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Cartilage to bone transitions; in health and disease

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Abstract

The aberrant redeployment of the ‘transient’ events responsible for bone development and postnatal longitudinal growth has been reported in some diseases in the inherently ‘stable’ cartilages. It is therefore plausible that lessons can be learnt as to disease aetiology from the molecular mechanisms underpinning transient chondrocyte differentiation and function. We herein discuss this evidence. We first review the developmental process of endochondral ossification and the cellular and physiological mechanisms controlling it and the postnatal growth plate. We then compare this transient cartilage anlagen to the inherently stable articular cartilage. Finally, we discuss the diseases in which the redeployment of these embryonic processes drive disease aetiology, with the foresight that deciphering those mechanisms surrounding pathological bone formation will aid future research into effective disease-modifying therapies.
**Introduction**

The transition of cartilage to bone is the basis by which most bones form. It is under tight regulation thereby ensuring both permissive foetal development through endochondral ossification, and fundamental postnatal longitudinal growth at the epiphyseal growth plate. However, emerging evidence has reported the aberrant redeployment of these ‘transient’ events in the inherently ‘stable’ articular cartilage in several pathological states. It is therefore plausible that lessons can be realised from the molecular mechanisms underpinning physiological transient chondrocyte differentiation and function. Certainly this knowledge may help decipher those mechanisms surrounding pathological bone formation and this will aid future research into effective disease-modifying therapies.

In the present Review, we will discuss aspects of both cartilage and bone physiology, as well as the complex cellular processes involved in the cartilage to bone transition during endochondral ossification and at the postnatal growth plate. Moreover, we will compare this transient cartilage phenotype with the stable phenotype adopted by articular chondrocytes in order to ostensibly safeguard their permanence, and will discuss disease pathology in which the ectopic re-induction of the transient phenotype is observed.

**Transient Cartilage**

*Endochondral ossification*

The majority of the skeleton is first formed during embryonic development as cartilage anlagen; models of future skeletal elements. The transient nature of this cartilage is reflected in its eventual replacement by bone in the fundamental process of endochondral ossification. Endochondral ossification is initiated by embryonic mesenchymal cells migrating to form pre-cartilage condensations, which then undergo differentiation into chondrocytes and secrete
cartilage extracellular matrix components (Fig. 1A). The chondrocytes of these cartilage condensations undergo an ordered process of proliferation, maturation, hypertrophy and cell death, which defines the outline of the endochondral bone (ref required maybe Mackie et al JOE 2011? Kronenberg Nature ??? Farquharson (book chapter) The molecular mechanisms which underpin the early patterning stages of bone formation are complex, but Homeobox (Hox), Sonic hedgehog (Shh), fibroblast growth factor (FGF), bone morphogenetic protein (BMP) and Wingless related (Wnt) signalling have been identified as critical in this process (Storm and Kingsley 1996; Tickle and Münsterberg 2001; Wang, et al. 2001; Davey, et al. 2006; Villavicencio-Lorini, et al. 2010). The apical ectodermal ridge and the zone of polarising activity are the two signalling centres of the growing limb bud controlling proximo-distal and anterior-posterior patterning, the regulatory signalling pathways of which have been comprehensively reviewed elsewhere (Summerbell, et al. 1973; Towers and Tickle 2009).

The cells on the outer surfaces of the cartilage condensations form the perichondrium of the future cartilage anlagen, while the chondrocytes in the centre of each cartilage anlage proliferate until the condensation reaches a specific size, at which point the chondrocytes become hypertrophic and will eventually undergo apoptosis (Fig. 1C). The perichondrial cells interact with the growth cartilage, secreting paracrine factors, including parathyroid hormone related peptide and Indian hedgehog, to regulate chondrocyte proliferation and differentiation (reviewed in Kronenberg, 2007).

