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TGF-ß and New Bone Formation

TGF- ß y Neoformación Ósea

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SUMMARY: The aim of this work is to present, by a literature review, the principal characteristics of TGF-beta, in the regulation and new bone formation.

KEY WORDS: TGF-beta; Bone.

INTRODUCTION

Fractured bones heal by a cascade of cellular events in which mesenchymal cells respond to unknown regulators by proliferating, differentiating and synthesizing extracellular matrix (Franceschi *et al.,* 2004). Current concepts suggest that growth factors may regulate different steps in this cascade (Proceedings of the Society for Experimental Biology and Medicine, 1992). Recent studies suggest regulatory roles for PDGF, αFGF, ßFGF, and TGF-ß in the initiation and the development of the fracture callus (Franceschi *et al*., 2004). Fracture healing begins immediately following injury, when growth factors, including TGF-ß 1 and PDGF are released into the fracture hematoma by platelets and inflammatory cells. TGF-ß1 and FGF are synthesized by osteoblasts and chondrocytes throughout the healing process. TGF-ß 1 and PDGF appear to have an influence on the initiation of fracture repair and the formation of cartilage and intramembranous bone in the initiation of callus formation (Vladimirov & Dimitrov, 2004).

Acidic FGF is synthesized by chondrocytes, chondrocyte precursors and macrophages. It appears to stimulate the proliferation of immature chondrocytes or precursors, and indirectly regulates chondrocyte maturation and the expression of the cartilage matrix (Takahashi et al., 2005). Presumably, growth factors in the callus at later times regulate additional steps in repair of the bone after fracture. These studies suggest that growth factors are central regulators of cellular proliferation, differentiation and extracellular matrix synthesis during fracture repair. Abnormal growth factor expression has been implicated as causing impaired or abnormal healing in other tissues, suggesting that altered growth factor expression also may be responsible for abnormal or delayed fracture repair (Tsubone *et al*., 2006). As a complete understanding of fracture-healing regulation involves, we expect new insights into the etiology of abnormal or delayed fracture healing and possibly new therapies for these difficult clinical problems.

Thus, the aim of this work is to present, by a literature review, the principal characteristics of transforming growth factor superfamily, in the regulation of the bone formation and wound healing bone.

DISCUSSION

Transforming growth factor ß in the regulation of bone formation and repair. Although nearly all cells synthesize and respond to TGF-ß, the highest levels of this peptide growth factor have found in bone and cartilage (Seyedin *et al.*, 1986). TGF- ß has also been found to be an important regulator of extracellular matrix synthesis and degradation, suggesting a potentially critical function of this growth factor in the regulation of bone formation and repair (Franceschi *et al*., 2004).

Transforming growth factor ß is present at sites of bone formation and repair. Using polyclonal antibodies raised against a synthetic peptide corresponding to the amino

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terminus of the mature TGF-b molecule, it has been possible to localize TGF-ß to areas of bone formation and repair in vivo. Two different polyclonal anti-TGF-ß antibodies have been reported to date, both raised against a similar amino acid sequence (Ellingsworth *et al.*, 1986; Flanders *et al.*, 1988). Interestingly, one antibody seems to recognize only intracellular TGF-ß, while the second antibody recognizes only extracellular peptides. The reason for this divergent pattern of localization has not been elucidated. Immunohistochemical studies of 11 to 18 day old mouse embryos using anti- TGF-ß antibodies have demonstrated positive staining for TGF-ß in tissues of mesodermal origin, such as connective tissue, cartilage and bone (Heine et al., 1987). In particular, intense staining for TGF- ß was observed in tissues actively participating in cartilage and bone formation. These include not only areas of intramembranous ossification, such as the developing calvarium, but also areas of endochondral ossification, including vertebral bodies and long bones (Heine *et al*.). TGF-ß immunostaining has also been demonstrated in the chondrocytes of the growth plates of more mature animals, which increase the length of long bones through the endochondral mechanism (Jingushi *et al*., 1990).

TGF- ß is also present at sites of bone repair. Immediately following fracture, TGF-ß staining can be appreciated in the area of the fracture hematoma and proliferating periosteal mesenchymal cells (Joyce et al., 1990a; Franceschi *et al*., 2004). At later stages of fracture healing, TGF-ß is evident within osteoblasts forming the intramembranous hard callus as well as within chondroprogenitor cells and chondrocytes comprising the soft callus (Chen *et al*., 2003). It is hypothesized that TGF-ß and perhaps other growth factors released from degranulating platelets in the fracture hematoma, initiates the cascade of cellular events resulting in bone and cartilage formation during fracture repair.

