MINI-REVIEWS AND PERSPECTIVES

The Importance of the Gastrointestinal Tract in the Control of Bone Mass Accrual

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One of the least anticipated and less heralded outcomes of mouse genetics has been to rediscover whole organism physiology. Among the many unexpected findings that it has brought to our attention has been the realization that gut-derived serotonin is a hormone-inhibiting bone formation. The importance of this discovery presented in this review is 2-fold. First, it provides a molecular explanation for 2 human genetic diseases—osteoporosis, pseudoglioma, and high bone mass syndrome; second, it suggests a novel and anabolic way to treat osteoporosis. These findings illustrate the importance of the gastrointestinal tract in the regulation of organ physiology at yet another extraluminal site.

One of the most unexpected and fertile advances in biology engendered through mouse genetics has been the rediscovery that physiology has to be studied ultimately at the level of the entire organism. Indeed, what mouse genetics has taught through the unraveling of the control of appetite by adipocytes,[1–3] the hematologic control of grooming behavior,[4] and the coordinated control of bone mass, energy metabolism, and reproduction[5–8] are illustrious of the premise that our knowledge of whole organism physiology is still rudimentary. The regulation of bone mass accrual by the gastrointestinal (GI) tract is a striking example of how mouse genetics has revealed unanticipated relationships between 2 organs that previously were rarely discussed in the same sentence.

The first evidence that the GI tract through one of its main functions, food absorption, can influence bone mass, came from the study of ATF4, a transcription factor enriched in osteoblasts and required for their terminal differentiation and function.[9] ATF4 affects all known activities of the osteoblasts: bone formation, including extracellular matrix synthesis, osteoclast differentiation, and energy metabolism. ATF4 achieves the latter 2 osteoblast functions in the most classical way, that is, by regulating the expression of genes needed for osteoclast differentiation and energy metabolism.[9–11] By contrast, ATF4 does not affect extracellular matrix synthesis by regulating the expression of extracellular matrix components. How does ATF4 do that? As it turns out, ATF4 is needed also for amino acid import into cells.[12,13] In osteoblasts that need to synthesize large amounts of protein, amino acid import is obviously important. Accordingly, adding amino acids to the ambient medium of cultured *Atf4*$^{-/-}$ osteoblasts suffices to restore collagen synthesis.[12] This cell culture observation led to the hypothesis that one could possibly correct the osteoporosis observed in *Atf4*$^{-/-}$ mice simply by adding proteins to the diet of these animals.[12] The biomedical relevance of what ATF4 does stems from the fact that ATF4 activity is affected in osteoblasts in 2 human diseases with skeletal manifestations. One disease is the Coffin–Lowry syndrome in which ATF4 transcriptional activity is decreased and the other is neurofibromatosis type I, in which ATF4 is increased.[10,12] Consistent with the hypothesis that dietary protein can compensate for an ATF deficiency, we have shown that increasing protein intake in pregnant *Atf4*$^{-/-}$ mice prevented the appearance of a low bone mass in their progeny.[10,12] The same was also true of *Rsk2*$^{-/-}$ pregnant mice.[10,14] Rsk2 is a kinase that phosphorylates ATF4, an event needed for its full activity. *RSK2* is the gene that is inactivated in the Coffin–Lowry syndrome. Conversely, decreasing protein intake in mice lacking *Nf1* in osteoblasts only prevented the appearance of skeletal manifestations. These observations were important because they demonstrated for the first time that, at least in the mouse, one can prevent the appearance of skeletal dysplasia through diet. Therapeutically beneficial or not, these observations were the first to reveal the influence that the GI tract can exert on bone physiology.

A second line of evidence that indicates that the GI tract influences bone remodeling came from a thorough analysis of the histologic manifestation of osteopetrosis, a group of diseases caused by a decrease in bone resorption. Looking systematically at different mouse mutations, all of which result in osteopetrosis, Schinke et al[15] elegantly showed that the hypocalcemia that accompanies some forms of osteopetrosis is not due to an osteoclast dysfunction, but rather to secondary hyperparathyroidism. Through a series of very clever analyses, the investigators showed that, in at least 1 form of osteopetrosis accompanied by hypocalcemia, the gene that is inactivated pro-

Abbreviations used in this paper: EC, enterochromaffin; GI, gastrointestinal; Lrp5, LDL receptor related protein 5.

