Extracellular matrix: Composition and Remodelling

Specific processes: Tumor metastasis, Wound healing, Liver fibrosis, Angiogenesis

Introduction

The extracellular matrix is a network of proteins and carbohydrates that supports and surrounds the cells in connective tissues. In addition to their structural-mechanical functions ECM molecules regulate diverse cellular functions, and play a significant role in several physiological and pathological processes, such as embryonic development, inflammation and wound healing, hemostasis, fibrosis of various organs, tumor growth and metastasis, and angiogenesis.
The interaction of ECM molecules with the cells is conducted through cell-matrix receptors including integrins (see. the seminar material on Cell adhesion).
The extracellular matrix is not static: it is remodelled constantly, which implies its constant breakdown by proteases, notably matrix metalloproteinases.
During the seminar, we will discuss this topic according to the following scheme:

1. Review the components of the extracellular matrix
2. Introduce the family of matrix metalloproteinases
3. Discuss selected figures from original papers on the role of MMPs in tumor metastasis and/or present a review on the role of MMPs in tumor metastasis
4. Review the process of wound healing and scarring

1. Components of the extracellular matrix

The extracellular matrix presents as two clearly identifiable structures:
1, the basement membrane: a condensed matrix layer formed adjacent to epithelial cells, and other covering cell sheets (e.g. mesothelium), or muscle cells, adipocytes, etc.
2, the interstitial matrix: a space-filling structure of huge variety that determines the main characteristics of a given connective tissue
Both of these structures are defined by an insoluble collagen scaffold to which soluble adhesive glycoproteins and proteoglycans adhere. (Reticular fibrils and elastic fibrils – other insoluble components of the ECM- are not discussed during this seminar)

Collagens

Collagens are ubiquitous proteins, most of them are right-handed triplehelices built from 3 polypeptide chains: the 3 alpha chains are left-handed helices, themselves. In the alpha chains every third amino acid is Gly, which has a small enough side-chain to fit into the triplehelix. The triplehelix domains are alternated with non-helical domains depending on the specific type of collagen. Proline and hydroxyproline represent 20 % of the amino acids in the general Gly-X-Y triplet. The hydroxyl group of hydroxyproline participates in the formation of hydrogen bonds, which stabilize the triple helix. Lysine and hydroxylysine in the helical or non-helical domains participate in the formation of covalent cross-bridges necessary for the maintenance of the supramolecular complex. The hydroxylysine residues are potential glycation sites too. The ratio hydroxylysine/lysine and the degree of glycation of hydroxylysine depends on the type of collagen and varies in different tissues and with ageing. Collagen alpha chains are

synthesized following the secretory pathway with several co-, and posttranslational covalent modifications and formation of the triplehelical structure in the ER. The supramolecular collagen structure is formed following the secretion of procollagen into the ECM and type-specific additional modifications.
More than 20 genetically distinct collagen types have been identified and classified based on their structures.
Fibrillar collagen types (I, II, III, V and XI) self-assemble into fibrils after their secretion into the ECM. The intracellular precursor, pro-collagen contains non-helical regions at the two terminals of the triple helical region (N- and C-telopeptides, N- and C-propeptides). Following secretion the propeptides are cleaved by specific N- and C-peptidases and thus the newly exposed telopeptides direct the axial arrangement in the course of collagen polymerization. The polymerization of the collagen monomers (300 nm long, 1.4 nm diameter) is a spontaneous process, in the course of which the monomers are arranged in a staggered manner with 234 amino acid overlap resulting in a 67-nm long repeats. The staggered arrangement optimizes the electrostatic and hydrophobic interactions and allows the formation of covalent cross-links between lysine/hydroxylysine residues of helical and neighboring non-helical regions. In the course of this cross-linking a lysyl oxidase enzyme oxidizes an ε-NH_2-group in the non-helical region, thereafter the newly formed aldehyde group forms a Schiff-base with the ε-NH_2-group of a lysine/hydroxylysine in the helical region of a neighboring monomer. These two-point cross-links further condense with near-by lysine/hydroxylysine or histidine, thus forming three-point cross-bridges. Fibrillar collagens are typically found in tissues that have to resist shear, tensile or pressure forces, such as tendons, bone, cartilage and skin.
Some collagens form networks (IV, VIII, X), a typical example is the basement membrane, mostly made of collagen IV.
Other collagens associate with fibril surfaces (Fibril Associated Collagens with Interrupted Triple Helix-FACIT- e.g. types IX, XII, XIV) and modify their supramolecular structure in a tissue-dependent way.
Type VI collagens form beaded structures, and type VIII collagens form the hexagonal network structure in the Descemet’s membrane of the cornea.