Primary ossification originates in the centre of the diaphysis of the developing skeletal element (Fig. 1D). The growth cartilage is invaded by blood vessels seemingly attracted by VEGF expression by hypertrophic chondrocyte (Zelzer, et al. 2002), and with this, infiltration of bone-resorbing osteoclasts and bone-forming osteoblasts occurs. Furthermore, the perichondrium becomes vascularised around the forming bone element to create the
periosteum. This process of blood vessel invasion facilitated via degradation of the calcified cartilage extracellular matrix around the hypertrophic chondrocytes is critically reliant on the activity of matrix metalloproteinase 13 (MMP13) (Stickens, et al. 2004). Cartilage matrix resorption is followed by the invasion of osteoblasts, which lay down the newly formed bone. This process spreads longitudinally from the primary ossification centre towards the ends of the bone (Fig. 1E). Eventually, secondary ossification centres form in the epiphyses of the bone which allows for the development of the transiet growth plate, the developmental region of cartilage located between the primary and secondary centres and the site for postnatal longitudinal bone growth (Fig. 1F) (Mackie, et al. 2008, 2011).

It should be noted that not all bone formation occurs via endochondral ossification; bones such as those of the skull form through intramembranous ossification. In this process skeletal elements form directly as bone from embryonic mesenchyme. Embryonic mesenchymal cells form condensations and differentiate directly to osteoprogenitor cells and later into osteoblasts. These osteoblasts secrete osteoid, unmineralised bone matrix, which is laid down around blood vessels. The compact layer of mesenchymal cells surrounding the skeletal element becomes the periosteum; osteoblasts on its inner surface deposit osteoid to form layers of bone (Hall 2005). These bones are primarily of neural crest origin and include the frontal, parietal, occipital and temporal bones of the skull and the lateral part of the clavicle in humans (Hall 2005). Various studies have indicated metabolic and morphogenetic differences between endochondral and membranous bones, though whether these differences are directly a result of their different modes of development, the origins of their precursors or whether they can be attributed to adaptations to diverse mechanical conditions in different skeletal elements is debatable (Hall 2005).
Embryological development of the skeleton

During mammalian embryological skeletal development the appendicular skeleton, comprising of the pectoral girdle, pelvis and the limbs, arises from the lateral plate mesoderm (Saito, et al. 2006). These structures form via endochondral ossification, as does much of the axial skeleton including the vertebrae and ribs, which arise from somites of paraxial mesoderm origin. The head skeleton arises from the ectodermal neural crest.

The ossification processes described previously are regulated during embryological development to produce the wide range of skeletal forms that we see in animals. A challenge of skeletal morphogenesis is the precision of growth regulation that is required to create functional skeletal proportions, which can vary so widely between species, and to produce paired skeletal elements which are near identical in size. Some of the major considerations in embryological skeletal development are how condensations get to be the right size, and how directional growth is achieved in limb patterning.

The planar cell polarity (PCP) pathway, a non-canonical Wnt pathway which is partially mediated by the cadherins Fat and Dachsous (Fat/Dchs) (Goodrich and Strutt 2011), gives directional information for cell movement and morphology; this is known to mediate the migration of neurites and the alignment of stereocilia in the ear (Dabdoub, et al. 2003; Vladar, et al. 2009; Wada and Okamoto 2009). Fat/Dchs signalling is involved in the control of organ size during development via the Hippo signalling pathway (Sharma and McNeill 2013). The PCP pathway and Wnt5a signalling have been implicated in regulation of cell shape, oriented cell division and directional cell movement which promote growth and morphogenesis in the limb bud, as in the growth plate cartilage proliferative zone (Romereim and Dudley 2011).
There is substantial evidence that mechanical loading of bone as a result of embryo movement also has a role in regulating the growth of developing skeletal elements. Pharmacological immobilisation of embryonic chicks with the use of neuromuscular blocking agents results in a reduction in longitudinal growth of hind limb elements, which is most pronounced in the more distal elements (Osborne, et al. 2002; Lamb, et al. 2003; Pitsillides 2006). Although highly likely to involve disrupted replacement of calcified cartilage by bone, it remains to be seen whether this reduction in longitudinal growth is due a deficiency in chondrocyte proliferation, differentiation or hypertrophy. Recent findings (Cooper, et al. 2013) implicate the terminal phase of chondrocyte hypertrophy in establishing species differences in the rate of longitudinal bone growth. It may be that mechanical loading exerts epigenetic regulation on this process and that mechanical plasticity in the regulation of bone growth confers an evolutionary advantage by acting as an additional source of phenotypic variation during development.

