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CARTILAGE CELL CLUSTERS

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Abstract

The formation of new cell clusters is a histological hallmark of arthritic cartilage but the biology of clusters and their role in disease are poorly understood. This is the first comprehensive review of clinical and experimental conditions associated with cluster formation. Genes and proteins that are expressed in cluster cells, the cellular origin of the clusters, mechanisms that lead to cluster formation and the role of cluster cells in pathogenesis are discussed.

Introduction

Osteoarthritis (OA) is the most prevalent joint disease and aging is among its major risk factors (1). Aging-associated deficits in cartilage cell function have been widely documented (2). Yet, the tissue destruction and remodeling process in OA-affected articular cartilage is due to cell activation with production of extracellular matrix (ECM)-degrading enzymes, inflammatory mediators as well as new synthesis of ECM components which includes some that are not present in normal adult articular cartilage (3). A histological hallmark of OA cartilage is cell clusters (4–8) and these clusters express a broad range of activation and abnormal differentiation markers, suggesting that they contribute to pathogenesis. The objective of this review is to summarize information on patterns and mechanisms of cluster formation and their role in disease.

Cell arrangements and clusters in normal cartilage

The basic cellular unit in normal articular cartilage is the chondron which is defined as consisting of one or more cells and the surrounding pericellular matrix (8,9). Multiple chondrons that are located in direct proximity to each other in a large lacuna are referred to as clusters. The pericellular matrix contains collagen types II, VI, IX and XI, hyaluronan, proteoglycans such as aggrecan, decorin and biglycan, and glycoproteins such as fibronectin, link protein and laminin (10). Cells bind via integrins and other receptors to these matrix components and this leads within the context of the territorial matrix structures to the different cell arrangements. The orientation and shape of the chondron reflects the local collagen architecture of the interterritorial matrix, which increases in thickness with depth from the tissue surface (11). Normal mature articular cartilage contains cell arrangements that are characteristic for each zone (Figure 1A). In the superficial zone cells are arranged in horizontal clusters parallel to the articular surface, in strings and pairs of

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cells and single cells dispersed among these patterns (12,13). The cell arrangements in the superficial zone vary among joints, possibly related to different mechanical loading mechanisms (13). The middle and deep zones contain double or multiple chondrons arranged as vertical columns of cells (Figure 1B) (14–16). These cell patterns are thought derive from cell proliferation, probably also cell migration and the formation and specific organization of the ECM during joint development and maturation (11,17,18).

Cell clusters in OA articular cartilage and other types of cartilage

Increased numbers and sizes of cell clusters are a hallmark histological feature of OA articular cartilage (Figure 2A) and can be detected in the majority of specimens (4, 5, 8, 19, 20). Clusters of chondrocytes are often localized near fissures and clefts of the upper cartilage layer. This shortens the diffusion distance for nutrients as well as for cell mediators from the synovial fluid but also of cluster-derived mediators to the synovial space. These chondrocyte clusters are characteristically round and located within a large lacuna, and can contain more than 20 cells (12). They are clearly different from the flattened, superficial and rounded, upper-middle zones chondrocytes from nonfibrillated human cartilage (13, 19). Chondrocytes in the middle and deep zones of severe OA have increased pericellular matrix with increased type X collagen (21–25). The concentration of type VI collagen may be reduced in the superficial zone of OA cartilage (22, 23) and a significant loss of mechanical properties is correlated with the loss of pericellular type VI collagen (26, 27).

Cell clusters not only form in OA-affected articular cartilage but also in the degenerated intervertebral disc (28–30), meniscus (31), fibrocartilaginous regions of tendons (32) and in cricoarytenoid cartilage (33).

Proliferation of cartilage cells in OA is a principal mechanism for the formation of clusters (34–40). This notion is supported by the incorporation of ^{3}H-tymidine, the presence of two nuclei within the same chondron (41) and detection of proliferation-associated antigens such as PCNA and Ki67 (31,42). The amount of proliferating chondrocytes increased during OA progression and cell division was activated specifically in cartilage with severe OA changes (43). Migration has been suggested as an alternative mechanism contributing to cluster formation based on changes in the chondrocyte cytoskeleton (19). Although chondrocytes can migrate in response to cytokines and growth factors in vitro (44,45) information on this process in adult articular cartilage is sparse. Establishing information on the extent and patterns of cell migration in mature cartilage will potentially open avenues to better understanding of cartilage homeostasis and responses to injury.

