The American Journal of Pathology, Vol. 184, No. 8, August 2014

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The American Journal of PATHOLOGY

ajp.amjpathol.org

# MINI-REVIEW

# Instructive Roles of Extracellular Matrix on Autophagy

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**CME Accreditation Statement:** This activity ("ASIP 2014 AJP CME Program in Pathogenesis") has been planned and implemented in accordance with the Essential Areas and policies of the Accreditation Council for Continuing Medical Education (ACCME) through the joint sponsorship of the American Society for Clinical Pathology (ASCP) and the American Society for Investigative Pathology (ASIP). ASCP is accredited by the ACCME to provide continuing medical education for physicians.

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**CME Disclosures:** The authors of this article and the planning committee members and staff have no relevant financial relationships with commercial interests to disclose.

Accepted for publication May 14, 2014.

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Autophagy plays an essential role in maintaining an intricate balance between nutrient demands and energetic requirements during normal homeostasis. Autophagy recycles metabolic substrates from nonspecific bulk degradation of proteins and excess or damaged organelles. Recent work posits an active and dynamic signaling role for extracellular matrix-evoked autophagic regulation, that is, allosteric and independent of prevailing nutrient conditions. Several candidates, representing a diverse repertoire of matrix constituents (decorin, collagen VI, laminin α2, endostatin, endorepellin, and kringle V), can modulate autophagic signaling pathways. Importantly, a novel principle indicates that matrix constituents can differentially modulate autophagic induction and repression via interaction with specific receptors. Most of the matrix-derived factors described here appear to control autophagy in a canonical manner but independent of nutrient deprivation. Because the molecular composition and structure of the extracellular matrix are dynamically remodeled during various physiological and pathological conditions, we propose that matrix-regulated autophagy is key for maintaining proper tissue homeostasis and disease prevention, such as cancer progression and muscular dystrophies. *(Am J Pathol 2014, 184: 2146–2153; http://dx.doi.org/10.1016/j.ajpath.2014.05.010)*

Macroautophagy (hereafter known as autophagy) is a catabolic process that nonselectively degrades bulk cytosolic components, proteins, and organelles, recycling liberated nutrients for the synthesis of new macromolecules.[1,2] Autophagic induction occurs via the reduced availability of nutrients. Intrinsically, autophagic initiation is a tightly coordinated and regulated process, involving the suppression of nascent peptide synthesis [via suppression of phosphoinositide 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) arm] and activation of the forkhead box subclass O3 (FoxO3) transcription factor that promotes recruitment of Vps34, a class III PI3K, to beclin 1-positive complexes.[3,4] Autophagy is linked to prevailing energetic demands, overall cellular metabolome, and cell growth programs.

The modulation of autophagy is emerging as a frontrunner in curtailing several diseases, including tumorigenesis[5] and muscular dystrophies.[6] The classification of autophagy as a tumor suppressive entity[7,8] further aligns with the amply documented function of soluble matrix constituents, such as decorin and endostatin, as oncostatic and angiostatic effectors.[9,10] Moreover, autophagy is gaining acceptance as a necessary prerequisite in maintaining tissue integrity and overall organismal homeostasis.[11–13] Aberrant autophagy, defined by either inadequate levels or hyperactive autophagic flux, is detrimental for normal tissue function and manifests as neurological disorders such as Parkinson disease[14] or congenital myopathies.[6]

Supported by NIH grants RO1 CA39481, RO1 CA47282, and RO1 CA164462 (R.V.I.) and the German Research Council SFB 815, project A5, SFB 1039, project B2, and Excellence Cluster ECCPS (L.S.).

Disclosures: None declared.


http://dx.doi.org/10.1016/j.ajpath.2014.05.010

Matrix-derived signals transduced from receptor engagement converge on a common core of autophagic machinery in a variety of tissues and specialized microenvironments and are necessary for germane biological phenotypes. A diverse collection of matrix constituents, including decorin, collagen VI, laminin α2, endostatin, endorepellin, and kringle V, are rapidly emerging as regulators of autophagy that are seemingly independent of the predominant nutrient concentrations. Although the concept of extracellular matrix (ECM) regulation of autophagy has been discussed before,[15] most of the previous studies focused on how detachment from the matrix (anoikis) would trigger autophagy, primarily through an integrin-mediated mechanism. In this review, we focus on soluble, matrix-derived products with an intrinsic ability to modulate catabolic metabolism. We propose that the ECM is a functional repository of autocrine and paracrine factors that dynamically modulate, couple, and wield autophagy as a potent tumor suppressor and function prominently in the prevention of several complex pathological states.

## Decorin Evokes Autophagy and Mitophagy

### Decorin Regulates Oncogenes and Tumor Suppressor Genes

Decorin, with the proposed title of guardian from the matrix, is a pan-receptor tyrosine kinase (RTK) inhibitor for tumorigenic and angiogenic suppression.[10,16] It binds RTK members via its protein core, concurrently elicits degradation, and attenuates downstream signaling.[10] Decorin suppresses critical oncogenes (β-catenin, *Myc*, and hypoxia inducible factor-1α) and simultaneously induces tumor suppressor genes (*p21*, thrombospondin-1, and mitostatin) that oppose oncogenesis and angiogenesis.[17,18] Moreover, decorin coordinates a pro-inflammatory cascade function in sepsis and tumorigenesis.[19]

### Decorin Induces *Peg3* Expression

A paradigm shift has occurred about the bioactivity of decorin after high-resolution transcriptome profiling of the tumor microenvironment.[20] Systemically administered decorin evokes a stromal-specific gene signature. Among the small subset of induced genes, decorin stimulates the expression of a putative tumor suppressor gene *Peg3*.[20] By exploiting the genomically stable nature of endothelial cells as a surrogate for a crucial component of the tumor microenvironment, paternally expressed 3 (Peg3) immunostaining with established autophagic markers has indicated the formation of subcellular structures reminiscent of autophagosomes.[21]

