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ESSENTIALS Synovial joints are complex functional elements of the vertebrate body that provide humans and animals with motion capabilities and hence the ability for locomotion and direct physical interaction with their environment. They are composed of different connective tissue structures that are derived from the same developmental structures in the embryo, but have distinct cellular and biochemical properties. Articular cartilage and synovial membrane are key components of synovial joints and show several peculiarities that makes them dif- ferent from other tissues. An in-depth knowledge of these features is important not only for understanding key features of articular func- tion, but also for providing explanations for important characteristics of both degenerative and inflammatory joint diseases. This chapter reviews the structure, biochemical composition, and function of articular cartilage and synovium, and points to important links be- tween physiology and pathologic conditions, particularly arthritis. Special emphasis is put on the interaction of resident cells with both the extracellular matrix and other neighbouring or invading cells. Introduction Articular joints are key components of the musculoskeletal system that provide flexible support to neighbouring bony structures and allow for the movement in different

parts of the body. This is par- ticularly true for synovial joints that are found mainly at the extremities and are the structural basis for key physiological activities such as walking, climbing, and gripping. Synovial joints are composed of different morphological structures, including bone, tendons, cartilage, the synovial membrane, and the synovial space with the synovial fluid. While functionally distinct and anatomically well separated under normal conditions, these tissue structures exhibit a high degree of communication, and growing evidence suggests that intertissue communication is essential not only for the developing joint during embryogenesis, but also for maintaining joint homeo- stasis in the adult organism. The notion that many joint diseases do not affect a single tissue component but the joint as a whole has been expanded recently, by the concept that intertissue communication is not merely an epiphenomenon of these diseases but a key driving element of their pathogeneses. Nonetheless, much of the recent biomedical research has been focused on the analysis of the individual tissue components of the joints rather than on their interactions. Thus, autoimmune diseases of the joints such as rheumatoid arthritis and psoriatic arthritis have been largely considered disorders of the synovial membrane, in which cartilage and bone are mere target tissues that suffer destruction from the inflamed synovium. In turn, osteoarthritis and osteoporosis as the main degenerative joint diseases have been looked at mainly as age-related disorders of cartilage and bone with only secondary involvement of adjacent tissues such as the synovium, tendons, and muscle. However, such an isolated view not only neglects important aspects of physiological joint development and function, but it also fails to provide answers to some key questions about the pathogenesis of these diseases. A key example is the unresolved question about the cellular and molecular basis of organ specificity of joint diseases (e.g. why does systemic autoimmunity under certain cir- cumstances specifically affect the joints, and what are the tissue determinants of joint manifestation?). Another important ques- tion regards mechanisms that link embryonic development and adult tissue remodelling, both during degenerative joint diseases and during regeneration. This chapter looks at synovial joints as complex organs with dis- tinct yet highly interconnected structures. We will try to demonstrate that this concept not only is key to understanding the development and function of articular joints, but becomes relevant particularly under pathological conditions such as arthritis. These not only affect joints as a whole, but are also based on complex pathological inter- actions between tissue components of the joints such as cartilage and synovium. Cartilage Cartilage is one, if not ‘the’ key component of synovial joints. Supported by the synovial fluid, cartilage provides a smooth and low-friction surface that is needed for gliding of both bony ends. At the same time the articular cartilage functions as an elastic and load-absorbing tissue. The biomechanical properties of articular cartilage are achieved by its unique structure. It is characterized 19.1 Joints and connective tissue—structure
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section 19 Rheumatological disorders 4380 by the presence of only one cell type, the chondrocytes, which are loosely embedded into a highly complex and organized extracel- lular matrix (ECM) (Fig. 19.1.1). The articular cartilage is or- ganized into at least four distinct zones that differ with respect to the density and orientation of the cartilage fibrils, as well as the number and shape of embedded chondrocytes. The super- ficial zone (which because of its structural organization is also called the tangential zone) makes up about 10% of the cartilage thickness. It is in direct contact with the joint cavity and char- acterized by densely packed cartilage fibrils with long-shaped chondrocytes in between. The middle zone makes up about 40% of the articular cartilage and is also called the transitional zone. It has more round-shaped chondrocytes, which are

