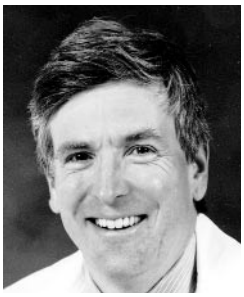
Editorial

What Does Cartilage Calcification Tell Us About Osteoarthritis?



Matrix calcification commonly develops in osteoarthritis (OA) cartilage. In this month's *Journal*, Derfus and colleagues have documented the particularly high frequency of positive knee joint fluids for calcium pyrophosphate dihydrate (CPPD) and/or basic calcium phosphate (BCP) crystals in a group of predominantly aged patients with advanced degenerative arthritis at joint arthroplasty[1].

BCP crystal presence, in particular, correlates positively with OA severity[1,2]. But clinically useful information from the presence of BCP or CPPD crystals may be difficult to obtain. First, radiologic evidence of chondrocalcinosis is far less sensitive than joint fluid analysis in detecting both CPPD and BCP crystal deposition[1]. Second, Derfus, *et al* detected BCP crystals by a specialized bisphosphonate binding assay. This methodology as well as other means to reliably identify microscopic and submicroscopic BCP crystals such as hydroxyapatite (HA) in synovial fluids[2,3] are not readily at hand for most rheumatologists.

The likely sources of joint fluid BCP particulates in advanced OA joint fluid include perichondrocytic HA crystal deposits (especially in the superficial zone) and bone shards embedded in cartilage and bony debris exposed by cartilage erosion[4,5]. Thus, detailed physical characterization of BCP particulates in joint fluids could add valuable information in clinically correlative studies.

Understanding the mechanisms driving cartilage calcification is valuable partly because CPPD and HA crystals can traffic to the synovium and can stimulate inflammation. CPPD and HA crystals also directly induce expression by chondrocytes and synoviocytes of matrix-degrading enzymes that worsen cartilage degeneration.

In the setting of OA, the processes leading to matrix calcification are widely viewed to reflect passive secondary consequences of advanced cartilage pathology. This editorial presents the argument that matrix calcification in OA, and conversely, cartilage degeneration in association with chondrocalcinosis, reflect mechanistically unified processes that actively drive stereotypical patterns of tissue injury culminating in calcification within degenerating cartilage (Figure 1).

ALTERED CHONDROCYTE DIFFERENTIATION IN OA: ROLE OF PARATHYROID HORMONE RELATED PROTEIN (PTHrP)

Articular cartilage, unlike growth plate cartilage, is specialized to resist matrix calcification. But regulated changes in chondrocyte differentiation and viability characteristically seen in growth plate chondrocytes, including proliferation, hypertrophy, and apoptosis, as well as changes in mitochondrial function, can occur in OA cartilage chondrocytes[6-9]. In endochondral development, PTHrP is a central regulator of spatial and temporal aspects of chondrocyte development as well as extent of matrix calcification[7,10]. Recently, it has been discovered that PTHrP expression is markedly upregulated in OA cartilage and PTHrP derived peptides are relatively abundant in joint fluid in OA[7,10].

In the growth plate, signaling through the PTH/PTHrP receptor stimulates chondrocyte proliferation. PTHrP, locally produced in large part in the perichondrium, and acting in conjunction with the Indian Hedgehog signaling system, also restrains the progression between prehypertrophic to hypertrophic differentiation in chondrocytes. In normal articular cartilage, PTH/PTHrP receptors are expressed by chondrocytes in all zones[7]. In this context, it appears likely that chondrocalcinosis secondary to hyperparathyroidism might be mediated in part by the ability of excess PTH to trigger increased chondrocyte proliferation and other promineralizing responses by a PTH/PTHrP receptor driven mechanism. But PTH/PTHrP receptor expression becomes limited principally to the superficial zone in OA[7], suggesting removal of a restraining mechanism for the development of chondrocyte hypertrophy in OA.