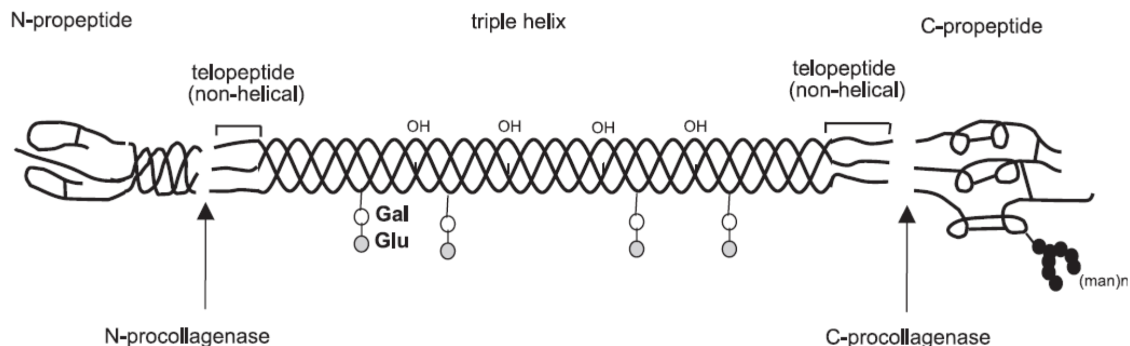


Fig. 1 Molecular structure of collagen

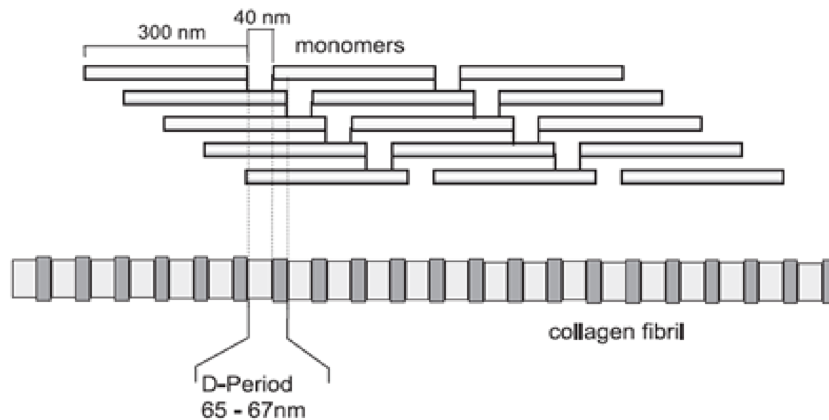


Fig. 2 Supramolecular assembly of collagen fibrils

Proteoglycans

Proteoglycans consist of glucosaminoglycan(GAG) chains posttranslationally added to a core protein by glycosyltransferases in the ER, followed by further elongation and modification of the GAG chain in the Golgi. The GAG chains are composed of repeated disaccharides. In *chondroitin-sulphates* the (beta1-3)glucuronic acid-N-acetyl-glucosamine unit is sulphated on C4 (chondroitin-sulphate A) or C6 (chondroitin-sulphate C) of the aminosugar moiety. *Dermatan-sulphate* (chondroitin-sulphate B) contains at least one iduronic acid, formed from glucuronic acid by postsynthetic epimerization. In *heparan-sulphate*, the repeated disaccharide is (beta1-4)glucuronic acid-N-acetyl-glucosamine, where glucuronic acid can be epimerized to iduronic acid, and both sugars can be sulphated, as well. *Keratan-sulphate* is composed of (beta1-4)galactose-N-acetyl-glucosamine. *Hyaluronic acid* contains (beta1-3)glucuronic acid-N-acetyl-glucosamine that are not sulphated, and it is the only GAG that is not attached to a core protein. Instead, hyaluronic acid can be found in tissues as an extremely long, free GAG chain, with Mw up to 10 000 kDa. Hyaluronic acids can form aggregates and networks, very frequently co-aggregate with proteoglycans, as well.

The sulphation of GAG chains frequently varies even within the same GAG molecule, and the same core-protein can carry different number of GAG chains, or GAG chains of various lengths, which results in the highly heterogeneous nature of proteoglycans.

Proteoglycans are the space fillers in the ECM: due to the presence of uronic acids and sulphate groups they are highly negative molecules that form a highly hydrated gel-like substance. This substance is responsible for the volume of the ECM and is also resistant to compression.

In addition to their structural functions, proteoglycans have been implicated in several processes, where they can modulate cell-cell, and cell-matrix interactions, regulate cellular behaviour including motility, proliferation, differentiation.

Based on their localization they are classified as *matrix proteoglycans* (e.g. aggrecan in cartilage, perlecan in the basal membrane, versican in vessel wall, or the decorin/biglycan/fibromodulin group in various tissues) or *membrane-associated proteoglycans* (e.g. syndecans and glypicans, CD44, thrombomodulin)

Adhesive glycoproteins

Adhesive glycoproteins connect the cells to the matrix structure, most of them are ligands for cell adhesion receptors and regulate cellular behaviour. They can also bind to the structural matrix components, thereby modulating the matrix structure and organizing the cells and ECM structures.
In addition to fibronectin and laminins described in the seminar material on Cell adhesion, glycoproteins, such as nidogen, tenascin, thrombospondin, osteopontin belong to this group.

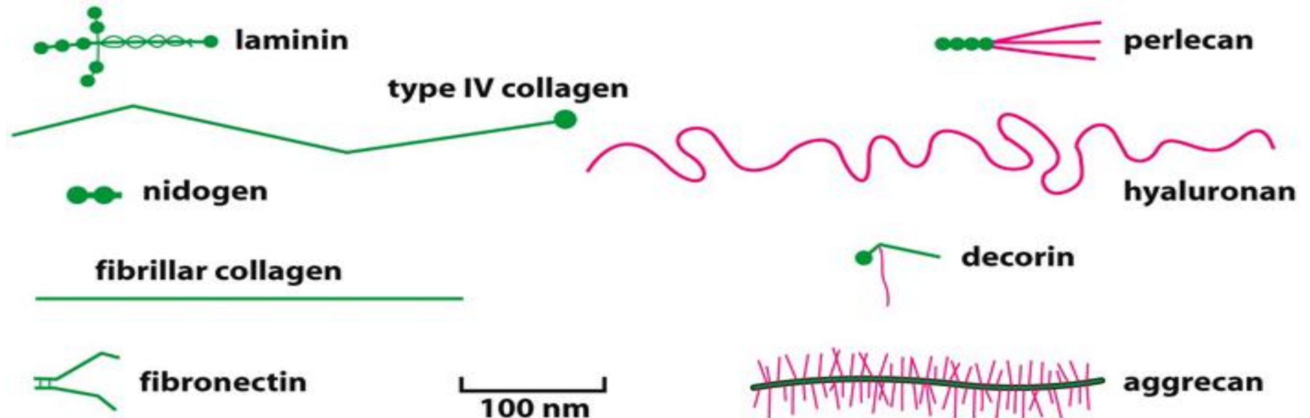


Fig. 3 Relative size of the core (green) and sugar (magenta) component of extracellular matrix proteins.