embedded into a less densely packed ECM. The deep or radial zone is the thickest layer of articular cartilage, and the chondrocytes here are oriented in perpendicular direction to the cartilage surface. The deep radial zone is separated by the tidemark from the calcified zone that is in direct contact with the subchondral bone. Development While articular cartilage is usually viewed as a covering tissue that is placed on top of the underlying bone, it actually constitutes a spe- cific part of the cartilaginous template, from which both the joints and the long bones are formed during embryogenesis through endochondral ossification. However, articular cartilage areas are not purely remnants of the bone formation process, in which ossifica- tion comes to a halt. Rather, it has been demonstrated that articular cartilage differs from growth plate cartilage and that articular chon- drocytes originate from a distinct population of mesenchymal pro- genitor cells that can be identified very early in limb development. These cells are derived from an area called the interzone and are characterized by a specific molecular pattern including the expres- sion of the growth factor Gdf5. The interzone is further subdivided into an intermediate and outer layer, which display a differential gene expression profile. The initial differentiation of the interzone appears to be regulated in a permissive manner by the concerted action of members of the Wnt-family of molecules such as Wnt9a (formerly known as Wnt14) and Wnt4 through activation of the Wnt/ß-catenin signalling pathway. Experimental evidence suggests that at the time of interzone formation, the Wnt/ß-catenin pathway is required to suppress the chondrogenic potential of the interzone cells, leading to a downregulation of the transcription factor Sox-9. Interestingly, the Sox-9 related transcription factors, Sox-5 and Sox-6, are both needed, cell-autonomously and nonautonomously for joint formation. Mechanisms, such as muscle contraction, are subsequently required in maintaining joint progenitor fate in the elbow joint, and one of the molecular mechanisms involved here is the stretch-activated upregulation of ß-catenin. The synovial joints differ by their anatomy and this is also reflected by differences in their transcriptome profiles, which points towards the existence of fundamental different mechanisms as to how individual joints de- velop after interzone establishment. The question whether this distinct environment and the early de- termination of cellular fate in articular cartilage areas results in a specific chondrocytic phenotype or rather constitutes a means that prevents articular chondrocytes from differentiating further into growth plate-like cells remains a matter of investigation and discus- sion. Cell-specific inactivation of ß-catenin in Gdf5 producing chon- drocytes of the interzone results in largely normal joint formation with alterations mainly in the superficial zone of articular cartilage. As such, it is not entirely clear whether the described ß-catenin de- pendent mechanism in early development affects the whole of the articular cartilage or predominantly the most superficial, tangential zone, or whether these differences arise because of temporal distinct functions of ß-catenin during development. Those cells of the mesenchymal condensations that are more distant from the interzone also undergo chondrogenic differenti- ation, and then longitudinal growth until the cartilage template is formed. Subsequently, these chondrocytes differentiate further to initiate endochondral ossification, which eventually results in the replacement of the cartilage template with trabecular bone. In add- ition to the transcription factor Sox-9 that is involved most prom- inently in chondrocyte differentiation, these steps are controlled by genes of the Hox family and regulated by the concerted action of different signalling proteins, particularly sonic hedgehog (shh), indian hedgehog (ihh), and growth factors of the fibroblast growth factor (FGF) family. Components of the ECM, particularly glyco- proteins such as fibronectin and protein-bound glycosaminoglycans such as heparan sulphate proteoglycans (HS) contribute signifi- cantly to chondrogenesis and thus to key properties of cartilage. They are synthesized by the differentiating chondrocytes and serve as a structuring scaffold for limb- and joint development. Moreover, they

are involved in creating gradients of growth factors through binding of soluble factors. Mutations in genes encoding for HS spe- cies such as perlecan or in enzymes that contribute to the assembly of HS side chains lead to severe skeletal defects. As a next step in endochondral ossification, chondrocytes from the centre of the car- tilage template start differentiating into hypertrophic chondrocytes, which is associated with fundamental changes in their biochemical profile. Hypertrophic chondrocytes switch their collagen profile to produce type X collagen and are able to calcify their surrounding matrix. Moreover, hypertrophic differentiation is associated with increased expression of matrix-degrading enzymes, particularly matrix metalloproteinase (MMP)-13, the vascular endothelial growth factor (VEGF), and transglutaminase 2, which acts a mo- lecular switch of chondrocytes into a calcifying phenotype. These changes are controlled prominently by hypoxia through expression of hypoxia-inducible factors (HIFs) and allow for the coordinated degradation of cartilage matrix and the subsequent invasion of blood vessels into the newly forming bone. Thus, while the mechan- isms that regulate the differentiation of hypertrophic chondrocytes and subsequent degradation of the ECM during bone development are only partly understood, an in-depth understanding of these pro- cesses helps explain the cellular and biochemical structure of adult Superficial zone Middle zone Deep or radial zone Tidemark with calcified zone Fig. 19.1.1 Zonal organization of cartilage (see text for explanation).

