Review
Fibroblast biology
Signals targeting the synovial fibroblast in arthritis

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Abstract

Fibroblast-like cells in the synovial lining (type B lining cells), stroma and pannus tissue are targeted by many signals, such as the following: ligands binding to cell surface receptors; lipid soluble, small molecular weight mediators (eg nitric oxide [NO], prostaglandins, carbon monoxide); extracellular matrix (ECM)–cell interactions; and direct cell–cell contacts, including gap junctional intercellular communication. Joints are subjected to cyclic mechanical loading and shear forces. Adherence and mechanical forces affect fibroblasts via the ECM (including the hyaluronan fluid phase matrix) and the pericellular matrix (eg extracellular matrix metalloproteinase inducer [EMMPRIN]) matrices, thus modulating fibroblast migration, adherence, proliferation, programmed cell death (including anoikis), synthesis or degradation of ECM, and production of various cytokines and other mediators [1]. Aggressive, transformed or transfected mesenchymal cells containing proto-oncogenes can act in the absence of lymphocytes, but whether these cells represent regressed fibroblasts, chondrocytes or bone marrow stem cells is unclear.

Keywords: fibroblast, rheumatoid arthritis, synovial membrane

Soluble mediators binding to cell surface receptors

Cytokine network and signal transduction

Cytokines bind to their receptors, activating signal transduction pathways such as adenylate cyclase/cAMP, phospholipase C/inositol trisphosphate, and Ca^{2+} and tyrosine kinases. Cytokines can stimulate random migration (chemokinesis), guided fibroblast migration along a concentration gradient (chemokinesis; Table 1) [2–15] and/or fibroblast proliferation (Table 2) [16–25]. Regulation of fibroblast migration and proliferation is not straightforward; the effect may be indirect or dependent on concentration and the cytokine network. Some cytokines act as competence rather than progression factors, some lack secretory signals, and some must be processed and released from the pericellular matrix or basement membranes (eg transforming growth factor beta [TGF-β] binding to chondroitin or the keratan sulfate of biglycan, decorin and fibromod-

COX = cyclooxygenase; ECM = extracellular matrix; EMMPRIN = extracellular matrix metalloproteinase inducer; HO = hemeoxygenase; IFN-γ = interferon gamma; IL = interleukin; iNOS = inducible nitric oxide synthase; MMP = matrix metalloproteinase; NADPH = nicotinamide adenine dinucleotide phosphate; NO = nitric oxide; NOS = nitric oxide synthase; TGF-β = transforming growth factor beta; TIMP = tissue inhibitor of metalloproteinase; TNF-α = tumor necrosis factor alpha.

Table 1

Soluble mediators regulating fibroblast migration

Effect	Factor	Cellular or tissue source	Reference
+	TNF-α	Macrophage, activated monocyte, B cell, T cell, fibroblast	[2]
+	IL-4	T cell, mast cell, bone marrow stromal cell	[3]
+	PDGF-AA, -BB, -AB	Platelet, macrophage, endothelial cell, skeletal muscle cell, fibroblast, vascular smooth muscle cell, glial cell, type I astrocyte, myoblast, kidney, epithelial cell, mesangial cell	[3]
+	TGF-β	Platelet, macrophage, T cell, skeletal muscle cell, fibroblast	[4]
+	bFGF	Brain, retina, bone matrix, endothelial cell, macrophage	[3]
+	EGF	Granulocyte, ectodermal cell, kidney, duodenal gland, platelet	[5]
+	Neurokinin A	Nerve cell	[3]
+	CGRP	Nerve cell	[6]
+	Endothelin-1 and -3	Endothelial cell, macrophage, fibroblast, many other cells	[3]
+	β-thromboglobulin	Platelet, megakaryocyte	[1]
+	Platelet factor 4	Platelet, megakaryocyte	[1]
+	LTB_4	Myeloid cells, from transported LTA_4 in many nonmyeloid and nonhematopoietic cells	[3]
+	IGF-I (SmC)	Fibroblast, skeletal cell, liver, endothelial cell, T cell	[7]
+	IGF-II (MSA)	Liver	[3]
+	Matrix proteins		
	Collagen	ECM	[8]
	Fibronectin	ECM	[9]
	Elastin	ECM	[10]
+	Serum derived chemotactic factor for fibroblasts	Complement (C_5)	[11,12]
–	Interferon	T lymphocyte, NK cell (interferon-γ), all cells (interferon-α)	[13]
–	Retinoids		[14]
–	Neutrophil factor	Neutrophil	[15]

