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Proteoglycan signaling in tumor angiogenesis and endothelial cell autophagy

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Abstract

The need for more effective cancer therapies is omnipresent as the ever-complex, and highly adaptive, mechanisms of tumor biology allow this disease to elude even the most stringent treatment options. The expanding field of proteoglycan signaling is enticing as a reservoir of potential drug targets and prospects for novel therapeutic strategies. The newest trend in proteoglycan biology is the interplay between extracellular signaling and autophagy fueled by the close link between autophagy and angiogenesis. Here we summarize the most current evidence surrounding proteoglycan signaling in both of these biological processes featuring the well-known suspects, decorin and perlecan, as well as other up-and-coming neophytes in this evolving signaling web.

Keywords

Proteoglycan; autophagy; angiogenesis; decorin; perlecan; biglycan; endostatin; tumorigenesis

1. Introduction

The role of proteoglycans as key signaling effectors is increasingly becoming mainstream [1–5]. Though these “glycanated gems” contribute to cell signaling in a variety of ways, including but not limited to immune regulation, modification of migration and proliferation, and promotion of cell survival [6–10], a niche role for proteoglycans in cancer biology is arising via their ability to both induce and inhibit angiogenesis and autophagy in the tumor parenchyma as well as the surrounding stromal cells.

The mechanism of action of proteoglycans in regulating tumor angiogenesis is intricate. The strong interactions of specific proteoglycans with vascular cells in the extracellular matrix alter their pathogenic phenotype. This process is fundamental in the control of vascular disease [11,12] and plays a significant part in regulating neovascularization. Angiogenesis can only happen when an orchestrated signaling cascade causes the breakdown of the basement membrane and extracellular matrix allowing proliferation and migration of capillary endothelial cells into the surrounding tissue, feeding the cancer cells. The switch

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from physiological vasculature to tumor neovascularization is evoked by systematic changes in the balance of pro- and antiangiogenic factors bound to heparin sulfate proteoglycans in the extracellular matrix and by a dysregulated expression of the major secreted proteoglycans in the tumor stroma. The binding of these proteoglycans to tyrosine kinase receptors, toll-like receptors and integrins on the surface of cancer and stromal cells leads to intracellular signaling events that finely regulate proliferation, migration and survival of malignant cells as well as vascular development through engagement of the endothelial stromal compartment [13]. Moreover, proteoglycans regulate tumor vascularization by simultaneously controlling angiogenesis and autophagy, a key homeostatic process in which cytosolic components are degraded and recycled through lysosomes.

Though autophagy was once believed to be a purely pro-tumorigenic driving force as it enables cell survival, the paradigm surrounding autophagy has shifted, or at least branched into two different schools of thought, regarding its exact function in cancer [14]. Growing evidence suggests that autophagy plays a role not only in cancer cells but also in the tumor microenvironment where it has been linked to both neovascularization and angiostasis. Surely it is a fine balancing act between these two roles, where proteoglycans and other extracellular components are at the epicenter of this intricate signaling hub. Indeed, extracellular matrix constituents can trigger or suppress autophagy, depending on the tissue or cell context [15–19].

Here, we summarize the major autophagic networks involved in proteoglycan signaling in cancer as they pertain to the most prominent players in this field: decorin, perlecan and its C-terminal fragment endorepellin, biglycan, syndecan, and endostatin. We also highlight some lesser studied, but equally significant, functions for other members of this proteoglycan family: lumican and glypican. Together, we hope to create a contemporary repository for these matrix components as a guide for current researchers to continue to investigate in the realm of cancer biology in hopes of identifying novel drug targets and therapeutic strategies.

2. Decorin, a long-standing catabolic champion

Decorin, a member of the small, leucine-rich family of proteoglycans, is the best characterized of all proteoglycans in terms of autophagy. From its humble beginnings as a regulator of collagen organization [20–22] to its long-storied history as a pan-receptor tyrosine kinase (RTK) inhibitor [23–27] and regulator of inflammation and fibrosis [28–32] decorin has a vast resume of outside-in signaling roles both in normal physiology and disease [33–36]. In the past decade, decorin has become a well-regarded pro-autophagic effector [16,17,37] (Fig. 1). We serendipitously discovered that decorin was capable of inducing canonical autophagy in endothelial cells via vascular endothelial growth factor receptor 2 (VEGFR2)-signaling (Fig. 1C) resulting in an adenosine monophosphate kinase (AMPK)-dependent induction of the autophagic proteins, microtubule-associated light chain protein 3 (LC3) and Beclin 1, with concurrent inhibition of the mammalian target of rapamycin (mTOR) complex [15] (Fig. 1C and 2B). This initial work jump-started an investigation that revealed a much more complex interplay between decorin and catabolism.

