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PROSPECTS

Fracture Healing as a Post-Natal Developmental Process: Molecular, Spatial, and Temporal Aspects of Its Regulation

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Abstract Fracture healing is a specialized post-natal repair process that recapitulates aspects of embryological skeletal development. While many of the molecular mechanisms that control cellular differentiation and growth during embryogenesis recur during fracture healing, these processes take place in a post-natal environment that is unique and distinct from those which exist during embryogenesis. This *Prospect Article* will highlight a number of central biological processes that are believed to be crucial in the embryonic differentiation and growth of skeletal tissues and review the functional role of these processes during fracture healing. Specific aspects of fracture healing that will be considered in relation to embryological development are: (1) the anatomic structure of the fracture callus as it evolves during healing; (2) the origins of stem cells and morphogenetic signals that facilitate the repair process; (3) the role of the biomechanical environment in controlling cellular differentiation during repair; (4) the role of three key groups of soluble factors, pro-inflammatory cytokines, the TGF-β superfamily, and angiogenic factors, during repair; and (5) the relationship of the genetic components that control bone mass and remodeling to the mechanisms that control skeletal tissue repair in response to fracture. J. Cell. Biochem. 88: 873–884, 2003.

Key words: fracture healing; BMP; angiogenesis; inflammatory cytokines

Ontogenetic development is characterized by a series of steps initiated at the time of fertilization and terminating with the differentiation, growth, and maturation of specialized tissues and organs. These developmental processes are characterized by both the molecular specialization that accompanies cellular differentiation and the way in which these cells are organized into functional structures during tissue morphogenesis. While developmental processes usually terminate when animals reach sexual maturity and adult size, some morphogenetic processes may be reinitiated in specific tissues as a consequence of injury. Thus, there is a subset of tissues that have the ability to recapitulate specific aspects of their initial developmental processes and thereby undergo regeneration. The repair of skeletal fractures is one such regenerative process [Vortkamp et al., 1998; Ferguson et al., 1999]. The postnatal tissue environment in which the regenerative processes of fracture healing takes place is different in a number of specific respects from those present during embryological development. These differences and their potential effects on the developmental process of fracture healing are essential to our understanding of bone morphogenesis and form the basis for the study of bone tissue engineering.

ANATOMY OF FRACTURE HEALING: THE ORIGINS OF POSTNATAL SKELETAL STEM CELLS AND MORPHOGENETIC SIGNALS

The end result of all developmental processes is the formation of tissues that have the appropriate morphological structures to carry out their physiological functions. In the case of fracture repair, the developmental processes of regeneration that are initiated in response

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to injury must reestablish both the original geometry and biomechanical competency of the damaged tissue structure. Like embryological development and skeletal growth, fracture repair involves the definition of specific morphogenetic fields and is thus dependent on instructive interactions between various proximate tissues.

A summary of these interactions is presented in Figure 1. The first conclusion that may be drawn from this anatomy is that the fracture line in the bone sets up the overall spatial relationships of the morphogenetic fields during tissue regeneration. This is shown by the development of two discrete crescent shaped centers of cartilage tissue formation that are symmetric with respect to the fracture line and taper proximally and distally along the cortices of the bone. Concurrently, a crescent shaped region of intramembranous bone formation is initiated at the proximal and distal ends of the area of periosteal response and tapers inward towards the fracture line deep to the ring of cartilage tissue. This morphology demonstrates that two distinct and interactive responses take place during fracture healing, endochondral and intramembranous bone formation. The instructive interactions between cells that are initiated within the repair process must therefore occur between the external soft tissues around the injured bone, the developing fields of endochondral bone formation, the intramembranous bone formation, and the underlying cortical bone and marrow. The specific questions that are currently unresolved are related to the origins of the skeletogenic cells that contribute to the endochondral field of development and the nature and origins of the initiating morphogenetic signals.

Three potential sources of cells and signals that set up these fields are indicated in the figure. These three sources are the periosteum, the surrounding soft tissues, and the marrow space at the site of the damaged cortical bone tissue. The primary tissue source of skeletogenic stem cells that give rise to the callus are believed to be from the periosteum [Nakahara et al., 1990], and studies have shown that there is diminished capacity for fracture callus development if the periosteum is removed [Buckwalter et al., 2001]. Other studies have also shown that cells within the periosteum robustly produce BMPs during the initial phases of fracture healing following injury