Structure of the basement membrane (also referred to as basal lamina)
The basement membrane is a thin (40-120 nm thick), though flexible sheet of ECM molecules that not only underlies all epithelia, but also surrounds certain individual non-epithelial cells, such as muscle cells, adipocytes, or Schwann cells. It separates these cells from the adjacent connective tissue, and provides a mechanical connection between the two layers, as well. *Epidermolysis bullosa*, a blistering, sometimes lethal skin disease is due to a genetic defect of certain basement membrane components, which leads to an improper anchorage of skin epidermis to the underlying dermis. In addition to its structural role, basal lamina components also influence cellular functions and serve as highways of cell migration. Like other extracellular matrices, the basement membrane contains fibrous proteins (collagen type IV), adhesive glycoproteins (laminins and nidogen), as well as proteoglycans (perlecan carrying heparan-sulphate side chains). *Laminin-1* is a large, flexible protein composed of three polypeptide chains (alpha, beta, and gamma) held together by disulfide bonds. It looks like a bunch of three flowers whose stems are twisted together at the foot, but whose heads remain separate. These heterotrimers self-assemble into a network through interactions between their heads and interaction between their foot and the cells producing them help to organize the network into an orderly sheet. Laminin anchorage occurs mostly via integrin receptors, another important receptor is dystroglycan, a proteoglycan with a transmembrane core protein and an extracellular GAG chain. This laminin network then presumably coordinates the assembly of the other basal lamina components, since laminin has multiple binding sites for nidogen and perlecan. *Type IV collagen*, like other collagens, is a superhelix of three polypeptide chains, however, its triplehelical structure is interrupted in more than 20 regions, allowing multiple bending of the molecule. Collagen type IV can bind to both

nidogen and *perlecan*, which can serve as linkers to connect collagen to the laminin network.

Basement membranes have diverse functions including a molecular filter in kidney glomeruli, or an organizer of neuromuscular junctions, we will see data on its role in tumor metastasis.

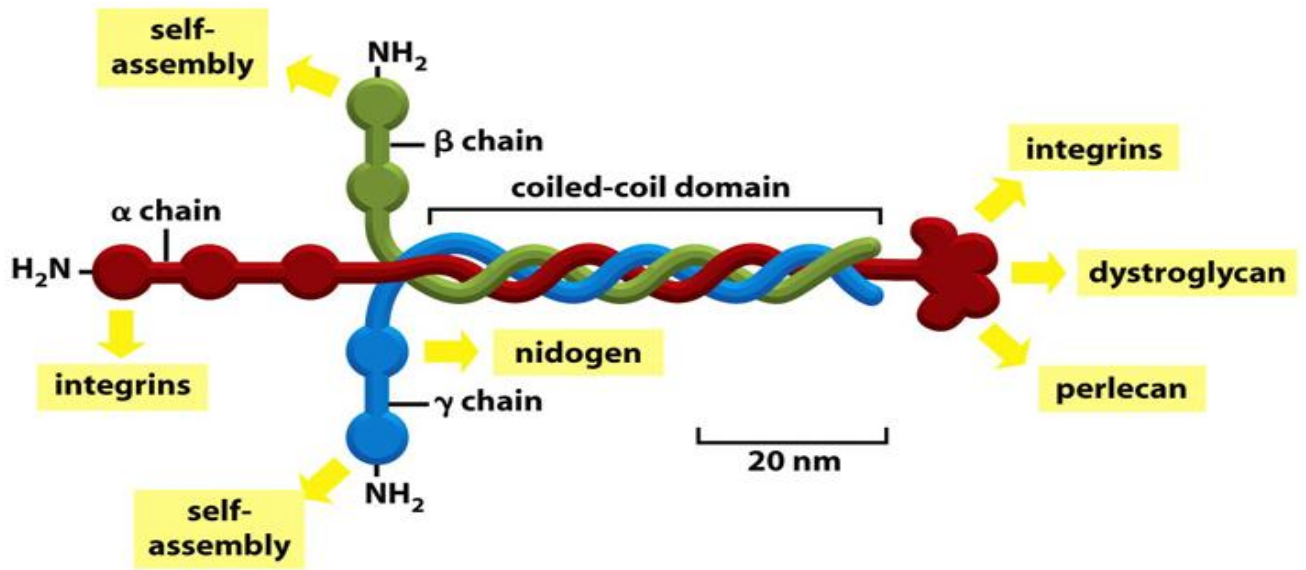


Fig. 4 Structure of laminin

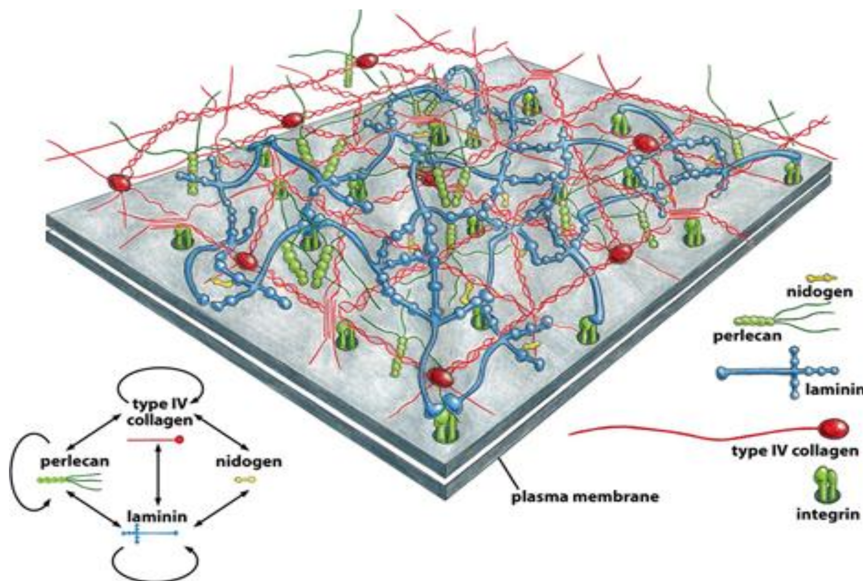


Fig. 5 Assembly of the basal membrane

2. ECM Remodelling: matrix metalloproteinases (MMPs) and tissue inhibitors of matrix metalloproteinases (TIMPs)

Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases that consist of more than 21 human MMPs. Based on their domain structure they can be divided into eight classes, three of which are membrane-bound (see Figure 2 in the review below). The MMPs can collectively degrade most of the components of the extracellular matrix (ECM), including collagens (collagenases), denatured or partially degraded collagens (gelatinases), laminins, as well as cell-adhesion molecules, growth factors, and growth factor receptors, through which they also influence cellular signalling and function.

