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TNAP, TrAP, Ecto-Purinergic Signaling, and Bone Remodeling

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ABSTRACT

Bone remodeling is a process of continuous resorption and formation/mineralization carried out by osteoclasts and osteoblasts, which, along with osteocytes, comprise the bone multicellular unit (BMU). A key component of the BMU is the bone remodeling compartment (BRC), isolated from the marrow by a canopy of osteoblast-like lining cells. Although much progress has been made regarding the cytokine-dependent and hormonal regulation of bone remodeling, less attention has been placed on the role of extracellular pH (pH_e). Osteoclastic bone resorption occurs at acidic pH_e. Furthermore, osteoclasts can be regarded as epithelial-like cells, due to their polarized structure and ability to form a seal against bone, isolating the lacunar space. The major ecto-phosphatases of osteoclasts and osteoblasts, acid and alkaline phosphatases, both have ATPase activity with pH optima several units different from neutrality. Furthermore, osteoclasts and osteoblasts express plasma membrane purinergic P2 receptors that, upon activation by ATP, accelerate bone osteoclast resorption and impair osteoblast mineralization. We hypothesize that these ecto-phosphatases help regulate $[ATP]_e$ and localized pH_e at the sites of bone resorption and mineralization by pH-dependent ATP hydrolysis coupled with P2Y-dependent regulation of osteoclast and osteoblast function. Furthermore, osteoclast cellular HCO_3^-, formed as a product of lacunar V-ATPase H^+ secretion, is secreted into the BRC, which could elevate BRC pH_e, in turn affecting osteoblast function. We will review the existing data addressing regulation of BRC pH_e, present a hypothesis regarding its regulation, and discuss the hypothesis in the context of the function of proteins that regulate pH_e. J. Cell. Biochem. 105: 655–662, 2008.

KEY WORDS: OSTEOBLAST; OSTEOCLAST; P2Y RECEPTORS; BONE REMODELING COMPARTMENT; BONE LINING CELLS; BICARBONATE; EXTRACELLULAR pH

Bone remodeling is a process of simultaneous formation/mineralization and resorption that occurs in localized areas of the bone matrix. Mineralization and resorption are inversely regulated by a number of factors, including cytokines, hormones, and substances such as osteoprotegerin (OPG) and receptor activator of NF-κB ligand (RANKL) [Blair et al., 2002; Martin and Sims, 2005; Pogoda et al., 2005]. One longstanding observation is that extracellular pH (pH_e) regulates the balance of resorption and formation/mineralization, since chronic systemic acidosis promotes resorption, whereas alkalosis promotes formation/mineralization [Arnett, 2003, 2008]. The pro-mineralization activity of osteoblasts is increased at alkaline extracellular pH [Kaysinger and Ramp, 1998; Brandao-Burch et al., 2005], whereas the pro-resorptive activity of osteoclasts, increased at acidic pH, is also dependent on the presence of HCO_3^-/CO_2 in the external medium [Carano et al., 1993; Arnett and Spowage, 1996]. Extracellular nucleotides such as ATP and UTP, released non-lytically from cells, interact with cell surface purinergic P2 receptors in an ecto-purinergic signaling system that is present in many organs [Bowler et al., 2001; Boeynaems et al., 2005; Kunzli et al., 2007; Yegutkin, 2008]. The ecto-purinergic system present in bone is thought to play an important role in the regulation of bone remodeling [Bowler et al., 2001; Gallagher, 2004].

We would like to present a hypothesis regarding the role of ecto-phosphatases in the regulation of bone mineralization and resorption. We propose that within the BRC proposed for cancellous bone [Hauge et al., 2001], two isolated extracellular compartments exist, consisting of the osteoclast lacunar resorption compartment, and the bulk pH_e of the BRC. We will further propose that the pH of the resorption and bulk compartments are regulated by extracellular purinergic nucleotides in a system comprised of an ATP release mechanism, a pH-sensitive ectophosphatase, and P2Y receptors that balance bone formation and resorption.

