Skeletal development and osteoarthritis

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The limited ability of articular cartilage to repair after traumatic insult or during arthritic degeneration is widely attributed to the fact that the tissue lacks both innervation and vascularisation. However, comparative studies of articular cartilage repair in load bearing and non-load bearing areas of the joint, in addition to some in vitro studies suggest more strongly that adult articular cartilage has, intrinsically, a reasonable repair potential if not ability.1 The possible reasons for this repair limitation will be discussed below, but it is not always the case and, indeed, there are many reports in the developmental literature of substantial repair and/or regenerative capacity of cartilage. An excellent example is the newt limb which, after amputation, not only regenerates the articular cartilage, but also the entire cohort of lost skeletal elements and in the correct spatial pattern. Whilst mammals do not possess such a regenerative potential, during embryology, there can be a considerable degree of regulation which allows for replacement of lost parts.2

At what point in development is the repair capacity of mammalian cartilage restricted and for what reasons? Repair mechanisms apart, we may also ask what can we learn about degenerative joint disease by studying the developing constituent tissues of a joint? Many of the processes which occur during both pathology and repair, seem to mimic some of those processes which occurred during embryonic development. In the following pages I will consider some of the features which appear common to both osteoarthritic cartilage and developing cartilage. Such features include cell proliferation, elevated matrix synthesis and morphogenesis; taken together, these processes constitute growth and in osteoarthritis this is manifest as osteophyte formation. Before addressing these aspects, it is worth considering the primary components of the development of a cartilaginous element of the appendicular skeleton.

Most work on the early development of the appendicular skeleton has been carried out on the embryonic chick. The chick is easily obtained, is cheap and readily accessible through holes cut in the shell. The incubation period of 21 days also facilitates experimental manipulation. In contrast, uterine development makes experimental manipulation of mammalian embryos very difficult. In addition, because the developing skeleton of the chick limb has been used widely as a model for pattern formation, there is a comprehensive literature on most aspects of early skeletogenesis. Whilst the relative lack of data on mammalian species is inconvenient, it is not restrictive since there is a tendency for the more fundamental processes in embryology to be conserved across vertebrate classes. Consequently, most of the processes and concepts mentioned below will have been obtained and formulated through studies on the chick embryo, but to our knowledge, most will hold for the mammalian counterpart.

The developing limb grows out from the flank of the embryo as an ectodermal outpushing containing mesenchyme which is derived from the somatopleure. At the tip of the early limb, a specialised thickening of the ectoderm develops which is known as the apical ectodermal ridge (AER).3 This ectodermal specialisation is essential for continued outgrowth through inductive influences and the underlying mesoderm, in turn, maintains the AER. Thus limb outgrowth is dependent on reciprocal ectodermal/mesodermal interactions4 which are required throughout the specification of the entire limb skeleton.5

For some time, the limb mesenchyme appears as a homogeneous population (histologically) fairly evenly distributed throughout the limb. About 12 hours before overt chondrogenesis (at 4 days of incubation), the presumptive cartilage cells condense to form prechondrogenic condensations which lie in the positions of the future skeletal elements and appear in a proximo-distal sequence. Consequently, the first to form in the leg are the condensations of the femur, tibia/fibula. The nature and significance of the prechondrogenic condensation has been the subject of some interest. Considerable cell/cell contact takes place within the condensation6 with gap junctions subsequently being identified7 and recent work has shown that the cells are able to transfer the dye lucifer yellow.8 Whilst some authors stress the importance of the interactions which occur within the prechondrogenic condensation it is, nevertheless, true that single mesenchymal cells isolated from the very early limb before condensation, can become chondrogenic when maintained under appropriate in vitro conditions.9 Consequently, the condensation process does not appear to be a prerequisite for chondrogenic differentiation, but may well facilitate the process by maintaining the cells in a rounded configuration which favours chondrogenesis.10 It is more likely that condensation is essential for accurate morphogenesis.

