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Endostatin in fibrosis and as a potential candidate of anti-fibrotic therapy

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ABSTRACT

Fibrotic diseases pose significant clinical challenges due to their broadness and complexity. Thus, a better understanding of fibrogenesis and the development of more effective treatments is imperative. Recent evidence suggests a significant antifibrotic potential of an endogenous glycoprotein, endostatin. While endostatin has been widely studied for its role as an anticancer adjuvant by inhibiting tumor angiogenesis, its possible implication in fibrosis remains largely unclear. Here, we review the role of endostatin in various cellular processes and highlight its antifibrotic activity. We hypothesize that endostatin conveys a homeostatic function in the process of fibrosis by regulating (a) TGF-β1 and its downstream signaling; (b) RhoA/ROCK pathway; (c) NF-κB signaling pathway; (d) expression of EGR-1; (e) PDGF/PDGFR pathway; (f) autophagy-related pathways; (g) pathways associated with cell proliferation and apoptosis. Finally, we propose a schematic model of the antifibrotic roles and mechanisms of endostatin; also, we outline future research directions of endostatin and aim to present a potential therapeutic approach for fibrosis.

1. Introduction

Fibrosis is a pathophysiological change characterized by excessive deposition of extracellular matrix (ECM) and is triggered by factors like trauma, infection, inflammation, blood circulation disorders, immune responses, and abnormal tissue repair (Wynn 2007; Lopez-de la Mora et al. 2015). The fibrotic process can be seen in almost all tissues and organs, examples including the liver, kidney, lung, pancreas, cardiovascular and cerebrovascular systems, bone marrow, skin, etc. (Luo et al., 2017) (Figure 1). The result of fibrosis seriously affects the function of tissues and organs, and fibrosis of important organs can endanger the lives of patients (Wynn 2007; Humphreys 2018; Jun & Lau 2018). The mechanisms underlying fibrosis development are complex and may be modulated by many factors and signaling pathways (Hu & Phan 2016; Klinkhammer et al. 2018; Tschumperlin et al. 2018; Wang et al. 2020) (Figure 2). However, the mechanisms of fibrosis are not fully understood and effective early diagnoses and treatments are still lacking in clinical practice.

Fibroproliferative disorders (FPDs) are characterized by excessive ECM accumulation and involve a variety of diseases, ranging from skin scarring and retroperitoneal fibrosis to life-threatening conditions like liver fibrosis/sclerosis, pulmonary fibrosis, glomerulosclerosis, renal interstitial fibrosis, pancreatic and gastrointestinal fibrosis, myelofibrosis, and arteriosclerosis (Huang & Ogawa 2012; Thannickal et al. 2014). The search for effective fibrosis treatments is a major research goal. Endostatin is a widely-known anticancer adjuvant that acts by inhibiting tumor angiogenesis and in recent years its anti-fibrotic effects have gained increasing attention, with cellular and animal model studies highlighting its therapeutic potential in FPDs.

2. Overview of endostatin

Endostatin, a bioactive product of hydrolysis of the carboxyl-terminal of extracellular matrix collagen XVII by protease (Figure 3), is an endogenous glycoprotein with remarkable anti-angiogenic effects (Pehrsson et al., 2019; Poluzzi et al., 2016). Endostatin's amino-terminal part has anti-angiogenic activity (O'Reilly et al. 1997; Tjin Tham Sjin et al. 2005), while the carboxyl-terminal part has the anti-fibrotic function (Yamaguchi et al. 2012). Structurally modified synthetic recombinant human endostatin (Endostar) is included in various cancer treatment guidelines (Rong et al. 2012; Xiao et al. 2015) and endostatin's anti-fibrotic effects have been validated in various FTD models.

Endostatin, of which the molecular weight is about 20 kDa, has many characteristics in a structure. Firstly, its structural stability depends partly on the integrity of the amino terminus sequence. Secondly, endostatin contains a zinc-binding motif, the capacity of zinc-binding also plays a key role in modulating the molecular stability and impacts the integral nature. Research has confirmed that zinc-binding ability takes the responsibility of condensing the structure of endostatin. Thirdly, the folding pattern is determined by the correct location of disulfide bonds in the endostatin
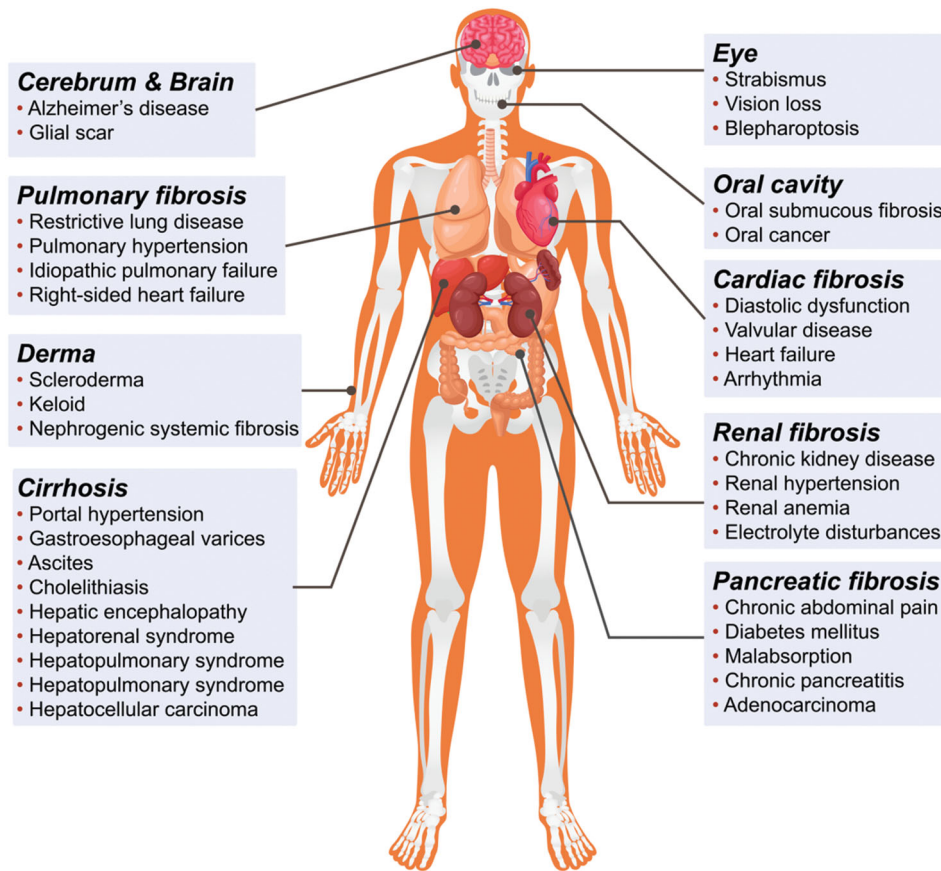
Figure 1. Distribution of fibroproliferative disorders in the human body. Nearly all organs and tissues can develop fibroproliferative disorders indicating the complexity and broadness of fibrotic diseases.

