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CONTROL OF TGF-BETA RECEPTOR EXPRESSION IN BONE

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1. ABSTRACT

Bone growth and remodeling are controlled by local and systemic growth factors. The first local bone growth factor purified to homogeneity was transforming growth factor type beta (TGF-beta). On skeletal cells, TGF-beta has multiple effects mediated through at least three distinct cell surface receptors. More recent evidence demonstrated hormone and growth factor dependent alterations in TGF-beta receptor expression on osteoblasts *in vitro*. Indeed, certain biological responses appear to depend on the proportional expression of the type I TGF-beta receptor. Studies defining the type I TGF-beta receptor gene promoter then revealed that it contained several binding sequences for a nuclear factor that varies in parallel with expression of the osteoblast phenotype. New observations linking these events appear to enhance our understanding of this pivotal growth factor during osteogenesis and systemic bone disease.

2. INTRODUCTION

Transforming growth factor beta (TGF-beta) is a potent and ubiquitous growth regulator. It was first isolated from tumor extracts where it was thought to induce a "transformation" between normal and neoplastic cell growth. Since then, TGF-beta expression or its activity has been examined in essentially every tissue in the body. Surprisingly, its effects diverge considerably. Stimulatory, inhibitory, or negligible effects have been reported on the same biochemical processes, and even within cells from the same tissue lineage. Some variations relate to its release from inactive complexes, some to its concentration-dependent biphasic actions, and others to the differentiation status of target cells. Therefore, subtle control mechanisms must exist that regulate TGF-beta expression or its activity within tissues. All of these processes have also been noted in bone. Still, little is known about how the very same molecule produces such divergent effects, and especially, how these events change during tissue differentiation.

TGF-beta binding to cell surface components is altered by certain agents that activate or suppress bone cell activity. Moreover, systematic differences in the TGF-beta receptor profile can be observed between less and more differentiated bone cells, suggesting that differentiation-dependent molecular regulators must co-ordinate these transitions. In the last several years new molecular tools were developed to address these questions. As a result, work on TGF-beta receptor expression has converged with other studies demonstrating the importance of a specific nuclear factor that appears to drive osteogenesis. In this review, we describe the progression of results, obtained by us and other investigators, that revealed new insight into the control of TGF-beta receptor expression and TGF-beta activity in bone.

3. BONE GROWTH AND REMODELING

Bone is a dynamic tissue that forms and remodels throughout life. Bone formation increases during early life and declines during senescence.

With many diseases or certain pharmacological interventions, osteoblast activity and bone formation decline. Bone loss then accelerates and its structural quality is reduced. This accompanies an increase in fracture and pain, and a decrease in mobility and function. Loss of bone integrity and much of its subsequent pathology may therefore follow an imbalance between osteoblastic bone formation and osteoclastic resorption that define the symmetrical sequence of normal bone remodeling (1-3).

4. BONE GROWTH REGULATORS

Bone cells are regulated by systemic (hormone) and local (bone-derived) factors (4-6). Hormonal effects are well appreciated, but local bone growth factors have only been identified and characterized in the last dozen years. By mass, bone is the largest reservoir of growth factors in the body (5-8). Many local bone growth factors are synthesized by osteoblasts. Also, bone matrix is a tenacious substrate, and some matrix-bound growth factors may originate from other tissues and produce important local actions after their release from remodeling bone. Initial efforts in this field described direct effects by local growth factors on bone cell activity. Other studies then centered on interactions that occur between systemic and local factors (9-19 among many others). Studies with fetal rat bone cultures first demonstrated TGF-beta in skeletal tissue. Cells derived from rat calvariae were then used to visualize TGF-beta receptors on bone cells (reviewed previously in reference 20). This culture model also provided the first evidence for detrimental effects by glucocorticoid on TGF-beta binding and function in any tissue, and new information for specific variations in TGF-beta receptors in parallel with expression of the osteoblast phenotype (19,21). Results from these and other similar *in vitro* studies therefore began to predict that the effects of some bone growth regulators could converge with those induced by TGF-beta, perhaps in large part by changing the levels of specific TGF-beta receptors.