Mechanistically, decorin engages vascular endothelial growth factor 2 (VEGFR2) for Peg3 redistribution on the autophagosomal membrane, concomitant with autophagosome formation in contrast to control conditions (Figure 1, A and B). Paramount with these observations is the requirement of Peg3 in maintaining basal *BECN1* levels. Further, silencing *Peg3* abrogates decorin-mediated induction of autophagic genes downstream of VEGFR2[21] (Figure 2). Concurrently, mitochondrial membrane potential and capillary morphogenesis are disrupted.[21]

Despite Peg3 being classified as a krupple-type zinc finger transcription factor, Peg3 is not observed within the nuclear compartment of endothelial cells, as shown for neural progenitors.[22] Because the architecture of zinc finger domains permits high-affinity RNA or DNA binding, Peg3 may bind mature *BECN1* mRNA for protection, stability, and/or enhanced translation for rapid synthesis. This scenario is congruent with the rapid kinetics of decorin-evoked autophagic induction. Further, despite decorin inducing *Peg3* expression in mammary fibroblasts cocultured with triple-negative breast carcinoma cells,[20] transcriptional induction with macrovascular and microvascular endothelial cells is not achieved. Therefore, it remains plausible that decorin directly stabilizes Peg3 via as-of-yet defined post-translational modifications.

### Decorin Is a Partial Agonist of VEGFR2

Molecular dissection of the downstream signaling apparatus reveals that decorin rapidly activates AMP-activated protein kinase, a master energy sensor and pro-autophagic kinase,[23,24] downstream of VEGFR2 signaling[25] (Figure 2).

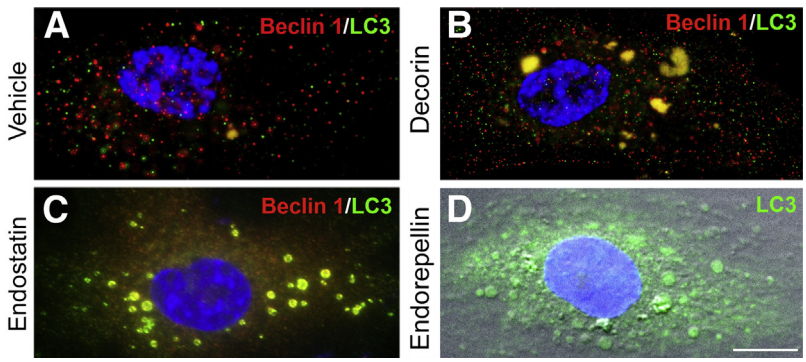


**Figure 1** Induction of endothelial cell autophagy by various soluble extracellular matrix constituents. Confocal images of human umbilical vein endothelial cells treated for 6 hours with vehicle (**A**) or with either 200 nmol/L human recombinant decorin (**B**) or 200 nmol/L human recombinant endostatin (**C**). Notice the presence of numerous autophagic vacuoles immunolabeled by antibodies against beclin 1 (red) and LC3 (green) after merging of the two fluorophores. Nuclei are stained in blue with DAPI. **D:** Immunofluorescence image of human umbilical vein endothelial cells treated with 200 nmol/L human recombinant endorepellin for 6 hours and stained with an antibody against LC3. The images were captured under differential interference contrast microscopy and show large autophagosomes stained by LC3. Scale bar = 10 μm (**A**–**D**).

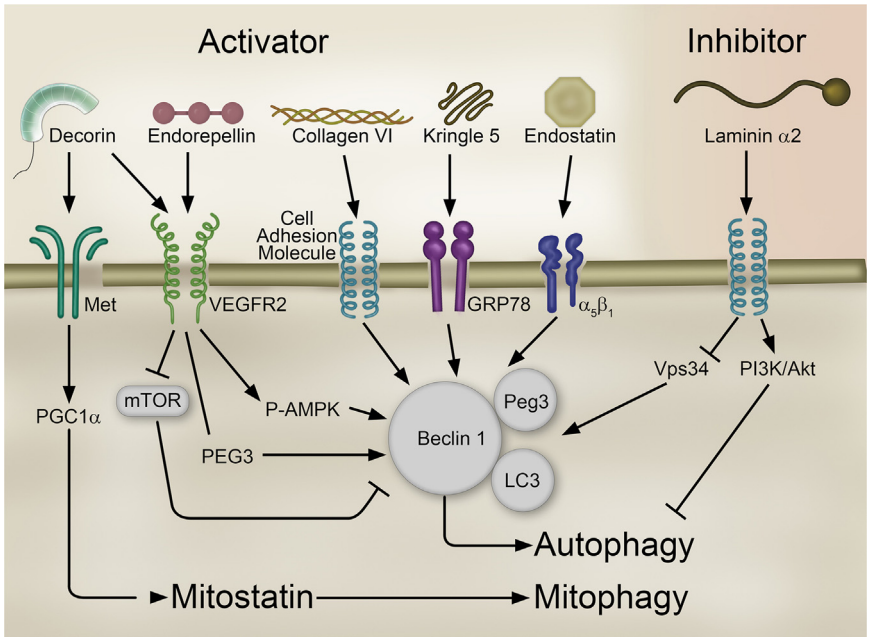


**Figure 2** Categorically, the regulatory matrix entities segregate into two functionally distinct classes, activators (decorin, collagen VI, kringle 5, and endostatin) and inhibitor (laminin α2). Binding of each discrete molecule to a cognate receptor permits a multitude of signaling cascades that terminate on shared autophagic components. Cell- and tissue-specific modulation of this common core (Peg3, beclin 1, and LC3) ultimately dictates a pro- or anti-autophagic response. Please see text, where indicated, for complete pathway analyses. P-APMK, phosphorylated adenosine 5′-monophsophate-activated protein kinase.