 joints. Recent data also suggest that joint pathologies such as osteo- arthritis are characterized by the (re)activation of certain parts of this embryonic programme in resting chondrocytes of the more superficial and embryologically distinct articular cartilage. Biology and metabolism The primary function of the chondrocytes in the articular cartilage is to maintain the structure of the surrounding matrix through coord- inated production and degradation of its components (Fig. 19.1.2). The exact mechanisms that ensure the homeostatic stability of these resting chondrocytes remain elusive, but it is important to empha-size that the term resting chondrocytes does not mean low metabolic activity. Rather, these cells are characterized by the high level pro- duction of cartilage matrix components, which not only allows for continuous cartilage remodelling but also provides the basis for the regenerative capacity of the cartilage. The ECM of cartilage consists of mainly three structural elem-ents: cartilage collagens, proteoglycans, and other noncollagenous proteins. These elements are strongly interconnected and show dif- ferences with respect to their amount and distribution between the area around the chondrocytes, termed territorial matrix, and the regions between territorial areas that are called the interterritorial matrix. Collagens The main collagen of cartilage is type II collagen, which makes up about half of all cartilage proteins and more than 90% of all car-tilage collagens. It has a triple helical structure and forms fibrillar suprastructures that includes type IX collagen as a fibril-associated collagen with interrupted triple helix (FACIT) as well as type XI col- lagen. As has been understood recently, these additional collagens within the collagen II fibrils are important for fibrillogenesis, that is, for the self-assembly of the fibrils, for the limitation of their lat- eral growth, and also for the stabilization of the mature fibrils. In addition, particularly type IX collagen—through binding of small leucine-rich proteoglycans such as decorin—appears to regulate the association of fibrils and is thus responsible for structural dif- ferences between territorial and interterritorial matrix. In addition to collagen types II, IX, and XI, other collagens such as collagen type VI are found in the cartilage. Collagens constitute the backbone of the ECM in the cartilage and their turnover rate is low. Thus, the half-life of type II collagen has been estimated to last more than a human lifetime, which explains why cartilage damage can be con- sidered largely irreversible once the integrity of the collagen network is lost. However, in contrast to what is

frequently believed, the regen- erative capacity of cartilage is considerable, and this is due largely to the constant renewal of other key components of its ECM, namely the glycosaminoglycans and proteoglycans. Glycosaminoglycans and proteoglycans Hyaluronan (HA) is a key glycosaminoglycan in cartilage. It con- stitutes the structural basis for the formation of large water binding aggregates with proteoglycans, mainly aggrecan. The amount of hyaluronan in the cartilage as determined by the rate of synthesis as well as the length of the individual hyaluronan molecules, therefore, is an important determinant of the cartilage structure and its func- tional properties. Other glycosaminoglycans in the cartilage include heparan sulphate, chondroitin sulphate, and keratan sulphate, which are usually bound to proteins in specific proteoglycans. Aside the collagen network, such proteoglycans constitute the most important components of the cartilage matrix with aggrecan being the most abundant proteoglycan of cartilage. It consists of three globular domains that are termed G1, G2, and G3. The G1 do- main interacts with the link protein of hyaluronan and is, thus, re- sponsible for the spatial distribution of aggrecan within the cartilage matrix. The interglobular protein domain between G1 and G2 can be cleaved by different metalloproteinases, including ADAMTS4 (aggrecanase-1) and ADAMTS5 (aggrecanase-2), as well as MMPs such as MMP-3 (stromelysin) and MMP-13 (collagenase-3). The interglobular domain between G2 and G3 harbours attachment sites for keratan sulphate and chondroitin sulphate side chains. These are attached and post-translationally modified in a multistep en- zymatic process. The specific function of the large number of highly sulphated chondroitin sulphate side chains is a matter for intense study, particularly because several lines of evidence suggest that the sulphation pattern not only is variable but changes significantly during aging. Proteoglycans are also found on the surface of chondrocytes. These cell surface proteoglycans include the families of syndecans and glypicans and are anchored to the cell either through a trans- membrane domain (as is characteristic for the syndecans) or through covalent binding of the C-terminus to glycosylphosphatidylinositol as found in glypicans (Fig. 19.1.2). They are endowed with indi- vidually characteristic numbers of heparan sulphate or chondroitin sulphate side chains that can bind a variety of molecules including components of the ECM such as fibronectin and a growing list of soluble mediators, particularly growth factors, but also cytokines and chemokines. Our knowledge about the role of these cell sur- face heparan sulphate proteoglycans (HSPGs) in the regulation of chondrocyte differentiation and behaviour has increased dramat- ically over recent years, and it has been shown that the effects of growth factors, such as FGF-2 and cytokines, such as osteopontin or chemokines (e.g. CXCL10) are regulated strongly by HSPGs such as syndecan-4. Integrins Syndecans Glypicans ECM Fig. 19.1.2 Cell-matrix interactions in cartilage. The interaction of chondrocytes with the extracellular matrix (ECM) plays a critical role in regulating the differentiation of chondrocytes during physiological development and in pathological conditions. Three classes of cell surface molecules contribute most prominently to the interaction of chondrocytes with ECM components: integrins, syndecans, and glypicans. The integrins and syndecans contain intracellular domains and can initiate signalling cascades, both independently and in collaboration with one another.

