Review

Fibroblast biology
Signals targeting the synovial fibroblast in arthritis

Yrjö T Konttinen*†‡, Tian-Fang Li*‡, Mika Hukkanen*, Jian Ma*†, Jing-Wen Xu*‡ and Ismo Virtanen*

*Institute of Biomedicine and †Institute of Dentistry, University of Helsinki, and ‡Surgical Hospital, Helsinki, Finland

Received: 24 February 2000
Revisions requested: 14 March 2000
Revisions received: 5 April 2000
Accepted: 27 April 2000
Published: 8 June 2000

Arthritis Res 2000, **2**:348–355

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 stimulating 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 α4β1 (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 α3β1 and α6β1, 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 α3, α6, β1 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 α5β1 downregulates the collagenase expression in human FLS	[47]
	RA-FLS bind more strongly to Fn than normal FLS; anti-α5, or β1 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 β1 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 β1 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 β1 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 messagers 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.

References

1. Konttinen YT, Saari H, Santavirta S, Antti-Poika I, Sorsa T, Nykanen P, Kemppinen P: **Synovial fibroblasts.** *Scand J Rheumatol* 1988, **76 (suppl)**:95–103.
2. Springer TA: **Adhesion receptors of the immune system.** *Nature* 1990, **346**:425–434.
3. Callard RE, Gearing AJH: *The Cytokine Facts*. London: Academic Press; 1994.
4. Ohgoda O, Sakai A, Koga H, Kanai K, Miyazaki T, Niwano Y: **Fibroblast-migration in a wound model of ascorbic acid-supplemented three-dimensional culture system: the effects of cytokines and malotilate, a new wound healing stimulant, on cell-migration.** *J Dermatol Sci* 1998, **17**:123–131.
5. Ware MF, Wells A, Lauffenburger DA: **Epidermal growth factor alters fibroblast migration speed and directional persistence reciprocally and in a matrix-dependent manner.** *J Cell Sci* 1998, **111**: 2423–2432.
6. Yule KA, White SR: **Migration of 3T3 and lung fibroblasts in response to calcitonin gene-related peptide and bombesin.** *Exp Lung Res* 1999, **25**:261–273.
7. Andresen JL, Ehlers N: **Chemotaxis of human keratocytes is increased by platelet-derived growth factor-BB, epidermal growth factor, transforming growth factor-alpha, acidic fibroblast growth factor, insulin-like growth factor-I, and transforming growth factor-beta.** *Curr Eye Res* 1998, **17**:79–87.
8. Postlethwaite AE, Seyer JM, Kang AH: **Chemotactic attraction of human fibroblasts to type I, II, and III collagens and collagen-derived peptides.** *Proc Natl Acad Sci USA* 1978, **75**:871–875.
9. Dean JW III, Blankenship JA: **Migration of gingival fibroblasts on fibronectin and laminin.** *J Periodontol* 1997, **68**:750–757.
10. Senior RM, Griffin GL, Mecham RP, Wrenn DS, Prasad KU, Urry DW: **Val-Gly-Val-Ala-Pro-Gly, a repeating peptide in elastin, is chemotactic for fibroblasts and monocytes.** *J Cell Biol* 1984, **99**:870–874.
11. Postlethwaite AE, Snyderman R, Kang AH: **Generation of a fibroblast chemotactic factor in serum by activation of complement.** *J Clin Invest* 1979, **64**:1379–1385.
12. Kondo H, Yonezawa Y, Ito H: **Interferon-beta, an autocrine cytokine, suppresses human fetal skin fibroblast migration into a denuded area in a cell monolayer but is not involved in the age-related decline of cell migration.** *Mech Ageing Dev* 1996, **87**:141–153.
13. Adelmann-Grill BC, Hein R, Wach F, Krieg T: **Inhibition of fibroblast chemotaxis by recombinant human interferon gamma and interferon alpha.** *J Cell Physiol* 1987, **130**:270–275.
14. Hein R, Mensing H, Muller PK, Braun-Falco O, Krieg T: **Effect of vitamin A and its derivatives on collagen production and chemotactic response of fibroblasts.** *Br J Dermatol* 1984, **111**:37–44.
15. Mensing H, Czarnetzki BM: **Generation and characterization of a neutrophil-derived inhibitor of fibroblast chemotaxis.** *Arch Dermatol Res* 1986, **278**:184–189.
16. Bitterman PB, Wewers MD, Rennard SI, Adelberg S, Crystal RG: **Modulation of alveolar macrophage-driven fibroblast proliferation by alternative macrophage mediators.** *J Clin Invest* 1986, **77**:700–708.
17. Basilico C, Moscatelli D: **The FGF family of growth factors and oncogenes.** *Adv Cancer Res* 1992, **59**:115–165.