Diseases and experimental in vivo models associated with cartilage cell clusters

In addition to OA, cluster formation has also been demonstrated in articular cartilage from patients with rheumatoid arthritis and aseptic necrosis (46) but this has not been analyzed in detail.

Kashin-Beck disease which is endemic in certain regions of Asia, features OA-like joint pathology, and is caused by a combination of different environmental factors and selenium deficiency (47). Cartilage cell death or chondronecrosis is a principal pathogenetic mechanism but clusters form in response to the cell injury and express type X collagen in human disease and in an experimental model (48). Chondrocyte clusters in cartilage from patients with Kashin-Beck disease exhibited pericellular staining for types I, II, III and VI collagen (49) and expressed several regulatory factors, including FGF-2, VEGF, TGFβ and PTHrP (50).

Experimental OA can be induced by various approaches such as transection of ligaments or meniscectomy, or injection of joints with collagenase, leading to instability due to ligament damage. Experimental OA can also be induced by intraarticular injection of agents such as iodoacetate or papain that cause chondrocyte damage or death. Chondrocyte cluster formation is a feature of all mechanical and chemical OA models (51,52). In a partial thickness cartilage defect model a repair response as indicated by chondrocyte proliferation in clusters and a decrease in defect size was observed in immature but not mature rabbits (53). Analysis of the temporal changes in a similar model in pigs showed that there was an initial phase of cell necrosis adjacent to the cut edges. This was followed by the formation of cell clusters that expressed MMP-13 (54). In the 'groove model' of OA experimental femoral cartilage lesions are induced by scraping grooves with a metal wire. This leads to fibrillations on the femoral condyle and the tibial plateaus. Histological analysis showed chondrocyte clusters around the cartilage lesions (55).

Deficient or excessive mechanical loading of cartilage is also associated with cluster formation. Joint immobilization in mature rabbits led to loss of glycosaminoglycans, and fissuring. There were acellular areas of cartilage and cell clusters at 28–42 days after immobilization (56). Cartilage from strenuously trained horses showed more fibrillation and chondrocyte clusters than did the more gently exercised animals (57). Inclusion of high impact exercise in a partial meniscectomy model in rats was associated with cluster formation (58,59). Impact loading of rat femoral head articular cartilage induced chondrocyte cluster formation as early as 4 hours after injury (60). Since chondrocyte doubling times are on the order of 24 hours (61) and this possibly suggests cell migration. Chronic repetitive stresses in vivo induced by knee ligament transection are also associated with cluster formation (62,63).

Osteochondral allograft transplantation is used to treat cartilage defects in younger individuals. A certain amount of chondrocyte death occurs during harvesting, cryopreservation and after implantation of the osteochondral grafts. In a goat model the formation of cell clusters in the implanted grafts was detected by 8 days and the clusters increased in size and cell numbers that exceeded 100 cells per cluster after one year. The cluster cells expressed type II collagen but not type I or type X (64,65). This model does not feature OA-like pathology or joint inflammation which may explain why cells did not show the abnormal differentiation with type X collagen expression seen in OA. The formation of such clusters expressing type II collagen may represent an initial stage of cartilage regeneration. In a rabbit allograft transplantation model there was also a marked increase in ECM and formation of round and polygonal clusters of chondrocytes in the middle and deep zones of the grafted cartilage. The adjacent host articular cartilage remained normal and did not show cell clusters (66).

Quinolone antibiotics induce an arthropathy in juvenile animals and this appears to be due to an increased sensitivity of immature chondrocytes to toxic effects of quinolones (67). Animals with the arthropathy showed cavities in the middle zone of the articular cartilage containing necrotic chondrocytes. After 14 days, many of the lacunae in the areas of the defects contained chondrocyte clusters. When treated for 14 days and allowed a 14-day recovery period, territorial matrix had been deposited around individual chondrocytes within the clusters, indicating that in immature joints there is a certain degree of spontaneous repair by cluster cells (68).

Thus, diverse types of clinical and experimental cartilage lesions are all associated with the formation of chondrocyte clusters that contain more cells than clusters in normal cartilage. In most examples of cartilage damage the cell clusters express abnormal activation and differentiation markers that appear to be due to concomitant inflammation.