One of 3 human PTHrP isoforms, PTHrP 1-173, is preferentially expressed by articular chondrocytes and PTHrP 1-

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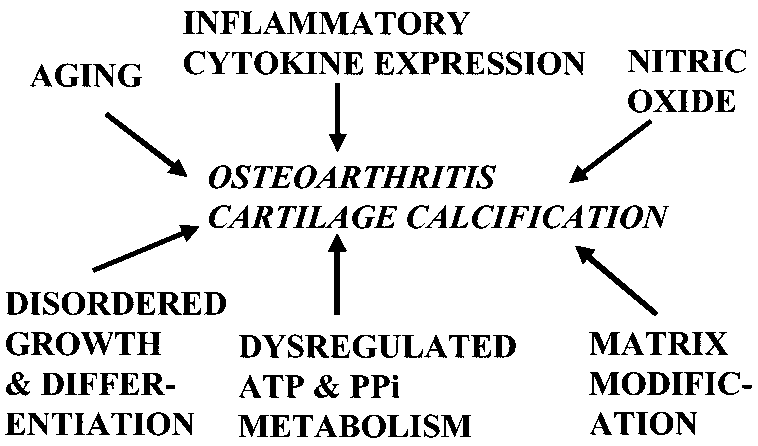

Figure 1. OA and matrix calcification: mechanistic convergence. Fundamental disease processes within cartilage that ultimately promote both OA and matrix calcification.

173 is upregulated by transforming growth factor-ß[7,10]. PTHrP 1-73 depresses matrix synthesis and regulates inorganic phosphate (Pi) metabolism via intracrine effects specific to this PTHrP isoform[7,10]. It appears likely that upregulated PTHrP 1-173 expression is a significant pathogenic factor linking OA with matrix calcification.

DYSREGULATED CHONDROCYTE ATP GENERATION

The development of decreased chondrocyte mitochondrial respiration signals the onset of calcification in the growth plate[8]. Compelling evidence, recently reviewed[8], suggests that mitochondrial damage and dysfunction also promote OA and cartilage calcification. For example, articular chondrocyte apoptosis, which is critically regulated by mitochondrial function, is promineralizing *in vitro* and regulates endochondral mineralization[8]. Chondrocyte apoptosis is enhanced in OA cartilage and directly associated with HA deposition therein[5]. Mitochondria also regulate chondrocyte matrix calcification by a variety of other fundamental activities beyond apoptosis, including regulation of intracellular calcium fluxes and chondrocyte ATP generation[8,9].

Purinergic receptor mediated signaling by extracellular ATP is increasingly recognized to exert physiologic and potentially pathologic effects on chondrocyte matrix synthesis and prostaglandin E_2 production, and intracellular ATP supports proteoglycan synthesis and viability. In this context, nitric oxide (NO) is a central mediator in OA, and NO suppresses mitochondrial respiration mediated ATP generation[9] and stimulates apoptosis in chondrocytes. Significantly, treatment of cultured chondrocytes with mitochondrial ATP synthesis inhibitors, and with sodium nitroprusside (a donor of NO and the mitochondrial complex IV inhibitor cyanide), recapitulates several features of OA, including decreased matrix synthesis and increased potential for calcification[9,11].

ABNORMAL PPi METABOLISM

PPi potently suppresses HA crystal deposition and propagation, and maintenance of relatively high extracellular PPi concentrations by chondrocytes is a vital physiologic mechanism to prevent articular cartilages from calcifying[12]. But paradoxically, in hyaline articular cartilage and meniscal fibrocartilages, excess extracellular PPi is dumped into the matrix, as illustrated in Figure 2.

Subject age directly correlates with PPi generation by chondrocytes. A large fraction of extracellular PPi appears to be transported from the cell interior by a mechanism involving the multiple-pass transmembrane protein ANK, a known regulator of cartilage calcification[13]. Activity of nucleotide pyrophosphatase phosphodiesterase (NTPPPH) family isozymes is a major component of chondrocyte PPi generation[14,15]. Both cartilage NTPPPH activity and extracellular PPi increase in aging and achieve a mean of roughly double normal levels in idiopathic CPPD deposition disease of the elderly. Of the NTPPPH isozymes, plasma cell membrane glycoprotein-1 (PC-1) has the greatest effect on extracellular PPi levels and matrix calcification in mineralizing cells (Figure 2)[14,15]. Major matrix calcification abnormalities have been described in both PC-1 deficient animals and a PC-1 deficient human (where ~50% depression of extracellular PPi has been observed)[16].