18. Brinckerhoff CE: **Morphologic and mitogenic responses of rabbit synovial fibroblasts to transforming growth factor beta require transforming growth factor alpha or epidermal growth factor.** *Arthritis Rheum* 1983, **26**:1370–1379.
19. Denk PO, Knorr M: **Effect of heparin on human corneal fibroblast proliferation *in vitro* with and without growth factor stimulation.** *Graefes Arch Clin Exp Ophthalmol* 1999, **237**:342–347.
20. Ludwig CU, Menke A, Adler G, Lutz MP: **Fibroblasts stimulate acinar cell proliferation through IGF-I during regeneration from acute pancreatitis.** *Am J Phys* 1999, **276**:193–198.
21. Kratz G, Lake M, Ljungstrom K, Forsberg G, Haegerstrand A, Gidlund M: **Effect of recombinant IGF binding protein-1 on primary cultures of human keratinocytes and fibroblasts: selective enhancement of IGF-1 but not IGF-2-induced cell proliferation.** *Exp Cell Res* 1992, **202**:381–385.
22. Gitter BD, Koehneke EM: **Retinoic acid potentiates interleukin-1- and fibroblast growth factor-induced human synovial fibroblast proliferation.** *Clin Immunol Immunopathol* 1991, **61**:191–120.
23. Haynes JH, Johnson DE, Mast BA, Diegelmann RF, Salzberg DA, Cohen IK, Krummel TM: **Platelet-derived growth factor induces fetal wound fibrosis.** *J Pediatr Surg* 1994, **29**:1405–1408.
24. Jutley JK, Wood EJ, Cunliffe WJ: **Influence of retinoic acid and TGF-beta on dermal fibroblast proliferation and collagen production in monolayer cultures and dermal equivalents.** *Matrix* 1993, **13**:235–241.
25. Yu F, Itoyama Y, Kira J, Fujihara K, Kobayashi T, Kitamoto T, Suzumura A, Yamamoto N, Nakajima Y, Goto I: **TNF-beta produced by human T lymphotropic virus type I-infected cells influences the proliferation of human endothelial cells and fibroblasts.** *J Immunol* 1994, **152**:5930–5938.
26. Hanemaaijer R, Sorsa T, Konttinen YT, Ding Y, Sutinen M, Visser H, van Hinsbergh VW, Helaakoski T, Kainulainen T, Ronka H, Tschesche H, Salo T: **Matrix metalloproteinase-8 is expressed in rheumatoid fibroblasts and endothelial cells.** *J Biol Chem* 1997, **272**:31504–31509.
27. Deleuran BW, Chu CQ, Field M, Brennan FM, Katsikis P, Feldmann M, Maini RN: **Localization of tumor necrosis factor receptors in the synovial tissue and cartilage-pannus junction in patients with rheumatoid arthritis. Implications for local actions of tumor necrosis factor alpha.** *Arthritis Rheum* 1992, **35**:1170–1178.
28. Gross SS, Wolin MS: **Nitric oxide: pathophysiological mechanisms.** *Annu Rev Physiol* 1995, **57**:737–769.
29. Sakurai H, Kohsaka H, Liu MF, Higashiyama H, Hirata Y, Kanno K, Saito I, Miyas N: **Nitric oxide production and inducible nitric oxide synthase expression in inflammatory arthritides.** *J Clin Invest* 1995, **96**:2357–2363.
30. McInnes IB, Leung BP, Field M, Wei XQ, Huang FP, Sturrock RD, Kinninmonth A, Weidner J, Mumford R, Liew FY: **Production of nitric oxide in the synovial membrane of rheumatoid and osteoarthritis patients.** *J Exp Med* 1996, **184**:1519–1524.
31. Nussler AK, Billiar TR: **Inflammation, immunoregulation, and inducible nitric oxide synthase.** *J Leukoc Biol* 1993, **54**:171–178.
