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COMMENTARIES

Osteoclasts Control Osteoblast Activity

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Commentary on: Lee SH, Rho J, Jeong D, Sul JY, Kim T, Kim N, Kang JS, Miyamoto T, Suda T, Lee SK, Pignolo RJ, Koczon-Jaremko B, Lorenzo J, Choi Y. v-ATPase V(0) subunit d2-deficient mice exhibit impaired osteoclast fusion and increased bone formation. *Nat Med*. 2006 Dec;12(12):1403-9.

In normal, healthy individuals, bone formation is tightly coupled to bone resorption, resulting in an equilibrium between these two processes. A recent paper (1) reports that genetic ablation of the d2 subunit of the V_0 v-ATPase leads to decreased osteoclast fusion, but increased bone formation, resulting in a mild form of osteopetrosis. The phenotype of these mice is surprising, since the osteoclasts have a reduced ability to fuse, whereas the acidification process, which is normally associated with the v-ATPase, remains unchanged. The impairment of pre-osteoclast fusion results in decreased numbers of multinuclear osteoclasts, but increased numbers of mononuclear pre-osteoclasts, and, all-in-all, no change in the number of TRACP-positive cells. Since the authors demonstrate that the ability of osteoblasts to form bone *in vitro* is unchanged in the d2 knockout mice, they speculate that increased bone formation is due to extrinsic factors released by the mutated osteoclasts. These findings support previous studies indicating that bone formation can occur independently of bone resorption. In addition, the results indicate that pre-osteoclasts could be the cells responsible for the recruitment/activation of osteoblasts, as formation is increased despite a reduced number of multinuclear osteoclasts. This osteoclast-derived signal that affects the initiation and perhaps quality of bone formation – the so-called and long-sought coupling factor – still remains to be identified.

Origins of the Concept of Coupling

The concept of coupling of bone formation to bone resorption originated from pioneering work pursued by Frost and co-workers (2). They demonstrated that bone formation in more than 97% of healthy adults occurred on bone surfaces that previously had undergone bone resorption and had a scalloped appearance (2). In addition, Baylink and colleagues demonstrated that the number of osteoblasts correlated with the number of nuclei in resorbing osteoclasts (3), indicating that the action of these two cell types were somewhat coordinated. Likewise, Howard *et al.,* in pivotal studies, demonstrated that bone resorption in bone organ cultures resulted in a bone formation response (4), thereby demonstrating a direct link between bone resorption and bone formation, referred to as coupling. These studies were the first to indicate that bone formation could be controlled by factors released from the bone matrix. Interestingly, recent studies have indicated that the secondary response of osteoblasts to osteoclastic bone resorption might not correlate directly with the resorptive activity of osteoclasts, but instead with the presence of osteoclasts, independent of the level of resorptive activity (5).

Evidence from Human Forms of Osteopetrosis

Osteopetrosis results from defective osteoclast function. The two most common reasons for osteopetrosis in humans are mutations in either the osteoclastic v-ATPase or in the chloride channel ClC-7. These mutations lead to decreased acidification of the resorption lacunae, reduced resorption, but also, interestingly, to increased numbers of osteoclasts (6-8). The increase in osteoclast number is caused by increased osteoclast survival, due to reduced dissolution of the inorganic matrix (5). However, the most interesting finding in these patients is that bone formation is maintained in the face of reduced bone resorption, and that increased bone formation, in some instances, correlates with increased numbers of non-resorbing osteoclasts (9-11). Thus, evidence from patients with osteopetrosis due to specific mutations indicates that non-resorbing osteoclasts still possess the ability to support bone formation.

Osteopetrotic Mice Shed Additional Light on the Uncoupling in Osteopetrosis

Two types of osteopetrotic mice have provided especially valuable information about the coupling of formation to resorption. *c-Src* knockout mice are characterized by increased numbers of non-resorbing osteoclasts and increased bone formation, although there is an intrinsic increase in bone formation in these mice (12). In contrast, *c-fos* and M-CSF receptor-deficient mice, which have no osteoclasts, are characterized by decreased levels of bone formation and disorganized formation (13;14) indicating that osteoclasts are important for control of the level, directionality and perhaps the quality of bone formation. In complete accordance with an anabolic role of osteoclasts in bone formation, a recent study demonstrated that the anabolic action of PTH was only present in osteoclast-rich osteopetrotic mice (*c-Src* knockout mice), but not in osteoclast-poor osteopetrotic mice (*c-fos* knockouts) (15). These data indicate that non-resorbing osteoclasts can mediate anabolic signals to osteoblasts, which then implicitly need to be non-bone derived. Figure 1 briefly summarizes these different signaling pathways and emphasizes that bone resorption, osteoclast number and bone formation are not always correlated. These summarized data strongly suggest that osteoclasts can control osteoblast activity in the absence of bone resorption, and thus are secreting non-bone derived signals.