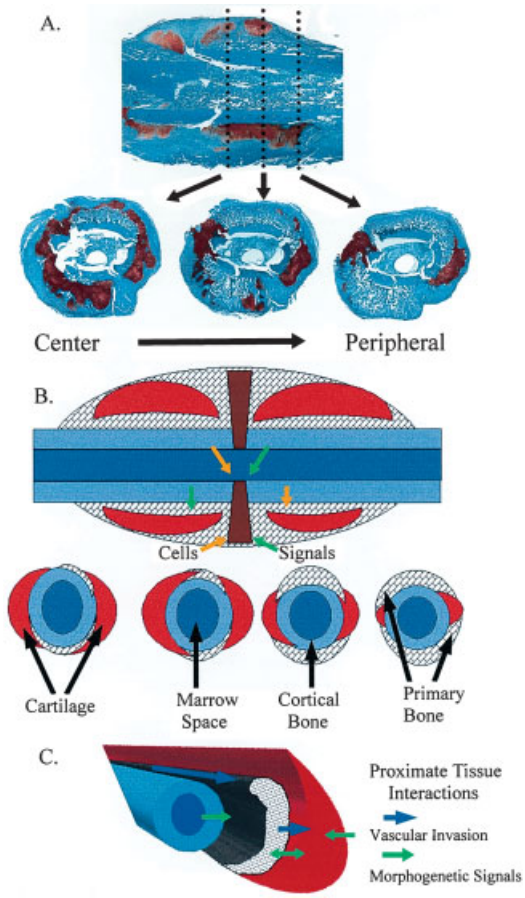

Fig. 1. Anatomic characterization of fracture repair. **A**: Representative sagittal and transverse histological sections are presented in upper and lower panels respectively of a mid-diaphysial transverse femur fracture at 14 days after. Transverse sections were taken at every 1,000 μm and the approximate positions of the transverse sections are denoted on the figure with a dotted line. The orientation of the each transverse section is denoted relative to the break site in the bone. Sections were stained with safranin O and fast green, and micrographic images are at 25 × magnification. Cartilage is stained bright red while bone is stained pale blue. **B**: Diagrammatic presentations of the morphogenetic fields of tissue development and the proximate tissue interactions. The sagittal view is presented in the upper panel and the transverse view is presented in the lower panel. Each of the tissue types is noted in the figure. The potential tissue origins of mesenchymal stem cells and morphogenetic signals are denoted by orange or green arrows, respectively. **C**: A Three dimensional rendering of spatial interactions of the developing tissue elements of the fracture callus. The orientation of vascular elements within the developing tissue are denoted with blue arrows. Major vessel in-growth is parallel to the cortices and from the proximal and distal edges of the periosteal surfaces. Co-lateral neo-vascular growth is from the nascent intramembranous bone into the cartilage.

[Bostrom et al., 1995], suggesting that these morphogens act locally to recruit and induce skeletogenic stem cells to differentiate. Considerable data are available which suggest that

mesenchymal stem cells might also be derived from either the surrounding muscle tissue, or the marrow space. Data to support a muscle origin comes from studies that have shown that either demineralized bone powder or purified BMPs implanted or injected into muscle tissue are capable of inducing the formation of ectopic bone [Urist, 1965; Iwata et al., 2002; Jingushi et al., 2002]. Other studies have shown that a number of different pre-myogenic cell lines are capable of being induced to differentiate into chondrogenic or osteogenic cells when treated with BMP(s) [Constantinides et al., 1978; Katagiri et al., 1994; Gerstenfeld et al., 2002]. A wealth of data also exists demonstrating that marrow stromal cells within the trabecular space are capable of undergoing differentiation into not only osteoblasts but chondrocytes as well [Jaiswal et al., 1997; Bianco et al., 2001; Sekiya et al., 2002]. The ultimate identification of the source of stem cells that give rise to the endochondral components of the callus are of considerable potential importance, for these cells contribute to the majority of the callus tissue and this mass of differentiated skeletal tissue can approach up to ~30% the volume of an injured long bone.

As stated earlier, the origins of the cells that contribute to intramembranous bone formation are more certain and appear to be derived from the underlying cortical bone and the periosteum. It may be speculated that at least some of these cells are likely to have arisen from the proliferative expansion of committed skeletal precursors. It is interesting to note that the ingrowth of vascular tissues into the developing callus proceeds with the development of the new periosteal bone and occurs from the proximal and distal edges where the periosteal response originates and progresses towards the fracture line. Thus, it may also be speculated that the interaction of the vascular elements and the initiation and propagation of the periosteal response are the primary driving mechanisms that facilitate the intramembranous bone formation. It has also been suggested that periovascular mesenchymal cells that exist in blood vessel walls contribute to fracture healing [Bouletreau et al., 2002].

Another important aspect of the anatomic geometry of the regenerative response is related to the origins and nature of the morphogenetic signals that initiate the developmental processes of bone regeneration and allow the developmental process to progress. In this context, the proximal/distal and medial/lateral asymmetry of the endochondral developmental process and the underlying fields of bone development may hold some clues to answering specific questions about the tissue origin and nature of the soluble signals that initiate and promote fracture healing. Since the tissue develops in an asymmetric manner that matches the observed development of the callus, it follows that there must be some underlying relationship between the gradients of the morphogens that promote the developmental process and the original anatomy of tissue or the anatomic characteristics of the injury to the tissue.