section 19 Rheumatological disorders 4382 Biochemistry and pathology in arthritis Osteoarthritis is the most common joint disease and a pathologic condition that is most closely linked to changes in cartilage biology. It is a debilitating disorder characterized by the progressive destruc- tion of articular cartilage through uncontrolled proteolysis of ECM and typically leads to a remodelling of affected joints. Osteoarthritis as a disease is discussed in Chapter 19.9, but it is important to note at this point that although the incidence of osteoarthritis increases with age, it is not a pure ageing

phenomenon. Rather, osteoarthritis is characterized by profound alterations to the biology of chondrocytes that show important similarities to endochondral ossification as it occurs during embryogenesis (previously described here). Recent data suggest that the triggers which initiate and drive the disease process in osteoarthritis may be distinct in some respect from those in embryonic development and more influenced by inflammation, which is why the term ‘osteoarthritis’ has been chosen. However, accumulating evidence supports the view that osteoarthritis consti- tutes a quite uniform way of how chondrocytes and cartilage react as a whole to stress that they are unable to compensate. This would mean that although the triggers that initiate osteoarthritis and the contribution of individual factors such as genetics, inflammation, mechanical stress, and others may vary considerably from patient to patient, there is ultimately a rather narrow path or programme that characterizes its pathophysiology. Chondrocyte de-differentiation During osteoarthritis articular chondrocytes revert to an immature chondrocytic stage and even undergo pre-hypertrophic to terminal chondrocyte maturation, indicating that their differentiation state, although normally permanent, is not terminal. Losing their differ- entiated phenotype, the chondrocytes in affected cartilage enter an endochondral ossification-like cascade of proliferation and hyper- trophic differentiation. This change is accompanied by marker ex-pression for the overt hypertrophic differentiation stage, such as alkaline phosphatase, Col10a1, with subsequent apoptotic death and mineralization of the diseased cartilage. Therefore, recent studies focus on the mechanisms that either stabilize the articular pheno- type or induce chondrocyte dedifferentiation. Osteoarthritic changes are associated with the re-expression of molecules and pathways in articular chondrocytes that are charac- teristic for different stages of endochondral ossification. The above- mentioned transmembrane heparan sulphate proteoglycans, and particularly syndecan-4 are a prime example for this concept. Thus, it was demonstrated that syndecan-4 gets re-expressed in both, in human osteoarthritis and in animal models of the disease, and correlates with its severity. Interestingly, the loss of synedcan-4 in genetically modified mice or its inhibition by specific antibodies prevented mice from the development of osteoarthritis-like changes suggesting that, indeed, interrupting the cascade that leads to the completion of the aberrant chondrocyte differentiation programme may be of therapeutic value. One pathway that has been proposed to regulate the chondrocytic differentiation during osteoarthritis (OA) is the Wnt signalling cascade and especially Wnt3a, because it can dose-dependently trigger either the canonical ß-catenin de- pendent pathway or the noncanonical pCamKII dependent pathway. Furthermore, both pathways react reciprocally inhibitory. However, both the constitutive activation and repression of the canonical ß-catenin dependent pathway let to osteoarthritis-like cartilage changes in mice. Likewise, it was demonstrated that the hypoxia-inducible factor HIF-2α, which as outlined here has been implicated strongly in endochondral ossification and in the regulation of genes involved in chondrocyte hypertrophy, also contributes to the development of osteoarthritis. Both, the overexpression of HIF-2α by gene transfer and the use of genetically modified mice that overexpress HIF-2α in their chondrocytes, resulted in an acceleration of osteoarthritis- like changes. In turn, genetic deletion of one allele of HIF-2α clearly reduced the severity of disease and reduced the expression of disease-relevant matrix-degrading enzymes. Destruction of articular cartilage It has been well established that matrix-degrading enzymes, mainly of metalloproteinases of the ADAMTS (A Disintegrin and Metalloproteinase with Thrombospondin motifs) and the MMP (matrix metalloproteinases) families, are ultimately responsible for the breakdown of ECM in osteoarthritis and thus for the progressive loss of articular cartilage. Among the different enzymes, particularly MMP-13 and ADAMTS-5 have been associated with chondrocyte- mediated cartilage damage and it has been demonstrated in animal models of osteoarthritis that the loss of these