Osteoblasts synthesize and respond to transforming growth factor ß *in vitro.* Both primary osteoblasts and transformed osteoblasts synthesize and respond to TGF-ß in culture. The synthesis and release of TGF-ß from these cells can also be modulated by systemic hormones known to regulate bone formation and resorption. Primary osteoblasts cultures have been used to demonstrate that these cells transcribe mRNAs for TGF-ß, translate this message at high levels and secrete TGF-ß protein in its latent form into the culture medium (Robey *et al.*, 1987).

Hormones involved in the systemic regulation of mineral metabolism also modulate the synthesis of TGF-ß by osteoblasts. Factors that are known to increase serum calcium by stimulating bone resorption, such as parathyroid hormone and 1,25-dihydroxyvitamin D3, increase the TGF-ß activity in the culture medium conditioned by osteoblasts (Pfeilschifter & Mundy, 1987). Estradiol has also been shown to increase the transcription of TGF-ß mRNA by osteoblasts, although levels of TGF-ß protein synthesis were not measured (Komm *et al.,* 1988).

TGF-ß has multiple actions in almost every cell type studied and bone-forming cells are no exception. As with other cells, these effects depend on the stage of differentiation of the target cells, the culture conditions and the presence or absence of other growth factors. In osteoblastic cells, conflicting results have been reported on the effect of TGF-ß depending on whether the target cells were primary osteoblasts or transformed osteoblasts. In general, however, TGF-ß has been found to have a biphasic mitogenic effect on cultured osteoblasts and stimulates synthesis of collagen and noncollagenous extracellular matrix proteins. In addition, TGF-ß inhibits some feature of the fully differentiated osteoblast phenotype, such as alkaline phosphatase activity and osteocalcin synthesis.

Although TGF-ß inhibits the growth of many cells types, it appears to be a biphasic stimulator of mitogenic activity by primary osteoblasts and MC3T3 cells (Centrella *et al.*, 1987). At low concentrations, TGF-ß stimulates osteoblasts chemotaxis as well as DNA synthesis and cell division (Pfeilschifter *et al.,* 1990). At higher concentrations, however, this mitogenic activity is decreased. In addition to its effects on cell growth and migration, TGF-ß also promotes the synthesis of bone matrix proteins by primary and transformed osteoblasts.

Although TGF-ß appears to promote osteoblastic synthesis of several of the proteins found in bone matrix, other markers of the terminally differentiated osteoblast are inhibited. These include synthesis of the osteocalcin, which is believed to be secreted late in osteoblast development, as well as alkaline phosphatase activity, which is necessary for matrix mineralization (Noda, 1989; Vukicevic *et al.*, 1990).

Whether TGF-ß exerts its effect on bone matrix synthesis by direct mechanism is controversial. Although increases in gene transcription for type I collagen and other matrix proteins have been observed, post-transcriptional and post-translational regulatory events have not been ruled out (Centrella *et al.*, 1991). Robey *et al.* also suggested that the increase in collagen accumulation seen in primary bovine osteoblast cultures in response to TGF-ß results from mitotic expansion of the population of collagen-producing cells. It is possible that multiple direct and indirect control mechanism may be utilized in the regulation of bone matrix synthesis by TGF-ß in vivo.

Although TGF-ß stimulates the growth of primary osteoblasts and promotes collagen synthesis by these cells, conflicting results have been obtained using transformed osteoblasts as the target cell population. In ROS 17/2 and UMR 106 cells, TGF-ß has been found to inhibit cellular proliferation and stimulate expression of the differentiated osteoblastic phenotype (Pfeilschifter *et al.,* 1987). The biological significance of these conflicting results is unclear. Transforming growth factor ß is a potential coupling factor linking bone formation and resorption.

Indirect evidence is accumulating to implicate TGF-ß as a potential coupling factor during bone remodeling, coordinately regulating the processes of matrix synthesis and matrix resorption (Zhu *et al.*, 2006).

TGF-ß is stored in the mineralized bone matrix in its latent form and can be activated by exposure to a low pH environment similar to that generated by the osteoclast during bone resorption. It has been demonstrated that latent TGF-ß added to osteoclastic cell cultures is activated to produce the biologically active peptide in vitro (Oreffo *et al.*, 1989).

TGF-ß has also been shown to promote bone matrix synthesis and inhibit matrix degradation. The positive effect of TGF-ß on osteoblast chemotaxis, proliferation and matrix synthesis has already been outlined. TGF-ß has also been shown to inhibit bone resorption by inhibiting both the formation and activity of osteoclastic cells. In several studies of osteoclast differentiation, TGF-ß blocks the conversion of circulating monocytes to multinucleated osteoclasts (Chenu *et al.*, 1988). TGF-ß also directly suppresses the bone-resorbing activity of osteoclasts in both avian and rodent models (Pfeilschifter *et al.*, 1988).