While it is clear that the signals that initiate and establish the symmetry of repair around the fracture line either arise from the marrow or are released from the injured bone matrix, many of these signals are part of the inflammatory processes [Einhorn et al., 1995; Barnes et al., 1999]. In terms of this aspect of the repair process, how the injury itself influences the responses in the tissues may be of considerable relevance since the field of inflammatory signals is propagated from the point of origin of the initial injury. Data supporting the role of inflammatory cytokines in the initiation of skeletal tissue repair are derived from our own studies demonstrating that in the absence of TNF-α signaling in receptor null animals, there is a loss of the symmetrical development of the callus around the fracture line. The absence of TNF-α signaling also leads to a delay in both intramembranous and endochondral bone formation. These data suggest that TNF-α signaling facilitates the repair process, perhaps by stimulating events necessary for mesenchymal stem cell recruitment or differentiation [Gerstenfeld et al., 2001].

The structural geometry of callus development might also be dependent on the muscular anatomy or vascularization of the tissue as well as the local biomechanical environment at the site of injury. Concerning the role of the biomechanical environment, our studies have shown that bending and shear loading at a bone defect site selectively drives chondrogenesis versus osteogenesis [Cullinane et al., 2002]. In other studies of unstable facture repair, cartilage tissue was shown to persist and molecular signals controlling chondrogenesis, such as Indian hedgehog, were shown to be induced earlier and have a more prolonged expression in

comparison to that observed in fixed fractures [Le et al., 2001]. These questions of how the morphogenetic fields are established and how factors such as the biomechanical environment drive both tissue differentiation and the anatomic geometry of the regenerative process are of considerable importance in identifying the molecular nature of the initiating signals and relating this to the origins of skeletogenic stem cells. The answers to these questions may have clinical importance, as the therapeutic responses to bioactive factors may be influenced by the timing and location of their placement into the correct morphogenetic field within the tissue. In addition, these answers will shed light on the temporal sequence of events so that the compounds can be introduced at the correct time and for the appropriate duration.

MULTIPLE CELLULAR AND MOLECULAR PROCESSES CONTRIBUTE TO FRACTURE REPAIR

The cellular and molecular processes that contribute to bone regeneration after fracture have many similar features to those which occur during embryonic and postnatal skeletal development. In Figures 2–5, a more detailed picture of the cellular and molecular features of fracture repair is presented. In Figure 2, a central area of the fracture callus at 14 days after injury is compared to that of a growth plate. The first and most striking feature from this comparison is the immense width of the central cartilage zone in the fracture callus relative to width of the cartilage in the distal growth plate within the same bone. The second difference is that the cells undergoing "hypertrophic differentiation" within the growth plate are much larger than those at the chondro/osseous junction in the fracture callus. These differences are interesting to note in the context of the observed variations in the mechanisms by which epiphyseal growth takes place in different species. Epiphyseal growth has been shown to be a combination of cellular proliferation, increasing cellular volume and increasing matrix deposition [Hunziker and Schenk, 1989; Breur et al., 1991], but different species use different combinations of these three mechanisms to achieve bone growth. In mammals, where growth is relatively slow, the primary rates of elongation occur by almost equal contributions of these three components. In contrast, within rapidly growing birds, the elongation occurs predominantly through cellular proliferation while increases in cell and matrix volume are more minor contributory factors to growth [Barreto and Wilsman, 1994]. It would appear then that fracture repair uses a more primitive mechanism to achieve the very rapid growth that is needed for the regenerative process and provides an example of ontogeny recapitulating phylogenenetic differences in a postnatal regenerative process. Such differences may also reflect on the differing functional role of the fracture callus in stabilizing the fracture site in addition to its role as a template for new bone formation. This basic difference in fracture repair compared to epiphyseal growth may also provide the means for identifying some of the molecular mechanisms that regulate the balance between chondrocyte proliferation, cellular volume control, and matrix deposition.

