[Frontiers in Bioscience 4, d731-742 October 15, 1999]

DEVELOPMENTAL PATTERNS OF CARTILAGE

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1. ABSTRACT

The current state of knowledge of cartilage differentiation leaves many questions unanswered. This review provides an up-to-date examination of current thinking on the subject of developmental patterns in cartilage formation. We will discuss the current model of limb elongation as well as the molecular aspects of chondrogenesis and growth plate formation. This will then be compared with the limited information currently known about the molecular aspects of osteoarthritis.

2. INTRODUCTION

Cartilage differentiation is a highly complex phenomena that remains incompletely understood. The purpose of this chapter is present an overview of current knowledge in the field of limb elongation and endochondral bone formation. Many of the topics that will be summarized here are covered in greater detail in other sections of this book. We will discuss the initial stages of limb elongation and initiation of chondrogenesis, describe the growth plate and formation of endochondral bone, and end with a brief look at how these developmental patterns may be related to osteoarthritis. Throughout, we will also examine some of the major families of proteins that play a role in limb development.

The sheer number of proteins that play a role in cartilage differentiation can be overwhelming. These proteins can be broken down into three major groups: growth factors, signaling molecules, and effector proteins. The first class, the growth factors, is composed of secreted proteins that initiate a cascade of events upon binding to receptors on cellular surfaces. They are generally highly conserved. Fibroblast Growth Factors, WNT proteins, and Hedgehog proteins, all which will be discussed below, fall within this category. Signaling molecules are the intracellular proteins that convert binding of the

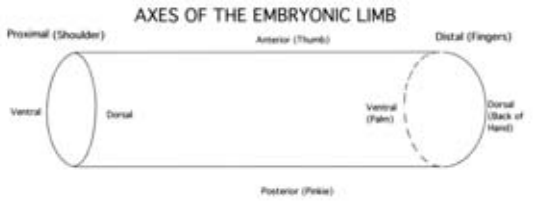


Figure 1. Conceptual Drawing of the Embryonic Limb.

growth factor into a cellular response. These proteins are often growth factor-specific, and include membrane-bound receptors as well as intranuclear transcription factors. Lastly, the effector proteins are the downstream result of growth factor signaling. Examples include extracellular matrix proteins. Interestingly, Bone Morphogenic Proteins can be a downstream result of growth factor signaling, although they in turn have effector molecules of their own. It is sometimes difficult to distinguish a growth factor's direct effect from that of its downstream effector molecules.

A factor complicating our understanding of cartilage differentiation is the diversity of experimental models employed in trying to elucidate its signaling pathways. For example, many molecules found to produce or inhibit cartilage formation, as well as their subsequent molecular cascades, were first discovered in invertebrates, such as Drosophila. Within vertebrates themselves, much of the research in this field has employed avian models, in particular chicken. Although the general patterns of gene activation are probably valid, individual effects of factors cannot necessarily be assumed to be identical across species. For example, Arvidsson *et al* reported in 1995 that FGF-2 induced proliferation while slowing differentiation of rat chondrocytes, an effect opposite to that of IGF-1 (1). By comparison, Dozin *et al* found in 1997 that FGF-2 required IGF-1 to cause proliferation in chicken embryo chondrocytes, while FGF-2 by itself had no effect (2).

Even intra-species experiments on cartilage differentiation may generate conflicting results, for they often use cartilage or bone of different origins, such as sternum, vertebrae or long bones; or different stages of development. Although many of the modulating compounds are often the same, in some cases their effects cannot be predicted in advance. For example, Douarin *et al.* recently reported that Sonic Hedgehog (Shh) and BMP-4 have an antagonistic relationship in the spine, whereby one promotes chondrogenesis in one part of the vertebrae while the other inhibits it. This is then reversed in a different section of the same bone. In the case of Shh the inhibition occurs directly through down-regulation of BMP-4 (3). By contrast, in the limb Shh is known to increase BMP-4 levels during limb elongation (4-5).

3. INITIAL STAGES

3.1. Limb Bud

3.1.1. Overview

The elongating limb bud has three axes; proximal to distal, anterior to posterior, and ventral to dorsal (figure 1). These axes are derived from embryologic anatomy, and therefore the anterior to posterior line is drawn from thumb to little finger. The establishment of these patterns is controlled by different sets of molecular signals. However, compounds involved with one axis may sometimes regulate factors responsible for a different axis, thereby making these three axes intimately linked. The proximal to distal axis is primarily controlled by Fibroblast Growth Factor-8 (FGF-8), the ventral to dorsal by WNT-7, and the anterior to posterior by Sonic Hedgehog (Shh) (figure 2).

