FIBROSIS

Guide to Insight into Fibrosis Development and Signaling Pathways

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INTRODUCTION

Purpose and Scope

WHAT TO EXPECT

Welcome to this Fibrosis Booklet, a document that helps scientists and researchers appreciate and navigate the cellular and signaling actors of the development of fibrotic pathologies and disorders. We hope the visuals provided in this document will help shed light on and clarify the understanding you have of these mechanisms. This document is organized around the cellular and molecular actors of fibrosis, and how they contribute to the disease develoment. An introductory section covers the fundamental notions and actors of fibrosis in terms of cells, activation pathways and extracellular matrix homeostasis. It is followed by a section dedicated to development of fibrotic disorders from wound-healing mechanisms. Details are given about the innate and adaptive arms of immunity that contribute to pro-fibrotic environments, as well as an overview of the current therapeutic approaches being explored to improve fibrosis management. The last section looks at two major organ-specific fibrotic disorders and reviews the specificity of each in terms of fibrosis development. It also gives an insight into fibrosis in the context of oncology as the fibrotic environment of tumor has become a key element in our understanding of cancer. The collection of molecular pathways presented in the document was prepared based on authentic and highly regarded articles and journals. Numbers in brackets throughout the booklet correlate with the references used, and all pathways have been curated for scientific knowledge and accuracy by Cisbio's scientific team.

WHY THIS GUIDE?

This new guide continues Cisbio-PerkinElmer's tradition of providing collections of specialized documents dedicated to different therapeutic areas such as immunology, autoimmunity, neurosciences, diabetes, and NAFLD. We also provide more practical guides covering the expertise of Cisbio's scientists in assay development and performance.

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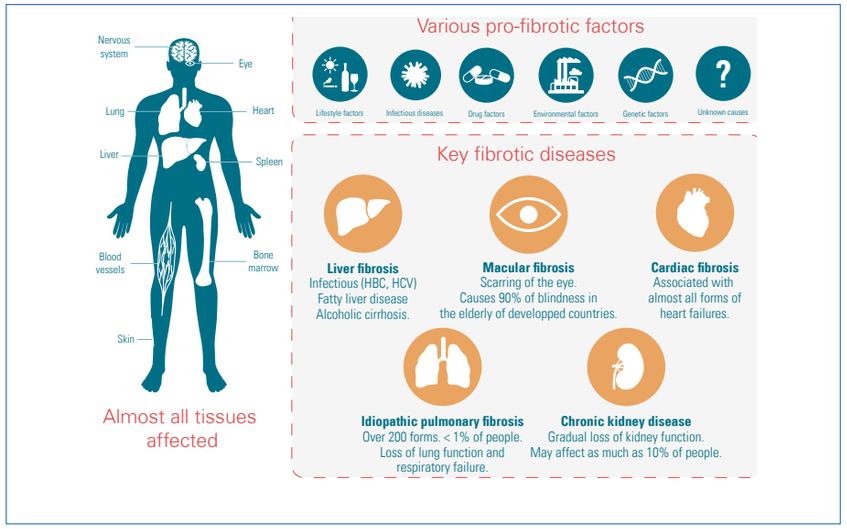
Fibrosis at a Glance

Upon injury or illness, the human body has an impressive repertoire of machinery in place for defense, self-preservation and self-healing. One important protective mechanism for tissue regeneration and wound healing is the formation of extracellular matrix (ECM), which acts as a glue holding broken tissues together, filling the space left after cellular death, and providing a medium suitable for the growth of new tissues. Fibrosis is a dangerous condition where an excessive accumulation of ECM, in response to chronic injury or illness, leads to organ disfunction and failure (1). The United States government estimates that 45% of deaths in the US can be attributed to fibrotic disorders and some examples of potentially fatal fibrotic diseases include idiopathic pulmonary fibrosis, renal fibrosis (chronic kidney disease), hepatic cirrhosis, and cardiac fibrosis. Currently, there are few treatments that can halt disease progression.

Fibrosis and its associated fibrotic disorders are prevalent pathologies that stem from inflammation mis regulation, which is a shared and central part of numerous pathologies including oncology disorders, autoimmunity, neurodegenerative diseases, and metabolic disorders such as diabetes or NAFLD. For this reason, fibrosis is an elusive disease whose causes and ramifications are multiple and make it a companion pathology to other disorders.



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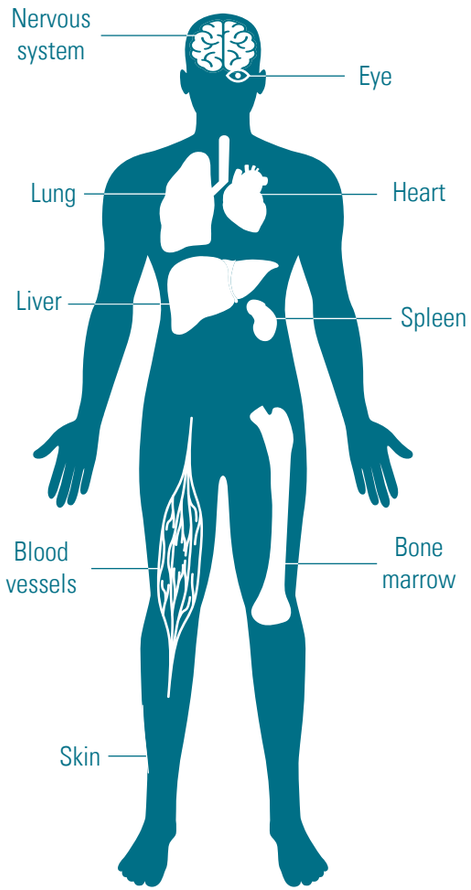

Almost all tissues affected

Various pro-fibrotic factors

Lifestyle factors | Infectious diseases | Drug factors | Environmental factors | Genetic factors | Unknown causes

Key fibrotic diseases

Liver fibrosis
Infectious (HBC, HCV)
Fatty liver disease
Alcoholic cirrhosis.

Macular fibrosis
Scarring of the eye.
Causes 90% of blindness in the elderly of developped countries.

Cardiac fibrosis
Associated with almost all forms of heart failures.

Idiopathic pulmonary fibrosis
Over 200 forms. < 1% of people.
Loss of lung function and respiratory failure.

Chronic kidney disease
Gradual loss of kidney function.
May affect as much as 10% of people.

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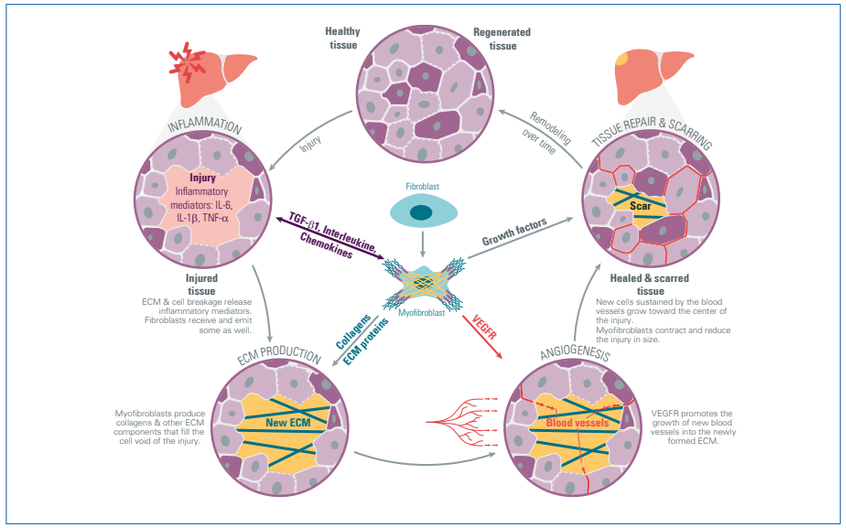
Fibroblasts and Myofibroblasts

FIBROBLASTS AT A GLANCE

Fibroblasts are one of the most abundant cell types in connective tissues, where they reside in a widely-spaced manner and anchor themselves via focal adhesion points involving integrin and fibronectin complexes connected to their actin cytoskeleton. They exhibit a typical elongated spindle shape or a stellate shape with indented nucleus morphology that is conserved throughout the whole body (2,3). Despite their conserved morphology, the gene expression profile of fibroblasts is dependent on their location and immediate environment, which results in a collection of phenotypically different subpopulations (4,5). The main variables between said subpopulations include surface markers, cytoskeletal characteristics and cytokine profiles (6). It is worth noting that fibroblasts are not a terminally differentiated cell type but rather quiescent precursors to myofibroblasts. The latter are promoted by TGF-β1 signaling and express significant amounts of cytokines, ECM proteins (extracellular matrix) and a-SMA (a-smooth actin). They have roles related to inflammation, connective tissue elaboration (ECM) and tissue mechanics (7,8,9). The involvement of fibroblasts and myofibroblasts in inflammation and ECM production made them hotspot in fibrosis research; the mechanism that regulate the differentiation step being one of their most investigated aspects.

Fibroblast are commonly split in two main groups depending on their surface expression of the glycoprotein Thy-1, which is a reliable marker for different cytokine profiles, propensity to differentiate into myofibroblasts and fibrotic properties. Thy-1-expressing fibroblasts exhibit less fibrotic properties than their Thy-1-negative counterparts. Actually, pro-inflammatory mediators IL-1 and TNF-α and pro-fibrotic TGF-β1 have respectfully be shown to induce the loss of Thy-1 expression in vitro (6,7,10).



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Fibroblasts and Myofibroblasts

ROLES OF FIBROBLASTS AND MYOFIBROBLASTS

Fibroblasts' best-known role is the production, maintenance and reabsorption of the ECM (extracellular matrix) that makes up connective tissues and holds tissues and organs together. They produce its components as well as the enzymes in charge of its degradation and remodeling. The ECM is a cell-free nutrient-rich media that connects cells and acts as an avenue for migration, differentiation and proliferation among other functions (see pages 19-22).

Fibroblasts are circumstantial actors and do not always differentiate to secrete and remodel the ECM. They work in coordination with inflammation signals which they are able to both respond to and emit via cytokines (TGF-β1, IL-1β, IL-6, IL-13, IL-33), protaglandins and leukotrienes (11,12).The processes of fibroblasts differentiation into myofibroblasts is partly under the control of those mediators, especially TGF-β1 (13). Once activated, fibroblasts and myofibroblasts are a source of cytokines and chemokines (TGF-β1, IL-1β, IL-33) on injury sites, which contribute to attract more fibroblasts and immune cells on site to resolve the injury. Additionally, the migration of those cells to the site is facilitated by the ECM secretion of fibroblasts. The inflammatory role of fibroblasts is such that they have grown to become a therapeutic topic of their own in chronic inflammation disorders (12,14,15).

In relation to their inflammatory properties and ECM secretion role, fibroblasts are major actors of the wound healing and tissue repair process. Their inflammation sensing abilities draw them to injury sites while the a-SMA-related contractile properties of myofibroblasts is key in sealing open wounds and the ECM they secrete serves as a new ground to both neutralize injury sources and grow new cells and tissues.

Finally, as part of their wound healing roles; fibroblast promote the establishment of new blood vessels through angiogenesis with two elements. First is the ECM they elaborate, which as cell-free and growth factor and nutrient-rich medium acts as an avenue for endothelial cell migration during angiogenesis. Second, fibroblasts express VEGF under the influence of TGF-β1; which targets endothelial cells' VEGFR receptor and promotes angiogenesis (16,12,17).

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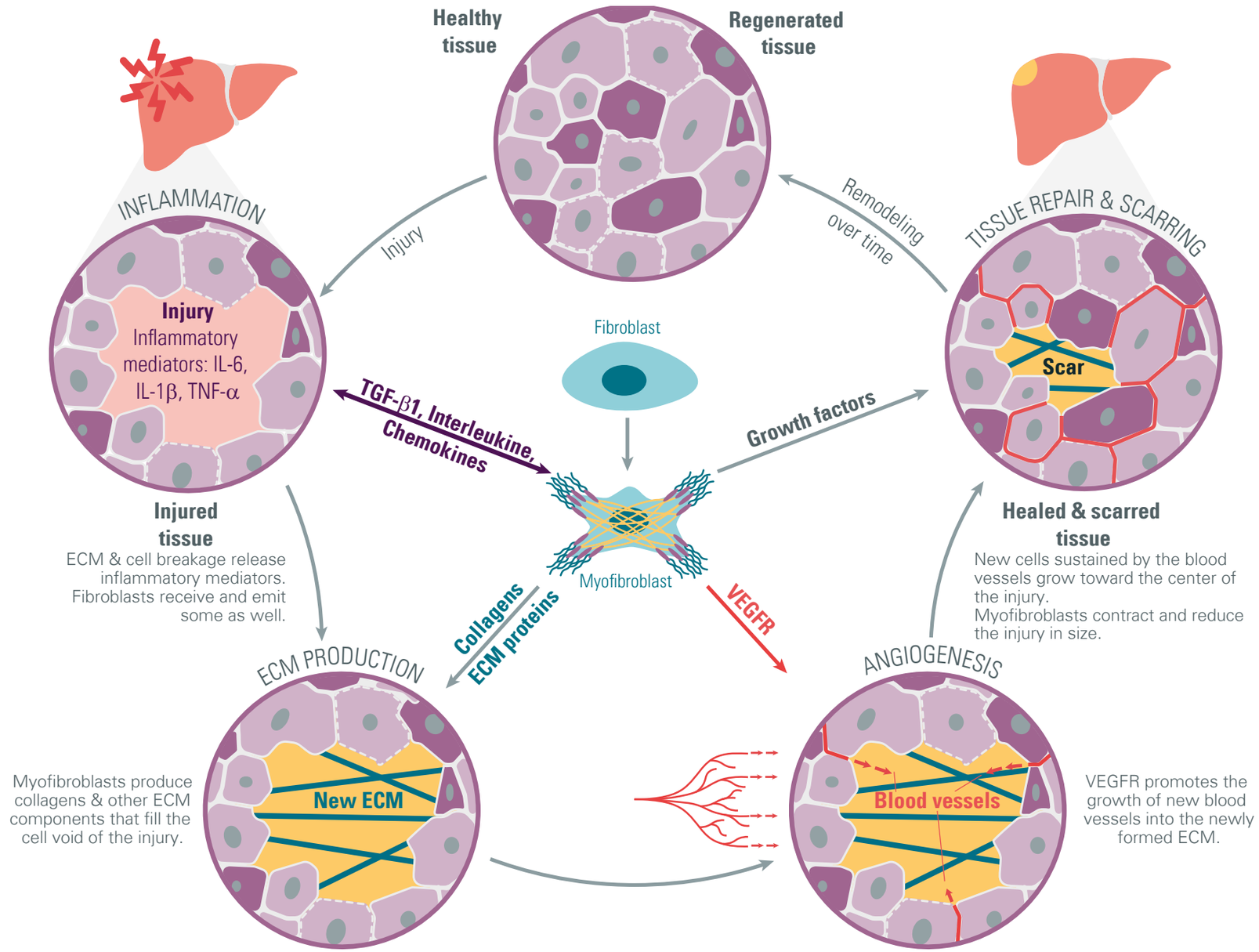

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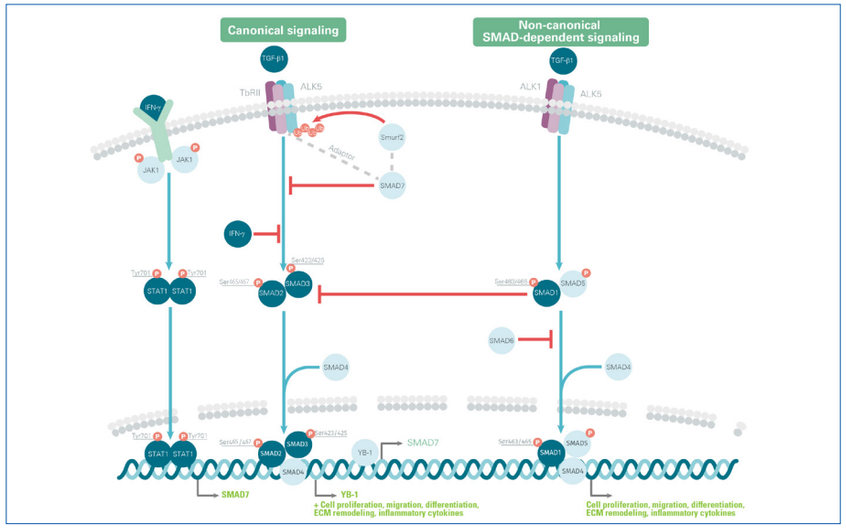
TGF-β1 Signaling

Transforming growth factor beta (TGF-b) and especially TGF-β1 is a growth factor responsible for several injury and inflammation-related mechanisms including fibroblast regulation, ECM management, immunity, immuno-regulation, proliferation, survival and differentiation. Multiple studies have pointed out TGF-β1 role in promoting fibrosis in most tissues, but its involvement in multiple systems makes it an unrealistic direct therapeutic target (18). For that reason, TGF-β1 signaling pathways and partners are usually considered better therapeutic approaches to fibrotic disorders. TGF-β1 signaling can be split in three parts: canonical SMAD-dependent, non-canonical SMAD-dependent and non-canonical SMAD-independent pathways such as ERK1/2, JNK, p38 MAPKs, JAK/STAT and ROCK kinases (19).