32. Borderie D, Hilliquin P, Hernvann A, Lemarechal H, Menkes CJ, Ekindjian OG: **Apoptosis induced by nitric oxide is associated with nuclear p53 protein expression in cultured osteoarthritic synoviocytes.** *Osteoarthritis Cartilage* 1999, **7**:203–213.
33. Mitchell JA, Larkin S, Williams TJ: **Cyclooxygenase-2: regulation and relevance in inflammation.** *Biochem Pharmacol* 1995, **50**:1535–1542.
34. Wu KK: **Inducible cyclooxygenase and nitric oxide synthase.** *Adv Pharmacol* 1995, **33**:179–207.
35. DiBattista JA, Martel-Pelletier J, Fujimoto N, Obata K, Zafarullah M, Pelletier JP: **Prostaglandins E2 and E1 inhibit cytokine-induced metalloprotease expression in human synovial fibroblasts. Mediation by cyclic-AMP signalling pathway.** *Lab Invest* 1994, **71**:270–278.
36. Takahashi S, Inoue T, Higaki M, Mizushima Y: **Cyclooxygenase inhibitors enhance the production of tissue inhibitor-1 of metalloproteinases (TIMP-1) and pro-matrix metalloproteinase 1 (proMMP-1) in human rheumatoid synovial fibroblasts.** *Inflamm Res* 1997, **46**:320–323.
37. Agro A, Langdon C, Smith F, Richards CD: **Prostaglandin E2 enhances interleukin 8 (IL-8) and IL-6 but inhibits GMCSF production by IL-1 stimulated human synovial fibroblasts *in vitro*.** *J Rheumatol* 1996, **23**:862–868.
38. Panchenko MV, Farber HW, Korn JH: **Induction of heme oxygenase-1 by hypoxia and free radicals in human dermal fibroblasts.** *Am J Physiol Cell Physiol* 2000, **278**:C92–C101.
39. Maines MD: **Heme oxygenase: function, multiplicity, regulatory mechanisms, and clinical applications.** *FASEB J* 1988, **2**:2557–2568.
40. Melillo G, Taylor LS, Brooks A, Musso T, Cox GW, Varesio L: **Functional requirement of the hypoxia-responsive element in the activation of the inducible nitric oxide synthase promoter by the iron chelator desferrioxamine.** *J Biol Chem* 1997, **272**:12236–12243.
41. Frisch SM, Vuori K, Ruoslahti E, Chan-Hui PY: **Control of adhesion-dependent cell survival by focal adhesion kinase.** *J Cell Biol* 1996, **134**:793–799.
42. Shiozawa K, Shiozawa S, Shimizu S, Fujita T: **Fibronectin on the surface of articular cartilage in rheumatoid arthritis.** *Arthritis Rheum* 1984, **27**:615–622.
43. Hino K, Shiozawa S, Kuroki Y, Ishikawa H, Shiozawa K, Sekiguchi K, Hirano H, Sakashita E, Miyashita K, Chihara K: **EDA-containing fibronectin is synthesized from rheumatoid synovial fibroblast-like cells.** *Arthritis Rheum* 1995, **38**:678–683.
44. Müller-Ladner U, Elices MJ, Kriegsmann JB, Strahl D, Gay RE, Firestein GS, Gay S: **Alternatively spliced CS-1 fibronectin isoform and its receptor VLA-4 in rheumatoid arthritis synovium.** *J Rheumatol* 1997, **24**:1873–1880.
45. Konttinen YT, Li T-F, Mandelin J, Liljestrom M, Sorsa T, Santavirta S, Virtanen I: **Increased expression of EMMPRIN in rheumatoid synovium.** *Arthritis Rheum* 2000, **43**:275–280.
46. Nozawa-Inoue K, Ajima H, Takagi R, Maeda T: **Immunocytochemical demonstration of laminin in the synovial lining layer of the rat temporomandibular joint.** *Arch Oral Biol* 1999, **44**:531–534.