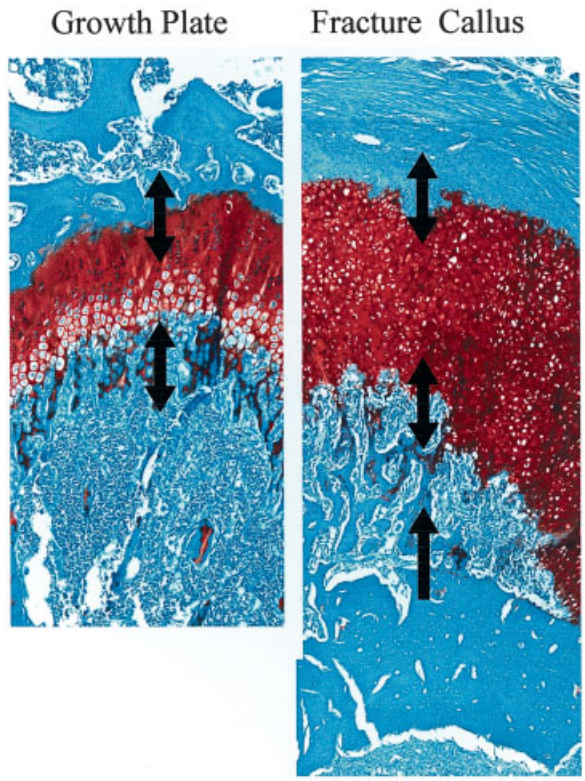

Fig. 2. Comparison of cellular characteristics of the endochondral progression in the postnatal growth plate and fracture calluses. Representative histological sections of a distal femur epiphyseal growth plate and a mid-diaphysial femur fracture at 14 days after injury are presented. Sections were stained with safranin O and fast green, and micrographic images are at 100 × magnification. Arrows denote potential sites of interactions with the various tssue types.

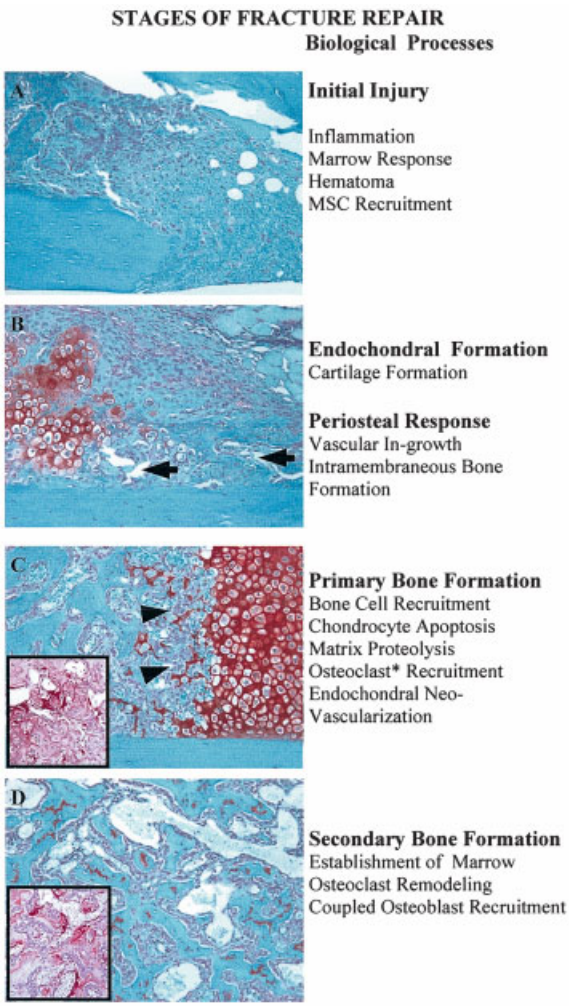

Fig. 3. Summary of the multiple stages of fracture healing. Histological sections are presented for each stage and a summary of the various processes that are associated with each stage is presented. All histological specimens are from sagittal sections of mouse tibia transverse fractures and were stained with safranin O and fast green, and micrographic images are at 200 × magnification. **A**: Section for the initial injury was taken from the fracture site 24 h post injury. **B**: Section depicting the initial periosteal response and endochondral formation is from 7 days post injury. Arrows denote vascular in growth from the peripheral areas of the periosteum. **C**: Section depicting the period of primary bone formation is from 14 days post injury. Arrows denote neo vascular in growth areas of the underlying new bone. Insert depict 400 × images of an osteoclast (*chondroclast) resorbing an area of calcified cartilage. **D**: Sections depicting the period of secondary bone formation are from 21 days post injury. Callus sites. Insert depicts 400 × images of an osteoclast resorbing an area of primary bone.

