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Mechanical and non-mechanical functions of filamentous and non-filamentous vimentin.

Alison E Patteson[1,2], **Amir Vahabikashi**[3], **Robert D Goldman**[3], **Paul A. Janmey**[4]

[1]Physics Department, Syracuse University, Syracuse, NY 13244

[2]BioInspired Institute, Syracuse University, Syracuse, NY 13244

[3]Department of Cell and Molecular Biology, Feinberg School of Medicine, Northwestern University, Chicago IL 60611

[4]Institute for Medicine and Engineering, Department of Physiology, University of Pennsylvania, Philadelphia, PA 19104

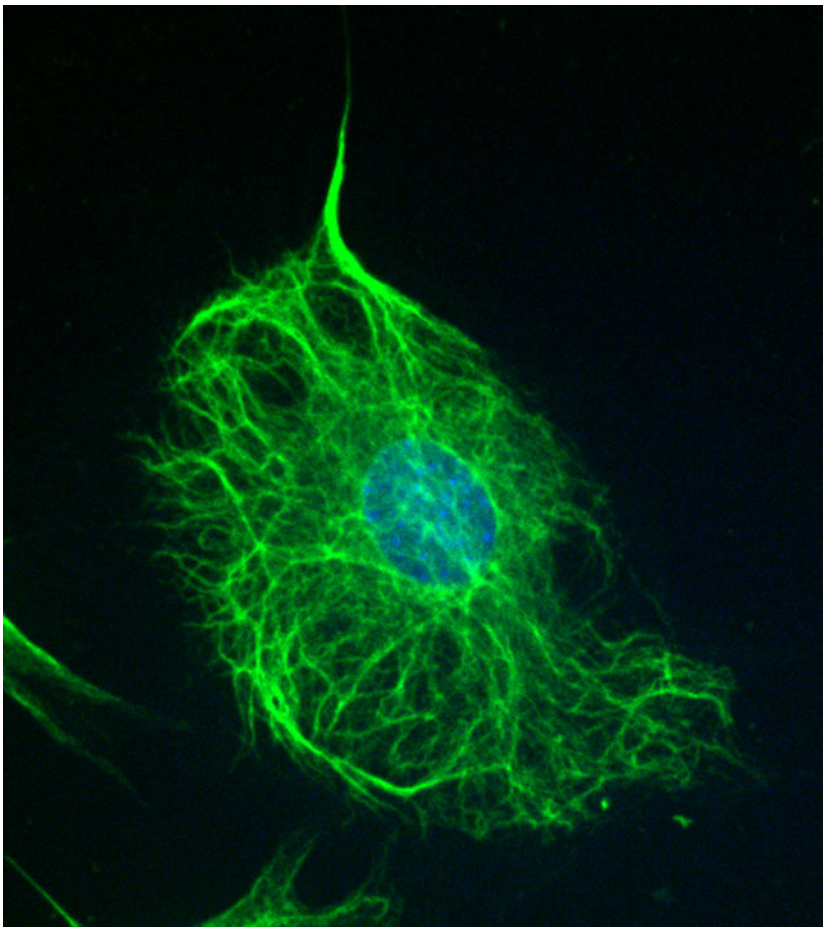
Abstract

Intermediate filaments (IFs) formed by vimentin are less understood than their cytoskeletal partners, microtubules and F-actin, but the unique physical properties of IFs, especially their resistance to large deformations, initially suggest a mechanical function. Indeed, vimentin IFs help regulate cell mechanics and contractility, and in crowded 3D environments they protect the nucleus during cell migration. Recently, a multitude of studies, often using genetic or proteomic screenings show that vimentin has many non-mechanical functions within and outside of cells. These include signaling roles in wound healing, lipogenesis, sterol processing, and various functions related to extracellular and cell surface vimentin. Extracellular vimentin is implicated in marking circulating tumor cells, promoting neural repair, and mediating the invasion of host cells by viruses, including SARS-CoV, or bacteria such as *Listeria* and *Streptococcus*. These findings underscore the fundamental role of vimentin in not only cell mechanics but also a range of physiological functions.