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motes acidification of the extracellular milieu and is expressed both in osteoclasts and in gastric parietal cells. Conversely, the same investigators showed that mice that are deficient in the gastrin receptor that stimulates parietal cell secretion of acid display hypocalcemia, secondary hyperparathyroidism, and osteoporosis. Remarkably, all these phenotypes could be corrected by calcium supplementation. These findings have immediate clinical relevance because they suggest that many patients suffering from hypochlorhydria or who chronically ingest proton pump inhibitors may be at risk to develop hypocalcemia and osteopenia if not a full-blown osteoporosis that could easily be prevented by supplementing their diet, an innocuous and inexpensive therapy. Indeed, long-term proton pump inhibitor use has been suggested to increase the risk of hip fractures.[16]

The third line of evidence indicating that the GI tract influences profoundly bone mass accrual has received the most attention lately because it came out of the molecular elucidation of human diseases and also because a therapeutic implication could be demonstrated. The surface molecule Lrp5 (LDL receptor related protein 5) is a gene of great interest to bone biology. The inactivation of Lrp5 leads to a very severe form of osteoporosis, which is observed in children, while a presumed activating mutation of Lrp5 results in a syndrome of high bone mass.[17,18] Although it was initially presumed that these 2 diseases would prove that canonical Wnt signaling regulates bone remodeling, attempts to demonstrate that altered Wnt signaling mediates the effects on bone of Lrp5 mutations were limited to cell culture experiments or provided conflicting results in vivo. This frustrating evidence led one of us (GK) to go back to square one and to carry out a microarray experiment to identify the genes that change expression as a result of loss-of-functions mutations in Lrp5.[19]

Surprisingly, the gene most highly expressed when *Lrp5* is absent is *Tph1*, which encodes tryptophan hydroxylase 1, the rate-limiting and initial enzyme in the synthesis of serotonin in enterochromaffin (EC) cells of the GI tract.[19–22] These data were consistent with the idea that gut-derived serotonin is a hormone that inhibits osteoblastic bone formation. Accordingly, serum serotonin levels are high in mice lacking *Lrp5* and low in mutant mice harboring an activating mutation in this gene. The biological relevance of these findings was further enhanced by observations made in different countries that human patients lacking *Lrp5* have high circulating serotonin levels in several studies, whereas patients with activating mutations in Lrp5 and the high bone mass syndrome have low levels of circulating serotonin. Beyond these 2 devastating but rare diseases, 1 experiment suggested a broader clinical implication for enteric serotonergic regulation of bone mass[19] (Figure 1). Mice that totally lack *Tph1* had virtually no detectable circulating serotonin and a high bone mass phenotype because of an increase in bone formation parameters. Remarkably, even gonadectomized female $Tph1^{-/-}$ mice did not develop osteoporosis because their increase in bone formation parameters outperformed the increase in bone resorption caused by the gonadectomy. The implication of this experiment is that inhibition of serotonin biosynthesis in EC cells may provide a treatment for osteoporosis. This observation is potentially important clinically because the therapeutic arsenal against osteoporosis that is currently in use is mostly geared toward inhibiting bone resorption. There is no anabolic drug that could be taken orally to prevent or

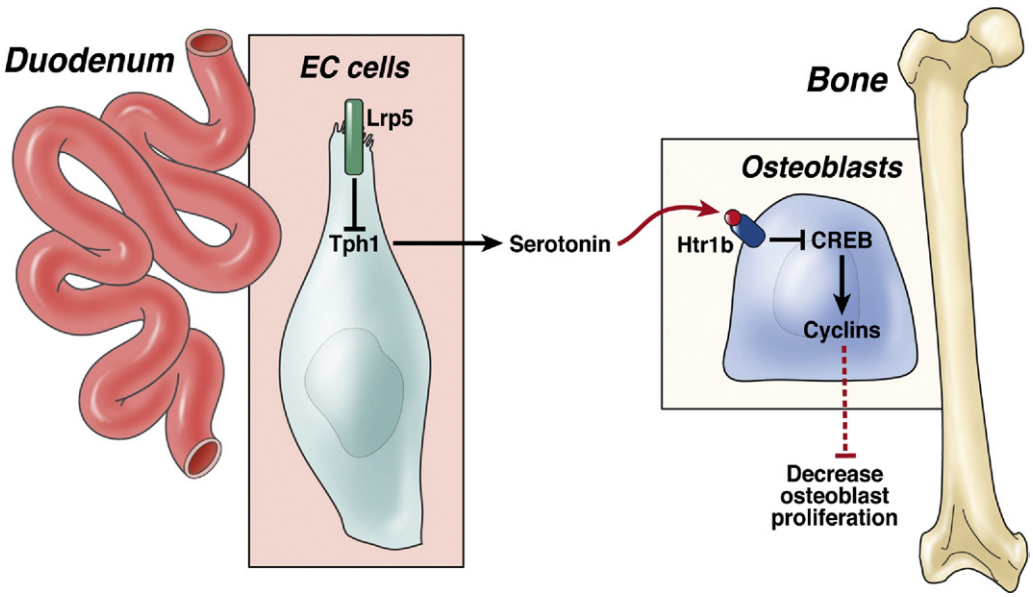

Figure 1. Gut-derived serotonin regulates bone mass accrual. LDL receptor related protein 5 (Lrp5) favors bone formation by inhibiting tryptophan hydroxylase 1 (*Tph1*) expression in enterochromaffin cells of the duodenum. Gut-derived serotonin, following its binding to Htr1B, inhibits *Creb* expression, which results in a decrease in *Cyclin* expression and osteoblast proliferation.

ameliorate osteoporosis. Fortunately, this potential importance can be tested in model organisms.