5. TGF-BETA SUPERGENE FAMILY AND BONE

Over 12 years ago, the growth factor isolated from rat bone culture medium, termed bone derived growth factor I, was identified as a homologue of TGF-beta type 1 (TGF-beta1) obtained from tumor extracts and blood platelets. Cartilage inducing factors from bovine bone were then recognized as TGF-beta isoforms, and it soon became clear that bone cells synthesize TGF-beta and that bone matrix is a major storage site for TGF-beta in the organism (20). The TGF-beta supergene family includes three isoforms of TGF-beta itself. Other less closely related gene family members are the bone morphogenetic proteins [BMPs]. The BMPs are stored in bone matrix and increase bone cell activity *in vivo* and *in vitro*, verifying a prominent role for TGF-betas and related molecules on bone formation (22-25). Many results now indicate independent roles for individual members of the TGF-beta supergene family and their receptors. The TGF-betas themselves are disulfide-linked dimers of 25 kilodaltons (kDa) (26,27). They are synthesized and released from cells in inactive complexes containing amino-terminal cleavage products, and in many cases, latent TGF-beta binding protein [LTBP]. LTBP is not found in medium from mouse bone cultures and may not be necessary for latency, but it could direct secreted TGF-beta to matrix storage pools (28,29). Latency might protect nascent TGF-beta from proteolysis and maintain a source of activatable TGF-beta. Activation of latent TGF-beta may be enzymatic. However, during bone resorption, TGF-beta might also be released from bone matrix and activated by the acidic micro-environment beneath the sealing zone of osteoclasts (26,30).

6. TGF- BETA RECEPTORS

On osteoblasts,TGF-beta binds to high affinity receptors like those found on many other cells (11,31). Studies in chemically mutated mink lung epithelial cells implicated TGF-beta receptor I [TGF-betaRI of 53 kDa] and TGF-beta receptor II [TGF-betaRII of 75 kDa] in signal transduction (32). Different mutant mink lung cells were then isolated that express various levels of TGF-beta receptors. When intact or mutant TGF-beta receptors were transfected back into the mutated cells, TGF-beta appears first to bind TGF-betaRII. This complex can recruit TGF-betaRI, which itself also associates directly with TGF-beta. In this context, the kinase domain of TGF-betaRII phosphorylates TGF-betaRI, and downstream signals follow (33-36). Thus, TGF-betaRI is indispensable for TGF-beta activity. TGF-beta also binds with lower affinity to proteoglycans of >250 kDa, first termed TGF-beta type III receptors [TGF-betaRIII], but now also called betaglycans (37-39). Cell surface betaglycans do not themselves initiate a biochemical event, but may facilitate extracellular storage of TGF-beta, control its activation, or regulate its binding to other TGF-beta receptors (40,41). Consequently, the proportion rather than the actual amount of each of TGF-beta receptor may determine the extent of ligand binding in total, or it may focus TGF-beta to inactive, potentially active, or active receptors or receptor complexes (21). Receptors for several of the TGF-beta supergene family members appear similar to TGF-betaRI and/or TGF-betaRII. While interactions between TGF-betaRI, TGF-betaRII and receptors for other TGF-beta supergene family members have been reported in reconstituted cell models, homologous ligand/receptor binding appears to be specific in the normal situation (42-51). For example, bone cells express receptors for several

TGF-beta supergene family members, but they preferentially bind TGF-beta at conventional TGF-betaRI, TGF-betaRII, and betaglycan (11,21,23,45). Other less well characterized TGF-beta receptors (20,31) have not been reported on osteoblasts. Again, many studies including those in bone predict changes in TGF-beta receptors during development, aging, or in response to specific hormones (11,19,21,52-58).