3.1.2. Initiation of Limb Bud Formation

The initial signal for outgrowth of the limb bud originates from intermediate or lateral plate mesoderm, and consists of FGF-8, FGF-10 (6), and perhaps retinoids (through induction of the ZPA, see below) (7). Whether the initial factor is FGF-10 or 8 might depend on how far back into embryogenesis one looks, and it is a subject of some debate (6, 8-10). While these two factors can cause limb bud formation and are the ones found *in vivo*, there have been many studies demonstrating that a variety of other FGF-soaked beads can cause limb induction when placed over the appropriate region. These include FGF –1, 2, and 4 (6, 8-9, 11-12).

3.1.3. Proximal to Distal Axis

The proximal to distal axis is regulated from a specialized section of ectoderm referred to as the apical ectodermal ridge (AER), a narrow piece of tissue oriented in an anterior to posterior direction on the distal part of the limb. It is thought that FGF-10 induces the expression of FGF-8 in the AER, which then becomes the main determinant of proximal-to-distal growth. This band of tissue continues to send signals to the undifferentiated mesenchyme behind it throughout elongation, thereby causing growth of the limb.

3.1.4. Fibroblast Growth Factors

FGF-8 is a member of the Fibroblast Growth Factor family, one of only a few groups of usually secreted proteins that govern the differentiation of most animals. Its name is misleading, of course, for its actions affect many cells in the body. There are currently 17 known FGF proteins (6), many of which are known to play a role in limb differentiation. For example, FGF's 2 (basic FGF) and 4, which are found in the AER, will sustain the development of an entire limb even after resection of the AER in chick (13-14).

The mechanism by which FGFs exert their effects is still currently being elucidated, although it is