+, Stimulation; –, inhibition. bFGF, basic fibroblast growth factor; CGRP, calcitonin gene-related peptide; ECM, extracellular matrix; EGF, epidermal growth factor; IGF, insulin-like growth factor; IL, interleukin; LTA, leukotriene A; LTB, leukotriene B; NK, natural killer; PDGF, platelet derived growth factor; TGF, transforming growth factor; TNF, tumor necrosis factor.

ulin, or basic fibroblast growth factor and platelet derived growth factor binding to the heparin sulfate of glypican, perlecan and syndecan).

Matrix deposition
The TGF-β family forms an important group of growth factors, consisting of three isoforms in man, and is important for matrix deposition because it modulates fibroblast recruitment and proliferation. This growth factor also stimulates production of collagens, proteoglycans, elastin, fibronectin, tenascin and thrombospondin, diminishes production of extracellularly active neutral endoproteinases belonging to the matrix metalloproteinase (MMP) and serine proteinase families, and stimulates production of endogenous MMP inhibitors (tissue inhibitor of metalloproteinase [TIMP]) and serpins (plasminogen activator inhibitor-1). Other profibrotic, collagen synthesis stimulat-

ing cytokines include endothelin, interleukin (IL)-1 and mast cell tryptase. Interferons and IL-4 decrease collagen synthesis. In addition to IL-4, 'biologicals' such as humanized anti-TGF-β antibodies and recombinant human interferons are, accordingly, being tested as a treatment for fibrotic diseases.

Matrix degradation
Fibroblasts produce proteolytic enzymes (in particular, MMPs). MMPs now comprise a group of 18 different enzymes in man, including the classic fibroblast collagenase MMP-1 (collagenase-1), the mesenchymal form of MMP-8 (collagenase-2) and MMP-13 (collagenase-3). MMP-8 was known as neutrophil collagenase until it was found to be produced by tumor necrosis factor α (TNF-α) stimulated fibroblasts, for example, although in a less glycosylated form (50 kDa instead of 75 kDa) [26]. Co-

Table 2

Soluble mediators regulating fibroblast proliferation

Effect	Factors	Examples of cellular and tissue source	Reference
+	AMDGF	Alveolar macrophage	[16]
+	aFGF, bFGF	Brain, retina, bone matrix, endothelial cells, macrophage	[17]
+	CTAP-III	Platelet	
+	CTAP-V	Platelet	
+	CTAP-PMN	PMN	
+	EGF and TGF-α	Granulocyte, ectodermal cells, kidney, duodenal gland, platelet	[18]
−	Interferon-γ	T lymphocyte, NK cell	[19]
+	IGF-I (SmC)	Fibroblast, skeletal cell, liver, endothelial cell, T cell	[20]
+	IGF-II (MSA)	Liver	[21]
+	IL-1α and IL-β	Monocyte/macrophage, Langerhans cell, other dendritic cells, T lymphocyte, B lymphocyte, NK cell, large granular lymphocyte, vascular endothelial cell, smooth muscle cell, fibroblast, thymic epithelial cell, astrocyte, microglia, keratinocyte, chondrocyte	
+	IL-1 inhibitor	Monocyte	[22]
+	PDGF-AA, PDGF-BB, PDGF-AB	Platelet, macrophage, endothelial cell, fibroblasts, vascular smooth cells, glial cell, type I astrocyte, kidney, epithelial cell, mesangial cells	
+	TCDGF	T cell	[23]
+	TGF-β	Platelets, macrophage, T cell, skeletal muscle cell, fibroblast	[24]
+	TNF-β	Lymphocyte	[25]