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THE BONE MULTICELLULAR UNIT (BMU)

FUNCTIONAL COUPLING

The concept of a multicellular unit was first advanced in the 1960s in order to explain how bone resorption and formation are so closely linked in the process of bone remodeling. The BMU consists of activated osteoclasts, osteoblasts, and osteocytes, arranged in a 3-D geometry, the exact nature of which remains elusive [Pogoda et al., 2005; Matsuo and Irie, 2008]. Observations that osteoclast activity was dependent on the presence of functioning osteoblasts have spawned numerous hypotheses to explain the observed functional coupling. One of the most prevalent involves the paracrine effect of the cytokines OPG and its ligand OPGL, RANKL, and macrophage colony stimulating factor (M-CSF). Hauge has further observed that the BMU is preceded by the formation of a functional unit: the BRC that is isolated from bone marrow by bone on three sides and by a "canopy" of lining cells expressing osteoblastic markers such as alkaline phosphatase (AP), osteonectin, and osteopontin. We will present a new hypothesis that BRC pH_e itself coordinates the acute regulation of resorption and formation/mineralization through the ecto-purinergic system.

EPITHELIAL AND EPITHELIAL-LIKE COMPONENTS OF THE BRC

Osteoblasts. Although bone is generally not thought of as being an epithelial tissue, the elements comprising the BRC have distinct epithelial or epithelial-like features. An epithelium is defined as a layer of polarized cells joined by tight junctions. Epithelial cells in culture usually form monolayers in which the cells spread, form tight junctions at the contact area, and then stop proliferating. Osteoblasts have distinct membrane domains, termed the vascular (dorsal), the lateral which apposes other osteoblasts, and the osteoid domain, facing the calcifying bone matrix. In the interest of simplicity, we will term the dorsal and lateral membranes the basolateral membrane. In cultured osteoblast-like UMR-106 monolayers plated on permeable supports, Green demonstrated that osteoblasts have polarized transport function, with amiloride-sensitive ^{22}Na uptake by the basolateral (vascular) membrane, consistent with NHE activity, and with DIDS-sensitive ^{36}Cl across the apical (osteoid) membrane, consistent with Cl^-/HCO_3^- anion exchange [Green, 1994]. The epithelial nature of osteoblasts, however, has never been reported in situ.

Osteoclasts. Activated osteoclasts have a pseudo-epithelial structure, as was suggested originally by Gluck [1992]. To resorb bone, the activated osteoclast firmly attaches to the bone matrix, sealing off the lacunar space from the external environment. This process is aided by integrins, which are cell matrix adhesion proteins [Akisaka et al., 2006]. Osteoclasts are divided into an acid-secreting, osteoid facing pole, and a bicarbonate-secreting interstitium-facing pole, which also serves to secrete the products of bone resorption into the interstitium. Osteoclasts secrete H^+ by a V-ATPase localized to the ruffled border [Baron et al., 1985] after activation, and HCO_3^- via anion exchange of a type which has not been positively identified [Baron et al., 1985; Akisaka et al., 2006]. Tartrate-resistant acid phosphatase (TrAP or TRACP) is also inserted in the ruffled membrane [Hayman et al., 2001; Sawyer et al., 2003]. The source of intracellular HCO_3^- and H^+ is CO_2 diffusing from the BRC, and HCO_3^- transported into the cell, possibly by an active mechanism [Pochhammer et al., 1979; Bouyer et al., 2007]. Osteoclasts also express the activity of the HCO_3^- transporter sodium: bicarbonate cotransporter n1 or NBCn1 which increases intracellular pH by actively transporting HCO_3^- into the cell. This is activated by colony-stimulating factor-1 or CSF-1 [Bouyer et al., 2007]. The osteoclast shares many similarities with acid-secreting gastric parietal cell or the renal intercalated cell, in which the ruffled membrane, elaborated by a massive, coordinated, rab protein-dependent membrane insertion event, is transformed into an HCl secreting organ [Anderson et al., 1986; Mulari et al., 2003; Harmey et al., 2004]. HCO_3^-, secreted across the opposite pole secretory domain, secretes the equivalent of "alkaline tide" HCO_3 secreted across the gastric parietal cell basolateral membrane. Although osteoclast HCO_3^- secretion has not been measured directly, its rate of secretion is presumed to be equivalent to that of V-ATPase mediated H^+ secretion. Since osteoclast resorptive activity, and presumably the V-ATPase H^+ secretory rate, is under purinergic control, we infer that the HCO_3^- secretory rate is also similarly regulated. We therefore hypothesize that HCO_3^- secreted across the osteoclast basolateral membrane is regulated by P2Y receptors, and that osteoclast HCO_3^- secretion is an important determinant of BRC pH_e.

