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PROSPECT

The Control of Chondrogenesis

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Abstract Chondrogenesis is the earliest phase of skeletal development, involving mesenchymal cell recruitment and migration, condensation of progenitors, and chondrocyte differentiation, and maturation and resulting in the formation of cartilage and bone during endochondral ossification. This process is controlled exquisitely by cellular interactions with the surrounding matrix, growth and differentiation factors, and other environmental factors that initiate or suppress cellular signaling pathways and transcription of specific genes in a temporal-spatial manner. Vertebrate limb development is controlled by interacting patterning systems involving prominently the fibroblast growth factor (FGF), bone morphogenetic protein (BMP), and hedgehog pathways. Both positive and negative signaling kinases and transcription factors, such as Sox9 and Runx2, and interactions among them determine whether the differentiated chondrocytes remain within cartilage elements in articular joints or undergo hypertrophic maturation prior to ossification. The latter process requires extracellular matrix remodeling and vascularization controlled by mechanisms that are not understood completely. Recent work has revealed novel roles for mediators such as GADD45β, transcription factors of the Dlx, bHLH, leucine zipper, and AP-1 families, and the Wnt/β-catenin pathway that interact at different stages during chondrogenesis. J. Cell. Biochem. 97: 33–44, 2006.

Key words: chondroprogenitor; chondrocyte differentiation; hypertrophy; transcription factors; bone morphogenetic proteins; GADD45β; endochondral ossification

Chondrogenesis is the process that results in the formation of the cartilage intermediate, or anlagen, and leads to endochondral ossification during skeletal development. This process is initiated by the differentiation of mesenchymal cells that arise from three sources: neural crest cells of the neural ectoderm that gives rise to craniofacial bones, the sclerotome of the paraxial mesoderm, or somite compartment, which forms the axial skeleton, and the somatopleure of the lateral plate mesoderm, which yields the skeleton of the limbs [Olsen et al., 2000]. In the human embryo, the appendicular skeleton develops from limb buds, first visible at around 4 weeks of gestation, and subsequent stages of human limb development were mapped in early studies by O'Rahilly and Gardner [1975]. The development of the joint is divided into two morphological events: formation of the cartilaginous anlagen that model skeletal elements and subsequent joint formation. The joint develops from the primitive avascular, densely packed cellular mesenchyme termed the skeletal blastema. The skeletal elements are prefigured in mesenchymal condensations, and common precursor mesenchymal cells divide into both chondrogenic and myogenic lineages that determine the differentiation of cartilage centrally and muscle peripherally. The surrounding tissues, particularly epithelium, influence the differentiation of mesenchymal progenitor cells to chondrocytes. The cartilaginous nodules appear in the middle of the blastema, and simultaneously, cells at the periphery become flattened and elongated to form the perichondrium. The differentiated chondrocytes can then proliferate and undergo the complex process of hypertrophic maturation. Much of our current understanding of chondrogenesis is based on early studies in chicken and later in mice. This review will focus

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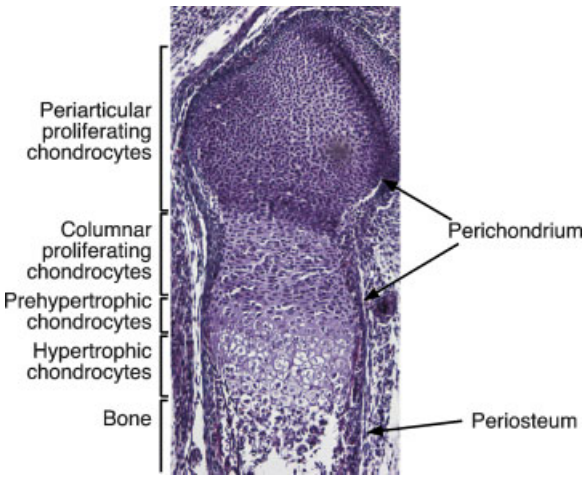

Fig. 1. Cellular organization of the growth plate of mouse femur at embryonic day 16.5 of endochondral development. Distinct morphologies are observed in the different zones by histological staining with hematoxylin and eosin. The periarticular proliferating chondrocytes are also called reserve chondrocytes. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

on the factors involved in chondrogenesis during the development of long bones (Fig. 1), which form as a result of replacement of the cartilage template by endochondral ossification.