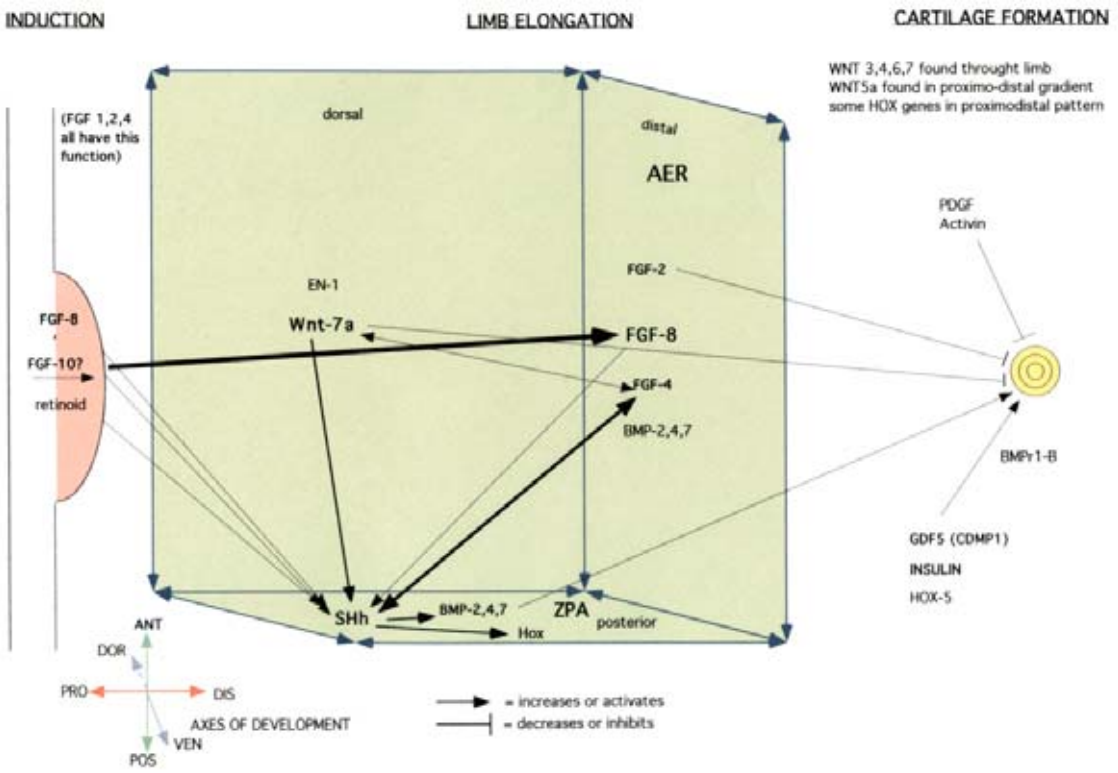


Figure 2. Schematic of Limb Elongation with the three Axis of Embryonic Limb. AER = Apical Ectodermal Ridge, ZPA = Zone of Polarizing Activity, FGF = Fibroblast Growth Factor, SHh = Sonic Hedgehog, BMP = Bone Morphogenic Protein, PDGF = Platelet Derived Growth Factor, HOX = Homeobox

known that several receptors exist which can bind FGF's, and therefore mediate their actions. Fallon *et al* reported in 1995 that different FGF receptors appeared in progression during the process of chondrocyte development in the chick (15): FGF Receptor (FGFR) 1 mRNA was found in undifferentiated mesenchyme, FGFR 2 mRNA in prechondrocytic cell aggregates, and FGFR3 in differentiating chondrocytes. FGFR 3 overexpression has also been found to inhibit endochondral bone growth by slowing the rate of chondrocyte differentiation (16). Additionally, these different receptors have several splice forms, which have varying characteristics such as specificity of binding and effect of activation. For instance, the IIIB form of FGFR3 exclusively binds FGF-1 (15,17). FGFs also bind heparan sulfate proteoglycans, which are obligatory co-factors of the FGFR's (17,18). The multitude of receptors may explain how the same factor can exert different, and sometimes opposite, effects during limb outgrowth.

FGF-8 is not the only FGF found in the AER; FGF 2 (19), 4 (20), and 9 (6) can be found as well. FGF- 4 plays an important role in the regulation of Sonic Hedgehog, and FGF-2, as already mentioned, is thought to be active in promoting proliferation of chondrocytes while inhibiting or slowing the progress of differentiation. Therefore, FGF's are involved not only in the proximo-distal axis, but in other axes as well.

3.1.5. Other Signals

Other compounds that have been found in the AER include WNT-11 (21), BMP-2 (22) and BMP-7 (OP-1) (23). The exact roles of these and other proteins in regulating the AER remain to be determined.