Implications for Novel Therapies

Pharmaceutical studies from several lines of investigation and many independent researchers also support the new findings. Osteoclastic bone resorption may be attenuated pharmaceutically without interfering with bone formation, or even while stimulating bone formation. This possibility is illustrated by recent studies using inhibitors of *c-Src*, the v-ATPase or ClC-7 in osteoclasts. In all cases, bone resorption was decreased, whereas bone formation was maintained or even increased (5,16-20). Whether the v-ATPase inhibitor used in previous studies affected osteoclast maturity in a manner correlating with the changes seen in the d2 knockout mice is unknown. However, these findings all indicate that bone resorption can be inhibited without negative effects on bone formation.

These data indicate that optimal manipulation of osteoclast activity may provide more benefit than abrogation of osteoclasts, and thereby their activity, as seen with either novel, potent bisphosphonates or anti-RANKL therapy (21). However, whether these important findings in animal models will translate into improved fracture efficacy in clinical trials remains to be seen. Even so, the prospect of using pharmaceutical intervention to inhibit bone resorption without inhibiting bone formation, in accordance with effects of the human osteopetrotic mutations in either the osteoclastic v-ATPase or in the chloride channel ClC-7 (9-11), is now a distinct possibility.

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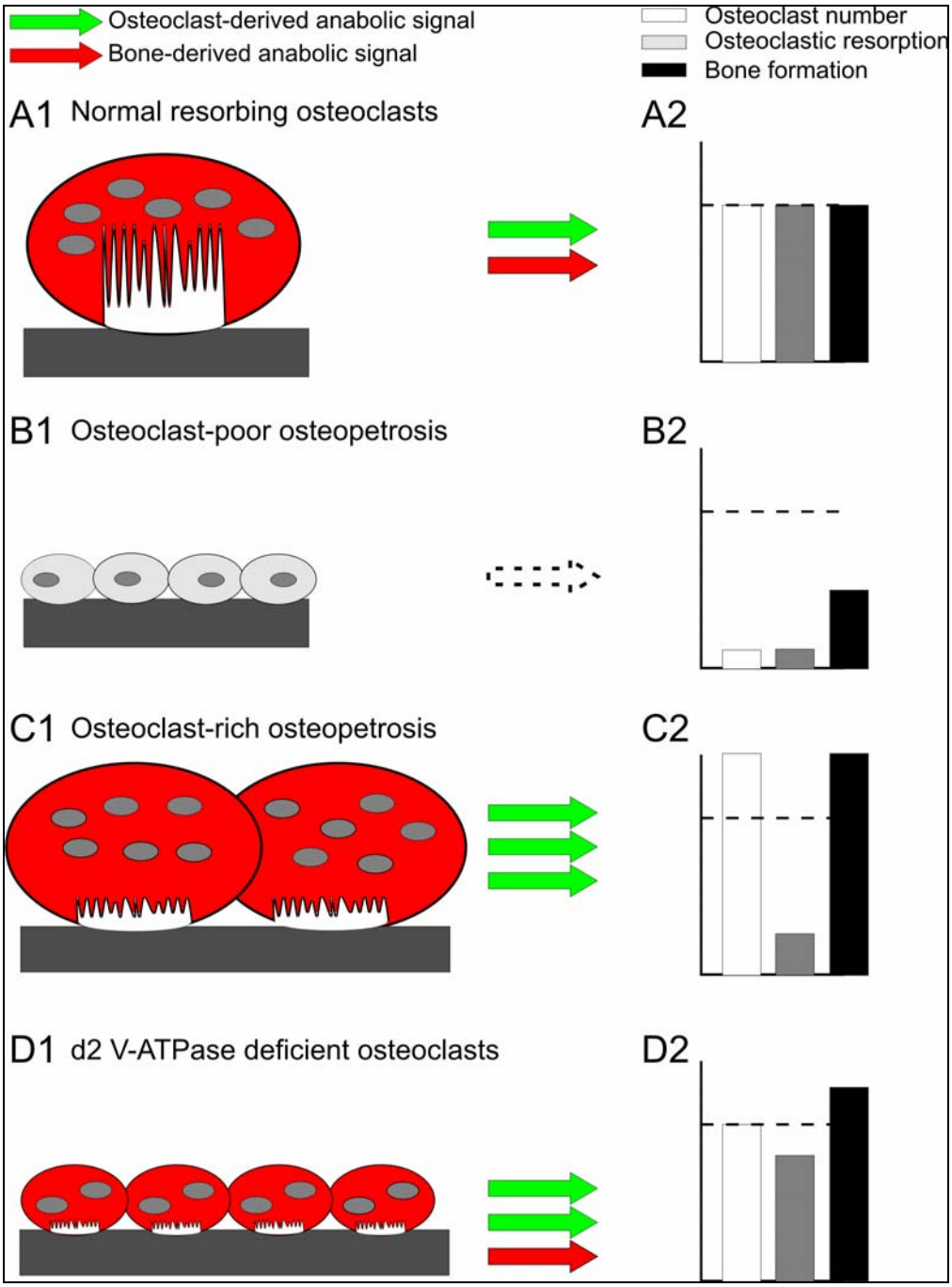