47. Sarkissian M, Lafyatis R: **Integrin engagement regulates proliferation and collagenase expression of rheumatoid synovial fibroblasts.** *J Immunol* 1999, **162**:1772–1779.
48. Rinaldi N, Schwarz-Eywill M, Weis D, Leppelmann-Jansen P, Lukoschek M, Keilholz U, Barth TF: **Increased expression of integrin on fibroblast-like synoviocytes from rheumatoid arthritis *in vitro* correlates with enhanced binding to extracellular matrix proteins.** *Ann Rheum Dis* 1997, **56**:45–51.
49. Wolf J, Carsons S: **Fibronectin mediates anchorage-dependent focus formation in cultured human synoviocytes.** *Semin Arthritis Rheum* 1992, **21**:387–392.
50. Carsons S, Wolf J: **Interaction between synoviocytes and extracellular matrix *in vitro*.** *Ann Rheum Dis* 1995, **54**:413–416.
51. Damsky C, Tremble P, Werb Z: **Signal transduction via the fibronectin receptors: do integrins regulate matrix remodeling?** *Matrix* 1992, **1 (suppl 1)**:184–191.
52. McCachren SS, Lightner VA: **Expression of human tenascin in synovitis and its regulation by interleukin-1.** *Arthritis Rheum* 1992, **35**: 1185–1196.
53. Tremble P, Chiquet-Ehrismann R, Werb Z: **The extracellular matrix ligands fibronectin and tenascin collaborate in regulating collagen gene expression in fibroblasts.** *Mol Biol Cell* 1994, **5**:439–453.
54. Asari A, Kuriyama S, Kominami E, Uchiyama Y: **Cytochemical localization of hyaluronic acid in human synovium with special reference to its possible process of degradation.** *Arch Histol Cytol* 1995, **58**:65–76.
55. Goldberg RL, Toole BP: **Hyaluronate inhibition of cell proliferation.** *Arthritis Rheum* 1987, **30**:769–778.
56. Huttenlocher A, Werb Z, Tremble P, Huhtala P, Rosenberg L, Damsky CH: **Decorin regulates collagenase gene expression in fibroblasts adhering to vitronectin.** *Matrix Biol* 1996, **15**:239–250.
57. Dodge GR, Boesler EW, Jimenez SA: **Expression of the basement membrane heparin sulfate proteoglycan (perlecan) in human synovium and in cultured human synovial cells.** *Lab Invest* 1995, **73**:649–657.
58. Schneider M, Voss B, Rauterberg J, Menke M, Pauly T, Michlke RK, Friemann J, Gerlach U: **Basement membrane proteins in synovial membrane.** *Clin Rheumatol* 1994, **13**:90–97.
59. Okada Y, Morodomi T, Enghild JJ, Suzuki K, Yasui A, Nakanishi I, Salvesen G, Nagase H: **Matrix metalloproteinase 2 from human rheumatoid synovial fibroblasts. Purification and activation of the precursor and enzymic properties.** *Eur J Biochem* 1990, **194**:721–730.
60. Seiki M: **Membrane-type matrix metalloproteinases.** *APMIS* 1999, **107**:137–143.
61. Wilson CL, Matrisian LM: **Matrilysin: an epithelial matrix metalloproteinase with potentially novel functions.** *Int J Biochem Cell Biol* 1996, **28**:123–136.
62. Dean MF, Cooper JA, Stahl P: **Cell contact and direct transfer between co-cultured macrophages and fibroblasts.** *J Leukoc Biol* 1988, **43**:539–546.
63. Dean MF, Rodman J, Levy M, Stahl P: **Contact formation and transfer of mannose BSA gold from macrophages to cocultured fibroblasts.** *Exp Cell Res* 1991, **192**:536–542.