The cDNAs for TGF-betaRI, TGF-betaRII, and betaglycan core protein, all prominently expressed by bone cells, are cloned (33,36,46,59-61), and specific antibodies are available to evaluate changes in TGF-beta protein. However, to understand how the expression of genes encoding the TGF-beta receptors are controlled during bone development, remodeling, and disease, promoter analysis is essential. Until recently only the TGF-betaRII promoter was cloned (62,63). Because TGF-betaRI is maintained with bone cell differentiation (21) and is the essential component for all known TGF-beta dependent effects (31), our lab cloned the rat TGF-betaRI promoter (64) to assess *cis*- and *trans*-acting elements that regulate TGF-betaRI synthesis by bone cells. Aspects of these studies are described later in this review.

7. EFFECTS OF TGF-BETA ON BONE CELL ACTIVITY

In vivo, local administration of TGF-beta increases bone formation (65-67). However, the molecular mechanisms of TGF-beta activity in bone have principally been determined by studies with primary and continuous cultures of bone cells that express varying degrees of the osteoblast phenotype. TGF-beta modestly enhances replication of fibroblasts and undifferentiated periosteal cells, but it is a potent mitogen in osteoblast-enriched cultures from fetal rat bone. The stimulatory effect of TGF-beta on DNA synthesis decreases at high TGF-beta concentrations and in cell cultures derived from more mature organisms, and it inhibits replication by certain osteosarcoma derived cells that are thought to represent highly differentiated osteoblasts. The mitogenic effect of TGF-beta therefore appears to be focused on cells that are first prominent at an intermediate stage of bone development. In order to allow effective skeletal tissue formation, cell populations like these must need to re-emerge during bone remodeling or fracture repair (20,21,68).

TGF-beta also alters the expression of many genes related directly to osteoblast activity. Consistent with its activity in many tissues, TGF-beta induces the synthesis of several matrix proteins by bone cells. Type I collagen comprises 90% of the organic matrix of bone and it is an essential element in skeletal calcification and structure. TGF-beta enhances type I collagen synthesis and the apposition of a collagen-containing matrix in cultures of isolated bone cells and in bone fragments (69-72). Studies in osteoblast-enriched cultures show that part of this effect is transcriptional, part is related to type I procollagen mRNA stability, and part derives from an increase in collagen secretion and its subsequent re-deposition to the cell layer (71). However, in cells prepared from human bone obtained after hip replacement surgery, TGF-beta only transiently increases type I procollagen mRNA and polypeptide levels, suggesting differences that could derive from disease state, age, or perhaps species variations (72). TGF-beta also induces the expression of other bone matrix components such as osteopontin and osteonectin. In contrast, it reduces the expression of a late stage osteoblast-derived protein, osteocalcin, in several osteoblast-like or osteosarcoma-derived cell cultures. Like the effect that occurs with most mitogenic factors, short term exposure to TGF-beta reduces the activity of alkaline phosphatase, a protein thought to be involved in matrix calcification, in fetal bone cells, but enhances its expression by some osteosarcoma and mature human bone cells (20,72). Furthermore, TGF-beta rapidly re-organizes its own receptor profile on osteoblasts (73). Overall, the response patterns that occur in osteoblasts derived from fetal, neonatal, mature and osteoporotic bone, and from osteosarcomas predict distinct changes in sensitivity to TGF-beta at specific stages of osteoblast differentiation.

Variations in TGF-beta activity could perhaps in part be related to one or another isform of TGF-beta. Indeed, in osteoblast-enriched cell cultures, TGF-beta3 is 3-10 fold more potent than TGF-beta1 or TGF-beta2. However, all three TGF-betas bind to the same three TGF-beta receptors, although with slightly different affinities, and in the end cause the same qualitative effects on osteoblast activity (74,75). Therefore, unlike the patterns that are found with endothelial cell cultures (41), dissimilarities in TGF-beta activity on bone cells do not seem to result from differences in the association of certain TGF-beta isoforms with only specific combinations of TGF-beta receptors.