Increased PC-1 expression also can be noxious. Specifically, PC-1 expression is markedly upregulated at sites of meniscal fibrocartilage matrix calcification, in association with apoptotic cell clusters[15]. Upregulated PC-1 expression directly promotes both matrix calcification by chondrocytes and chondrocyte apoptosis *in vitro* in a NTPPPH isozyme selective manner[15,17]. Thus, excess extracellular PPi generation (and possibly PC-1 catalyzed extracellular ATP consumption) could compromise chondrocyte viability in aging and OA.

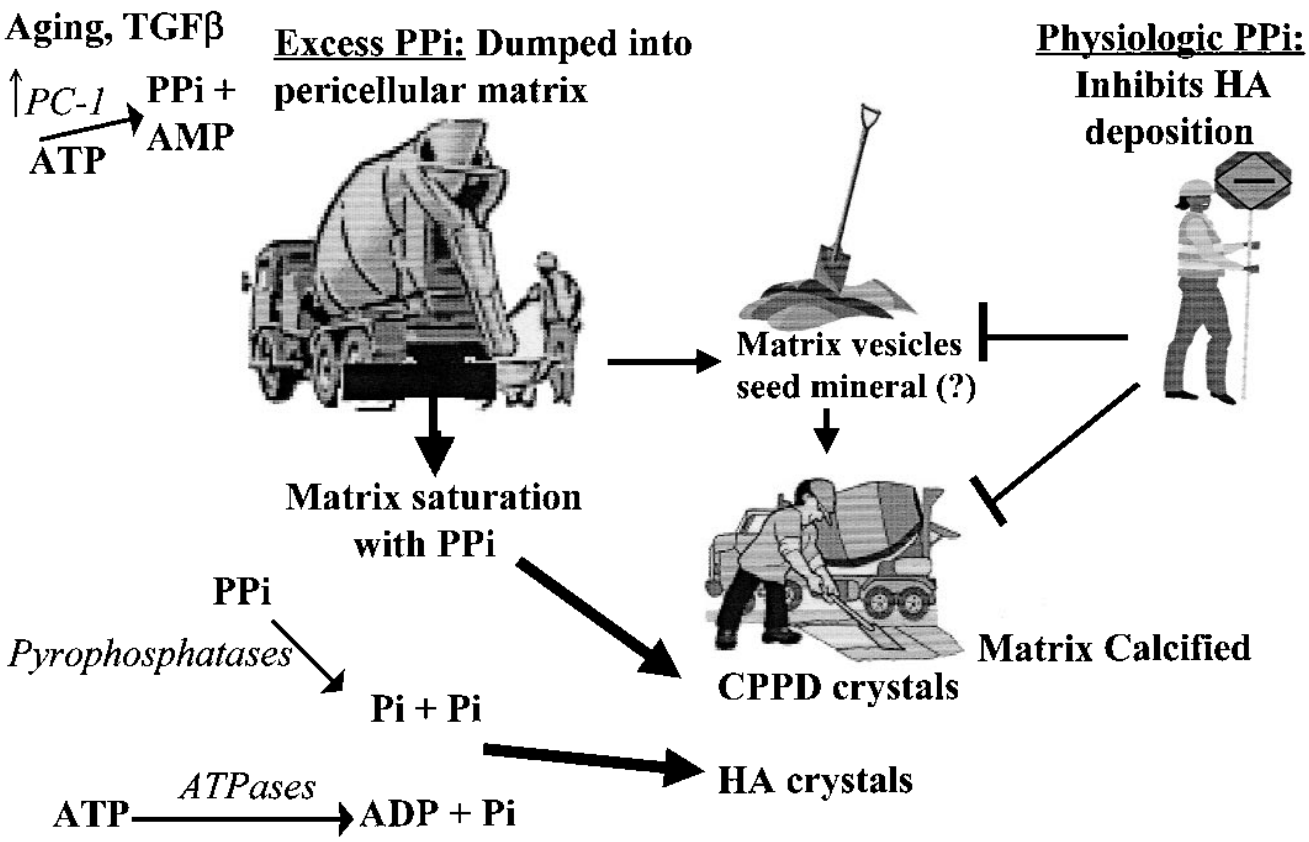

Figure 2. How chondrocytes build CPPD and HA crystal deposits: roles of PC-1, ATP, and PPi metabolism and inorganic phosophate (Pi) generation. This paradigm and the additional potential of Pi to stimulate calcification by effects on expression of mineralization regulatory genes are discussed in detail in the text.

It should be noted that the ability of chondrocytes to calcify the matrix (Figure 2) can be regulated not only by the solubility products of PPi, Pi, and calcium, but also by chondrocyte signaling via Pi and free calcium (through specific membrane transport mechanisms and receptors, respectively). Pi, which can be generated by several distinct pyrophosphatases and ATPases, is promineralizing, in part via effects on gene expression, differentiation, and function[12].

TRANSFORMING GROWTH FACTOR-ß RESPONSIVENESS AND CHONDROCYTE HYPERTROPHY

Active TGF-ß is markedly increased in the OA joint and has the potential to modulate OA by effects including stimulation of matrix metalloproteinase-13 (MMP-13) expression. TGF-ß also markedly elevates extracellular PPi in chondrocytes[12], mediated partly by induction of PC-1 expression and translocation to the plasma membrane[14]. In normal chondrocytes, insulin-like growth factor-1 (IGF-1) inhibits TGF-ß from elevating chondrocyte extracellular PPi[18]. Thus, the decreased IGF-1 responsiveness characteristic of chondrocytes in OA may contribute to sustained elevation of PPi generation.