Graphical Abstract

Vimentin forms a wickerwork network that provides mechanical protection of the nucleus and elasticity to the cytoskeleton. New work highlights vimentin's life outside the cell. It is secreted by multiple cell types and can provide positive signals for wound healing and act as a cofactor for pathogen infection.

Corresponding author: Paul A. Janmey, janmey@mail.med.upenn.edu.



Keywords

cytoskeleton; intermediate filaments; vimentin; nucleus; pathogenesis

1. Introduction

Many studies of the intermediate filament (IF) system have focused on the mechanical properties of the networks formed by this cytoskeletal polymer. The non-cytoplasmic intermediate filaments, which compose structural materials such as nails and the slime secreted by hagfish [1] have evolved to perform specific mechanical tasks. This would initially suggest that the cytoplasmic IFs would as well have a predominantly mechanical role. Our attention in this review is focused on recent discoveries on both mechanical and non-mechanical roles of the protein vimentin and the filaments it forms (VIFs) in cell biology. Vimentin is expressed mainly in mesenchymal cells. Its basic structure is mostly in the form of αhelical coiled-coil dimers that assemble into 10 nm diameter filaments through a series of intermediate oligomers. Vimentin's N-terminus is important for controlling filament assembly and both N- and C-terminal domains mediate many interactions with binding partners. The structure and assembly of VIFs are reviewed elsewhere [2]. Previous studies have shown significant contributions of vimentin to cell mechanics and contractility

[3–5]. However, many studies also point to non-mechanical roles for this abundant protein, with cellular functions ranging from lipogenesis to regulating GTPase signaling and the transduction of signals from transmembrane receptors [6–10]. This brief summary highlights three distinct aspects of recent research related to vimentin and VIFs.

We will first focus on the expanding list of proteins, nucleic acids, and other molecules that bind vimentin, often with a change in their ability to transmit a signal or react with their ligands. In some respect this section is an update of earlier reviews that pointed to the importance of intermediate filaments in cell signaling and other non-mechanical functions [10,11].

We will then emphasize recent findings on how the mechanical properties of the vimentin network provides a function that cannot be fulfilled by either of the other two cytoskeletal elements, actin filaments and microtubules. We will discuss how VIFs create a cage around the nucleus, increasing the cell's ability to withstand large strains during locomotion in confined 3D spaces without damage to the nucleus. This effect of the perinuclear VIF network is not evident in studies of cells on flat surfaces but leads to large effects on motility and nuclear shape in 3D culture systems.

Finally, we will discuss the rapidly emerging field of extracellular vimentin. Early studies suggested that the reaction between anti-vimentin antibodies and antigens on the external surfaces of cells might be due to cytoskeletal debris released from neighboring cells, or perhaps local and transient rupture of the plasma membrane [12]. However, it is now clear that cell surface vimentin appears in the absence of detectable cell damage and is important for infection by bacteria or viruses or signaling from one cell type to another [13].

2. Vimentin binds to diverse cellular targets

There is already a large catalog of proteins that link VIFs to F-actin and microtubules, and the physical properties of composite networks formed by multiple cytoskeletal network systems promote mechanical responses that cannot be achieved by any single network system. These topics have been recently reviewed in [14,15]. Here we summarize only a few of the most recently reported cytoskeletal ligands for vimentin, which illustrate how broadly vimentin can affect cell mechanics and other functions in ways that cannot be inferred simply from the mechanical properties of VIFs alone.