8. REGULATION OF TGF-BETA RECEPTORS ON BONE CELLS

Variations in TGF-beta receptor levels appear to parallel the changes in TGF-beta activity that occur as bone cell differentiation proceeds. Notably, the relative amounts of betaglycan, measured by both radio-iodinated TGF-beta binding assay and by mRNA analysis, are significantly lower on differentiated osteoblasts (11,21,68); this predicts that betaglycan expression is reduced as osteoblasts mature. The proportions of TGF-betaRI and TGF-betaRII also vary, and do so independently from each other, during this process. Specifically, in fetal

rat bone cells, the relative amount of TGF-beta binding to TGF-betaRII also decreases when osteoblast-like activity increases and betaglycan levels fall, while the proportion of TGF-beta binding to TGF-betaRI increases significantly. These patterns too are consistent with changes in steady state mRNA levels for TGF-betaRI and TGF-betaRII (21).

Some studies in the pre-osteoblastic neonatal murine MC3T3-E1 cell line suggest large decreases in all three TGF-beta receptors and in TGF-beta function during the processes of collagen matrix deposition and mineralization (76,77) *in vitro.* Changes like this do not appear to occur in primary osteoblast-enriched cell cultures from fetal rat bone (45, and other unpublished studies). These differences may relate to the phenotype of MC3T3-E1 cells that endogenously express very little TGF-betaRI (68). Thus, in the absence of specific osteogenic inducers, changes in the proportion of TGF-betaRI may be difficult to note when its levels are initially so low. In contrast, and perhaps more consistent with results in primary bone cell cultures, immunohistochemical staining during mouse organogenesis showed a more ubiquitous staining pattern for TGF-betaRI than for TGF-betaRII. In these studies, staining for TGF-betaRII was preferentially found in regions of undifferentiated cells that thereafter differentiated into bone (78), much like the changes that occur in fetal rat cell cultures (21). Furthermore, in human osteosarcoma derived MG-63 cell cultures, differentiation induced by 1,25 dihydroxyvitamin D3, suppresses the levels of TGF-betaRII in parallel with a decrease in certain aspects of TGF-beta activity (79).

Agents that increase TGF-beta binding to betaglycan tend to reduce the stimulatory effects of TGF-beta on osteoblast function. Small but significant increases in binding occur in response to parathyroid hormone, and larger and more sustained effects occur with glucocorticoid treatment (11,19,80). However, although betaglycan levels can vary dramatically on bone cells with differentiation (described above), with these osteotropic hormones, or with growth factor treatment (see below), lack of promoter information has severely limited our ability to understand how these events occur at the molecular level. Glucocorticoid decreases TGF-beta binding to a small extent to TGF-betaRII, an effect that increases with longer exposure to hormone (19). Nonetheless, initial studies with TGF-betaRII promoter constructs (63) show no consistent changes in promoter activity when bone cells are treated with glucocorticoid , or with BMP-2, TGF-beta, PGE2, or retinoic acid (unpublished data). These results and earlier studies showing large changes in betaglycan in response to these agents (19,21,73, and unpublished data) support that TGF-beta binding to TGF-betaRII is in part regulated indirectly by the proportion of betaglycan present on the cell surface (21,41,81). Little else is currently known about the molecular mechanisms that mediate changes in betaglycan or TGF-betaRII expression in bone cells or in any tissue.

In contrast to small or negligible changes in TGF-betaRII, the proportion TGF-beta binding to TGF-betaRI is rapidly and significantly affected by positive and negative regulators of osteoblast activity (19,21). New studies, described in more detail below, predict how changes in TGF-betaRI expression may occur by variations in the level of a more recently appreciated, osteoblast-restricted nuclear factor, CBFa1.