Deciphering the complex regulatory network reveals that decorin suppresses anti-autophagic effectors (Akt, mTOR, p70S6K) while simultaneously evoking the physical and recombinatorial association of Bcl-2, beclin 1, and Vps34[25] Decorin-induced association of beclin 1/Vps34 complexes and the concurrent disengagement of the autophagic inhibitor Bcl-2 further reinforce a dynamic network for autophagic initiation that is wholly coordinated by an ECM constituent.[25] These findings reveal a new function for decorin. Because decorin requires the VEGFR2 kinase for downstream autophagic induction, the view of decorin as strictly an RTK inhibitor erodes; decorin does indeed function as a partial agonist of VEGFR2 and a modulator of a novel master regulator of autophagy, Peg3, which controls beclin 1 and LC3 expression.

### Decorin Attenuates EGFR and Downstream Effectors

Decorin has an avid proclivity for attenuating epidermal growth factor receptor (EGFR) and downstream effectors. Notably, a recent study indicates that EGFR and Akt phosphorylate beclin 1, inactivating autophagic progression and promoting tumorigenesis.[26,27] Attenuation of EGFR and Akt signaling by decorin[28] represents a viable signaling pathway for autophagic induction in cancer cells. Decorin exerts its anti-oncogenic activities indirectly via endothelial autophagy and directly by attenuating the angiogenicity of the tumor proper. Moreover, decorin functions as a partial agonist of Met and evokes tumor cell mitophagy in mammary carcinoma cells.[29]

By dynamically regulating peroxisome proliferator-activated receptor-γ coactivator-1α, stabilized mitostatin mRNA permits accumulated mitostatin and mitophagic induction independent of beclin 1 (Figure 2).[29] Crucially, loss of mitostatin abrogates canonical mitophagic stimuli (rapamycin and nutrient deprivation) and decorin-evoked mitophagy.[29] These observations define mitostatin as a novel and fundamental mitophagic effector. Importantly, failure of mitophagic induction prevents decorin from suppressing vascular endothelial growth factor A–mediated angiogenesis. Thus, mitostatin (and mitophagic initiation) is critical for decorin-mediated angiostasis.[29]

Autophagic induction may represent a general phenomenon for related SLRP members. Decorin may represent a novel class of matrix components that are released for autophagic induction after a pathological insult. A recent study indicates that FoxD1 transcriptionally represses *Dcn* expression.[30] Derepression of *Dcn* via inactivation of FoxD1 would stimulate pro-autophagic processes mediated by decorin. FoxD1 may be downstream of anti-autophagic PI3K/Akt/mTOR effectors, or may be directly antagonized by the pro-autophagic AMP-activated protein kinase pathway. Thus, soluble decorin elicits autophagy within the stroma and mitophagy within the tumor and is postulated as a soluble factor for tumorigenic and angiogenic suppression.

## The Matrix Evokes Autophagy for Cancer Suppression

### Role of Autophagy in Cancer

The role of autophagy in modulating tumorigenesis currently subsumes a dichotomous function by either promoting or restricting progression, although the precise function of autophagy in cancer remains unknown.[31] However, the critical importance of autophagy operating within the initial stages of tumorigenic development is rapidly becoming evident.[32] Data support the existence of a

common core of signaling pathways that are responsible for regulating cell proliferation, angiogenesis, cell growth, and apoptosis that are subverted for controlling autophagy throughout malignant transformation.[32] Several opportunities for clinical intervention and novel chemotherapeutic targets (eg, mTOR, tuberous sclerosis 2, beclin 1) for modulating autophagy have come into focus.[32]

### Role of Autophagy in Tumorigenesis

Hyperactive PI3K/Akt/mTOR/p70S6K signaling with the consequent activation of Ras via a class I PI3K typifies many solid tumors, including breast, lung, liver, and prostate carcinomas.[31] Collectively, the above signaling cascades inhibit autophagic progression and function while simultaneously driving cancer in a growth factor–dependent manner.[32] Moreover, the down-regulation of autophagy (and to a larger extent, specialized autophagy, such as chaperone mediated and mitophagy) is associated with and considered necessary for competent tumorigenesis.[31]

Metabolically, the stimulation of proliferation, migration, and metastasis strictly relies on various anabolic processes for macromolecule assembly. Conversely, as a whole, autophagy is a catabolic process that seemingly opposes molecular and biochemical synthesis.[32] Furthermore, in the context of growth factor signaling, stimulatory ligands regulate glucose and amino acid influx and production, respectively, both of which are potent autophagic inhibitors.[33]

### Autophagy in Cancer Therapeutics

Stimulation of autophagy compromises several aspects of continued tumorigenic growth such as immune-evasion and angiogenesis.[33] Intriguingly, specific classes of antineoplastic chemotherapeutics (eg, anthracyclines and oxaliplatin) trigger immunogenic cell death.[34] Importantly, these drugs require and depend on autophagic induction.[34] Tumors experiencing autophagy recruit T lymphocytes and antigen-presenting dendritic cells directly into the tumor parenchyma.[34] Moreover, pharmacological agents that suppress the anti-autophagic mTOR signaling cascade (eg, rapamycin) suppresses angiogenesis in various mouse models.[33]

Autophagic induction is directly relevant for chemotherapeutic efficacy and tumor responsiveness.[31,35] However, predictions for patient response to cancer therapeutics are compounded by our limited knowledge on forecasting autophagic flux *in vivo* in response to the stimulus.[35] As such, four distinct forms of autophagy have been identified, cytoprotective, cytostatic, cytotoxic, and nonprotective,[35] each offering unique cellular viability responses to chemotherapeutic agents.