2.1. Novel cytoskeletal and focal adhesion links

The most clearly established role of intracellular vimentin is to form a three-dimensional network as one of the three cytoskeletal systems. As this network system is increasingly studied, numerous new interacting proteins and regulators of VIFs are emerging. Here we summarize a few recent examples to illustrate the range of cytoskeletal structures that involve vimentin. In addition to the crosslinker plectin and multiple plus and minus end directed molecular motors [16] that link VIFs to microtubules, the protein Rudhira/Breast Carcinoma Amplified Sequence 3 (BCAS3) also links vimentin to microtubules and leads to MT stabilization, an effect that is essential for endothelial cell motility, focal adhesion dynamics, and angiogenesis [17]. Carmil, a protein that interacts with actin capping protein

and hence affects actin dynamics also binds VIFs, suggesting a novel link between these two filament systems [18]. Another actin regulatory protein, girdin, that links AKT signaling to cytoskeletal dynamics was shown to bind vimentin by mass spectrometry and immunoprecipitation from pancreatic cancer cells [19]. Hic-5, a focal adhesion scaffold protein stabilizes the VIF network by modulation of RhoGTPases, and its ablation leads to the disassembly of VIF [20]. Filamin A, an actin crosslinker, directs the kinase PAK1 to vimentin, altering its assembly into VIFs [21]. A mechanical link from VIFs to the LINC complex mediates transmission of forces from the cell surface to the nucleus [22]. Linkage of VIFs to cortical actin by the crosslinker plectin, one of the first known integrators of the cytoskeleton, has now been seen to be essential for the cellular restructuring required for mitosis [23]. These and other examples of novel ligands for vimentin are summarized in Figure 1.

2.2. Regulation of lipid droplet formation.

Lipid droplets in adipocytes and other cell types have long been known to be surrounded by a network of VIFs [24]. This network is implicated in adrenal steroidogenesis [25] and its disruption inhibits lipid drop formation in 3T3L1 cells [26]. Proteomic analysis shows that vimentin is enriched in cholesterol-containing lipid droplets compared to triglycerol-based lipid droplets [27], and the binding of hormone sensitive lipase, the major neutral cholesterol esterase, to vimentin facilities delivery of free cholesterol to mitochondria for steroid hormone production [28]. Vimentin can bind directly to some phospholipids [29], and the binding to lipid droplets is proposed to be at least in part mediated by perilipin, which binds directly to the hydrophobic lipid droplet and has an acidic surface-exposed domain to which cationic arginine-rich sites in vimentin’s N-terminus can dock [30]. A mutation in human vimentin that disrupts VIFs leads to decreased perilipin and lipid accumulation in adipocytes, and is associated with progeroid syndrome [31]. Phosphorylation of the oxysterol binding protein 4L (ORP4L) leads to its binding to vimentin and facilitates cholesterol extraction from membranes, further implicating vimentin in lipid droplet formation and processing [32].

2.3. Mechanisms of vimentin degradation.

The stability of vimentin depends to a great extent on its phosphorylation state, and at least some forms of soluble vimentin are a target for degradation. The ring finger protein 208 (RNF208), an estrogen-inducible E3 ligase, binds to vimentin that is phosphorylated at Ser39 and polyubiquitinates the Lys97 residue of vimentin, leading to its degradation. Downregulation of RNF208 in triple negative breast cancer is associated with greater malignancy and with increased vimentin stability [33]. Ubiquitination by gigaxonin, an E3-ligase targeting factor encoded by the giant axonal neuropathy (GAN) gene also leads to vimentin degradation, and mutations in GAN are associated with the accumulation of VIFs in GAN as well as other types of intermediate filaments in the nervous system [34]. VIFs are also subject to proteolysis by calpain during osmotic shock [35,36] and are substrates for some bacterial proteases [37]. VIFs are also cleaved by calpain during pyroptosis of inflammatory cells. The resulting disruption of the VIF network leads to fluidization of the cytoskeleton and softening of the cell [35].