MESENCHYMAL CELL CONDENSATION AND DETERMINATION OF CHONDROPROGENITORS

The process of chondrogenesis occurs in stages beginning with mesenchymal cell recruitment and migration, proliferation (Fig. 2), and condensation, which are regulated by mesenchymal-epithelial cell interactions [Hall and Miyake, 2000; Tuan, 2004]. The aggregation of chondroprogenitor mesenchymal cells into precartilage condensations was first described by Fell [1925] and represents one of the earliest events in chondrogenesis. This process is dependent upon signals initiated by cell–cell and cell–matrix interactions and is associated with increased cell adhesion and formation of gap junctions and changes in the cytoskeletal architecture. Zwilling [1972] proposed that positional information for organization of the limb bud was imparted by diffusible agents generated at the tip of the limb bud and along its posterior margin, promoting the development of a cartilaginous anlage along proximal-distal and anterior-posterior axes, respectively. Limb buds develop from the lateral plate mesoderm [Tickle and Munsterberg, 2001]. The patterning

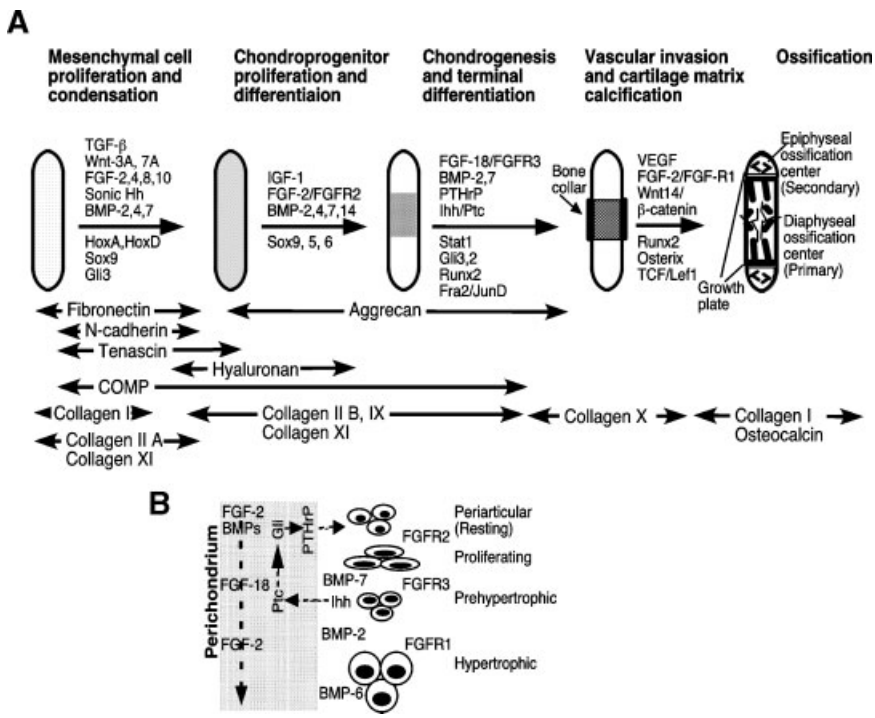

Fig. 2. Sequence of events of chondrogenesis during the development of long bones. **A**: The different stages are represented schematically, showing the temporal patterns of growth and differentiation factors (above the arrows) and the transcription factors involved below the arrows. The extracellular matrix proteins that distinguish the different stages are indicated below. **B**: Model of the regulation in the growth plate shows the sites of expression and action of the Ihh/PTHrP, FGF, and BMP pathways in the different zones and perichondrium.

of limb mesenchyme is due to interactions between the mesenchyme and the overlying epithelium [Capdevila and Izpisua Belmonte, 2001]. The embryonic limb possesses two signaling centers, the apical ectodermal ridge (AER) and the zone of polarizing activity (ZPA), which produce signals responsible for directing the proximal-distal outgrowth and anterior-posterior patterning, respectively [DeLise et al., 2000; Olsen et al., 2000]. Prior to condensation, the prechondrocytic mesenchymal cells produce extracellular matrix rich in hyaluronan and collagen type I, as well as type IIA collagen containing the exon 2 encoded aminopropeptide found in noncartilage collagens [Sandell et al., 1994]. The initiation of condensation is associated with increased hyaluronidase activity and the appearance of the cell adhesion molecules, neural cadherin (N-cadherin), and neural cell adhesion molecule (N-CAM). TGF-β, which is among the earliest signals in chondrogenic condensation, stimulates the synthesis of fibronectin, which in turn regulates N-CAM. Syndecan binds to fibronectin and downregulates N-CAM, thereby setting the condensation boundaries. The extracellular matrix molecules, which also include tenascins and thrombospondins, including cartilage oligomeric protein (COMP), interact with the cell adhesion molecules to activate intracellular signaling pathways involving focal adhesion kinase and paxillin, to initiate the transition from chondroprogenitor cells to a fully committed chondrocyte [DeLise et al., 2000]. N-cadherin and N-CAM disappear in differentiating chondrocytes and are detectable later only in perichondrial cells.