+, Stimulation; −, inhibition. aFGF, acidic fibroblast growth factor; AMDGF, alveolar macrophage-derived growth factor; bFGF, basic fibroblast growth factor; CTAP, connective tissue-activating peptide; EGF, epidermal growth factor; IGF, insulin-like growth factor; IL, interleukin; NK, natural killer; PDGF, platelet derived growth factor; PMN, polymorphonuclear cell; TCDGF, T cell derived growth factor; TGF, transforming growth factor; TNF, tumor necrosis factor.

localization of TNF and its receptors in synovial tissue and at the cartilage–pannus junction may play a role in the pathogenesis of rheumatoid arthritis [27]. Fibroblasts produce TIMPs (1–4), which were previously called human fibroblast collagenase inhibitors. TIMP-1 is induced by inflammatory cytokines IL-1 and TNF-α, but also by TGF-β, progesterone and estrogen. IL-6, interestingly, does not seem to stimulate the production of collagenase, but is a potent inducer of TIMP-1.

Lipid soluble mediators penetrating the cell membrane

NO is a freely diffusible radical gas, which is a product of the catalytic conversion of L-arginine to L-citrulline by nitric oxide synthases (NOS) (EC 1.14.13.39) via the chemical reaction between the guanidino-nitrogen of L-arginine and dioxygen. The activity of the inducible NO synthase (iNOS) requires pro-inflammatory cytokines such as IL-1 and TNF-α for upregulation of mRNA and protein. The activity of iNOS, in turn, is under strict control of nicotinamide adenine dinucleotide phosphate (NADPH), flavine adenidine dinucleotide, flavin mononucleotide, heme and 5,6,7,8-tetrahydrobiopterin for activity [28]. The iNOS is highly expressed in the rheumatoid synovium, particularly in synovial fibroblasts [29,30]. The mRNA initiation site of the iNOS gene is preceded by a promoter sequence box, along with two distinct regions upstream containing consensus sequences for the binding of various transcription factors. Region 1 contains lipopolysaccharide responsive elements such as the binding sites for nuclear factor-1, IL-6 and NF-κB, indicating a locus for LPS induced synthesis of iNOS. Region 2 contains motifs for interferon gamma (IFN-γ)-regulated transcription factors but does not directly regulate induction of iNOS; instead, it subserves region 1. LPS therefore stimulates iNOS synthesis directly, and IFN-γ acts in synergy with LPS to augment iNOS synthesis and NO production. This synergy also extends to the cytokines IL-1 and TNF-α, which, in combination with IFN-γ, augment the synthesis of iNOS and NO [31]. Apoptosis induced by NO is associated with nuclear p53 protein expression in cultured human fibroblasts [32]. NO can also induce the synthesis and activity of cyclooxygenase (COX)-2 and hemeoxygenase (HO)-1.

Prostaglandin H_2 (PGH_2) synthase (EC 1.14.99.1) has two activities, COX and peroxidase, and occurs in two isoforms, known as COX-1 and COX-2 [33]. The inducible COX-2 mRNA and protein are stimulated largely by the

same factors as iNOS, such as IL-1, TNF-α and IFN-γ [34]. The promoter region of COX-2 contains binding sites for NF-κB, NF-IL6 and two motifs for IFN-γ activated sequences [34]. PGE_2 and PGE_1 inhibit cytokine-induced metalloproteinase expression in human synovial fibroblasts [35]. COX inhibition conversely enhances the production of pro-MMP-1 in human rheumatoid synovial fibroblasts [36]. PGE_2 also enhances the synthesis of IL-8 and IL-6, but inhibits granulocyte–macrophage colony-stimulating factor production by IL-1 stimulated synovial fibroblasts [37].

