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Review

Endostatin's emerging roles in angiogenesis, lymphangiogenesis, disease, and clinical applications

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ABSTRACT

Background: Angiogenesis is the process of neovascularization from pre-existing vasculature and is involved in various physiological and pathological processes. Inhibitors of angiogenesis, administered either as individual drugs or in combination with other chemotherapy, have been shown to benefit patients with various cancers. Endostatin, a 20-kDa C-terminal fragment of type XVIII collagen, is one of the most potent inhibitors of angiogenesis.
Scope of review: We discuss the biology behind endostatin in the context of its endogenous production, the various receptors to which it binds, and the mechanisms by which it acts. We focus on its inhibitory role in angiogenesis, lymphangiogenesis, and cancer metastasis. We also present emerging clinical applications for endostatin and its potential as a therapeutic agent in the form a short peptide.
Major conclusions: The delicate balance between pro- and anti-angiogenic factors can be modulated to result in physiological wound healing or pathological tumor metastasis. Research in the last decade has emphasized an emerging clinical potential for endostatin as a biomarker and as a therapeutic short peptide. Moreover, elevated or depressed endostatin levels in diseased states may help explain the pathophysiological mechanisms of the particular disease.
General significance: Endostatin was once sought after as the 'be all and end all' for cancer treatment; however, research throughout the last decade has made it apparent that endostatin's effects are complex and involve multiple mechanisms. A better understanding of newly discovered mechanisms and clinical applications still has the potential to lead to future advances in the use of endostatin in the clinic.

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1. Introduction

Research over the last three decades has greatly clarified the process of angiogenesis and its role in cancer, with more than 78,000 articles published on this concept. Angiogenesis is the process of neovascularization from pre-existing vasculature and is involved in various basic physiological and pathological processes [1–4]. The discovery of endostatin in a murine hemangioendothelioma cell line in 1997 was a major breakthrough in our understanding of angiogenesis [5]. Endostatin, a 20-kDa C-terminal fragment of type XVIII collagen, is one of the most potent endogenously produced inhibitors of angiogenesis [5]. After 18 years of research on endostatin, the paradigm of bench-to-bedside research has not culminated in an effective FDA-approved drug. However, the State Food and Drug Administration of China did approve Endostar, a modified recombinant human endostatin, in 2005 for the treatment of non-small-cell lung carcinoma [6]. Since then, phase II studies of recombinant endostatin in the United States have failed to show any activity related to the inhibition of angiogenesis [7]. The evidence is inconclusive and circumstantial regarding why Endostar failed in clinical trials in the United States, but was approved quickly in China. Despite extensive preclinical and clinical studies on endostatin therapy, the specific mechanisms responsible for its anti-angiogenic and anti-tumoral activities are far from completely understood. Originally, endostatin was thought to only block new blood vessel growth, but emerging data suggests that various mechanisms and roles account for the efficacy of endostatin in in vitro and in vivo models. Greater insight into the mechanisms and physiological roles associated with the anti-angiogenic activity of endostatin may help improve the current treatments, uncover other factors with similar activities, identify predictive markers for therapy, and potentially help with the discovery of novel therapeutics. In this review, we will present the various receptors to which endostatin binds and the related mechanisms of action. We will discuss the biological actions and production of endostatin and briefly mention other peptides with activity similar to that of

Abbreviations: NC, noncollagenous; COL, collagenous; MMP, matrix metalloproteinase; bFGF, basic fibroblast growth factor; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; SPARC, secreted protein acidic and rich in cysteine; TG-2, transglutaminase-2; mP, mini peptide; ES, endostatin; mEP, mini endostatin peptide; HUVEC, human umbilical vein endothelial cell

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endostatin. Other anti-angiogenic peptides derived from collagen include arresten, canstatin, tumstatin, and restin, which were recently reviewed [8] and will therefore not be the focus of this review. Endostatin and the fragments of collagen IV are referred to as matrikines [8] or as matricryptins [9,10]. After establishing a framework for endostatin biology, we present current in vitro and in vivo models for studying angiogenesis and the potential that endostatin has for clinical application. Understandably, the hype surrounding endostatin in the early 2000s as the cure-all for various cancers is gone [11], but several research groups have persisted in their efforts to achieve a better understanding of endostatin. The scope of this review is to discuss endostatin's role in regulating angiogenesis, lymphangiogenesis, and cancer metastasis in the hopes of better explaining why continued research into the application of endostatin is worthwhile.

2. The roles of various collagen types in angiogenesis

The basement membrane (BM), a specialized form of the extracellular matrix (ECM), has been recognized for its multi-faceted functions as a regulator of cell interactions, cell structure, and cell assembly. For example, specific components of the vascular BM have been found to regulate angiogenesis. The vascular BM contains collagens, a heterogeneous family of proteins, which contain at least one triple-helical domain made of the repeating Gly-X-Y sequence with the presence of a glycine residue as every third residue [12]. To date, 28 different collagen types have been identified and described in mammalian species, and six of these, type I, type IV, type VIII, type XV, type XVIII, and type XIX, have been implicated in the regulation of angiogenesis.