peptides, and the correct folding of endostatin peptides is   crucial for obtaining the most stable structure and biological
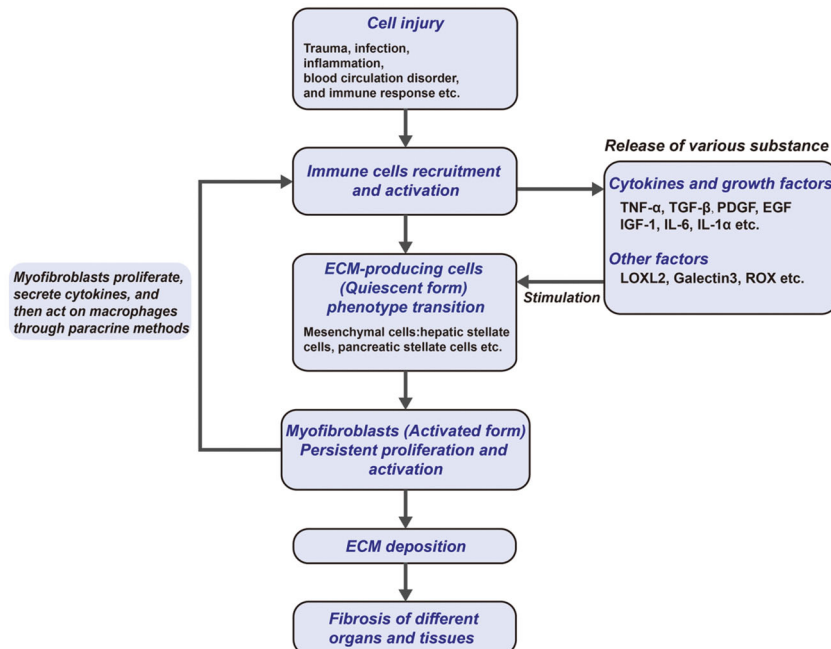

Figure 2. Core steps of fibrogenesis. The formation mechanism of fibrosis is very complex and shares several common core steps.
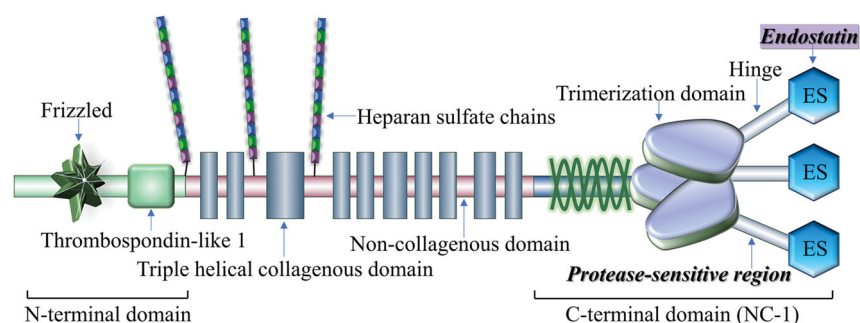
Figure 3. Structure of endostatin and collagen type XVIII. Endostatin is a bioactive fragment produced by hydrolysis of carboxyl-terminal of collagen type XVIII in the extracellular matrix by proteases.

activity to the maximum extent (Folkman 2006; Han et al. 2007; Fu et al. 2009).

The molecular mechanism of endostatin is complicated and involves many targets and pathways. Endostatin acts on numerous factors, including α5 integrins, glypicans, nucleolin, flk1, and tropomyosin (MacDonald et al. 2001; Karumanchi et al. 2001; Sudhakar et al. 2003; Shi et al. 2007). Although endostatin is widely studied, most studies have focused on its anti-angiogenic and cancer adjuvant effects and its anti-fibrosis effects remain unclear. This review focuses on the mechanisms underlying endostatin as a potential anti-fibrosis agent.

3. Anti-fibrotic mechanisms of endostatin

3.1. TGF-β1 signaling

Transforming growth factor β1 (TGF-β1) is a multifunctional cytokine, it has the functions of regulating cell proliferation, differentiation, and the production of ECM, and plays an important role in the process of organism development, wound healing, organ fibrosis, tumor generation, and metastasis. Therefore, it has always been a research hotspot in the above fields (Massague et al. 2000; Meng et al. 2016; Stewart et al. 2018). To date, three mammalian isoforms have been identified, TGF-β1, TGF-β2, and TGF-β3 (Yu et al. 2003). On the cellular membrane lies two kinds of serine/threonine kinase-type receptors, which are TGF-β targets. TGF-β signals through a heterologous receptor complex of type I and type II receptors and in most cases transmits the signal via Smads (Xu et al. 2012; Miyazawa & Miyazono 2017). The Smad family includes three categories, receptor-regulated Smads (R-Smad, including Smad1/2/3/5/8), inhibitory Smads (I-Smad, including Smad6/7), and common-mediator Smad (Co-Smad, including Smad4) (Hill 2016). R-Smads are activated by TGF-β receptor I. Smad2 and Smad3 are two main R-Smads phosphorylated by TGF-β receptor I. Phosphorylated R-Smad form heterodimeric complexes with Co-Smad. The complexes then translocate to the nucleus and regulate target gene transcription by binding to specific DNA sequences (Massague & Wotton 2000; Xu et al. 2012).