A small molecule inhibitor of Tph1 has been reported that can be taken orally and that, remarkably, had already been tested in humans at a high dose without reported deleterious adverse effects. This compound, called LP533401, interacts with the key amino acid in the hydroxylase moiety of TPH1 and inhibits the function of the enzyme.[23] A proof-of-concept experiment was thus conducted in a stepwise manner in mice and rats to determine whether TPH1 inhibition has potential in the treatment of osteoporosis. First, it was shown that when given orally once a day LP533401 dose dependently prevents the appearance of osteoporosis in gonadectomized mice. Remarkably, prevention of osteoporosis was achieved using a minimal dose and by reducing circulating serotonin levels only 30%. The second step in testing the serotonin hypotheses was to determine whether moderate decreases in gut serotonin biosynthesis is able to cure a preexisting osteoporosis in mice or rats. Osteoporosis was produced for this purpose by ovariectomizing animals 6 or 12 weeks before inhibiting TPH1. Here again, the answer was yes. Because of a purely anabolic mechanism of action, LP533401 therapy culminated in an increase in the number of osteoblasts. Because the rat is the animal of choice to test novel anti-osteoporotic drugs and injectable parathyroid hormone is the only available anabolic treatment for this disease, we asked whether inhibiting gut serotonin synthesis could cure osteoporosis in rats ovariectomized 3–12 weeks before the start of the treatment with LP533401 to inhibit TPH1 and whether it was at least as effective as parathyroid hormone in doing so.[23] Here again the answer was yes. LP533401 cured ovariectomy-associated osteoporosis in rats and was at least as effective in the regard as parathyroid hormone.

It has been demonstrated that gut-derived serotonin has additional hormonal and paracrine effects than that on bone. As mentioned, gut-derived serotonin is synthesized and secreted by EC cells. This cell population of the of the GI tract had first been described in 1897,[24] and, by 1937, they had been observed to contain a biogenic amine, which was initially called "enteramine,"[25] but turned out to be serotonin.[26] EC cells had long been recognized to be a component of a system of intestinal epithelial cells, now known as enteroendocrine cells that secrete hormones. Enteroendocrine cells are sensors that transduce luminal stimuli to the secretion of hormones and/or paracrine factors.[27,28] Experiments by Edith Bülbring and her colleagues soon confirmed that EC cells secrete serotonin in response to increases in intraluminal pressure.[29–31] Because the release of serotonin from EC cells also stimulated the peristaltic reflex, the concept that EC cells are paracrine regulators of GI motility became established.[32] Many studies over the years have confirmed this linkage and have expanded the list of luminal stimuli that cause EC cells to secrete. This list now includes acids, bile salts, tastants, olfactants, and nutrients.[33–35] Nerves presumably acting on basolateral surface of EC cells also regulate their secretion.[36–38]

The gut-derived serotonin has, at least, 3 other hormonal functions than that exerted on the bone. First, during pregnancy maternal production of serotonin is critical for embryonic morphogenesis.[39] Second, gut-derived serotonin can have effects on liver. Normal hepatic regeneration is distributed in mice lacking *Tph1* and restored when the animals are treated with 5-hydroytryptophan to overcome the block in serotonin biosynthesis.[40] 5-HT2A and 5-HT2B receptors seem to mediate the effect of serotonin on liver regeneration.[40] The lung is a third organ that gut-derived serotonin effects, sometimes resulting in adverse effects. Serotonin has been implicated as an important pathophysiologic contributor to pulmonary hypertension.[41]

Future Directions

The discovery of EC cells[24] was instrumental in the discovery that a hormone, initially secretin[27] and now many others, could be released in 1 organ and travel via the circulation to signal in another organ. The idea that serotonin served a paracrine function in the gut that was critical for motility and the high prevalence of irritable bowel syndrome riveted scientific attention to the local actions of gut-derived serotonin to the bowel. These ideas were not wrong, but it is now clear that serotonin has more than 1 function. This of course is only the beginning of a new chapter. The potential therapeutic importance of inhibiting gut-derived serotonin synthesis for the treatment of bone mass diseases makes it necessary to continue to understand how the synthesis of this hormone is regulated. In particular, what are the relevant ligands of Lrp5? A second question to be addressed has to do with serotonin itself, because most important hormones have more than 1 function, and thus, is it the case for serotonin? If yes, what are those functions? Answering these questions will contribute even further to underscore the importance of gut-derived serotonin.

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Conflicts of interest

The authors disclose no conflicts.