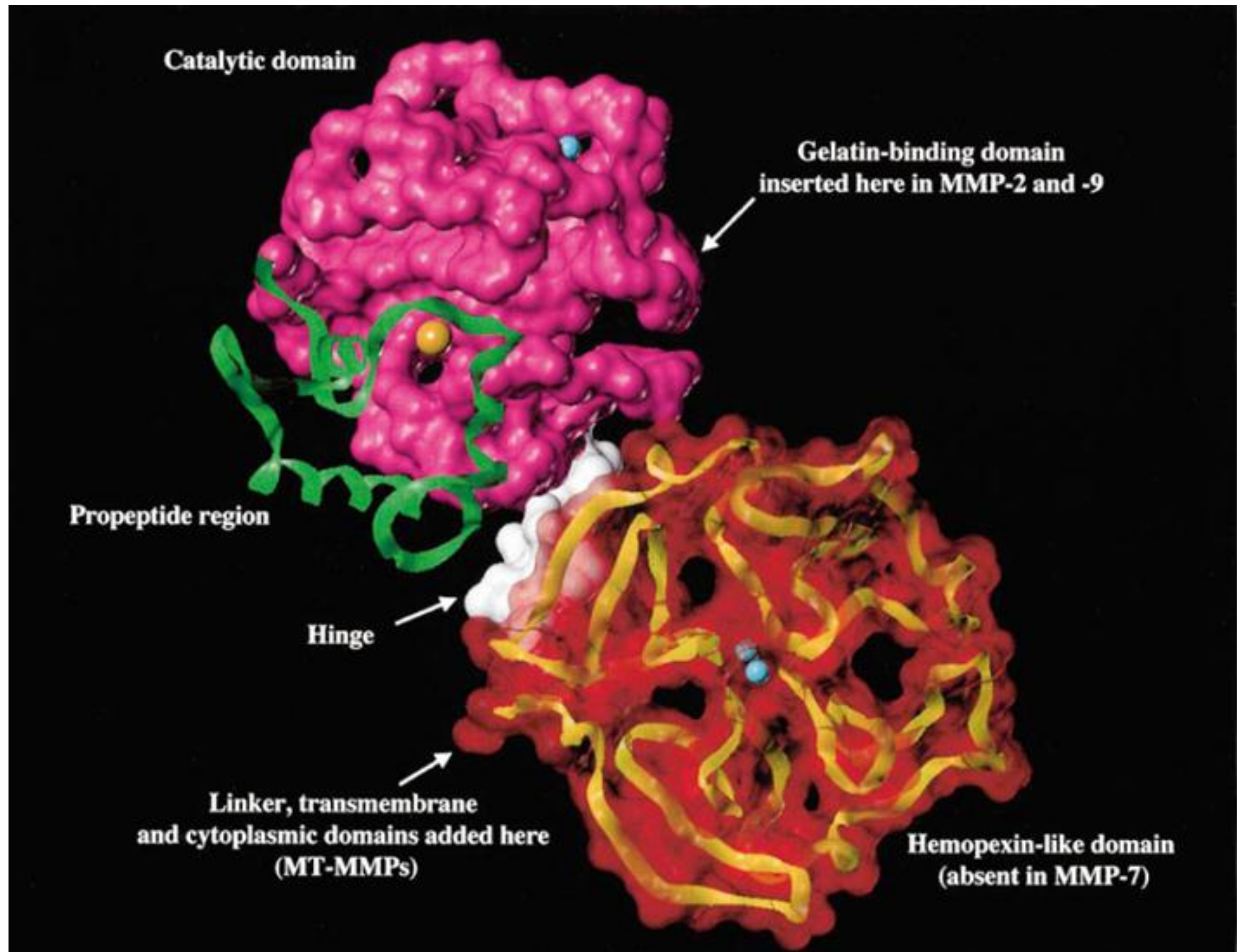


Fig. 6 Tertiary structure of MMPs (the catalytic site Zn is shown in yellow)

The MMPs are synthesized as inactive zymogens, where a Cys residue in the propeptide domain forms a bridge with the zinc in the catalytic center, and prevents enzymatic activity. Activation occurs when this Cys-Zn2+ interaction is disrupted and the propeptide is proteolytically removed. The activation occurs in two steps: in the 1st step, the Cys-Zn2+ interaction is perturbed as a result of a conformational change (induced chemically by e.g. APMA-amino phenyl mercuric acid-) or proteolysis by a serine-

protease (e.g. furin, plasmin), and a partially active enzyme is formed. In the 2nd step, the propeptide domain is completely removed by autocatalytic cleavage or by another MMP yielding the fully activated form of the enzyme. The mechanism of MMP activation resembles the cascade-like proteolytic activation of the coagulation and thrombolytic systems.