In vitro models and inducers of cluster formation

Chondrons, the cells with their intact pericellular matrix can be isolated using modifications of protocols that are used for cell isolation from cartilage (69,70). When chondrons were cultured in alginate beads or agarose constructs, various types of cell clusters formed over time. The clusters differed in morphology and ECM deposition and these differences were related to the cartilage zone from which the chondrons were isolated (69). Chondrons cultured as pellets increased in size and weight over a 6-week period without apparent cell proliferation. Chondron pellets accumulated significantly more proteoglycan and type II collagen than did chondrocyte pellets, indicating an anabolic effect of the native pericellular matrix. After 5 weeks in culture, ECM remodeling was evident in the chondron pellets. Cells that had been uniformly distributed throughout the pellets began to cluster between large areas of interterritorial matrix rich in type II collagen. After 12 weeks, clusters were stacked in columns, suggesting cell migration (70). Such enzymatically-isolated chondrons cultured in a three-dimensional matrix serve as a useful model of cluster formation.

Chondrocytes cultured as suspensions in agarose or alginate adopt a round morphology and form clusters of cells reminiscent of chondrocyte clusters in intact cartilage (71,72). In alginate scaffolds a higher cell seeding density led to formation of larger cell clusters that expressed type II but not type I collagen (73).

Several growth factors can stimulate chondrocyte cluster formation in vitro and FGF2 appears the most important. In a genetic screen where chondrocytes were transduced with genes that are expressed in OA cartilage and then cultured in agarose, FGF2 was the most potent inducer of cluster formation. Interestingly, all other genes that induced clusters also upregulated FGF2 expression, suggesting a common FGF2-dependent pathway (74). Similar effects of FGF2 were observed in other culture models. Addition of FGF2 to cartilage explants with surgical wound incisions induced proliferation with cluster morphology, expression of Notch-1 and the proteinases MMP-13 and ADAMTS-4 (75). In mandibular condylar cartilage of aging mice TGF-β induced numerous clusters (76) while TGF-α stimulated proliferation of chondrocytes and formation of cell clusters in rat osteochondral explants. In the same model TGF-α reduced type II collagen and aggrecan while increasing MMP-13 and cathepsin C (77). Mechanical stress on chondrocyte suspension cultures also stimulated cluster formation (78).

These observations from culture models suggest that isolated chondrons and chondrocytes spontaneously form cell clusters and this can be enhanced by the addition of mitogens such as FGF2 or TGF or by mechanical stress. An important unanswered question is whether cells can migrate from the clusters and populate acellular areas or form new interterritorial matrix similar to that in normal articular cartilage.

Cytoskeleton and cilia of cluster cells

In normal chondrocytes F-actin is typically organized as a dense cortical structure, predominantly located just inside the cell membrane. The cells in fibrillated OA cartilage displayed rearranged actin filaments (19). The vimentin intermediate filaments in superficial flattened chondrocytes assemble in the form of perinuclear bundles while in the middle zone chondrocyte vimentin was perinuclear as well as in the cell periphery. On the other hand, the intermediate filaments in the cluster cells in human OA cartilage were more disorganized and sometimes completely absent (19). In experimental OA in rat knees, all three major cytoskeletal elements underwent distinct reduction in stain intensity ranging from 5% for actin to almost 40% for vimentin (79). There was also significant cytoskeletal disorganization (80). The cytoskeletal changes in OA chondrocytes are presumably linked to their abnormal activation and differentiation patterns as a close association between

cytoskeletal organization and chondrocyte differentiation has been documented in various in vitro models (81,82).

The contractile actin isoform, α-smooth muscle actin mediates the contractile behavior of cells and may be beneficial in tissue repair responses. The original observation that chondrocytes were able to contract three-dimensional matrix structures (83) led to the discovery of α-smooth muscle actin in certain types of chondrocytes (84). In normal articular cartilage 75% of cells in the superficial zone but only 10% in the deep zone express this actin isoform. In OA cartilage clusters the majority of cells expressed α-smooth muscle actin (85). It is at present unknown whether normal chondrocytes can de novo express α-smooth muscle actin or whether the increase in cells expressing α-smooth muscle actin in OA is the result of proliferation of this subset. The strong differentiation potential of α-smooth muscle actin positive cells indicates that they may represent immature chondrocytes or chondroprogenitor cells (86).