Source of osteoclast CO_2. Since the BRC is isolated from circulation by a canopy of osteoblast-like cells, CO_2 presumably originates in the vascular marrow space, diffusing into the BRC. We have recently found that the apical membrane-expressed CA XIV facilitates CO_2 diffusion across the duodenal brush border [Akiba et al., 2006; Mizumori et al., 2006]. Possibly, CA XIV expressed on the bone lining cells facilitates CO_2 entry into the BRC [Riihonen et al., 2007].

REGULATION OF FORMATION/MINERALIZATION BY EXTRACELLULAR pH AND BY PURINE NUCLEOTIDES

Numerous observations extending over several decades have documented that systemic acidosis is associated with demineralization. Ramp et al., and more recently, the Arnett laboratory, have confirmed that lowering medium pH in cultured osteoblasts inhibits bone formation while increasing bone resorption in cultured osteoclasts [Ramp et al., 1994; Arnett, 2003, 2008; Brandao-Burch et al., 2005]. Raising medium $[CO_2]$ additionally increases bone resorption. Thus, pH_e and $[CO_2]$ regulate the balance between mineralization and resorption [Carano et al., 1993].

Recently, several reports of expression of P2Y receptors on osteoclasts have documented the important role of extracellular nucleotides in bone remodeling. Indeed, several osteoblast functions appear to be regulated by an ecto-purinergic system in which ATP is non-lytically released from the cytoplasm [Buckley et al., 2003], where it can interact with surface P2 receptors. Through phospolipase C (PLC)-dependent signaling mechanisms, P2 receptors can potentiate the physiologic effects of parathyroid hormone (PTH) on bone remodeling [Bowler et al., 2001]. Activation of $P2Y_2$ receptors expressed on osteoblasts by extracellular nucleotides and extracellular PPi inhibited bone mineralization in vitro [Orriss et al., 2007]. Cell-surface purinergic activation increases intracellular Ca^{++} through activation of protein kinase C (PKC), which is associated with impairment of mineralization

[Shimegi, 1996]. Furthermore, the extracellular nucleotides ATP and ADP can activate $P2Y_1$ receptors on the osteoclast surface, increasing bone resorption, at likely physiologic concentrations and at acidic pH [Hoebertz et al., 2001; Gallagher, 2004]. Thus, at low concentrations, extracellular nucleotides profoundly decrease mineralization by osteoblasts and increase bone resorption by osteoclasts. This ecto-purinergic system, consisting of released ATP binding to membrane P2Y receptors, is present in many epithelia where surface and localized pH affects physiological processes such as bile secretion, sperm capacitation, ovum implantation, pancreatic HCO_3^- secretion, renal HCO_3^- reabsorption, and airway mucus secretion [Litosova et al., 1969; McAlroy et al., 2000; Schreiber and Kunzelmann, 2005; Bakst and Akuffo, 2007; Lu et al., 2007].

ECTO-PHOSPHATASES AS pH SENSORS?

Tissue non-specific alkaline phosphatase (TNAP) and TrAP are glycosylphosphatidylinositol (GPI) anchored ecto-enzymes expressed in alkaline and acidic compartments, respectively. Although useful as marker enzymes for organelles and cell membranes and types [Doty and Schofield, 1976; Andersson and Marks, 1989], and subject to much speculation about their function, prior inability to measure enzyme activity in intact tissue in vivo has hindered the understanding of their function.