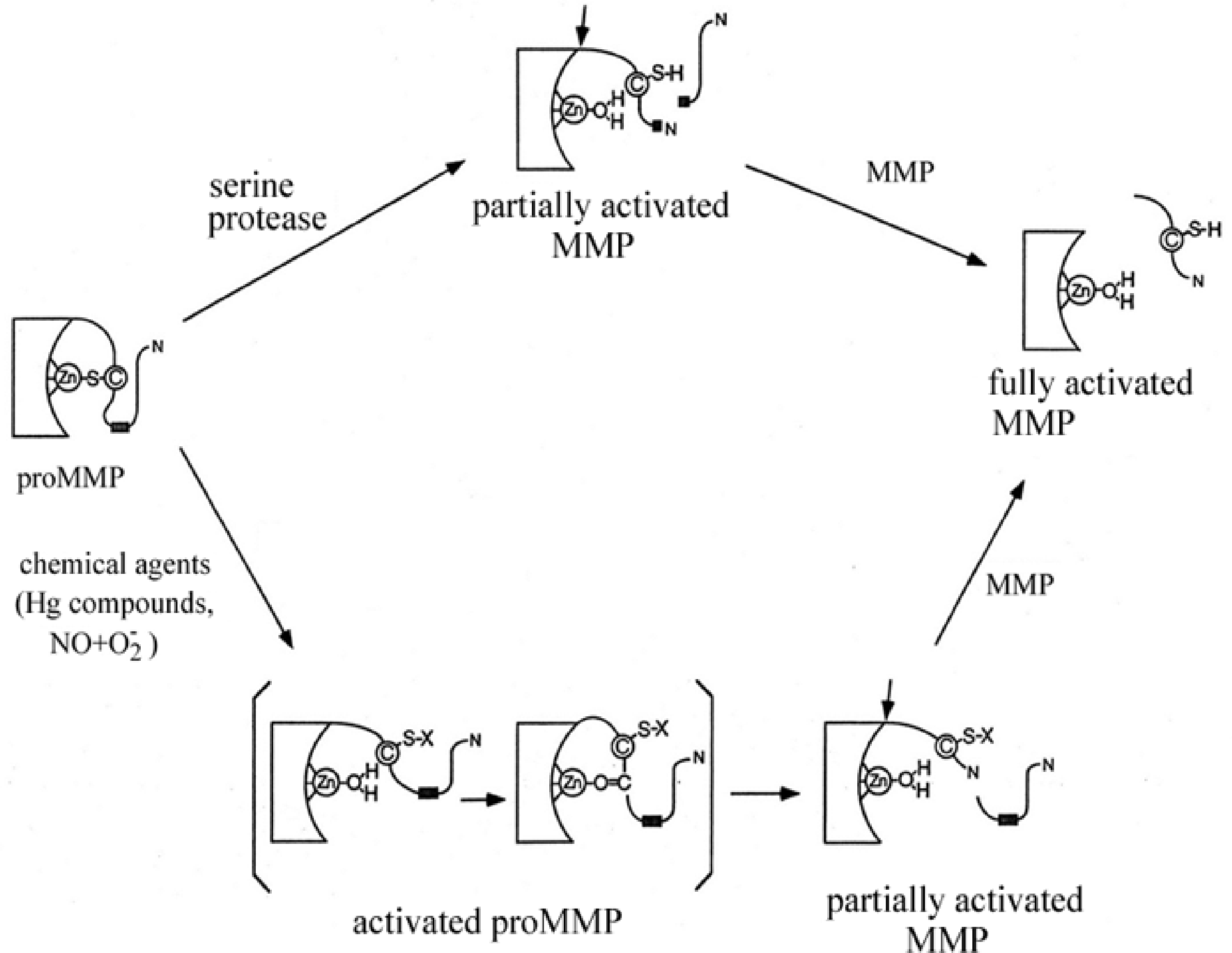


Fig. 7 MMP activation

MMP activity is regulated by endogenous inhibitors, including the tissue inhibitors of metalloproteinases (TIMPs), alpha-2-macroglobulin from the plasma, and the membrane-bound inhibitor RECK (reversion-inducing cystein-rich protein with kazal motifs). Alpha-2-macroglobulin, an abundant plasma protein, binds to MMPs, and the enzyme-inhibitor complex is irreversibly cleared by scavenger receptor-mediated endocytosis in macrophages.

TIMPs act by forming a reversible 1:1 TIMP-MMP complex with the catalytic zinc in the MMP. Surprisingly, most of the intermolecular contacts between the two proteins are restricted to the N-terminal five amino acids of TIMP, which binds to the MMP active site in a substrate-like manner. The C-terminal domain of TIMP confers the inhibitor with some proteinase specificity, e.g. TIMP-1 binds to MMP-9 with higher affinity, as compared to MMP-2, whereas TIMP-2 is a better inhibitor for MMP-2, than for MMP-9.

In addition to acting as a reversible MMP inhibitor, TIMP-2 plays a cofactor role in the activation of proMMP-2 (for details, discuss Figure 4. in the recommended review below, and the paper on pericellular proMMP-2 activation by MT1-MMP).

Many of the MMP genes are inducible, and the effectors include not only the soluble growth factors, cytokines and chemicals, but cell-matrix and cell-cell interactions have been shown to influence MMP gene expression, as well. Enhanced MMP gene expression may be down-regulated by suppressive factors (e.g. TGFβ1, glucocorticoids).

3. Matrix metalloproteinases in tumor metastasis

MMPs have been implicated in cancer invasion and metastasis as proteases degrading the ECM barrier in the direction of invasion. To carry out such a function, MMPs are expected to act at the leading edge of invading cancer cells. MT1-MMP was identified as the first membrane-anchored type MMP acting as a key enzyme responsible for the degradation of the pericellular ECM. Unlike the soluble MMPs, MT1-MMP activation occurs in the trans-Golgi by furin, during the secretory pathway, so a catalytically active enzyme reaches the cell surface. In addition to MT1-MMP, MMP-2 and MMP-9, two types of type IV collagenases have been implicated in cancer invasion. Even though these MMPs are soluble enzymes produced by fibroblasts located in the stroma surrounding cancer cell nests, type IV collagenase activity was found to associate with the cancer cell surface. As it turns out, the expression of MT1-MMP confers the cells the ability to bind and to activate proMMP-2.

MT1-MMP, in addition to its role in proMMP-2 activation, is a membrane-bound collagenase, itself. “Clearing a path” in the ECM, however, is not the only mechanism that promotes tumor cell invasion. MT1-MMP also interacts with cell adhesion receptors, such as CD44, a hyaluronic acid receptor, as well as with syndecan-1, a transmembrane proteoglycan, which results in enhanced cell migration. MT1-MMP cleaves laminin-5, a basal lamina protein, and the cleaved laminin-5 also promotes cell migration through the basement membrane.

Angiogenesis is the formation of new vessels from existing vessels and is required for an adequate blood supply in tumor growth. During this process endothelial cells need to detach from neighbouring cells, invade into the surrounding stroma, proliferate and generate a tube structure. These steps include the degradation of the basal lamina, and the collagen-rich stroma. Several other MMPs have been shown to play a role in angiogenesis, however, in an experimental collagen-rich matrix, only the MT1-MMP knock out mice failed to show neovessel formation. MT1-MMP also up-regulates the gene expression of VEGF by tumor cells, a growth factor important for angiogenesis.