64. Shock A, Laurent GJ: **Adhesive interactions between fibroblasts and polymorphonuclear neutrophils *in vitro*.** *Eur J Cell Biol* 1991, **54**:211–216.
65. Behzad AR, Chu F, Walker DC: **Fibroblasts are in a position to provide directional information to migrating neutrophils during pneumonia in rabbit lungs.** *Microvasc Res* 1996, **51**:303–316.
66. Burns AR, Simon SI, Kukielka GL, Rowen JL, Lu H, Mendoza LH, Brown ES, Entman ML, Smith CW: **Chemotactic factors stimulate CD18-dependent canine neutrophil adherence and motility on lung fibroblasts.** *J Immunol* 1996, **156**:3389–3401.
67. Murakami S, Hino E, Shimabukuro Y, Nozaki T, Kusumoto Y, Saho T, Hirano F, Hirano H, Okada H: **Direct interaction between gingival fibroblasts and lymphoid cells induces inflammatory cytokine mRNA expression in gingival fibroblasts.** *J Dent Res* 1999, **78**:69–76.
68. Rezzonico R, Burger D, Dayer JM: **Direct contact between T lymphocytes and human dermal fibroblasts or synoviocytes down-regulates types I and III collagen production via cell-associated cytokines.** *J Biol Chem* 1998, **273**:18720–18728.
69. Shock A, Rabe KF, Dent G, Chambers RC, Gray AJ, Chung KF, Barnes PJ, Laurent GJ: **Eosinophils adhere to and stimulate replication of lung fibroblasts '*in vitro*'.** *Clin Exp Immunol* 1991, **86**:185–190.
70. Greenberg G, Burnstock G: **A novel cell-to-cell interaction between mast cells and other cell types.** *Exp Cell Res* 1983, **147**:1–13
71. Trautmann A, Krohne G, Brocker EB, Klein CE: **Human mast cells augment fibroblast proliferation by heterotypic cell–cell adhesion and action of IL-4.** *J Immunol* 1998, **160**:5053–5057.
72. Oliani SM, Girol AP, Smith RL: **Gap junctions between mast cells and fibroblasts in the developing avian eye.** *Acta Anat (Basel)* 1995, **154**:267–271.
73. van der Plas A, Nijweide PJ: **Cell–cell interactions in the osteogenic compartment of bone.** *Bone* 1988, **9**:107–111.
74. Ritchlin C, Dwyer E, Bucala R, Winchester R: **Sustained and distinctive patterns of gene activation in synovial fibroblasts and whole synovial tissue obtained from inflammatory synovitis.** *Scand J Immunol* 1994, **40**:292–298.
75. Zvaifler NJ, Tsai V, Alsalameh S, von Kempis J, Firestein GS, Lotz M: **Pannocytes: distinctive cells found in rheumatoid arthritis articular cartilage erosions.** *Am J Pathol* 1997, **150**:1125–1138.

Authors' affiliations: Yrjö Konttinen (Department of Anatomy, Institute of Biomedicine, and Department of Oral Medicine, Institute of Dentistry, University of Helsinki, and Department of Oral Medicine, Surgical Hospital, The Helsinki and Uusimaa Health Care Concern, Helsinki, Finland), Tian-Fang Li and Jing-Wen Xu (Department of Anatomy, Institute of Biomedicine, and Department of Oral Medicine, Surgical Hospital, The Helsinki and Uusimaa Health Care Concern, Helsinki, Finland), Mika Hukkanen and Ismo Virtanen (Department of Anatomy, Institute of Biomedicine, University of Helsinki, Helsinki, Finland), and Jian Ma (Department of Anatomy, Institute of Biomedicine, and Department of Oral Medicine, Institute of Dentistry, University of Helsinki, Helsinki, Finland),

Correspondence: Professor Yrjö T Konttinen, Department of Oral Medicine, Surgical Hospital, The Helsinki and Uusimaa Health Care Concern, Kasarmikatu 11-33, FIN-00130 Helsinki, Finland.
Tel: +358 9 1918477; Fax: +358 9 1918576;
E-mail: yrjo.konttinen@helsinki.fi