Many of the genes that pattern the distribution and proliferation of mesenchymal condensations at sites of future skeletal elements and control subsequent limb development have been described [DeLise et al., 2000; Hall and Miyake, 2000; Olsen et al., 2000; Eames et al., 2003] (Fig. 2). The current view is that a series of patterning systems functions sequentially over time [Tickle, 2003]. Fibroblast growth factor (FGF), hedgehog, bone morphogenetic protein (BMP), and Wnt pathways coordinate signaling along the three axes of the limb to insure correct patterning along the dorso-ventral and anteroposterior axes [Tickle, 2002]. Wnt signals, including Wnt2a and Wnt2c, are among the earliest signals required to induce FGFs, such as FGF-10 and FGF-8, which act in positive feedback loops [Niswander, 2003]. Thus, FGF-10 induces Wnt3a, which acts via β-catenin to increase FGF-8, which then maintains FGF-10 expression [Tickle and Munsterberg, 2001]. FGFs are involved in both limb initiation and limb bud outgrowth [Tickle, 2002]. The homeobox (Hox) transcription factors encoded by the HoxA and HoxD gene clusters are critical for the early events of limb patterning in the undifferentiated mesenchyme, and are required for the expression of FGF-8 and Sonic hedgehog (Shh) [Kmita et al., 2005]. Hoxd11 and Hoxd13 modulate proliferation of cells within the condensations [Hall and Miyake, 2000]. Wnt7a is expressed early during limb bud development where it acts to maintain Shh expression [Tickle, 2003]. Shh signaling, which is required for early limb patterning, though not for limb formation, is mediated by the Shh receptor Patched (Ptc1), which activates another transmembrane protein Smoothened (Smo) and inhibits processing of the Gli3 transcription factor to a transcriptional repressor [Niswander, 2003; Barna et al., 2005]. The hedgehog pathways are regulated by intraflagellar transport proteins that control both the activator and repressor functions of Gli family members [Liu et al., 2005].

BMPs, which were identified originally as molecules that induce ectopic endochondral ossification [Urist, 1965], set the stage for bone morphogenesis by initiating chondroprogenitor cell determination and differentiation, but also regulate the later stages of chondrocyte maturation and terminal differentiation to the hypertrophic phenotype. BMP-2, -4, and -7 coordinately regulate the patterning of limb elements within the condensations depending upon the temporal and spatial expression of BMP receptors and BMP antagonists, such as noggin and chordin [Pizette and Niswander, 2000; Niswander, 2002; Tickle, 2003]. In vitro and in vivo studies have shown that BMP signaling is required both for the formation of precartilaginous condensations and for the differentiation of precursors into chondrocytes [Yoon and Lyons, 2004; Yoon et al., 2005].

CHONDROCYTE DIFFERENTIATION

The differentiation of chondroprogenitors is required for subsequent stages of skeletogenesis and is characterized by the deposition of cartilage matrix containing collagens II, IX,