The primary cilium was initially described in motile cells but is now known to be a highly conserved, single cytoplasmic organelle in virtually all eukaryotic cells. The chondrocyte primary cilium projects into the pericellular matrix of the chondron and through integrins and other receptors interacts with matrix components such as collagens type II and VI (87–90). In addition to cell migration, the chondrocyte cilium is thought to play a role in mechanotransduction (91). Defects in perichondrial, chondroblast, and chondrocyte primary cilia have recently been implicated in both skeletal patterning and growth plate abnormalities (92–96).

In normal cartilage, the length of superficial zone chondrocyte cilia was shorter than deep zone cilia. Cilia showed a clear orientation in relation to the zone of origin; superficial zone chondrocyte cilia were consistently oriented away from the articular surface. In both mild and severe OA tissue, the proportion of ciliated cells increased markedly from the articulating surface to the tidemark. The percentage of cilia detected on chondrocytes at the joint surface increased with OA severity (97). There were also significant differences between the incidence of cilia in deep zone cells, both between normal and mild OA and mild and severe OA. The increased presence of cilia, centrioles and filopodia in and near clusters under the fibrillated region of the OA cartilage suggests that they could be motile cells (7,19). The filopodia may also be involved in cell-to-cell contact and communication between paired chondrocytes in the superficial zone of articular cartilage (98). Small cytoplasmic projections were detected between some chondrocyte pairs in the superficial zone of adult rabbit articular cartilage. Such cell-cell interactions are likely to also occur in cell clusters but this has not been formally demonstrated.

Proteins and genes expressed in clusters

Immunohistochemical analyses of OA cartilage have drawn attention to clusters because they express a very large number of induced proteins that are not detected in normal cartilage cells (99–118). A representative list of induced proteins is shown in Table 1. Although a systematic analysis of gene or protein expression in OA clusters such as using clusters that are isolated and separated from other cartilage cells has not yet been performed, the available information suggests an activation pattern that prominently includes ECM-degrading enzymes and inflammatory mediators (Figure 2B). A variety of growth factors that is expressed in the clusters (50,119) presumably drive the cell proliferation in the clusters and possibly in the adjacent cartilage areas. Cell clusters in OA cartilage also show changes in cell signaling proteins that mediate the cell activation. An additional pattern is that of abnormal differentiation. This is based on hypertrophy markers such as collagen X (120), osteocalcin (21), osteopontin (121), MMP-13 and Runx2 that are co-localized in

nearly all chondrocytes in clusters in fibrillated OA cartilage (122). Cluster cells also express markers of immature or de-differentiated chondrocytes. Pleiotrophin is abundant in fetal or juvenile cartilage, but rarely detected in normal adult tissues. In human OA cartilage pleiotrophin was expressed in clusters of superficial chondrocytes (123). It needs to be determined whether these activation markers are shared by all cluster cells or whether some cells express inflammatory mediators while others express growth factors.

These findings indicate that OA cluster cells are activated and represent an important source of pathogenetic mediators. At present the relative contribution of cells in the original cartilage areas versus of those in the expanded clusters is unknown but it is apparent that cluster cells are a major contributor to the overall increase in gene and protein expression observed in OA cartilage as detected in DNA array and proteomics analyses (124–126).

Cell death and crystal deposition in clusters

A series of studies examined apoptosis in cartilage from human and experimental OA and consistently reported apoptotic cells in the clusters (127–129). It is unclear what induces cluster cell apoptosis but the selective death of only some cells in each cluster argues against general mechanisms such as nutrient or oxygen deprivation. Potential mechanisms are expression of programmed cell death receptors and ligands that may in part be related to the abnormal hypertrophic differentiation program.

A scenario that encompasses cluster cell hypertrophy, cell death and matrix calcification can be proposed. Crystal deposition was described in the pericellular regions near the surface zone of OA articular cartilage and this was coupled with increased numbers of matrix vesicles and alkaline phosphatase activity (130). There is abundant evidence for the expression of hypertrophic markers in the clusters (see above). In addition, cluster cells express pyrophosphate-generating enzymes (131). Matrix calcification in growth plate physiologically involves cell death and the generation of matrix vesicles and chondrocyte-derived apoptotic bodies can serve similar functions (132). Apoptotic chondrocytes in OA cartilage showed abundant alkaline phosphatase-rich matrix vesicles budding from the plasma membrane with hydroxyapatite microcrystals on their surface (133). Staining of cartilage sections showed co-localization of clusters containing apoptotic cells adjacent to calcium deposits (131) (Figure 3). In OA-affected menisci the presence of type X collagen and deposition of calcium as detected with alizarin red also suggested the possibility that mineralization of the extracellular matrix surrounding the cell clusters was occurring (31).