Carbon monoxide is produced by two homologous microsomal HO (EC 1.14.93) isoenzymes: inducible HO-1 (heat shock protein-32) and constitutively expressed HO-2. The latter is widely expressed in fibroblasts, and HO-1 can be induced in these and other cell types by hypoxia and free radicals [38]. HO-1 prevents cell death by regulating intracellular iron levels. HO functions by cleaving heme to biliverdin and carbon monoxide, in the presence of NADPH and NADPH-cytochrome P_{450}, with equimolar iron released from the heme as a co-product [39]. There is a regulatory loop between iron metabolism and the NO pathway: intracellular ferric iron (Fe^{3+}) levels can significantly decrease iNOS mRNA transcription, and iron chelating agents like desferrioxamine can increase iNOS transcription and NO production [40]. NO itself, conversely, can directly control intracellular iron metabolism by activating the iron-regulatory protein involved in ferritin translocation. Therefore, the interplay between iNOS and HO-1 activities may have far-reaching consequences in situations characterized by oxidative stress.

Extracellular matrix, and integrin and nonintegrin receptors

The ECM–cell interactions are coupled to cytoskeletal elements, such as α-actinin, talin and tensin, and affect various tyrosine kinases, for example focal adhesion kinases, Src (the protein product of the *src* gene of the Rous sarcoma virus) family kinases and Crk (the protein product of the *crk* gene from chicken retroviruses CT10 and ASV-1). Focal adhesion kinases provide a potent anoikis resistance factor [41], anoikis referring to apoptosis caused by loss of ECM–cell adhesion. ECM–fibroblast interactions are important because the synovial lining cells and the pannus are subjected to shear stress. Synovial cells are also subjected to cyclic mechanical loading during the movement of the joint.

The synovial ECM provides hydraulic resistance, preventing rapid seepage of synovial fluid out of the joint cavity, and modifies the traffic of macromolecules. It may trap antigens, which contribute to inflammation. The three main classes of synovial structural polymers are collagen (scaffolding), extrafibrillar glycosaminoglycans/proteoglycans and structural glycoproteins. Under normal circumstances, collagen is hidden in a matrix created by the latter two

classes. Fibronectin guides fibroblast migration as an immobilized substrate and attractant in the leading edge of the pannus (haptotaxis) [42]. The extra domain-A fibronectin isoform is associated with the activated, transformed state of type B lining cells [43]. The interaction between connecting sequence-1 fibronectin (or vascular cell adhesion molecule-1) and $\alpha 4\beta 1$ (very late activation antigen-4) may play a role in the proliferation of synovial lining and lymphocyte migration [44]. EMMPRIN ($M_r \approx 58\,000$) is an integral plasma membrane glycoprotein of the pericellular matrix belonging to the immunoglobulin superfamily, previously referred to as tumor-cell derived collagenase stimulatory factor. It is identical to the M6 leukocyte activation antigen, and highly homologous to rat OX-47 or CE9, mouse basigin or gp42, and chicken HT-7 or neurothelin molecules. Reciprocal immunoprecipitation, cell surface crosslinking and immunofluorescence co-localization experiments demonstrated that EMMPRIN can form a complex with integrins $\alpha 3\beta 1$ and $\alpha 6\beta 1$, which may play a role in the synovial membrane [45]. Many other ECM–fibroblast interactions are of potential relevance in the synovial membrane (Table 3) [46–61].