and XI and aggrecan (Fig. 2). The nuclear transcription factor Sox9, one of earliest markers expressed in cells undergoing condensation (Fig. 3), is required for the expression of the type II collagen gene (Col2a1) and certain other cartilage-specific matrix proteins, including Col11a2 and CD-RAP, prior to matrix deposition in the cartilage anlagen [Ng et al., 1997; Lefebvre et al., 2001; Eames et al., 2003]. Two additional Sox family members, L-Sox5 and Sox6, which are not present in early mesenchymal condensations, but are co-expressed with Sox9 during chondrocyte differentiation [Lefebvre et al., 1998], have a high degree of sequence identity with each other, but have no sequence homology with Sox9 except in the HMG box. They can form homo- or heterodimers, which bind more efficiently to pairs of HMG box sites than to single sites, and unlike Sox9, they contain no transcriptional activation domain. The expression of SOX proteins is dependent upon BMP signaling via BMPR1A and BMPR1B, which are functionally redundant and active in chondrocyte condensations but not in the perichondrium [Yoon et al., 2005]. L-Sox5 and Sox6 are required for the expression of Col9a1, aggrecan, and link protein, as well as Col2a1, during overt chondrocyte differentiation [Smits et al., 2001]. The runt-domain transcription factor, Runx2 (also known as Core binding factor, Cbfa1, and Osf2), is also expressed in all condensations, including those that are destined to form bone [Ducy et al., 1997; Komori et al., 1997; Otto et al., 1997] (Fig. 3).

BMPs transduce signals through the formation of heteromeric complexes of types I and II receptors, which have serine-threonine kinase activity. Upon BMP binding, the type II receptor, BMPRII phosphorylates type I receptors, ALK-2, BMPRIA/ALK-3, and BMPRIB/ALK-6. Signaling is mediated by the canonical SMAD pathway, which involves the signal-transducing acceptor proteins, Smads 1, 5, and 8 and inhibitory Smads 6 and 7 [Derynck and Zhang, 2003; Wan and Cao, 2005]. Other BMP-induced transcription factors include JunB, JunD and ID, and DLX family members [Xu et al., 2001; Li and Cao, 2003] (Fig. 3). BMPs can also signal by activating TGFβ-activated kinase 1 (TAK1), which interacts with MEKK1 and activates p38 and JNK cascades, or by activating Ras/ERK1/2 or RhoA/ROCK signaling [Derynck and Zhang, 2003; Yoon and Lyons, 2004]. The p38 pathway contributes to the initiation of chondrogenic cellular condensation [Nakamura et al., 1999], and ERK1/2 activation cross interacts with

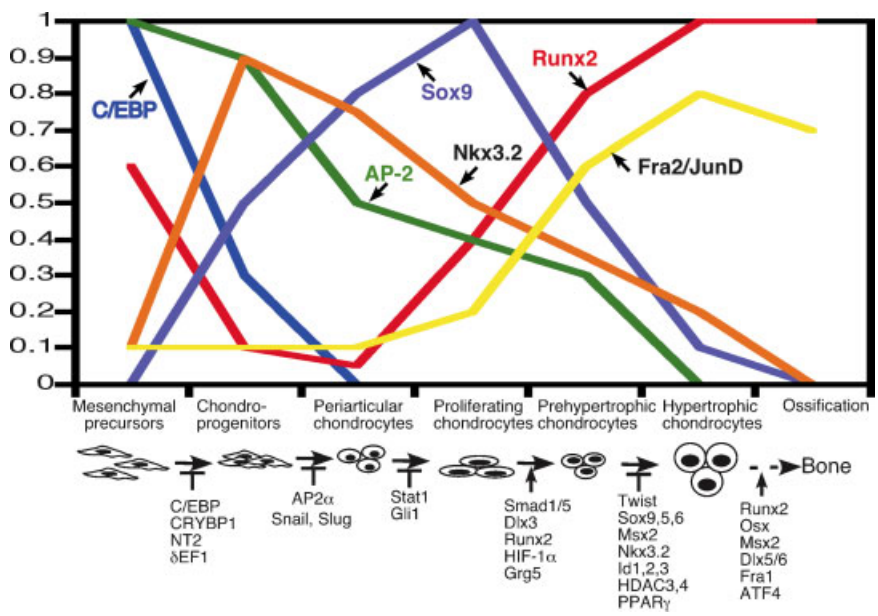

Fig. 3. Schematic representation of the expression transcriptional regulators at different stages of chondrogenesis and endochondral ossification. The graphical representation is based on findings of published studies referenced in the text. Additional transcription factors that are inhibitors or activators at different stages are indicated below the scheme of cellular transitions. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

BMP-2-induced signaling to regulate chondrogenesis in a positive manner [Seghatoleslami et al., 2003]. After BMP-induced phosphorylation of receptor-activated (R)-SMADs, they form complexes with the common Smad4 and translocate to the nucleus, where they bind to SMAD elements in the promoters of target genes. They can also interact with a large number of other DNA-binding transcription factors, coactivators such as CBP/p300 and repressors [Derynck and Zhang, 2003].