Besides canonical Smad-dependent pathways, TGF-β1 can also modulate fibrosis through Smad-independent signaling pathways, the key events of which reside in the activation of myofibroblasts, suppression of ECM degradation, and excessive deposition of ECM (Katz et al. 2016; Meng et al. 2016; Chen et al. 2018). Different Smad proteins may have distinct effects on fibrosis. There is a complex interaction between TGF-β/Smads and other signaling pathways (Dennler et al. 1998; Piek et al. 2001; Yuan & Varga 2001). Recent studies have identified many other mechanisms that modulate TGF-β1/Smads signaling in fibrosis, including epigenetic histone modifications, non-coding RNAs (Sun et al. 2010; Bian et al. 2014; McClelland et al. 2015; Yu et al. 2015). Additionally, AP1, a key pro-fibrotic transcription factor, is modulated by the non-Smad pathway via JNK activation (Jin et al. 2016). The facts above suggest that there may be a complex and huge regulation network that needs further research.

Endostatin is reported to downregulate TGF-β1 expression, thereby exerting an anti-fibrotic effect on TGF-β1-induced fibrosis. Endostatin was shown to alleviate peritoneal sclerosis by reducing TGF-β1 and α-SMA expression (Tanabe et al. 2007) in a study in which researchers induced peritoneal sclerosis via intraperitoneal injection of chlorhexidine gluconate (Tanabe et al. 2007). Immunohistochemical analysis showed that the number of TGF-β (+) and α-SMA (+) cells in the endostatin-treated peritoneum were significantly lower than in the control group. Besides, the western blotting analysis showed that endostatin suppresses TGF-β1 expression. However, whether endostatin influences processes downstream of TGF-β like the canonical Smad pathway or MAPK signaling is unknown (Yamaguchi et al. 2012).

3.2. Rhoa/ROCK pathway

Mammalian Rho GTPases fall into 8 families and the most studied subfamilies are Rho, Rac, and Cdc42. Rho GTPases are involved in cell migration, phagocytosis, contraction, and adhesion. RhoA, the best described Rho GTPase is associated with FPDs. RhoA promotes stress fiber formation and elongation, actin bundle contraction, and directional adhesion (Heasman & Ridley 2008; Hall 2012). ROCK (Rho-associated kinase) is a serine/threonine-protein kinase and direct RhoA effector. There are two subtypes of ROCK, namely ROCKI and ROCKII which differ in functions and distribution in the human body (Loirand 2015). ROCK targets include cytoskeleton-related proteins like myosin light chain and LIM kinase (LIMK) (Maekawa et al. 1999).

The RhoA/ROCK pathway is closely associated with pathological fibrosis. Hepatic stellate cells (HSCs), which play a pivotal role in liver fibrosis/sclerosis, are regulated by ROCK via multiple pathways, including p38 MAPKs, JNK, and ERK1/2 (Fukushima et al. 2005). Fasudil and Y27632 are selective ROCK inhibitors that suppress HSC proliferation and collagen synthesis *in vitro* (Tada et al. 2001; Fukushima et al. 2005). Fasudil ameliorates renal fibrosis in rats (Satoh et al. 2002; Han et al. 2020), while Y-27632 attenuates tubulointerstitial renal fibrosis in unilateral ureteral obstructed mice (Nagatoya et al. 2002). Rho influences α-SMA production and TGF-β1-induced cytoskeleton remodeling during epithelial-mesenchymal transition (EMT). Combined inhibition of ROCK and TGF-βRI reverses EMT (Masszi et al. 2003).

Endostatin is reported to inhibit the RhoA/ROCK pathway. Recent findings confirm that endostatin suppresses HSCs activation by inhibiting RhoA/ROCK1 signaling pathway (Ren et al. 2019). HSCs can be triggered by factors like inflammation or injury. Once activated, HSCs may turn into myofibroblasts and synthesize excessive ECM. In the study above, researchers established liver fibrosis models *in vitro* by treating HSCs with TGF-β1 or PDGF-BB and found that endostatin significantly reverses upregulation of collagen I, α-SMA, and F-actin by TGF-β1 or PDGF-BB. Similar results that endostatin suppresses the expression of RhoA and ROCK1 were obtained from PCR and western blot analyses.

3.3. NF-κB pathway

Nuclear factor-κB (NF-κB), a transcription factor present in the cytoplasm in inactive heterodimeric or homodimeric form, regulates multiple cellular processes. Particularly, NF-κB has crucial roles in immune cell activation, inflammation, stress response, and apoptosis. Many stimuli can activate NF-κB, which translocates from the cytoplasm to the nucleus, driving the expression of genes that contain κB binding sites (Oeckinghaus & Ghosh 2009; Hoesel & Schmid 2013). The human NF-κB family is comprised of five cellular DNA-binding subunits: p50, p52, crel, relA, and relB. In general, NF-κB binds to its inhibitory IκB partner (IκBα, IκBβ, and IκBε) and does not show transcriptional activity. The IKK protein kinase complex (IKKα, IKKβ, and IKKγ) is the core link that regulates NF-κB signaling. NF-κB must be released from IκB for activation. The activation of NF-κB mainly comprises two pathways: the canonical pathway, which is initiated due to cytokines like IL-1(interleukin-1) and TNF-α; and the alternative pathway, which plays a crucial role in B lymphocytes (Hoesel & Schmid 2013).

NF-κB signaling is implicated in fibrosis in various tissues and organs. NF-κB activation in HSCs/hepatic myofibroblasts (HMFs) promotes fibrosis in various ways, including direct pro-fibrotic function, antiapoptotic effects, and secretion of inflammatory mediators (Luedde & Schwabe 2011). A recent study showed that NF-κB signaling activation promotes pulmonary fibrosis by mediating the transactivation of fibrogenic genes in fibroblasts and myofibroblasts, suggesting a framework for further NF-κB signaling studies. Two NF-κB-related genes encoding pro-fibrotic proteins, osteopontin (OPN), and tissue inhibitor of metalloproteinase 1 (TIMP1) have been identified (Dong & Ma 2019).