ECM molecules that function as autophagic inducers counteract growth factor signaling and suppress anti-autophagic signaling relays in favor of pro-autophagic outcomes (via Vps34). Intriguingly, decorin suppresses class I PI3K/mTOR signaling in physiologically normal endothelial cells, leading to autophagic programs.[21] Moreover, decorin elicits mitophagy in tumor cells.[29] Collectively, induction by decorin (and related autophagic matrikines) may represent a mechanism for tumorigenic and angiogenic suppression or for quelling homeostatic imbalances relevant for human pathologies (ie, muscular dystrophies). These functions may depend on the form of autophagy evoked and in the pathological microenvironment by which it is evoked.

## Collagen VI Is Required for Maintaining Skeletal Muscle Homeostasis via Autophagy

### Collagen VI Distribution and Function

As a major structural constituent of the ECM, collagen VI is widely distributed and highly expressed in multiple tissues, including skin, cornea, adipose tissue, lung, brain, nerves, blood vessels, intervertebral disks, and skeletal muscle.[36] Collagen VI forms a lattice composed of beaded microfilaments that mediate a plethora of protein–protein interactions that provide structural support within the local microenvironment for the surrounding tissues.[36] However, collagen VI promotes and regulates active signaling processes for a variety of cell functions, including proliferation, angiogenesis, apoptosis, tumorigenesis,[36] and maintaining a stem cell niche for satellite cell renewal and muscle regeneration.[37]

### Collagen VI Maintains Skeletal Muscle Homeostasis

Collagen VI is a major ECM protein localized within the endomysium of skeletal muscle and is chiefly responsible for regulating muscle cell homeostasis and function.[6] Balancing anabolic and catabolic processes in skeletal muscle is essential for achieving homeostasis. A prime mechanism for ensuring an appropriate response from skeletal muscle during times of physiological activity, stress, and exercise occurs via autophagy.[6,38] The importance of autophagy is underscored by the findings that defective or hyperactive autophagic responses are ultimately detrimental for skeletal muscle and often manifest as muscle dystrophies.[6,39]

Recently, maintaining autophagy in skeletal muscle has been linked to proper collagen VI structure, expression, and function.[38,40] Several congenital muscular dystrophies, including Bethlem myopathy and Ullrich congenital muscular dystrophy, have been causatively associated with mutations in the genes that encode the three chains of collagen VI.[40] For instance, deficiencies of collagen VI trigger morphological aberrations in the ultrastructure of mitochondria and sarcoplasmic reticulum.[41] Further, muscle fiber death via activation of the intrinsic apoptotic cascade occurs spontaneously in myopathic mice with collagen VI deficiency (*Col6a1*$^{-/-}$).[41] Combined, both abnormalities precede and result in myofiber failure and degeneration while presenting clinically as a collagen VI-type muscular dystrophy.

Interrogating the molecular underpinnings of this phenotype reveals impaired autophagic induction and flux in *Col6a1*$^{-/-}$ mice.[40] Indeed, skeletal muscle from *Col6a1*$^{-/-}$mice exhibits compromised Akt/FoxO3 signaling, increased-activated protein kinase activation, lower amounts of lipidated LC3, and decreased autophagosome formation, indicating an energy anomaly concomitant with decreased basal autophagy.[40] Further, Bnip3 and beclin 1, two key effectors of mitochondrial removal[42] and autophagic induction,[4] respectively, are disproportionately deregulated in *Col6a1*$^{-/-}$ skeletal muscle[40] (Figure 2). Restoration of beclin 1 activity or dietary restriction in *Col6a1*$^{-/-}$ mice reactivates autophagy and ameliorates the dystrophic phenotype.[40] Moreover, pharmacological modulation of key targets, such as the autophagic inhibitor mTOR, with either rapamycin or cyclosporin A diminishes myofiber degeneration, promotes the turnover of malfunctioning organelles, and reinstates normal mitochondrial function by re-establishing physiologically relevant levels of Bnip3 and beclin 1.[40]

## Collagen IV and Decorin May Function as an Outside-In Pro-Autophagic Complex

Functionally, collagen VI is an autophagic inducer and prime regulator for skeletal muscle that circumvents muscular dystrophies by fine-tuning the autophagic flux that is optimal for functional myofibers. Soluble decorin, which engages VEGFR2 for endothelial cell autophagy and Met for tumor cell mitophagy, is a canonical collagen VI binding partner.[10] Thus, decorin-bound collagen VI may act synergistically for regulatory control of autophagic initiation within the myofiber microenvironment. Notably, the C-terminal portion of collagen VI interacts with tumor endothelial marker 8.[43] Because tumor endothelial marker 8 physically interacts with VEGFR2, it is possible that collagen VI might also induce autophagy in endothelial cells by using these two receptors. Moreover, the colocalization and physical interaction of decorin and collagen VI, together with the potential use of common receptors and core signaling pathways, suggests that these two matrix constituents are part of an outside-in signaling network that controls intracellular catabolism via autophagic induction and modulation.

## Basement Membrane Laminin α2 Functions as an Endogenous Autophagic Inhibitor

In contrast to the pro-autophagy molecules discussed in the previous section, the laminin α2 chain subsumes a unique position insofar as it has anti-autophagic properties.[44] Laminin α2 comprises the α2 subunit of laminin 211, a key basement membrane component. Mutations in the α2 subunit cause merosin-deficient congenital muscular dystrophy (MDC1A), a progressive muscular dystrophy characterized by muscle weakness due to generalized skeletal muscle atrophy, peripheral neuropathy, and joint contractures.[39,44]