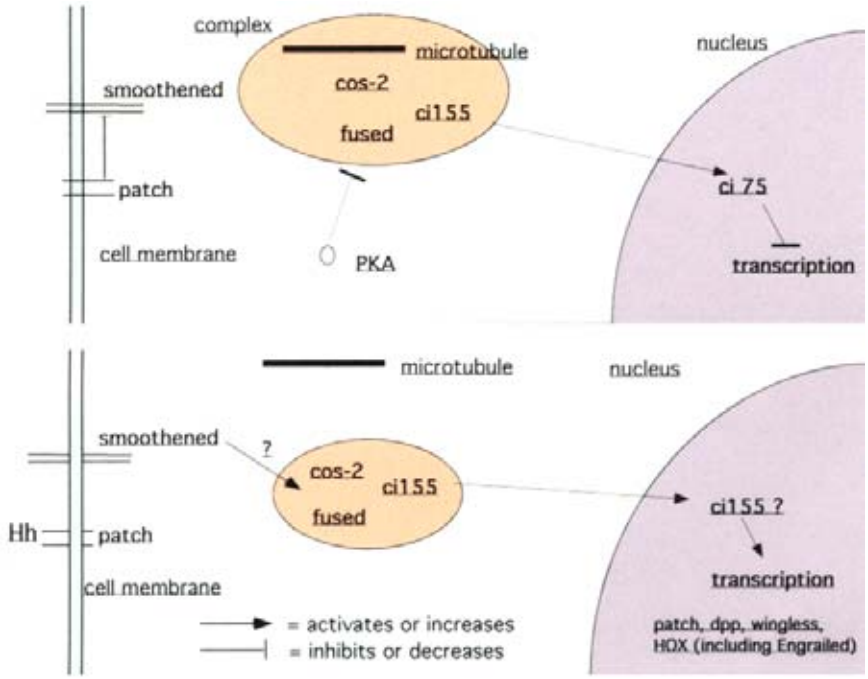
3.2. Anterior - Posterior Axis

The anterior-posterior axis is regulated from the Zone of Polarizing Activity (ZPA), a section of tissue located in the posterior mesenchyme (4,24). The ZPA is defined based on its activity and the expression of Sonic Hedgehog, rather than on anatomical landmarks. Transplantation of the ZPA onto the anterior surface of a limb will result in the development of additional digits, which mirror that of normal limb development (24-25). As mentioned previously, Sonic Hedgehog is the mediator of the ZPA's ability to organize tissue, and it is thought that many of the other signaling molecules found in the ZPA are downstream effectors of Shh binding (24).

3.2.1. Hedgehog

Hedgehog (Hh) genes were first identified in Drosophila (26), and it is in that model system that

Figure 3. Hedgehog Signaling Pathway in Drosophila and Correlates in Vertebrate Development. Hh = Hedgehog,



PKA = Protein Kinase A, Ci = cubitus interruptus

many of the components of its subsequent pathway have been elucidated. Hh protein in the fruit fly consists of only a single species, but in vertebrates four distinct variants have been isolated, Sonic (Shh), Desert, Indian (Ihh), and Tiggywinkle (4). They are all secreted proteins, with the same active 19KD amino terminal fragment, a product of an autoproteolitic cleavage (27), presumably mediated by the carboxy terminal. This active fragment is linked during processing to cholesterol, which anchors the protein to the cell membrane (28). Recent work has also shown that the human Shh becomes posttranscriptionally palmitoylated (29). The attachment to lipid is thought to be integral to the signaling activity of Hh (29-30), and several inhibitors of cholesterol synthesis have been shown to block the Shh signaling pathway (31).

The intracellular effects of HH proteins have not been entirely confirmed in vertebrates (figure 3). It is known that the different Hedgehog proteins bind to a receptor known as Patch (Ptc) (32). At least in Drosophila, Ptc then releases inhibition on the constitutively activated Smoothened (33), which dissociates a microtubule bound complex consisting of several proteins, namely Cos-2, Fused, and Cubitus interruptus (Ci) (33-34) . Ci has two forms, a larger size (155KD) which activates transcription, and a proteolytically cleaved smaller size (75KD) which inhibits it (33,35). Binding of Hh releases the 155KD Ci, which then activates transcription and mediates Hh's effects. In vertebrates, Ci's functions are handled by several distinct proteins; named Gli 1, 2, and 3 derived from different genes (36-37) .

Sonic Hedgehog is known to upregulate several molecules, including BMP's (homologs of Decapentaplegic in Drosophila), HOX genes, and wingless (WNT in vertebrates, although this association has only been shown in Drosophila). Sonic Hedgehog also upregulates Patch, thereby creating a negative feedback loop by increasing the quantity of a negative modulator of its own activity.

In the limb, Sonic Hedgehog is known to increase production of several BMPs (4-5,24,38-39). It also increases FGF-4 (40) in the AER. An interesting positive feedback loop is created, by which FGF-8 in the AER, in a retinoid sensitive manner (7,13,24), activates Shh in the ZPA, which in turn increases production of FGF-4 in the AER. FGF-4 then regulates Shh production in a feedback loop (20,40-41). The method by which Sonic Hedgehog exerts its effects on patterning from the ZPA is not entirely clear. Some of its actions may be related to the molecules that it upregulates, which then diffuse in a gradient like fashion. There have been indications, however, that Shh might have a more direct effect. However a problem arises in that, as previously mentioned, Shh is covalently linked to cholesterol and therefore attached to the cell membrane. On a cellular level, it is difficult to see how the ZPA can affect differentiation of cells on the other side of the limb. A pathway by which it could directly affect tissue some

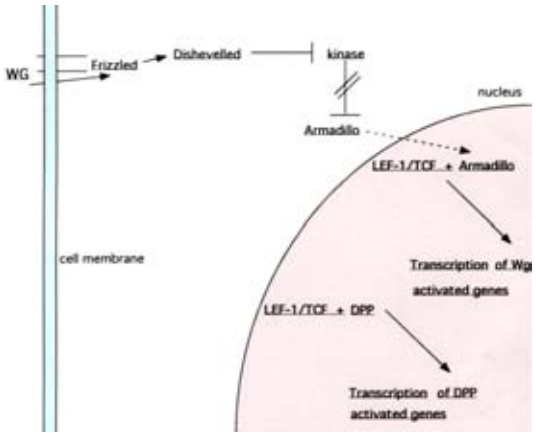


Figure 4. Wingless (WNT) Signaling in Drosophila and Correlates in Vertebrate Development. WG = wingless, DPP = decapentaplegic, LEF = lymphoid-enhancer factor, TCF = T-cell Factor

distance away has yet to be found.