enzymes or inhibition of their activation results in less severe cartilage damage. However, not only is it likely that the relevance of individual members of the MMP and ADAMTS family varies during different phases of osteo- arthritis, but it may also be hypothesized that other MMPs (e.g. MMP-3 or MT1-MMP), which themselves are a critical activators of other MMPs and cathepsins contribute to the loss of articular car- tilage in osteoarthritis. As during embryonic development, the induction and unbalanced action of these enzymes results from the specific differentiation of the chondrocytes and, therefore, these processes are very closely linked. It needs to be emphasized, however, that cartilage matrix can be degraded by these enzymes not only when they are released from the chondrocytes themselves, but also when there is an increased and unbalanced expression in neighbouring tissues such as the syn- ovial membrane. The most prominent example for a disease where cartilage destruction is mediated mainly by an invading synovial membrane is rheumatoid arthritis. In this disease, which is covered in detail in Chapter 19.5 of this book, the inflamed and hyperplastic synovial membrane that attaches to the articular cartilage and deeply growth into and destroys the cartilage matrix. Data from dif- ferent animal models including the severe combined immunodefi- ciency (SCID) mouse model of RA-like cartilage destruction and the human TNF-α transgenic (hTNFtg) mouse suggest that cartilage destruction by the inflamed and hyperplastic synovium requires the attachment and, thus, the direct contact of the synovial tissue with the cartilage. Also, the early loss of proteoglycans from the cartilage, which is most likely driven by the chondrocytes themselves, appears to constitute an important prerequisite for the pannus tissue to in- vade. This notion has been, among others, derived from studying the sequence of destructive events in the hTNFtg mouse. It was demonstrated in this study that the loss of cartilage components not only is an early event in destructive TNF-α-dependent arthritis, but that it facilitates the attachment of the inflamed synovial membrane to the cartilage matrix and, thus, initiates matrix degradation and

 inflammation through cell-matrix interactions. Also, experiments in which these hTNFtg mice were crossed with animals lacking Il-1, an important mediator of early proteoglycan loss, showed that the resulting hTNFtg/IL-1 knockout mice develop inflammatory arth- ritis with pannus formation. However, their cartilage is largely pro- tected, showing no significant loss of proteoglycans and also no attachment or invasion of the pannus tissue. Synovial membrane The synovial membrane is a second specific component of syn- ovial joints. Normally, it is only a few (i.e. about four to five) cell layers thick and consists mainly of fibroblast-like cells that through cell-cell contacts form a layer that lines up the synovial membrane against the joint cavity and the synovial fluid. The fibroblast-like synoviocytes have a key function in providing the joint cavity and the adjacent cartilage with lubricating molecules such as hyalur- onic acid, as well as with plasma-derived nutrients. Unlike other bordering membranes in our body, the synovial membrane has no basal membrane, which means it differs significantly from the clas- sical composition of an epithelium. Also, cellular contacts between the fibroblast-like synoviocytes lack tight junctions and desmo- somes, which together with the absent basal membrane on the one hand facilitates the efflux of serum components from the small ca- pillaries into the synovial space, but on the other hand also means that the most superficial layer of the synovial membrane, the intimal lining layer, has to take over these functions. In this context, the ad- hesion molecule cadherin-11 has been shown to mediate a strong homophilic adhesion between synoviocytes and to be largely re- sponsible for their organization into a tissue. Consequently, the lack of cadherin-11 in genetically modified mice results in greatly dis- turbed synovial architecture. This most superficial lining layer of the synovium can be separated histologically from the looser network of