2.4. Notch, NOGO, and PI 3-kinase signaling

Numerous signaling pathways interact with vimentin [7,8,10]. A study of shear stress effects on arterial remodeling show that shear stress increases phosphorylation of vimentin at serine 38, and this leads to binding of the Notch pathway component jagged 1 to vimentin. The resulting increase in Notch signaling is required for appropriate arterial remodeling [38]. Intracellular vimentin binds the NOGO receptor (NgR) which in mature form is trafficked to the plasma membrane of glioblastoma cells where it can inhibit their migration. When bound to vimentin in the cell interior, maturation of NgR is suppressed [39]. This finding may be related to the increased malignancy of glioblastomas expressing high levels of vimentin. Vimentin is also a ligand for mitogen-activated protein kinase kinase 4 (MAP2K4), and this interaction is reported to modulate the effect of MAP2K4 on phosphoinositide-3-kinase (PI3K)/AKT signaling, resulting in changes in breast cancer cell proliferation and migration [40].

2.5. Polysaccharides and nucleic acids

Proteins are not the only ligands for vimentin. On the cell surface vimentin can bind polymers containing N-acetylglucosamine (GlcNAc) [41]. This binding has been exploited to create improved methods for isolating mesenchymal stem cells with surface-exposed vimentin by use of GlcNAc-containing polymer-coated dishes [42]. Vimentin can itself be post-translationally modified by ligation of GlcNAc to serine [43], and this modification affects the assembly of vimentin into filament networks with subsequent effects on cell motility and pathogen invasion (see 4.5.1) [44]. Two non-coding RNA’s have also been identified as ligands of vimentin. The long non-coding RNA LncRNA BC088259 is upregulated after sciatic nerve injury and modulates the migration of Schwann cells. This effect on migration has been proposed to involve binding of LncRNA BC088259 to vimentin [45]. A different non-coding RNA, named down-regulated in its expression by hepatitis B virus X (dreh) is involved in glucose transport, and exogenous downregulation of dreh in 3T3-Li adipocytes increases glucose transport by increasing GLUT4 expression in the plasma membrane by a mechanism thought to involve vimentin [46]. This result supports the idea that vimentin is important for lipogenesis, as discussed in 2.2.

3. Perinuclear vimentin

3.1. What is a vimentin intermediate filament cage?

A robust feature of VIFs is their formation of a cage-like network that encircles the nucleus VIF with densely packed filaments (Figure 2) and radiates to the cell periphery [47]. This organization of VIFs depends on substrate stiffness. When cells are grown on soft substrates, the perinuclear vimentin cage is collapsed and localized closely around the nucleus. When cells grow on stiffer substrates, the spread area of the cell increases, and the VIF network extends more toward the cell periphery. These trends have been observed in multiple cell types, including human mesenchymal stem cells, endothelial cells, and mouse fibroblasts (3T3 cells) [47].

There is evidence that VIFs establish indirect physical connections to the outer nuclear membrane through interactions with the linker of the nucleoskeleton and cytoskeleton

(LINC) complex [48]. The LINC complex also connects to the nuclear lamina, a thin filamentous layer surrounding the nuclear periphery that is mainly composed of the type V IF proteins, the nuclear lamins [49,50]. While actin and microtubules also form indirect links to the nuclear envelope through the LINC complex, they do not form the cage-like structure around the nucleus, which seems to be unique to IFs. Recent high resolution studies [51] show that vimentin rings that form around the nucleus during initial cell spreading on a 2D surface do not appear to link with actin nor require tubulin. It is noteworthy that these latter VIF structures were originally identified as birefringent spheres with isotropic cores using polarized light microscopy. They form rapidly in live cells in response to substrate attachment and exhibit positive birefringence with respect to their circumferential axes which correlates with highly organized parallel arrays of VIFs, excluding both microtubules and [52].

Here we argue that the functional implications of the perinuclear cage are distinct from those of the cytoplasmic VIF network that extends into the cytoplasm, associates with focal adhesions, microtubules and forms links with the actin-rich cell cortex (5).