3.2.2. Other Signals

The majority of other molecules that have been suggested as possible mediators of the ZPA's organizing capabilities are downstream products of Shh cell activation. Particular attention has been paid to the BMP's in this regard.

3.3. Dorsal-Ventral

The dorsal-ventral axis (from back of hand to palm) is the one about which least is known. The main controlling protein is WNT-7a (42). Unlike the anterior-posterior and proximal-distal axes, there is no central location where this protein is produced. In fact, in chicken, the sites of WNT-7a expression change during embryogenesis, beginning in the entire dorsal ectoderm and ending in the dorsal ectoderm overlying interdigital spaces (43).

3.3.1. WNTs

As mentioned previously, WNT is the vertebrate homolog of the Drosophila gene wingless, which in fruit flies is upregulated by Hh. The WNT family is composed of 15 members (44). Although the most carefully studied WNT factor in vertebrates, WNT-1, is not found in the limb, there are at least six WNT members which are. Four of these, WNT- 3, 4, 6, and 7b are uniformly distributed in the limb ectoderm, while WNT 5a is found in a proximo-distal pattern in the limb mesenchyme (45-46). WNT-7a, in addition to dorso-ventral patterning, is thought to possibly play a role in the FGF-4/ Shh positive feedback loop (43,47).

Like Hh, most of the activating pathway of the WNT family has been identified in Drosophila, and has yet to be verified in vertebrates (figure 4). Wingless binds to the transmembrane receptor Frizzled (48-49), which in turn activates a second protein known as Dishevelled (50), see figure 4. Activated Dishevelled then inactivates a kinase that is a repressor of the Armadillo protein (51,52). Armadillo then enters the nucleus where along with yet another factor called T-Cell Factor (TCF) causes transcription of wingless sensitive genes (52) (TCF might be acting as a repressor that becomes inactivated or as an activator (49)). In vertebrates the homolog of Armadillo is named B-catenin, whereas TCF is known either as TCF or a related protein called Lymphoid Enhancing Factor (LEF-1).

3.3.2. Other Signals

Another possible candidate for a role in dorso-ventral patterning is EN-1, a homolog of the Drosophila gene Engrailed (53). En-1 is a Hox gene, which, as mentioned previously, is sometimes upregulated by Shh, thereby possibly further linking the three axis of limb development. Not surprisingly, Engrailed is upregulated by wingless in Drosophila, although EN-1 has not yet been shown to be downstream of WNT-7a in vertebrates (45). En-1 might be a ventral differentiation factor, thereby creating a gradient between the two compounds that controls dorso-ventral differentiation during embryogenesis.

4. INITIATION OF CHONDROGENESIS

There has been a concerted effort to elucidate the signals that initiate chondrogenesis. Most of this research has concentrated on molecular aspects, but there has also been data indicating that biomechanical forces and cell-cell interactions may play a role as well.

4.1. Chemical Factors

Many compounds have been shown, usually *in vitro*, employing different cell cultures, to induce or inhibit chondrogenesis. The limitation to this approach, of course, is that one does not see interactions between different cell types, thereby possibly missing some molecular effects. Many of these molecules may also play roles in growth plate development. One such group of factors is the Bone Morphogenic Proteins (BMPs).

4.1.1. Bone Morphogenic Proteins

The BMP family consists of at least 13 proteins, all members of the TGF-B superfamily with the exception of BMP-1 (54). As with the FGF's, BMP's are misleadingly named, for their actions have been implicated in the development of many tissues, including heart and gut. Because BMP's have been isolated from different species, and due to the lack of standardization of these purifications, several of the BMP's have alternate names that are often used interchangeably. For instance, BMP-7 is OP-1, BMP-8 is OP-2, BMP-12 is Growth and Differentiation Factor 7 (GDF-7), and BMP-13 is both GDF-6 and CDMP-2 (54-55). The Cartilage Derived Morphogenic Proteins (CDMP) are also TGF-B family

members, and have similarities with the BMP's (or in some cases are the same proteins) (55).