fibroblast underneath: the sublining. In addition to the fibroblast- like synoviocytes, which are also termed type B synoviocytes, there are macrophages present in the synovial membrane, which are also called type A synoviocytes. In the normal synovium, these are few in number and mainly have phagocytic functions. In addition to providing the synovial cavity with nutrients and lubricating factors, production of ECM components and cross-linking these into the specific three-dimensional structure is the main function of the cells in the synovial membrane, mainly the fibroblast-like synoviocytes. This is an active process in which the fibroblasts have to migrate to sites of tissue remodelling and to interact with ECM molecules through specific surface receptors. Through these receptors, fibro- blasts have to sense changes in the structure and composition of the surrounding synovial tissues and they have to respond dynamically by adjusting the production and cross-linking of ECM components. Cell-matrix interactions For the interaction with ECM components, particularly collagens, integrin receptors are of special importance. They are heterodimeric molecules that consist of one α and one β chain, of which at least 16 different α and 8 different β chains have been identified so far. These can combine into at least 24 different integrins. Normally, fibroblast-like synoviocytes use their integrins receptors to sense and interact with ECM components within the synovial membrane, but under pathological conditions, they have been shown to mediate also the interaction with cartilage collagens and other cartilage com- ponents. For the attachment of synovial fibroblasts to ECM compo- nents of connective tissue and cartilage, β-1-integrins are especially important. It is important to emphasize that the engagement of in-tegrin receptors on the surface of fibroblast-like synoviocytes, just like on other cells, does not merely establish a cell-matrix contact but also leads to the formation of adhesive cell structures that are called focal adhesions. These focal adhesions are characterized by a specific rearrangement of the actin cytoskeleton as well as the re- cruitment of a variety of adaptor molecules. As a consequence of their formation, intracellular signalling cascades are being activated that regulate the transcription of genes and thus, control cell prolif- eration and survival, secretion of certain cytokines and chemokines, as well as matrix deposition and resorption. Among the molecules that transmit the signals from integrin clusters to the interior of fibroblast-like synoviocytes, the focal adhesion kinase (FAK) plays a central role. Focal adhesion kinase is a tyrosine kinase that is re- cruited into newly established focal contacts and in turn recruits other adaptor proteins such as p130Cas and Grb2. These then lead to the activation of PI3-kinase and Src-kinase and promote the ini- tiation of a variety of signalling cascades, such as the Raf-MEK-Erk pathway. However, many of these signalling pathways can also be activated by focal adhesion kinase-independent signalling mechan- isms, particularly through growth factors but also by cytokines and chemokines. The question of how the signals from different sources, including those from focal adhesions, are coordinated into a specific cellular response is a matter of intense research. The analysis of cell-matrix interactions of fibroblast-like synoviocytes is complicated by two observations. First, other and even more complex adhesive and invasive structures have been iden- tified and characterized over the past years and the question what role such podosome-like structures play in normal and pathological joint remodelling remains unclear. While classical podosomes are found mainly on macrophages and related cells, it has been re- ported that podosome-like structures such as invadopodia are also found on other cells such as fibroblasts, particularly when they are transformed. The second important observation has been that in addition to integrins, other cell surface molecules are involved in the attachment of fibroblasts to ECM components as well as in their response to growth factors and cytokines. Among those, the above-mentioned HSPGs and particularly syndecans have gained special interest recently. In the joint, syndecans are expressed not only on chondrocytes but also on fibroblasts. Data from

syndecan knockout mice have taught us important lessons on their general role in tissue remodelling and demonstrated that syndecan-4 is par- ticularly important for the response of fibroblasts to tissue injury. While syndecan-4 deficient mice show no major abnormalities, they exhibit alterations in wound healing when challenged in dif- ferent ways. Moreover, it could be demonstrated that the response of syndecan-4 deficient fibroblasts to fibronectin attachment is sig- nificantly altered and that syndecan-4 deficient fibroblasts have a re- duced ability to differentiate into α-smooth muscle actin (a-SMA) positive myofibroblasts. The exact mechanisms by which syndecans are involved in the specific functions of different fibroblast popu- lations remain to be determined, but it appears that syndecans are