Stem cell markers in cartilage and clusters

The abnormal activation and differentiation pattern of cluster cells in OA cartilage has been interpreted as chondrocyte de-dedifferentiation (3) where the differentiated articular chondrocytes change gene expression patterns in response to the different extracellular signaling environment (134). An alternative scenario is that cluster formation is the function of a unique subpopulation of progenitor cells. A series of recent studies suggests the presence of cells with phenotypic markers and functions of progenitor cells in mature articular cartilage (135–137). Cell surface markers such as Stro-1 or Notch-1 were most strongly expressed in the superficial zone (136–138). Notch-1 was also densely localized in the deeper zone of articular cartilage (138,139). A prominent feature of OA cartilage is the strong expression of stem cell markers in the cell clusters (138) (Figure 4). Surgical injury to articular cartilage is also associated with proliferation of progenitor cells that produce new extracellular matrix (140). Migratory chondrogenic progenitor cells were recently described in OA joints (141) and they resemble cells that are seen in the clusters. A systematic analysis of the fate of cells expressing progenitor markers during the development of OA is required to address this hypothesis.

Summary and Conclusions

Cell organization in clusters represents an interesting and as yet not well-characterized phenomenon in normal articular cartilage, in tissue responses to chemical or mechanical injury and prominently in cartilage affected by diseases such as OA. Cluster formation also occurs readily in culture models suggesting an intrinsic tendency and capacity of certain cartilage cells to assume this cellular organization.

It can be proposed that repair of cartilage lesions that include both ECM damage and cell death would require replication of some of the cells adjacent to the damaged area, followed by migration, differentiation and new matrix formation (140). It appears that cluster formation represents the first phase of this response. Clusters are seen with a remarkable uniformity in cartilage exposed to a broad range of injuries that are always associated with some degree of cell death. Whether any surviving cell or only certain subsets of cells are able to proliferate and form clusters is unknown. An interesting hypothesis is that it is a function of immature or progenitor cells. The triggers for cell proliferation may include loss of cell-cell interactions due to cell death. Liberation of growth factors such as FGF2 from damaged tissue appears to be an important mechanism for the generation of mitogenic stimuli at least during the initial stages of cluster formation. The rate and size of cluster formation depend on the type of injury and maturity of cartilage. Cells within clusters have certain features suggesting that they can migrate but whether cells do indeed migrate from the clusters to acellular areas of cartilage is an important unresolved question of importance to understanding intrinsic cartilage repair as well as to cartilage engineering approaches. Movements of single cells or collective movements of interconnected groups or clusters of cells have recently been characterized as important mechanisms in tissue and organ development (142). Cell-cell adhesions, cell-matrix interactions, contractility and the ability of cells to form protrusions and migrate as described for the cluster cells contribute to active motility.

Tissue engineering of chondral and osteochondral grafts is being actively pursued as a potential method of surgically repairing cartilage lesions. Cluster formation occurs in three-dimensional cultures of chondrocytes and needs to be carefully evaluated in engineered constructs using scaffolds with stem cells. Spontaneous chondrocyte cluster formation has also been noted in osteochondral allografts in several animal models (66). These clusters did not appear to express the abnormal markers seen in OA clusters. However, it must be noted that these studies were conducted in tissue or cells from young adult animals and in joints that did not have an inflammatory response. Collectively, these findings raise a concern for tissue engineering during the pre-implantation cell proliferation and matrix generation phases as well as the postoperative phase after implantation in the chondral lesion.