Endostatin has been shown to inhibit NF-κB signaling, exerting antifibrotic activity. This is attributable to several mechanisms. Recent studies show that endostatin blocks NF-κB pathway due to its anti-inflammatory properties. Endostatin is also reported to bind to α5β1 integrin, a chondrocyte mechanoreceptor or transmembrane integrin receptor that transmits high-intensity mechanical signals. These signals are stimuli that activate NF-κB. Therefore, the interaction between α5β1 integrin and endostatin suppresses mechanoreceptor function, thereby preventing NF-κB activation (Tabruyn et al. 2009; Marcu et al. 2010). Recombinant human endostatin has been shown to antagonize the formation of mouse osteoclasts by inhibiting NF-κB and MAPKs signaling pathways (Chen et al. 2016). In summary, although there is no direct evidence, it is likely that endostatin has anti-fibrotic effects via NF-κB signaling.

3.4. EGR-1

Human EGR-1 (early growth response 1) is located on chromosome 5q31 and encodes an 80 kDa DNA binding transcription factor. EGR-1 expression levels are low or undetectable in resting cells but are elevated by many extracellular stimuli. The EGR-1 consists of an activation domain, inhibition domain, and a DNA binding domain. DNA binding domain is comprised of three zinc fingers targeting GC-rich DNA sequences. EGR-1 modulates the expression of various growth factors, cytokines, and adhesion molecules, including TGF-β, bFGF (basic fibroblast growth factor), PDGF-A, PDGF-B, IL-2, TNF-α, M-CSF (macrophage colony-stimulating factor), ICAM-1 (intercellular adhesion molecule 1), and CD44, thereby modulating signal transduction (Bhattacharyya et al. 2011; Li et al. 2020).

Numerous studies have shown that EGR-1 drives fibrosis (Bhattacharyya et al. 2011) and that EGR-1 acts downstream of Smad-independent TGF-β signaling. For example, TGF-β1 induces the phosphorylation of MEK1, upregulating ERK1/2, and its substrate Elk-1, triggering EGR-1 expression in normal human fibroblasts (Bhattacharyya et al. 2008). Ectopic EGR-1 expression drives collagen gene expression, mediating fibrosis. Similar observations have been made in a mouse model of hepatic fibrosis using TGF-β1. The results showed that TGF-β1 stimulates EGR-1 expression by activating p38 MAPK/Elk-3 signaling cascade, converging on Elk-3, and promoting liver fibrosis by modulating EMT in liver cells (Li et al. 2017). EGR-1 is a ROS-sensitive transcription factor and is activated by reactive oxygen species (ROS) produced by phagocytes at sites of tissue injury. EGR-1 activation by the Ras/Raf/ERK signaling cascade is oxidants/ROS-dependent (Roy et al. 2004).

EGR-1 downregulation is proposed as one of the endostatin's mechanisms of action. Yamaguchi and colleagues described the potent anti-fibrotic effects of E4, a peptide derived from endostatin. Intriguingly, E4 peptide suppresses fibrosis in cell culture, human skin organ culture, and animal models. Specifically, it suppressed EGR-1 expression in fibroblasts, thereby lowering fibronectin (FN), collagen I (ColI),

and lysyl oxidase (LOX) production. Lower LOX levels reduce collagen cross-linking, making the ECM more fragile (Yamaguchi et al. 2012; Nishimoto et al. 2015). It is reported that oral E4 administration has anti-fibrotic properties in a bleomycin-induced mouse model of pulmonary fibrosis, indicating that E4 has potential as an oral therapy for fibrotic disorders (Nishimoto et al. 2015).

3.5. PDGF/PDGFR pathway

Platelet-derived growth factor (PDGF) is a major mitogenic agent. There are five PDGF subtypes: PDGF-AA, PDGF-BB, PDGF-AB, PDGF-CC, and PDGF-DD. PDGF receptor (PDGFR) is a transmembrane glycoprotein with tyrosine-protein kinase activity, including three kinds of dimers, $\alpha\alpha$, $\alpha\beta$, and $\beta\beta$, which are composed of PDGF-α and PDGFR-β subunits in a certain way. PDGF-A and PDGF-C predominantly bind to PDGFR-α chain, PDGF-B binds to both -α and -β chains, and PDGF-D binds to the β chain only. PDGF functions depend not just on the concentration of the ligand but also on ligand binding to specific receptors. Mesenchymal cells are the principal site of PDGFRs expression. Once bound by ligands, PDGFRs are phosphorylated to activate transcription factors and diverse cytoplasmic signaling cascades, like Janus kinase, mitogen-activated protein kinase, p38, phospholipase C, and Ras GTPase activating protein, driving downstream gene expression and exerting biological effects (De Donatis et al. 2008; Klinkhammer et al. 2018; Papadopoulos & Lennartsson 2018; Sil et al. 2018).

PDGF modulates fibrosis and is a promising target for anti-fibrotic therapy. PDGF promotes fibrosis in wound healing. Recombinant PDGF-BB promotes healing of chronic foot and leg ulcers (Barrientos et al. 2014). Moreover, PDGFs exert mitogenic and anti-apoptotic effects on fibroblasts and myofibroblasts that synthesize ECM, thereby facilitating the progression of fibroproliferative disorders (Klinkhammer et al. 2018).

A recent paper demonstrated that endostatin downregulates PDGFR and p-ERK protein levels, reducing scar formation in a fibroblast model of fibrosis. ERK, which is highly phosphorylated in scar fibroblasts, is located downstream of the PDGFR signaling pathway and modulates cell proliferation and migration. It is reported that endostatin does not affect ERK expression but suppresses its phosphorylation, suggesting that endostatin may alleviate dermal fibroblast fibrosis by regulating PDGFR/ERK signaling (Li & Ren 2017). Additionally, endostatin attenuates activation of hepatic stellate cells in a liver fibrosis model upon induction with PDGF-BB and TGF-β1 by inhibiting RhoA/ROCK1 signaling.