AP

AP is expressed as several paralogs, with TNAP being the predominant bone paralog. TNAP is expressed on the basolateral plasma membrane of osteoblasts and osteoblast precursors [Bernard, 1978; Hoshi et al., 2001; Miao and Scutt, 2002; Nakano et al., 2004], on bone lining cells [Pillai and Santhakumari, 1984; Miao and Scutt, 2002], and on matrix vesicles (MVs) [Majeska and Wuthier, 1975], the initial site of mineralization [Anderson et al., 2004]. Due to its broad substrate specificity, TNAP functions to promote mineralization, by liberating Pi from ATP, and also by hydrolyzing PPi, which inhibits mineralization. Evidence supporting the latter role includes studies of transgenic mice null for Akp2, the gene encoding TNAP, which, similar to the human ortholog, have deficient mineralization. Double knockouts null for Akp2 and proteins that reduce PPi partially corrected the mineralization defects, emphasizing the important of TNAP in PPi metabolism [Harmey et al., 2004]. TNAP also hydrolyzes AMP to adenosine, which could have effects of bone P1 receptors [Millán, 2006].

The AP paralog intestinal AP (IAP) is also highly expressed in the duodenal brush border. Recent studies from our laboratory have helped define the physiological role of IAP. When IAP activity was measured in intact tissue in vivo using the fluorogenic phosphatase substrate ELF-97 in live animals, we found that duodenal mucosal IAP activity is dependent not only on medium pH as would be expected, but also on the rate of duodenal epithelial HCO_3^- secretion [Akiba et al., 2007]. This observation suggested that the exceedingly high pH optimum of AP (>9) might exist in a microenvironment at the surface of HCO_3^--secreting tissues, with regulation of AP function dependent on its microclimate pH, which in turn is dependent on bulk extracellular pH and the rate of HCO_3^- secretion.

Since extracellular purine phosphatases such as ATP increase the rate of HCO_3^- secretion, and since AP activity degrades ATP at alkaline pH to adenosine which does not activate P2Y receptors [Majeska and Wuthier, 1975; Humphreys and Chou, 1979; Corbic et al., 1985], we hypothesized that IAP serves as a sensor that regulates local pH by adjusting the balance between phosphorylated, P2Y receptor active, and dephosphorylated, P2Y receptor inactive nucleotides. Since TNAP and IAP share similar pH optima and ATP hydrolytic activity, we hypothesize that ATP increases the rate of osteoclast HCO_3^- secretion into the BRC, which, when it nears the pH optimum of TNAP, is degraded by TNAP, decreasing $[ATP]_e$, and augmenting the rate of mineralization. Thus, TNAP helps maintain a suitably alkaline bulk pH_e in the BRC, and a low $[ATP]_e$, enhancing mineralization.

TrAP

TrAP, like AP, is a GPI-anchored ectoenzyme. Unlike AP, it is expressed in the osteoclast ruffled membrane, with its catalytic site presumably located in the acidic lacunar space between the bone matrix and the apical membrane [Hayman et al., 2001; Sawyer et al., 2003]. TrAP, a useful osteoclast marker, exists in many forms. The form localized to the ruffled membrane has been distinguished by the presence of ATPase activity [Andersson and Marks, 1989], which corresponds to the TrAP paralog TrAP-5b [Hayman et al., 1996]. TrAP is a protease-activated protein; since it exists in an acidic milieu containing proteases secreted by the osteoclast it is assumed to be activated by lysosomal proteases secreted into the lacunar space. Protease-cleaved TrAP hydrolyzes ATP at a pH optimum of 4.9 [Mitic et al., 2005].

THE OSTEOCLAST LACUNA

Within the resorptive lacuna, we propose that a separate purinergic system regulates bone resorption, again through pH-dependent ATPase activity and P2Y receptors. This proposed mechanism is more speculative than the regulation of osteoblast and osteoclast activity through BRC $[ATP]_e$, in that the detailed localization of P2Y receptors or measurement of lacunar $[ATP]_e$ on osteoclasts has never been reported.