Of note, prior to the discovery of the decorin-autophagy connection, a unique microarray investigating genes affected by decorin *in vivo* in both the tumor proper and tumor stroma unearthed a novel transcription factor, paternally expressed gene 3 (Peg3) as a decorin-inducible gene unique to the tumor microenvironment [38]. After noting the link between decorin and autophagy, further study revealed that Peg3 is a necessary intermediary in autophagic progression in endothelial cells and is absolutely required for activation of the Beclin 1 promoter as well as the secretion of the anti-angiogenic factor, thrombospondin-1 [39], thereby providing the first connection between decorin-induced autophagy and angiostasis (Fig. 1C).

It is important to note that these initial studies connecting decorin and autophagy were performed solely in endothelial cells, a key component of the tumor stroma. Given its promiscuity in RTK-binding, decorin is distinctive among proteoglycans and other signaling molecules in that it interacts with both the tumor stromal cells and the tumor cell surface [27]. Thus, the focus next turned toward the possibility that decorin might also induce autophagy in the tumor proper. Interestingly, rather than evoking canonical autophagy as it does in the tumor vasculature, decorin instead induces mitophagy, or mitochondrial autophagy, in a triple negative breast carcinoma model (Fig. 1B). [40]. This signaling pathway is spearheaded via a decorin/Met receptor interaction resulting in induction of the tumor suppressor and mitophagic inducer, mitostatin (Fig. 1B) [40]. This mitochondrial depolarization likely impacts tumor cell metabolism, curtailing tumor growth. The importance of these findings is underscored by the fact that loss of mitostatin results in the inability of decorin to suppress pro-angiogenic factors, namely VEGFA [41], resulting in a complex interplay between the tumor proper, its secretome, and the tumor microenvironment, particularly the tumor blood supply.

Additionally, in a glioma model, the ability of decorin to inhibit cell migration has been mechanistically linked to autophagy induction [42]. Though this study was the first documentation of decorin-provoked canonical autophagy in cancer cells, it is highly probable that other tumors can undergo this process in response to decorin provided they have the proper cell surface receptors and downstream signaling machinery. Interestingly, one of the first RTKs implicated in decorin-induced tumor stasis was the epidermal growth factor receptor (EGFR) [43–45] (Fig. 1A). Work done by an independent group illustrates that EGFR-inhibition is coupled with autophagic induction [46]. Given the ability of decorin to inhibit EGFR [43] (Fig. 1A), it would be surprising if decorin treatment did not induce autophagy in tumor types that express high levels of this RTK.

Furthermore, decorin itself is the first proteoglycan to be deemed “autophagy-inducible” [47]. Specifically, decorin transcript and protein expression are significantly increased in the setting of nutrient deprivation as well as following direct inhibition of mTOR using the small molecule inhibitor, Torin 1 [47]. Moreover, decorin itself is a metabolic regulator affecting the glucose metabolism in the heart. Specifically, *Dcn*^{-/-} mice display impaired cardiac autophagy following fasting [48] with resultant aberrations in glucose metabolism and cardiac function, which can be rescued with exogenous decorin treatment where these mice appear to be exquisitely sensitive to decorin given their native decorin-null state [48]. Though these anomalies in metabolism were observed in the heart, it is likely that some of

these differences attributed to lack of decorin expression may also hold true in the setting of cancer, as most tumor cells do not express this proteoglycan.

Along with its effect on the tumor vasculature and tumor cells and, given its ability to interact with countless RTKs, we believe that decorin may also induce autophagy in other stromal cell types, including fibroblasts. Thus, the implications are vast for the utility of channeling the pro-autophagic capability of decorin in terms of cancer treatment. Both decorin itself and any of its numerous downstream partners are ideal targets for novel drug design. As we participate in the ever-expanding research in this field, it is likely that we will see much more innovation in the coming years with advancement in patient prognosis and outcome.