section 19 Rheumatological disorders 4384 important cell surface receptors that due to their unique properties integrate signals from ECM components and soluble factors. In this context, most recent data have demonstrated that fibroblast-like synoviocytes are regulated in their specific response by proteogly- cans, and this mechanism has been termed ‘proteoglycan switch’. Specifically, it was shown that RPTPσ, a transmembrane tyrosine phosphatase, is reciprocally regulated by interactions with chondro- itin sulphate or heparan sulphate containing extracellular proteo- glycans and that this proteoglycan switch regulates fibroblast-like synoviocyte function This may be particularly important under con- ditions such as arthritis, in which either cell-anchored proteoglycans get overexpressed or in which fibroblast-like synoviocytes are ex- posed to such proteoglycans. In line with this notion, administration of a RPTPσ decoy protein ameliorated the severity of rheumatoid arthritis-like disease in the K/BxN serum transfer model. Degradation of extracellular matrix The fibroblasts of the synovial membrane not only are responsible for the deposition of ECM molecules and their assembly, but they are also involved prominently in their destruction and removal. For the degradation of ECM components, fibroblast-like synoviocytes produce a diversity of matrix-degrading enzyme. These include members of the metalloproteinase family, including MMPs and ADAMTS, but also different cathepsins. Most MMPs are not ex- pressed constitutively but only upon induction by specific stimuli such as cytokines, growth factors, or ECM molecules. The most prominent exceptions are MMP-2 and MT-MMPs, which are also regulated by these factors but expressed constitutively at significant levels. Among the cytokines, IL-1 is perhaps the most potent inducer of a variety of MMPs and has been shown to strongly regulate the expression of MMP-1, -3, -8, -13, and -14. Growth factors such as FGF and platelet-derived growth factor (PDGF) can also induce MMPs in fibroblasts and, thus, act synergistically with cytokines such as IL-1 on MMP expression. The third group of MMP inducers are matrix proteins (collagen, fibronectin), and especially their deg- radation products activate MMP expression in fibroblasts, providing the possibility for a site-specific MMP activation in regions of matrix breakdown. There are several intracellular signalling pathways that are responsible for the transcriptional activation of MMPs. Thus, bindings sites for the activator protein-1 (AP-1) are present in the promoters of nearly all MMPs, suggesting that the transcription factors jun/fos transcription contribute prominently to the regula- tion of these enzymes. Indeed, there is ample evidence that all three mitogen/stress-activated protein kinase (MAPK/SAPK) families, ERK, JNK, and p38 kinase, are involved in the regulation of MMP expression by integrating signals upstream of jun/fos. This has been shown particularly for MMP-1, -9, and -13. Biochemistry and function in arthritis The composition, molecular pattern, and also the biochemistry of the synovial membrane changes dramatically during inflam- mation. This is true for acute inflammatory episodes where the accumulation of inflammatory cells with subsequent stimulation of the local mesenchyme occurs for a limited period of time, but even more so for chronic inflammation. The mechanisms that

regulate the switch from acute to chronic inflammation within the synovium are not very well understood, but it has been shown that fibroblast-like synoviocytes contribute significantly to this process. This has become particularly evident for rheuma- toid arthritis, where these cells are a key part of the local im- mune system in the joints and integrate signals form different sources into a pathological tissue response (Fig. 19.1.3). While responding to the stimuli in the chronically inflamed synovium, rheumatoid arthritis fibroblast-like synoviocytes undergo fun- damental changes, and multiple lines of evidence suggest that these result in a stable activation, which is maintained even in the absence of continuous stimulation by inflammatory triggers. As a consequence of this stable fibroblast-like synoviocyte ac- tivation, the disease process is perpetuated and might progress when inflammation ameliorates. The underlying mechanisms are not entirely clear, but the chronic exposure of fibroblast-like synoviocytes to inflammatory cytokines, growth factors, and the ECM appears to result in the imprinting of an aggressive pheno- type. To this end, they not only express a variety of cytokine re- ceptors but also toll-like receptors (TLRs) through which they can react to damage-associated molecular pattern (DAMP) and Fc- receptors to respond to immunglobulins. Most of the cytokines that synovial fibroblasts respond to are derived from invading inflammatory cells such as macrophages or lymphocytes. TNF-α, IL-1, and interferon-γ are prominent ex- amples, and a critical role for these inflammatory cytokines in cel- lular activation is supported by in vivo animal models of rheumatoid arthritis in which the constitutive overexpression of inflammatory cytokines results in a RA-like disease. One most prominent example is the human TNF-α transgenic (hTNFtg) mouse. On the other hand, the activation of fibroblast-like synoviocytes also results in a vicious circle in which the activated fibroblasts themselves pro- duce inflammatory factors including cytokines, growth factors, and lipid mediators that act in an autocrine and paracrine fashion to Chemokines Growth factors (Pre-damaged) ECM Synovial fibroblasts Transformed appearing RA synovial fibroblasts Stable imprinting (epigenetic changes) Cytokines Fig. 19.1.3 Fibroblast-mediated cartilage destruction in rheumatoid arthritis. Under normal conditions, the physiological function of synovial fibroblasts is to build a lining membrane that secretes lubricants, provides the joint cavity and the adjacent cartilage with nutritive plasma proteins, and to be involved in matrix remodelling. In rheumatoid inflammation, cellular alterations following activation through inflammatory stimuli, chemokines, and growth factors result in a tumour-like transformation of these cells, which exhibit a special phenotype with unique features such as reduced susceptibility to apoptosis, expression of cartilage adhesion molecules, and the increased production of matrix-degrading enzymes. The mechanisms that imprint these changes into transformed-looking fibroblasts are not entirely clear, but epigenetic changes have been shown to constitute an important factor in this process.