Transforming growth factor ß induces bone and cartilage formation *in vivo*. The conflicting data on the effect of TGF-ß on cultured osteoblasts has necessitated in vivo experiments to clarify further the role of this peptide in bone formation. Several reports now demonstrate that TGF-ß injected into the area of periosteum will result in bone formation by osteoblasts in vivo (Joyce *et al.*, 1990a; Noda and Camilliere, 1989; Marcelli *et al*., 1990). This induction of bone formation is apparent 3 days after two daily injections of TGF-ß in doses ranging from 50ng to 5µg. The bone that is formed in response to injection of TGF-ß appears to mineralize normally and persist for at least 40 days following injection.

The pathway of bone formation resulting from TGF-ß injection depends on the type of bone injected. Injection of flat bones results in intramembranous ossification, whereas injection of long bones results in both intramembranous and endochondral ossification. It is interesting to note that these are the same pathways of bone formation involved in the development and repair of these respective types of bone. In fact, when TGF-ß is injected into long bone periosteum for 14 days, the resulting bone and cartilage tissue bears a striking histological resemblance to the pattern of bone and cartilage formation associated with fracture healing. This suggests that TGF-ß may be an important regulator of bone repair by initiating the sequence of cellular events that lead to bone formation following injury (Celeste *et al*., 1990; Tsubone *et al.*).

Members of the TGF-ß superfamily involved in the molecular bone repair:

PDGF. Platelet rich plasma has been produced, yielding a four time higher number of thrombocytes than normal plasma. Degranulation of platelets has been performed by means of calcium and thrombin. Plasma has served as a control, whereas plasma in combination with calcium and thrombin was used to distinguish the difference between calcium and/or thrombin mediated effects and growth factor induced effects on the cells. The observed enhanced proliferation and migration of endothelial cells towards the platelet derived growth factors was driven by the plasma component of these preparations. However PDGF solely stimulated the migration and proliferation of mesenchymal stem cells. The increased osteogenic differentiation of growth factor treated mesenchymal stem cells was mostly driven by the high level of calcium used for the platelets degranulation. In summery, the different components of platelet derived growth factors work together to influence human endothelial and mesenchymal stem cells (Kilian *et al.,* 2004; Nagai *et al.,* 2005).

FGF. Four fibroblast growth factor receptors (FGFR1-4) constitute a family of transmembrane tyrosine kinases that serve as high affinity receptors for at least 22 FGF ligands. Gene targeting in mice has yielded valuable insights into the functions of this important gene family in multiple biological processes. These include mesoderm induction and patterning; cell growth, migration, and differentiation; organ formation and maintenance; neuronal differentiation and survival; wound healing; and malignant transformation. Furthermore, discoveries that mutations in three of the four receptors result in more than a dozen human congenital diseases highlight the importance of these genes in skeletal development (Coumoul & Deng, 2003; Franceschi*,* 2005).

IGF. Insulin-like growth factor-I (IGF-I) plays important roles in the anabolic regulation of bone and cartilage metabolism (Bolander, 1992; Canalis). Osteoblasts and chondrocytes produce this growth factor, express its receptor, and respond to it (Canalis, 1993; Laron, 2001). IGF-I appears essential for normal bone development because deletion of IGF-I or its

receptor leads to a reduction in bone size at birth (Liu *et al*., 1993; Powell-Braxton *et al*., 1993). Clinically, patients with Laron syndrome caused by IGF-I deficiency exhibit growth retardation and osteoporosis (Laron *et al.*, 1999). IGF-I is also reported to be expressed during fracture healing and to stimulate it, suggesting a role as an autocrine/paracrine factor potentiating bone regeneration (Trippel, 1998; Shukunami *et al*., 1996). Insulin also plays important roles in the anabolic regulation of bone and cartilage metabolism (Thomas *et al*., 1997). Although the anabolic effect of insulin on bone may be primarily related to its ability to stimulate osteoblast proliferation, that on cartilage may involve the acceleration of chondrocyte differentiation (Kato & Gospodarowicz, 1984; Shukunami *et al*., 1996). Patients with insulin deficiency as exemplified by type 1 diabetes mellitus are associated with osteoporosis (Krakauer *et al.*, 1997; Piepkorn *et al.*, 1997). Diabetes has also been shown to impair fracture healing, which is restored by treatment with insulin in both humans and animals (Kawaguchi *et al.*, 1994; Loder, 1988; Macey *et al*., 1989).