*Morphology of the postnatal growth plate*

The growth plate is a highly specialised developmental region, located in the metaphysis, which is responsible for postnatal linear bone growth. It consists of chondrocytes arranged in columns that parallel the axis of the bone surrounded by their collagen- and proteoglycan-rich extracellular matrix (Fig. 2A) (Farquharson et al CTI 1994; Ballock and O'Keefe 2003; Mackie, et al. 2011). These chondrocytes undergo a series of tightly regulated differentiation and maturation processes, as is reflected by their changing morphology and matrix production, whilst maintaining their spatially fixed locations (Hunziker, et al. 1987).

Interference with the maturational progression of growth plate chondrocytes leads to abnormal cartilage formation and ossification and modifications in rates of bone formation, for example rickets and dwarfism. Details of the many syndromes and diseases, and possible
therapeutic strategies are outwith the scope of this review but have been the subject of
discussion by Krakow and Rimoin (Krakow and Rimoin 2010).

The first zone of the growth plate, often known as the resting or germinal zone, consists of
the resting chondrocytes and undifferentiated progenitors. Unlike the rest of the growth plate,
the chondrocytes of the resting zone are distributed sporadically and have low proliferation
potential (Hunziker et al. 1987; Ballock and O'Keefe 2003; Mackie et al. 2011). From the
resting zone, the chondrocytes progress to a proliferative phenotype in which they adopt a
flattened, oblate shape and arrange themselves into longitudinal columns, controlled by the
chondrocytes in the resting zone which have been postulated to produce a growth plate-
orientating factor (Hunziker et al. 1987; Abad, et al. 2002). The high mitotic activity of these
proliferating chondrocytes is controlled by numerous factors including endocrine/autocrine
regulation, circadian rhythm and age and undergo a period of high secretory activity as they
produce a collagen type II and proteoglycan rich matrix (Farnum, et al. 2002).

The chondrocytes undergo major phenotypic changes as they gradually become hypertrophic
however, until recently the mechanisms by which this occurs is unclear but may involve
membrane transporters in the chondrocyte membrane (Bush PG et al JBMR 2010; Loqman et
al J Cell Biochem 2013). Furthermore, an elegant paper by Cooper et al., has defined three
distinct phases through which mammalian chondrocyte volume increases are achieved: (i)
true hypertrophy, indicating a proportionate increase in dry mass production and fluid uptake,
responsible for approximately three-fold increases in volume, (ii) a four-fold enlargement
solely by cell swelling and (iii) a second distinct phase of true hypertrophy with proportionate
dry mass and fluid volume increases. These authors show that this final stage is dependent
upon insulin-like growth factor-1 (IGF-1), a well-recognised regulator of longitudinal bone
growth and chondrocyte hypertrophy (Wang J et al FASEB J 1999; Mushtaq T et al
Endocrinology 2004 Cooper et al. 2013). More recent emerging evidence has detailed its
regulation by the Wnt signalling pathway, more specifically by Wnt-induced secreted protein 3 (WISP3) (Ahmed and Farquharson 2010; Farquharson and Ahmed 2013; Rao, et al. 2013). Indeed, it appears that the Wnt pathway is critical in growth plate function, as we have previously discussed in our recent review (Staines, et al. 2012).

As well as changes in cell morphology, the chondrocytes of the hypertrophic zone also change the composition of their surrounding extracellular matrix to be predominantly collagen type X rich. The matrix also includes chondrocalcin, osteonectin and osteopontin, as well as having increased membrane activity of alkaline phosphatase, indicative of the final maturation phase (Sommer, et al. 1996; Shen 2005). Together these changes facilitate mineralisation of the matrix, a complex process reliant upon levels of calcium and phosphate (Castagnola, et al. 1988). Mineralisation of the extracellular matrix also enables vascular invasion, through which osteoclasts and osteoblasts and gain access via migration to replace the cartilage template with bone (Gerber, et al. 1999; Horner, et al. 1999; Engsig, et al. 2000; Zelzer et al. 2002).