Given its low pH optimum, we hypothesize that since osteoclast P2Y receptor activation increases the rate of pit excavation, presumably related to increased H^+ secretion, and if P2Y receptors are expressed on the ruffled membrane, TrAP could also serve as a servo mechanism to regulate lacunar fluid pH. This low pH activates acid phosphatase activity, hydrolyzing ATP, decreasing P2Y receptor activation, thus decreasing the rate of acid secretion. We speculate that like TNAP, the unusual pH optimum of TrAP activity also functions as a surface pH sensor, regulating H^+ secretion to adjust the pH of its milieu.

PURINERGIC REGULATION OF BONE REMODELING

Incorporating the elements described in the preceding sections, we hypothesize that the bulk pH_e of the BRC is regulated by the pH-dependent activity of TNAP though ATP hydrolysis and activation

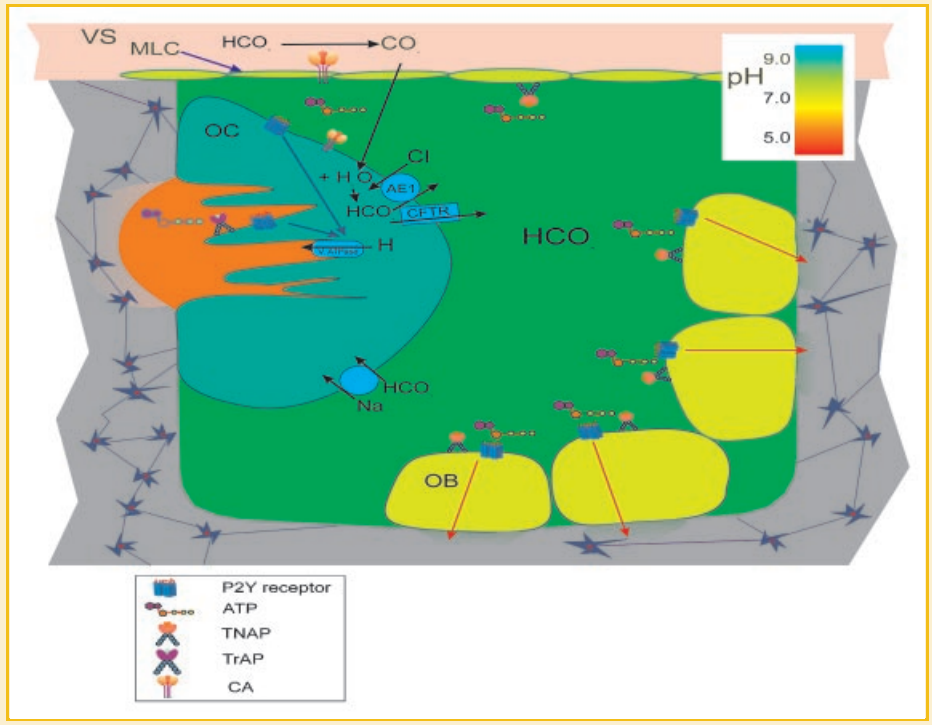

Fig. 1. Model of hydrolase-dependent purinergic extracellular pH (pH_e) regulation during bone remodeling. The bone remodeling compartment (BRC) is proposed to have two extracellular compartments in disequilibrium with systemic pH: the lacunar space and the bulk fluid of the BRC. CO_2, derived from vascular HCO_3^- by CA activity expressed on the bone lining cells, diffuses into the BRC, where it enters the osteoclast cytoplasm. The CO_2 is reconverted to H^+ and HCO_3^- with the H^+ secreted into the lacuna by the V-ATPase; HCO_3^- is secreted across the osteoclast basolateral membrane by a combination of an anion exchanger, the CFTR and possibly by NBCn1. HCO_3^- in the BRC main compartment increases pH_e, osteoblast precursor cell plasma membrane, the bone lining cells, and on the surface of matrix vesicles, which then increases the rate of hydrolysis of ATP released from the cytoplasm of BRC component cells. Lowered $[ATP]_e$ de-inhibits osteoblast-mediated formation/mineralization through reduced P2Y signaling and also decreases osteoclast-mediated bone resorption through a $P2Y_1$-mediated mechanism, decreasing osteoclast HCO_3^- secretion, lowering TNAP activity and increasing $[ATP]_e$. Lacunar pH and $[ATP]_e$ are regulated by TRAP, expressed on the osteoclast ruffled membrane, which has ATPase activity with a pH optimum of 4.9. TNAP, tissue nonspecific alkaline phosphatase; TrAP, tartrate-resistant acid phosphatase; CA, carbonic anhydrase; VA, vascular space; OC, osteoclast; OB, osteoblast; MLC, marrow lining cells; NBC, sodium bicarbonate cotransporter; red arrows, inhibition; blue arrows, augmentation.