TGF-β1 canonical pathway is SMAD-dependent and signals through the transmembrane serine/threonine kinases TGF-β1 type II (TbRII) and type I (ALK5) receptors. TGF-β1 binds TbRII, which in turn recruits and associates with ALK5. The newly formed complex phosphorylates SMAD2 and SMAD3 which associate in the SMAD2/3 complex before recruiting SMAD4. The resulting SMAD2/3/4 complex acts as a transcription factor that promotes the expression of a pro-fibrotic phenotype (ECM genes including collagen genes COL1AL1, COL3A1, TIMP1 and 60 other ECM-related genes). SMAD7 has been identified as a regulatory feedback inhibitor for this pathway. Along with pro-fibrotic genes, SMAD2/3/4 also promotes the expression of YB-1, a transcription factor for SMAD7, which then acts as a regulatory inhibitor by competing with SMAD2 and SMAD3 for binding to ALK5. SMAD7 also acts as an adaptor between the TbRII/ALK5 complex and the ubiquitin ligase Smurf2 (SMAD ubiquitin regulatory factor), which labels said complex for degradation by proteasomes (20–25). IFN-g is also an inhibitor of this pathway that proved efficient in research on IPF (26,27). In vivo studies found it directly inhibits collagen production by the myofibroblasts via an inhibition of the TGF-β1-induced phosphorylation of SMAD3 and a promotion of SMAD7 through JAK1/STAT1 (28,29,30).

Non-canonical SMAD-dependent TGF-β1 signaling involves ALK1, another version of the TGF-b type I receptor. The occurrence of that pathway is not fully understood but seems to be cell-type-dependent. TGF-β1 binds ALK1, which in turn recruits and associates with ALK5. The newly formed complex phosphorylates SMAD1 and SMAD5 which associate in the SMAD1/5 complex before recruiting SMAD4. This step has an inhibitory effect on SMAD2/3. The resulting SMAD1/5/4 complex acts as a transcription factor that promotes the expression of a pro-fibrotic phenotype. Part of that pathway regulation is made by SMAD6, which competes with SMAD4 to associate with SMAD1/5 complex (20,25,31–33).



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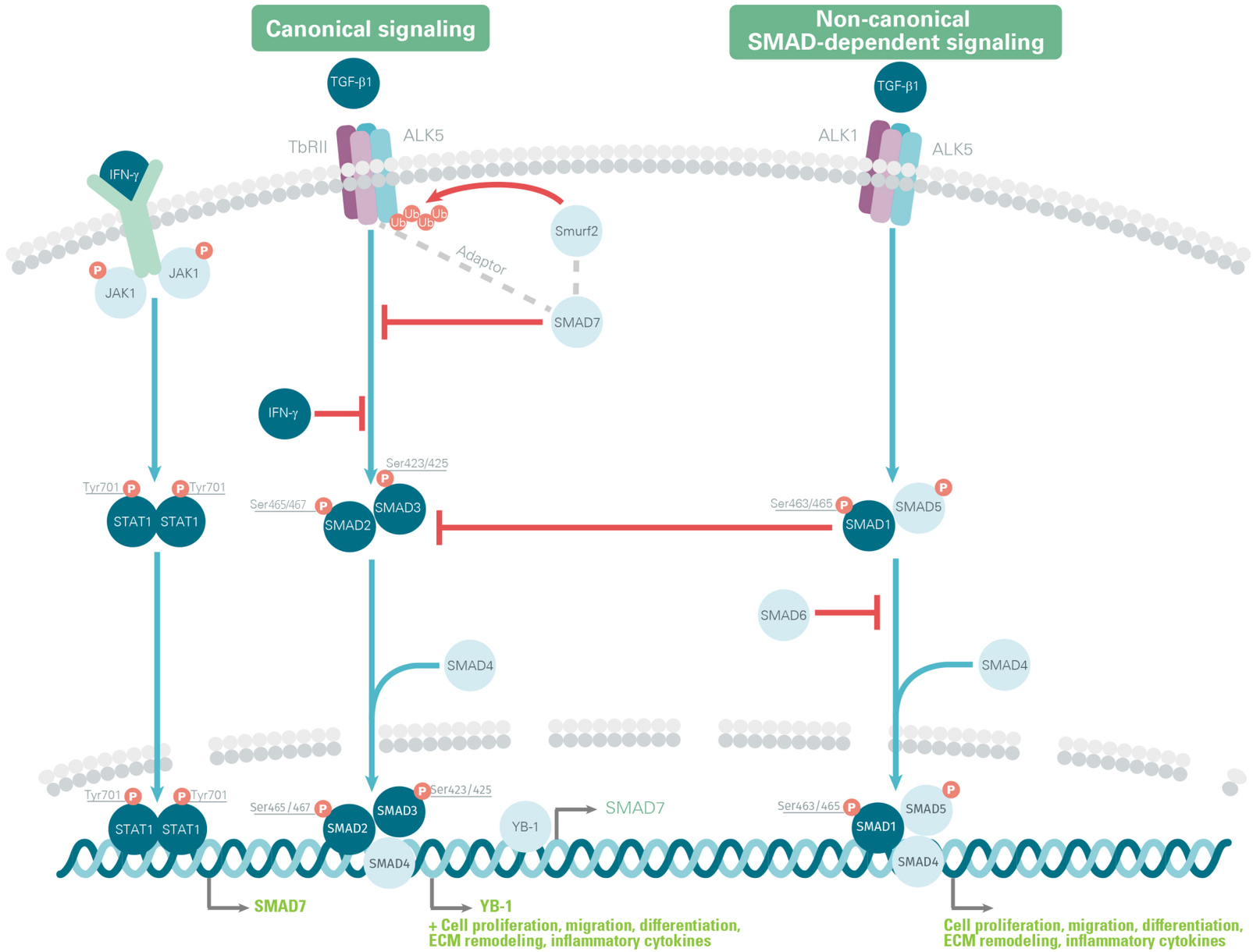

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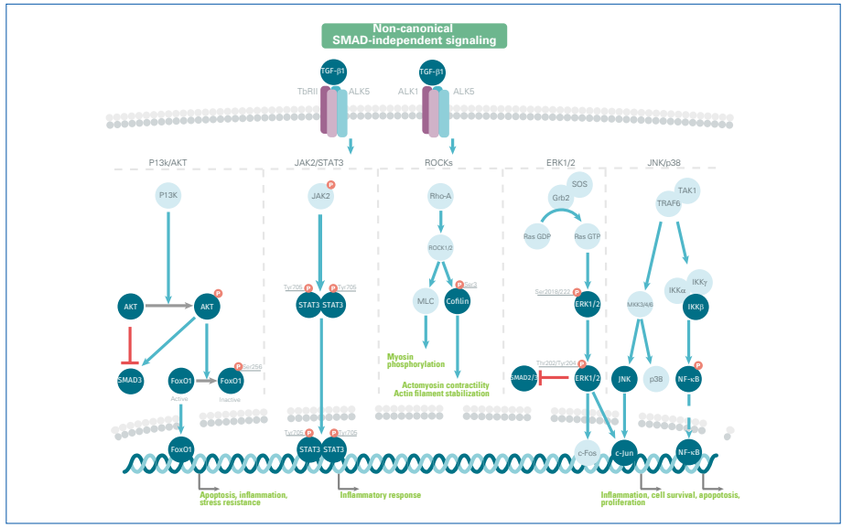
TGF-β1 Signaling

Non-canonical smad-independent pathways are also activated with results in the range of cell survival, proliferation, increase in cell size, inflammation and sometimes apoptosis (20,34–36)

- ERK: TbRII/ALK5 complex recruits Grb2/SOS complex which converts Ras GDP in Ras GTP. Raf is activated and phosphorylates MEK1/2 which phosphorylate ERK1/2. ERK goes on to promote transcription factors Fos and c-Jun. ERK can directly phosphorylate SMAD proteins in linker regions which prevents their association in complex and downregulates SMAD signaling.
- JNK/p38 MAPK: TbRII/TbRI complex recruits and signals through TRAF6/TAK1 which activates MKK4, MKK3/6 and IKK (SMAD6 acts as an inhibitor of that step). These respectively activate JNK (then c-Jun), p38 (then its transcription factors) and NFkB. JNK phosphorylate linkers in SMAD2/3 proteins which prevents association in complex and downregulates SMAD signaling.
- P13K-AKT: P13K phosphorylates AKT which inactivates FoXO (downregulation of cell proliferation). Non-phosphorylated AKT regulates SMAD3 activity by inhibiting its phosphorylation while phosphorylated AKT promotes SMAD3 activity by inhibiting the SMAD3 inhibitor GSK3.
- JAK2/STAT3: ALK5 activates JAK2, vwhich phosphorylates STAT3, which dimerizes and translocates to the nucleus where it acts as a transcription factor. The stimulation of this JAK2/STAT3 axis by TGF-β1 has been found to correlate which fibrotic phenotype in cell models. Additionally, STAT3 was found to also be phosphorylated by other kinases associated with pathological fibrotic patterns (SRC, JNK).
- ROCK kinases (Rho-associated coiled-coil containing kinase): Rho-like GTPase RhoA (also promoted by the action of SMAD2/3/4 complex on DNA) activates ROCKs (ROCK1/2), which phosphorylate MLC and cofilin. The latters respectively promote myosin phosphorylation and actomyosin contractility and actin filament stabilization, which regulates cell morphology, proliferation, adhesion and mechanical properties. This cytoskeletal regulation is thought to be linked to myofibroblast differentiation due to the importance of mechanical and cytoskeletal properties in that process (see pages 15-18).



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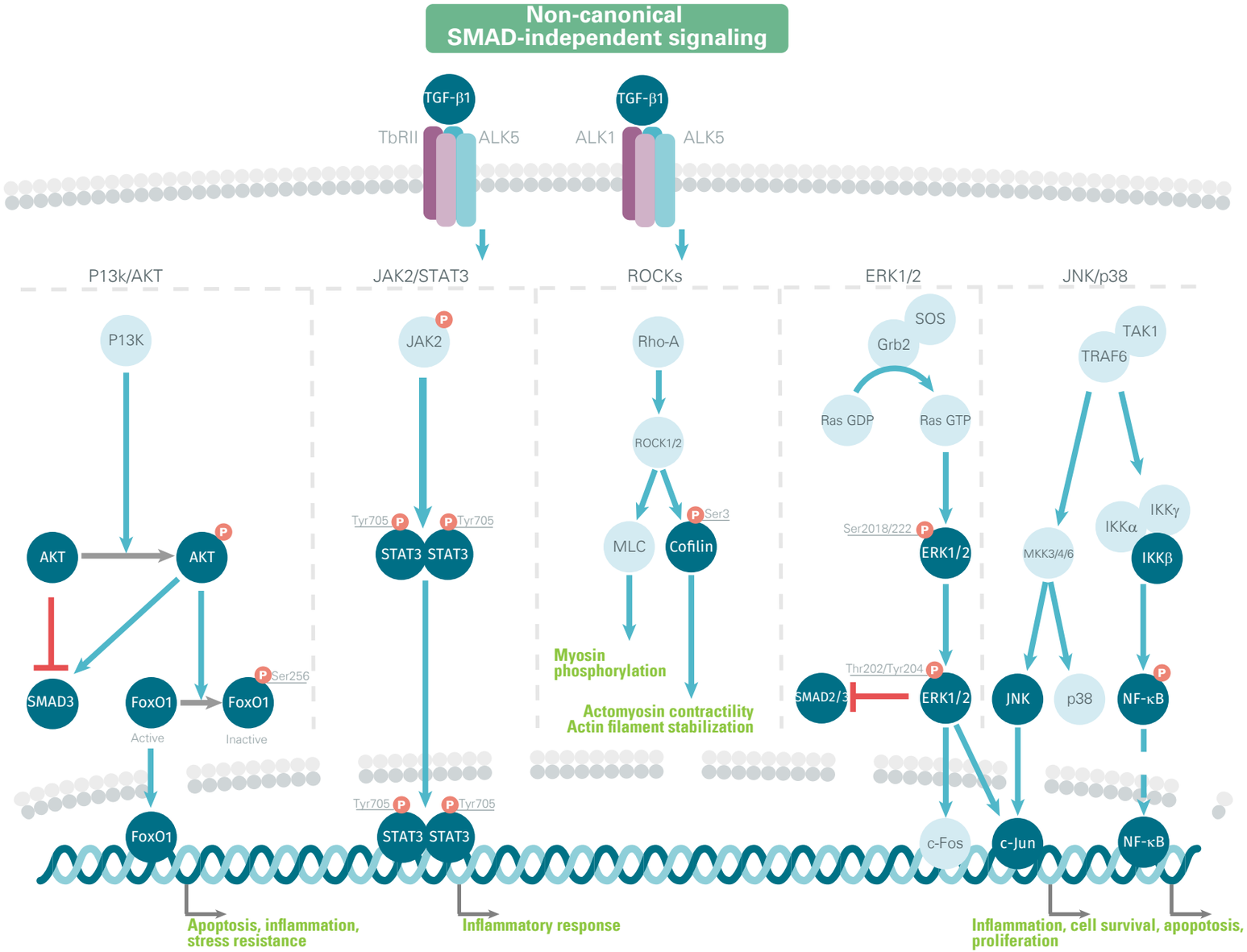

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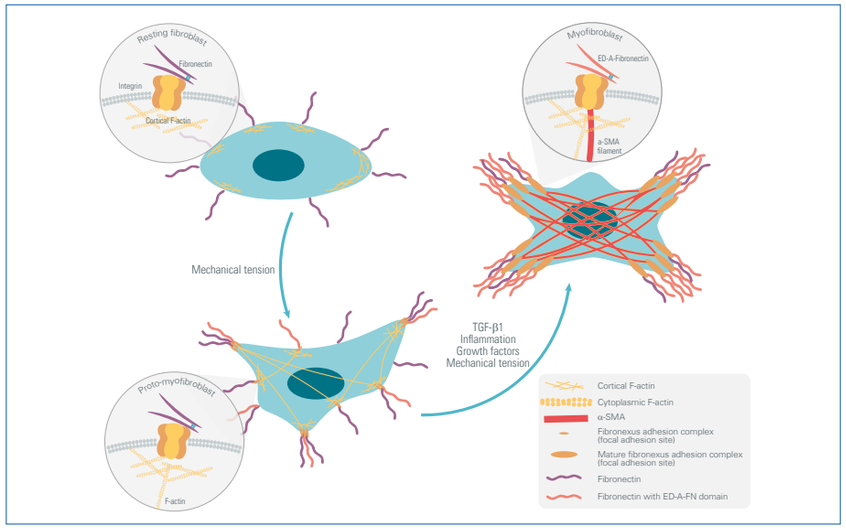
Myofibroblasts and Their Differentiation

Myofibroblasts are the terminally differentiated stage of fibroblasts, with increased ECM secretion abilities, a-SMA-rich cytoskeleton, wound healing and inflammatory properties. They appear to mostly differentiate from quiescent fibroblasts, but it has also been found they can have multiple progenitors depending on their location and therefore may arise from epithelial cells, endothelial cells, bone-marrow-borne fibrocytes, hepatocytes or other organ specific cells (37). Myofibroblasts are one of the main topics of interest in fibrosis research due to their significantly enhanced ECM secretion, which in spite of being the foundation of wound-healing, also seems to be the main out-of-control mechanism that promotes fibrotic scarring and disorders.

With the notable exception of few other mediators like IL-6, the myofibroblast ECM-production phenotype requires TGF-β1 to express itself. Additionally, in the case of IL-6, collaboration with autocrine TGF-β1 has not been ruled out. Once differentiated, that phenotype is not dependent on TGF-β1 to persist (13,38). TGF-β1 also appears to be necessary for the a-SMA expressing phenotype (39,40). It is not entirely clear in which proportions the different TGF-β1 signaling pathways contribute to myofibroblast differentiation, but non-canonical ones are believed to be involved along the canonical signaling (7,37,41).

Interestingly, ECM protein production and a-SMA expression are not distinct phenotypes but work together to achieve a fully differentiated stage characterized by an a-SMA rich cytoskeleton and outstanding secretion abilities. Some studies have found that a-SMA is necessary for the full expression of the ECM production phenotype, which suggests cytoskeletal re-organization to be a milestone on the way to fibrosis development (7,42). Additional research found the mechanical environment is critical to the full phenotype with implications of the ECM stiffness and structure. A damaged or broken ECM promotes the phenotype by introducing intracellular tension, which promotes a-SMA as a reaction (43,44).