The fate of the terminally differentiated chondrocyte at the chondro-osseous junction is still a matter of debate. It is well accepted that it does require, however, to be removed so as to maintain the steady-state thickness of the growth plate. There has been significant evidence to suggest that the chondrocytes die by apoptosis and this appears the most accepted mechanism (Magne, et al. 2003; Shapiro, et al. 2005). Indeed the activation of caspases and the decreased expression of the anti-apoptotic factor, Bcl-2 in hypertrophic chondrocytes certainly suggest this (Amling, et al. 1997; Adams and Shapiro 2002). The distinct lack of typical apoptotic morphological changes in the terminal hypertrophic chondrocytes does, however, challenge this theory (Emons, et al. 2009; Carames, et al. 2010). It would be expected that the condensation of cellular chromatin and the fragmentation of the cell nucleus would be visible, associated with the eventual break down of the cell into several vesicles which are then
phagocytosed. Instead, the presence of autophagic vacuoles and the expression of autophagy-regulating genes by growth plate chondrocytes suggest these cells undergo processes more similar to autophagy than apoptosis (Roach and Clarke 2000; Shapiro et al. 2005). Furthermore, the transdifferentiation of chondrocytes has also been proposed (Descalzi Cancedda, et al. 1995; Roach 1997). This involves the division of the terminal hypertrophic chondrocyte to produce one daughter cell which undergoes apoptosis and one which trans-differentiates into an osteoblast phenotype. However this theory has yet to be fully ratified.

Longitudinal bone growth occurs at the growth plate until, in large mammals at least, it closes once sexual maturity has been reached. This is initiated through the formation of mineralised tethers between epiphyseal and diaphyseal bone promoting the fusion of the primary and secondary ossification centres (Haines et al. J Anat 1975). The chondrocytes of the growth plate reach a state of senescence as they exhaust their proliferative potential, and longitudinal bone growth is ceased. In humans, gonadal steroids like oestrogen and androgen mediate these effects (Nilsson, et al. 2005; Mackie et al. 2011).

It is interesting to note that in mice, as well as in rats, whilst growth will eventually slow, the growth plate rarely closes completely in healthy animals. This difference in growth plate physiology means that some caution should be applied to the use of the rodent growth plate as a model for human disease. Nevertheless, the similarities between the underpinning signalling and cellular processes in murine and human growth plates means this model still stands as effective and can be easily genetically manipulated to provide insights into growth plate physiology.
Stable cartilage

Articular cartilage formation

The earliest emergence of ‘stable’, articular cartilage during skeletal patterning occurs with the formation of interzones; regions of undifferentiated mesenchyme which separate developing skeletal elements in which the joint will later form. There has been some debate as to whether individual skeletal elements are discrete from the outset of their development, or whether these elements form from a single cartilage condensation which is later divided by interzones (ref).

Transient growth cartilage and articular cartilage differ in their collagen composition during their development; in embryonic growth cartilage, expression of collagen type IIA precedes collagen type IIB expression, which is not detectable at later stages of development. However in articular cartilage, collagen type IIA is never present in the interzones, which provides evidence for the belief that the cartilage anlagen that form individual skeletal elements are not continuous (Ng, et al. 1993; Pitsillides and Ashhurst 2008). This suggests that the populations of cells which will become the growth and articular cartilage chondrocytes are discrete. Evidence for these discrete origins is also provided by the elegant Gdf5-mediated tracking of interzonal cells (Pacifici, et al. 2005; Pacifici, et al. 2006).

Interzones first appear as densely cellular, homogenous regions with GDF-5, Wnt9a, autotaxin and chordin being known interzone markers (Pacifici et al. 2005). Non-canonical Wnt9a signalling is particularly important early in development as it acts to inhibit chondrocyte differentiation at the presumptive joint site (Hartmann and Tabin 2001). As joint development progresses, the interzone differentiates into three recognisable layers; two chondrogenic layers which cover the articular surfaces of the developing skeletal elements and an intermediate layer which separates them. There is evidence to suggest that the cells
derived from this intermediate layer differentiate to become articular chondrocytes, while the outer layer chondrocytes are incorporated into the growing epiphysis (Ito and Kida 2000).

Joint cavity formation is thought to occur due to a combination of extrinsic mechanical factors and intrinsic factors. Cell death is not thought to be primarily responsible for the formation of the joint cleft; rather, changes in extracellular matrix composition including hyaluronan synthesis, mediated by mechanical activation of the MEK-ERK pathway, which result in a loss of tissue cohesion have been implicated (Archer, et al. 1994; Ito and Kida 2000; Bastow, et al. 2005).