Angiogenesis-a brief review

Angiogenesi*s* is the process of vessel growing and branching throughout the body. The formation of the first rudiments of vessels from the early embryonic endothelial cells in the course of the embryonic development is called *vasculogenesis*, but further branching and maturation of the vessel structure is a similar process throughout our lives. Eventually, almost every cell is located within 50-100 μm of a blood capillary to ensure adequate blood supply. The renewal and development of the vasculature is required not only during embryonic development and body growth, but is also necessary in tissue

repair, as well as in tumor growth and metastasis. Angiogenesis follows a carefully orchestrated sequence of events: 1, an endothelial tip cell is selected, that will start to form a new capillary branch; 2, the endothelial tip cell, with many filopodia, grows into the surrounding tissue; 3, endothelial stalk cells trail behind the tip cell, and hollow out to form the lumen of the new vessel; 4, the newly formed vasculature is stabilized by the recruitment of mural cells (e.g. smooth muscle cells, fibroblasts) and the deposition of an extracellular matrix, which altogether ensure that parenchymal cells receive adequate nutrient supply.

The signals controlling this whole event are rather complex, but *vascular endothelial growth factor (VEGF)* is a key factor. VEGF is a relative of platelet-derived growth factor (PDGF), and a survival factor for endothelial cells (its main target cells). VEGF receptors belong to the group of receptor-tyrosine kinases, and activate the phosphatidyl-inositol-3-kinase/protein kinase B pathway, as well as the MAP-kinase pathway. The biological effects of VEGF on endothelial cells include proliferation, production of proteases (MMPs and serine proteases) that digest the surrounding ECM to help sprouting. The endothelial tip cells detect the VEGF gradient and move toward its source that signalled the need for angiogenesis. Increased vascular permeability and vasodilation (NO-mediated) lead to the formation of a provisional matrix around the new branch, and integrin receptor-matrix molecule interactions help the endothelial cells to migrate along these matrix molecules. *Angiopoetins* in the presence of VEGF facilitate sprouting and the formation of a new “immature” vascular network. The maturation of newly formed vessels is required to reach branching and vessel wall structure appropriate for the site and vessel-type (e.g. capillary, arteriole, artery, vein, or lymphatic vessels). *PDGF* secreted from endothelial cells in response to VEGF recruits mural cells and promotes their proliferation. Angiopoetins secreted by mural cells and endothelial cells facilitate endothelial cell-mural cell interactions, as well as endothelial cell-matrix interactions. *TGF*β promotes the production of ECM and proteases by mural cells and endothelial cells and regulates the final differentiation of vessel wall cell-types. An excessive formation of blood vessels is undesired, inhibitors of angiogenesis limit the number of vessels and the deposition of ECM molecules. Such inhibitors include *angiostatin*, a cleavage product of plasminogen, and *endostatin*, a cleavage product of collagen type XVIII.

There are several situations that may necessitate angiogenesis, a well-studied example is hypoxia, and the *hypoxia-inducible factor-1(HIF-1)*. HIF-1 is an alpha-beta heterodimer, the beta subunit is a constitutively expressed nuclear protein, whereas HIF-1alpha is induced by a shortage of oxygen. HIF-1 alpha/beta was first recognized as a DNA-binding protein that up-regulates the transcription of erythropoietin, and later turned out to be an oxygen-sensor in a wide variety of cell types. In well-oxygenated cells the concentration of HIF-1alpha is low because of its continuous degradation. Non-heme, iron-, and 2-oxoglutarate-dependent dioxygenases, that require molecular oxygen, can hydroxylate HIF-1alpha on two Pro, and an Asn residues. One oxygen atom of the O_2 molecule creates the hydroxyl group, and the other one oxidizes 2-oxoglutarate to succinate with the release of CO_2. Hydroxylation at the Pro residues mediates interactions with the von Hippel-Lindau (VHL) E3 ubiquitin ligase complex that targets HIF-1alpha for proteasomal degradation. The second hydroxylation-dependent control is the beta-hydroxylation of an Asn residue, which blocks the interaction of the HIF-1alpha C-

terminus with the transcriptional coactivator, p300 (this is a histone acetyl-transferase). In oxygenated cells, this dual mechanism (proteolytic destruction and inhibition of transcriptional activity) inhibits the HIF-dependent transcription of proangiogenic factors, e.g. VEGF.
The role of the HIF-pathway in the regulation of angiogenesis is underlined in von Hippel Lindau syndrome. People affected carry just one functional copy of the VHL gene, and when the other one gets mutated, the lack of a functional protein leads to high HIF-1alpha levels, regardless of oxygen availability, which leads to the development of hemangioblastomas, tumors with dense masses of blood vessels.
VEGF mRNA is up-regulated in solid tumors, (both cancer cells and stromal cells produce VEGF) and angiogenesis is stimulated in order to supply the growing tumor mass. The structure of the vasculature, however, is abnormal with uneven wall thickness, leaky endothelial cell lining, altered expression of endothelial cell markers and adhesion molecules. These abnormalities are considered to result from an imbalance between pro-, and anti-angiogenic molecules. Inhibition of angiogenesis may limit tumor growth and metastasis formation, e.g. anti-VEGF treatments potentiate the anti-tumor effects of conventional radiation therapy and chemotherapy.