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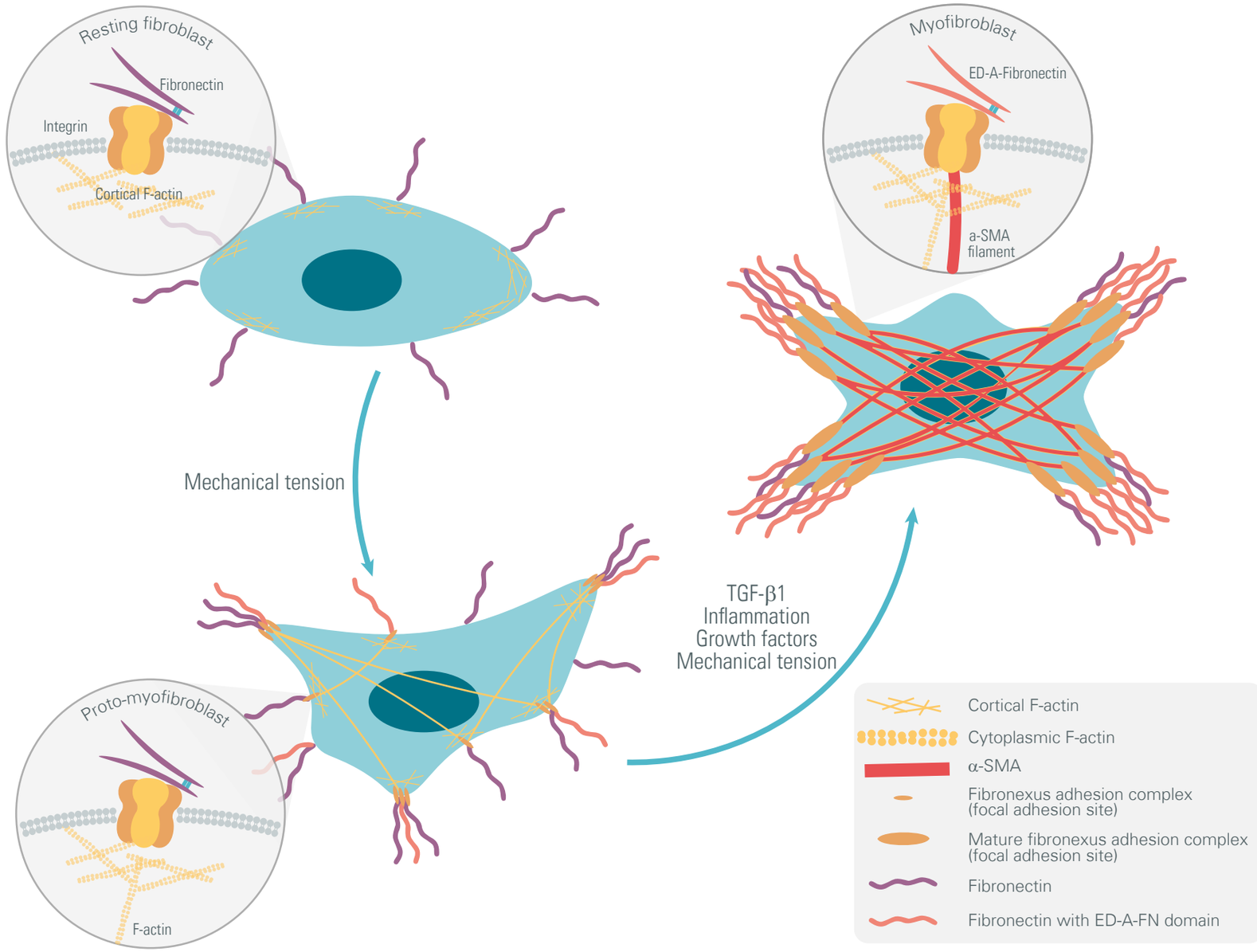

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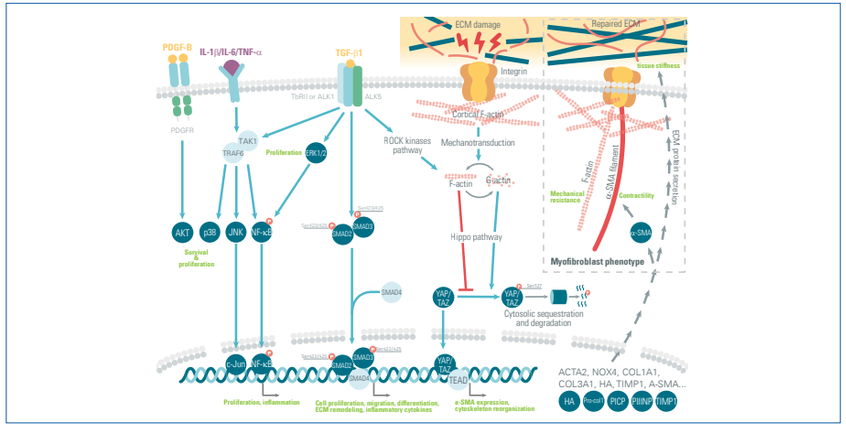
Myofibroblasts and Their Differentiation

Fibroblasts differentiation into myofibroblasts is understood as a two-stage process (37,45)

- Resting fibroblasts are usually stranded in the ECM and have low levels of ECM protein secretion and no a-SMA incorporated in their structure. They are activated by stress, either inflammatory in the form of mediators and mechanical in the form of an open wound or damaged matrix, which pull on their cortical actin cytoskeleton via the fibronectin complex that connect them the ECM. When activated, fibroblasts are drawn along inflammatory mediator gradients and migrate from the ECM to injury sites where they initiate differentiation under the influence of growth factors, especially TGF-β1, but also FGFs (22 fibroblast growth factors targeting FGFRs) or PDGF (46,47). Said growth factors are usually stored in the ECM via binding proteins, the rupture of the ECM integrity releases those and make them available for activated fibroblasts (48). At that point, fibroblasts become proto-myofibroblasts that start to organize their cytoskeleton differently and incorporate more cytoplasmic actin filaments. They also start to organize the extracellular fibronectin into fibronexus-adhesion complex (extracellular fibronectin associated to cytoplasmic actin filaments) (49). The cell begins to include focal adhesion sites to which it ties both its new actin filaments (intracellular) and fibronectin (extracellular).

- The primed proto-myofibroblast is now susceptible of getting fully activated. Three elements have been identified as critical to the full differentiation. First, TGF-β1 remains necessary at that stage. Second, the mechanical tension proto-myofibroblasts received is significantly increased by the more rigid actin cytoskeleton and anchorage points in the form of fibronexus-adhesion complexes; as the tension triggers the increased polymerization of actin filaments, those modulate the Hippo pathway and YAP/TAZ signaling, which in turn promotes the TEAD transcription factor for a-SMA and other cytoskeletal modifications (48,50,51). Some recent evidence pointed at GPCR activity for additional regulation of that mechanical trigger and found Gs and Gi proteins to have opposite pro-fibrotic and anti-fibrotic action respectively, via their control over the cAMP/PKA axis, which is associated to the cytosolic filament or globular actin state. Gq proteins are assumed to weigh in fibrosis regumation via the activation of ROCK kinases that ultimately stabilize actin filaments (52). Third, in the resting fibroblast state, the fibronectin is usually expressed without its ED-A domain (ED-A FN). When in proto-myofibroblast form, this ED-A FN is expressed and interacts with latent TGF-b binding protein-1 (LTBP-1) which in turns stores TGF-β1 in the immediate proximity of the the proto-myofibroblast and increases its availability (53). Once fully differentiated, the new myofibroblast exhibits an a-SMA-rich cytoskeleton with multiple fibronexus-adhesion complexes as anchorage points, strongly expressed ED-A-FN and enhanced ECM secretion abilities as well as secretion of MMPs inibitors TIMPs.



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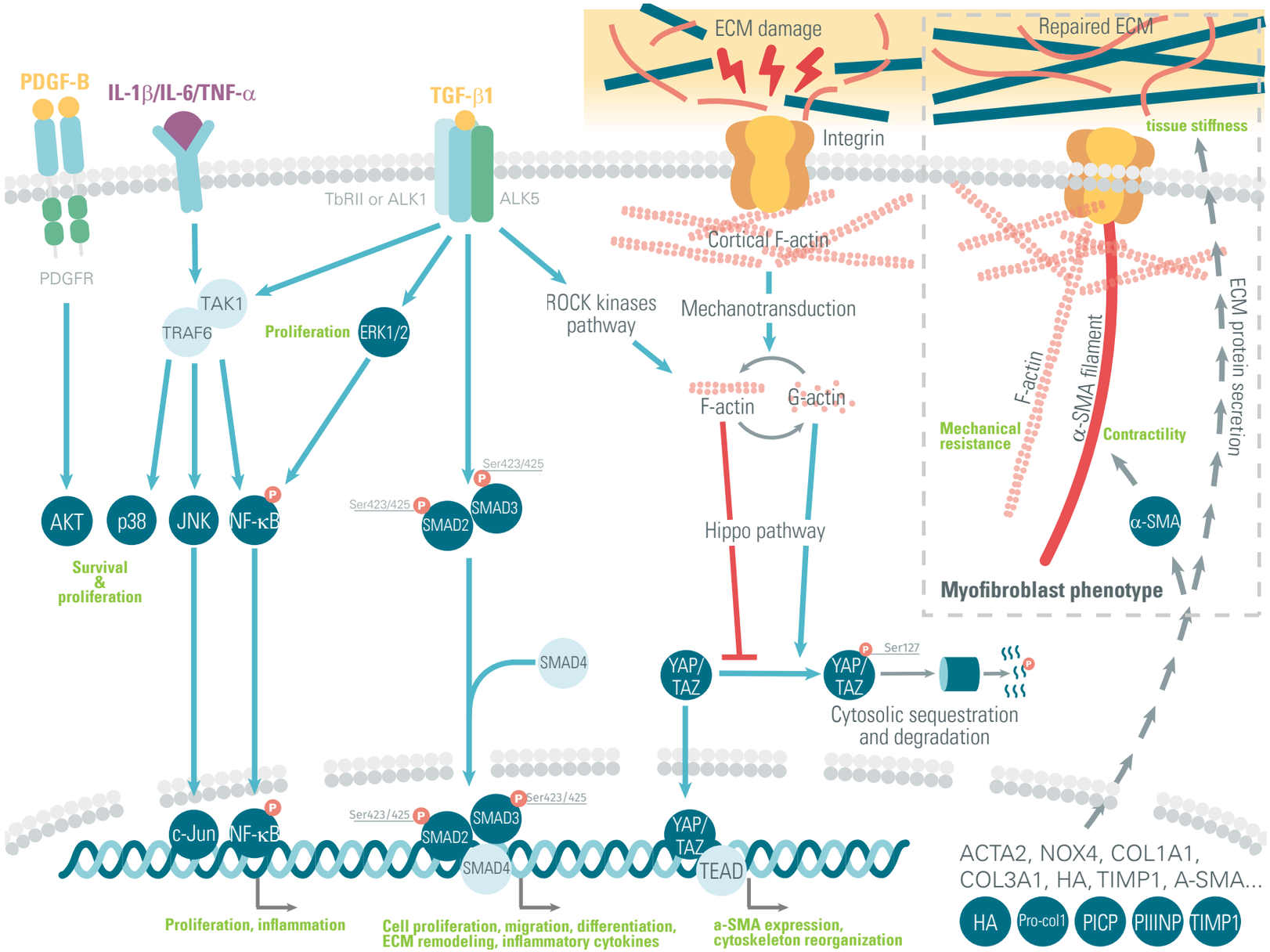

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The Extracellular Matrix (ECM)

DESCRIPTION AND COMPONENTS

The extracellular matrix (ECM) is a complex tri-dimensional and non-cellular network, made of a spectrum of proteins and organic molecules organized in a stable structure that confers parts of their mechanical properties to tissues and organs. It is a dynamic construction that is constantly elaborated and remodeled in regard to the critical role it plays in a number physiological mechanism that include but are not restricted to cell adhesion, mobility, proliferation, and differentiation (54). The ensemble of ECM components are called "matrisome" and include over 300 distinct molecules.

Structural proteins

Structural proteins are the ECM components that give the matrix its structural properties and support its architecture. Fibroblasts have a large portfolio of structural proteins they can express in an environment-dependent regulated way, which allows the mobilization of proteins with appropriate properties in different tissues and organs. The most abundant of those proteins are the collagens and elastins, which respectively confer rigidity and strength and stretchiness and flexibility. The ratios of those changes accordingly across organs. Some studies found structural proteins of the ECM may positively regulate fibrotic mechanism as they give more stiffness to the ECM, which is a mechanical trigger of the myofibroblast phenotype (see pages 15-18) (55).

- Collagens are trimeric multidomain molecules that confer stiffness and strength to tissues and the ECM and are the most abundant proteins in mammals, accounting for about 30% of the total protein mass. Collagen II was first discovered in 1962 (56) and the collagen family now includes some 28 variations (collagen I, II, III, IV, etc.) that have various properties and are expressed in different tissues across the body. Of those 28, collagen I is the most abundant and account for up to 90% of all collagens (57). They are synthetized as individual alpha chains that assemble in intracellular trimeric pro-collagen. Pro-collagens are elongated proteins that contain at least one triple helical domain with loosely spinned ends called pro-peptides (see page 22). Once secreted, pro-collagens are cleaved into mature collagens by extracellular enzymes that remove the pro-peptides. Depending on their type and tissues, collagens assemble in four supramolecular structures that are used to divide them in subfamilies (see page 22). These structures are fibrils (II, XI, IX, III,I, V), beaded filaments (VI), anchoring fibrils (VII) and networks (IV). Collagens are not only constituents of the ECM, they also contribute to cell-matrix adhesion by forming direct interaction with some cell receptors. The most significant collagen receptors are transmembrane integrins. Others include dimeric discoidin receptors which negatively regulate collagen fibrillogenesis (58), and some glycoproteins. Collagens are some of the most robust components of the ECM and substrates of the MMPs (matrix metalloproteinases), a specific set of enzymes that is responsible for their degradation in matrix remodeling (57,59).
- Elastins account for up to 90% of all ECM elastic fibers and confer tissues their elasticity and stretchiness. These proteins are essential for tissue resistance to mechanical tension (60) and are especially involved in the expansion properties of tissues like blood vessel, lungs and skin (46(61). Elastin is a highly insoluble protein and is therefore synthetized as soluble tropoelastin, which can be handled by cellular transportation means. Once secreted at the membrane, tropoelastin is converted into elastin fibers with no apparent cleavage but hydroxylation of proline residues may be at play (61,62). Elastin fibers are assembled from multiple elastin strings connected by covalent bonds (see figure on page 22).

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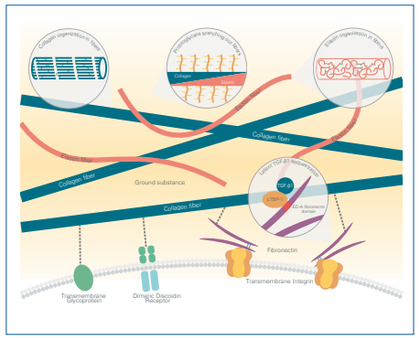
Adhesive proteins

Adhesive proteins bridge the gap between cells and the ECM and contribute to connect both. The main family of these proteins are fibronectins, which are dimers of almost identical units connected with two disulfide bonds near their C-terminal extremities. Fibronectins have a structure made of repeated FN domains of three types and may incorporate alternatively spliced domains (12,63). The repeated and alternative domains respectfully include RGD domains that interact with the transmembrane protein integrin for adhesion; and the ED-A-FN domain, which is expressed under the influence of stress and inflammation, and interacts with LTBP-1 (latent TGF-β1 binding protein 1) to binds TGF-β1 in close proximity of the cells and promotes its signaling that way (53). In relation to its TGF-β1 storage abilities, fibronectin may be involved in regulating the availability of TGF-β1 in fibrotic context (64). Another family of adhesive proteins are the laminins, which also interacts with integrins to anchors cells to basal laminas (deep skin layer type of ECM incorporating collagen IV).

Ground substance

The ground substance of the ECM refers to an ensemble of hydrated gels constituted of proteoglycans, that fill the space surrounding structural protein fibers. It is a very permeable medium that acts as an avenue for biomolecules like nutrients and mediator. Its cell-free and nutrient-rich nature makes it a medium in which cells can move freely, whether for migration purposes in the cases of fibroblasts and immune cells, or for proliferation purposes in the context of wound healing, angiogenesis or invading tumors (12). Proteoglycans are structured around a core protein that carries multiple glycosaminoglycans (GAGs) branching out. The GAGs are of four types: hyaluronan, chondroitin sulfate and dermatan sulfate, heparan sulfate and heparin, keratan sulfate. They all exist in the ECM attached to a core protein except for hyaluronan that may exist on its own. Proteoglycans are highly hydrated and hold significant amounts of water in a sponge-like way, making the ECM a water-rich medium (90% of the ECM is water held by proteoglycans) and providing additional compression resistance (65).