Clusters that form in arthritic articular cartilage contain cells that express a large number of pathogenic mediators and thus appear the site of much of the increased biosynthetic activity that characterizes OA cartilage. The activation patterns are those of a disordered or mixed differentiation phenotype. This may be due to the influence of inflammatory mediators produced in the clusters or by other inflamed joint tissues. The activation of cluster cells and their products appear to contribute to the manifestations of cartilage diseases such as ECM degradation and calcification, and joint inflammation. The cluster cells illustrate the ability of a subpopulation of cartilage cells to undergo activation and proliferation even in older individuals. Correcting the abnormal differentiation and harnessing the reparative potential of these cells pharmacologically may offer new approaches to cartilage repair and OA therapy.

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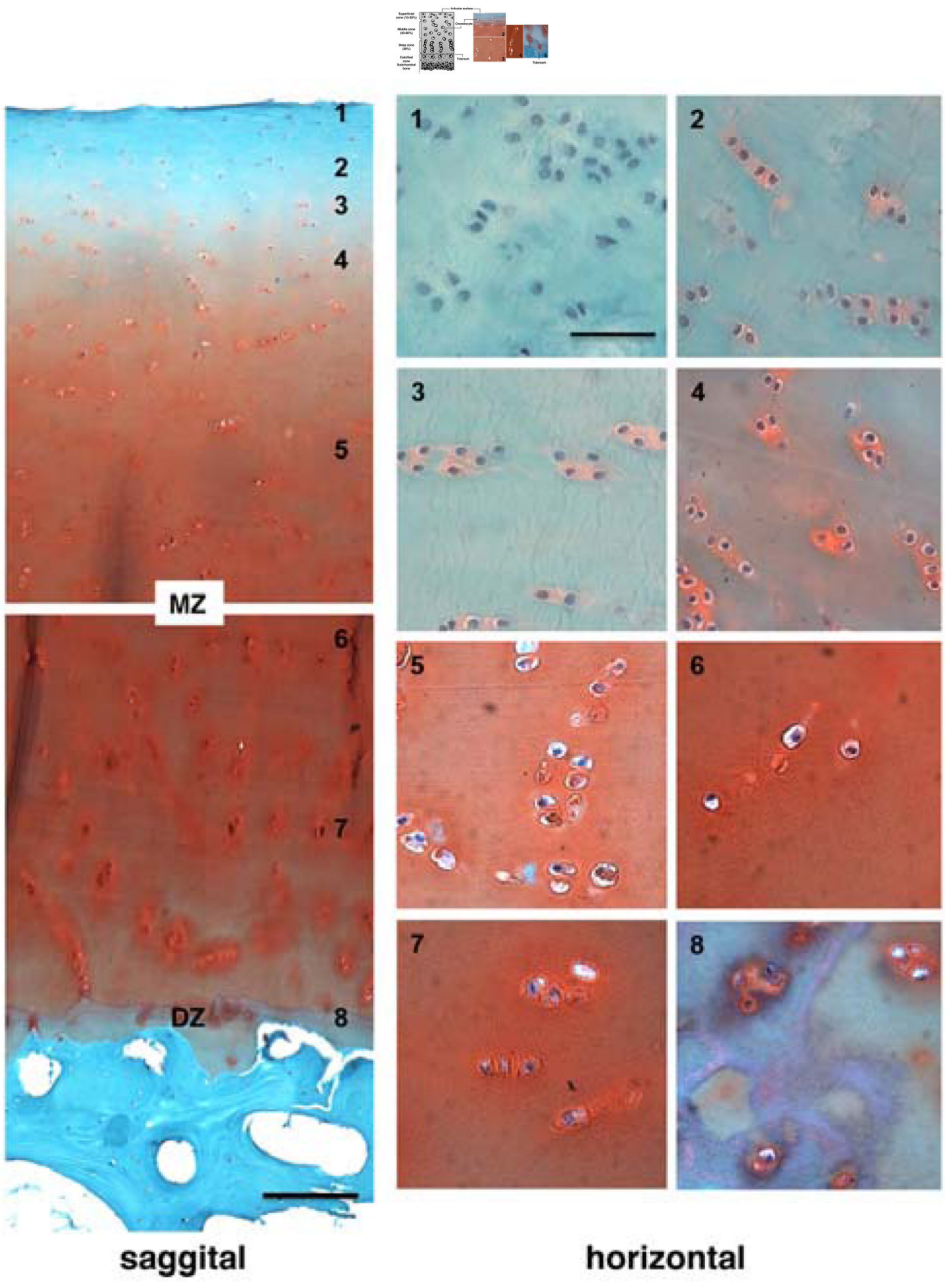

Figure 1. Schematic drawing and microscopic image of cell arrangements in normal cartilage

A. Overview of cartilage zones.

1) Each zone has characteristic cell shape, morphology, orientation, and pericellular matrix (PM) deposition.