The most important receptor family binding and responding to ECM is formed by integrins, which are heterodimeric molecules comprising, to date, 16 alpha and 8 beta subunits. The β_1 integrins bind collagens, laminins, entactin/nidogen, fibronectin, tenascin and vascular cell adhesion molecule-1, whereas β_2 integrins are mainly expressed in blood leukocytes and perform a role in both immune inflammation, and in heterotypic interactions of fibroblasts with other cells. The α_V integrins mediate adhesion to provisional matrix molecules, such as fibrinogen, fibronectin, vitronectin, thrombospondin and osteopontin. In addition to these three major subclasses, $\alpha_6\beta_4$ integrin, as a component of hemidesmosomes, forms a receptor for laminin-5 and laminin-10. The $\alpha_{IIb}\beta_3$ and $\alpha_4\beta_7$ integrins, as well as $\alpha_E\beta_7$ integrin, perform roles in platelet function and vascular adhesion, respectively [41]. Integrin subunits α_3, α_4, α_5, α_6, α_V and β_1 are overexpressed in synovitis [48]. Nonintegrin receptors, such as CD44, binding hyaluronan, and also other ligands (such as collagens I and VI), are of importance in this respect. CD44–hyaluronan interaction modulates the migration of inflammatory leukocytes into the extravascular compartment of the synovial membrane.

Cell–cell interactions

Direct cell–cell interactions are typical for epithelial cells, but direct cell–cell contacts have been considered rare in connective tissue. Connective tissue cells such as fibroblasts were thought to be regulated not only by soluble factors, but also by effects resulting from ECM–fibroblast interactions. However, time-lapse cinephotomicrography and light and electron microscopy have been used to show close physical apposition and adhesion between fibroblasts and other cells. This adhesion is not only a

Table 3

Interactions between extracellular matrix (ECM) proteins and fibroblast-like synoviocytes (FLS)

ECM	Effect	Reference
Laminin (Ln)	Ln is synthesized in rat and human FLS, and is involved in FLS adhesion	[46]
	FLS adhesion to Ln shows enhanced proliferative ability in response to PDGF	[47]
	RA-FLS bind to Ln more strongly than normal FLS, with monoclonal antibodies to integrin $\alpha 3$, $\alpha 6$, $\beta 1$ subunits partly blocking this adhesion	[48]
Fibronectin (Fn)	FLS plated on the substrate containing Fn show extensive focus formation, and enhanced adhesion and proliferation	[49]
	CS-1 Fn correlates with FLS proliferation	[50]
	ED-A Fn is associated with activation of FLS	[44]
	FLS adhering to Fn show higher proliferative ability in response to PDGF	[43]
	Adhesion to Fn through integrin $\alpha 5\beta 1$ downregulates the collagenase expression in human FLS	[47]
	RA-FLS bind more strongly to Fn than normal FLS; anti-$\alpha 5$, or $\beta 1$ monoclonal antibodies block the adhesion	[48]
	Rabbit FLS cultured on the substrate containing Fn fragment show upregulated expression of procollagenase and prostromelysin	[51]
Vitronectin (Vn)	FLS adhering to Vn shows higher proliferative ability in response to PDGF. Adhesion to Vn through integrin αv downregulates collagenase expression in human FLS	[47]
Tenascin (Tn)	FLS synthesize Tn	[52]
	RA-FLS bind more strongly to Tn than normal FLS; monoclonal antibodies to integrin $\beta 1$ block adhesion	[48]
	Rabbit FLS cultured on Tn/Fn mixed substrate show increased expression of collagenase, stromelysin, the 92 kDa gelatinase, and c-*fos*	[53]
Hyaluronan (HA)	Synthesized by FLS, degraded by macrophage-like lining cells	[54]
	HA inhibits proliferation of FLS	[55]
Decorin	Modulates MMP-1 gene expression of rabbit FLS when present on the substrate with Vn or Fn fragment	[56]
Perlecan	Involved in adhesion and growth of FLS	[57]
Collagen type I	FLS adhering to collagen type I show higher proliferative ability in response to PDGF. Adhesion to collagen type I through integrin $\beta 1$ downregulates the collagenase expression in human FLS	[47]
Collagen type IV	Synthesized by FLS	[58]
	RA-FLS bind more strongly to collagen type IV than normal FLS	[48]
	monoclonal antibodies to integrin $\beta 1$ block adhesion	[59]
	Degraded by MMP-2, MT-MMP	[60]
	Degraded by matrilysin	[61]