CHONDROCYTE PROLIFERATION

Throughout chondrogenesis, the balance of signaling by BMPs and FGFs determines the rate of proliferation, thereby adjusting the pace of the differentiation [Minina et al., 2002] (Fig. 1). In the long bones, long after condensation, BMP-2, -3, -4, -5, and -7 are expressed primarily in the perichondrium and only BMP-7 is expressed in the proliferating chondrocytes [Minina et al., 2002]. BMP-6 is found exclusively later in hypertrophic chondrocytes along with BMP-2. Of the more than 22 FGFs identified thus far [Itoh and Ornitz, 2004], the ligands that activate FGFRs during chondrogenesis in vivo have been difficult to identify, but the signaling depends upon the temporal and spatial location of not only the ligands, but also the receptors [Ornitz, 2005] (Fig. 2B). FGFR2 is upregulated early in condensing mesenchyme and is present later in the periphery of the condensation along with FGFR1, which is expressed in surrounding loose mesenchyme. FGFR3 is associated with proliferation of chondrocytes in the central core of the mesenchymal condensation and may overlap with FGFR2. Proliferation of chondrocytes in the embryonic and postnatal growth plate is regulated by multiple mitogenic stimuli, including FGFs, which converge on the cyclin D1 gene [Beier, 2005].

In the growth plate (Fig. 2B), FGFR3 serves as a master inhibitor of chondrocyte proliferation via phosphorylation of the Stat1 transcription factor, which increases the expression of the cell cycle inhibitor p21 [Sahni et al., 1999]. Recent studies suggest that FGF-18 is the preferred ligand of FGFR3, since Fgf18-deficient mice have an expanded zone of proliferating chondrocytes similar to that in Fgfr3-deficient mice, and that FGF-18 can inhibit Ihh expression [Liu et al., 2002]. FGF18 and FGF9 are expressed in the perichondrium and periosteum and form a functional gradient from the proximal proliferating zone, where FGF18 acts via the FGFR3 to downregulate proliferation and subsequent maturation [Liu et al., 2002; Ohbayashi et al., 2002]. Both FGF18 and FGF9 interact with FGFR1 in the prehypertrophic and hypertrophic zones, where recent evidence indicates that they regulate vascular invasion by inducing the expression of vascular endothelial growth factor (VEGF) and VEGFR1, respectively. As the epiphyseal growth plate develops, FGFR3 disappears and FGFR1 expression is upregulated in prehypertrophic and hypertrophic chondrocytes, suggesting a role for FGFR1 in the regulation of cell survival and differentiation, and possibly cell death [Ornitz, 2005].

The proliferation of chondrocytes in the lower proliferative and the prehypertrophic zones is under the control of a local negative feedback loop involving signaling by PTHrP and Indian hedgehog (Ihh) (Fig. 2B). Ihh expression is restricted to the prehypertrophic zone and PTHrP receptor is expressed in the distal zone of periarticular chondrocytes. The adjacent, surrounding perichondrial cells express the Hedgehog receptor patched (ptc), which upon Ihh binding, similar to Shh in the mesenchymal condensations, activates Smo and induces Gli transcription factors, which can feed back regulate Ihh target genes in a positive (Gli1 and Gli2) or negative (Gli3) manner [Ingham and McMahon, 2001; McMahon et al., 2003; Vokes and McMahon, 2004; Tyurina et al., 2005]. Early work indicated that Ihh induces expression of PTHrP in the perichondrium [Vortkamp et al., 1996] and that PTHrP signaling then stimulates cell proliferation via its receptor expressed in the periarticular chondrocytes [Lanske et al., 1996]. These interactions are modulated by a balance of BMP and FGF signaling that adjusts the pace of chondrocyte terminal differentiation to the proliferation rate [Minina et al., 2002]. FGF-18 or FGFR3 signaling can inhibit Ihh expression [Liu et al., 2002] and BMP signaling upregulates the expression of Ihh in cells that are beyond the range of the PTHrP-induced signal [Minina et al., 2002]. Recent evidence indicates that Ihh acts independently of PTHrP on periarticular chondrocytes to stimulate differentiation of columnar chondrocytes in the proliferative zone, whereas PTHrP acts by preventing premature differen-

tiation into prehypertrophic and hypertrophic chondrocytes, thereby suppressing premature expression of Ihh [Kobayashi et al., 2005]. Thus, Ihh and PTHrP, by transiently inducing proliferation markers and repressing differentiation markers, function in a tempero-spatial manner to determine the number of cells that remain in the chondrogenic lineage versus those that enter the endochondral ossification pathway.