2) Superficial zone (SZ) cells are small, elongated in shape, parallel to the surface, and lack an extensive PM. These cells predominate the first 50 μm.

3) The middle zone (MZ) is distinguishable by rounded cells that do not exhibit an organized orientation relative to the surface, are within ECM rich in proteoglycans and show presence of PM.

4, 5) Deep zone (DZ) cells show an extensive PM deposition with chondrons in groups of three or more cells arranged in columns perpendicular to the surface. Modified from (138).

B. Horizontal sections of normal cartilage. Horizontal sections of normal human articular cartilage were prepared from the SZ to tide mark and stained with safranin O. As indicated on the saggital plane cross-section, image numbers 1–4 were cut at 20μm intervals starting from superficial to middle zone. Image numbers 5–8 represent sections from the MZ, DZ and tidemark at 400μm intervals. Bars: 200μm (large). 50μm (small).

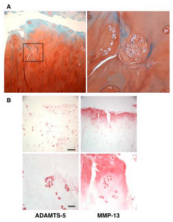

Figure 2. OA clusters

Articular cartilage from a 75-year old male was stained with safranin O (A). The magnification was ×100 (left) and ×400 (right). (B) Articular cartilage from a 57-year old male was stained with ADAMTS-5 and MMP-13. Bar: 200μm (upper) and 50μm (lower).

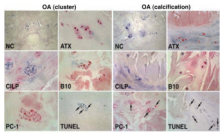

Figure 3. OA cluster cell apoptosis and calcification

Localization of TUNEL-positive cells, calcium deposits and pyrophosphate-generating enzymes in menisci from OA-affected human knees. The left panel shows apoptotic cells, many in clusters, in the vicinity of (alizarin red-positive) calcified areas. The right panel shows cells immediately bordering calcifications. Staining for plasma cell membrane glycoprotein (PC-1), autotaxin (ATX) and B10 is also prominent at sites of calcification and in areas with TUNEL-positive cells. CILP: cartilage intermediate layer protein. Modified from (131).

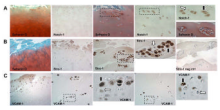

Figure 4. OA cluster staining for stem cell markers

A majority of cells in clusters (69 to 79%) are positive for Notch-1, Stro-1 and VCAM-1. Clusters located in the DZ had significantly reduced frequencies of Stro-1 positive cells. (A) Safranin O and Notch-1 staining in clusters (×10 and ×40). (B) Safranin O and Stro-1 staining of OA cartilage sections (×10 and ×40). (C) OA cartilage sections immunostained for VCAM-1 (×10 and ×40). Positive staining indicated by black arrows and negative with white arrows. Modified from (138).