Many of the BMP's have been found to be active in promoting either chondrogenesis or bone formation, either alone or in combination with other molecules. The same compound may even have varying effects, based on cell type or cell age. Hiraki *et al*, for example, showed that BMP-2 initiated chondrogenesis in undifferentiated ATDC5 cells (56), while Hurle *et al* showed that BMP-2 and BMP-7 caused apoptosis of undifferentiated mesenchyme while supporting already differentiated chick chondrocytes (57). BMP-2 has also been shown to induce the undifferentiated mouse mesenchymal cell line 10T1/2 down the osteogenic pathway, particularly when combined with Shh (58). BMP-4 (59) and CDMP-1/GDF-5 (55-60) have also been implicated in the initiation of chondrogenesis.

BMP's 2 and 4 are thought to support the transition to chondroblasts, as previously mentioned, but they may also promote proliferation and hypertrophy of chondrocytes (57). Not surprisingly, Noggin, a BMP antagonist, slows cartilage formation, and mice lacking Noggin develop hyperplasia (61), although this effect may be due to lack of inhibition at the differentiation stage.

One reason for the variety of results observed with BMP's may be the existence of several BMP receptors. Type I and II receptors function closely together, and upon ligand binding to both the type II phosphorylates the type I receptor (62-63), thereby activating the protein. There are two BMP type I receptors, a and b, which differ in their spatial and temporal distribution. IB is found in precartilaginous tissue, and is thought to possibly regulate apoptosis, while IA is specific for prehypertrophic chondrocytes and perichondrium, and is thought to regulate the pace of chondrogenesis (63). Thereby a difference in receptor, and not necessarily the secreted molecule itself may be the difference between cells differentiating or dying.

4.1.2. Other Signals

There have been many other compounds and genes tied to the initial steps of chondrogenesis. Among these are insulin (2), IGF - 1 (2), Inhibin (64), dexamethasone (65), and possibly products of Hox genes (66). For instance, IGF-1 is known to encourage both differentiation of mesenchyme into chondroblasts and proliferation of those cells (2,67). There are also several agents that inhibit chondrogenesis, such as FGF-2. Likewise, WNTs - 7a and 1 (which is not found in the limb) (44) block chondrogenesis when *in vitro*. It is thought possible that WNT-5a replaces WNT-1 in the limb, given its proximo-distal gradient. Lastly, Activin (64) and PDGF (68) also have inhibitory properties on chondrocyte development.

There are also extracellular markers that characterize different stages of chondrogenesis; some more specific than others. For example, type II collagen is typically associated with cartilage differentiation. In actuality, there are two splice forms of type II collagen, A and B, with exon 2 being cleaved out of the type IIA mRNA in order to get type IIB (69). Type IIA collagen is found in chondroprogenitor cells, as well as certain other tissues, possibly acting as an inducer of chondrogenesis (70-71). Type IIB (72) is expressed in chondrocytes. Additionally, type X collagen is expressed only by hypertrophic chondrocytes, while type I collagen is known as a marker of both the mesenchymal phenotype and also of osteocytes. Intranuclear proteins such as SOX-9 have also been proposed as markers for chondrogenesis, particularly in less mature chondrocytes (73-74).

4.2. Biomechanical Factors

There have been some indications that stress applied to certain tissue may result in initiation of or increase in chondrogenesis (75). Most of this type of research, however, has dealt with mature articular cartilage, rather than embryonic tissue. One exemption is a recent paper by Slavkin *et al.* (76), which found that a static compressive force increased both the rate and quantity of collagen II and aggrecan expression in embryonic tissue. While there remains much work to be done before the effect of mechanical stress on limb development is fully understood, such forces could play a significant role in the initiation and maintenance of chondrogenesis.