Before the synthesis of type II collagen (at 4-5 days incubation), the prechondrogenic condensation is strongly immunoreactive for type I collagen,11 fibronectin12 and PG-M.13

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Concomitant with the secretion of type II collagen and aggregan, the cells assume distinctive orientations (figure). Cells in the centre of the rudiment flatten so that their long axes lie perpendicular to the axis of growth. Those cells residing at the epiphyseal ends of the rudiment remain rounded. Hypertrophy begins at the centre of the flattened cell zone (at 7 days incubation) which now represents the mid-diaphysis. Thus the three chondrocyte types, rounded, flattened and hypertrophic are established and will remain until the closure of the epiphyseal growth plate. The formation of the secondary centre of ossification (in mammals) delineates the epiphyseal plate cartilage from what now can be called articular cartilage rather than epiphyseal cartilage. There have been few studies from this point in development to the establishment of the recognised histological zones of articular cartilage. Nevertheless, there are some publications of note. Mankin\textsuperscript{14,15} studied the pattern of cell division in the neonate rabbit knee. Briefly, it was found that two bands of proliferation were evident. One above the sub-chondral plate and the other beneath the articular surface. It can be easily envisaged that such a pattern facilitates the expansion of the tissue. We do not know at what point proliferation becomes restricted to two bands from the more generalised pattern of proliferation within the embryonic epiphysis.

There are also a number of fundamental questions remaining to be resolved which may have a direct bearing on the ability of cartilage to repair during pathology later in life. Such questions include: how do articular chondrocyte sub-populations (for example, a surface chondrocyte versus a chondrocyte which occupies the calcified cartilage zone) evolve from what appears as a rather homogeneous population of epiphyseal chondrocytes in the embryonic rudiment? Are they specified purely by position within the tissue, or do epigenetic influences such as loading have a major role? Clearly there is not a simple progression of a differentiative maturation process as in the epiphyseal plate. Furthermore, even during OA when the calcified tide-mark advances through the tissue depth, it never progresses as far as an intact unfibrillated articular surface. Do the discoid surface articular chondrocytes have the ability to calcify? Certainly, recent evidence from our laboratory has shown that the surface chondrocytes can synthesise alkaline phosphatase and type X collagen under appropriate culture conditions,\textsuperscript{16} but we have yet to demonstrate calcification in these cultures.

When considering the relationships between development and pathological change such as OA, it is useful to identify possible common features present in both situations and also how these may differ from each other particularly in their regulation.

Relevant features include:

1. **Cell division** The mechanisms whereby chondrocytes re-enter the cell cycle remain to be elucidated. Is the new division driven solely by growth factor availability in a matrix depleted of proteoglycans? Does collagen or other matrix components have a role—particularly those involved in cell/matrix interactions? To what extent do epigenetic factors such as mechanical loading affect the process?

2. **Elevated matrix synthesis and turnover** To what extent is the newly-synthesised matrix in OA similar qualitatively to that synthesised during development? What are the differences (and similarities) between matrix degradation? This latter issue is complex since turnover of matrix may be controlled at several levels which include, amount of proteinase enzymes synthesised, the relative degree of activation or inhibition and the specificity of the enzymes synthesised.

3. **Growth** During embryonic development, cartilage growth incorporates morphogenesis. In OA, this renewed growth and morphogenesis may be manifest as osteophyte formation and advancement of the tidemark. To what extent is the morphogenesis in this renewed growth epigenetically controlled particularly in relation to functional use of the joint?