3.2. Separate roles for cytoplasmic versus perinuclear vimentin in cellular mechanics

3.2.1. VIF cytoplasmic network—There has been an emerging interest in the role of intermediate filaments in the mechanical properties of cells and their role in transmitting forces to the nuclear envelope. VIFs crosslink with actin and microtubules through plectin and other crosslinkers, creating the cytoskeletal links that connect the cell surface to the nucleus [23,53–55]. Vimentin is a major cargo for the microtubule motors kinesin and dynein, so motors may also be part of the bridging between VIFs and the microtubule network [56–59]. One of the first demonstrations of vimentin's role in this process was done through the application of forces at the cell surface using micropipettes or manipulation of surface bound microbeads [60]. Pulling at the cell cortex distorts the cell nucleus and moves it in the direction of pull, indicating the direct transmission of forces through molecular connections between integrins, cytoskeletal filaments, and the nuclear envelope. Pulling on cells that lacked both microfilaments and microtubules still produced nuclear deformation, suggesting that the intermediate filament network alone is sufficient to transmit mechanical stress to the nucleus [60].

The transmission of forces through the cytoskeletal network of all three filament types depends on the mechanical properties of the network. Studies involving reconstituted cytoskeletal networks show that the mechanical properties of VIFs differ from those of actin and microtubules [61]. VIF networks are soft but have an extraordinary ability to stiffen when under strain and can withstand much larger strains without breaking as compared to actin and microtubules [61,62]. A number of studies have now shown that VIFs modify the mechanical properties of the cell itself, enhancing the cell elastic behavior [3–5,63], particularly under conditions of large cell strains [64,65] and in regions close to the perinuclear VIF network [66].

In the simplest physical picture, we can model the cytoskeleton as a spring with a spring constant k that is proportional to its stiffness. When a force F is applied to the boundary of the cell, it displaces the cell boundary a distance x to produce an equal and opposite force F,

$F = -kx$, that is transmitted to the nucleus through the spring. For a soft cell, the nucleus feels little force as k is small whereas the larger k in a stiffer cell makes the nucleus feel a greater force. This simple picture suggests that the increased cell stiffness due to VIF facilitation of the transmission of forces applied at the cell boundary to the nucleus, allowing the nucleus to better 'feel' external forces. This also implies that it takes little force to displace the nucleus in a soft cell lacking VIF as compared to a stiffer cell that possesses a VIF network. A dramatic illustration of this point is the much easier isolation of nuclei from vimentin deficient cells with a micromanipulator as compared to their wild type counterparts that require depolymerization of F-actin for successful nucleus isolation [67].

The cell, of course, has means of generating its own forces. Vimentin is not the engine that drives the cell. That is the role of the contractile machinery of the cell, whose main components are the acto-myosin complex. However, there exist bidirectional interactions between the two networks. For instance, contractile actomyosin arcs mediate subcellular localization of the VIF network to the perinuclear region [68], and there is now significant evidence that VIFs assist in the transmission of the contractile actomyosin forces to the nucleus. When a cell adheres to a flat 2D substrate, the nucleus becomes more compressed over time as the cell spreading area increases [69,70]. One model for this behavior is that contractile forces generated by apical stress fibers push down on the nucleus, compressing it [70–72]. Several studies have now shown that disruption or deletion of the VIF network results in changes to nuclear shape [22,73], even when the F-actin network remains unchanged [74]. In the absence of VIF, the nucleus rounds up and is no longer compressed [69,74]. Using nuclear shape as a read-out for the forces applied to the nuclear surface, these studies indicate that VIFs facilitate nuclear deformation and assist the actomyosin network in pushing down on the nucleus. The exact mechanism by which VIFs apply forces to the nuclear envelope remains unclear, but it seems to require the LINC complex [22]. Another possibility is through vimentin's crosslinks with actomyosin filaments where cutting these links by removing VIFs would decrease the force felt by the nucleus.