4.3. Cell-Cell Interactions

Certain cell lines begin to express chondrocytic markers when plated at high concentrations *in vitro*. One example of this is the C3H10T1/2 cell line, which will begin to differentiate when placed in micromass cultures. A possible explanation for this comes from Lau *et al.* (77), who have recently discovered a heparin binding protein, Cyr61, which promotes cell aggregation and adhesion in mesenchymal limb tissue. This protein promotes chondrogenesis in micromass cultures, and inhibition of Cyr61 prevents it. Additional data from Tuan *et al* showed that increased calcification of ECM can inhibit chondrogenesis in embryonic calveria. Interestingly, soluble calcium appeared not to play a role (78-79). Other possibilities include N-CAM and N-cadherin, which have been found on the plasma membranes of clumping prechondrocytic cells as they differentiate to chondrocytes (80). Both cell-cell and cell-ECM interactions may be crucial to cartilage development.

5. GROWTH PLATE AND BONE FORMATION

5.1. Introduction

The cartilage rudiments which form templates of the future bones of the skeleton undergo

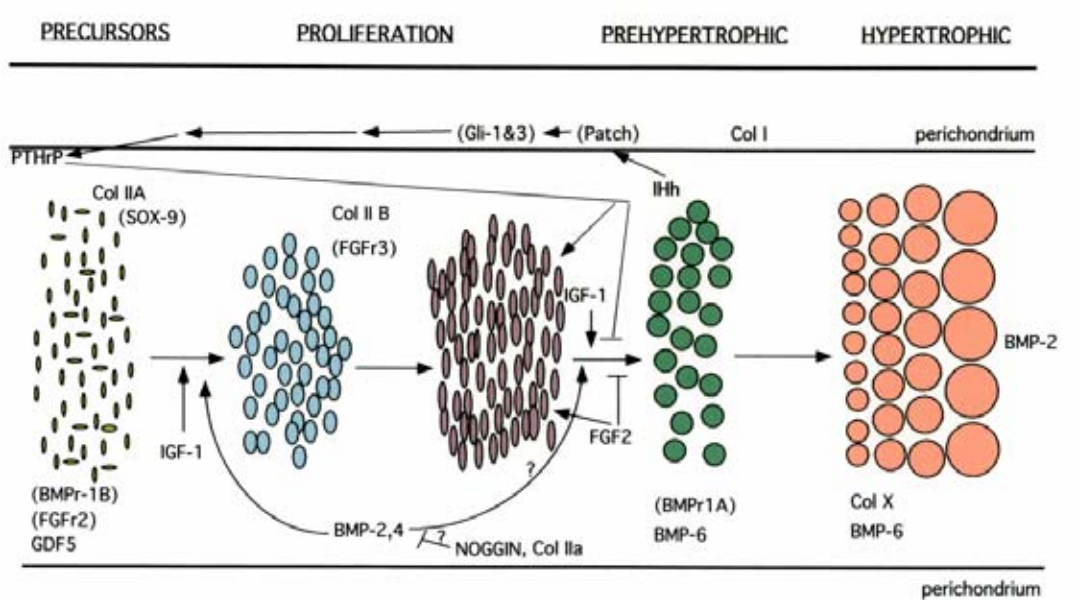


Figure 5. Embryological Development of Cartilage. IGF-1 = Insulin-like growth factor 1, PTHrp = Parathyroid Hormone related protein, Col IIA = Collagen IIA, Col IIB = Collagen IIB, BMPr = Bone Morphogenic Protein receptor, FGFr = Fibroblast Growth Factor receptor, Ihh = Indian Hedgehog, BMP = Bone Morphogenic Protein, please refer to text for any other abbreviations

the normal sequence of cell proliferation, maturation, hypertrophy, calcification and ossification. The growth plate, or physis, allows bone extension to occur by a similar process, substituting one tissue substance (bone) for another (cartilage). Histologically, several zones, reminiscent of the formation of cartilage templates in the embryo, may be identified (81), (figure 5). Closest to the epiphysis one finds undifferentiated mesenchymal cells, sometimes named the resting zone. These cells appear unorganized. Moving towards the metaphysis, the cells become progressively more chondrocytic, until they are identifiable as chondroblasts. During this maturation, the cells are in a proliferation stage. Finally, the chondroblasts become rounder and more linearly organized until they become hypertrophic chondrocytes. This event is followed vascular invasion and bone formation.

5.2.Markers

One example of a molecule often used as a marker of growth plate development is Indian Hedgehog, which is found in the proliferative and prehypertrophic areas. Further along, BMP-6 is seen in prehypertrophic and hypertrophic cells, along with articular cartilage (82-84). As mentioned previously, type X collagen is found in the hypertrophic zone (85). Both collagen X and BMP-6 are often used as signs of progression to the hypertrophic phenotype.