3.6. Regulating autophagy-related pathways

Autophagy is the physiological catabolic mechanism that uses lysosomes to break down harmful cellular components, including misfolded proteins, damaged organelles, and excessive lipids, to achieve the clearance and reuse of cytoplasmic waste (Mizushima et al. 2008). Autophagy is a self-survival mechanism that occurs under special circumstances such as external stress, hunger, hypoxia, and endoplasmic reticulum stress. The disruption of the autophagy mechanism is closely related to the pathogenesis of tumors, neurodegenerative diseases, metabolic-related diseases, immune diseases, and other diseases (Levine & Kroemer 2019; Morishita & Mizushima 2019). Depending on the route of cargo transport into lysosomes, autophagy pathways are divided into three main categories: macroautophagy, microautophagy, and chaperone mediated autophagy; currently, macroautophagy has been the most extensively studied. Macroautophagy is characterized by autophagosomes that swallow excess cytoplasmic components and fuze with lysosomes; it is a multistep process that involves initiation, nucleation, expansion, fusion, and degradation (Dikic & Elazar 2018). In microautophagy, the lysosome membrane directly wraps the small cytosolic components without the assistance of autophagosomes (Oku & Sakai 2018). Chaperone-mediated autophagy refers to the process in which the proteins containing a specific motif are identified and combined by a chaperone and then translocated across the lysosomal membrane (Kaushik & Cuervo 2018).

Studies have found that fibrotic diseases have a deep connection with autophagy. EMT is recognized as an important mechanism of fibrosis, and autophagy participates in the occurrence and development of fibrosis by regulating epithelial damage and activating myofibroblasts, which are the core steps of EMT (Nieto et al. 2016; Stone et al. 2016; Takagaki et al. 2020). Dysregulation of autophagy is found in pathological processes such as PF (pulmonary fibrosis), liver fibrosis, cardiac fibrosis, and peritoneal fibrosis. Specifically, autophagy slows down the pathological process of PF by regulating the apoptosis of fibroblasts and the senescence of alveolar epithelial cells. Additionally, autophagy may be an important therapeutic target for a variety of intrapulmonary fibrosis diseases, including idiopathic PF, cystic fibrosis lung disease, silicosis, and smoking-induced PF (Zhao et al. 2020). Furthermore, inhibition of autophagy can break the homeostasis of proximal tubular epithelial cells, thereby, aggravating kidney damage and further inducing renal fibrosis (Kimura et al. 2011; Tang et al. 2020). Intriguingly, autophagy upregulation can also promote fibrosis under certain circumstances. For example, studies have shown that Gα12 overexpression resulting from miR-16 dysregulation can activate HSCs by facilitating autophagy (Kim et al. 2018). In addition, PGc-1α is upregulated in systemic sclerosis (SSC) and promotes autophagy to foster TGF-β-induced fibroblast activation (Zhang et al. 2020). In summary, autophagy acts as a double-edged sword: on one hand, it can inhibit the occurrence of fibrotic diseases in most cases; on the other hand, excessive autophagy will aggravate the fibrotic process; the specific mechanism remains to be further explored.

Recent studies have indicated a new activity for endogenously-released fragments of the ECM: they can not only exert anti-angiogenic effects but also induce autophagy (Goyal et al. 2016; Neill et al. 2021). Endostatin is an autophagy inducer in endothelial cells. Soluble endostatin evokes autophagy by directly binding to the α5β1-integrin and regulating Beclin 1 and β-catenin levels (Nguyen et al. 2009).

Specifically, endostatin treatment reduces Bcl-2 and Bcl-xL by interacting with α5β1-integrin and increasing the levels of Beclin 1 by inhibiting the Wnt/β-catenin pathway, thereby, disrupting the physiological Bcl-2 (or Bcl-xL)/Beclin 1 ratio and finally leading to the upregulation of autophagy. In addition, the levels of Beclin and β-catenin are negatively correlated; thus, it is reasonably speculated that β-catenin modulates Beclin 1 either by influencing its stability or by transcriptional repression (Nguyen et al. 2009). Although there is no direct evidence, it is reasonable to speculate that endostatin can also induce autophagy through similar mechanisms on epithelial cells and mesothelial cells. A reasonable explanation of the antifibrotic effects of endostatin is that it can help cells remove cytotoxic waste by increasing autophagy levels and maintaining the homeostasis of epithelial or mesothelial cells so that they can resist external negative stimuli, thereby, inhibiting EMT and reducing fibrosis. However, this mechanism still needs further experimental verification.

3.7. Regulating pathways associated with proliferation and apoptosis

Fibrosis is characterized by excessive formation and deposition of ECM by activated and expanded tissue mesenchymal stromal cells like fibroblasts, pericytes, and myofibroblasts. Fibroblast activation by various pathophysiological factors is a central link in fibrosis.

Based on the above knowledge, the possibility that endostatin has a straightforward effect on fibroblasts is worth consideration. Numerous studies have identified a potential role for endostatin indirectly modulating fibroblasts. Recombinant human endostatin (rhEndostatin) prevents synovial thickening in adjuvant arthritis (AA) rats by inhibiting synovial fibroblast proliferation and promoting apoptosis (Huang et al. 2008; Huang et al. 2014). Using MTT analysis

and flow cytometry, a recent study found that rhEndostatin inhibits the proliferation of HSFs and in parallel, reduced levels of proliferative markers like p53, p27, CDK4 (Cyclin-dependent kinase 4), cyclinD1, and PCNA (proliferating cell nuclear antigen) was observed (Gong et al. 2016). In another study, transmission electron microscopy and flow cytometry revealed that rhEndostatin induces apoptosis of HSFs and significantly downregulates apoptosis-related biomarkers like bcl-2, NF-κB, c-jun, and c-fos (Gong et al. 2017).

