells and ceiling culture-derived preadipocytes to better understand their contrasting osteogenic capabilities and to identify the precise mechanisms that direct these multipotent cells toward skeletal lineages. Epigenetic markers are the structural adaptation of chromo-somal regions to register, signal, or perpetuate altered functional states. In addition to regulating transcriptional programs, the epigenome also coordinates lineage differentiation.^{11,12} There are at least three fundamental mechanisms by which epigenetic control is implemented: DNA methylation, posttranslational modification of histone tails, and the various roles played by noncoding RNAs. In the article by Sasahara et al., the DNA methylation pattern was shown to potentially contribute to enhanced osteogenic capacity of adipose tissue progenitors. Furthermore, they identified enhanced trimethylation of histone H3 at lysine 4 at the *RUNX2* P2 promoter of ceiling culture-derived preadipocytes, which is commonly associated with gene activation. Interestingly, we have also recently demonstrated that long noncoding RNAs may play a role in the regulation of adipose-derived stromal cell differentiation,¹² and emerging studies have shown direct interaction of various long noncoding RNAs with adaptor proteins such as WDR5, which can drive trimethylation of histone H3 at lysine 4.¹³

Epigenetic mechanisms are reversible and are therefore attractive targets for molecular treatments. Thus, defining the epigenome of adipose progenitors with enhanced osteogenic capabilities may reveal new ways of manipulating regulator pathways of these candidate progenitor cells to direct stem cell programming and promote osteogenesis. “Epidrugs” are drugs that act on epigenetic modifiers to influence gene expression and activate differentiation pathways¹⁴ and have already shown promise in cancer treatments. Numerous groups have created epigenetic editing platforms to induce a wide variety of changes, including DNA methylation and demethylation and histone posttranslational modifications. The results presented by Sasahara et al. reveal epigenetic landscapes that may promote osteogenesis in adipose tissue progenitors, and this is a first step toward producing epidrugs able to enhance this process for therapeutic benefit.

In summary, the authors take a novel approach toward identifying and defining the subpopulations of progenitor cells in adipose tissue with enhanced osteogenic capacity. This work has important clinical implications, as adipose tissue progenitors can be easily harvested in abundance with minimal donor-site morbidity. Understanding which subfractions of cells in adipose tissue are most useful for skeletal reconstruction holds huge promise for clinical medicine. Further investigations could compare this ceiling culture-derived preadipocyte population with the subpopulations of adipose-derived stromal cells with enhanced osteogenic potential (e.g., CD105⁺ adipose-derived stromal cells), to

ascertain whether adipose-derived stromal cell subpopulations have similar epigenetic profiles conducive to osteogenesis.

REFERENCES

1. Sasahara Y, Kubota Y, Kosaka K, et al. Adipose-derived stem cells and ceiling culture-derived preadipocytes cultured from subcutaneous fat tissue differ in their epigenetic characteristics and osteogenic potential. *Plast Reconstr Surg*. 2019;144:644–655. [PubMed: 31461020]
2. Zielins ER, Paik K, Ransom RC, et al. Enrichment of adipose-derived stromal cells for BMPRII facilitates enhanced adipogenesis. *Tissue Eng Part A* 2016;22:214–221. [PubMed: 26585335]
3. Brett E, Zielins ER, Chin M, et al. Isolation of CD248-expressing stromal vascular fraction for targeted improvement of wound healing. *Wound Repair Regen*. 2017;25:414–422. [PubMed: 28464475]
4. Levi B, Wan DC, Glotzbach JP, et al. CD105 protein depletion enhances human adipose-derived stromal cell osteogenesis through reduction of transforming growth factor β 1 (TGF- β 1) signaling. *J Biol Chem*. 2011;286:39497–39509. [PubMed: 21949130]
5. McArdle A, Chung MT, Paik KJ, et al. Positive selection for bone morphogenetic protein receptor type-IB promotes differentiation and specification of human adipose-derived stromal cells toward an osteogenic lineage. *Tissue Eng Part A* 2014;20:3031–3040. [PubMed: 24854876]
6. Chung MT, Liu C, Hyun JS, et al. CD90 (Thy-1)-positive selection enhances osteogenic capacity of human adipose-derived stromal cells. *Tissue Eng Part A* 2013;19:989–997. [PubMed: 23216074]
7. Zuk PA, Zhu M, Mizuno H, et al. Multilineage cells from human adipose tissue: Implications for cell-based therapies. *Tissue Eng*. 2001;7:211–228. [PubMed: 11304456]
8. Sugihara H, Yonemitsu N, Miyabara S, Toda S. Proliferation of unilocular fat cells in the primary culture. *J Lipid Res*. 1987;28:1038–1045. [PubMed: 3655558]
9. Asada S, Kuroda M, Aoyagi Y, et al. Ceiling culture-derived proliferative adipocytes retain high adipogenic potential suitable for use as a vehicle for gene transduction therapy. *Am J Physiol Cell Physiol*. 2011;301:C181–C185. [PubMed: 21471463]
10. Nalliah RP, Allareddy V, Kim MK, Venugopalan SR, Gajendrareddy P, Allareddy V. Economics of facial fracture reductions in the United States over 12 months. *Dent Traumatol*. 2013;29:115–120. [PubMed: 22487364]
11. Bird A Perceptions of epigenetics. *Nature* 2007;447:396–398. [PubMed: 17522671]
12. Luan A, Paik KJ, Li J, et al. RNA sequencing for identification of differentially expressed noncoding transcripts during adipogenic differentiation of adipose derived stromal cells. *Plast Reconstr Surg*. 2015;136:752–763. [PubMed: 26090763]
13. Wang KC, Yang YW, Liu B, et al. A long noncoding RNA maintains active chromatin to coordinate homeotic gene expression. *Nature* 2011;472:120–124. [PubMed: 21423168]
14. Ivanov M, Barragan I, Ingelman-Sundberg M. Epigenetic mechanisms of importance for drug treatment. *Trends Pharmacol Sci*. 2014;35:384–396. [PubMed: 24993164]