3. Perlecan: a double-edged sword in autophagy

Perlecan, a gigantic basement membrane heparan sulfate proteoglycan, has been a key contender in promoting carcinogenesis for ages given its function as a repository of pro-angiogenic growth factors to provoke neovascularization and tumor development [49–51]. Furthermore, the dichotomy between parent perlecan and its C-terminal fragment endorepellin in angiogenesis is striking [49,52,53]. This opposition also holds true in the relationship between whole perlecan and endorepellin with autophagy. As an entire proteoglycan, perlecan acts in an anti-autophagic manner [54]. Specifically, studies involving conditional *Hspg2*^{-/-} mice revealed that muscle lacking perlecan houses increased numbers of autophagosomes and exhibited significant inhibition of the mTOR pathway when compared with their wild-type counterparts [54]. Functionally, these findings result in muscle atrophy due to enhanced catabolism and highlight the role of perlecan in the maintenance of muscle mass by suppressing autophagy [54].

In complete juxtaposition, endorepellin, domain V of perlecan is anti-angiogenic [55–59] and undertakes a wholly different function in autophagy. Like decorin, endorepellin binds to and signals through VEGFR2 to induce the pro-autophagic intermediaries AMPK, Beclin 1, LC3, and p62 [60] while contemporaneously inhibiting mTOR (Fig. 3A) overall resulting in the formation of large autophagosomes in endothelial cells (Fig. 4A). Interestingly, this activity via VEGFR2 is reliant on the phosphorylation of Tyr¹¹⁷⁵ as prevention of its activity blocks these pro-autophagic effects [60]. Unlike decorin, endorepellin is unique in that it engages in dual receptor antagonism by binding simultaneously to VEGFR2 and $\alpha 2\beta 1$ integrin (Fig. 3A). [52]. However, this endorepellin-integrin axis appears to be completely uninvolved in its ability to evoke autophagy [60]. Moreover, this signaling avenue may even be antithetical to the endorepellin-VEGFR2 pathway as treatment with the laminin globular domain 3 (LG3) region of endorepellin, which solely and selectively binds to the integrin rather than VEGFR2, actually inhibits autophagic gene expression [60]. Thus, there may be opposing roles within a single proteoglycan entity which likely serve to fine-tune physiologic autophagy to prevent excessive or insufficient catabolism necessary for normal cellular homeostasis.

Like decorin, endorepellin markedly induces the expression of Peg3 [60], the newly discovered master regulator of autophagy [15], while inhibiting VEGFA [39]. Peg3 plays a

vital role in the initiation of autophagy as it is relocated and considerably expressed in large autophagosomes in the cytoplasm of endothelial cells (Fig. 4D) where it also recruits the autophagic markers Beclin 1 and LC3.

Recently, the ability of endorepellin to induce autophagy in endothelial cells has taken a more directly physiologic role. Using aortic ring assays as a proxy for tumor angiogenesis, current work demonstrates that endothelial sprouting is exquisitely sensitive to endorepellin [61] (Fig.2 A, B). This endorepellin-induced angiostasis is directly linked to its pro-autophagic capabilities as blocking autophagy using the AMPK-inhibitor, Compound C prevents the endorepellin-mediated reduction in blood vessel growth [61]. Even more, telling is the finding that Torin 1 similarly inhibits vessel sprouting via direct mTOR inhibition [61]. Hence, the ability of endorepellin to curb tumor neovascularization is an attractive target for novel adjuvant chemotherapeutic options.

Most recently, the aptitude of endorepellin to induce not only canonical autophagy but also mitophagy, has been explored and the results were quite fruitful. Using novel Nanostring technology, the gene signature of endothelial cells treated with endorepellin demonstrated a proclivity for mitophagy vis-à-vis vehicle-treated cells [62]. Subsequent investigation revealed that this pro-mitophagic transcriptome results in enhanced mitostatin and Parkin expression, once again downstream of VEGFR2, and consequent mitochondrial depolarization [62]. Therefore, endorepellin, like decorin, alters both canonical autophagy in a nutrient-independent manner while also affecting the mitochondrial energy balance. Taken together, these combined forces act as powerful mediators of endothelial cell dynamics resulting in a reduction in vessel growth. Therefore, future work will elucidate a more specific and translational role for endorepellin in antiangiogenic therapy.