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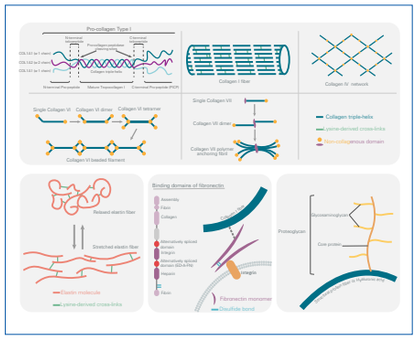

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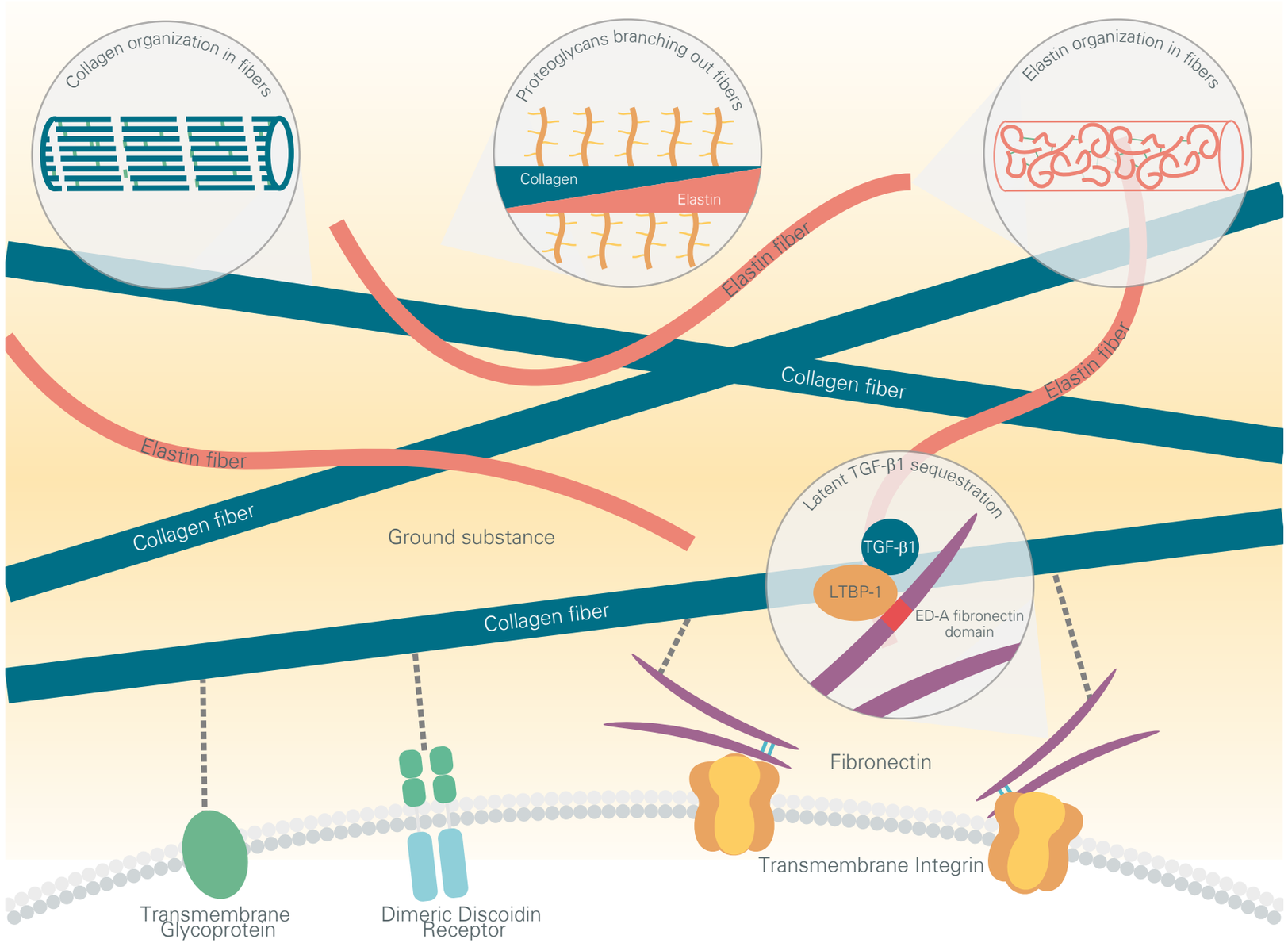

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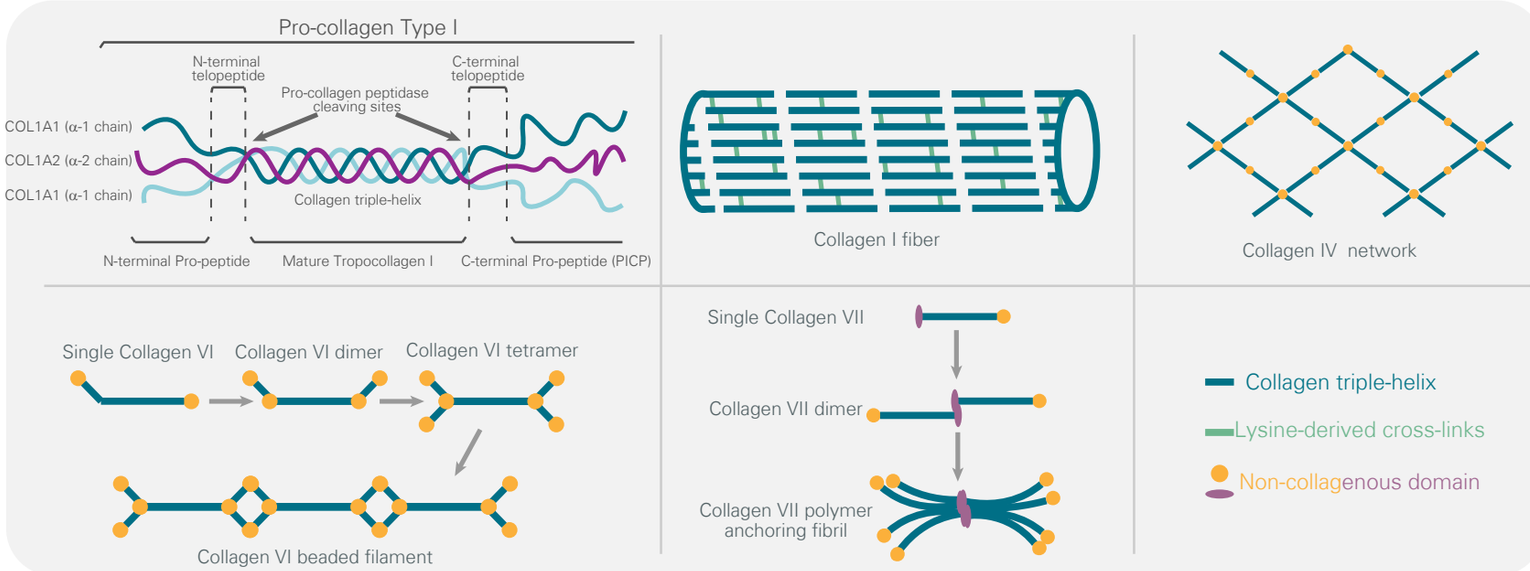

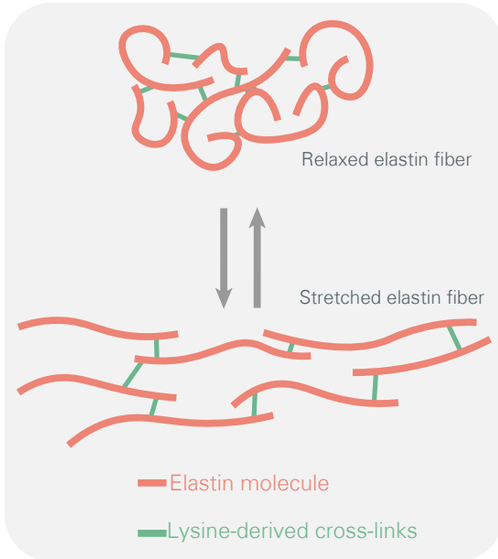

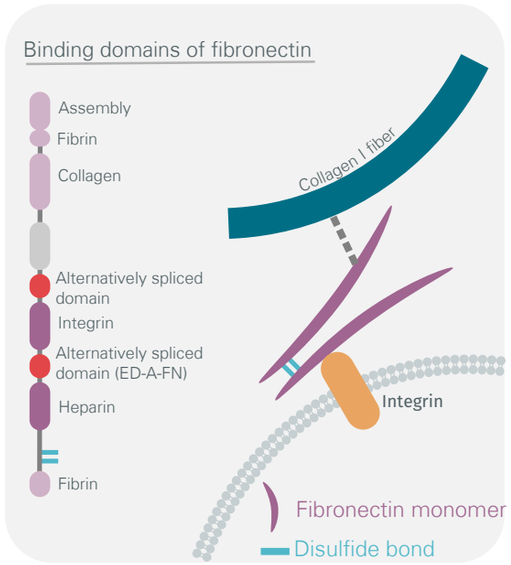

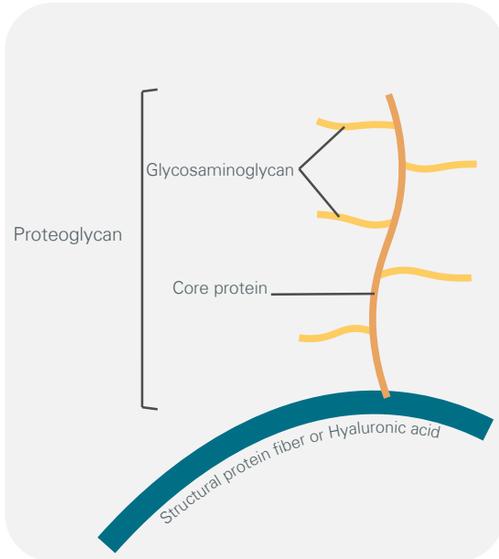

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Homeostatic regulation of the ECM

The homeostatic regulation of the ECM is the collection of mechanisms by which the elaboration, degradation and subsequent reabsorption of the ECM is performed in a balanced way that allows for the healthy maintenance and remodeling of the matrix when prompted to by cellular events (differentiation, migration, apoptosis, etc.). The current understanding is that fibroblasts/myofibroblasts are solely responsible for both sides of that balance, being the ones to elaborate and degrade the ECM (12).

On the elaboration side, the secreted structural proteins need to be assembled into fibers. For collagens and elastins, this assembly step is performed by LOX enzymes (Lysyl oxidase), which catalyze lysine-derived cross-links between collagens or elastins (see page 22), resulting in the supramolecular structures that can support the ECM (66,67). The regulation of LOX is not fully described but it is understood to be upregulated as a result of TGF-β1 signaling, both canonical (SMAD2/3) and non-canonical (p13 MAP kinase pathway). Inflammatory cytokines IL-1β and TNF-α were also found to promote its expression (68).

On the degradation side, ECM components and especially structural proteins are robust and may only be degraded by specific enzymes. These are split in the three main families of MMPs (Matrix Metalloproteinases), ADAMs (A Disintegrin And Metalloproteinases) and ADAMTS (ADAMs with Thrombospondin motifs), plus a few specialized proteases such as elastase (54,69,70). While the ADAMs and ADAMTs are essentially “shedding” proteases that mostly deal with cell-matrix adhesion structures and proteins, the MMPs are potent proteases involved in the deep deconstruction of the ECM.

There are about 28 MMPs that are split in six subtypes depending of their organization, location and substrates (see page 24); the MT-MMPs are notably membrane bound (Membrane Type-MMPs) (71). The regulation of MMPs is complex and multifactorial, even though it is not fully understood, all pathways involved seem to converge toward JNK and its resulting activation of transcription factors c-Jun and c-Fos. The main regulation axis seems to be TGF-β1 non-canonical signaling via the ERK and JNK/p38 MAP kinase pathways but other triggers such as various growth factors and cytoskeleton-related mechanical tension may be involved too, particularly in the expression MMP2 and MMP9 (72). TGF-β1 canonical signaling may be at play as some SMAD proteins are known to participate in the upregulation of MMP9 through an undescribed mechanism. Cytokines are also involved in MMPs regulation. Pro-inflammatory IL-1β in particular promotes them via the NF-kB pathway or via p38 MAP kinase, which on top of promoting NF-kB directly, also mediates transcription factors c-Jun and c-Fos activation (73). MMPs are secreted or expressed at the membrane as precursors and their activity is regulated by the TIMPs (tissue inhibitors of metalloproteinases) and by themselves, as they activate each other in a complex regulatory network (see figure on page 24).

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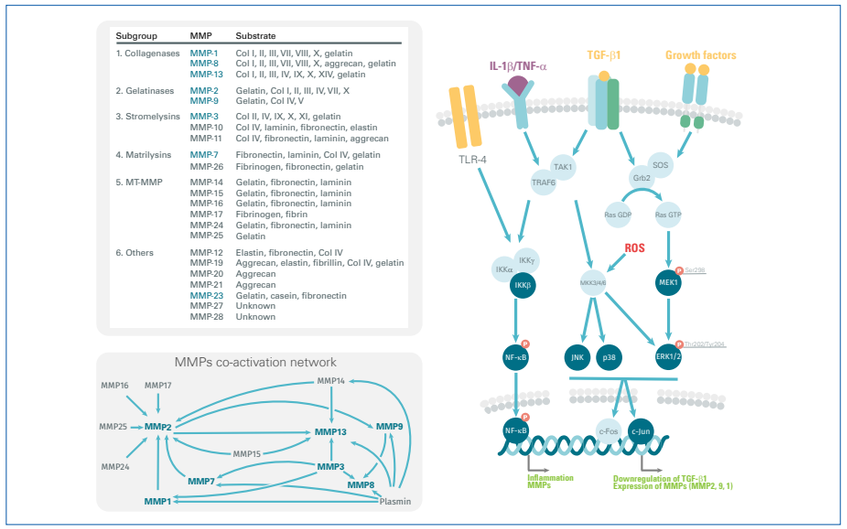
In relation to their ECM degradation role, MMPs are credited with a responsibility for releasing the growth factors that are stored within. These include TGF-β1, VEGF, PDGF and FGFs (fibroblast growth factors). In the case of TGF-β1; MMP2, 9 ,13 and 14 are known to directly release it from its ECM-associated sequestration complex with latent TGF-b binding proteins (74). Along with ADAMs and ADAMTs, the MMP family proteases also play a role in altering cell-matrix adhesion by shaving transmembrane proteoglycans of the syndecan family off of cells (74). Finally, along their contribution to ECM management, MMPs fulfil roles in the range of inflammatory response. Pro-inflammatory TNF-α and TLR4 signaling have been identified as promoters of MMPs, which in turn become promoters of immune T-cells and apoptosis via the caspase pathway.



Subgroup	MMP	Substrate
1. Collagenases	MMP-1	Col I, II, III, VII, VIII, X, gelatin
	MMP-8	Col I, II, III, VII, VIII, X, aggrecan, gelatin
	MMP-13	Col I, II, III, IV, IX, X, XIV, gelatin
2. Gelatinases	MMP-2	Gelatin, Col I, II, III, IV, VII, X
	MMP-9	Gelatin, Col IV, V
3. Stromelysins	MMP-3	Col II, IV, IX, X, XI, gelatin
	MMP-10	Col IV, laminin, fibronectin, elastin
	MMP-11	Col IV, fibronectin, laminin, aggrecan
4. Matrilysins	MMP-7	Fibronectin, laminin, Col IV, gelatin
	MMP-26	Fibrinogen, fibronectin, gelatin
5. MT-MMP	MMP-14	Gelatin, fibronectin, laminin
	MMP-15	Gelatin, fibronectin, laminin
	MMP-16	Gelatin, fibronectin, laminin
	MMP-17	Fibrinogen, fibrin
	MMP-24	Gelatin, fibronectin, laminin
	MMP-25	Gelatin
6. Others	MMP-12	Elastin, fibronectin, Col IV
	MMP-19	Aggrecan, elastin, fibrillin, Col IV, gelatin
	MMP-20	Aggrecan
	MMP-21	Aggrecan
	MMP-23	Gelatin, casein, fibronectin
	MMP-27	Unknown
	MMP-28	Unknown

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	MMP-16	Gelatin, fibronectin, laminin
	MMP-17	Fibrinogen, fibrin
	MMP-24	Gelatin, fibronectin, laminin
	MMP-25	Gelatin
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	MMP-19	Aggrecan, elastin, fibrillin, Col IV, gelatin
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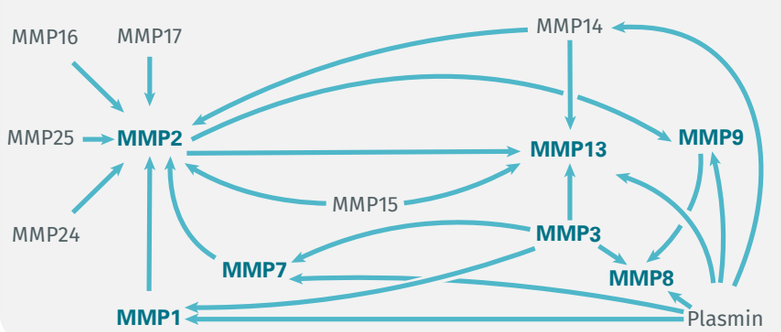
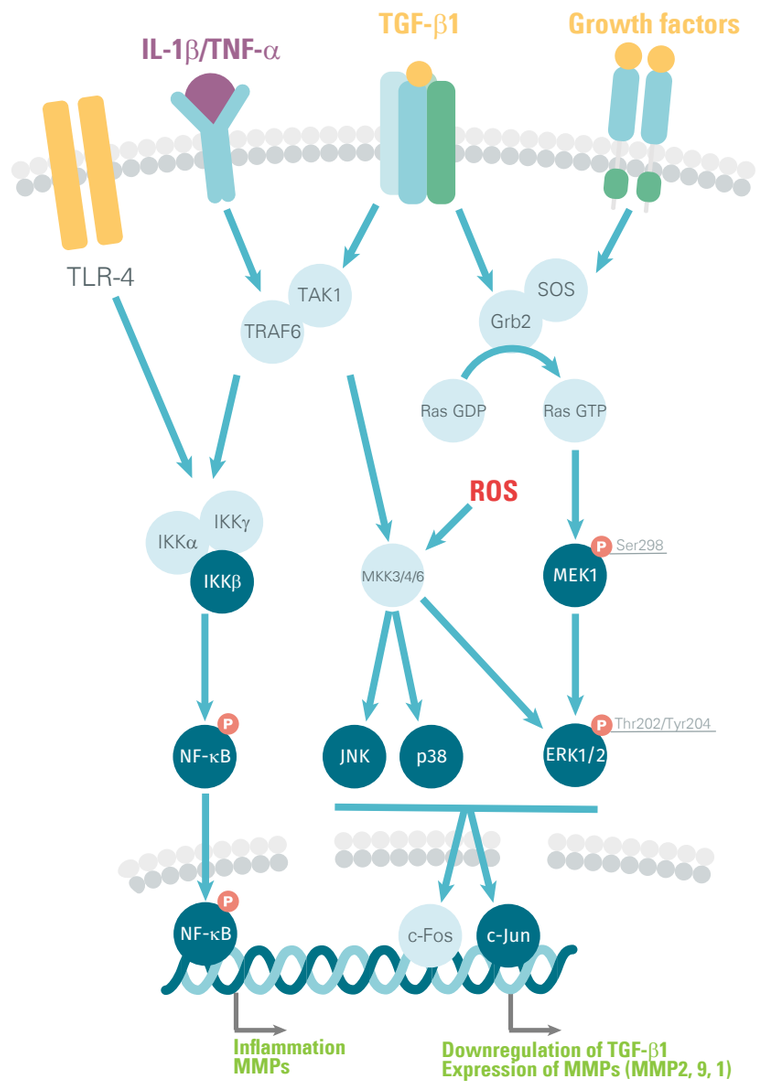
MMPs co-activation network





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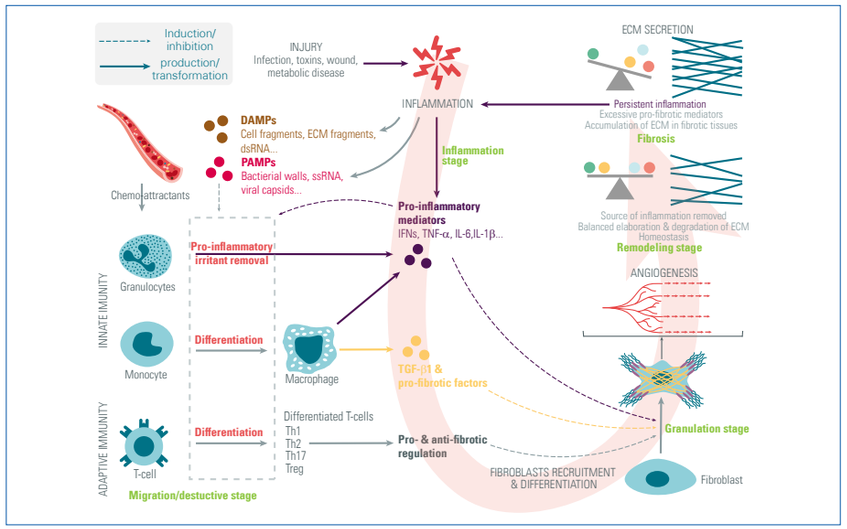
WOUND-HEALING, TISSUES REPAIR AND FIBROSIS

Overview

Fibrosis as a pathology is defined as an abnormal and excessive deposition of ECM (extracellular matrix) in tissues to the point where connective tissues replace parts of organs' functional tissues, which, among other things, can result in organ disfunction and makes fibrosis a main contributor to wide-ranging organ failures: systemic sclerosis (SSc), idiopathic pulmonary fibrosis (IPF), liver cirrhosis, kidney fibrosis, cardiac fibrosis observed in cardiac hypertrophy, etc. Fibrosis as a process, however, is not a systematically pathological event and is an important part of wound-healing and tissue repair in response to injuries (open wounds, infection, inflammation, etc.).