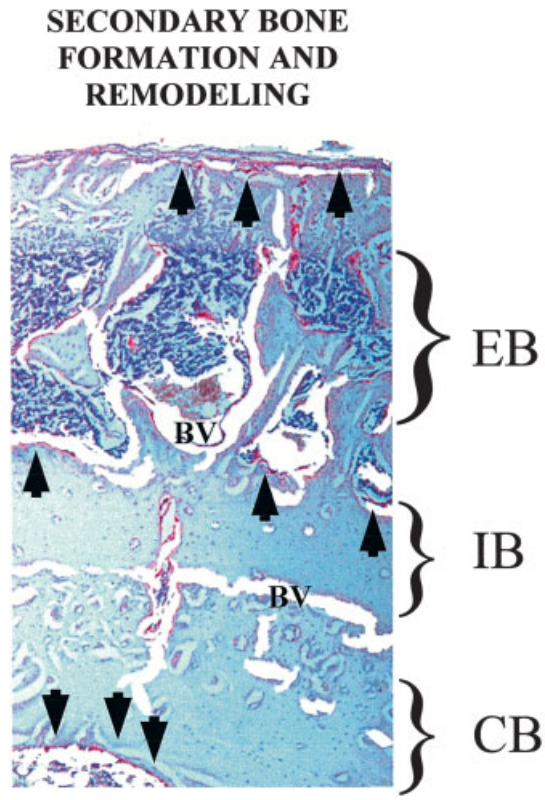

Fig. 4. Composite transverse cross section of the complexity of late stage bone remodeling of the fracture callus. Composite section of transverse sections of a rat femur fracture site at 35 days post injury. Specimens were stained with hematoxylin and fast red violet for TRAP positive cells. Micrographic images are at 100 × magnification. **A**: The arrows denote the three separate surfaces undergoing resorption. The two sets of vascular elements (BV = blood vessels) are indicated. One set of vessels are derived the original vascular in-growth parallel to the cortical surfaces within the periosteum. The second set of vessels are those that arose as from neovascular growth into the endochondral areas of bone formation. These later vessel surfaces show the infiltration of osteoclasts into the bone. The areas of tissue that were originally derived from the zones of endochondral bone formation (EB), intramembranous bone (IB) at the cortical surface, and the original cortical bone (CB) that is now being remodeled are denoted.

A review of the various stages of fracture repair and the biological processes associated with these stages is presented in Figures 3–5. Since an understanding of the basic stages of fracture repair have been well established, this section will focus on placing the various biological processes in the context of three key groups of soluble factors (pro-inflammatory cytokines, the TGF-β superfamily, and angiogenic factors) that regulate these processes (Fig. 5).

Pro-Inflammatory Cytokines

As described above, the role of inflammatory cytokines in initiating the repair response is only now becoming fully appreciated, yet the role these molecules play in the regulation of