As described earlier, other TGF-beta supergene family members that regulate skeletal cells include the BMPs. These agents were first defined by a functional assay where they initiated cartilage formation that was then replaced by bone at ectopic sites *in vivo* in the rat (20,22). Some BMPs are more effective on osteoblasts, and others are more effective on less differentiated bone cells. Certain effects induced by several BMP family members are distinct from those induced by TGF-betas, and in some instances differ even from each other. BMPs enhance the synthesis of cartilage proteoglycan, alkaline phosphatase, osteocalcin, and PTH receptors in cultures of uncommitted stromal cells, chondrocytes, and/or osteoblast-like cells (21,45,81-92). Mutations in the mouse *short ear* gene (that is genetically related to human BMP-5) correlate with abnormal growth, skeletal formation, and fracture repair in these animals (93). BMPs act through specific BMP receptors and do not directly compete for binding at high affinity TGF-beta receptors. Nevertheless, BMP-2 and BMP-4 potently alter TGF-beta binding on osteoblasts. In direct contrast to the effects of glucocorticoid, these BMPs rapidly decrease TGF-beta binding to TGF-betaRII and betaglycan, and increase its binding to TGF-betaRI. In so doing, BMP treatment alters the effects of TGF-beta on bone cell replication, matrix protein synthesis, and alkaline phosphatase activity in patterns that are consistent with progressive increases in osteoblast differentiation (21). Other studies recently showed that BMP-2 can oppose the inhibitory effect of glucocorticoid on TGF-beta binding and activity. Surprisingly, BMP-2 also suppresses the stimulatory effect of glucocorticoid on a gene promoter containing a positive glucocorticoid response element (94).

Some osteoblast regulators may therefore alter TGF-beta induced osteoblast activity by re-distributing TGF-beta binding among its various receptors. Changes in TGF-beta binding may in part depend on new TGF-beta receptor synthesis (19,20,73). While these findings suggest that effects by several regulators of bone cell activity converge at

the molecular level on TGF-beta receptor expression, detailed information requires the new molecular tools that have become available only recently.

9. TGF-BETA RECEPTOR I PROMOTER

Consequently, variations in TGF-beta receptors can define the extent or the nature of TGF-beta activity for bone cells. Initial studies to understand how this occurs suggested a very short half-life of 2-6 h for cell surface TGF-beta receptor proteins in untreated bone cell cultures. In some instances, there is a rapid recovery after hormone or growth factor treatments. Measurements of transcript half-lives of 6-20 h for TGF-beta receptor mRNAs show reasonable turnover rates, and predict that constitutive expression is needed to maintain an adequate supply of cell surface TGF-beta receptor protein (73). Again, TGF-betaRI is essential for TGF-beta activity. Its mRNA half-life is short (6-7 h) by comparison to TGF-betaRII and betaglycan (17-20 h), and its expression varies in appropriate ways on bone cells with differentiation and in response to specific bone growth regulators. To examine transcriptional control of TGF-betaRI in better detail, its promoter was cloned in our lab from a rat genomic library (64). Consistent with the widespread importance of TGF-beta in most tissues, the organization of the TGF-betaRI promoter is like many constitutively expressed genes. The rat TGF-betaRI promoter is very similar to the human TGF-betaRI promoter that was cloned by others simultaneously (95). However, reporter constructs used to analyze the human TGF-betaRI promoter failed to include important upstream and downstream control elements that our lab established by sequence, reporter, gel shift, and mutation analyses. Deletions and point mutations showed that two downstream CCAAT boxes in the TGF-betaRI promoter do not contribute to its expression in osteoblasts. In contrast, the promoter contains multiple binding sequences for transcription factor Sp1, a condition that is often associated with constitutive gene expression (96,97). The many Sp1 binding sequences may contribute in part to multiple transcription start sites (64). Also, at least one Sp1 site is essential for basal TGF-betaRI promoter activity (96). Even so, and consistent with initial observations of TGF-betaRI mRNA and protein (21), the TGF-betaRI promoter is significantly more active in more differentiated bone cells (64). This predicted the presence of tissue specific *cis*-acting elements within the TGF-betaRI promoter itself, and specific *trans*-acting factors within osteoblast nuclei (96,98). Consistent with this possibility, there are four separate binding sequences for members of the CBFa transcription factor family within the maximally active 1.0 kb region of the TGF-betaRI promoter, and two others within the next 5' 0.1 kb (98).

