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Endostatin and endorepellin: A common route of action for similar angiostatic cancer avengers☆

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ABSTRACT

Traditional cancer therapy typically targets the tumor proper. However, newly-formed vasculature exerts a major role in cancer development and progression. Autophagy, as a biological mechanism for clearing damaged proteins and oxidative stress products released in the tumor milieu, could help in tumor resolution by rescuing cells undergoing modifications or inducing autophagic-cell death of tumor blood vessels. Cleaved fragments of extracellular matrix proteoglycans are emerging as key players in the modulation of angiogenesis and endothelial cell autophagy. An essential characteristic of cancer progression is the remodeling of the basement membrane and the release of processed forms of its constituents. Endostatin, generated from collagen XVIII, and endorepellin, the C-terminal segment of the large proteoglycan perlecan, possess a dual activity as modifiers of both angiogenesis and endothelial cell autophagy. Manipulation of these endogenously-processed forms, located in the basement membrane within tumors, could represent new therapeutic approaches for cancer eradication.

Contents

Abbreviations: PCD, programmed cell death; NC1, non-collagenous sequence 1; VEGFR1/2, vascular endothelial growth factor receptor 1/2; CAM, chick chorioallantoic membrane; MMPs, matrix metalloproteinases; GAP, GTPase-activating protein; FAK, focal adhesion kinase; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; HIF-1α, hypoxia-inducible factor 1-alpha; VEGFA, vascular endothelial growth factor A; VEGF, vascular endothelial growth factor; Wnt, wingless-type MMTV integration site family; eNOS, endothelial nitric oxide synthase; Akt, protein kinase B; PP2A, serine/threonine-protein phosphatase 2A; GSK-3β, glycogen synthase kinase 3 beta; TNF-α, tumor necrosis factor alpha; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; STAT, signal transducer and activator of transcription; AP-1, activator protein-1; Bcl-2, B-cell lymphoma 2; Bcl-xL, B-cell lymphoma-extra large; SIPS, stress-induced premature senescence conditions; EGF, epidermal growth factor; LG, laminin-like globular domain; GAG, glycosaminoglycan; EHS, Engelbreth–Holm–Swarm; HSPG2, heparan sulfate proteoglycan 2; SEA, sperm protein, enterokinase and agrin; LDL, low-density lipoprotein; FGF, fibroblast growth factor; PDGF, platelet-derived growth factor; HGF, hepatocyte growth factor; TGF-β, transforming growth factor beta; Trol, terribly reduced optic lobes; HS, heparan sulfate; cDNA, complementary deoxyribonucleic acid; LG3, laminin-like globular domain 3; BMP-1, bone morphogenetic protein 1; tPA, tissue plasminogen activator; ECM1, extracellular matrix protein 1; AMP, adenosine monophosphate; PKA, protein kinase A; FAK, focal adhesion kinase; HSP27, heat shock protein 27; SPR, surface plasmon resonance spectroscopy; TIMP-2, tissue inhibitor of metalloproteinase 2; SHP-1, Src homology-2 protein phosphatase-1; RTK, receptor tyrosine kinases; EGFR, epidermal growth factor receptor; PLCγ, phospholipase C gamma; PI3K, phosphatidylinositide-3 kinase; PDK1, phosphoinositide-dependent kinase 1; mTOR, mammalian target of rapamycin; PIP2, phosphatidylinositol 4,5-bisphosphate; IP3, inositol trisphosphate; NFAT1, nuclear factor of activated T-cells 1; JNK, c-Jun N-terminal kinase; PKC, protein kinase C; Vps34, vacuolar protein sorting-associated protein 34; LC3, microtubule associated light chain 3; Peg3, paternally expressed gene 3; LMWH, lower molecular weight; PEG, polyethylene glycol; NGR, asparagine–glycine–arginine; RGD, arginyl–glycyl–aspartic acid; SCID, severe combined immunodeficiency; NSCLC, non-small-cell lung carcinoma; FDA, food and drug administration; PBDC, platinum-based doublet chemotherapy; PET, positron emission tomography; CT, computed tomography; EGFP, enhanced green fluorescent protein.

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1. Introduction

The development of new blood vessels from pre-existing vasculature, known as angiogenesis, is a complex mechanism involving the concerted actions of endothelial cells, smooth muscle cells and pericytes. Due to the intrinsically high proliferative rate of cancer cells, the supply of nutrients and oxygen via angiogenesis is a *sine qua non* for the overall expansion of cancers [1]. Conventional therapy, exerting a cytotoxic action, has been commonly focused on targeting the mass of growing cancer cells; however, drug resistance to single agent therapies is often an adverse outcome [2].

