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Connexins in the skeleton

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ABSTRACT

Shaping of the skeleton (modeling) and its maintenance throughout life (remodeling) require coordinated activity among bone forming (osteoblasts) and resorbing cells (osteoclasts) and osteocytes (bone embedded cells). The gap junction protein connexin43 (Cx43) has emerged as a key modulator of skeletal growth and homeostasis. The skeletal developmental abnormalities present in oculodentodigital and craniometaphyseal dysplasias, both linked to Cx43 gene (*GJA1*) mutations, demonstrate that the skeleton is a major site of Cx43 action. Via direct action on osteolineage cells, including altering production of pro-osteoclastogenic factors, Cx43 contributes to peak bone mass acquisition, cortical modeling of long bones, and maintenance of bone quality. Cx43 also contributes in diverse ways to bone responsiveness to hormonal and mechanical signals. Skeletal biology research has revealed the complexity of Cx43 function; in addition to forming gap junctions and "hemichannels", Cx43 provides a scaffold for signaling molecules. Hence, Cx43 actively participates in generation and modulation of cellular signals driving skeletal development and homeostasis. Pharmacological interference with Cx43 may in the future help remedy deterioration of bone quality occurring with aging, disuse and hormonal imbalances.

Contents

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1. Introduction

Bone is constantly formed and degraded in order to adapt to mechanical and metabolic demands. The ability to coordinate the function of bone forming and resorbing cells is essential for translating tissue level mechanical and metabolic inputs to single cell effectors for the maintenance of bone quality. Gap junctional intercellular communication is an important mechanism of cell-to-cell communication in bone as in many other tissues. Gap junctions form by the pairing (docking) of two hexameric channels (connexons, or "hemichannels") on cell membranes of opposing cells to form an aqueous pore that permits the direct exchange of small molecules between the cytoplasm of the coupled cells. Connexons are composed by six subunits, connexins, which dictate the size and charge selective permeability of the gap junction channel [1,2] (Fig. 1). There are 20 connexin genes in the mouse and 21 (plus one pseudogene) in humans [3]. Although most commonly monomeric, connexons can form heteromeric connexons composed of different connexins. Heterotypic channels (docking of two connexons with different connexin composition) can also occur. This allows for large plasticity in the biophysical properties of gap junctions, dictating not only which molecules can be communicated, but also how the open/closed state of the channel is regulated, and what downstream signaling molecules can be recruited to the gap junction [4–6].

A metaphor of a computer network can describe the multicellular ensemble of gap junction-coupled cells. In this analogy, the cells represent the computers and the gap junctions loosely represent the modem/router. Connexin abundance determines the bandwidth, which can be fine-tuned through modulation of the open/closed state or abundance of the gap junction channels to affect actual bandwidth. Much like a computer network, the information exchanged across the intercellular network "administered" by gap junctions is diverse and dictates many functions. Hence, gap junctions can specifically restrict or permit certain types of information being exchanged among cells in the network. As discussed in this review, regulation of bone homeostasis by connexins exemplifies this concept (Fig. 2). Via different molecular mechanisms, connexin43 (Cx43)—the most abundant connexin present in bone cells (Figs. 3 and 4), modulates bone modeling and remodeling, the response to hormonal and mechanical stimuli, and the expression of osteo-anabolic and osteo-catabolic genes. While much less is known about other bone connexins, Cx40, Cx45 and Cx46, very recent data reveals distinct roles for Cx37 in bone development and homeostasis.

2. Connexins in human skeletal disease

In the last decade, mutations of Cx43 gene (*GJA1*) have been identified in patients with skeletal dysplastic syndromes. The increasing number of disease-causing Cx43 mutations and the breadth of the clinical spectrum related to such mutations provide a genetic demonstration that the skeleton is a major site of Cx43 action, a notion that had already emerged from animal studies. Advances in human genetics have also inspired the development of mouse models of human Cx43 disorders.

2.1. Oculodentodigital dysplasia

A large number of *GJA1* mutations have been found in patients with oculodentodigital dysplasia (ODDD), a disease affecting multiple organs but primarily the skeleton, with characteristic craniofacial abnormalities (skull hyperostosis, pointed nose, enamel hypoplasia), aplastic or hypoplastic middle phalanges, syndactyly, and broad tubular long bones [7–9]. Underscoring a predominantly autosomal dominant inheritance, Cx43 mutants found in ODDD typically act as dominant negative; they are assembled in gap junctions but the intercellular channel is functionally defective [10–12]. However in some families, the ODDD phenotype has a recessive inheritance pattern. Further, the ODDD clinical spectrum can be variable, underscoring the complexity of Cx43 function in the skeleton (reviewed in Ref. [9]).

Mouse models of ODDD have been developed. A germline mutant generated through *N*-ethyl-*N*-nitrosourea mutagenesis, called $Gja1^{Jrt/+}$, harbors a heterozygous *Gja1* mutation (G60S). Although this mutation is not found in humans, the skeletal features of $Gja1^{Jrt/+}$ mice reproduce many of the features of ODDD patients, including syndactyly, enamel hypoplasia, and craniofacial anomalies [10]. Additionally, bone mineral density is abnormally low in $Gja1^{Jrt/+}$ mice, with decreased trabecular bone volume and reduced mechanical strength, features not yet described in the human disease. $Gja1^{Jrt/+}$ mice also exhibit thin cortical bone and enlarged marrow cavity in the femoral diaphysis. In another approach, the *Gja1* G138R point mutant – found in several ODDD families – was used to replace one wild type *Gja1* allele using the Cre/*loxP* method. Induction of a global *Gja1* to $Gja1^{G138R}$ gene replacement using the ubiquitously expressed PGK-Cre in the mouse ($cODDD^{PGK}$) produced many of the multi-organ phenotypic features of human ODDD, including craniofacial abnormalities, but also decreased trabecular bone volume [12]. Conditional replacement of one wild type allele with the $Gja1^{G138R}$ allele in cells of the chondro-osteogenic lineage by *Dermo1/Twist2-Cre*, which is expressed starting at E9.5 ($cODDD^{TW2}$) recapitulates all the skeletal defects seen in the global $cODDD^{PGK}$ and $Gja1^{Jrt/+}$ mice, thus demonstrating that the osteogenic lineage is central for Cx43 modulation of skeletal development and homeostasis [13]. Consistent with the other two mouse ODDD models, whole body bone mineral density is reduced in $cODDD^{TW2}$ mice, and this is associated with cortical thinning and a pronounced widening of diaphyseal cross sectional area; while trabecular bone is essentially unaffected [13]. As discussed more in depth later, expansion of the marrow cavity and cortical thinning are also seen in mice with conditional *Gja1* ablation in the osteogenic lineage. Thus, ODDD mutations in the mouse phenocopy most of the human disease, but they also reveal additional features not described in humans. The most prominent differences are in the cranial vault. While the skull is thickened in the human disease, mineralization of the skull is delayed and defective at birth in $cODDD^{TW2}$ mice. Furthermore, low bone density has not been described in humans with ODDD, although the skeletal features of these patients have not been studied in detail. Notably, both the $Gja1^{Jrt}$ and the $Gja1^{G138R}$ mutants are dominant negative for channel function [10] and thus may also interfere with other connexins (e.g., Cx37, Cx40, or Cx45) in forming functional channels in cells that express multiple connexins, such as osteolineage cells. In so doing, they may also interfere with gap junctional communication between osteoblasts (or osteocytes) and other cells

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