*Articular cartilage phenotype*

Fully developed articular cartilage can be divided into superficial, intermediate and deep zones. These different zones all consist of chondrocytes which unlike transient chondrocytes, maintain a stable phenotype characterised by small cell size and expression of tenascin-C, and do not undergo a sequence of proliferation, maturation, hypertrophy, apoptosis and ossification (Pacifici et al. 2005; Pacifici et al. 2006). It has been suggested that the transcription factor ERG is expressed (? necessary early in joint development to establish the articular chondrocyte phenotype (Pacifici et al. 2006). It is the organisation of cells and their collagen type II rich matrix in the differing zones however which makes them distinct from one another. Whilst the superficial zone consists of elongated chondrocytes orientated parallel to the surface of the cartilage, in the deep zone the chondrocytes are more rounded and are aligned along the collagen fibrils (Fig. 2B). The collagen fibrils in the intermediate zone of the articular cartilage are arranged in arches which allows the transition from the deep and superficial zones (Minns and Steven 1977).

The deep zone of the articular cartilage forms an interface, termed the tidemark, with calcified cartilage which forms through mechanisms that are not quite understood. Within
this calcified cartilage, the chondrocytes are hypertrophic as shown by their expression of collagen type X and alkaline phosphatase (Heinegard and Oldberg 1989; Gannon, et al. 1991; Stephens, et al. 1992). The calcified cartilage layer is semipermeable and whilst it acts as a physical barrier for vascular invasion of the overlying articular cartilage, it does permit the passage of small molecules from the underlying subchondral bone (Arkill and Winlove 2008). In humans, the calcified cartilage thickness varies widely, from approximately 20-250µm however, like the tidemark, this a dynamic structure which can be remodelled during ageing and disease, as highlighted by observed morphological changes in osteoarthritis including tidemark duplication and increased calcified cartilage thickness (Lane and Bullough 1980; Oettmeier, et al. 1989; Oegema, et al. 1997; Hunziker, et al. 2002; Burr 2004). This therefore leads us to suggest that future research should place an emphasis on understanding the molecular regulation of this osteochondral interface so as to provide insights into its dysregulation in disease and to identify potential targets for therapeutic intervention.

The ectopic initiation of cartilage-bone transitions

The transition of cartilage to bone in the healthy individual is under tight regulation so as to prevent disturbed development and/or longitudinal bone growth. This regulation is also observed in repair of fractured bone tissue in which there is a deliberate re-initiation of the endochondral processes previously discussed in this Review. There has been much recent focus upon the Wnt signalling pathway in fracture repair and, in particular, the recent discovery of enhanced repair through the administration of neutralizing antibodies against sclerostin, a known Wnt inhibitor (Secreto, et al. 2009; Ominsky, et al. 2011; Virk, et al. 2013). In contrast to this desired acceleration in endochondral ossification processes, in certain diseases their ectopic redeployment is detrimental and considered to be at least contributory to the observed disease pathology. Herein we discuss conditions in which this
occurs and touch upon current understanding regarding the role of these processes in their aetiology.

Osteoarthritis

Osteoarthritis is a degenerative joint disease and a massive world-wide healthcare burden. Characterised by articular cartilage loss, subchondral bone thickening and osteophyte formation, the osteoarthritic joint afflicts much pain and disability on its sufferers. Its underpinning molecular mechanisms are, nevertheless, not fully understood. However there is increasing evidence implicating the re-initiation of the transient chondrocyte phenotype in osteoarthritic aetiology and pathology (Fosang and Beier 2011; Pitsillides and Beier 2011). This hypothesis is based upon the previously discussed common embryonic development of cartilage and bone, and although it has been met with controversy, some of the evidence certainly is appealing (Brew, et al. 2010).