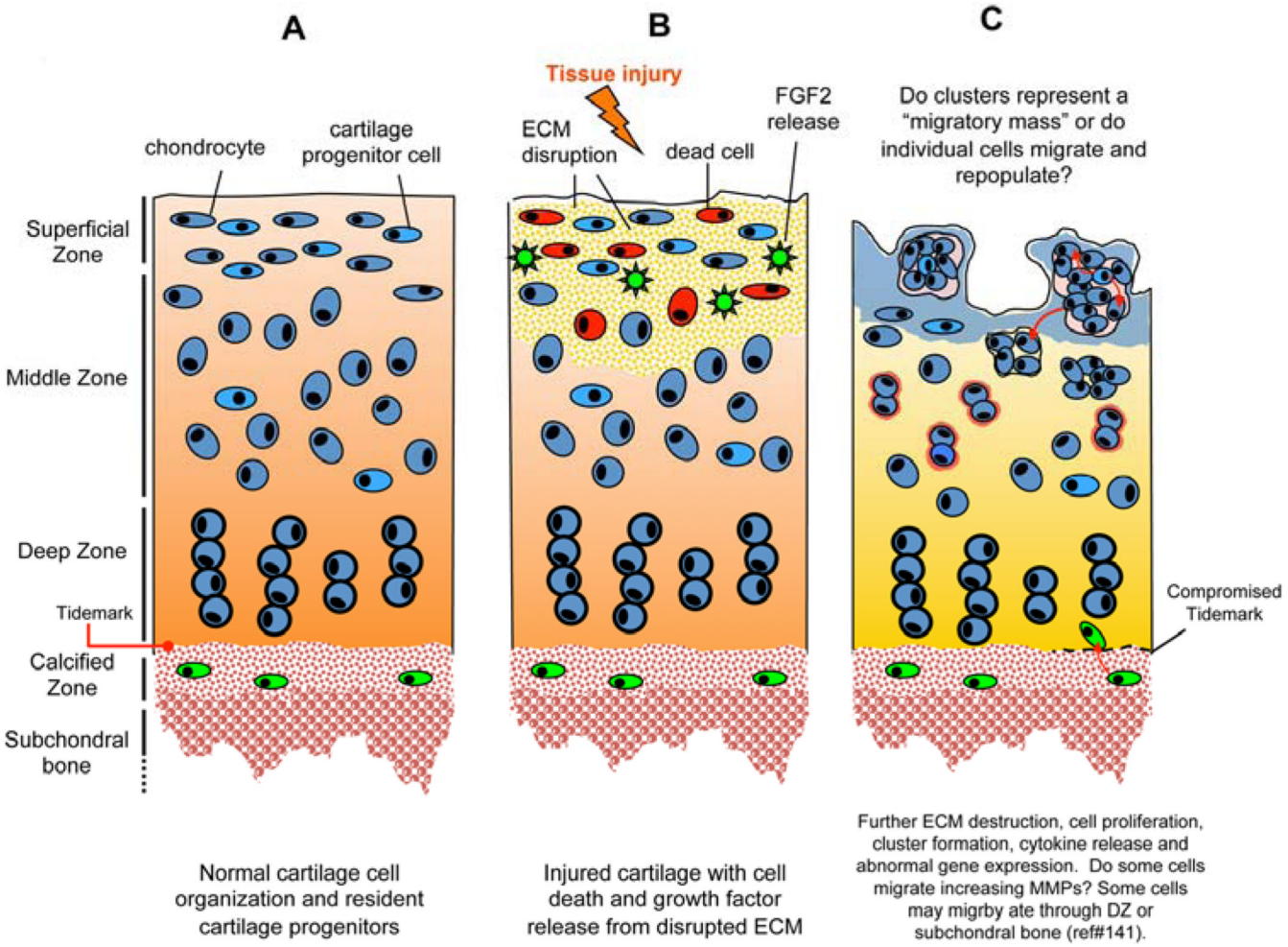

Figure 5. Schematic drawing of mechanisms and phases of cluster formation

(A). Normal cartilage cell organization and resident cartilage progenitors. (B). Injured cartilage with cell death and growth factor release (e.g. FGF2) from disrupted ECM. (C). Further ECM destruction, cell proliferation, cluster formation, cytokine release and abnormal gene expression. Some cells may migrate through DZ or subchondral bone (141).

Table 1

Gene and protein expression patterns in OA cartilage cell clusters.

Receptors, Kinases, Transcription factors	Sox9(73,138)
	Runx2(122)
	C-maf (99)
	Leptin/ Leptin receptor (28)
	c-Fos,c-Jun(AP-1)(30)
	HMGB1 (100)
Cytokines, Growth factors	BMP-7(OP-1)(101)
	FGF2(102)
	BMP-2,−4,−6(103)
	PGE2, COX-1,−2(104)
	Histamine (105)
	S-100(106)
	IL-1β,TNFa(107)
	Pleiotrophin(123)
	WISP3 (110)
ECM, ECM-degrading Enzymes	Aggrecan (72,73)
	Procollagen (64)
	Col I (72)
	Col II (64,69,72,73)
	Col VI (23)
	Col X (120)
	TIHP-1 (54)
	5ZP(12)
	MMP-1,−8,−13(107)
	MMP-2, −3, −8,−9(108)
	ADAMTS-10(109)
	Cathepsin K (113), D (114), B (115)
	Suits (116)
	Syndecan-4(116)
Cell Death	Apoptosis (103,111)
	Autophagy (111,112)
Progenitor Cell Markers	Notch-1 (118,138)
	Stro-1 (138,116,141)
	VCAM-1 (138)
	Ann ex in VI (42)
	Plelotrophln(123)