Following an initial inflammation stage where cytokines (interleukins, chemokines, TNFs, IFNs) and growth factors are released by damaged and dead cells at the injury site. Innate immunity cells are drawn to the injury and activated by coagulation factors in open wounds and/or by the inflammatory cytokines secreted by the local tissue cells (IFNs, TNF-α, IL-6, IL-1β). This is the migration/destructive stage. Innate immunity cells come in two waves: the first is the local macrophages residing in the injured tissues. the second is drawn to the injury by gradients of chemokines (notably CCL2) (75) and involves granulocytes (mostly neutrophils), dendritic cells and monocytes from the blood, the latter of which differentiate into more macrophages on site. All those cells have a dual role. On the one hand they remove the source of the injury (infection, necrotic cell fragments, foreign bodies) and clean the wound via phagocytosis. They also digest the remnants of broken cells, ECM, and other biological remains that could promote inflammation in the tissues.

On the other hand, they aim to restore the tissues to their homeostasis state. The macrophages are key in that process due to the spectrum of phenotypes they can express, which range from potent pro-inflammatory and pathogen-removing cells to growth-factor-secreting and wound-healing promoting agents (See pages 29-30). Tissue damage and the abundance of cytokines can also directly activate cells of the adaptive immunity and more specifically T-cells which differentiate into helper and regulatory cells (Th and T-reg) with the purpose of stimulating and regulating other immune cells.



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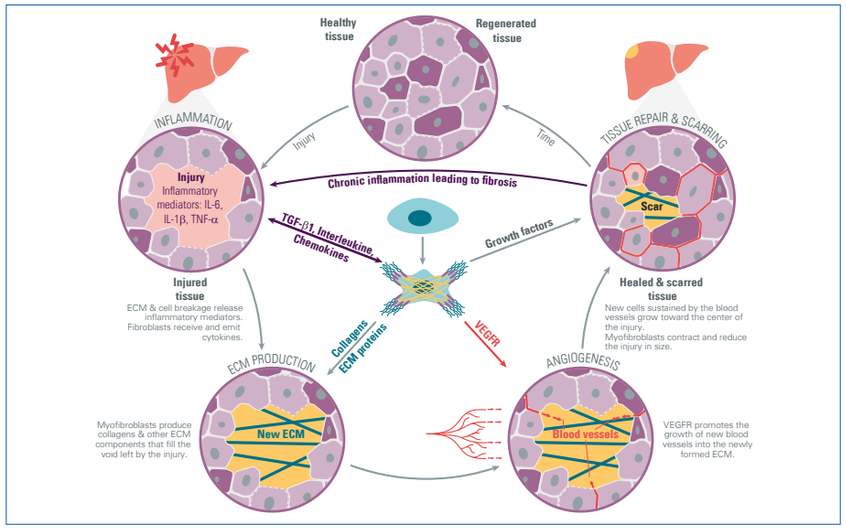
This mobilization is followed by the granulation/proliferation stage and the recruitment of a new cell type on site. Fibroblasts residing in the ECM surrounding the injured tissues are activated by the TGF-β1 produced by macrophages and/or released on site by the injury-related ECM breakdown. Following that gradient, they migrate from the ECM to the injury where they differentiate into myofibroblasts (see pages 11-18). Myofibroblasts then secrete new ECM at the site, which effectively ties and provides mechanical strength to the injured tissues. The addition of ECM is critically important for growing new blood vessels and cells as it is a favorable medium for both. Along with macrophages, myofibroblasts also promotes angiogenesis (new blood vessels) via VEGF (vascular endothelial growth factor) secretion.

At the end of that whole process, tissues repair with a mix of two things: regeneration, which is the ideal like-for-like replacement of damaged and dead cells. And fibrosis/fibrotic scar tissues, which are the remains of the ECM produced at the injury due to structural proteins of the ECM (collagens) being long-lived robust molecules. Over time, in a balanced and inflammation-free environment, these scar tissues are removed and re-organized while the persistence of myofibroblasts on site promotes the injury reduction in size thanks to their contractility. This remodeling stage is the last of tissue repair.

Pathological fibrosis, however, occurs when the long-term remodeling and removal of ECM suffers from a higher production than removal rate. This event is associated with chronic inflammation, which keeps myofibroblasts active for extended periods, thus making them and their ECM protein secretion the main effectors of pathological fibrosis. Causes for pro-fibrotic chronic inflammation are varied and include failure to eliminate the initial inciting factors (infection, foreign bodies), chronic inflammatory metabolism or behavior (sugar/fat metabolism in diabetes and NAFLD/NASH, smoking habits, etc.), or mis-activation and regulation of inflammatory and pro-fibrotic signals by immune cells (macrophages, adaptive immunity regulatory or helper T-cells, etc.) (75,76).



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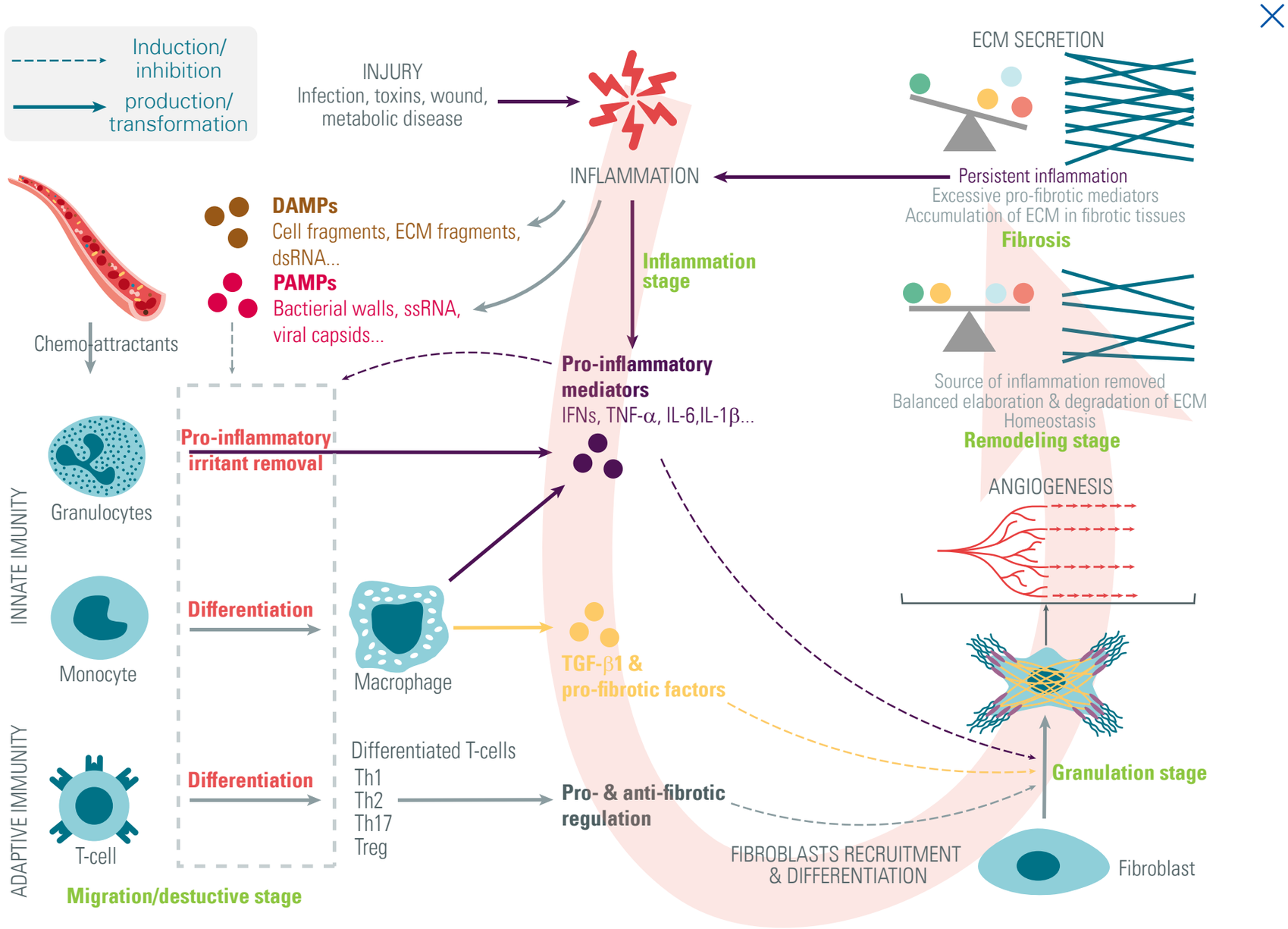

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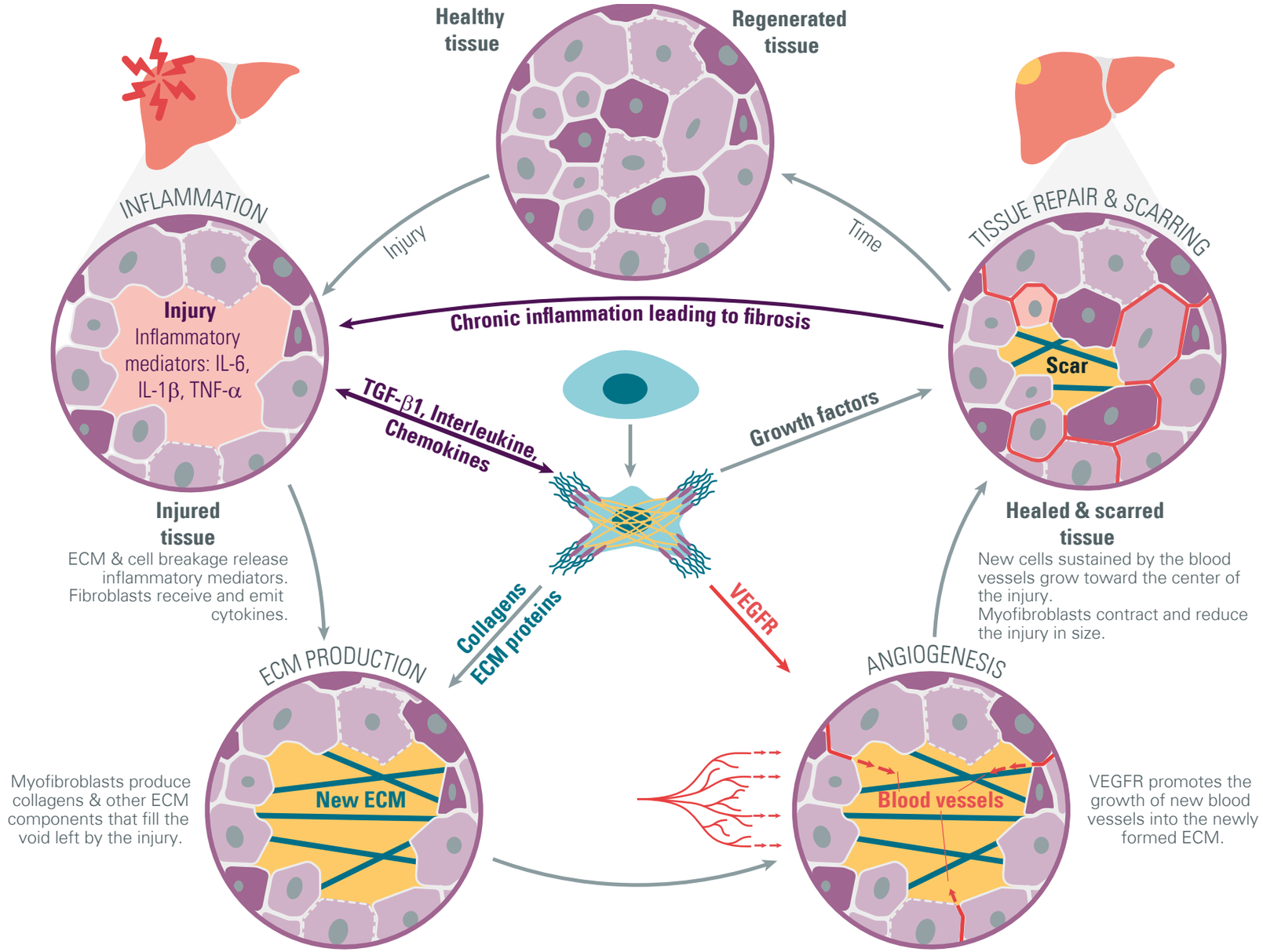

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WOUND-HEALING, TISSUES REPAIR AND FIBROSIS

Innate Immunity Actors of Fibrosis

MACROPHAGES

Macrophages are professional phagocytic immune cells that arise from bone-marrow precursors differentiating into parent monocytes in the peripheral blood before they infiltrate tissues where they become resident macrophages. They are plastic cells that respond to their microenvironment and fulfill homeostasis-maintenance missions that include tissue modeling, recycling, and clearance of dead cells and the removal of pathogens such as necrotic debris, bacteria, toxins, etc. Because of their tissue-resident nature, they are usually the first immune cells to confront an injury and are responsible for initiating the recruitment of other specialized cells.

Macrophages are divided in two main categories that exhibit opposed and complementary phenotypes. Classically activated macrophages (CAM) or M1 are induced by a combination of TLR signaling and IFN-g. They are potent antigen-presenting cells with strong pro-inflammatory cytokine expression (IL-6 and TNF-α), making them key promoters of T-cell expansion and further enhancing their own phenotype. They also exhibit high levels of lysis compounds and regulator nitric oxide that empower them against pathogens. They are the pathogen-clearing pro-inflammatory population of macrophages.

Alternatively activated macrophages (AAM) or M2 are induced by IL-4 or IL-13, and are especially involved in anti-parasite and anti-fungus responses. The fatty acid receptor CD36 has also been identified as a promoter of this phenotype over M1. They express high levels of arginase (enzyme required for DNA synthesis), TGF-β1, and the immunosuppressive cytokine IL-10. The M2 designation encompasses a third subtype worth mentioning: regulatory macrophages, induced by IL-10 and expressing higher levels of that cytokine than conventional M2 (77,78). It is important to note that due to their highly plastic nature, macrophage categories of phenotypes are not homogeneous and clearly defined, but rather exist in a spectrum of populations which are not restricted to the characteristics of their most prominent phenotype. Additionally, these main categories are divided into multiple subsets of tissue-specific macrophages whose phenotypes are dependent on the tissues they reside in (microglia in central nervous system, Kupffer cells in liver, osteoclasts in bones, alveolar macrophages in lung, etc.)(77,79).