**Cell division**

During early development of the cartilage rudiment, there is widespread division within the epiphyseal cartilage and particularly the rounded chondrocytes.\textsuperscript{17} After the formation of the secondary centre of ossification, division becomes restricted to the proliferative cells of the epiphyseal plate, and in the developing articular cartilage, to a band of cells in the basal region adjacent to the subchondral plate and a second band of cells beneath the articular surface as described above.\textsuperscript{14,15,18} Interestingly,
during development, there is a transition of the main proliferative zone from the rounded cells of the epiphysis to the flattened cells of the epiphyseal plate. We do not know what the cellular basis of this transition is, but in vitro, we know that a flattened morphology favours proliferation.20 Gradually, as growth ceases, so too does proliferation.20-22

In mature normal articular cartilage, there is no chondrocyte division. During degenerative disease including animal models of arthritis and experimentally damaged cartilage, chondrocyte proliferation is re-initiated.23-29 In human osteoarthritis at the time of arthroplasty, the renewed division appears as cell clusters or chondrones which normally reside in the transitional zone. However, the arthritic lesion by this time is advanced and the surface layers highly fibrillated or lost altogether. In cases of repair due to experimental lesions, such as, whole depth defects into the subcondral plate, then the proliferative response can also occupy the full cartilage depth.20 In such model systems, however, it appears that surface chondrocyte proliferation or clusters are rare.30

In full-depth articular cartilage explants maintained in organ culture, proliferation is most common in the basal regions of the cartilage (and near the cut edges) but only if the culture medium contains serum and hence a variety of growth factors31 or exogenously added growth factors.32 Thus there are distinct differences between the patterns of proliferation in developing and repairing cartilage and, whilst during development, transitional zone chondrocytes do not normally divide, they clearly retain the potential to do so after traumatic insult. The other important difference is that during early development of the cartilage anlagen, mitosis is followed by the separation of daughter cells by intercellular matrix partitions. In later development of the articular cartilage, mitosis appears directional and can give rise to cell columns as seen in the transitional zone. During repair, however, large clusters or chondrones result from proliferation which can comprise over 100 cells with little or no partitioning between the cells. The observed differences between the two systems may reflect quite distinct epigenetic influences on morphogenesis; for example, during early repair, the cartilage is usually under normal functional loading.

We know little about the regulation of proliferation during cartilage development and repair. However, two quite separate but possibly related questions are—what regulates the cessation of proliferation during development and what initiates proliferation in repair? We do not know the answers to either of these questions, but in relation to development, there is now a considerable literature on the effects on chondrogenesis and cartilage growth of three major families of growth factors: the insulin-like growth factors (IGFs), the transforming growth factors (TGFs) and the fibroblast growth factors (FGFs). Most of these studies address the distributions of growth factors during development and the effects of exogenously applied growth factors both on chondrogenic differentiation by mesenchyme (in vivo and in vitro) and the maintenance of phenotype by differentiated chondrocytes in vitro.33 There are a number of useful generalisations that can be drawn: TGFβ peptide is present in all areas of the chondrifying condensation during chondrogenesis,34 but once the tissue has differentiated, then there is little mRNA for TGFβ, present in the chondrocytes.35 It appears there is great variation in the distribution of messages for the various isoforms of TGFβ which suggests multi-functional roles particularly in concert with other factors. For example, it has been suggested that the TGFβ1 isoform has a central role in chondrogenic induction.35 In vitro studies using chick limb mesenchyme micro-mass cultures have shown that all members of the TGF superfamily have the ability to stimulate chondrogenesis36-37 including bone morphogenetic proteins38 and activin.39 The latter comprises a family of growth factors implicated in specification of axis pattern in Xenopus40 and induction of mesoderm in the same animal.41 However, all of the above in vitro studies have included at least insulin (or insulin-containing serum) in the medium (to facilitate cell adhesion), which is also known to bind to the IGF receptor. Consequently, we cannot rule out a synergistic effect. Indeed, in the presence of trace amounts of serum, O’Keefe et al.42 found TGFβ to be inhibitory to the proliferation of articular chondrocytes. More recently, it has been shown that TGFβ can lead to chondrocyte modulation in vitro with similar characteristics to dedifferentiation, that is, expression of type I collagen.43

The effects of IGFs are well documented, and in addition to promoting chondrogenesis through stimulation of matrix secretion, have also been shown to be mitogenic for chondrocytes.35 Ralphs et al.44 showed that during the development of the chick humerus, most chondrocytes stained positively for IGF1 peptide but this distribution became restricted to the hypertrophic cartilage region during later development. Again, there appears to be complex patterns of distribution which are little understood. Most authors concur that there appears to be differential potency between IGF1 and IGF2 depending on the chondrocyte location, species and conditions under which the factors are applied.