Proteoglycans are large molecules with complex modular structures that reside in strategic positions, within the extracellular matrix and basement membranes, and are in close contact with vascular endothelia. By virtue of their particular architecture, they directly interact with ligands and receptors involved in the regulation of tumor growth and new vasculature formation [3]. The modular nature of proteoglycans results in their susceptibility to proteolytic attack by diverse enzymes in the extracellular environment thereby releasing individual modules with biological activity, often with opposite effects than the parental protein core [4,5].

Autophagy is an emerging field in the context of cancer progression. It is a mechanism exerted through the action of lysosomes that allows cells to maintain a homeostatic balance between *de novo* generated and degraded molecules, under normal conditions. Often, it is physiologically induced to counteract the lack of available nutrients in high metabolic situations, where an energetic supply is needed [6–10]. Autophagy can evoke apoptotic cell death [11–13] but, in response to cytotoxic stimuli, can promote autophagic programmed cell death (PCD) in cells that are instead protected against apoptosis [14]. Hence, autophagy exhibits duality, in that it may be cytoprotective or cytotoxic.

Many factors combine to orchestrate and regulate angiogenesis and autophagy, and since aberrations of these programs are often seen in tumors, its modulation holds clinical value in cancer therapy [15]. Recent evidence suggests that several constituents of the extracellular matrix can regulate autophagy via interaction with cell surface receptors [16]. Thus, together with the ability to regulate angiogenesis [17], proteoglycans and other matrix constituents can harbor pro-autophagic activity that can be beneficial in suppressing cancer growth [18–22]. Recent discoveries have pointed out a new activity for endogenously-released fragments of the extracellular matrix, not only as anti-angiogenic factors but also as autophagy inducers [23–25].

In this review, we will critically assess the role of two well-known fragments derived from heparan sulfate proteoglycan (HSPG) protein core, namely endostatin derived from collagen XVII and endorepellin, derived from perlecan. After several years of investigating the biological effects of these two anti-angiogenic factors there is new evidence indicating that both bioactive molecules converge on a common theme of action: dual receptor antagonism leading to angiostatic and pro-autophagic activity.

2. Collagen XVIII

Collagen XVIII belongs to a group of collagen-like proteins of the extracellular matrix also known as multiplexins, which include collagen XV as its closest relative [26]. It was subsequently discovered that collagen XVIII is substituted with HS chains and thus it is a true HSPG [27]. Collagen XVIII possesses a trimeric structure with a central area of three homologous α1 chains, and it harbors ten collagen regions interrupted by eleven non-collagenous (NC) domains [27,28] (Fig. 1A). Collagen XVIII and XV share an N-terminal thrombospondin-like module. In addition, the N-terminus of collagen XVIII can contain a cysteine-rich domain related to the frizzled module of *Drosophila* and/or an acidic segment A, based on alternative splicing. These multiplexin components can be modified by chondroitin sulfate chains, on collagen XV, or HS side chains, on collagen XVIII [27,29,30]. They share not only structural homology but also a C-terminal NC1 module containing the endostatin protein with intense angiostatic activity (Fig. 1A). Localized to chromosome 21 [31], the gene of human collagen XVIII possesses 43 exons and two promoters. Variants of its transcription generate a total of three different isoforms. One short form of this collagen is NC11-303, whereas another promoter activity is responsible of the other two longer isoforms [32–36].

Collagen XVIII is widely distributed and it is one of the main constituents of epithelial and vascular basement membranes [26]. Mice deficient in *Col18* show abnormal eye development [37] and abnormal ocular vessel formation and maturation [38–40]. Additionally, during atherosclerosis collagen XVIII plays a role in neovascularization and in preserving the permeability of blood vessels [41,42]. Collagen XVIII has been suggested not only as an anti-atherosclerotic factor but also as a negative regulator of angiogenesis. Indeed, aortic explants isolated from *Col18a1*$^{-/-}$ mice show increased angiogenesis compared to wild-type mice [43]. Recently, collagen XVIII has been implicated in the pathogenesis of renal ischemia/reperfusion as a mediator of leukocytic influx [44], and in hyperlipidemia associated with fatty liver and visceral obesity, suggesting that it might play a role in the adipose tissue formation [45].

2.1. Prognostic relevance of collagen XVIII in cancer

In humans, a mutation in *COL18A1* gene results in an autosomal recessive disease, the Knobloch syndrome, which in turn leads to blindness at birth because of abnormal retinal development [36,46]. Similarly, a pathology in which the retina is not well vascularized has been also reported in *Col18a1*$^{-/-}$ mice [47]. Notably, in *Caenorhabditis elegans*, deletion of the NC1 domain of *cle-1*, the orthologue of human collagen XVIII, induces defects in cell migration and axonal guidance, and this phenotype can be rescued by ectopic expression of this domain [48].

In spite of an accumulating wealth of information on the role of collagen XVIII in various pathological states, the biological role of collagen XVIII in human cancer is not well defined. There are several studies reporting abnormal levels of endostatin (see also below) in

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