4. Brief summary of the possible mechanisms of endostatin in fibrotic diseases

Potential endostatin targets are shown in Figure 4 but it is not clear how they are modulated by endostatin. Endostatin is reported to exert its antiangiogenic effect on endothelial cells via multiple targets and pathways (Wickstrom et al. 2005). We speculate that it may have similar effects on fibroblast-type cells and mesothelial cells, which may lead to antifibrotic effects. The mechanism of endostatin action can be summarized by three major downstream effects: inhibition of HIF-1α/VEGFA and Wnt signaling-dependent down-regulation of β-catenin (Hanai et al. 2002), suppression of FAK/Ras/p38-MAPK/ERK signaling cascade through α5β1-integrin binding (Rehn et al. 2001; Shichiri & Hirata 2001; Wickstrom et al. 2001), and actin disassembly via Src-dependent-p190RhoGAP activation (Wickstrom et al. 2002; 2003). Additionally, endostatin competitively inhibits VEGFR2 binding to its ligand, thereby suppressing VEGF-mediated downstream effector pathways involving p38-MAPK, p125FAK, and ERK (Kim et al. 2000; Hajitou et al. 2002; Eriksson et al. 2003). Based on the information above, we propose a schematic model of the antifibrotic roles of endostatin (Figure 5), however, there are still a number of details awaiting further investigation and validation.
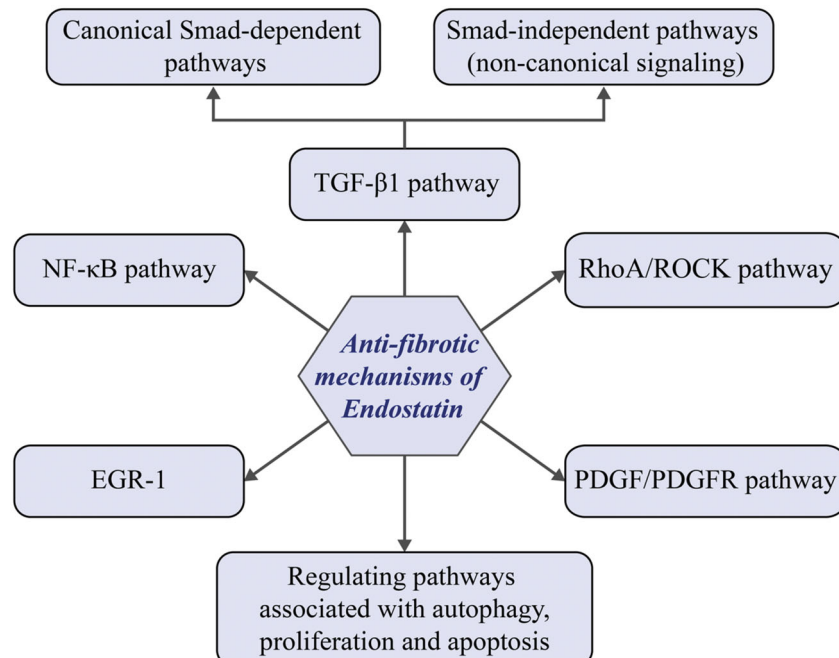

Figure 4. Overview of different roles of endostatin in fibrotic diseases.
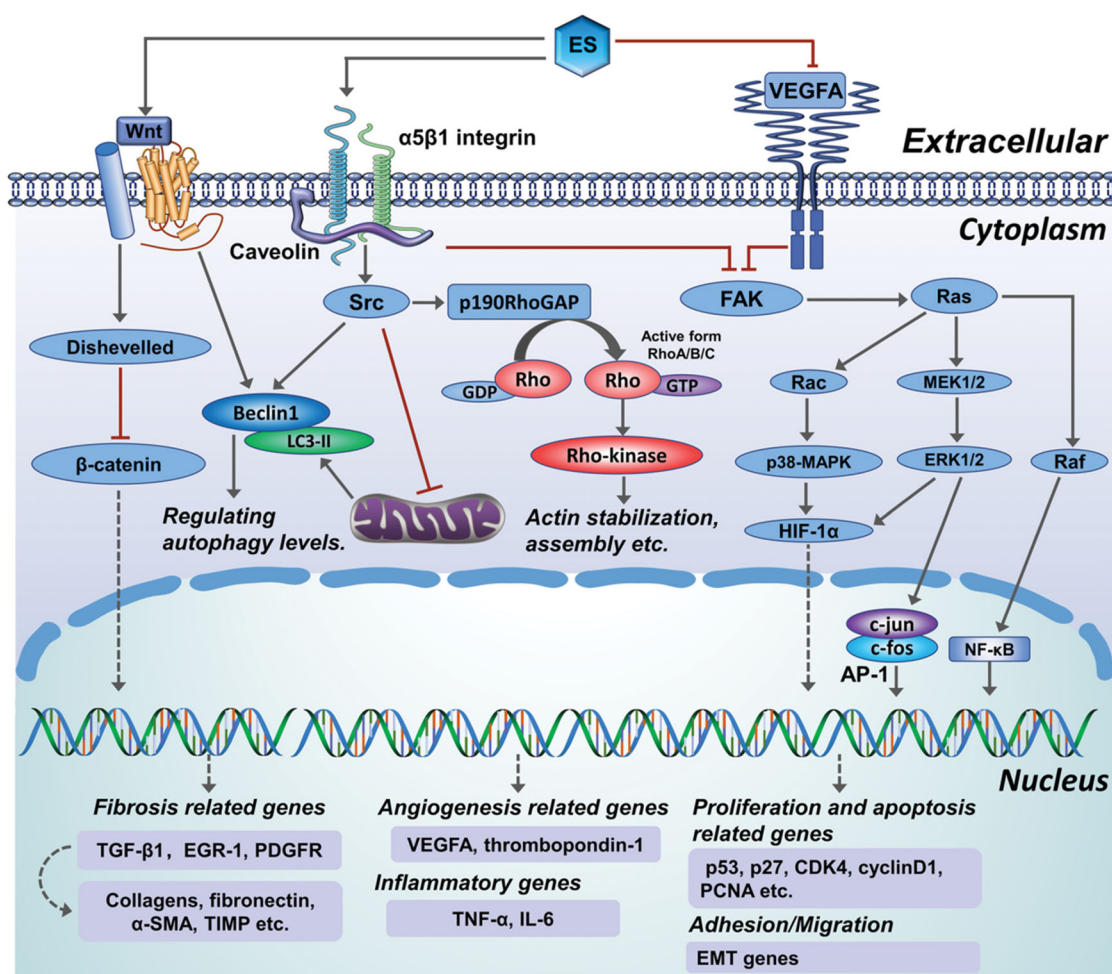
Figure 5. An elaborate scheme showing the anti-fibrotic mechanisms of endostatin and a comprehensive illustration of potential antifibrotic effects of endostatin.