In general, FGF promotes chondrocyte proliferation probably in concert with some of the TGFβ factors.33 Unfortunately, we know very little about the distribution of FGF in the developing cartilage. However, in addition to maintaining the proliferative state, FGF also inhibits terminal differentiation, that is, hypertrophy.45 Consequently, this factor may have a role in the establishment and delineation of the calcified cartilage and subchondral plate.

We know much less about the distributions and roles of these growth factors in the regulation of the mature normal tissue and during degenerative disease. However, we do know that the extracellular matrix can act as a pool of bound growth factors which may be
released and activated during degeneration. This would appear to be the case for TGFβ since the concentration in the tissue has been calculated to be some ten times that required to elic a stimulatory response when exogenously applied to explants. A variety of matrix components are known to selectively bind growth factors and in vitro experiments suggest that for TGFβ, decorin or biglycan may be key players. In the mature articular tissue, it is suggested that whilst TGFβ has the ability to promote synthesis generally, it nevertheless may play more of a regulatory role with IGF1 maintaining aggrecan levels. Consequently, it can be envisaged that degenerative changes in the matrix may release TGFβ which can then stimulate cells to up-regulate synthesis as part of a repair response. Similarly, it has been shown recently by Middleton and Tyler that OA chondrocytes have elevated IGF1 mRNA levels when compared with normal cartilage chondrocytes, and again this may be linked to both a proliferative and synthetic repair response. Furthermore, it appears that the degree of IGF1 gene expression may be proportional to the severity of the lesion. In relation to OA, there is also evidence that ageing tissue responds less vigorously than younger cartilage to exogenous IGF and TGF which may indicate differential receptor expression, growth factor affinity or availability. This may partly explain the poor reparative response of cartilage in older patients.

The role of FGF in the mature tissue has been little studied but has been identified within the tissue. It is likely to play a considerable role in the control of the proliferative response and may act as a proliferative progression factor. In this context, it is interesting that a reduction in the bFGF receptor is coupled to the terminal differentiation of rabbit chondrocytes, that is, the non-proliferative hypertrophic state. One study on rabbits has shown that FGF can potentiate repair of intra-articular defects (which do not penetrate the sub-chondral plate). To place all of the above data in context, we still need to know what are the stimuli which initiate a proliferative repair response. If adult articular cartilage is explanted to culture, then there is little proliferation other than adjacent to cut edges. Most authors have attributed the onset of division as a response to matrix breakdown whilst others have specifically distinguished loss of proteoglycans as an initiating factor. Although there is no good evidence to substantiate this. Indeed, by selectively depleting the matrix of cartilage explants in culture, we have shown that bacterial hyaluronidase depletion of proteoglycan did not stimulate DNA replication, but cleavage of collagen with purified collagenase did. We interpreted our data to suggest that collagenase treatment allowed the constituent chondrocytes to undergo shape changes, particularly flattening which facilitates proliferation in vitro. However, in relation to the in vivo situation, the role of mechanical stimulation in the initiation of proliferation needs to be investigated.

Nevertheless, the above and other data all suggest that considerable disruption to the tissue architecture accompanies renewed proliferation. An interesting corollary is ageing. During normal ageing, there is a decline in cellularity of the superficial zones and, since mitotic figures are not observed, we can assume that cell loss is not compensated for by proliferation. Thus the natural decline in cell numbers in the superficial region during ageing would appear to be insufficient stimulus for a significant proliferative repair response. In contrast, during OA superficial cells are lost and is followed by proliferation of chondrocytes in the deeper tissue. Whether these two events are related, however, remains to be established.