CS-1, connecting sequence 1; ED-A, extra domain-A; MMP, matrix metalloproteinase; MT, membrane type; PDGF, platelet derived growth factor; RA, rheumatoid arthritis.

passive event, but can affect one or both of the interacting cells. Such events have been proven to be dependent on cell–cell contact by the lack of effect of cell culture supernatant (ie in the physical absence of one of the interacting cells). Similar conclusions have been drawn based on the inhibition of the observed effect upon use of physical barriers between the interacting cells (eg their separation by membranes). This abolishes cellular events dependent on cell–cell interaction. Many of these heterotypic interactions are dependent on the β_2 (CD18) integrins, shown by the use of blocking antibodies. Adherens junctions have been reported between fibroblasts. Another relatively new and unexpected finding is that gap junctions are present in fibroblasts. Built up from transmembrane proteins, connexons, gap junctions allow the spread of small molecular second messengers like Ca^{2+} and cAMP from one cell to another. Transfection of fibroblasts with the 'receptor for hyaluronic acid-mediated motility' regulates gap junctional intercellular communication and connexin-43 expression, affecting focal adhesion and cytoskeleton organization, with various secondary effects on motility, growth and transformation (Table 4) [62–73].

Table 4

Direct cell–cell interactions between fibroblasts and other cell types

Cell type	Effect	Reference
Macrophage	Direct transfer (of FITC-dextran, mannose BSA gold) from macrophages to fibroblasts	[62,63]
Neutrophil	Neutrophil adhesion to fibroblasts is increased by PMA treatment of neutrophils and by IL-1α or TNF-α treatment of fibroblasts	[64]
	Fibroblasts provide directional guidance to adhering neutrophils	[65]
	PAF and IL-8 enhance neutrophil adhesion to and motility of adhered neutrophils along fibroblasts, respectively, in an integrin β_2 dependent process	[66]
Lymphocyte	Fibroblasts synthesize IL-1α, IL-1β, IL-6 and ICE	[67]
	Fibroblast mediated synthesis of collagen type I and type III is decreased	[68]
Eosinophil	Activated eosinophils adhere to fibroblasts: this adhesion is inhibited with RGDS	[69]
Mast cell	Formation of mast pseudopods and their translocation to fibroblast surface	[70]
	Mast cell stimulates fibroblast proliferation after cell–cell contact in an IL-4 dependent manner	[71]
	Gap junctions between the mast cell and fibroblast are possible	[72]
Osteoblast-like cells	Osteoblast-like cells stimulate fibroblast proliferation (regulation of osteoprogenitor cell proliferation?)	[73]

BSA, bovine serum albumen; ICE, interleukin-1β-converting enzyme; IL, interleukin; FITC, fluorescein isothiocyanate; PAF, platelet-activating factor; PMA, phorbol myristate acetate; RGDS, arginyl-glycyl-aspartyl-serine; TNF, tumor necrosis factor.

Conclusion

It has been claimed that the rheumatoid arthritis synovial fibroblasts differ from their nonrheumatoid counterparts in terms of growth rate, life span, glycolytic metabolism, synthesis of hyaluronan and sulfated glycosaminoglycans, acid hydrolase activities, and metabolic and structural mitochondrial proteins [1]. The rheumatoid fibroblasts show a sustained and distinct morphology and pattern of gene activation [74,75], and might represent nonrheumatoid fibroblasts, but might also be phenotypically altered chondrocytes or bone marrow derived stem cells. These differences between the normal and the inflammatory synovium may be due to a selection pressure in the synovial mileau, where water- and lipid-soluble stimuli, cyclic loading, shear stress, ECM contacts and direct cell–cell contacts more or less permanently modulate the phenotype and function of fibroblast-like cells in the synovial lining, stroma and pannus.

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