4. Wound healing and tissue repair

Damage to tissues can result from various acute and chronic stimuli, including mechanical injury, infections, metabolic abnormalities, and autoimmune reactions. The repair process involves two stages: a first, regenerative phase, where injured cells are replaced by cells of the same type; and a second phase in which connective tissue is deposited with the possible restoration of the original organ structure. If this process goes out of control, it results in substantial remodelling of the ECM, and the formation of permanent scar tissue. In some cases, such as liver fibrosis, parenchymal tissue cannot be regenerated, and is replaced by connective tissue, instead.
Following tissue injury, damaged epithelial/endothelial cells release inflammatory mediators that initiate blood clot formation including platelet aggregation and fibrin deposition. *Activated platelets* release chemokines and growth factors, such as PDGF (platelet-derived growth factor) and TGFβ (tumor growth factor-beta). *Leukocytes* from the circulation are recruited to the site and activated by chemokines (e.g. PDGF, TGFβ). Neutrophils (arriving the earliest) and macrophages (arriving after 1-2 days) eliminate tissue debris, dead cells, invading organisms. Activated leukocytes secrete profibrotic cytokines, such as IL-13 and TGFβ, which further activate macrophages and fibroblasts. Activated fibroblasts transform into *myofibroblasts* expressing α-SMC (alpha-smooth muscle cell actin- the histological marker of myofibroblasts) that combine the contractile properties of smooth muscle cells with the fibroblasts' ability to produce ECM components including fibrillar collagens.
At the molecular level, *TGFβ (transforming growth factor β)* is a cytokine vital to tissue repair, however, its excessive action has also been implicated in fibrotic scarring leading to organ damage. TGFβ is a dimer of a 12 kDa polypeptide, and belongs to the superfamily of cytokines regulating embryonal development, cell differentiation, and tissue repair. Platelets contain high concentrations of TGFβ, which is released upon their degranulation. TGFβ is chemoattractant for leukocytes, induces angiogenesis, control the production of several cytokines and inflammatory mediators. Two additional features of

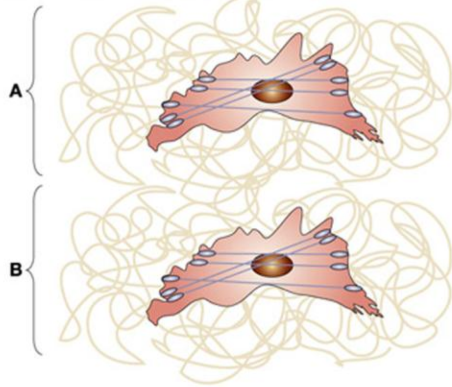
TGFβ action may easily lead to an out-of-control healing process: 1, the autoinduction of TGFβ-production in myofibroblasts, which may lead to persistent activation, and 2, the induction of ECM deposition by TGFβ via increased synthesis of all major ECM components and a simultaneous blockage of matrix degradation (increased TIMP, decreased MMP expression). Supporting the potential role of TGFβ in fibrosis, anti-TGFβ antibodies capable of blocking the binding of TGFβ to its cell surface receptor have been successfully applied in some cases.

The term wound healing covers the *restoration of the ECM integrity* following mechanical injury. This is not a simple task, because in the basal state the structural elements of the ECM form a network, which is anchored under strain on the parenchymal cells. The existence of mechanical strain in the tissues is supported by the fact that the length of blood vessels and nerves is 25 – 30 % smaller in isolated state compared to their length *in situ* in the tissues. This mechanical strain provides an elastic protective shield around the parenchymal cells and in certain tissues it is a prerequisite for the basic organ function (e.g. in the lung alveoli). Thus, in the course of wound healing not only the ECM continuity should be restored, but the strain of the structure should be tuned accordingly. This process called *mechano-regulation* requires the coordinated function of many molecular and cellular components. The changing mechanic strain at the site of injury initiates the secretion of transforming growth factor β1 (TGF- β1) in the neighboring fibroblasts, whereas the platelets arriving from the damaged blood vessels secrete platelet-derived growth factor (PDGF). Both cytokines can initiate the *differentiation of myofibroblasts* manifested primarily in the massive production of α-SM actin. As a result of this the myofibroblasts can exert long-lasting contraction forces coupled to the events of the extracellular structural rearrangements. The contraction is based on the interactions of actin microfilaments and myosin (non-muscle type), which are enabled by the phosphorylation of the myosin light chain (MLC). This phosphorylation can be catalyzed by two kinases: Ca^{2+}-dependent MLC kinase (MLCK) and Rho-kinase (RhoA small G-protein activated kinase). The elevation of the intracellular Ca^{2+}-level results in fast (the affinity of MLCK for MLC is high), but short-lasting contraction (the myosin phosphatase removes the phosphate groups from the phosphorylated MLC also with high affinity). In contrast, MLC is not so good substrate of Rho-kinase and thus the contraction provoked by it is slow. However, the myosin-binding subunit of myosin phosphatase is also a substrate of Rho-kinase and in phosphorylated state it does not bind myosin, the Rho-kinase initiated contraction is long-lasting and energetically more suitable to maintain the isometric strain the newly forming connective tissue. The exact mechanisms, which activate the Rho-kinase and adjust the strength of contraction, are not known. The initial active contraction is followed by structural rearrangement of ECM around the myofibroblasts (Fig. 8). These cells express transmembrane adhesion complexes, which connect the extracellular collagen fibers with the intracellular microfilaments. Consequently, the cell contraction results in stretching of the initial loose collagen meshwork (Fig. 8b, “B” fibroblast). This deformation of the initial collagen matrix is accompanied by mechanical strain in the opposite direction compared to the final structure. This is resolved by the *matrix metalloproteinases* produced by the myofibroblasts (MMP-1,-2,-3,-9). MMP-1 removes the outer collagen layer, and n accordance with their substrate specificity (see MMP chapter), the MMP-2 and 9

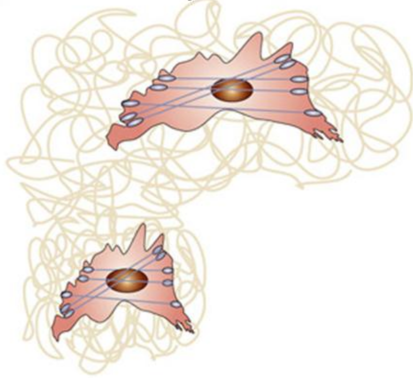
solubilize the excess partially degraded fibers. Thereafter the formation and crosslinking of a single new collagen layer is sufficient to rejoin the remnant fibers in the post-contraction state and allow the restoration of the regular fiber structure. Thus, the newly formed collagen stabilizes the generated mechanical strain (Fig. 8c). Following injury the cycle illustrated in Fig. 8 is repeated multiple times and thus the original mechanical state of the tissue is restored. Thereafter the myofibroblasts disappear from the scar area as a result of apoptosis.