**Elevated matrix synthesis and turnover**

We know very little about the role of the rate of matrix synthesis and turnover either during development or repair. There are obvious intrinsic differences between the two situations. During development, both synthesis and turnover are intimately linked to morphogenesis and growth whereas in repair, elevated synthesis is likely to be related to (in the first instance) supplementation of lost matrix with any morphogenetic consequence a secondary effect. Nevertheless, there are several similarities between the two processes. However, before consideration of these facets it is worth remembering that in discussing repair during osteoarthritis, we mean the initial repair response during the early stages of the disease (and probably preclinical). Those responses which occur during the later stages of the disease progression are impossible to interpret and, in any case, are the final throes of a failed reparative response.

Most of the data which have accrued concerning the quantitative and qualitative changes in matrix composition during early OA has been derived from animal models particularly the dog. It has been shown that there is a significant up-regulation of proteoglycan synthesis although at the same time increased enzymatic degradation which in concert with mechanical loading leads to overall loss of proteoglycan from the matrix. The newly synthesised proteoglycans have many similarities with those from immature animals in that they have a reduced chondroitin 6:4-sulphate ratio and the glycosaminoglycan chain length is increased. Alterations in sulphation reminiscent of those found in more embryonic tissues have been reported recently. Using a panel of antibodies which recognise a number of epitopes including sulphated regions on the GAG chain, Caterson et al have shown that early OA cartilage in the Pond-Nuki model expresses epitopes recognised by antibodies 7D4 and 3B3 which are absent or expressed at very low levels in non-AO cartilage. The same epitopes are, however, present in embryonic cartilage and pre-chondrogenic mesenchyme.
We know a lot less about collagen both in development and in OA. We know again from various animal models that collagen type II synthesis is elevated in early OA. More recently, it has been shown that type IX collagen, which is covalently linked to the surface of type II fibrils and is thought to control the fibril diameter of type II collagen is not correspondingly upregulated. There appears therefore to be an overall depletion of type IX during early OA. The significance of these observations is not clear, and since we know so little about the roles of the various minor collagen types in development, relevant parallels are impossible to draw. However, we are now beginning to gain better insights using transgenic models which include gene knock-out experiments. Very recently, it has been reported that mice which do not express the type IX collagen gene develop normally, appear phenotypically normal but develop osteoarthritis after about six months (Olsen, personal communication). Conversely, mice in which the alpha 1 (II) collagen chain gene is over-expressed develop abnormally thick type II collagen fibrils within the cartilage matrix. Intriguingly, such animals die at birth.

More easily appreciated is the contribution of another minor collagen, type X. This collagen has an association with calcification and is produced mainly by hypertrophic chondrocytes. In normal cartilage, it is found in the calcified cartilage zone which intergrades with the sub-chondral plate. From late adolescence it is not detected in articular cartilage at all. However, during OA the calcified tide-mark moves up through the tissue depth with a consequent expression of type X collagen both in the newly calcifying cartilage and in the upper transitional zone. Such a response can be regarded as a re-initiation of growth. However, perhaps the pertinent question in relation to development is how the zone of calcified cartilage is regulated during development and how is the tide-mark positionally specified? What prevents the entire articular cartilage from calcifying? Studies in our laboratory have shown that human articular surface chondrocytes have the ability to synthesise both type X collagen and alkaline phosphatase under culture conditions although in short term culture conditions (at two weeks) they have little ability to calcify. Clearly, there is considerable scope for further investigation in this important area, and closer study of developing systems will prove fruitful.

In relation to turnover, we know little about either development or repair. We know that during repair (that is, early OA), turnover is elevated and is consistent with the increased levels of synthesis. However, whether these levels of degradative enzymes generate a 'credit' or 'deficit' in newly elaborated matrix is unknown. Again, this is an important area of investigation and wholesome to know more about the various matrix pools which may be differentially turned over and the consequent effects this may have on matrix function and integrity.