Hypertrophic chondrocytes colocalize with CPPD crystal deposition in articular cartilage[19]. In this context, hypertrophic chondrocytes generate not only more extracellular PPi[20] but also produce more mineralization competent matrix vesicles *in vitro*[21]. It appears likely that CPPD crystal deposition in OA may be stimulated by heightened, persistent matrix synthetic reparative responses conducted by hypertrophic chondrocytes. Altered TGF-ß signal transduction in aging and OA may be at play. Specifically, experimental expression of a truncated, kinase defective TGF-ß receptor type II promotes hypertrophy and terminal chondrocyte differentiation along with cartilage degeneration *in vivo*[22]. Moreover, aging human knee chondrocytes show decreased growth responses to TGF-ß, yet produce more extracellular PPi in response to TGF-ß than do chondrocytes from young donors[23].

Upregulation of chondrocyte expression of interleukin 1ß (IL-1ß) is one of several proinflammatory pathways that modulate the pathogenesis of OA[24]. Significantly, TGF-ß and IL-1 exert a variety of opposing effects on growth, matrix synthesis, matrix degradation, and other chondrocyte functions pertinent to the pathogenesis of OA. Correspondingly, IL-1 blocks TGF-ß effects on PC-1 expression and extracellular PPi[25]. IL-1 itself also blunts PC-1 expression and markedly decreases extracellular PPi

in chondrocytes[25]. These activities may transduce effects of IL-1 on the form and extent of matrix calcification by chondrocytes.

INFLAMMATORY MEDIATORS IN MATRIX MODIFICATION FOR CALCIFICATION

Degradation of the articular cartilage matrix, a central feature in OA, also is likely a fundamental factor in preparing the matrix for calcification. This interplay between OA and calcification extends beyond simple physical-chemical modulation of crystal deposition in the matrix. For example, MMP-13 appears to promote chondrocyte hypertrophy. In addition, stabilization of calcium-binding pericellular matrix proteins that are substrates for the cross-linking enzymes of the transglutaminase (TGase) family may stimulate calcification. Cartilage matrix proteins that are substrates for TGases include the S100 family calcium binding proteins[19], the hypertrophic, chondrocyte expressed osteoblastic matrix proteins osteonectin and osteopontin, and several collagen subtypes.