5. Prospects of endostatin

5.1. Future research perspective: postoperative adhesion

Endostatin has been shown to be an exact anti-fibrotic molecule and exerts anti-fibrotic effects in multiple manners, which has been verified in many fibrosis models (Table 1). Considering the fact that many physiological and pathological conditions are characterized by fibrosis, endostatin may potentially be used on more fibrosis-related diseases. For example, postoperative adhesions, which is recognized as one of the most common clinical complications following diversified operations, such as abdominal and pelvic operation, plastic surgery, repair procedure of tendon, and the like.

Postoperative adhesion (POA) is a manifestation of assorted aberrant histologic proliferation, characterized by hyperplastic fibrous tissue adherence to adjacent tissues and organs (Figure 6). Fibrotic adhesion bands may have various appearances, ranging from thin-layer slices to thick bands with neovascularization. More than 90% of patients undergoing abdominal surgery develop varying degrees of abdominal adhesions, which lead to complications like chronic abdominal pain, intestinal obstruction, and female infertility (Arung et al. 2011; Hellebrekers & Kooistra 2011). Besides, adhesion formation secondary to ligament and tendon healing may restrict joint movement. Moreover, POA secondary to open-heart surgery may make the pericardium fibrotic and thickened, resulting in constrictive pericarditis, which critically influences cardiac function. Furthermore, adhesions that occur after plastic surgery may seriously diminish therapeutic efficacy and fail to achieve desired outcomes. These complications frequently require multiple hospital admissions, increasing the burden on the patient and the healthcare system (ten Broek et al. 2013). These facts suggest that POA is an important clinical problem with potentially serious outcomes. Existing anti-adhesion strategies include reducing intraoperative damage, physical barriers, and anti-adhesion drugs (Moris et al. 2017; Kou et al. 2020; Li et al. 2020). However, currently, there are few effective anti-adhesion drugs.

The process of POA is very similar to fibrosis (Chegini 2008; Capobianco et al. 2017), and in both the core steps are the activation of fibroblasts, production of large amounts of fibrous collagen, other matrix proteins, and α-smooth muscle actin (α-SMA). Broadly speaking, the formation of POA is also a fibrotic process. The transforming growth factor-β system has been proved to be the main pro-fibrotic mediator of POA formation, and TGF-β1 can induce the formation of postoperative peritoneal adhesion model (Chegini 2008; Jin et al. 2016). Besides, studies have confirmed that the

Table 1. Fibrotic diseases in which endostatin may have an effect.

Fibrotic diseases	Target cell	Possible role in diseases	References
Liver fibrosis	Hepatic stellate cells	RhoA/ROCK1 signal pathways; inhibiting the expression of α-SMA, collagen-1, and TGF-β1	(Chen et al., 2014; Ren et al. 2019)
Pulmonary fibrosis	Alveolar type II cells	Reducing the levels of TNF-α and TGF-β1; decreasing alveolar type II cell apoptosis	(Nishimoto et al. 2015; Richter et al., 2009)
Systemic sclerosis	Fibroblasts	Reducing levels of Egr-1, LOX	(Yamaguchi et al. 2012)
Hypertrophic scar	Skin fibroblasts/hypertrophic scar fibroblasts (HSFs)	PDGFRβ/ERK pathway; inhibiting HSFs proliferation and inducing its apoptosis	(Gong et al. 2016; Gong et al. 2017; Li & Ren 2017)
Arthritis	Synovial fibroblasts	Downregulating the level of cyclin-D1 and PCNA; inhibiting the NF-κB and MAPKs signaling pathways	(Chen et al. 2016; Huang et al. 2008; Huang et al. 2014)
Myocardial remodeling	Myofibroblasts	Stimulating proliferation and migration of myofibroblasts	(Isobe et al., 2010; Sugiyama et al., 2018)

ROCK1: Rho-associated kinase 1; α-SMA: α-smooth muscle actin; TNF-α: tumor necrosis factor α; TGF-β1: transforming growth factor β1; Egr-1: early growth response-1; LOX: lysyl oxidase; PDGFRβ: platelet-derived growth factor receptor β; ERK: extracellular signal-regulated kinase; PCNA: proliferating cell nuclear antigen; NF-κB: nuclear factor-κB; MAPK: mitogen-activated protein kinase.

Etiologies

Intraoperative injuries
Postoperative surgical site complications (e.g., inflammation, ischemia, foreign body reaction)
Other causes: postoperative radiotherapy, ect.

Possible mechanisms

- imbalance of inflammatory, coagulation and fibrinolytic systems
- mesenchymal transition of peritoneal mesothelial cells
- activation of TGF-β signaling pathway, ect.