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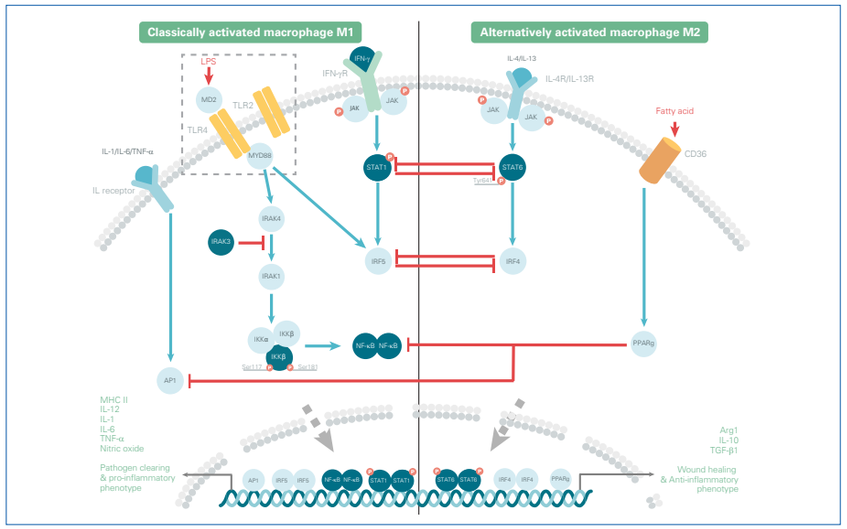
Innate Immunity Actors of Fibrosis

In relation to fibrosis, macrophages have controversial roles which may result from the spectrum of phenotypes they express, and that puts them on either sides of pro- and anti-fibrotic actors. They are sources of strong chemoattractants like CCL2, which are essential for recruiting granulocytes and monocytes from the blood and fibroblasts from the ECM (75,80). This, at terms, promotes the presence of inflammatory cells on site and the fibrotic activity of myofibroblasts. There does not seem to be a consensus on M2 macrophages in particular. They are a key source of TGF-β1 in inflamed tissues, which indicates pro-fibrotic outcomes (81). They also secrete other growth factors that make them fundamental drivers of fibroblasts differentiation and contributor to the wound-healing and tissue repair process (16,17). However, they express IL-10 and the subtype of IL-10-induced M2 macrophage is a major source of that anti-inflammatory cytokine. Some studies have concluded on the antifibrotic nature of that phenotype due to it being a source of said IL-10 and arginase, which also has anti-fibrotic properties (82,83). Along with the action of arginase, the anti-fibrotic role of this phenotype would be mediate in collaboration with the differentiation of IL-10-dependent regulatory T-cells (T-reg), whose anti-inflammatory properties are anti-fibrotic (84).



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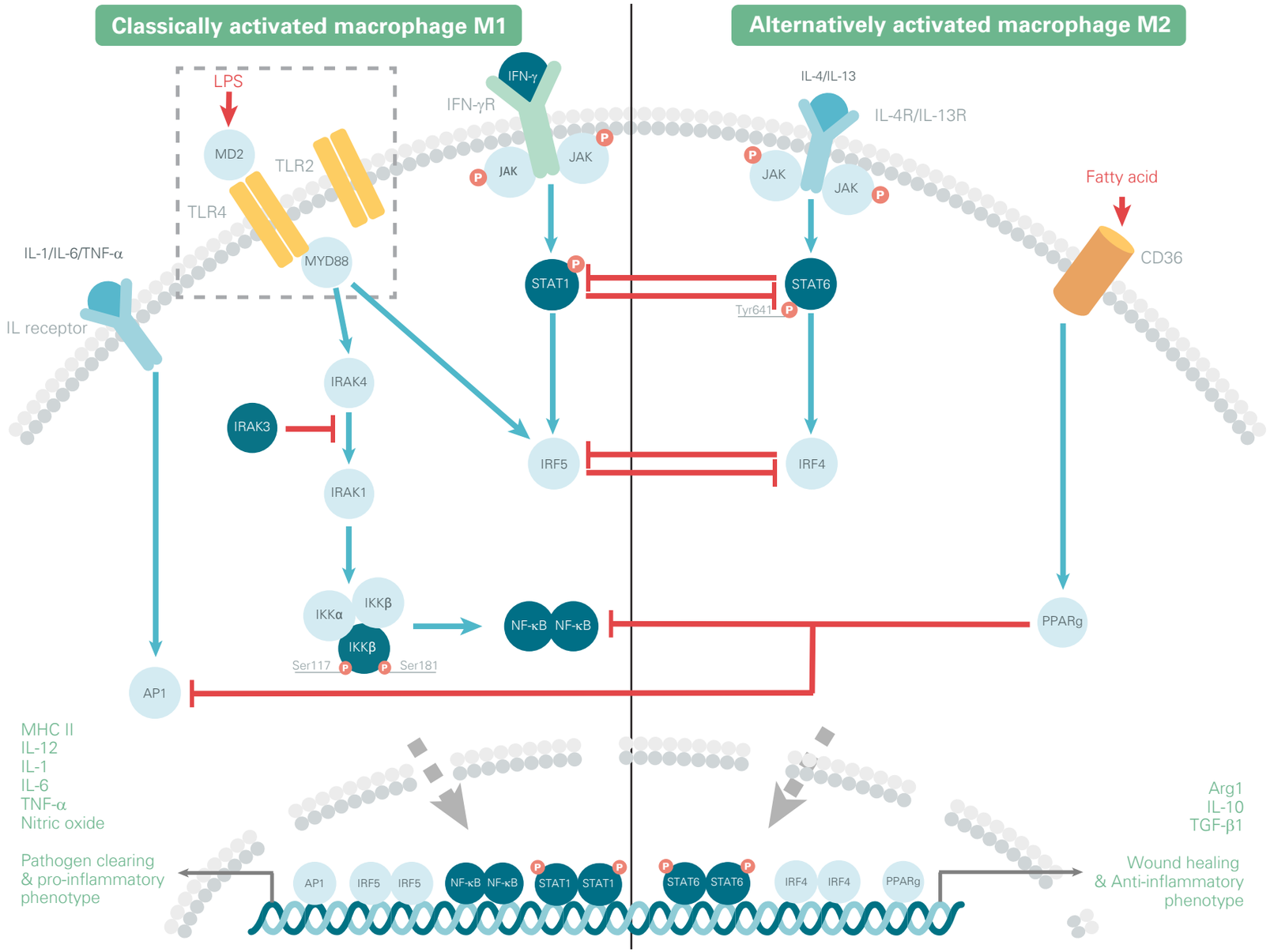

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Innate Immunity Actors of Fibrosis

OTHER INNATE RESPONDERS

Monocytes and differentiated macrophages have key roles in the development of fibrosis but other innate immunity cells have also been identified as relevant actors of the disease and investigated as such.

Neutrophils are short-lived cells (few days) circulating in blood. They are phagocytic cells and clear small pathogens via intracellular lysosomal degradation. They are also capable of attacking all kinds of large pathogens with the proteolytic enzymes, cytotoxins, antimicrobial peptides, and other molecules their granules contain (85). Neutrophils are typically the first immune cells recruited on inflammation sites after resident macrophages activation. In inflammatory contexts their lifetime extends and they accumulate on site, which greatly elevates the local inflammation and promotes the recruitment and stimulation of more immune cells. Neutrophils can perform an unusual type of cellular death in the form of NETosis, which is the release and assembly of DNA in extracellular NETs (Neutrophill Extracellular Traps) that act as pathogen traps (86,87). In relation to fibrosis, neutrophils are understood to have pro-fibrotic effects for several reasons. Their fast recruitment on inflammation site where they degranulate cytotoxic content may sustain the inflammation in time and aggravate it. This cytotoxicity-induced inflammation is thought to promote chronic inflammation in collaboration with Th17 cells that promote neutrophil accumulation on site via IL-17A (75,88). Additionally Recent research suggested the cellular content they release by NETosis could result in pro-fibrotic, pro-inflammatory effects that outlast them in tissues (89). They have been observe to accumulate in the lungs of IPF patients, where they are a reliable marker of poor outcomes (90).

Eosinophils mostly reside in epithelia in normal conditions. Their granule content includes cytotoxic compounds (eosinophil cationic protein (ECP), eosinophil peroxidase (EPX), and major basic protein (MBP)) that enable the extracellular destruction of most kinds of pathogens; and cytokines such as IL-4, IL-8, IL-10, IL-13 and TGF-β1, which modulate both innate and adaptive immunity. As phagocytes, they also remove pathogens via intracellular digestion (91–94). Relatively to fibrosis, eosinophils are both a source of pro-fibrotic TGF-β1 and IL-13 as well as a source of cytotoxic pro-inflammatory compounds (95,96). They have been identified as contributors to IPF, liver fibrosis and myofibroblasts activation in skin and the IL-5 upon which depends their differentiation and recruitment is also considered a pro-fibrotic mediator. Studies however suggest they are not drivers of fibrosis of their own but rather exacerbating agents (96–98).

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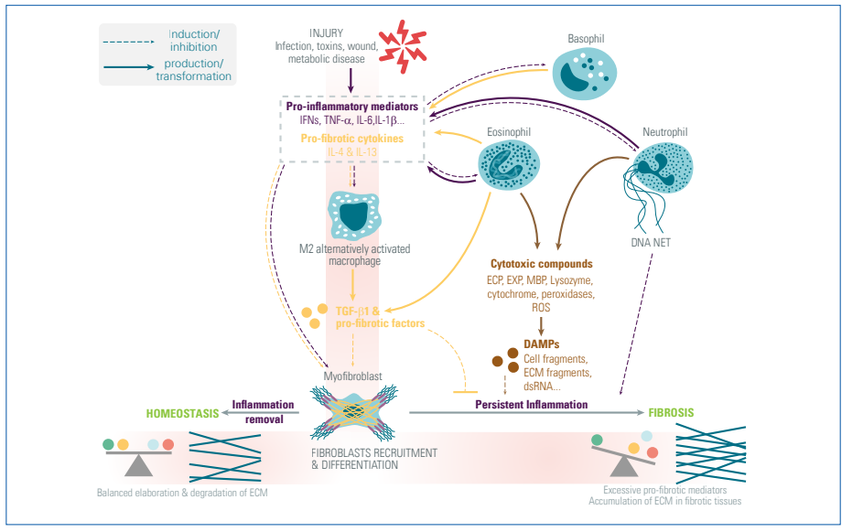
Innate Immunity Actors of Fibrosis

Basophils circulate in peripheral blood, have the shortest lifetime among granulocytes (1 to 2 days) and are usually recruited in tissues upon inflammation and allergic disorders. Their granules have no pathogen-killing content but make them strong modulators of inflammation in allergic contexts (anticoagulant heparin, chemoattractant leukotriene (LTs), and platelet activating factors) (94,99). They retain a phagocytic activity which is especially efficient against IgE immune complexes they bind with their FceRI receptors. When active, basophils express high levels of IL-4 and IL-13 (42), which modulate allergic response, dendritic cell activity, and macrophage polarization toward tissue repair (TGF-β1-secreting phenotype). They have been observed to contribute to Th2 differentiation but are not required for that process (94,101). They also have been linked to myelofibrosis (102) and their secretion of IL-4 and IL-13 suggest pro-fibrotic effects.



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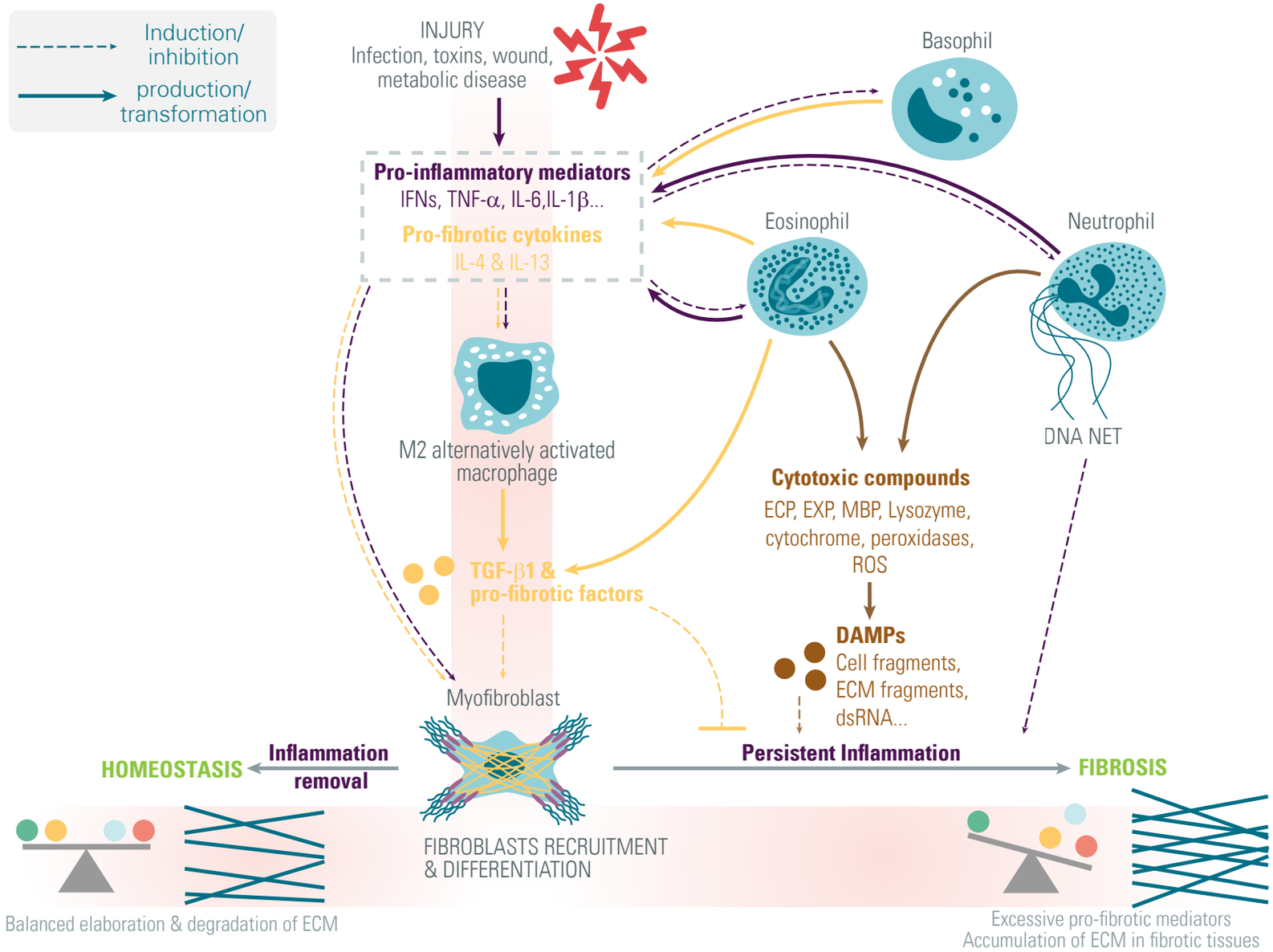

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Adaptive Immunity Actors of Fibrosis

Helper T-cells are a family of differentiated effector CD4+ T-cells whose activity supports, activates, and promotes the actions of other immune cell types. Their contribution to the development of an adaptive and humoral immune response is critical, as antibody-secreting B-cells and cytotoxic CD8+ T-cells are unable to perform the full extent of their missions without their support. They exist in several subtypes with different phenotypes and roles.

- Th1 helper cells differentiate in presence of IL-12 and promote the activity of phagocytic cell macrophage through a consistent expression of IFN-g. They also play a key role in adaptive cytotoxicity, by providing the IL-2 necessary for the activation of CD8+ T-lymphocytes. In the context of fibrosis, Th1 appear to have anti-fibrotic properties mediated via their secretion of IFN-g, which disrupts TGF-β1 canonical signaling and inhibit collagen production (30). As the Th1 phenotype promoter, IL-12 was found to have antifibrotic properties in in vivo models (75,103).
- Th2 helper cells arise from stimulation by IL-4, and enhance pathogen clearing through stimulation of eosinophils, basophils, and mastocytes with IL-4, IL-5 and IL-13. They also contribute to the humoral response by providing IL-2 for B-cell activation and sustain the overall inflammation with the pro-inflammatory IL-6. It is worth noting that they mediate their own differentiation in a positive IL-4 feedback loop. In fibrosis development, Th2 are believed to be drivers of fibrotic disorders where chronic inflammation is sustained by an immune response against persistent immunogens. In this process they would promote the wound-healing TGF-β1-secreting macrophage phenotype M2 via their production of cytokines IL-13 and IL-4 (104,105) and promote fibroblasts growth via IL-13-induced JAK2/STAT6 signaling that promotes PDGF-AA (106) . Consequently, IL-4 is also investigated as a pro-fibrotic mediator due to its role in Th2 differentiation (107).
- Th17 helper cells are induced by IL-6, IL-21, and TGF-b, and are potent pro-inflammatory cells that drive neutrophils and macrophage to infection sites with their secretion of IL- 17A, IL-17F, IL-21, IL-22, and CCL20. Due to their inflammatory functions, defects in their behavior are suspected of contributing to many autoimmune diseases (108). In relation to fibrosis, Th17 are thought to create lasting pro-inflammatory environment via their secretion of IL-17A, which is a pro-inflammatory cytokine promoting long-term neutrophil presence on site, which in turns sustain inflammation via the degranulation of their cytotoxic content and are known to correlate with poor outcomes in IPF patients (75,88,90).

Regulatory T-cells or T-reg are a subtype of effector CD4+ T-cells whose phenotype and role make them down-regulators of the activation and proliferation of other CD4+ or CD8+ effector T-cells. It has been reported that they could also have downregulation behaviors toward B-cells and dendritic cells (109). Their proliferation and function are sustained by IL-2, which they do not express but is however produced by other effector T-cells. This results in a regulating loop that ensures T-reg deactivation and decrease as they downregulate effector T-cells, effectively starving themselves in IL-2 (110).