Cartilage morphogenesis

It is evident from the preceding account that there is a considerable potential for articular cartilage to repair. It appears, however, that the repair matrix for some reason is functionally unstable invariably progressing towards total degeneration. In a similar vein, attempts at experimental repair of articular cartilage have resulted largely in failure even though defects may be filled with a cartilaginous-like material. The most likely reason for this failure lies in morphogenesis. The morphogenetic mechanisms which give rise to articular cartilage are unknown. We do not know how both the structural and biochemical heterogeneity, which characterises articular cartilage, emerges from the rather homogeneous rounded chondrocyte zone of the embryonic epiphysis. During early development, cartilage morphogenesis proceeds through a combination of intrinsic and extrinsic factors. Cell shape and matrix organisation are key intrinsic features (reviewed by Thorogood) whilst mechanical influences are important extrinsic factors. These considerations do not provide insights into the development of the gross organisation of articular cartilage such as Benninghoff arcades and collagen leaf-structure or even of the smaller micro-structure of cartilage such as the chondron. Clearly, these structural facets must play a crucial role in tissue function. It is likely therefore that if repair cannot reproduce the precise morphogenesis (either intrinsically or under extrinsic influences) of the cartilage in the region of degeneration, then the replacement tissue will ultimately fail under functional loading.

There is some evidence that the chondrocyte has an ability to reconstruct a functional matrix which closely resemble chondrons under in vitro conditions. In this respect, it seems possible that chondrocytes would be able to repair adequately in response to limited tissue disruption. It seems less likely, however, that the same cells could repair larger lesions occurring in the interterritorial matrix.

OSTEOPHYTE FORMATION

A common feature of OA joints is the formation of osteophytes or cartilaginous lips which eminate from the marginal transitional zone, particularly in joints such as the hip. Such outgrowths represent renewed growth which clearly incorporate morphogenesis. The structure of osteophytes resembles immature cartilage growth to an extent in that endochondral ossification occurs in the basal region but no secondary centre forms. However, the tissue is both structurally and biochemically heterogeneous (for example, containing both collagen types I and II) and lacks the order of normal growth cartilage. Consequently, osteophytes may contain both hyaline and fibrocartilage, and are also characterized by collagen types identified above. The heterogeneous nature of osteophytes has prompted Sokoloff to liken them with the histological events observed in fracture callus formation. The
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During unknown osteophyte formation is impossible. Features of this process, primarily because it occurs in a region where a number of different tissue types converge (articular cartilage, fibrocartilage of insertions, synovium and peristeum) and it is unknown to what extent the various tissue or cell types contribute to the renewed growth. Similarly, our lack of knowledge of the development of these histologically complex regions such as the marginal transitional zone make interpretation or drawing of comparisons impossible.

Conclusions

The repair ability of adult articular cartilage is limited even though there appears to be a considerable repair potential. There are several features which are shared by both developing and repairing cartilage such as elevated matrix synthesis and cell proliferation. Equally, there are obvious differences between the two processes particularly in relation to morphogenesis, which during development is intimately related to growth. It is likely that control of morphogenesis is the key to successful cartilage repair. If we return to one of our original questions, which posed 'at what point during development is the repair capacity of mammalian cartilage restricted and for what reasons?', it is clear that we do not know the answer. Equally, it is also clear that the cohort of chondrocytes which are responsible for the growth of the articular cartilage is different to those which respond to degeneration during repair. Consequently, we need more information on repair mechanisms in foetal and immature cartilage and a much deeper understanding of tissue regulation and how this may change with ageing and disease. Further studies are also required to determine morphogenetic effects of a number of growth factor families. In addition, we need to know more about the factors which regulate the formation and maintenance of the calcified tide mark and underlying subchondral plate.

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