In osteoarthritic cartilage, there have been observed decreases in collagen type II and aggrecan integrity in comparison to normal articular cartilage (Helminen, et al. 1993; Garnero, et al. 2002; Jalba, et al. 2011; Henrotin, et al. 2013). Furthermore, markers previously thought to be unique to the hypertrophic chondrocytes of the growth plate have been detected in both animal models of osteoarthritis, and in patients with the disease. The best recognised of these are MMP13 and collagen type X, however also detected are alkaline phosphatase, osteopontin, Indian hedgehog, and osteocalcin (Hoyland, et al. 1991; Aigner, et al. 1993; Pullig, et al. 2000; Appleton, et al. 2007; Studer, et al. 2012). Like in the transient growth plate cartilage, chondrocyte hypertrophy is the prerequisite for matrix mineralisation and in osteoarthritis, the increased formation of hydroxyapatite has been documented and is consistent with hypertrophic chondrocyte changes (Fuerst, et al. 2009).
MMP13, a key marker of chondrocyte hypertrophy, is proving a critical target in osteoarthritis research due to its potent role in the degradation of collagen type II, proteoglycans, collagen types IV and IX, osteonectin and perlecan (Wang, et al. 2013). Indeed, MMP13-deficient mice predictably have an endochondral bone growth defect with altered growth plate microarchitecture and increased trabecular bone (Stickens et al. 2004). Nevertheless, whilst the surgical induction of osteoarthritis in this mouse causes chondrocyte hypertrophy and osteophyte formation, the graded score of cartilage degradation was intriguingly significantly reduced (Little, et al. 1999). Deletion of the MMP13 gene specifically in chondrocytes also produces similar deceleration of osteoarthritis disease progression following meniscal-ligamentous injury in a mouse model (Wang et al. 2013). Consistent with this crucial role for MMP13, its overexpression was found to result in pathological osteoarthritis-like changes in the articular cartilage of mice (Neuhold, et al. 2001).

Epigenetics has been strongly implicated in osteoarthritis in recent years with all three currently known mechanisms- DNA methylation, histone modifications and non-coding RNAs - showing evidence of controlling the chondrocyte phenotype. This too has highlighted a role for MMP13. More specifically, MMP13 promotor methylation is altered in osteoarthritic cartilage, suggesting that these epigenetic changes can therefore drive the chondrocyte hypertrophy observed in the pathological state (Roach, et al. 2005). It should be noted that the precise initiation and control of these events is yet to be established. However, with the surge in epigenetic studies in recent years it is certainly an exciting and promising time for this field.

Taken together, this highlights the significant role of this catabolic enzyme in osteoarthritis however the mechanisms underpinning the expression and function of MMP13 in osteoarthritic chondrocytes is yet to be fully established. At the recent OARSI world
congress, tantalising data was presented showing the molecular regulation of MMP13 by osteoarthritic chondrocytes, with the Wnt signalling pathway and its inhibitors playing a common central role. This is not particularly unsurprising considering the current focus and development in implicating the Wnt signalling pathway as an important therapeutic target in bone and cartilage disease.

An understanding of the molecular mechanisms surrounding this apparent recapitulation of the articular cartilage chondrocytes to a developmental state will improve understanding into the pathophysiology of osteoarthritis. The evidence discussed here ultimately begs the question; can we manipulate the transition of the stable articular cartilage to a transient phenotype for therapeutic benefit?

Certainly the field of tissue engineering would benefit from such understanding. Combining the use of cells and biomaterials, this approach has emerged as a promising target for cartilage repair. The first repair of cartilage defects was described in 1994 and used autologous chondrocytes (Brittberg, et al. 1994). Despite the limitations associated with this including the need to disrupt healthy cartilage and problems with culturing chondrocytes, this has certainly set the platform from which cartilage tissue engineering has greatly progressed in recent years. The use of human mesenchymal stem cells has been repeatedly reported in osteoarthritis with their ability to overcome the limitations defined by the use of chondrocytes providing much excitement in the field (Luyten 2004; Coleman, et al. 2010). However their expression of the chondrocyte hypertrophy marker collagen type X still limits their effectiveness as a candidate for tissue engineering, especially as a recent study showed that these mesenchymal stem cells upon culture express higher levels of the genes associated with osteoarthritis than chondrocytes derived from the osteoarthritic joints themselves (Mwale, et al. 2010). Nevertheless, the field of tissue engineering is unequivocally set to make great advances in the forthcoming years with
Intervertebral disc calcification