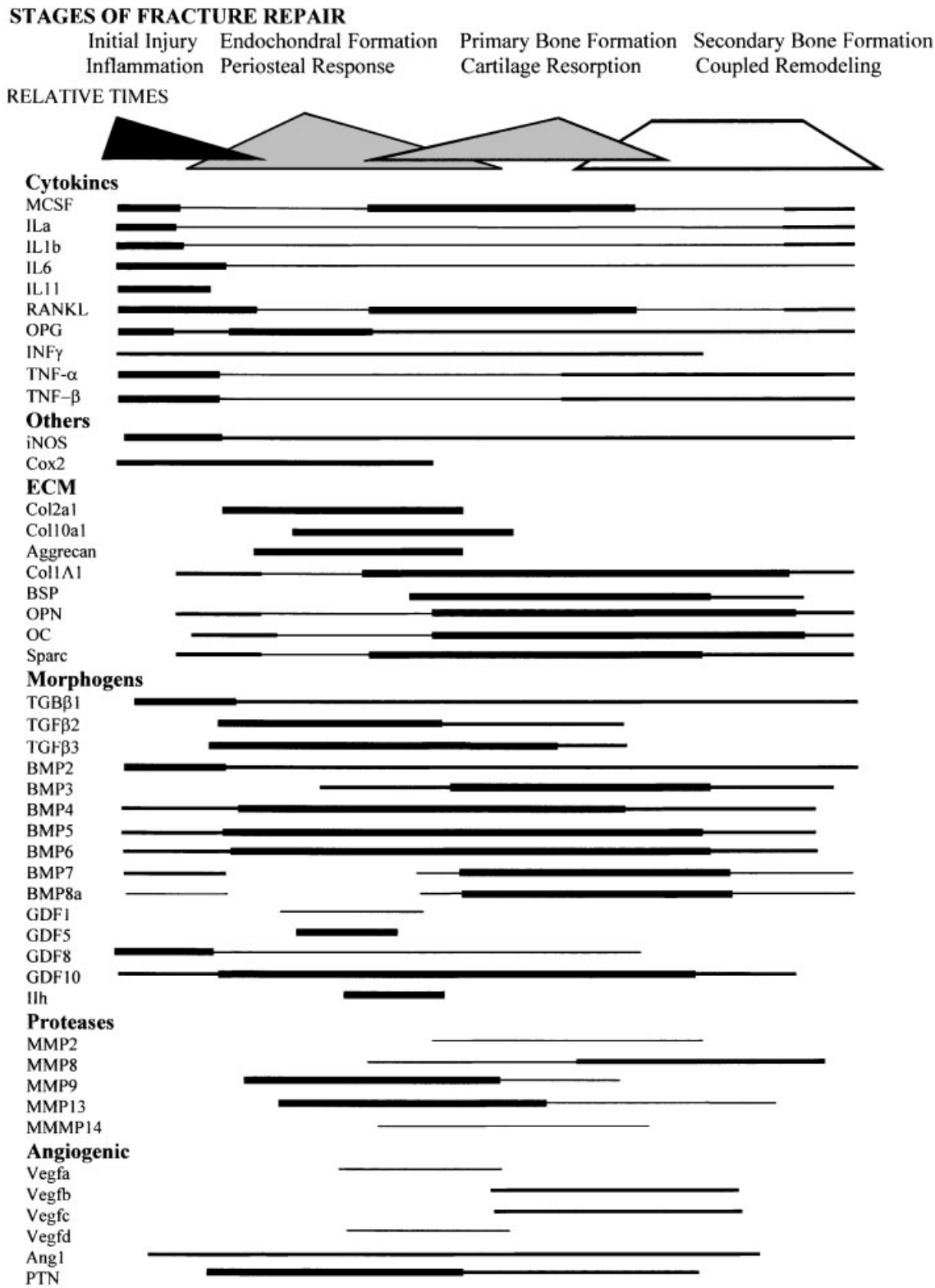

Fig. 5. Schematic summary of the stages of fracture repair and their associated molecular processes. The relative temporal aspects of each of the stages of the fracture healing process are denoted by basic geometric shapes that also connote the relative intensity of the molecular processes that define each of the stages. The relative levels of expression of various mRNAs that have been examined in our laboratories are denoted by three line widths. The levels of expression are by percent over baseline for each and are not comparable between individual mRNAs. Data for expression levels for the pro-inflammatory cytokines and the ECM mRNAs was from Kon et al., 2001; TGF-β family members was from Cho et al., 2002; Proteases and Angiogenic factors is from Lehmann et al., 2002; Cox2 is from Gerstenfeld et al., 2002. Data pertaining to Ihh and iNOs expression are from unpublished data.

bone remodeling has long been known [Gowen et al., 1983; Kimble et al., 1994, 1997; Barnes et al., 1999]. Two discrete types of resorption take place during fracture repair. The first occurs at the end of the endochondral period in which mineralized cartilage is removed and primary bone formation takes place. M-CSF, RANKL, and OPG are elevated, yet most of the cytokines that have been associated with bone remodeling, including IL1α, IL1β, and IL-6, are absent during this period [Kimble et al., 1997; Kon et al., 2001]. The exception for this group of cytokines is TNF-α, which begins to increase at the end of the period of endochondral resorption. The second type of resorption occurs during secondary bone formation (Fig. 4) and is driven solely through the coupled process of remodeling. IL-1 and IL-6 again begin to show increased levels of expression while OPG, M-CSF, and RANKL show diminished expression levels. These data suggest that the processes that mediate endochondral bone resorption and bone remodeling phases are different and that the resorption of the mineralized cartilage is more dependent on the activities of M-CSF, OPG, and RANKL and less affected by the activities of these other cytokines. In contrast, bone remodeling would appear to be dependent on the levels of RANKL as well as co-regulated by the activities of IL-1, IL-6, and TNF-α that are found in bone marrow. The conclusion that the mechanisms which regulate calcified cartilage resorption are different from that of bone are further supported by the studies of RANKL (TRANCE) deficient mice or mice in which RANKL expression was rescued by the engineering of RANKL expression in lymphocytes. In these studies, RANKL expression by lymphocytes was able to promote osteoclast development and rescue the osteopetrosis in both the marrow space and woven bone replacement in the cortical shafts. It did not, however, correct the chondrodysplasia of the epiphyseal and metaphyseal areas. This observation led these authors to conclude that RANKL was under differing mechanisms of local tissue control in cartilage as opposed to bone [Kim et al., 2000]. The role of resorptive activities in re-shaping fracture callus to normal bone anatomy is poorly understood. Moreover, the role of bone remodeling in restoring bone integrity requires further investigation. Defining both the similarities and differences between fracture healing and bone development are essential to our further understanding of skeletal growth and its relationship to skeletal biomechanics.