4. Biglycan: bridging inflammation and autophagy

Like decorin, biglycan is a member of the family of small, leucine-rich proteoglycans that has carved a place for itself not only in skeletal-muscle diseases [63,64] but also in inflammation [65,66] and angiogenesis [67]. Following an insult to tissue, biglycan is proteolytically released from the extracellular matrix and is freely soluble to instigate pro-inflammatory signaling pathways [68,69]. Alternatively, macrophages can synthesize this proteoglycan *de novo* [65]. Either scenario results in biglycan acting as a signaling ligand for the Toll-like receptors (TLR) 2 and 4 to activate the ERK and NF- κ B pathways, thereby modifying the immune response and recruiting leukocytes to the area of interest [70,71] (Fig. 1D). Moreover, in macrophages, soluble biglycan engages TLR2/4 and the co-receptor CD14 to activate the p38/NF- κ B/MAPK pathway, thereby triggering inflammation [72] (Fig. 1D).

With regard to neovascularization, biglycan takes an opposing role to decorin, despite their structural homology and close family ties. Specifically, biglycan binds VEGFA and activates signaling via VEGFR2 to promote angiogenesis [73]. Moreover, biglycan overexpression in colon cancer cells can directly upregulate VEGFA expression, thereby promoting tumor neovascularization and cancer growth [74]. Interestingly, biglycan levels are markedly increased in tumor endothelial cells, especially in highly-metastatic tumors, due to

hypomethylation of the *BGN* promoter in tumor endothelial cells [75]. Thus, epigenetic control of the *BGN* gene could play a role in enhancing the structure and function of the tumor blood vessels in the metastatic niche [76].

Regarding catabolic pathways linked to biglycan, emerging data suggest that this leucine-rich proteoglycan is capable of acting in a pro-autophagic manner [77]. In an ischemia/reperfusion model using cardiomyocytes, biglycan is able to provide cytoprotection following reperfusion by increasing levels of autophagy [77]. Mechanistically, these findings appear to be related to biglycan/TLR-4 signaling, as inhibition of downstream effectors of this pathway results in less cardioprotection following biglycan treatment [77]. Most recently, biglycan was found to induce autophagy in M1-type macrophages by engaging both TLR-4 and CD44, a receptor that is a critical binding partner for hyaluronan and is also involved in angiogenesis [78] (Fig. 1D). Physiologically, this biglycan-mediated autophagic induction was related to increased M2 macrophage recruitment to the kidney and reduced renal tubular damage following ischemiareperfusion injury [78]. Though these findings were not observed in a cancer setting, the role of tumor-associated macrophages (TAM) in the tumor stroma as modulators of metastasis and chemo-resistance makes this novel information attractive for future malignancy-related applications.

On the other hand, despite the emergence of these novel data supporting biglycan-induced autophagy, there is also evidence that biglycan may counteract this process as it inhibits the activity of endostatin, the C-terminal fragment of collagen XVIII, which exhibits pro-autophagic properties via binding and signaling through $\alpha 5\beta 1$ integrin to increase Beclin 1 expression [79,80] (Fig. 3B). Thus, like perlecan, the role of biglycan in autophagy is complex and possibly context-dependent, and its exact role in tumorigenesis and neovascularization is yet to be seen.

5. Syndecan and endostatin: new players in autophagy

A single transmembrane heparan sulfate proteoglycan, syndecan wears many hats in terms of function [24]. As syndecan is expressed at the cell surface, the composition of its glycosaminoglycan chains is dependent on its localization to ensure that the proper growth factors are presented to their respective receptors. Along with growth factor presentation, syndecan family members are also crucial for matrix and cell-cell adhesion and tumorigenesis [81]. Heparanase, an enzyme that selectively cleaves heparan sulfate molecules, is especially important for linking syndecan shedding to tumor growth and metastasis [82–84]. With regard to autophagy, heparanase acts as an autophagic inducer as heparanase overexpression reduces mTOR activity whereas reduction of heparanase conversely increases this pathway [85]. As the heparan sulfate side chains of syndecan are prominent targets of this enzyme and promote angiogenesis via growth factor presentation, it is likely that there exists a deeper role for syndecan in this autophagic induction and resultant tumor progression.