5.3. Regulation

There are many compounds that are thought to regulate the growth plate, although separating the effects on specific zones is very difficult. IGF-1 and FGF-2 have stimulatory effects on proliferation, although these actions seem to be animal and age-dependent. FGF-2 might increase proliferation by inhibiting cells from progressing to the hypertrophic state (39).

Perhaps the most important, and certainly the most studied regulators of the growth plate are Indian Hedgehog and Parathyroid Hormone Related Protein (PTHrp). PTHrp is known to decrease the rate of differentiation of chondrocytes in the growth plate. Vortkamp *et al* have proposed that there exists a local loop to control PTHrp expression thereby controlling the change from proliferative to hypertrophic chondrocytes (84). In this model, Ihh, secreted by proliferating and prehypertrophic cells bind to a protein called Patch in the perichondrium. Several Hedgehog associated proteins, such as Patch and Gli-1 and 3 are expressed in the perichondrium (82,84). It is thought that Patch and Gli are not present in the sections of growth plate where Ihh is found (84). Patch, then, through an as yet unclarified pathway, increases the local secretion of PTHrp from perichondrial cells near the undifferentiated mesenchyme. PTHrp then binds to its receptor, which has been found on chondrocytes prior to Ihh expression. Through this event a negative feedback loop is established, for cells beginning to hypertrophy send signals to the remaining cells that block further differentiation, and thereby increase proliferation.

It is not known whether Indian Hedgehog acts directly or indirectly to increase PTHrp release. As mentioned previously, hedgehog proteins are attached to the cell membrane, so their ability to diffuse over distance is questionable. There has been some evidence that the BMP-IA receptor is a downstream mediator of Ihh in the perichondrium

(63,84). Given that BMP's are known to be upregulated by Hedgehog proteins, it is attractive to think that BMP-IA receptor might be involved in posttranscriptionally the pathway by which Ihh exerts its effects on limb development.

6. CHONDROGENESIS AND OSTEOARTHRITIS

The process of chondrogenesis is relevant to osteoarthritis (OA) in two ways. First, evidence is beginning to emerge that osteoarthritic chondrocytes are quite metabolically active and reinitiate synthesis of some proteins that are characteristic of early developmental stages. Second, an understanding of cartilage differentiation and development will provide guiding principles for tissue engineering of neo-cartilage, and may, therefore, play a part in new therapies for this common disease.

In the early phase of OA, the pathologic processes that occur seem to indicate that mechanisms of cartilage repair, rather than degradation, are at work. For instance: (1) Chondrocytes in OA cartilage are stimulated to undergo cell replication, which is the converse of the nonproliferating chondrocytes of normal cartilage (86). (2) An increase in the synthesis of ECM molecules has been observed in OA cartilage (87). (3) Chondro-osteophyte nodules form. Similar changes have also been reported in experimental canine OA, an important model in the study of early disease (88). The process of remodeling in OA cartilage seems to mimic those of cartilage development during embryogenesis. Sorrell and associates (89) and Caterson and associates (90) demonstrated that when monoclonal antibodies to specific patterns of sulfation infrequently found on chondroitin sulfate were used as probes, the appearance of these patterns were associated with different cell types and were differentially regulated in chick embryogenesis. Interestingly, the same patterns of sulfation were increased canine OA knee cartilage compared to non-operated contralateral controls (90).

The most definitive studies showing the types of molecules synthesized during the attempts at repair during OA come from Aigner and associates (91-93). They showed that many of the chondrocyte characteristic molecules are reexpressed, such as type II collagen, aggrecan, and link protein. Increased type II collagen mRNA is a particularly abundant hallmark of OA tissue. Recently, we have shown that some of this type II collagen is of the IIA variety, which is an embryological form of the molecule. In contrast, type I collagen, a marker for mesenchymal cells and bone cells, is not expressed. Interestingly, type X collagen, characteristic of hypertrophic chondrocytes, was also found, suggesting that the cells may be undergoing further differentiation. Consequently, there is substantial evidence to indicate that chondrocytes are activated in OA and potentially could be stimulated to synthesize appropriate cartilage ECM, or even recapitulate developmental patterns.

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Key Words: Hedgehog, Cartilage, Development, BMP, FGF, Limb, Review

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Received 8/24/99 Accepted 8/31/99