VEGF. Many degenerative processes in the skeletal system are induced by mechanical overload. Osteoarthritis and spontaneous tendon ruptures are two examples of mechanically influenced diseases. Incubator-housed compression apparatuses and cyclic strain chambers are adequate models to investigate the cellular processes. Recent studies have shown that growth factors are involved in the transduction pathways of mechanical overload leading to tissue degradation. Vascular endothelial growth factor (VEGF) is a dimerized, 45 kDa peptide that normally attracts endothelial cells in wound healing. VEGF can be detected in the superficial zone of the tibial plateau in osteoarthritic (OA) patients with degenerative changes but not in healthy articular cartilage. Blood vessels are only rarely observed in OA cartilage suggesting that there are other roles for VEGF in cartilage. VEGF is also detectable in ruptured but not in normal tendons. The mechanically induced expression of VEGF in avascular tissues like articular cartilage or fibrocartilage of contact areas from gliding tendons initiates degenerative processes. Chondrocytes from OA cartilage also express the VEGF receptor 2. In vitro assays have shown that VEGF binds the VEGFR-2 leading to a phosphorylation of MAP kinases (ERK1/2) with subsequent transcription factor accumulation (activator protein 1 = AP-1). One of the antagonists of VEGF is endostatin. Endostatin, a fragment of collagen type XVIII, is expressed in avascular tissues and has the potency to decrease VEGF induced effects (ERK1/2 phosphorylation). The increase in matrix metalloproteinase (MMP) production and the decrease in tissue inhibitor metalloproteinase (TIMP) synthesis is a result of the signal transduction cascade activation. MMPs participate in the degradation processes of osteoarthritis whereas TIMPs are inhibitors of the MMPs. Taken together mechanically induced VEGF is involved in the destruction and endostatin in the maintenance of avascular tissues of the bone and joint system (Pufe *et al.*, 2005).

BMPs. The BMPs are subset of the transforming growth factor (TGF) ß superfamily of dimeric, disulfide cross-linked growth and differentiation factors. To date at leats six human BMPs have demonstrated osteogenic activity: BMP-2, 4, 5, 6, 7 (also referred to as osteogenic protein [OP-1]) and 8 (OP-2) (Wozney, 1993; Cook *et al*., 2005). In addition, a number of newly discovered molecules, including growth differentiation factor (GDF) 1, 6 and 7 as well as BMP-9, dorsalin and Vg1, can be considered to fall within the BMP family (Wozney, 1995). While the BMPs are similar to other factors in the TGF-ß superfamily and other proteins such as TGF-ß, fibroblast growth factor and platelet-derived growth factor can be found in bone and have various effects on bone and cartilage cells in vitro, only BMPs have been demonstrated to induce either cartilage or bone formation in vivo. Moreover, the osteogenic BMPs all appear to have qualitatively similar activities. All induce only transient cartilage and result in complete bone formation.

The osteogenic activity present in bone matrix consists of a complex mixture of BMP proteins. Amino acid sequences of these BMPs have been used to construct oligonucleotide probes, which were then employed to obtain genomic or complementary (c) DNA clones for each of the proteins. These cDNAs have been transferred into expression vectors and expressed in several cell types including CHO cells that are currently being used to prepare rhBMP-2 for clinical trials (Wozney *et al*., 1988). The ability to synthesize recombinant, highly pure BMPs has rapidly advanced our understanding of the osteoinductive activity of several of the proteins in this family.

Like other members of the TGF-ß superfamily, the actions of BMPs are mediated by specific receptors, which are members of the serine/threonine kinase family (Wozney, 1993). Detailed characterization of BMP receptors and determination of how many different receptors and determination of how many different receptors exist for the BMP family of proteins requires further experimentation, as these receptors, like their ligands, form a complicated family of molecules.

Despite of BMPs are members of the TGF-ß superfamily, they have a few effects in common proven by in vitro studies in different cellular types. An example, TGF-ß inhibit the phosphatase alkaline production in most cellular types, including C26, W-20-17 e MC3T3-E1 cells (Noda & Rodan, 1986; Katagiri *et al*., 1990), while BMPs have been increased in these cellular types. In contrast of BMPs, TGF-ß suppresses the sulphate incorporation in any specific cellular types.

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RESUMEN: Este trabajo tiene como objetivo presentar, por medio de una revisión de la literatura, las principales características de la TGF-beta en la regulación de la neoformación ósea.

PALABRAS CLAVE: TGF-beta; Hueso.

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