Moreover, a recent study suggests that the role of autophagy as it pertains to syndecan is even more complex. In glioblastoma multiforme, glioma stem cells (GSCs) provide a means for resistance to chemotherapy as well as a breeding ground for tumor recurrence [86]. A

mechanism by which they cause these effects is via protective autophagy, rendering them resistant to anoikis [86]. This protective autophagy is thought to be mediated in part by syndecan binding protein (SDCBP) [86]. Intriguingly, suppressing SDCBP promotes autophagic cell death [86]. Thus, the interchange between protective autophagy to promote tumor survival and autophagic cell death appears to be mediated by a binding partner of syndecan, and therefore more work needs to be done to completely unravel this proteoglycan-related signaling pathway and how best to exploit it in reducing tumor burden.

Endostatin, the N-terminal hinge domain of collagen XVIII is detected in various human tissues and sera [80]. A 22 kDa globular domain, endostatin is cleaved from collagen by the proteases cathepsin L and elastase and released as a monomer. Endostatin has a broad spectrum of angiostatic activities on endothelial cells mediated by the direct binding with VEGFR2 (Fig. 3B). The interaction of the two molecules inhibits VEGF activity by blocking VEGF-induced VEGFR2 phosphorylation and consequent activation of ERK, p38 MAPK, and p125FAK [87]. In cancer, endostatin inhibits local invasion and tumor vascularization, overall causing a decrease in tumor growth due to downregulation of the mRNA levels of VEGFA [88]. The robust angiostatic activity of endostatin is also mediated by the binding to its first ever-identified receptor, the integrin $\alpha 5\beta 1$ on the surface of endothelial cells. Soluble endostatin acts like an antagonist by inhibiting integrindependent endothelial cell functions, such as cell migration [89]. Moreover, endostatin induces autophagy in endothelial cells by interacting with $\alpha 5\beta 1$ -integrin and modulating Beclin 1 and β -catenin levels and downregulating endogenous levels of the anti-apoptotic Bcl-2 [79] (Fig. 4B). Overall, the autophagic and angiostatic activity of endostatin in endothelial cells could represent a novel therapeutic agent in the tumor microenvironment, used to counteract tumor growth and spreading.

6. A proteoglycan portfolio: other autophagic players

As proteoglycan-regulated autophagy is becoming more popular, a collection of these extracellular signaling effectors in the setting of cancer and targeted therapy is rapidly growing. For example, lumican, a small, leucine-rich proteoglycan structurally similar to decorin and biglycan, has been shown to increase the efficacy of chemotherapy partly via inhibiting AMPK and consequent autophagy [90]. Moreover, like decorin, lumican also promotes mitochondrial damage and reactive oxygen species production, which has been thought to result in increased sensitivity to the cancer drug, gemcitabine [90].

Glypican-3, another cell surface-associated heparan sulfate proteoglycan and solidly established marker of hepatocellular carcinoma, may also be a contender in the autophagic web of cancer as p62, a well-known autophagic substrate, is increased in these cancer cells due to a catabolic defect preventing its autophagic clearance [91]. Importantly, mTOR inhibition using the small molecule inhibitor, Torin 1, reduces expression of glypican-3 and p62 in these hepatocellular carcinoma cells [91], suggesting that the autophagic defect may be to blame for the increased glypican levels in these tumors and also suggests that there may be a feedback mechanism for the catabolic degradation and/or induction of proteoglycans, particularly in the background of cancer.

7. Conclusions and perspectives

The extracellular matrix is becoming a hotspot of potential diagnostic indicators, prognostic markers, and therapeutic targets, particularly when it comes to proteoglycans. These matrix components are extremely attractive as druggable targets given their active roles in both angiogenesis and autophagy, two biological processes highly linked to tumorigenesis, metastasis, and chemo-resistance. This material is even more important as we are now uncovering the links between these two pathways and learning better how to influence one by swaying the other. Though the nuances are still enigmatic, it does appear that altering either autophagy or angiogenesis has a direct effect on the other. Moreover, though the literature is most extensively focused on a select subset of proteoglycans, particularly small, leucine-rich and heparan sulfate proteoglycans, in autophagy and angiogenesis, it is categorically conceivable to believe that there are others that will soon be added to this ever-growing list.