In contrast to collagen VI-mediated autophagic induction, loss of *LAMA2*, which encodes the laminin α2 subunit, causes increased expression of autophagy genes[44] (Figure 2). Among the up-regulated genes are *FOXO3*, a pioneer transcription factor involved in regulating autophagy genes, *BECN1*, *MAPLC3B*, and *p62/SQSTM1*.[44] Interestingly, MDC1A myotubes have induced protein levels of Vps34, beclin 1, LC3, and cathepsin L.[44] The cysteine protease cathepsin L liberates endorepellin, the angiostatic C-terminus of perlecan, a major basement membrane constituent.[45]

In analogy with the pharmacological (rapamycin) rescue of deficient autophagy on loss of collagen VI in Bethlem myopathy and Ullrich congenital muscular dystrophy, systemic delivery of 3-methyladenine, a Vps34 inhibitor, restores autophagy gene expression programs and normalizes MDC1A skeletal muscle.[4,44] As tissue morphology improves, the regenerative capacity of the muscle is stimulated and overall survival increases.[4,44] Further, the phosphorylation of Akt, an autophagic inhibitor that simultaneously activates mTOR and attenuates the pro-autophagic FoxO3 forkhead transcription factor, phenocopies (eg, diminished phosphorylation at Thr308 and Ser476) wild-type littermates.[44] Therefore, basement membranes are composed of functionally opposite members that can fine-tune autophagic induction in a temporal and spatial manner.

## Endostatin, Endorepellin, and Kringle 5 Evoke Endothelial Cell Autophagy and Apoptosis

### Endostatin

The proteolytically processed C terminus of collagen XVIII-α1 yields a potently angiostatic module known as endostatin, which mediates antiproliferative and antimigratory effects. Through high-affinity interactions with the α5β1 integrin, soluble endostatin induces endothelial cell autophagy in multiple models[46,47] and human umbilical vein endothelial cells (Figure 1C). Thus, endostatin could be also considered as a unique member of pro-autophagic members. Moreover, a mutated form of endostatin (P125A endostatin) that exhibits higher affinity binding and enhanced inhibitory effects evokes autophagy in a Vps34/beclin 1/LC3-dependent manner[46,47] (Figure 2). Autophagosome nucleation and maturation are pharmacologically inhibited by 3-methyladenine treatment in the presence of P125A endostatin,[47] suggesting that, as with decorin, a canonical pathway is followed. In accordance with increased differential binding of beclin 1 with pro-autophagic effectors and simultaneous disengagement from anti-autophagic partners, both Bcl-2 and Bcl-$_{xL}$ are reduced.[47] Further, in analogy to decorin, endostatin suppresses Wnt/β-catenin signaling concurrent with decreased β-catenin levels.[47] Functionally, β-catenin opposes beclin 1 signaling,[47] underscoring the biological relevance of β-catenin suppression for autophagic induction in endothelial cells downstream of the α5β1 integrin for effective anti-angiogenic properties.[47]

### Endorepellin

Proteolytically released endostatin from the collagen XVIII-α1 chain and ensuing endothelial cell autophagy may represent a general mechanism for processed basement membrane heparin sulfate proteoglycans. Perlecan, a major heparin sulfate proteoglycan of cell surfaces and vascular basement membranes, may be processed for endorepellin release, resulting in angiostasis and endothelial cell autophagy, downstream of simultaneous ligation of VEGFR2 and the α2β1 integrin.[48] Indeed, endorepellin stimulates autophagy in endothelial cells (Figure 1D) downstream of VEGFR2 in a Peg3-dependent manner.[49] Notably, endorepellin promotes autophagy via the first two laminin-like globular domains LG1/2, whereas the terminal LG3 domain interacts with the α2β1 integrin to induce dissolution of actin cytoskeleton.[49]

### Kringle 5

Kringle 5, an internal domain of plasminogen, can induce endothelial cell autophagy via GRP78 (glucose-regulated protein, 78 kDa) another cell surface-expressed receptor.[50] Kringle 5 evokes autophagy in a beclin 1-dependent manner via differential binding to the Bcl-2/beclin 1/Vps34 complex[50] (Figure 2). Concurrent with autophagic induction, Kringle 5 also evokes the intrinsic apoptotic cascade via potentiation of caspase-3/-7 activity.[50] It is probable that mitochondrial insufficiency via increased autophagy is responsible for apoptotic induction. Notably, soluble decorin and endostatin increase plasminogen expression.[51,52] Thus, these established and multifunctional agents might induce expression of additional members of the autophagic cell machinery.

A universally recurring theme shared among decorin, collagen VI, endostatin, endorepellin, and kringle 5 involves the convergence of pro-autophagic signals onto a common core of autophagic effectors, including Vps34, beclin 1, and lipidated LC3 (Figure 2). Despite signaling from a diverse array of receptors, soluble pro-autophagic molecules engage a common set of relays for endothelial autophagy. The recent identification of Peg3 as an autophagic kingpin[21] may represent a common mode for autophagic signaling and recruitment of the downstream transduction apparati.

## Perspectives

Regulatory networks that govern autophagic induction, maintenance, and termination are rapidly being uncovered as key cellular mechanisms for a variety of processes, including metabolism, growth, proliferation, differentiation, genome stability, and apoptosis. Because intracellular checkpoints and supramolecular complexes responsible for executing autophagy are being elucidated, signals derived from the extracellular milieu are beginning to be more appreciated. The ECM, as a whole, is undergoing a paradigmatic shift that not only provides structural cues for cells and tissues but also actively functions as a signaling hub that dictates cell behaviors, identities, and phenotypes. This critical role is executed by matrikines and is exemplified in the scenario of EGFR regulation.[53] A multitude of matricellular proteins act as either endogenous activators (tenascin C, laminin 322, secreted protein acidic and rick in cysteine, thrombospondin, osteopontin, and fibulins) or inhibitors (decorin) that spatially and temporally modulate EGFR signaling plasticity.[53]