CHONDROCYTE HYPERTROPHY

The development of long bones from the cartilage anlagen occurs by a process termed endochondral ossification, which involves terminal differentiation of chondrocytes to the hypertrophic phenotype, cartilage matrix calcification, vascular invasion, and ossification [Ferguson et al., 1998; Colnot and Helms, 2001; Ballock and O'Keefe, 2003; Provot and Schipani, 2005] (Fig. 1). This process is initiated when the cells in the central region of the anlage begin to hypertrophy, increasing cellular fluid volume by almost 20 times. Ihh, which is required for endochondral bone formation [St-Jacques et al., 1999] and synchronizes skeletal angiogenesis with perichondrial maturation, is expressed in prehypertrophic chondrocytes as they exit the proliferative phase, enter the hypertrophic phase, and begin to express the hypertrophic chondrocyte marker, type X collagen (Col10a1) and alkaline phosphatase. Runx2, which serves as a positive regulatory factor in chondrocyte maturation to the hypertrophic phenotype [Enomoto et al., 2000], is expressed in the adjacent perichondrium and in prehypertrophic chondrocytes but less in late hypertrophic chondrocytes [Kim et al., 1999; Takeda et al., 2001], overlapping with Ihh, Col10a1, and BMP-6 [Ferguson et al., 1998; Colnot, 2005]. BMP-induced Smad1 and interactions between Smad1 and Runx2/Cbfa1 are important for chondrocyte hypertrophy [Enomoto et al., 2000; Leboy et al., 2001; Zheng et al., 2003] (Fig. 3). In Runx2-deficient mice, the late stages of chondrocyte hypertrophy are blocked [Komori et al., 1997; Otto et al., 1997]. Matrix metalloproteinase (MMP)-13, a downstream target of Runx2, is expressed by terminal hypertrophic chondrocytes [Inada et al., 1999; Jimenez et al., 1999; Porte et al., 1999; Colnot et al., 2004; Ortega et al., 2004]. MMP-13 deficiency results in significant interstitial collagen accumulation leading to the delay of endochondral ossification in the growth plate with increased length of the hypertrophic zone [Inada et al., 2004; Stickens et al., 2004]. In contrast, both Col10a1 knockout mice and transgenic mice with a dominant interference Col10a1 mutation have subtle growth plate phenotypes with compressed proliferative and hypertrophic zones and altered mineral deposition [Jacenko et al., 2001]. The dwarfism observed in human chondrodysplasias with COL10A1 mutations involves skeletal elements that are under great mechanical stress due to disruption in the pericellular matrix in the hypertrophic zone, although a role for defective vascularization has been proposed [Gress and Jacenko, 2000]. Other ECM proteins, including osteocalcin and osteopontin, are known to play functional roles in cell–matrix interactions during endochondral ossification. The ECM remodeling that accompanies chondrocyte terminal differentiation is thought to induce an alteration in the environmental stress experienced by hypertrophic chondrocytes, which eventually undergo apoptosis [Ferguson et al., 1998; Vu et al., 1998; Gerber et al., 1999]. Together these studies indicate that ECM remodeling is the dominant rate-limiting process for chondrocyte hypertrophy, angiogenesis, and osteoblast recruitment during endochondral ossification [Ortega et al., 2004].

Angiogenesis, involving the invasion of perichondrium and hypertrophic zone by blood vessels, is required for the replacement of cartilage by bone [Colnot et al., 2004; Colnot, 2005]. Removal of angiogenic stimuli by ablating VEGF [Gerber et al., 1999] or VEGF receptors [Maes et al., 2002] results in increased length of the hypertrophic zone. *Mmp*-$9^{-/-}$ mice have a similar phenotype [Vu et al., 1998]. The angiogenic factor, VEGF promotes vascular invasion via specifically localized receptors, including Flk expressed in endothelial cells in the perichondrium or surrounding soft tissues, neuropilin (Npn) 1 expressed in late hypertrophic chondrocytes, or Npn 2 expressed exclusively in the perichondrium [Colnot and Helms, 2001]. VEGF is expressed as three different isoforms: VEGF188, a matrix-bound form, is essential for metaphyseal vascularization, whereas the soluble form, VEGF120 (VEGFA), regulates chondrocyte survival and epiphyseal cartilage angiogenesis [Maes et al., 2004; Zelzer and Olsen, 2005]. VEGF164 can be

