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Commentary

VPS35: Two Ways to Recycle the Parathyroid Hormone Receptor (PTH1R) in Osteoblasts

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The recombinant parathyroid hormone (PTH) Teriparatide is the only therapy for postmenopausal osteoporosis that increases bone mass (i.e., is anabolic). Its therapeutic action depends on a short "burst" of PTH in the circulation followed by a rapid return to normal levels; if PTH levels remain high, bone loss occurs (i.e., the effect is catabolic) (Frolik et al., 2003). This occurs because PTH not only stimulates the bone forming activity of osteoblasts, but also stimulates their ability to support osteoclast formation by producing RANKL. If PTH-induced RANKL production persists for longer than usual, anabolic intermittent PTH treatment can be switched to a catabolic effect (Walker et al., 2012). This may explain why PTH therapy is not effective in all patients, and why PTH anabolic effect is not sustained with long-term treatment. Identifying mechanisms that control how PTH signalling is terminated within the cell may provide new ways to design PTH-based therapies to overcome this problem. Three recent papers including work in *EBiomedicine* by Xiong et al. (Xiong et al., 2016; Chan et al., 2016; McGarvey et al., 2016) indicate that PTH receptor signalling duration is controlled by VPS35 and SNX27, two components of the retromer complex.

PTH signals through PTH receptor (PTH1R). This G protein-coupled receptor is shared with PTHrP (PTH-related protein), a paracrine factor produced by osteoblasts essential for normal physiological bone formation (Martin and Sims, 2013). PTH or PTHrP binding to PTH1R initiates intracellular cyclic adenosine monophosphate (cAMP) signalling that continues as the receptor is internalised into endosomes. The signal is terminated by the acidic endosomal environment, which causes ligand dissociation, and by subsequent receptor recycling or degradation mediated by the multi-protein complex termed retromer (Feinstein et al., 2011).

Retromer has two known intracellular protein-transport functions. It carries protein cargo within endosomes, including internalised transmembrane receptors such as PTH1R, to the *trans*-Golgi network (Feinstein et al., 2011); once the cargo reaches that destination it is recycled into components used by the cell to manufacture new proteins. The second function is to shuttle proteins within endosomes back to the plasma membrane, thereby restoring the transmembrane receptor availability. Retromer contains a heterotrimer of vacuolar protein sorting-associated proteins VPS26, VPS29 and VPS35, and a dimer of membrane-associated sorting nexin (SNX) family members. These SNX family members sort proteins to the appropriate intracellular actin-mediated transport pathway. For instance, SNX27 directs traffic to the plasma membrane. SNX27 binds to the PTH1R through the SNX27 PDZ domain and tethers PTH1R to the VPS35-containing retromer complex (Chan et al., 2016; McGarvey et al., 2016). PTH and PTHrP signalling could be modified by both functions of retromer.

VPS35 knockdown in kidney cells (Feinstein et al., 2011) and osteoblasts (Xiong et al., 2016) resulted in only a very slight elevation in the cAMP response to PTH. What is more convincing is an extended cAMP response duration reported in both studies, with cAMP levels remaining high until at least 40 min after initial exposure to PTH. VPS35 deficiency might limit retrograde PTH1R transport, or impair trafficking to the plasma membrane: which of these is required to limit the cAMP response? VPS35 overexpression increased, and depletion reduced, PTH1R trafficking to the Golgi (Xiong et al., 2016; Feinstein et al., 2011). In addition, VPS35 or SNX27 depletion decreased PTH1R recycling rate at the plasma membrane following PTH treatment (Chan et al., 2016; McGarvey et al., 2016). Both VPS35 and SNX27 knockdown increased cAMP accumulation (Chan et al., 2016). Slower PTH1R recycling may therefore sustain cAMP accumulation without VPS35.

VPS35 and SNX27 transport many intracellular receptors with broad functions. Those identified to date that regulate bone metabolism include the β1 and β2 adrenergic receptors, GPR177 — a receptor that

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regulates Wnt family member sorting (Klinger et al., 2015) — and degradative RANKL transport in osteoclasts (Xia et al., 2013). There are likely to be additional cargoes. Unsurprisingly, global VPS35 knockouts are early embryonic lethal, and global SNX27 knockouts exhibit a severe skeletal phenotype (Chan et al., 2016). Specific VPS35 knockdown in the osteoblast lineage, using osteocalcin-targeted-Cre, resulted in mildly lowered bone mass in the primary spongiosa, assessed at a single time point (Xiong et al., 2016). The cellular mechanism responsible is unclear – serum biochemistry showed elevated markers of bone formation and elevated serum calcium, suggesting that both bone formation and resorption are high with an imbalance in favour of resorption; no histomorphometry is reported. Further work is needed to determine the basal effect of VPS35 deletion in osteoblasts.

Due to retromer's multiple functions, the key question from a therapeutic perspective is how PTH anabolic action is modified in mice with specific VPS35 or SNX27 deletion in osteoblasts; this must be performed in vivo because it is not possible to model PTH anabolic action in vitro. Mice with VPS35 deletion targeted to osteoblasts showed a greater increase in bone mass in the primary spongiosa in response to PTH compared to controls (Xiong et al., 2016). It must be noted that the primary spongiosa is the region where new trabeculae are formed from mineralized cartilage at the growth plate, and bone mass in this region is controlled by many biological processes in addition to bone formation and resorption, such as chondrocyte differentiation and angiogenesis (Poulton et al., 2012). Increased bone mass in this region does not relate directly to bone remodelling in mature bone. For this reason it is not yet clear whether VPS35 depletion in osteoblasts shifts the balance of PTH treatment to favour an anabolic effect by reducing its pro-osteoclastic action. Although an impaired osteoclastic response to PTH treatment was observed when osteoclast precursors were co-cultured with osteoblasts deficient in VPS35 and analysis at a single time point showed low RANKL mRNA levels (Xiong et al., 2016), this is suggestive only. It remains to be clarified whether this reflects reduced RANKL production in response to PTH, or a shorter duration of RANKL response, or if the effect in vivo is osteoclast-mediated at all.

We can conclude that VPS35, at least through SNX27-mediated PTH1R recycling, controls the duration of cAMP response to PTH1R in both osteoblasts and kidney cells. How this pathway can be exploited to improve PTH anabolic action remains to be determined.

Conflicts of Interest

The author has no conflicts of interest to declare.

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