Complications & Disadvantages

- bowel obstruction, some severe cases needs reoperation
- chronic pelvic pain
- infertility
- significantly increasing the difficulty of reoperation

Ophthalmic operation
Cardiac operation
Abdominal operation
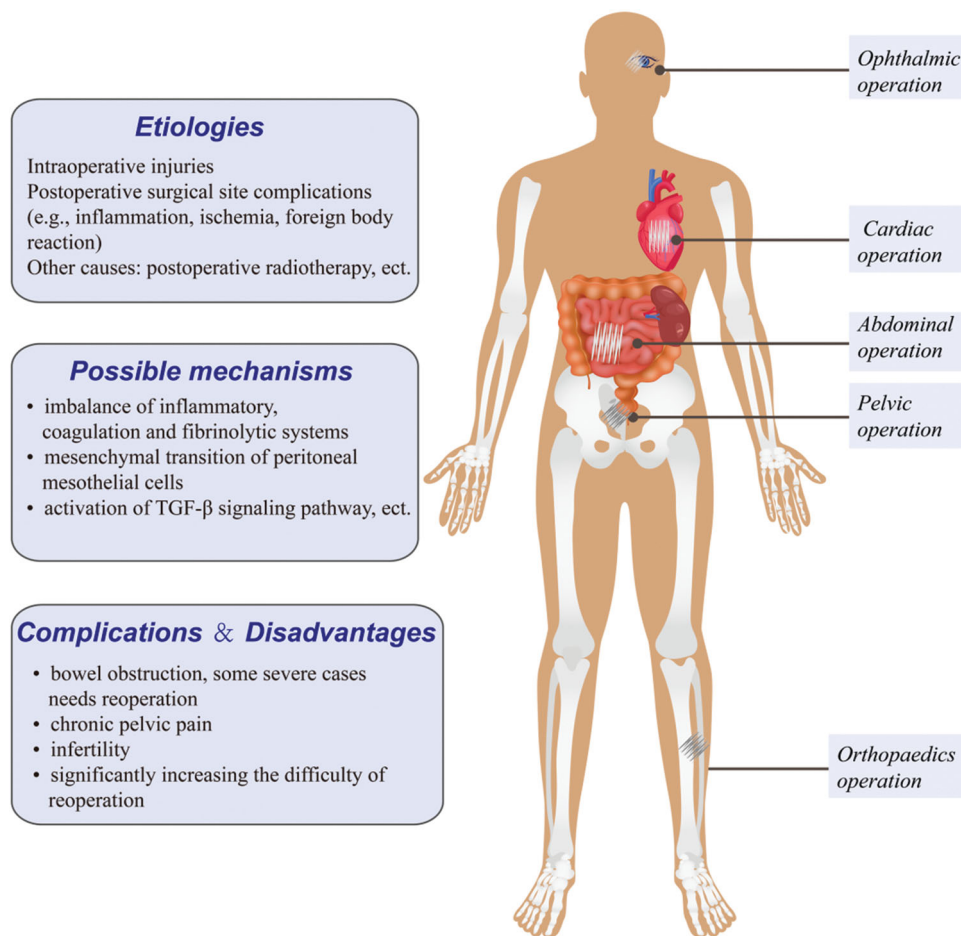Pelvic operation
Orthopaedics operation

Figure 6. Representative postoperative adhesion, and a brief overview of its etiology, mechanisms and complications. Postoperative adhesion (POA) is a comprehensive manifestation of assorted aberrant histologic proliferation, characterized by hyperplastic fibrous tissues adhering to adjacent tissues and organs.

formation of postoperative peritoneal adhesions involves activation of RhoA/ROCK signaling pathway and the EGR-1 pathway (Roy et al. 2004; Liu et al. 2020). These mechanisms are consistent with the anti-fibrotic mechanism of endostatin described above. Thus, we have sufficient reasons to speculate that endostatin can also inhibit the formation of postoperative adhesions, but this hypothesis still needs further investigation. The application of anti-fibrotic drugs to POA is a promising research direction. On top of the existing anti-adhesion formation strategy, combining anti-fibrotic strategy may more effectively inhibit POA formation, reducing fibrotic disease burden and benefiting patients.

5.2. Future challenges

Despite its good anti-fibrotic effects, the use of endostatin has many challenges. First, it is an endogenous molecule and its structural stability needs improvement. Future studies

should improve the structure of endostatin to give it stronger anti-fibrotic activity while reducing its antiangiogenic properties and other side effects. Many studies have yielded good results in this regard, including the development of recombinant human endostatin (Endostar) by Chinese researchers. Compared with natural endostatin, Endostar has an added 9 amino acid sequence at the N-terminus, which prolongs its half-life, significantly improving biological activity and stability (Han et al. 2007; Xiao et al. 2015). Moreover, Endostar nano-carriers are reported to maintain it at good concentrations in plasma and tissues (Hu & Zhang 2010; Tong et al. 2010; Chen & Hu 2011). Endostar is now widely used in anticancer therapy. A Japanese study also found that E4, an endostatin-derived peptide, may suppress organ fibrosis. Relative to natural endostatin or recombinant endostatin, E4 has only the C-terminal structure and no N-terminal antiangiogenic properties, which minimizes side effects. The molecular structure becomes more stable due to C-terminus chemical modification (Yamaguchi et al. 2012; Nishimoto et al. 2015).

There are many obstacles in anti-fibrotic treatment and it is nearly impossible to completely reverse pathological fibrosis. Many reasons contribute to this condition, including the fact that there are too many etiologies of fibrosis; the mechanisms of fibrosis are complex and involve many pathophysiological processes; and last but not least, single-target therapy may not be effective. Future research should consider combining treatment with endostatin and other anti-fibrotic drugs targeting distinct fibrosis pathogenic factors, which may have better therapeutic effects. Currently, endostatin treatment of fibrotic diseases has only been tested in cells and animal models, which do not recapitulate *in vivo* human conditions. Moreover, fibrosis mechanisms may differ across organs and tissues. Thus, further research is needed to determine if endostatin can effectively inhibit fibrosis in humans.

6. Conclusion

Pathological fibrosis causes many diseases and a heavy medical burden worldwide. However, there are few effective anti-fibrosis treatments. In this review, we have discussed the anti-fibrotic mechanisms of endostatin and its therapeutic value based on fibrosis disease models and highlight potential future research directions. Further comprehensive studies on the anti-fibrotic mechanisms of endostatin and its derivatives or analogs will uncover new strategies for the treatment of fibrosis-related diseases.

Disclosure statement

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