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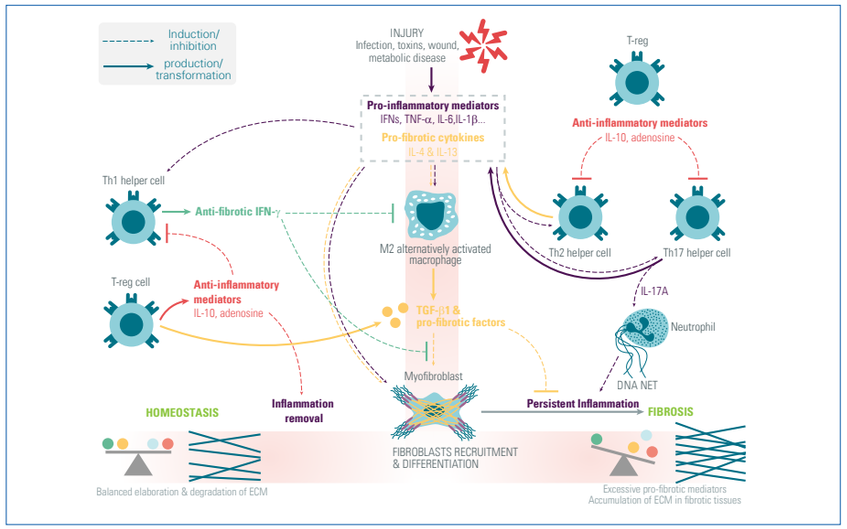
Adaptive Immunity Actors of Fibrosis

The contribution of T-reg to fibrosis remains unclear and seems to depend on the situation. It is, though, commonly admitted that they are more anti- than pro-fibrotic. T-reg have anti-fibrotic effects via their anti-inflammatory properties and effectively promote the de-escalation from an inflammatory state to homeostasis by inhibiting the activity of other activated T-cells and removing them from the circulation. The main suppressive messengers T-regs express and secrete is IL-10 (111). T-regs also strongly promote the immunosuppressive metabolite adenosine (109) by expressing the transmembrane CD39 and CD73, which respectively convert ATP to AMP and AMP to adenosine (112,113). More radically, T-reg directly reduce effector T-cell populations via the cytotoxic granzyme/perforin axis that induces cellular death (114,115). Finally, T-reg consume IL-2 via CD25 which serves as a pump, reducing its availability for effector T-cells, leading them to an anergic state (116). However, T-reg produce significant amounts of pro-fibrotic TGF-β1, and some studies found they could induce fibrosis rather than inhibit it (75,117,118).



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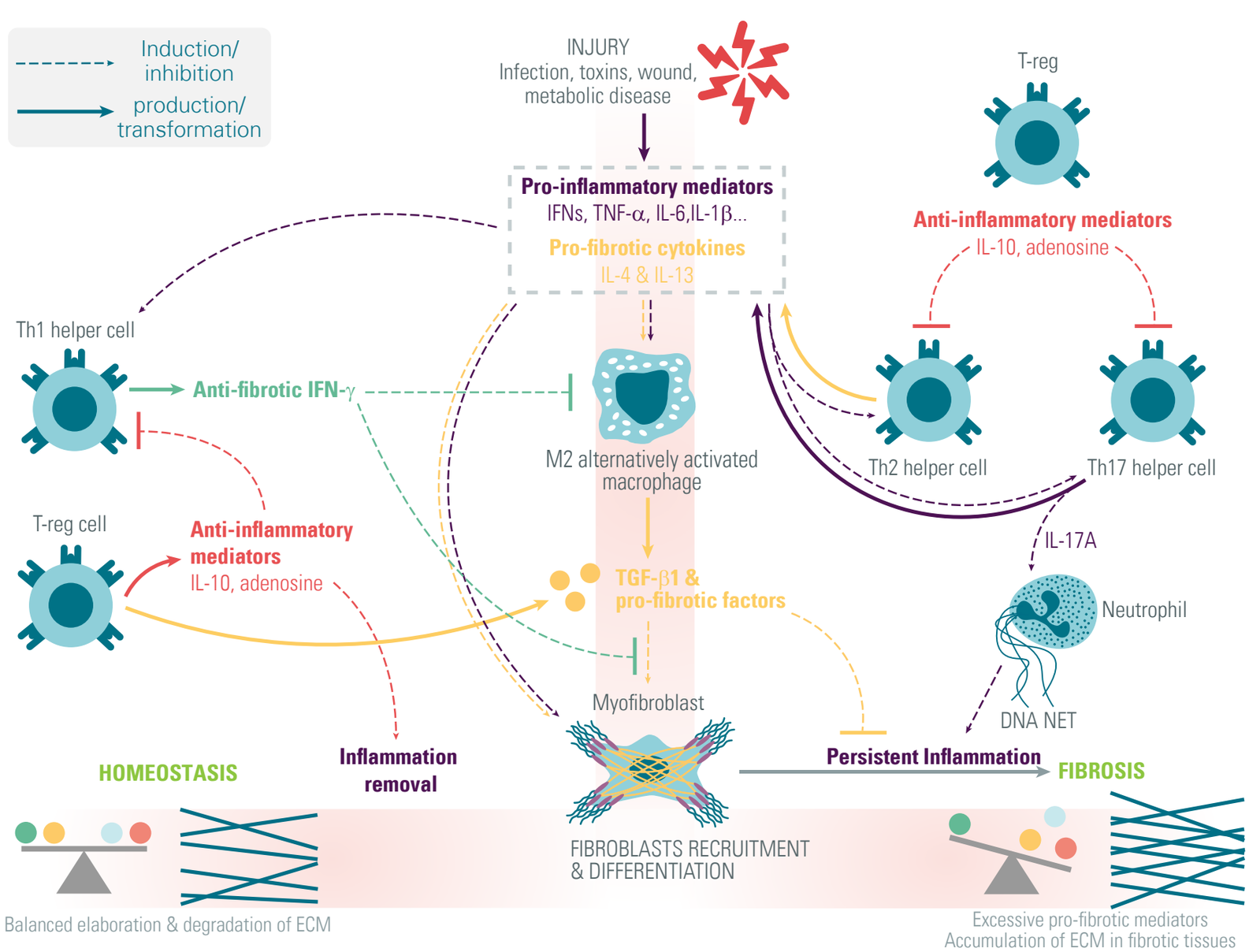

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Treating and Reversing Progressive Fibrosis

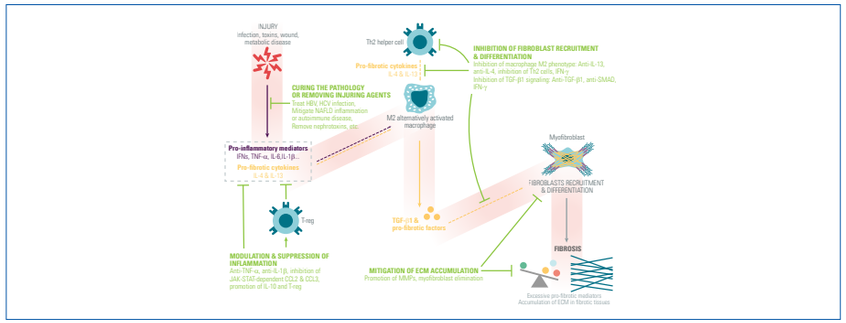
The regression of ECM deposit in fibrotic tissues (and the resulting reversal of fibrosis) is an event that is always associated with the removal of chronic inflammation. For this reason, curing the pathology or removing the injuring agents that originated the inflammation is the most effective way to reverse fibrosis, as shown in the case of viral-infection-induced liver fibrosis (HBV, HCV), which reverses after the infection is cured (119). Regression or reversal of fibrosis is possible to an extent and documented for different pathologies where immunosuppressive therapies have been key (120–123). However, the complete reversal of advanced fibrosis with restoration of the original tissue architecture remains discussed, with current knowledge suggesting it is not possible.

On top of the removal of chronic inflammation, fibrosis reversal is associated with a number of events, including decrease in TGF-β1, reversed unbalance between pro-fibrotic and anti-fibrotic signals and declines in myofibroblasts populations (8,119,121). Anti-fibrotic therapies therefore target pro-fibrotic signaling at multiple levels: inhibition of fibroblasts migration, differentiation, proliferation, promotion of myofibroblasts cellular death, inhibition of collagen secretion, promotion of collagen degradation (increase MMPs, decrease TIMPs), etc. (121,123).

Current therapeutic approaches being investigating and deemed credible are numerous. Modulation and suppression of inflammation is one. In that regard, Pro-inflammatory cytokine antagonists (anti- TNF-α, anti- IL-1β) have had moderate success in experimental mouse/rat models (124,125) and TNF-α antagonist Etanercept showed promising result in IPF progression in humans (126). Inhibition of the pro-inflammatory JAK-STAT pathway is explored (127), as well as inhibitors of chemokines that attract monocytes and fibroblasts (CCL2 and CCL3) (80). Finally, the use of anti-inflammatory IL-10 is also an alternative being investigated with research showing that its absence in mouse models promotes more severe fibrotic outcomes (128,129).

Inhibition of fibroblast activation and differentiation is another major area of research in fibrosis; with studies looking to inhibit TGF-β1 expression, activity or signaling. As ECM protein-secreting myofibroblasts are the main effectors in all types of fibrotic pathologies, any approach that successfully regulate them would have wide-ranging anti-fibrotic applications (76). With fibroblast activity, Th2-produced IL-13 (and IL-4 in a lesser way) is a potential target due to it increasing fibrotic outcomes via TGF-β1 signaling and being a promoter of the TGF-β1-secreting macrophage phenotype (104,105). IL-4 is investigated too as a promoter of T-cells differentiation into Th2, which then express IL-13 and are pro-fibrotic (107). The use of IFN-g is also investigated for its anti-fibrotic disruption of TGF-β1 signaling (28,29), but with weak results in patients (130).

Other approaches include the elimination of collagen sources (myofibroblasts) via pro-apoptosis or anti-survival signaling (inhibitors of ERK), modulation of the expression of MMPs (matrix metalloproteinases) as they are the direct mediators of matrix degradation and fibrosis reversal, and promotion of T-reg recruitment and activity for their anti-inflammatory properties and anti-Th2 effects.



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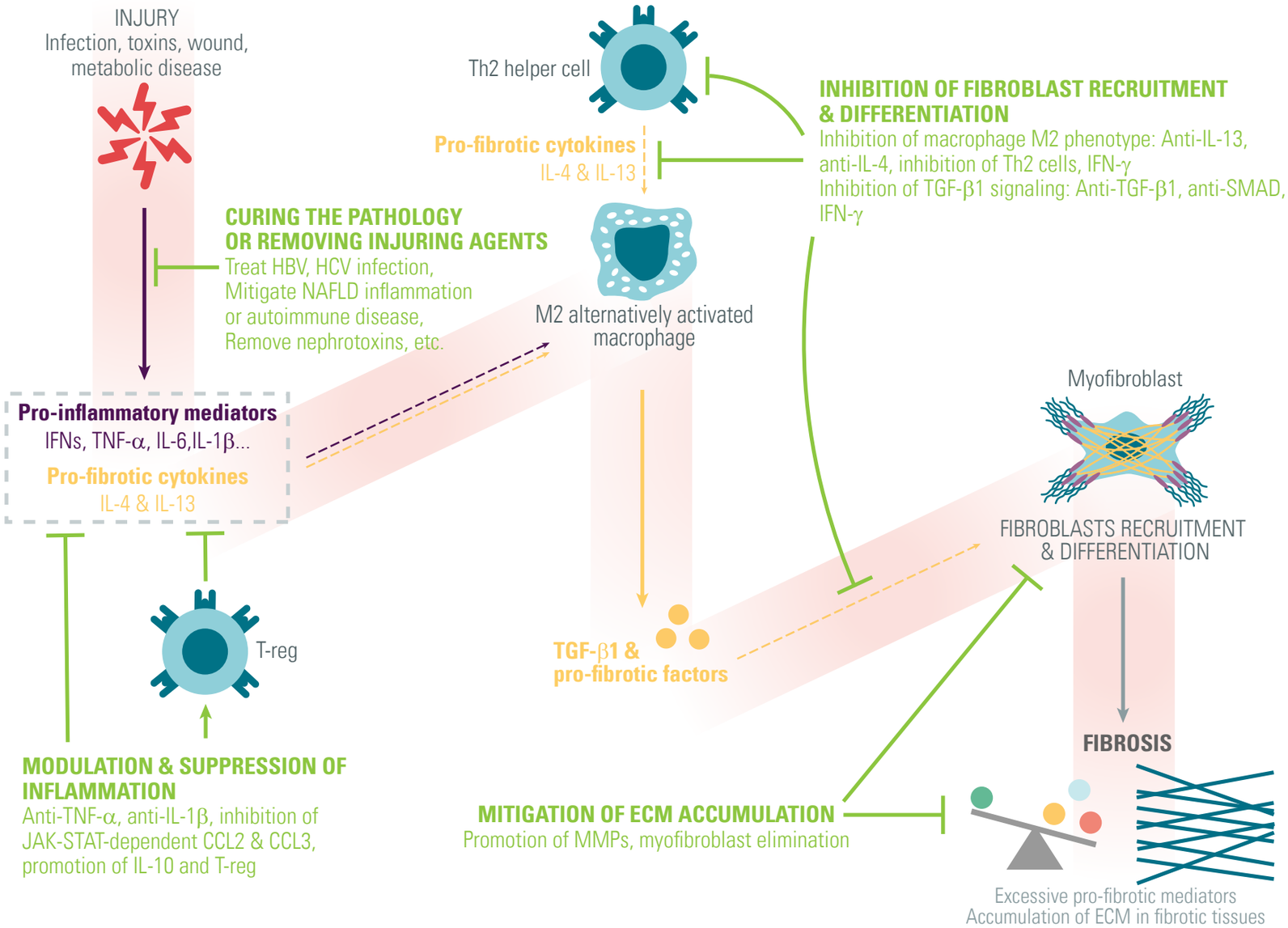

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KEY FIBROSIS-RELATED PATHOLOGIES

NAFLD-Associated Liver Fibrosis

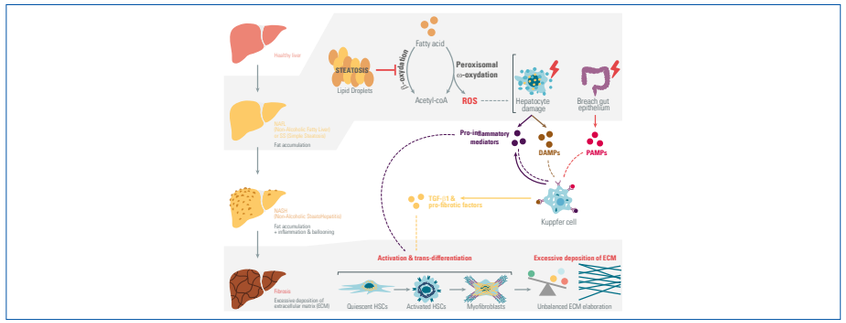
The liver is the most vital organ in the body. It governs whole-body energy homeostasis by performing many essential functions related to nutrient digestion, metabolism and storage, as well as detoxification and immunity. All nutrients along with drugs and other potentially harmful substances from the small intestine, stomach, pancreas, and spleen are absorbed by the liver through the portal vein. As the main detoxifier of the organism, the liver is inherently exposed to most irritant agents the body encounters, which makes it an organ especially susceptible to scarring in long term exposure to toxic environments and practices.

In the early 2000s, changes in alimentation and physical inactivity in developed countries have laid the ground for the collection of disorders designed by non-alcoholic fatty liver disease (NAFLD) (131). NAFLD is a term used to cover a spectrum that ranges from fatty liver to non-alcoholic steatohepatitis to fibrosis, and finally cirrhosis. Lipid accumulation, or steatosis, marks one end of the spectrum and is generally considered benign. Simple accumulation of fat in the liver is sometimes referred to as "Simple Steatosis" and does not include inflammation. Lipid accumulation is considered a hepatic manifestation of obesity and its related metabolic disorders such as diabetes and insulin resistance. The other end of the spectrum is characterized by the development of hepatocellular carcinoma (HCC), the most prevalent type of primary liver cancer. Non-alcoholic steatohepatitis (NASH), fibrosis and cirrhosis are intermediary steps between simple steatosis and HCC. In NASH, the build-up of fat in the liver is accompanied by inflammation, which, if left untreated can progress to fibrosis. Over time, fibrotic tissues come to replace a significant portion of the heathy tissues and impair the function of the liver.

In NAFLD-associated fibrosis, the triggering agents and source of inflammation come from the dysregulation of carbohydrate and lipid metabolism induced by lasting unbalances in diet. While β-oxidation is the usual process by which fatty acids (FAs) are converted into Acetyl-CoA, the continuous lipogenesis (lipid storage) that accompanies over-alimentation with diets rich in lipids and carbohydrates (or obesity and insulin resistance) inhibits β-oxidation. The conversion of lipids is then performed through abnormally increased peroxisomal ω-oxidation, which generate ROS. The cytotoxicity that results from ROS both create local inflammatory stress and cellular death with release of DAMPs (damage-associated molecular patterns), which are picked up by Kuppfer cells' TLRs (liver resident macrophages) and trigger the trickle down process of local inflammation and scarring processes.

However, the escalation of NAFLD to NASH then fibrosis is understood to incorporate other factors along carbohydrate and lipid metabolism. The current model coined 'multiple parallel hits' describes a multitude of events occuring in parallel, including genetic predisposition, insulin resistance, adipose tissue dysfunction, abnormal lipid metabolism, lipotoxicity, altered production of inflammatory mediators, and dysregulation of the gut-liver axis and innate immunity (132–136).

More information on NAFLD/NASH and associated signaling pathways in our dedicated Guide.