either soluble or matrix bound and may act directly on chondrocytes via NPN-2. VEGF is released from the ECM by MMPs such as MMP-9 expressed by endothelial cells that migrate into the central region of the hypertrophic cartilage coincident with Flk-positive cells. MMP-13 appears to cooperate with MMP-9 by degrading nonmineralized matrix in the late hypertrophic zone during primary and secondary ossification [Vu et al., 1998; Ortega et al., 2004]. The membrane-bound MT1-MMP (MMP-14), which has a broader range of expression than MMP-9, is essential for chondrocyte proliferation and secondary ossification [Zhou et al., 2000]. These events of cartilage matrix remodeling and vascular invasion are prerequisite to migration and differentiation of osteoclasts and osteoblasts, which remove mineralized cartilage matrix and replace it with bone.

Recent studies indicate that Runx2 can also interact with AP-1 family members during cytokine-induced or PTHrP-dependent MMP-13 expression in chondrocytes [Mengshol et al., 2001] or osteoblasts [Hess et al., 2001; D'Alonzo et al., 2002]. Several genetic studies have provided insight into the roles of AP-1 family members, including c-Fos, Fra1, Fra2, c-Fos, FosB, JunB, and ATF-2, in skeletal development in vivo [Grigoriadis et al., 1993; Reimold et al., 1996; Jochum et al., 2000; Jochum et al., 2001; Hess et al., 2003; Karreth et al., 2004] (Fig. 3). MMP-13 is also a target of c-Maf, which can form heterodimers with AP-1 family members and regulates the differentiation of hypertrophic chondrocytes [MacLean et al., 2003].

NOVEL MEDIATORS OF CHONDROGENESIS AND ENDOCHONDRAL OSSIFICATION

The molecular mechanism of MMP-13 expression in hypertrophic chondrocytes remains unclear. Since Runx2 is expressed mainly in prehypertrophic and less in late hypertrophic chondrocytes [Kim et al., 1999; Takeda et al., 2001], whereas MMP-13 is expressed only in the late hypertrophic zone [Colnot et al., 2004; Ortega et al., 2004; Colnot, 2005], the spatial and temporal discrepancy suggest that unknown intermediate molecules may synchronize with Runx2 to regulate MMP-13 gene expression. A candidate intermediate molecule is GADD45β, which has been implicated in the stress response and cell survival during terminal differentiation of different cell types. In a recent study, we identified GADD45β as a prominent early response gene induced by BMP-2 through a Smad1/Runx2-dependent pathway [Ijiri et al., 2005]. Since this pathway is involved in skeletal development, we examined embryonic growth plates and observed expression of GADD45β mRNA coincident with Runx2 protein in prehypertrophic chondrocytes, whereas GADD45β protein was localized in the nucleus in late stage hypertrophic chondrocytes where Mmp13 mRNA was expressed. In GADD45β knockout mouse embryos, defective mineralization, and decreased endochondral growth accompanied deficient Mmp13 and Col10a1 gene expression in the hypertrophic zone. Transduction of siRNA-GADD45β in epiphyseal chondrocytes in vitro blocked terminal differentiation and the associated expression of Mmp13 and Col10a1 mRNA. Finally, GADD45β stimulated MMP-13 promoter activity in chondrocytes through c-Jun N-terminal kinase (JNK)-mediated phosphorylation of JunD partnered with Fra2 in synergy with Runx2. Thus, GADD45β has a previously undiscovered role as a critical mediator required for MMP-13 expression during the later stages of chondrocyte hypertrophy (Fig. 4). Our findings on Runx2 synergy provide a new concept regarding a temporal and spatial link between BMP-2-induced GADD45β and the induction of MMP-13 gene transcription via the JNK/JunD pathway at terminal stages. The nuclear localization of GADD45β in hypertrophic chondrocytes also suggests a direct mechanism by which AP-1 family members could regulate terminal differentiation. Since GADD45β is associated with cell cycle G2-M arrest [Vairapandi et al., 2002],