The most established pan-RTK inhibitor and potent tumoricidal agent, decorin, is once again a harbinger for a novel regulatory archetype that embodies a potentially new class of matrix molecules. Autophagy may represent the underlying and unifying mechanism for defending against tumor and disease progression. A new category of matrikines has emerged that differentially modulate autophagic flux in a wide variety of cell and tissue microenvironments. Importantly, decorin, collagen VI, endostatin, endorepellin, and kringle 5 represent pro-autophagic matrix constituents that engage a diverse array of cell surface receptors for autophagic initiation. However, the laminin α2 chain acts as an endogenous autophagic inhibitor.[44] Absence of laminin α2 permits excessive autophagy and yields a progressive muscular dystrophy that is suppressed by pharmacological agents.[44] Collectively, these matrix members provide a molecular counterweight for maintaining and activating autophagy that is requisite for preventing and combating disease progression (eg, cancer, neurodegeneration, and muscular dystrophies). This unique class of matrix molecules can function as a safeguard against inappropriate and hyperactive autophagy (as seen in MDC1A) that, if unchecked, can manifest as homeostatic abnormalities.

Decorin-mediated autophagic induction of endothelial cells occurs via a Peg3/beclin 1/LC3-mediated pathway,[21,54] analogous to the role that beclin 1 and LC3 have for endostatin-, collagen VI-, and kringle 5-mediated autophagosome formation.[40,47,50] However, decorin-evoked tumor cell mitophagy progresses in a beclin 1-independent manner but relies on a new general mitophagic effector, mitostatin.[29] Interestingly, endothelial cells appear exquisitely sensitive because decorin, endostatin, endorepellin, and kringle 5 evoke autophagy and/or apoptosis. Autophagic induction by these soluble matrix constituents plays a critical role in suppressing tumor angiogenesis.

A key tenet of pro-autophagic activity is the requirement of positive signaling via RTKs, integrins, and coreceptors for the protracted suppression of the anti-autophagic PI3K/Akt/mTOR pathway.[23] Attenuated PI3K/Akt/mTOR signaling, with simultaneous-activated protein kinase activation, alleviates Bcl-2 inhibition of beclin 1,[13] beclin 1/LC3/Peg3 binding,[21] and FoxO3.[3] Intriguingly, de-repression and consequent stabilization of FoxO3 downstream of Akt effectively transactivates several pro-autophagic loci.[3,44,55] It is plausible that FoxO3 and Peg3 may physically assemble and synergize at proximal promoters of autophagy genes for induction by autophagic stimuli. FoxO3 and Peg3 may directly target these specialized matrix elements such as decorin and collagen VI, for transcriptional

activation, resulting in sustained autophagic signaling. Moreover, ligand binding to cognate receptors may stimulate further expression of FoxO3 and Peg3, thereby constituting a positive feed-forward loop. Analysis of endogenous autophagic inhibitors, such as Rubicon,[1] has not yet been performed. Matrikine-regulated autophagy may differentially affect endogenous inhibitors (eg, laminin α2 and Rubicon) for feedback inhibition and autophagic cessation. Moreover, a recent study has identified a chromatin switch mediated by hMOF histone acetyltransferase at H4K16 that dictates the outcome of autophagic signaling.[56] Thus, matrix-derived signals might directly influence this epigenetic switch in the decision of autophagy-evoked cell death.

## Conclusions

We have critically evaluated the role of several novel candidates for autophagic regulation. The aggregated themes discussed distill into a new theory about matrix signaling and the resulting effect on intracellular processes for disease prevention. These members bind either one or multiple signaling receptors and perhaps include coreceptors for signal transduction, acting as partial agonists for downstream autophagic activation. Moreover, the molecular profile of the matrix dictates a microenvironment-specific autophagic response. Thus, autophagic regulation further reinforces the idea of matrix-based therapeutics as novel modalities for combating a range of pathologies. We predict that in the near future other ECM products will be found to possess the biological function of affecting intracellular self-eating and modulating catabolic metabolism.

## Acknowledgment

We sincerely apologize for not citing several publications that have made significant contribution in the fields of autophagy and matrix biology because of space constraints.