Role of the TGF-β Superfamily in Fracture Healing

Since the discovery that implanted demineralized bone induces the de novo formation of cartilage and bone at extraskeletal sites [Urist, 1965] and the subsequent purification of the osteoinductive activity and cloning of the individual bone morphogenetic proteins (BMPs) [Wozney et al., 1988; Celeste et al., 1990], the TGF-β superfamily of morphogenetic proteins has been perhaps the most intensively studied group of factors in skeletalgenesis and fracture repair [Joyce et al., 1990; Rosier et al., 1998]. Several studies have attempted to define the role of endogenous BMPs in normal fracture healing. Using reverse transcriptase PCR amplification, the temporal and spatial distribution BMP-4 mRNA expression was defined in murine fracture healing [Nakase et al., 1994]. In an investigation of BMP-2 and BMP-4 protein expression by immunological techniques, Bostrom et al. (1995) delineated the expression of these BMPs over a 4-weeks period of fracture healing. Recently, our laboratory has shown that specific members of the transforming growth factor-β superfamily, including multiple BMPs (1–8), GDFs (1, 5, 8, and 10), and TGF-β1–3, act in combinations to promote the various stages of intramembranous and endochondral bone formation observed during fracture healing [Cho et al., 2002]. A summary of these studies is seen in Figure 5. This examination of the temporal patterns of mRNA expression for members of the TGF-β superfamily over a 28 day period of fracture healing in mouse tibiae showed that BMP 2 and GDF 8 were maximally expressed on day 1 after fracture, suggesting roles as early response genes in the cascade of healing events. In light of its known actions as a negative regulator of skeletal muscle growth, the restriction of GDF 8 expression to day 1 also suggests that it may similarly regulate cell differentiation early in the fracture healing process. GDF 5, TGF-β2, and TGF-β3 showed maximal expression on day 7, when type II collagen expression peaked during cartilage formation. In contrast, BMPs 3, 4, 7, and 8 showed a restricted period of expression from days 14 through 21, when the resorption of calcified cartilage and osteoblastic recruitment were most active. TGF-β1, BMP5,

BMP6, and GDF10 were constitutively expressed from days 3–21. During the same time period, GDFs 3, 6, and 9 could not be detected while GDF1 was expressed at extremely low levels. These findings suggest that several members of the TGF-β superfamily are actively involved in fracture healing. Although they are closely related both structurally and functionally, each has a distinct temporal expression pattern and a potentially unique role in fracture healing. Other recent studies have shown that the expression of the BMP antagonists also play an important role in fracture repair. In one recent study, expression of noggin mRNA was shown to be enhanced in the early phase of fracture callus formation, and its temporal expression was similar to that of BMP-4. These authors suggested that the noggin/BMP-4 balance could be an important factor in the regulation of callus formation during fracture healing [Yoshimura et al., 2001]. In aggregate the numerous studies reviewed here define many of the temporal and spatial features of the expression of the TGF-β superfamily during fracture healing. However, since they potentially induce more than one cellular event, further work is clearly needed to define their specific functional roles in the repair process. In addition, the molecular events that initiate the expression of the BMPs after injury and how the overlapping multiple members of this family coordinately regulate the various stages of the fracture repair represent a fruitful area of future investigation. BMP proteins heterodimerize [Brunet et al., 1998; Chang and Hemmati-Brivanlou, 1998; Reddi, 2001a], and as a result, some may have antagonizing functions targeted to specific cell types. In addition, there may be specificity of action dependent on the expression of specific BMP antagonists [Reddi, 2001b]. Future research will give insight as to how combinations of BMPs interact to either enhance or diminish BMP activity.

Role of Metalloproteinases and Angiogenic Factors in Fracture Healing

Fracture healing creates a demand on the surrounding tissues to increase blood flow so that induction of bone regeneration can occur within the callus. Such dependency of optimal bone healing on the development of an adequate blood flow has been well established in a number of studies of fracture repair and extensively reviewed [Glowacki, 1998; Rowe et al., 1999; Einhorn and Lee, 2001; Gerber and Ferrara, 2000]. Furthermore, endochondral ossification in normal fracture healing also requires the coordination of both the molecular mechanisms that regulate the extracellular matrix remodeling and the vascular penetration of new blood vessels into the resorbing matrix [Vu et al., 1998]. Thus, matrix degradation and angiogenesis are either correlated or concurrent processes during endochondral bone formation. The final stages of endochondral ossification and bone remodeling are dependent on the action of specific matrix metalloproteinases to degrade the cartilage and bone, allowing the invasion of the blood vessels.