 further promote inflammation, angiogenesis, chemoattraction, and misbalanced tissue homeostasis in a vicious circle. In rheumatoid arthritis, invading B cells also promote the acti- vation of synovial fibroblasts through immunoglobulin G (IgG) binding to the high-affinity FcgammaR (FcgammaR I) on their sur- face. Through this interaction the production of IL-16 and RANTES (chemokine (C-C motif) ligand 5) is increased, which in turn at- tracts T cells. It has also been shown that in rheumatoid arthritis, the direct interaction between fibroblast-like synoviocytes and inflam- matory cells through CD40L (CD154) with CD40 contributes to the disease process. Of note, synovial fibroblasts are not only stimulated by inflam- matory and immune cells and their mediators, but in turn also con- tribute to the accumulation of these cells. Thus, the influx of CD4+ T cells into the proliferating synovium is enhanced by rheumatoid arthritis synovial fibroblasts due to their production of CXCL16 and IL-16. Rheumatoid arthritis fibroblast-like synoviocytes are also an important source of cytokines with IL-

2-like activity, IL- 15, and IL-7, in rheumatoid arthritis joints. Rheumatoid arthritis synovial fibroblasts have been shown to contribute to the attrac- tion and accumulation of B cells in inflamed tissue. For example, the secretion of SDF-1 by synovial fibroblasts has been shown to facilitate B cell migration and activation to the synovium. In summary, the interaction between mesenchymal, immune, and inflammatory cells is mediated by a tightly regulated, extremely complex network of cytokines that results in the maintenance of inflammation. FURTHER READING Archer CW, Dowthwaite GP, Francis-West P (2003). Development of synovial joints. Birth Defects Res C Embryo Today, 69, 144–55. DeLise AM, Fischer L, Tuan RS (2000). Cellular interactions and signaling in cartilage development. Osteoarthritis Cartilage, 8, 309–34. Eyre D (2002). Collagen of articular cartilage. Arthritis Res, 4, 30–5. Fuerst M, et al. (2009). Calcification of articular cartilage in human osteoarthritis. Arthritis Rheum, 60, 2694–703. Hunziker EB, Michel M, Studer D (1997). Ultrastructure of adult human articular cartilage matrix after cryotechnical processing. Microsc Res Tech, 37, 271–84. Knudson CB, Knudson W (2001). Cartilage proteoglycans. Semin Cell Dev Biol, 12, 69–78. Korb-Pap A, et al. (2012). Early structural changes in cartilage and bone are required for the attachment and invasion of inflamed syn- ovial tissue during destructive inflammatory arthritis. Ann Rheum Dis, 71, 1004–11. Mundlos S, Olsen BR (1997). Heritable diseases of the skeleton: part I: molecular insights into skeletal development-transcription factors and signaling pathways. Faseb J, 11, 125–32. Pearle AD, Warren RF, Rodeo SA (2005). Basic science of articular car- tilage and osteoarthritis. Clin Sports Med, 24, 1–12. Poole AR, et al. (2001). Composition and structure of articular car- tilage: a template for tissue repair. Clin Orthop Relat Res, (391 Suppl), S26–33. Zwerina J, et al. (2007). TNF-induced structural joint damage is medi- ated by IL-1. Proc Natl Acad Sci U S A, 104, 11742–7.

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