## References

1. Mizushima N, Komatsu M: Autophagy: renovation of cells and tissues. Cell 2011, 147:728−741
2. Wang K, Klionsky DJ: Mitochondria removal by autophagy. Autophagy 2011, 7:297−300
3. Zhao J, Brault JJ, Schild A, Cao P, Sandri M, Schiaffino S, Lecker SH, Goldberg AL: FoxO3 coordinately activates protein degradation by the autophagic/lysosomal and proteasomal pathways in atrophying muscle cells. Cell Metab 2007, 6:472−483
4. Funderburk SF, Wang QJ, Yue Z: The Beclin 1-VPS34 complex−at the crossroads of autophagy and beyond. Trends Cell Biol 2010, 20:355−362
5. Koren I, Kimchi A: Cell biology. Promoting tumorigenesis by suppressing autophagy. Science 2012, 338:889−890
6. Grumati P, Bonaldo P: Autophagy in skeletal muscle homeostasis and in muscular dystrophies. Cells 2012, 1:325−345
7. Liang J, Mills GB: AMPK: a contextual oncogene or tumor suppressor? Cancer Res 2013, 73:2929−2935
8. Komatsu M, Ichimura Y: Physiological significance of selective degradation of p62 by autophagy. FEBS Lett 2010, 584:1374−1378
9. Iozzo RV, Sanderson RD: Proteoglycans in cancer biology, tumour microenvironment and angiogenesis. J Cell Mol Med 2011, 15:1013−1031
10. Neill T, Schaefer L, Iozzo RV: Decorin: a guardian from the matrix. Am J Pathol 2012, 181:380−387
11. Mizushima N, Yoshimori T, Levine B: Methods in mammalian autophagy research. Cell 2010, 140:313−326
12. Choi AMK, Ryter SW, Levine B: Autophagy in human health and disease. N Engl J Med 2013, 368:651−662
13. Rubinsztein DC, Codogno P, Levine B: Autophagy modulation as a potential therapeutic target for diverse diseases. Nat Rev Drug Discov 2012, 11:709−730
14. Dagda R, Cherra SJ 3rd, Kulich SM, Tandon A, Park D, Chu CT: Loss of PINK1 function promotes mitophagy through effects on oxidative stress and mitochondrial fission. J Biol Chem 2009, 284:13843−13855
15. Lock R, Debnath J: Extracellular matrix regulation of autophagy. Curr Opin Cell Biol 2008, 20:583−588
16. Schaefer L, Iozzo RV: Small leucine-rich proteoglycans, at the crossroad of cancer growth and inflammation. Curr Opin Genet Dev 2012, 22:56−57
17. Neill T, Painter H, Buraschi S, Owens RT, Lisanti MP, Schaefer L, Iozzo RV: Decorin antagonizes the angiogenic network: concurrent inhibition of Met, hypoxia inducible factor-1α, vascular endothelial growth factor A, and induction of thrombospondin-1 and TIMP3. J Biol Chem 2012, 287:5492−5506
18. Neill T, Jones HR, Crane-Smith Z, Owens RT, Schaefer L, Iozzo RV: Decorin induces rapid secretion of thrombospondin-1 in basal breast carcinoma cells via inhibition of Ras homolog gene family, member A/Rho-associated coiled-coil containing protein kinase 1. FEBS J 2013, 280:2353−2368
19. Merline R, Moreth K, Beckmann J, Nastase MV, Zeng-Brouwers J, Tralhão JG, Lemarchand P, Pfeilschifter J, Schaefer RM, Iozzo RV, Schaefer L: Signaling by the matrix proteoglycan decorin controls inflammation and cancer through PDCD4 and microRNA-21. Sci Signal 2011, 4:ra75
20. Buraschi S, Neill T, Owens RT, Iniguez LA, Purkins G, Vadigepalli R, Evans B, Schaefer L, Peiper SC, Wang ZX, Iozzo RV: Decorin protein core affects the global gene expression profile of the tumor microenvironment in a triple-negative orthotopic breast carcinoma xenograft model. PLoS One 2012, 7:e45559
21. Buraschi S, Neill T, Goyal A, Poluzzi C, Smythies J, Owens RT, Schaefer L, Torres AT, Iozzo RV: Decorin causes autophagy in endothelial cells via Peg3. Proc Natl Acad Sci U S A 2013, 110:E2582−E2591
22. Relaix F, Weng X, Marazzi G, Yang E, Copeland N, Jenkins N, Spence SE, Sassoon D: Pw1, a novel zinc finger gene implicated in the myogenic and neuronal lineages. Dev Biol 1996, 177:383−396
23. Alers S, Löffler AS, Wesselborg S, Stork B: Role of AMPK-mTOR-Ulk1/2 in the regulation of autophagy: cross talk, shortcuts, and feedbacks. Mol Cell Biol 2012, 32:2−11
24. Lee JW, Park S, Takahashi Y, Wang HG: The association of AMPK with ULK1 regulates autophagy. PLoS One 2010, 5:e15394
25. Goyal A, Neill T, Owens RT, Schaefer L, Iozzo RV: Decorin activates AMPK, an energy sensor kinase, to induce autophagy in endothelial cells. Matrix Biol 2014, 34:46−54
26. Wei Y, Zou Z, Becker N, Anderson M, Sumpter R, Xiao G, Kinch L, Koduru P, Christudass CS, Veltri RW, Grishin NV, Peyton M, Minna J, Bhagat G, Levine B: EGFR-mediated Beclin 1 phosphorylation in autophagy suppression, tumor progression, and tumor chemoresistance. Cell 2013, 154:1269−1284
27. Wang RC, Wei Y, An Z, Zou Z, Xiao G, Bhagat G, White M, Reichelt J, Levine B: Akt-mediated regulation of autophagy and tumorigenesis through Beclin 1 phosphorylation. Science 2012, 338:956−959