Angiogenesis is believed to be regulated by two separate pathways: a vascular endothelial growth factor (VEGF)-dependent pathway and an angiopoietin-dependent pathway [Suri et al., 1996]. It may be speculated that both sets of regulatory pathways are functional during fracture repair. The VEGF related family of molecules are essential mediators of neo-angiogenesis and are endothelial-cell specific mitogens [Ferrara and Davis-Smyth, 1997]. This family of proteins binds to two receptor tyrosine kinases, Flt-1 (VEGFR1) and Flk-1/KDR (VEGFR2). The other set of regulators that directly control vascular growth contains angiopoietin 1 and 2 and their receptors, Tie 1 and 2. These regulatory vascular morphogenetic molecules and their receptors are related to the formation of larger vessel structures and the development of co-lateral branches from existent vessels. It has been shown that treatment with an anti-VEGF chimeric protein significantly inhibits blood vessel formation during endochondral growth in long bones and impedes trabecular bone formation [Gerber et al., 1999]. In a recent study, fracture repair was shown to be enhanced by the exogenous administration of VEGF during the fracture repair process [Street et al., 2002]. These data demonstrate the critical role of VEGF related signaling in neo-angiogenesis and in the endochondral process of new bone formation. The role of the angiopoietin pathway and its contributions in bone repair are not as well understood. Our recent studies show that Ang 1 and the Tie-2 receptor are induced 3 to 5 fold during the initial periods of fracture healing [Lehmann et al., 2002]. This indicates that initial vascular ingrowth from feeding vessels in the periosteum may play an important role in the repair

process. In this context it is interesting to note that neo-angiogenic vessels that infiltrate the endochondral regions of the callus appear to be fed from the underlying larger vessels that have grown along the cortical surfaces (Figs. 1, 3, and 4).

A number of recent studies have shown that BMPs will stimulate the expression of VEGF by osteoblasts and osteoblast-like cells [Yeh and Lee, 1999; Deckers et al., 2002] and also express VEGF related receptors and proteins [Harper et al., 2001; Deckers et al., 2002]. The tissue specific regulation of VEGF expression during bone development has been shown to be dependent on the expression of Cbfa1/Runx2, which is known to be a key transcriptional factor that regulates the commitment of mesenchymal cells to the skeletal cell lineage [Zelzer et al., 2001]. Studies by Harada et al. [1995] and Goad et al. [1996] have implicated osteoblasts as the primary regulators of angiogenesis in fracture healing. These cells are also known to express elevated amounts of VEGF [Harada et al., 1995]. VEGF has also been shown to be a crucial component in the coupling of hypertrophic cartilage remodeling and bone formation to the processes of angiogenesis in endochondral growth of long bones [Gerber et al., 1999]. These data demonstrate that the BMP and VEGF mediated pathways are essential for skeletal regeneration and suggest that there may be an intimate relationship between them, which would allow for the coordinated regulation of events that initiate new bone formation.

RELATIONSHIP OF GENETIC DIFFERENCES THAT EFFECT SKELETAL DEVELOPMENT, GROWTH, AND BONE QUALITY TO THE PROCESSES OF FRACTURE HEALING

As has been described above, fracture healing as a recapitulation of a developmental process entails the complex interaction of multiple cell types and cellular processes. Aging and postnatal processes that maintain tissue homeostasis may also be considered as an extension of a developmental program that is initiated at the time of conception. Thus fracture healing may provide a unique model to assess mechanisms of aging. Recent epidemiological studies have shown that bone mineral density is controlled, in part, by a multifactorial set of genetic factors [Heaney et al., 2000]. Initial experiments in congenic strains of mice have identified specific outcome measurements that define BMD, including bone geometric parameters (cortical thickness vs. diameter) and defined alterations in metabolic measures of endosteal rates of formation [Beamer et al., 1999; Richman et al., 2001]. Subsequently these traits have been mapped to specific gene loci in various strains in mice [Beamer et al., 2001]. Most recently, using a cDNA microarray approach, variations in the expression of specific mRNAs were identified and can be related to quantitative variations in BMD [Gu et al., 2002]. In human studies, biochemical and kindred analyses of an autosomal dominant syndrome characterized by high bone mineral density identified a mutation in the LDL-receptor related protein-5 and mechanistically correlated this to aberrant Wnt signaling activity within the affected kindred [Boyden, 2002]. Furthermore, a preliminary report of functional mRNA expression analysis in fracture repair has suggested that the Wnt signaling system is uniquely activated at defined times during fracture healing [Hadjiargyrou et al., 2002]. In this context, two recent studies, one examining soft tissue wound healing [Li et al., 2001a,b; Masinde et al., 2001] and one examining skeletal repair in a non critical size defect within different strains of mice, suggest that combining genomic mapping of complex traits with full genome RNA analysis offers a merging of two very powerful technologies that may identify many of the underlying genetic mechanisms that control bone repair. It may also be hypothesized that variations in genomic profiles that are linked to BMD will be reflected in variations in the genomic expression profiles expressed during skeletal tissue healing. Thus if strain variation in BMD is developmentally controlled, these genes should be recapitulated during the developmental processes that are found in skeletal tissue healing.

In conclusion, fracture healing offers a unique window into many of the developmental processes that form the skeleton, but in a postnatal context. This may be informative to our further understanding of skeletal growth and repair, as well as those processes which influence skeletal aging.

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