Intervertebral disc degeneration is a major cause of back pain world-wide with complex, expensive surgery which is often prone to failure (Urban and Roberts 1995). Located between the vertebral bodies of the spine, the cartilaginous intervertebral disc (IVD) functions to resist compressive loads (Broberg 1983). Anatomically it consists of a central nucleus pulposus contained within a fibrocartilage ring, the annulus fibrosus, laterally and the cartilage end plates inferiorly and superiorly (Roberts, et al. 2006). The nucleus pulposus shares many similarities with articular cartilage, both in their matrix composition and their metabolism (Raj 2008). IVD disc degeneration is a major cause of back pain and a huge financial burden worldwide. Associated with ageing and with abnormal mechanical loading playing a substantial role, its pathogenesis is yet to be fully elucidated. There is, however, emerging evidence for the occurrence of hypertrophic differentiation in this process.

The IVDs of patients with degenerative disc disease have increased alkaline phosphatase activity, and also in contrast to healthy IVDs express significant levels of hypertrophic differentiation markers, including collagen type X, OPG, MMP13 and Runx2 (Boos, et al. 1997; Nerlich, et al. 1997; Sato, et al. 2008; Rutges, et al. 2010; Hristova, et al. 2011). Associated with this, microCT analysis clearly shows increased levels of IVD calcification with increasing degeneration (Rutges et al. 2010). A recent in vitro study sought to examine the mineralization potential of the cells of the annulus fibrosus, and concluded that under certain conditions these cells can induce mineralization as indicated by their increased von kossa staining and alkaline phosphatase expression and other markers including MMP13 and Runx2 (Nosikova, et al. 2013).

Understanding of the mechanisms supporting induction of such hypertrophy in IVD cells is somewhat lacking. However, it was shown recently that parathyroid hormone (PTH) can
enhance disc repair through its inhibition of collagen type X and alkaline phosphatase expression, and through the promotion of collagen type II production. These regenerative properties of PTH are mediated through mitogen-activated protein kinase (MAPK) signalling (Madiraju, et al. 2013). The authors of this study suggest that the supplementation of PTH to degenerated discs may prevent further calcification and as such may enhance cellular based therapies. This is certainly promising due to the known critical role for PTH signalling in chondrocyte hypertrophy and matrix mineralization (Mackie et al. 2011; Yano, et al. 2013).

The major current problem hindering IVD tissue engineering, like in osteoarthritis, is the expression of collagen type X by human mesenchymal stem cells. PTH can, however, also inhibit this expression and may thus open additional avenues for the treatment of degenerative IVDs (Mwale et al. 2010).

**Heterotopic ossification**

Heterotopic ossification (HO) is a common occurrence in muscle, tendon and ligaments following trauma by injury, disease or surgery. Initiated by cartilage formation, the endochondral ossification and ectopic bone formation in these tissues can produce severe functional impairment (Medici and Olsen 2012).

Besides the trauma-induced HO described, a rare disease called fibrodysplasia ossificans progressiva (FOP) is a hereditary form of HO, presenting itself as painful and highly inflammatory soft tissue swellings which progressively ossify rendering the sufferer immobile (Cohen, et al. 1993; Shore, et al. 2006; Medici and Olsen 2012). Similarly to trauma-induced HO, the aberrant bone formation in patients with FOP occurs via endochondral ossification processes. Emerging evidence has strongly implicated increased bone morphogenetic protein (BMP) signalling in the pathogenesis of FOP, as well as in trauma-induced HO (Cohen et al. 1993; Kwapisz, et al. 2013). The identification of
ACVR1/ALK2, one of the four type I receptors that mediate BMP signalling, as the mutated gene in FOP in 2006 has further highlighted the BMP signalling pathway as an attractive target for future therapy (Shore et al. 2006).

**Conclusions**

The aberrant redeployment of embryonic processes in diseases such as osteoarthritis and intervertebral disc degeneration is now well established, with emerging evidence further fuelling the hypothesis that lessons for limiting disease progression can be acquired from the regulators of transient cartilage biogenesis and development. This Review provides some pointers as to potential targets for future drug therapies or tissue engineering approaches which will only further our understanding of the underpinning molecular mechanisms involved in these diseases, and will provide advances towards patient benefit.

**Declaration of interest**

The authors have no conflicts of interest

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