Figure 1: Schematic figure illustrating the signals from osteoclasts to osteoblasts. A). Normal, healthy individuals. B). Osteoclast-poor osteopetrosis. C). Osteoclast-rich osteopetrosis. D). d2 v-ATPase-deficient animals. The figure shows osteoclasts (red) and their nuclei (gray spots), smaller osteoclast precursors with fewer nuclei, and macrophages/monocytes (gray). The figure indicates the resorptive activity of the osteoclasts through the presence of a more or less developed ruffled border. A1). In normal healthy individuals, bone formation signals arise from both the resorbed matrix (red arrow) and the osteoclasts themselves (green arrow). B1). In osteoclast-poor situations, only a low level of the bone formation signal (dotted arrow) is present. C1). In osteoclast-rich situations, the number of non-resorbing osteoclasts is increased due to

increased survival and production of the osteoclast-derived signal (green arrows), which is increased accordingly. D1). The d2 v-ATPase-deficient animals have decreased fusion, and therefore smaller and immature osteoclasts, as well as lower resorption, but the osteoclast-derived signal (green arrows) is increased, and the bone-derived signal (red arrow) is likely also present as some resorption remains. A2-D2). Schematic illustration of osteoclast number, osteoclastic resorption and level of bone formation in the four different situations. The dotted line represents the values in normal, healthy individuals.

Remaining Questions

The osteoclast-derived coupling signal, the so-called and long-sought coupling factor, remains to be identified. However, important findings by Lee et al. (1) may provide insight into this enigma. Osteoclasts from d2 knockout mice have reduced surface MMP activity due to impaired expression of ADAM family proteins. Thus the coupling factor may be degraded or somewhat modulated by MMP activity. Other preliminary studies have shown that non-resorbing osteoclasts secrete bone-anabolic signals (22). Taken together, these studies suggest that protease activity may be an integral part of adequate processing of the "anabolic signal" from osteoclasts, *i.e.*, the coupling factor.

With respect to the actual coupling factors, there are several candidates among the "usual suspects", which include IGF-1, TGF-β or a member of the TGF-β super family (23). In addition to the "classical" players, a recent study showed that EphrinB2 on osteoclasts activated bone formation by osteoblasts through activation of EphB4. The inhibition of osteoclastogenesis by EphB4 establishes a forward-reverse signaling system between osteoclasts and osteoblasts (24), which is still under investigation. Identification of the osteoclast phenotype involved in the production of this factor is of high importance, since the d2 knockouts display reduced osteoclast surface and increased bone formation (1), whereas human forms of osteopetrosis display increased osteoclast surface, which correlates directly to osteoblast surface (11).

Taken together, the signaling from osteoclasts to osteoblasts may involve many possible signals, only some of which were discussed in this brief commentary, including secreted signals, modulation of the resorption surface, and sequestration of osteoclast-derived signals into cement lines. In the normal, healthy individual, all of these important routes of communication result in an equilibrium between bone resorption and bone formation. In postmenopausal osteoporosis, this balance is shifted toward continuous bone loss. Based on the collective findings presented in the field as of late, future interventions to alter osteoclast activity may be able to shift back, or even completely reverse, postmenopausal bone loss by using inhibitors of bone resorption that also stimulate bone formation.

Conflict of Interest: The authors report that they are full-time employees of Nordic Bioscience.

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