J Musculoskelet Neuronal Interact 2006; 6(4):349-350

Hylonome

Perspective Article

TGF-β regulation of osteoblast differentiation and bone matrix properties

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Keywords: TGF-beta, Bone Matrix Material Properties, Runx2, Hearing Loss

With the promise of harnessing stem cells to generate skeletal tissue, remains the challenge of creating tissue of sufficient mechanical quality. Bone matrix material properties, in addition to bone mass and architecture, determine the ability of bone to resist fracture[1]. Several hormones and growth factors that control bone mass and architecture have been identified, including parathyroid hormone and female reproductive hormones[2,3]. However, little is known about the mechanisms that control the material properties of skeletal matrices. The material properties of the bone matrix are products of both the organic and mineral content. For example, individuals with osteogenesis imperfecta suffer from a high incidence of bone fracture because of collagen mutations that disrupt the organic and mineral structure of the bone matrix[4].

TGF-β regulation of bone matrix material properties

We have recently identified TGF-β as a key regulator of bone matrix mechanical properties and composition[5]. Bone matrix properties were tested in mice with genetic alterations in TGF-β signaling. These data were the first to show that the mechanical properties of bone matrix are regulated, specifically through a pathway including TGF-β, the TGF-β receptor, and Smad3. Thus, a partial reduction of TGF-β signaling increases several mechanical and compositional properties of bone, including increased bone matrix mechanical properties, mineral concentration, cortical thickness, trabecular bone volume, and fracture resistance. These conclusions were derived using atomic force microscopy (AFM), X-ray topographic microscopy (XTM), and 3-point bending.

The author has no conflict of interest.

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Accepted 30 July 2006

TGF-β repression of Runx2 function and terminal osteoblast differentiation

We previously showed that TGF-β inhibits terminal osteoblast differentiation by repressing Runx2, a critical transcriptional regulator of osteoblast differentiation[6]. Specifically, TGF-β activates Smad3 to bind and inhibit Runx2 function. We have since shown that histone deacetylases 4 and 5 (HDAC4/5) are required for this transcriptional repression[7] overexpression or knockdown of Smad3 and HDAC4/5 activity in retrovirally infected cells or by siRNA altered osteoblast differentiation. Therefore, we showed that Runx2, Smad3 and HDAC4/5 are required for TGF-β to inhibit terminal osteoblast differentiation. These findings are supported by the phenotypes of Smad3 and HDAC4 null mice, which exhibit premature chondrocyte and osteocyte terminal differentiation[8-10].

TGF-β regulation of Runx2 function *in vivo*

That Runx2 is downstream of TGF-β *in vivo* is supported by the appearance of a cleidocranial dysplasia-like phenotype in both Runx2+/- mice and in D4 mice that overexpress TGF-β in osteoblasts under control of the osteocalcin promoter[11-13]. Both mouse lines exhibit dysplastic or absent clavicles and patent cranial sutures. The similarity in the Runx2+/- and D4 phenotypes is consistent with the ability of TGF-β to repress Runx2 function, as was observed *in vitro*[6]. We are further investigating the extent to which TGF-β regulates Runx2 expression and function *in vivo*. Specifically, we are investigating whether Runx2 is also downstream of TGF-β in the control of bone matrix material properties.

Bone disease-associated hearing loss: bone matrix material properties in the ear

Though each bone has "signature" matrix mechanical properties, the functional significance of this local regulation remains unclear. However, the composition and mechanical properties

Disease	Observed defect	Refs.
Otosclerosis	Inappropriate bone remodeling, gene(s) unknown	19
Osteogenesis Imperfecta	Poor bone matrix mechanical properties due to collagen mutation	18
Paget's Disease	Reduced cochlear bone mineral concentration, gene(s) unknown	14
Camurati-Engelmann Disease	TGF-β mutation	17
Cleidocranial Dysplasia	Heterozygous loss of function mutation of Runx2	15,16

Table 1.

of bone are clearly important for normal hearing. Very little is known about the role of bone in hearing. Hearing loss is associated with a number of human bone diseases including otosclerosis, osteogenesis imperfecta, Paget's disease, Camurati-Engelmann disease, and cleidocranial dysplasia[14-19]. The defects in some of these human bone diseases are related to impaired bone matrix properties, TGF-β signaling, and Runx2 function (Table 1). This hearing loss can be conductive or sensorineural in origin. Although bone in the ear has several unique properties and is critical for the development of the neural structures of the ear, the role of bone in sensorineural hearing loss is not understood. We are currently investigating the role of bone matrix properties in hearing, as well as identifying the pathways that control these properties in the ear. Specifically, auditory brainstem response testing is used to measure hearing in mice with mutations that affect TGF-β or Runx2 function. The bone matrix material properties of the cochlear capsule are measured using atomic force microscopy with nanoindentation, while ear structure is examined histologically and radiologically. These studies provide an insight into the functional role of bone matrix material properties in auditory structure and function.

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