of P2Y receptors. CO_2, derived from vascular HCO_3^- by CA activity expressed on the bone lining cells, diffuses into the BRC, where it enters the osteoclast cytoplasm. The CO_2 is reconverted to H^+ and HCO_3^- with the H^+ secreted into the lacuna by the V-ATPase; HCO_3^- is secreted across the osteoclast basolateral membrane by a combination of an anion exchanger, the CFTR and possibly by NBCn1. HCO_3^- in the BRC main compartment increases pH_e, activating TNAP expressed on the osteoclast basolateral membrane, osteoblast precursor cell plasma membrane, the bone lining cells, and on the surface of MVs, which then increases the rate of hydrolysis of ATP released from the cytoplasm of BRC component cells. Lowered $[ATP]_e$ de-inhibits osteoblast-mediated formation/mineralization through reduced P2Y signaling and also decreases osteoclast-mediated bone resorption through a $P2Y_1$-mediated mechanism, decreasing osteoclast HCO_3^- secretion, lowering TNAP activity and increasing $[ATP]_e$. Lacunar pH and $[ATP]_e$ are regulated by TrAP, expressed on the osteoclast ruffled membrane, which has ATPase activity with a pH optimum of 4.9 (Fig. 1). This system would thus be one means out of many by which the balance of formation/mineralization and resorption is acutely maintained through pH-dependent TNAP-mediated regulation of BRC $[ATP]_e$. At high concentrations, ATP also serves a role as Pi donor for bone formation at the surface of MVs [Nakano et al., 2007]. Indeed, the activity of TNAP, and other ecto-phosphohydrolases such as nucleotide pyrophosphatase phosphodiesterase 1 (NPP1) and PHOSPHO1 are essential for normal mineralization to occur [Gluck, 1992; Del Fattore et al., 2008].

This model predicts the reciprocal regulation of osteoclasts and osteoblasts by BRC pH_e. High pH_e, such as during alkalosis, increases $[HCO_3^-]_e$, impairing HCO_3^- secretion from osteoclasts. This in turn impairs acidification of the lacunar space, impairing resorption, while increasing TNAP activity, decreasing BRC $[ATP]_e$. The net effect is inhibition of resorption (increased lacunar pH) and increased formation (decreased osteoblast P2Y signaling). Low pH_e, as would occur in acidosis, increases resorption and decreases formation by reversing these changes.

TESTING THE HYPOTHESIS

This hypothesis predicts that any abnormality that would alter lacunar or BRC pH_e or that would affect the rate of extracellular ATP hydrolysis would affect bone remodeling. To illustrate our point, we will choose loss-of-function mutations of several of the proposed components of this hypothesized ecto-purinergic regulatory system.