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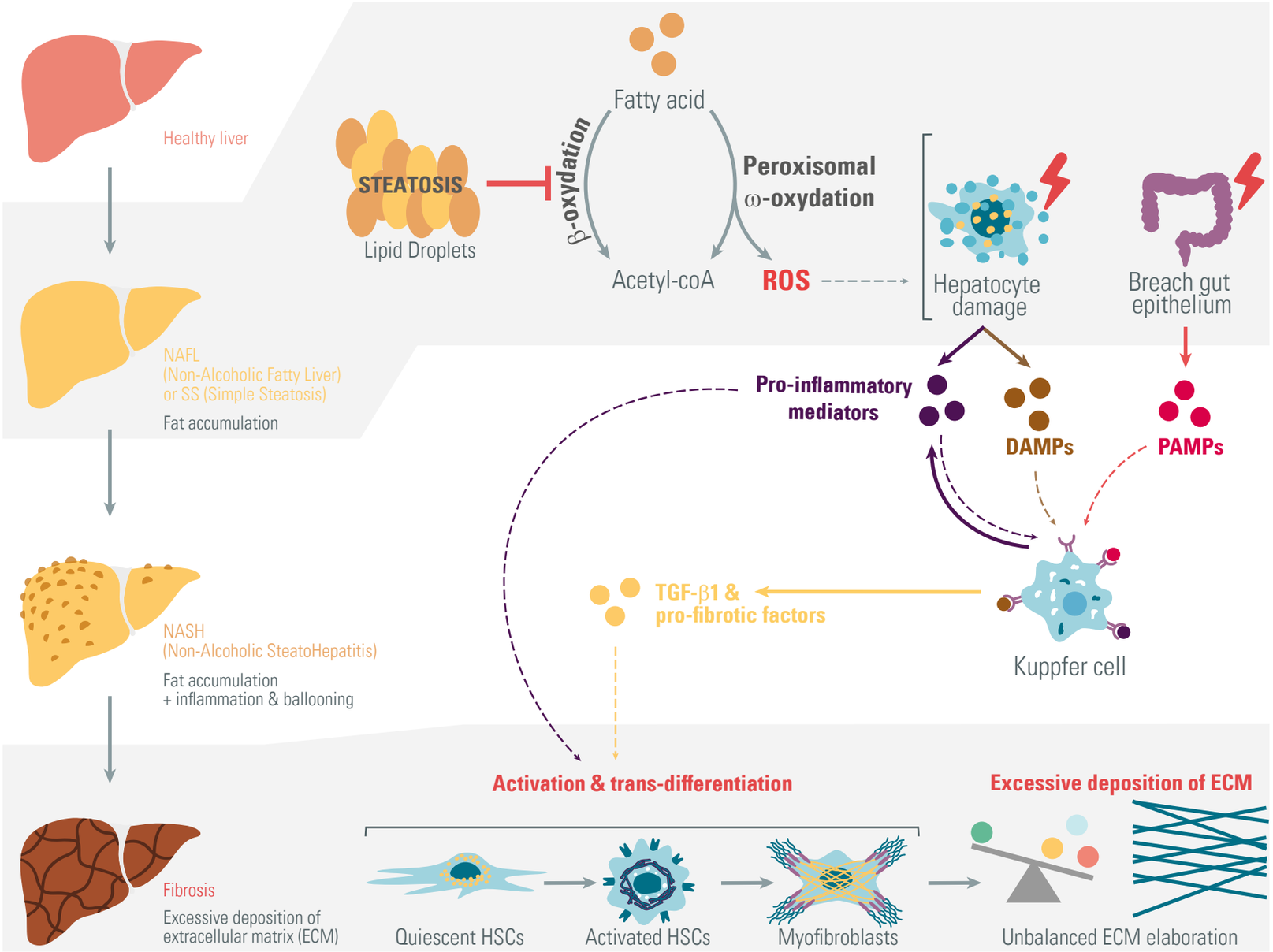

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KEY FIBROSIS-RELATED PATHOLOGIES

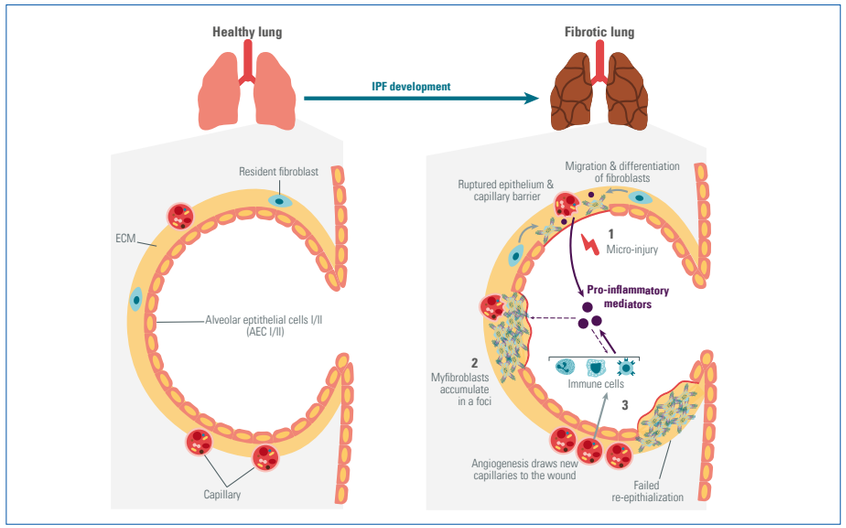
Idiopathic Pulmonary Fibrosis

IPF is the most prevalent form of the larger family of interstitial lung diseases (ILDs), which are a group of parenchymal lung diseases of different inflammation and fibrosis levels. IPF is considered an aggressive disease with chronic, progressive, fast-growing fibrosis of the lung tissues, which result in irreversible loss of lung function, respiratory failure and high mortality rates.

While environmental factors have been identified as recurrent promoters of the disease, genetic and epigenetic predisposition at the individual level are the most critical elements for IPF development. The current understanding is that the contribution of external factors and repetition of micro-injury of the alveolar epithelium in such a susceptible genetic context leads to the chronic wound-healing and tissue repair process that results in the abnormal accumulation of scar tissues that characterizes fibrosis. The unclear nature and respective contribution of the genetic background and different environmental, autoimmune or infectious factors that lead to the development of IPF originated the idiopathic denomination of the disease.

At the alveolar scale, the development of IPF is described as starting with a micro-injury of the alveolar epithelium, which is ruptured locally along with the adjacent capillary barrier. Bleeding from the capillary into the wound brings fibrin and coagulation factors from platelets which initiate the inflammatory reaction by activating local alveolar macrophages. Said macrophages also receive inflammatory signals from their TLRs, which pick up the DAMPs (damage-associated molecular patterns) released upon epithelial rupture. This is followed by the unfolding of a usual wound-healing process; with the recruitment of other immune cells, fibrocytes and fibroblasts which proliferate and differentiate under the influence of growth factors (TGF-b1, PDGF, VEGF, FGF), which brings the myofibroblast phenotype to the injury.

IPF is characterized by the accumulation of fibroblasts and myofibroblasts into a fibrotic foci at the micro-injury site, which causes the abnormal local secretion of ECM proteins with resulting loss of alveolar elasticity, distortion of the alveoli architecture and exclusion of alveolar epithelial cells I/II (AEC I/II) which prevent re-epithelialization of the injury. Additionally, the proliferation of myofibroblasts and ongoing inflammation promote angiogenesis which sustain the local swelling and facilitate future micro-injury-related bleeding (137–139).



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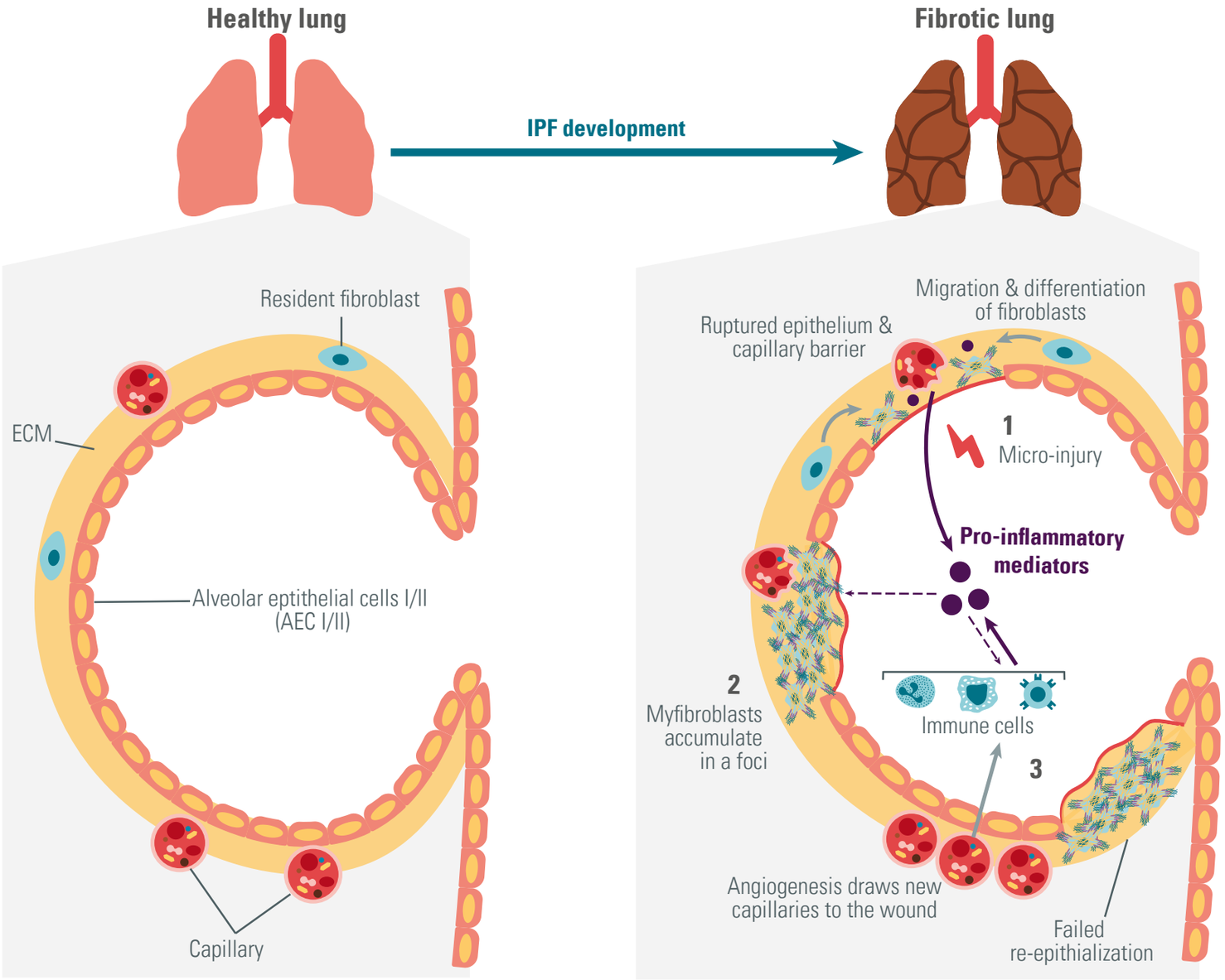

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KEY FIBROSIS-RELATED PATHOLOGIES

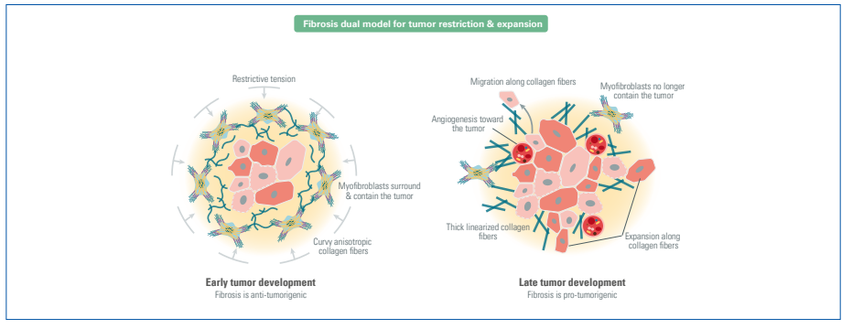
Fibrosis Relation to Tumor Development

Fibrosis as a mechanism related to the ECM, inflammation and tissue repair is closely associated to tumor development and growth, with evidence for chronic scarring acting a laying ground for cancer being observed as early as 1850 (140). In the eighties, the notion that tumors are treated like wounds that do not heal, and exhibit all the characteristics of tissue repair processes (leucocytes infiltration, neovascularization, fibroblast accumulation) (141,142) introduced fibrosis as a companion process and potential pathology to cancer. In particular, later evidence showed that both tumor progression and development of fibrosis are associated with myofibroblast proliferation and activity (143,144), and that stiffer ECM remodeling is associated with tumors (145).

The definitive pro- or anti-tumorigenic nature of fibrosis is controversial, with evidence in favor of both and it is likely not the same at all stages of tumor development. In early stages of tumor growth, fibrotic behaviors appear to contribute to the containment of cancer cells and the reduction of tumor expansion. This is achieved in similar ways to wound-closing, with myofibroblasts surrounding and crossing the inflamed area with anisotropic collagens, then contracting around it. The application of mechanical force around the tumor results in a tighter stroma that limits tumor growth. However, later events of ECM reorganization shift these anti-tumorigenic effects (146).

While collagen fibers in normal tissues are usually curly and not organized in any particular direction, they come to be altered, thicker and linearized around tumors. The suspected mechanisms at play in these changes are an increase in lysine-derived cross links between collagen catalyzed by LOX enzymes, and some contractility of tumor cells which pull on the collagen fibers and cause them to align perpendicularly to the tumor surface. The thick linearized collagen fibers pointing away from the tumor then act as “highways” along which tumor cells migrate, which promotes tumor expansion and metastasis (147–149). This assumption of contractility and motility on tumor cells part is corroborated by the increased ROCK pathway signaling in cancer, which regulates cytoskeletal composition and architecture to those ends (150). Integrins, as regulators of cytoskeletal properties of cells, are also actors of that process with their expression being tied to transmembrane mechanical tension. As tissue stiffness increases around tumors, integrin expression is promoted which in turn results in contractility and motility via cytoskeletal alterations (151,152). In this model, fibrosis and cancer support each other in an amplification loop, where the tumor acts as the permanent wound providing the inflammatory environment in which myofibroblasts thrive and promote fibrotic phenotypes, which in turn facilitate tumor progression (153).

Additionally to that reorganization, the whole regulation of the ECM is altered in tumor environment with collagen and MMP turnover increasing greatly, which both facilitates the reorganization in a linear architecture and creates spaces for cancer cells to invade (149,154).This increased turnover, is also a source of growth factors which get released from the ECM and promote invading tumor cell growth in this already cell-free and nutrient rich medium (12).



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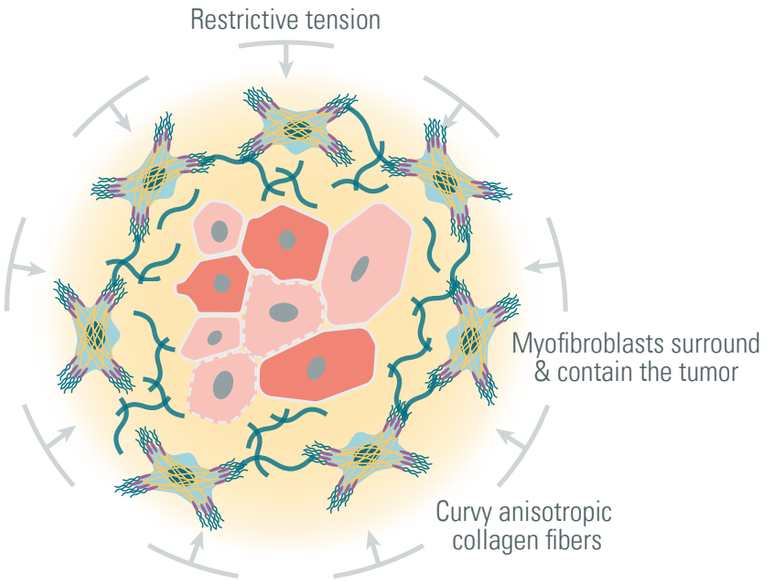
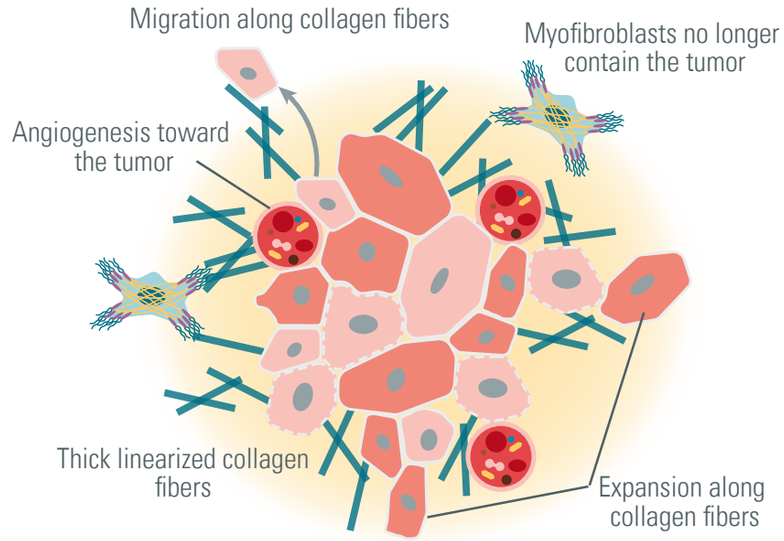
Fibrosis dual model for tumor restriction & expansion



Early tumor development
Fibrosis is anti-tumorigenic



Late tumor development
Fibrosis is pro-tumorigenic

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