10. NUCLEAR FACTOR CBFa

The CBFa transcription factors were independently identified by several labs and termed CBFs, PEBP2 alphas, AMLs, or NMPs. Due to confusion with names of diseases or other transcription factor families, it has been suggested that the two subunits for these factors should be termed as CBFa and CBFb. Thus, active nuclear factor contains one CBFa subunit (CBFa1, CBFa2 or CBFa3), and a common CBFb subunit that greatly increases CBFa subunit binding to DNA (99-102). CBFa subunits contain a so-called *Runt* homology domain, derived from its similarity to a transcription factor involved in cell fate associated with body segmentation, sex determination, and neurogenesis in *Drosophila*. The CBFa *Runt* domain contains binding sites for DNA and for the CBFb subunit. The COOH-terminal region of the CBFa subunit contains a transactivation domain required for CBFa dependent gene transcription. CBFa1 and CBFa2 also contain sites that can be phosphorylated by components of the mitogen activated protein (MAP) kinase system that are activated in response to certain growth factors and cytokines, and perhaps phorbol esters. Phosphorylation may have a potent stimulatory effect on CBFa dependent gene transcription (103,104). CBFb subunits tend to be ubiquitous and over expressed, and uncomplexed CBFb subunits accumulate in the cytoplasm (99). Gene expression regulated by CBFa can therefore result from: 1) variations in three different CBFa subunits that themselves may distinguish several *cis*-acting DNA binding sequences in subtle ways; 2) by the amount or the intracellular location of the common CBFb subunit; 3) or by growth regulators that activate or suppress osteogenic cell function.

In addition, other proteins can associate with the CBFa or CBFb subunits independently. For example, transcription factors termed C/EBPs physically interact with CBFa2 through the *Runt* domain common to all CBFa subunits, and synergistically enhance M-CSF receptor (c-*fms*) gene expression in macrophage-like cells (105). Other interactions have also been noted with nuclear factors c-Myb and with Ets family members in the neutrophil elastase and myeloperoxidase promoters (106,107). Functional levels of C/EBP beta and C/EBP delta are found in osteoblasts (108) predicting that similar interactions may occur in bone cells. CBFb subunits have wider tissue distribution than CBFa subunits. This and the existence of a large CBFb cytoplasmic pool, suggests that other binding partners exist, or modifications are needed to promote nuclear localization or association with CBFa subunits, but how this occurs is not yet clear (100, and personal communications from Dr. Yoshiaki Ito, Kyoto University, Japan; and Dr. J. Peter Gergen, State University of New York at Stonybrook).

11. CBFa, TGF-BETA RECEPTOR I, AND BONE

The function of CBFa as a nuclear regulator initially seemed restricted to the expression of genes important to blood cell differentiation in higher organisms. Compatible with this, some leukemias correlate well with translocations in the CBFa or CBFb subunit genes. In two cases, breaks occur in the COOH-terminal transactivation region of the CBFa subunit. This perhaps allows strong binding by the intact *Runt* domain to the CBFb subunit and to DNA, even while it prevents effective transactivation at target gene promoter sites. Another translocation occurs at a site upstream of the *Runt* domain, and another within the CBFb subunit. While each effectively alters target gene

expression in lymphoid cells, the molecular details of these events are not yet resolved (109,110). For example, new studies from Dr. Ito's lab reveal that a splice variant of CBFa2, lacking a portion of the *Runt* domain, inhibits CBFa dependent T cell receptor promoter activity, myeloid cell proliferation and differentiation independently of its ability to bind DNA (111). Consistent with the widespread effects of CBFa-like transcription factors in *Drosophila,* other recent studies reveal the presence of these factors in muscle (112).