28. Morrione A, Neill T, Iozzo RV: Dichotomy of decorin activity on the insulin-like growth factor-I system. FEBS J 2013, 280:2138–2149
29. Neill T, Torres A, Buraschi S, Owens RT, Hoek J, Baffa R, Iozzo RV: Decorin induces mitophagy in breast carcinoma cells via peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) and mitostatin. J Biol Chem 2014, 289:4952–4968
30. Fetting JL, Guay JA, Karolak MJ, Iozzo RV, Adams DC, Maridas DE, Brown AC, Oxburgh L: FOXD1 promotes nephron progenitor differentiation by repressing decorin in the embryonic kidney. Development 2014, 141:17–27
31. Hu YL, Jahangiri A, Delay M, Aghi MK: Tumor cell autophagy as an adaptive response mediating resistance to treatments such as anti-angiogenic therapy. Cancer Res 2012, 72:4294–4299
32. Kondo Y, Kanzawa T, Sawaya R, Kondo S: The role of autophagy in cancer development and response to therapy. Nat Rev Cancer 2005, 5:726–734
33. Botti J, Djavaheri-Mergny M, Pilatte Y, Codogno P: Autophagy signaling and the cogwheels of cancer. Autophagy 2006, 2:67–73
34. Michaud M, Martins I, Sukkurwala AQ, Adjemian S, Ma Y, Pellegatti P, Shen S, Kepp O, Scoazec M, Mignot G, Rello-Varona S, Tailler M, Menger L, Vacchelli E, Galluzzi L, Ghiringhelli F, di Virgilio F, Zitvogel L, Kroemer G: Autophagy-dependent anticancer immune responses induced by chemotherapeutic agents in mice. Science 2011, 334:1573–1577
35. Gewirtz DA: The four faces of autophagy: implications for cancer therapy. Cancer Res 2014, 74:647–651
36. Chen P, Cescon M, Bonaldo P: Collagen VI in cancer and its biological mechanisms. Trends Mol Med 2013, 19:410–417
37. Urciuolo A, Quarta A, Morbidoni V, Gattazzo F, Molon S, Grumati P, Montemurro F, Tedesco FS, Blaauw B, Cossu G, Vozzi G, Rando TA, Bonaldo P: Collagen VI regulates satellite cell self-renewal and muscle regeneration. Nat Commun 2013, 4:1964
38. Grumati P, Coletto L, Schiavinato A, Castagnaro S, Bertaggia E, Sandri M, Bonaldo P: Physical exercise stimulates autophagy in normal skeletal muscles but is detrimental for collagen VI-deficient muscles. Autophagy 2011, 7:1415–1423
39. Bonaldo P, Sandri M: Cellular and molecular mechanisms of muscle atrophy. Dis Model Mech 2013, 6:25–39
40. Grumati P, Coletto L, Sabatelli P, Cescon M, Angelin A, Bertaggia E, Blaauw B, Urciuolo A, Tiepolo T, Merlini L, Maraldi NM, Bernardi P, Sandri M, Bonaldo P: Autophagy is defective in collagen VI muscular dystrophies, and its reactivation rescues myofiber degeneration. Nat Med 2010, 16:1313–1320
41. Irwin WA, Bergamin N, Sabatelli P, Reggiani C, Megighian A, Merlini L, Braghetta P, Columbaro M, Volpin D, Bressan GM, Bernardi P, Bonaldo P: Mitochondrial dysfunction and apoptosis in myopathic mice with collagen VI deficiency. Nat Genet 2003, 35:367–371
42. Zalckvar E, Berissi H, Mizrachy L, Idelchuk Y, Koren I, Einsenstein M, Sabanay H, Pinkas-Kramarski R, Kimchi A: DAP-kinase-mediated phosphorylation on the BH3 domain of beclin 1 promotes dissociation of beclin 1 from Bcl-XL and induction of autophagy. EMBO Rep 2009, 10:285–292
43. Nanda A, Carson-Walter EB, Seaman S, Barber TD, Stampfl J, Singh S, Vogelstein B, Kinzler KW, St Croix B: TEM8 interacts with the cleaved C5 domain of collagen alpha 3(VI). Cancer Res 2004, 64:817–820
44. Carmignac V, Svensson M, Körner Z, Elowsson L, Matsumura C, Gawlik KI, Allamand V, Durbeej M: Autophagy is increased in laminin α2 chain-deficient muscle and its inhibition improves muscle morphology in a mouse model of MDC1A. Hum Mol Genet 2011, 20:4891–4902
45. Iozzo RV: Basement membrane proteoglycans: from cellar to ceiling. Nat Rev Mol Cell Biol 2005, 6:646–656
46. Chau YP, Lin JY, Chen JH, Tai MH: Endostatin induces autophagic cell death in EAhy926 human endothelial cells. Histol Histopathol 2003, 18:715–726
47. Nguyen TM, Subramanian IV, Xiao X, Ghosh G, Nguyen P, Kelekar A, Ramakrishnan S: Endostatin induces autophagy in endothelial cells by modulating Beclin 1 and beta-catenin levels. J Cell Mol Med 2009, 13:3687–3698
48. Goyal A, Poluzzi C, Willis CD, Smythies J, Shellard A, Neill T, Iozzo RV: Endorepellin affects angiogenesis by antagonizing diverse vascular endothelial growth factor receptor 2 (VEGFR2)-evoked signaling pathways: transcriptional repression of hypoxia-inducible factor 1α and VEGFA and concurrent inhibition of nuclear factor of activated T cell 1 (NFAT1) activation. J Biol Chem 2012, 287:43543–43556
49. Poluzzi C, Casulli J, Goyal A, Mercer TJ, Neill T, Iozzo RV: Endorepellin evokes autophagy in endothelial cells. J Biol Chem 2014, 289:16114–16128
50. Nguygen TM, Subramanian IV, Kelekar A, Ramakrishnan S: Kringle 5 of human plasminogen, an angiogenesis inhibitor, induces both autophagy and apoptotic death in endothelial cells. Blood 2007, 109:4793–4802
51. Reijerkerk A, Mosnier LO, Kranenburg O, Bouma BN, Carmeliet P, Drixler T, Meijers JC, Voest EE, Gebbink MF: Amyloid endostatin induces endothelial cell detachment by stimulation of the plasminogen activation system. Mol Cancer Res 2003, 1:561–568
52. Davies JE, Tang X, Bournat JC, Davies SJ: Decorin promotes plasminogen/plasmin expression within acute spinal cord injuries and by adult microglia in vitro. J Neurotrauma 2006, 23:397–408
53. Grahovac J, Wells A: Matrikine and matricellular regulators of EGF receptor signaling on cancer cell migration and invasion. Lab Invest 2014, 94:31–40
54. Neill T, Torres AT, Buraschi S, Iozzo RV: Decorin has an appetite for endothelial cell autophagy. Autophagy 2013, 9:1626–1628
55. Mammuccari C, Milan G, Romanello V, Masiero E, Rudolf R, Del Piccolo P, Burden SJ, Di Lisi R, Sandri C, Zhao J, Goldberg AL, Schiaffino S, Sandri M: FoxO3 controls autophagy in skeletal muscle in vivo. Cell Metab 2007, 6:458–471
56. Füllgrabe J, Lynch-Day MA, Heldring N, Li W, Struijik RB, Ma Q, Hermanson O, Rosenfeld MG, Klionsky DJ, Joseph B: The histone H4 lysine acetyltransferase hMOF regulates the outcome of autophagy. Nature 2013, 500:468–471