Thus, we affirm that this area of research is particularly exciting and we are hopeful that there will be many more discoveries in the near future that will bring us closer to bridging the bench to the bedside by exploiting proteoglycans or their derivatives as both markers of disease as well as drug targets and/or adjuvant therapies.

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Abbreviations

AMPK	adenosine monophosphate kinase
BGN	biglycan
DCN	decorin
EGFR	epidermal growth factor receptor
ERK	extracellular signal-regulated kinase
GSC	glioma stem cells
Hspg	heparan sulfate proteoglycan
LC3	microtubule-associated light chain protein 3
LG3	laminin globular domain 3
Met	hepatocyte growth factor or scatter factor receptor
mTOR	mammalian target of rapamycin
NF-κb	nuclear factor kappa-light-chain-enhancer of activated B cells

PEG3	paternally expressed gene 3
PGC1-α	peroxisome proliferator-activated receptor gamma coactivator 1 α
RTK	Receptor Tyrosine Kinase
SDCBP	syndecan binding protein
TAM	tumor-associated macrophage
TLR	Toll-like receptor
VEGF-A	vascular endothelial cell growth factor A
VEGFR2	vascular endothelial cell growth factor receptor 2

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Fig. 1.

A. View on decorin's angiostatic and pro-autophagic signals following binding of decorin to different receptors on the surface of cancer and stromal cells of the tumor microenvironment. Notice the tumor-suppressive action of decorin upon binding to EGFR and Met directly on tumor cells (A, B), its anti-angiogenic and pro-autophagic activity through binding to VEGFR2 on endothelial cells (C), and its relative, biglycan's TLRs-mediated ability to induce inflammation and autophagy in tumor-associated macrophages (D).

Fig. 2.

Decorin and endorepellin-induced angiostasis and autophagy in *ex vivo* and *in vitro* conditions, respectively (A) Representative confocal z-stack images of aortic rings treated with vehicle (PBS), decorin (200 nM) or endorepellin (200 nM), respectively. Treatment of vehicle/decorin/endorepellin was given on alternating days for a total of seven days. Note the dense-network of sprouts seen emanating from vehicle-treated rings whereas minimal sprouting is seen under decorin and endorepellin-treated conditions. Rings were labeled with IB4 (red), Isolectin B4 which is an endothelial cell marker, scale bar = 500 μm. (B) Immunofluorescence images of HUVEC cells treated with vehicle, decorin (200 nM, 6 h) or endorepellin (200 nM 6 h). Cells were stained for (top panel): LC3β (green) and DAPI (blue) or (bottom panel): mTOR (green) and DAPI (blue), with prominent autophagosome formation (white arrows), scale bar = 10 μm.

Fig. 3.

A comprehensive model of angiostatic and pro-autophagic activities exerted by endorepellin (A) and endostatin (B) in endothelial cells. Endorepellin evokes autophagy by binding to VEGFR2 and inhibiting mTOR. Endostatin, instead, prevents binding of VEGFA to VEGFR2, therefore inhibiting its downstream signaling and evokes autophagy via the integrin $\alpha_5\beta_1$.

Fig. 4. Gallery of fluorescence micrographs depicting autophagy in endothelial cells. A) Differential interference contrast microscopy images of human endothelial cells exposed to vehicle, endorepellin or rapamycin. Notice the formation of large autophagosomes in both experimental conditions (white arrows), Nu = nucleus labeled by DAPI. (B) Effect of endostatin on autophagy as shown by the convergence of Beclin 1 and LC3 into autophagosomes (yellow arrows). (C) Differential interference contrast microscopy of porcine endothelial cells transiently transfected with *Peg3-tdTomato*. Notice the formation of large autophagosomes in the cells expressing *tdTomato* and little or no autophagosomal activity in the untransfected cells (white asterisks). Nuclei labeled by DAPI.