CONDITIONS ASSOCIATED WITH IMPAIRED ECTO-PHOSPHATASE ACTIVITY

Hypophosphatasia. Hypophosphatasia is a rare mineralization disorder associated with several TNAP loss-of-function mutations [Whyte, 1994; Mornet, 2007]. Depending on the nature of the mutation, phenotypes can vary between severe hypomineralization with pathologic fractures first observed in mid-life to marked impairment of the mineralization of a stillborn fetus. Bone pain and pathologic fractures predominate in all forms. One of the most commonly used biochemical markers of hypophosphatasia is a low plasma concentration of AP, which has been attributed to the loss of TNAP function. The lack of success of therapeutic attempts to replete AP activity systemically suggests that localized AP function most likely contributes to the AP phenotype [Whyte, 1994]. Accordingly, multiple hypotheses have been advanced regarding the nexus between TNAP activity and bone mineralization. Several compelling hypotheses regarding the effect of AP activity on mineralization have been advanced. Phosphoethanolamine, pyridoxal 5′ phosphate, and PPi have been proposed as endogenous TNAP substrates, although the endogenous concentration of these substrates is far lower than the mM K_m measured for TNAP biochemically; furthermore, the putative pH at the catalytic site is thought to be much lower than the enzyme's pH optimum [Whyte, 1994].

Dysfunctional osteoblast TNAP activity associated with the disease decreases ATP hydrolysis and increases $[ATP]_e$, increasing P2Y-dependent purinergic signaling on osteoblasts, and impairing mineralization. Elevated $[ATP]_e$ would also increase osteoclast activity, through osteoclast $P2Y_1$ receptors. Since TNAP, unlike other ecto-phosphohydrolases can hydrolyze inorganic phosphates, higher interstitial concentrations of PPi might also be present, in particular in the vicinity of the TNAP expressing MV. The impairment of calcification around the growth plate in TNAP knockout mice attests to the importance of TNAP expressed on the MV membrane for mineralization [Anderson et al., 2004]. This hypothesis is testable by the demonstration of elevated $[ATP]_e$ in hypophosphatasia, accompanied by an elevated BRC pH_e. Why hypophosphatasia has such a predominant bone phenotype given the broad tissue distribution of TNAP remains a further mystery, although biochemical studies suggest that AP variants are expressed in other organs and that TNAP activity is retained in liver and kidney through compensatory expression of IAP [Mueller et al., 1983].

TrAP NULL MICE

As discussed above, TrAP is highly expressed in the osteoclast ruffled membrane [Gothlin and Ericsson, 1971] in addition to dendritic cells and macrophages. Deletion of the *Tracp* gene in mice is phenotypically characterized by impaired osteoclast activity in vitro and in vivo, accompanied by increased mineralization [Hayman et al., 1996]. Our model predicts that TrAP dysfunction lowers lacunar pH by decreasing TrAP-mediated lacunar ATP hydrolysis. This lack of purinergic lacunar pH control excessively acidifies the lacunar space, impairing resorption, presumably by decreasing lacunar pH_e below the pH optimum for the secreted resorptive enzymes. Increased lacunar acidification is accompanied by increased osteoclast HCO_3^- secretion, increasing BRC pH_e, in turn increasing TNAP-mediated ATP hydrolysis, lowering bulk BRC $[ATP]_e$, and increasing mineralization, consistent with the observed phenotype.

CONDITIONS ASSOCIATED WITH IMPAIRED HCO_3^- TRANSPORT

Our model predicts that impairment of any component of the HCO_3^- secretory pathway in osteoclasts decreases mineralization by preventing adequate alkalinization of the BRC bulk pH, increasing bulk BRC $[ATP]_e$. We will discuss two loss-of-function mutations of components of the HCO_3^- secretory pathway which have bone phenotypes.

Cystic fibrosis (CF). CF is a relatively common autosomal recessive condition associated with loss-of-function mutations of the CF transmembrane regulator (CFTR), a protein cloned in 1989. Bone disease commonly complicates CF, usually manifest as low bone density and increased susceptibility to fractures. Chronic pulmonary infections with elaboration of pro-inflammatory cytokines, corticosteroid therapy, vitamin D malabsorption, malnutrition, and sex hormone insufficiency are considered contributory to the CF bone phenotype [Aris et al., 2005]. Despite these many risk factors, multivariate analysis indicates that the CFTR mutation in and of itself is an independent risk factor for the development of the CF bone phenotype, an observation supported by the marked bone phenotype manifested in mice homozygous for the common CFTR ΔF508 mutation [Gawenis et al., 2001; Dif et al., 2004; Haston et al., 2008]. CFTR mutant mice typically display an intestinal phenotype characterized by preventable obstruction, not manifesting the pulmonary disease or growth retardation characteristic of the clinical disease. Recent editorials have implicated the osteoblast in the pathogenesis of CF bone disease, although little is known about CFTR expression in bone [Aris and Guise, 2005]. Recently, immunohistochemical evidence of CFTR expression was found in osteoclasts, osteoblasts, and osteocytes [Shead et al., 2007]. Furthermore, isolated osteoblasts have a CFTR-like, cAMP-activated Cl^- current [Chesnoy-Marchais and Fritsch, 1989]. Therefore, CFTR is expressed in the BRC, and probably facilitates HCO_3^- secretion across the osteoclast basolateral membrane, maintaining bulk BRC pH_e.