Two distinct TGases, the tissue form of Factor XIIIa (a latent enzyme) and Type II or tissue TGase (tTGase), are expressed in temporal and spatial association with chondrocyte hypertrophy and matrix calcification in the growth plate. Recently, we described marked upregulation of tTGase and Factor XIIIa expression hypertrophic cells in the superficial and deep zones of knee OA articular cartilage and the central (chondrocytic) zone of OA menisci[26].

IL-1ß modulates the pathogenesis of OA in part through induction of NO synthase expression and increased NO generation. Significantly, IL-1ß induces Factor XIIIa and tTGase expression in human cartilages[26]. Moreover, IL-1 (as well as tumor necrosis factor-α and donors of NO and the potent oxidant peroxynitrite) induce increased chondrocyte TGase activity, likely via NO and oxidant mediated TGase posttranslational modifications[26].

Increased Factor XIIIa and tTGase activities directly induce calcification by chondrocytic cells[26]. TGases also promote activation from latency of TGF-ß[27], and tTGase, a guanosine triphosphate-binding protein, can modulate several intracellular signaling pathways. The aforementioned findings potentially implicate inflammation induced TGase activity as an amplification factor for not only OA but also cartilage matrix calcification. These observations are particularly pertinent because OA severity related, donor age dependent, and marked age dependent IL-1 induced increases in TGase activity occur in chondrocytes from human knee menisci, a major site for CPPD deposition disease in aging and OA[26].

SUMMARY AND CLINICAL-PATHOLOGIC RELEVANCE

This editorial has pointed out that fundamental mechanisms, including disordered chondrocyte differentiation and viability, dysregulated ATP and PPi metabolism, and the effects of specific inflammatory mediators, converge to actively drive both OA and matrix calcification. It should also be noted that crystal deposition, while common, is not a universally detected feature in joints with advanced cartilage degeneration[1-5]. Conversely, cartilage crystal deposition may occur without advanced cartilage degeneration, particularly in idiopathic CPPD deposition disease. Mechanistic divergence of OA and matrix calcification could result from predominance of a minority of pathogenic mechanisms relative to the majority illustrated in Figure 1. For example, prominent MMP activation without substantial chondrocyte hypertrophy and derangements in ATP and PPi metabolism would favor development of OA without matrix calcification. Conversely, sustained chondrocyte hypertrophy without progression to apoptosis would be expected to drive both cartilage reparative and matrix calcification responses, and favor CPPD deposition over further progression of OA.

CPPD or BCP crystals (or both) may be recovered from joints with advanced degenerative arthritis, as illustrated by Derfus, *et al*[1]. CPPD and BCP crystal deposition can develop differentially, such as in different zones of articular cartilage and probably in distinct phases of cartilage degenerative disease (e.g., as hypertrophic chondrocytes lose viability). Differences in pathogenesis likely promote differential crystal deposition in degenerative arthritis. For example, abundant cartilage NO production may promote chondrocyte extracellular ATP depletion that consequently favors HA over CPPD crystal deposition[28]. But it is also noteworthy that differences exist between HA and CPPD crystal types in ease of detection in joint fluids, and synovial fluid crystal analyses also are clearly less informative and sensitive than direct study of cartilage.

Could more systematic evaluation of joints for evidence and forms of cartilage calcification, done prior to endstage disease, provide clinicians with practical, cost effective information to weigh working pathogenic factors and prognosis in degenerative arthritis? Could clinical measurements of one or more of the intraarticular mediators discussed here (such as PTHrP, TGF-ß, PPi, PC-1/NTPPPH, IL-1, NO, and transglutaminase activity) provide useful additional correlative data for the same purpose in a cost effective manner? Longterm outcome studies to test these questions in depth would be intriguing. The results could facilitate development of rationally targeted and more effective early treatment strategies for both OA and matrix calcification.

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