Work from Drs. Jane Lian and Gary Stein (University of Massachusetts Medical Center, Worcester), and Dr. Gerard Karsenty (MD Anderson Cancer Center, Houston) further reveal that CBFa may be an important regulator of osteocalcin expression by osteoblasts (113-118). One CBFa binding site may contribute as much as 75% to the activity of the osteocalcin promoter in osteoblast-like cells, perhaps accounting in part for the tissue-specific expression of this protein (116). Most importantly, after the osteocalcin studies and work from our lab with the TGF-betaRI promoter were reported (1996 meeting of the American Society for Bone and Mineral Research, abstract #56), Dr. Toshihisa Komori (Osaka University, Japan) produced mice in which the gene for the CBFa1 subunit was "knocked-out". Animals died very soon after birth and suffered severe osteogenic deformities, while cartilage formation appeared intact (119). Analogous results were seen by Dr. Michael J. Owen's group (IRCF, London) (120). Mineralized skeletal elements consistent with osteoblast-dependent bone formation were not evident in these animals, and few if any cells with osteoblast morphology were apparent. Consistent with insertion, deletion or missense mutations in CBFa1 that occur in humans, these mice are considered models for the skeletal disorder cleidocranial dysplasia (119-121). However, genes directly effected by CBFa1, especially those important for skeletal development, are difficult to determine when the factor is absent or dysfunctional, and when osteoblasts are absent or hard to detect. Hormone dependent decreases in CBFa1 could also challenge skeletal integrity and more readily indicate important downstream targets. In this regard, our lab noted changes in CBFa1 expression with osteoblast differentiation (98), and that glucocorticoid rapidly and potently suppressed CBFa1 levels, CBFa1 binding to the TGF-betaRI promoter, TGF-betaRI promoter activity, TGF-betaRI mRNA and protein levels, and TGF-beta function in primary osteoblast cultures (122). We also find increases in CBFa1 expression by osteoblasts in response to BMP-2 (unpublished studies). These studies establish new molecular links in osteoblasts to CBFa1 with regard to important extracellular bone cell regulators and to a new genomic target, TGF-betaRI. These findings, changes in the expression of TGF-betaRI during osteoblast differentiation, and the importance of TGF-beta and TGF-betaRI for osteoblast activity suggest that our unique new observations with the TGF-betaRI promoter will significantly influence our understanding of TGF-beta biological activity in skeletal tissue, and of CBFa1-dependent control of bone cell function.

12. PERSPECTIVE

TGF-beta is abundant in bone and is a potent regulator of osteoblast activity. The TGF-beta receptor profile varies with the state of osteoblast differentiation. Notably, the proportion of TGF-betaRI fluctuates in response to important systemic and local agents, consistent with changes in osteoblast function. To understand this in molecular detail, the TGF-betaRI promoter was cloned, and found to contain several binding sequences for tissue-restricted transcription factors termed CBFa. Several labs have now shown enrichment for one CBFa subunit, CBFa1, with osteoblast differentiation. Loss of CBFa1 by gene elimination limits skeletal tissue formation essentially to chondrogenesis. Furthermore, initial evidence indicates specific variations in CBFa1 in response to positive and negative regulators of the osteoblast phenotype. Studies in nonskeletal cells predict important interactions between CBFa and other nuclear proteins, and regulation of CBFa activity by transcriptional and post-transcriptional events. Our observations revealing molecular links between CBFa1 and TGF-betaRI provide further evidence that TGF-beta is an important factor in osteogenesis. Changes in TGF-betaRI expression provide a tight and phenotype dependent gatekeeper system that controls how the effects of TGF-beta are perceived. New information to understand the normal mechanisms that control TGF-betaRI through CBFa1 expression and activity in bone cells will improve our understanding of its important role in skeletal tissue growth, remodeling and repair.

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