2.1. Collagen types involved in regulation of angiogenesis

In 1994, O'Reilly et al. discovered the first anti-angiogenic peptide, angiostatin [13]. Angiostatin is a 38-kDa fragment from plasminogen that was first extracted from murine urine and shown to mediate the suppression of murine tumor metastasis by inhibiting endothelial cell proliferation [13]. Since the discovery of angiostatin, other anti-angiogenic peptides associated with collagen have been found and are the topic of this section. Collagens can be categorized as fibrillar or nonfibrillar. Fibrillar collagens form collagen fibrils and are composed of an uninterrupted collagenous domain. Collagen fibrils contribute to the structure, strength, and tensile properties of tissues. This is in contrast to nonfibrillar collagens, which have interruptions in their collagenous domain and structurally do not form fibril bundles. The three primary collagens that have been implicated in the regulation of angiogenesis (type IV, type XV, and type XVIII collagens) are non-fibrillar collagens. In addition, type XV and type XVIII collagens form the multiplexin subfamily of nonfibrillar collagens because both contain multiple alternating collagenous (COL) and noncollagenous (NC) domains [14]. Type I collagen, a fibrillar collagen, has been shown to stimulate angiogenesis in vivo and in vitro [15–17]. Type VIII collagen, a network-forming collagen that forms hexagonal networks, releases vastatin, an anti-angiogenic fragment located at its C-terminus NC1 domain [18]. Type XIX collagen, a member of the fibril-associated collagens with interrupted helices (FACIT) family, also possesses anti-angiogenic properties at its C-terminus NC1 domain [19]. See Table 1.

2.1.1. Type IV collagen

Type IV collagen is the most abundant component of the BM and serves as the scaffold that binds to laminin, fibronectin, entactin, and proteoglycans to form the mesh-like structure of the BM [20,21]. Type IV collagen is composed of six different α chains (α1–α6) that are encoded on six different genes (COL4A1–COL4A6) [22]. The three primary anti-angiogenic fragments released from the α1, α2, and α3 chains of type IV collagen are arresten, canstatin, and tumstatin, respectively. Further information on matrikines released from type IV collagen can be found in a review published by Monboisse et al. [8].

2.1.2. Type XV collagen

Type XV collagen is classified as a chondroitin sulfate proteoglycan and a member of the multiplexin and non-fibrillar collagen subgroups [23]. Type XV is highly homologous to type XVIII collagen; the two share homology in seven of their COL domains, in their NC11 domains, and in their NC1 domains [24–28]. Cleavage of the C-terminal NC1 domain of type XV collagen on its α_1 chain results in the production of restin, a 22-kDa anti-angiogenic factor similar to endostatin [28]. Similar to endostatin, restin inhibits bFGF-induced endothelial cell migration in vitro and exhibits anti-angiogenic properties in vivo in xenograft carcinoma mouse models [28,29]. Endostatin and restin are both capable of suppressing tumor growth, but endostatin has a stronger antitumorigenic effect [28].

2.1.3. Type XVIII collagen

Type XVIII collagen is the only heparan sulfate proteoglycan collagen and is found in various epithelial and vascular BMs. Type XVIII collagen is a non-fibrillar collagen and a member of the multiplexin subfamily [30]. Type XVIII collagen contains 10 collagenous domains interspersed in 11 non-collagenous domains as shown in Fig. 1 [31]. Overall, this structure is flanked by an N-terminal NC11 domain and a C-terminal NC1 domain [31]. The NC1 domain is composed of an association domain involved in oligomerization of three α_1 chains to form a

Table 1
Anti-angiogenic peptides released from collagen in basement membranes.

Collagen chain	C-terminal domain	MW	Function	Reference
α1(IV)	Arresten	26 kDa	Inhibits endothelial cell proliferation and migration Suppresses tumor growth Stimulates apoptosis	Colorado et al. [38] Nyberg et al. [39]
α2(IV)	Canstatin	24 kDa	Inhibits endothelial cell proliferation and migration Suppresses tumor growth Stimulates apoptosis.	Kamphaus et al. [40] Panka and Mier [41]
α3(IV)	Tumstatin	28 kDa	Inhibits endothelial cell proliferation Suppresses tumor growth Stimulates apoptosis	Maeshima et al. [42,43]
α1(VIII)	Vastatin	18 kDa	Inhibits endothelial cell proliferation Stimulates apoptosis	Xu et al. [18]
α1(XV)	Restin	22 kDa	Inhibits endothelial cell migration Suppresses tumor growth	Ramchandran et al. [28] John et al. [29]
α1(XVIII)	Endostatin	20 kDa	Inhibits endothelial cell proliferation and migration Suppresses tumor growth	Sasaki et al. [32]
α1(XIX)	NC1 domain	2 kDa[a]	Inhibits endothelial cell migration Suppresses tumor growth Inhibits endothelial cell pseudotube formation	Ramont et al. [19]

[a] Predicted using 19 amino acid sequence of type XIX collagen's NC1 domain [19] with ProtParam [44].