If scar formation is impaired because of abnormal apoptosis, a hypertrophic scar is retained. The latter contains a lot of myofibroblasts and abnormal amount of ECM, a state designated as fibrosis. The hypertrophic scar results in malformation and malfunction of the organ. Clinical manifestations of such abnormal tissue repair include burning scar contracture, malformations following mammary or abdominal surgery, Dupuytren contracture (severe lesions of the palmary fascia with immobilization of one or more fingers). In the course of an internal organ disease abnormal tissue repair can lead to potentially lethal organ damage, such as liver fibrosis, pulmonary fibrosis, renal fibrosis, and restenosis after balloon angioplasty in the coronary arteries.

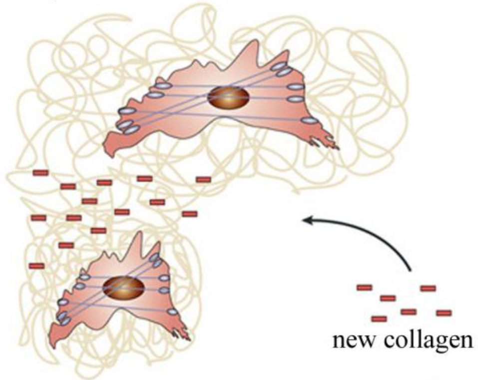
a Adhesion of myofibroblasts to collagen



b Contraction of myofibroblast “B”



c Synthesis of new collagen stabilizes the compact structure



d A new cycle starts after cell relaxation

Fig. 8 Mechano-regulation in the course of wound healing

<u>Recommended presentations:</u>

1, Please, summarize the **components of the extracellular matrix**, based on your previous studies in anatomy and biochemistry.

2, Summarize the structure and function of the **MMP family**, the mechanism of proMMP activation and their TIMP-type inhibitors. Use the scheme above and relevant parts of the following reviews:

2.1 Brinckerhoff CE, Matrisian LM. Matrix metalloproteinases: a tail of a frog that became a prince. Nature Rev Mol Cell Biol 2002, 3:207-214 (Figs 2, 4)

2.2 Nagase H, Woessner JF Jr. Matrix metalloproteinases. J Biol Chem 1999, 274:21491-21494.

3, Discuss the roles of **MMPs in tumor metastasis** formation. You may choose to present the relevant parts of the review article (3.1) or present and discuss a few selected figures from the original papers illustrating the experimental nature of research in this field (3.2-3.4). If the latter use Figure 1 from the review paper for a scaffold.

3.1 A selected review on pericellular proteolysis and its role in tumor metastasis and angiogenesis:
Itoh Y, Seiki M. MT1-MMP: A potent modifier of pericellular microenvironment. J Cell Physiol 2006, 206:1-8 Note Figures 1, and 2.

3.2 An original paper on proMMP-2 activation:
Will H, Atkinson SJ, Butler GS, Smith B, Murphy G. The soluble catalytic domain of membrane type 1 matrix metalloproteinase cleaves the propeptide of progelatinase A and initiates autoproteolytic activation. J Biol Chem 1996, 271: 17119-17123.

Figures 2 and 3 present kinetic and gel electrophoretic data on the activation of proMMP2 by MT1-MMP, and its inhibition by TIMP-2

3.3 MMPs promote cell migration-original paper
Gianelli G, Falk-Marzillier J, Schiraldi O, Stetler-Stevenson WG, Quaranta V. Induction of cell migration by matrix metalloproteinase-2 cleavage of laminin-5. Science 1997, 277:225-228

Summary:
Cell migration across ECM boundaries is required in several important processes, including tissue remodelling and tumor invasion. Laminin-5 is a component of basement membranes. Cells adhere to and migrate on ECM molecules by means of integrin receptors, and laminin-5 interaction with integrins has been shown to be essential for the adhesion of epithelial cells to basement membranes. In an experimental model, the so-called transwell migration assay, the migration of epithelial cells through filters was promoted specifically by laminin-5 predigested by MMP2. Pretreatment of laminin-5 by

MMP9 or plasmin (two other potentially relevant proteases) was not promigratory, nor were other basement membrane components, such as type IV collagen, laminin-1 or fibronectin. Using monoclonal antibodies that specifically block various epitopes on laminin-5, as well as on integrin receptors, the adhesion of the breast epithelial cells to MMP2-cleaved or uncleaved laminin-5 occurs via integrin a3b1, a laminin receptor. Cleavage of laminin-5 by MMP2 results in the appearance of a new epitope on the alpha3 subunit, which is not involved in cell adhesion, but directly stimulates cell motility. Analysis of various mouse tissue samples with immunoblotting demonstrated the presence of this new epitope in tissues undergoing remodelling (mammary tissue from a pregnant rat) and mouse carcinoma, whereas it was undetectable in quiescent tissues, (such as tongue or mammary tissue from a sexually immature female rat).

3.4 ECM degradation by MMPs-original paper
Several MMPs have been shown to be able to degrade several components of the extracellular matrix, here we recommend just one original paper with a few simple Figures as an illustration.

Ohuci E, Imai K, Fujii Y, Sato H, Seiki M, Okada Y. Membrane type 1 matrix metalloproteinase digests interstitial collagens and other extracellular matrix macromolecules. J Biol Chem 1997, 272: 2446-2451.

Figures 4, and 5 present data on the MT1-MMP cleavage patterns of collagen types I, II, and III, type I gelatine, fibronectin, vitronectin and laminin-1 followed by SDS-gel electrophoresis. Figure 6 shows that MT1-MMP and MMP2 synergistically cleave fibrillar collagens. Kinetic parameters are summarized in Table I.

4, Summary of the basic **wound healing** process and the role of TGFβ in it. Use the following figures from reviews.

4.1 Wynn TA. Common and unique mechanisms regulate fibrosis in various fibroproliferative diseases. J Clin Invest 2007, 117:524-529. Figure 1.

4.2 Border WA, Ruoshlati E. Transforming growth factor-β in disease: the dark side of tissue repair. J Clin Invest 1992, 90:1-7. Figure 2.

Liver fibrosis as a clinical example for abnormal tissue repair:
4.3 Iredale JP. Cirrhosis: new research provides a basis for rational and targeted treatments. Br Med J 2003, 327:143-147.