Published in final edited form as:

Bone. 2007 June ; 40(6): 1671–1674.

Letter re: “The 3.6 kb DNA fragment from the rat Col1a1 gene promoter drives the expression of genes in both osteoblast and osteoclast lineage cells” by Boban et al. (*Bone* 39:1302–1312, 2006)

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Letter to the Editor

Dear Editor:

In the paper by Boban and colleagues [1], the authors used a parabiosis mouse model to test whether osteoblast-lineage cells traversed the circulation of the parabiosed pair. Using mice expressing GFP driven by the col3.6 promoter, they found that there were GFP-positive cells lining bone surfaces in the non-GFP-expressing parabiosed mouse, indicating extensive transfer of GFP-positive cells. In addition, when they infused Lin[−] Sca-1⁺ c-kit⁺ cells from a col3.6-GFP mouse into a non-GFP-expressing mouse, these cells also appeared on bone surfaces as GFP-positive lining cells. However, based on immunohistochemistry for TRAP and *in vitro* bone marrow cultures, the investigators concluded that the GFP-positive cells lining bone surfaces in the two experiments described above were osteoclasts (or osteoclast-lineage cells), and that there was no transfer of osteoblast-lineage cells between the parabiotic pairs or differentiation of the Lin[−] Sca-1⁺ c-kit⁺ cells into osteoblastic cells.

There are some concerns with the interpretation of the findings, and the authors are perhaps overlooking another important cell type, the bone lining cell, which is likely closely related to the osteoblast, but probably serves a very distinct functional role [2]. The alternate explanation for the findings in this paper are that the GFP-positive cells that are lining the bone surfaces in the parabiosis model and in the mice infused with the Lin[−] Sca-1⁺ c-kit⁺ cells are, in fact, bone lining cells. These cells lie in close proximity to osteoclasts [2], express bone-related proteins such as alkaline phosphatase, low levels of osteocalcin and type I collagen (i.e., they are not highly active in synthesizing matrix as are the osteoblasts on bone surfaces), and are also positive for expression of intercellular adhesion molecule-1 (ICAM-1) [2,3], which appears to be necessary for binding to osteoclast precursors and the subsequent support of osteoclastogenesis [4]. By contrast, matrix-synthesizing osteoblasts express high levels of type I collagen and are ICAM-1 negative [2]. Moreover, since bone lining cells lie adherent to bone surfaces, they are unlikely to be present in the bone marrow cultures used in the paper to support the argument that osteoblastic cells did not transfer in the parabiotic mice.

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The first point in support of the alternate hypothesis that at least a substantial subset of the GFP-positive cells on bone surfaces in the parabiosis model used by Boban et al. [1] are osteoblast lineage cells is the observation in the paper that while treatment of a non-parabiosed col2.3ΔTK mouse with ganciclovir led to dramatic bone loss, this apparently did not occur in the parabiosis setting. Although these findings are stated only in passing in the results, and no data regarding this potentially very important observation is presented in the paper, it is difficult to envision that transfer of osteoclasts (or osteoclast lineage cells) alone would be sufficient to prevent bone loss following ablation of osteoblasts and bone lining cells.

A second point indicating that the GFP-positive cells in the parabiosed mouse and the mouse infused with Lin⁻ Sca-1⁺ c-kit⁺ cells are bone lining cells is the level of GFP expression by these cells. As shown in Figure 6 of the paper, TRAP-positive osteoclasts are very faintly GFP-positive. By contrast, the GFP-positive lining cells in the non-GFP expressing parabiotic mouse in Figure 1B and the bone lining cells in the mouse receiving the Lin⁻ Sca-1⁺ c-kit⁺ cells from the col3.6-GFP mouse in Figure 4B are robustly GFP-positive, suggesting that these cells are not osteoclasts.

The final issue is the use of TRAP staining as the sole method of identifying osteoclast lineage cells. Thus, there is now a fairly extensive literature documenting expression and staining of osteoblasts/osteocytes/bone lining cells for TRAP [5–9]. Indeed, osteoblastic cells (MSCs) analyzed in Figure 7 of the current paper clearly express the TRAP mRNA. Moreover, cells in close proximity to osteoclasts or monocytes (such as bone lining cells) actively endocytose TRAP [6,9]. As such, the GFP-positive cells on the bone surfaces in the models used by Boban and colleagues [1] may be TRAP positive, but these cells (or certainly many of them) may not be osteoclasts and/or monocytes/macrophages.

Resolution of these concerns is, in fact, fairly straightforward. Co-staining of the same sections shown in Figures 1B and 4B with alkaline phosphatase and demonstrating that none of the GFP-positive cells express this marker (which is present on bone lining cells) would conclusively support the authors' conclusion that in these models, osteoblast-lineage cells (at least as defined by GFP expression from the col3.6 promoter) do not transfer between the parabiotic pairs and that Lin⁻ Sca-1⁺ c-kit⁺ cells from col3.6-GFP mice do not give rise to osteoblast-lineage cells lining bone surfaces.

The issues raised here also highlight how little we understand about bone lining cells, which may not simply be quiescent osteoblasts. They may have a different lineage from functional osteoblasts, and their principal role may be in the support of osteoclastogenesis and perhaps of hematopoiesis, since the hematopoietic stem cell niche is present not contiguous to active matrix-synthesizing osteoblasts or to stromal cells in the marrow, but rather next to quiescent cells that are likely bone lining cells [10,11]. These cells also appear to be important for the initiation (by digesting non-mineralized collagen protruding from the bone surface prior to osteoclast attachment) and conclusion (by digesting the collagen left by osteoclasts) of the resorptive process [2]. Finally, bone lining cells form the roof of the bone-remodeling compartment (BRC), where they may play a key role in regulating both osteoblastogenesis and osteoclastogenesis [3,12].

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