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A New Osteocytic Cell Line, Raising New Questions and Opportunities

Ivo Kalajzic, MD, PhD

Reconstructive Sciences, School of Dental Medicine, UConn Health, Farmington, CT, USA

Bone biology has experienced an "osteocyte-rich" decade. In the process of discovering new targets for improving bone health, and following a "we should think better" strategy on innovating and developing new experimental tools, research on osteocyte biology has seen a dramatic expansion. New discoveries have allowed tremendous inroads in our understanding of how bone tissue is organized and how osteocytes regulate osteoblastogenesis, osteoblast function, osteoclastogenesis, bone turnover, and metabolism.

Studies on osteocytes are performed using in vitro and in vivo approaches, often by overexpressing or deleting target genes.(1) Importance has been given to in vivo studies because they provide direct evidence for the relevance on how genes affect biological processes in bone as an organ. The most commonly used in vivo models are based on the dentin matrix protein-1 (DMP1) promoter to direct expression of fluorescent proteins or Cre recombinase in a population of cells at preosteocyte and osteocyte stage.(2–4) Use of these transgenic mice have produced a large amount of data on osteocyte function, and have already been utilized to generate osteocytic cell lines. However, DMP1-driven Cre recombinase has been detected not only in osteocytes, but in late osteoblasts (cells on bone surfaces), as well as in some cells of other tissues and organs.(5–8) Despite this broader than expected expression, DMP1Cre and DMP1CreERT2 lines remain the most effective tool available to target late osteoblasts and osteocytes. However, these observations outline the need for developing specific ways to target osteocytes more selectively, but also to use novel cell lines in which an osteocyte phenotype would be easy to identify.

Development of osteocyte-like cell lines has propelled investigation of osteocyte biology. Until recently, the most widely used cell lines were MLO-Y4 and MLO-A5 generated by the Bonewald group, and the HOB-01-C1, a human preosteocyte-like cell line.(9–12) More recently, two additional lines were generated from cells derived from transgenic mice in which the DMP1 promoter drives green fluorescent protein (DMP1-GFP) in preosteocytes/osteocytes.(2,13,14) The IDG-SW3 and Ocy454 cell lines were made using crosses of DMP1-GFP mice with Immortomouse, in which an IFN-γ–inducible promoter drives expression of thermolabile large T antigen, allowing for conditional immortalization of cells derived as outgrowths from bone chips. In these models, cells are expanded at 33°C in the presence of IFN-γ and then allowed to acquire their original phenotype at 37°C in the absence of IFN-γ. The cells are DMP1-GFP-negative and T-antigen-positive under immortalizing conditions, but are DMP1-GFP-positive and T-antigen-negative under osteogenic conditions. These cell lines are particularly useful as they express Sost/Sclerostin and are responsive to PTH. They have been widely used and proven valuable to understand osteocyte biology.

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The major expectation from these cell lines is that they develop osteocytic-like cells within a mature bone matrix, form lacunocanalicular structures, and allow 3D organization of osteocytes. In this issue of the *Journal of Bone and Mineral Research* (*JBMR*), Wang and colleagues[15] report a novel cell line that meets most of these morphological criteria. Two novel cell lines were derived from osteoblast-rich calvarial digests from mice, using membrane-bound GFP (AcGFP-mem; Clontech) driven by the DMP1 promoter as selection mechanism. From more than 100 single-cell clones, two lines were established based on the expression of GFP and ability to mineralize (OmGFP66 and OmGFP10). Similar to other osteocyte-like cell lines, these cells proliferate and express alkaline phosphatase and *Col1a1* before undergoing mineralization, suggesting that these cells are transformed at their early stage of osteogenic differentiation. A rather unique feature of the OmGFP66 line is the ability to form 3D mineralized structures resembling features of cortical bone with highly organized lacunae and dendritic connections. Besides morphological similarity with osteocytes, once OmGFP66 cells express DMP1-GFP, they also express *Phex*, *Mepe*, *Fgf23*, and *Gja1*, while at a later differentiation point Sost/sclerostin is produced. They also express *Rankl* and *Opg*, *Hif1a*, as well as osteoblast genes, and are responsive to PTH by downregulating expression of Sost and Dmp-1. Thus, this cell line progresses through stages of osteogenic differentiation in a similar way as do primary cultures of calvarial osteoblasts. They also share the heterogeneity of calvarial cell populations. Indeed, only a portion of cells in the differentiated cultures form mineralized structures. Although this is expected, such heterogeneity complicates interpretation of results from whole-cell RNA and protein extracts. Using enzymatic digestion of monolayered cells from differentiated areas, the authors clearly show that only cells embedded within mineralized structures express a characteristic osteocyte gene profile. The other cell line, OmGFP10, exhibits less organized mineralized areas that are similar to the pattern of mineralization seen in previously developed lines, Ocy454 and IDG-SW3.

In contrast to Oc454 and IDG-SW3 where Immortomouse was bred into the DMP1-GFP mice making every cell suitable for immortalization, to generate OmGFP66, calvaria derived cells were in vitro transfected with pSV3neo plasmid to introduce SV40 T-antigen. One point that is not addressed in the article is why the organized mineralization pattern of bone-like structures is seen in just one line out of hundreds of clones. One reason may be insertion of the SV40 T-antigen in a specific gene locus critical for osteogenic differentiation. Another explanation may be due to the stage of differentiation at which the cells were transformed by the SV40 T-antigen. Cell clones can arise from a transformed osteocyte, a mature osteoblast, or even a progenitor cell present within the calvarial bone.[8] Isolated and in vitro expanded cells are very heterogeneous and after immortalization a number of clones will be able to mineralize and express DMP1-GFP. However, this does not make them osteocytes by their origin. It is therefore possible that various populations of progenitors are targeted by transformation, and in the case of the OmGFP66 a progenitor cell that had been destined to form functional bone-like structures and exhibit osteocyte phenotype was targeted. Indeed, these new cell lines expand very well in culture, and this is not a characteristic of fully formed osteocytes. It would be interesting to identify which cells among those residing in the calvarium have the potential of differentiating into osteocytes and generating bone-like structures.

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Despite these limitations, there are several advantages to this cell line. The most important one is the ability to identify cells that reach the osteocyte stage by DMP1-GFP expression. In addition, this new cell line provides a valuable 3D model for studying dendrite formation, osteocyte interconnectivity, and live imaging of cell-to-cell interactions.

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