CFTR function is unequivocally needed for HCO_3^- secretion in duodenum, airway, cholangiocyte, pancreatic duct, the male reproductive tract, and in several other organs [Ulrich, 2000; Hug et al., 2003; Wang et al., 2003; Chan et al., 2006]. Indeed, the CFTR clinical phenotype is comprised almost exclusively of dysfunction of HCO_3^- secreting organs. We propose that hypomineralization is also part of the CF phenotype due to impairment of HCO_3^- across the osteoclast basolateral membrane, lowering bulk BRC pH_e. Impaired osteoclast secretion of HCO_3^- into the BRC bulk compartment would impair mineralization by the same mechanism described with TrAP dysfunction.

CA II deficiency. Carbonic anhydrase (CA), which exists in cytoplasmic and in membrane-bound forms, is universally expressed in HCO_3^- secreting tissues. Although its expression in bone has not been widely studied, its dysfunction clinically is accompanied by osteopetrosis and cerebral calcifications, the latter finding confirmed in transgenic mice null for CA II [Margolis et al., 2008]. Impaired bone resorption observed in CA II null mice can be explained by impairment of osteoclast formation of cellular HCO_3^- and H^+ from CO_2 and H_2O. Lacunar H^+ secretion would be impaired

by less H^+ available for secretion into the lacuna by the V-ATPase, in turn impairing resorption. Gastric H^+ secretion is also impaired by CA inhibitors, presumably by a similar mechanism [Puscas et al., 1989].

FINAL THOUGHTS

The proposed model is likely to be most important for the acute regulation of bone remodeling on a minute-to-minute basis, with the other tested and more accepted regulatory mechanisms acting on longer time scales. We realize that many aspects of our proposed model are speculative, although our proposed hypothesis would likely complement the existing established cytokine and hormone-dependent mechanisms. Nevertheless, no satisfactory explanation for the unusual pH optima of acid or alkaline phosphatases has been advanced, which, coupled with our observations of duodenum in vivo, add plausibility to our conjecture that these GPI-anchored, highly conserved phosphohydrolases with unusual pH optima serve as part of a pH-sensing mechanism for small compartments in pH disequilibrium with plasma, such as in lysosomes, synapses, and epithelial brush borders, in addition to the two extracellular compartments proposed for the BRC. The model also predicts that conditions that primarily affect lacunar acidification such as TrAP and CA II dysfunction will produce an osteopetrosis predominant phenotype, whereas primary impairment of osteoclast HCO_3^- secretion, such as in CF, or with bulk BRC $[ATP]_e$ regulation, such as in TNAP deficiency, produce predominant hypomineralization phenotypes. The bone phenotypes associated with dysfunction of the other components of the proposed BRC ecto-purinergic pH regulatory system (e.g., NBCn1, CA XIV, and P2Y receptors) are either too severe due to global expression, or as yet undescribed.

CONCLUSION

Regulation of lacunar and BRC pH_e by alteration of TrAP function, and BRC and lacunar $[ATP]_e$ by bulk pH_e by the ecto-purinergic system and TNAP and can help explain the mechanism by which loss-of function of TNAP, TrAP, CAII, and CFTR can produce bone phenotypes. If the hypothesis is confirmed by independent studies, we hope that it will lead to the discovery of new and useful therapeutic targets for the treatment of bone disease.

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