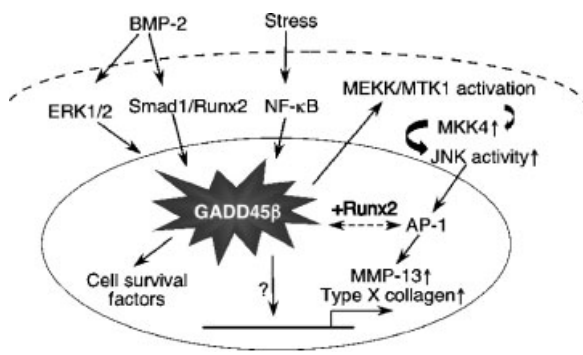

Fig. 4. Scheme of the potential roles of GADD45β in chondrocyte hypertrophy during chondrogenesis.

it may be involved in the inhibition of chondrocyte proliferation in the hypertrophic zone while promoting cell survival during a time sufficient to permit matrix remodeling, vascularization, and mineralization. The process of death of hypertrophic chondrocytes is also one of the most poorly understood steps during endochondral ossification. Since GADD45β is essential for JNK-mediated blockade of TNFα-induced apoptosis in fibroblasts [Papa et al., 2004], it may play a role as a survival factor that maintains expression of critical genes prior to apoptosis.

Other novel mediators of chondrogenesis influence gene transcription by promoting or inhibiting gene expression at different stages (Fig. 3). The hypoxia-inducible factor (HIF) 1α is required for chondrocyte survival during hypertrophic differentiation, partly due to its regulation of VEGF expression [Schipani et al., 2001; Provot and Schipani, 2005]. A long form of c-Maf interacts with Sox9 at early stages to upregulate Col2a1 expression [Huang et al., 2002], whereas C/EBPβ and γ and AP-2α may inhibit chondrocyte differentiation by blocking transcription of Col2a1, aggrecan, and other cartilage-specific genes by direct or indirect mechanisms [Davies et al., 2002; Huang et al., 2004; Imamura et al., 2005]. Many factors that have been defined by whether they inhibit or enhance function of Runx2 coordinate chondrocyte and osteoblast differentiation [Komori, 2005]. Since there is no SMAD site on the Runx2 promoter, it has been proposed that homeobox genes of the Dlx family such as Dlx3 could activate Runx2 signaling in response to BMP-2 during endochondral ossification, whereas Dlx5 and Msx2 are known to inhibit Runx2-mediated activation of genes such as osteocalcin at later stages [Balint et al., 2003; Hassan et al., 2004]. The homeodomain protein Nkx3.2, which is an early BMP-induced signal required at the onset of chondrogenesis, is a direct transcriptional repressor of Runx2 promoter activity [Lengner et al., 2002]. The bHLH factor Twist transiently inhibits Runx2 function and prevents premature osteoblast differentiation [Bialek et al., 2004], whereas cooperation of the Groucho homolog Grg5 or the leucine zipper protein ATF4 with Runx2 promotes chondrocyte maturation [Wang et al., 2004] or osteoblast differentiation [Xiao et al., 2005], respectively. Histone deacetylase 4 (HDAC4), which is expressed later in prehypertrophic chondrocytes, prevents premature chondrocyte hypertrophy by interacting with Runx2 and inhibiting its activity [Vega et al., 2004]. Recent reports from several groups indicate that Wnt signaling, via the canonical β-catenin pathway and activation of TCF/Lef transcription factors, functions in a cell autonomous manner to induce osteoblast differentiation and suppress chondrocyte differentiation in early chondroprogenitors [Day et al., 2005; Gaur et al., 2005; Glass et al., 2005; Hill et al., 2005; Hu et al., 2005]. During chondrogenesis, Wnt/β-catenin acts at two stages, at low levels to promote chondroprogenitor differentiation and later at high levels to promote chondrocyte hypertrophic differentiation and subsequent endochondral ossification [Day et al., 2005; Tamamura et al., 2005].

In conclusion, the interplay of positive and negative factors controls the rate and progression of chondrogenesis. The discovery and elucidation of several novel pathways have increased our knowledge of the complex gene networks during the different stages of chondrocyte differentiation, proliferation, and maturation. It will be exciting and challenging to dissect how all of these networks interact to generate the normal skeleton.

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