3.2.2. Vimentin perinuclear cage—New evidence shows that in addition to transmitting forces to the nucleus, VIFs can also resist the transfer of forces and reduce nuclear deformations. A clear demonstration of this was done using micropipette aspiration to directly apply pulling forces on adherent fibroblasts [75]. Cells transfected with siRNA to decrease vimentin expression had increased nuclear deformation from pulling forces. These results were reproduced using SW13 adrenal carcinoma clones that do not express vimentin. Interestingly, neither actin nor microtubules were required to resist local pulling forces, whereas lamin A was. The effects of vimentin did not require mechanical linkages to the nucleus, as was shown by altering the LINC complex protein KASH4. These results suggest that the VIF perinuclear cage confers stiffness to the nucleus.

Consistent with this picture are 3D confining cell motility experiments in wild type and vimentin-null mEFs [66,74]. We found that loss of vimentin increased cell motility through small pores, suggesting that the VIF network impedes confined motility. Cells lacking vimentin exhibited higher nuclear deformations after migrating through pores of 3 um diameter, which is a much smaller size than the effective diameter of an unstressed mEF nucleus (~10 um). Loss of vimentin also increased the rates of nuclear damage associated

with confined motility, manifesting itself through enhanced nuclear blebs, nuclear envelope rupture, and DNA damage repair. These results reveal that the perinuclear VIF cage may have a distinct role in increasing the effective stiffness of the nuclear envelope.

Further studies are helping to paint a more complete picture of VIFs in 3D cell motility amongst varied cell lines. Recent studies indicate that VIFs also decrease confined cell motility in amoeboid cancer cells [76]. In dendritic cells vimentin is seen to enhance 3D movement [76] and provide mechanical resilience to protect the nucleus [77]. More studies are needed to determine how VIF network assembly and its interactions with the other cytoskeletal components help to establish different modes of confined cell motility.

Does vimentin facilitate or resist force transmission to the nucleus? We have laid out evidence for both cases. One plausible explanation that incorporates both effects is that the cytoplasmic VIF network and the perinuclear VIF cage might have separate roles in maintaining nuclear shape. The main mechanical function of the cytoplasmic VIFs might be to increase cytoskeletal stiffness and to assist with the transmission of forces to the nucleus. This is consistent with the decreased 3D motility rates of cells with VIFs [66,74,76]. On the other hand, cells containing a perinuclear VIF cage seems to serve as a protective structure that increases the effective stiffness of the nuclear envelope, as seen by reduced nuclear deformability in cells expressing vimentin [74,77]. In this way, VIFs not only enhance the cell stiffness but also the effective stiffness of the nuclear envelope. The cytoplasmic VIFs assist in transmitting forces to the nucleus, and the perinuclear cage resists them. How cells might regulate perinuclear versus cytoplasmic VIF assembly is unclear but could be in part established through connections with microtubules. Microtubules are required for moving VIFs away from the perinuclear region to the cell surface. When cells are treated with nocodazole which drives depolymerization of microtubules, VIFs retract to the nuclear surface with very few VIFs in the peripheral regions [56].

4. Vimentin appears on the surface of cells and is secreted by multiple cell types.

The presence of extracellular vimentin has been documented for at least 35 years, but a functional role for the surface exposure and the mechanisms by which vimentin is released from the cell interior are only recently beginning to be identified (Table 1). One early study of antibodies generated against surface-exposed antigens in malignant monocytes showed that these antibodies were specific to vimentin [12]. Since that time extracellular or cell surface vimentin has been documented in a wide array of settings in which it appears to play a role in both normal physiology and pathologic states, with a particular emphasis on circulating cancer cells and on infection by bacteria and viruses.

4.1. Sources of extracellular vimentin.

Extracellular vimentin can be generated by multiple mechanisms in addition to simple release of vimentin from necrotic cells or disrupted cell membranes, and autoantibodies to circulating, covalently modified vimentin are commonly found in inflammatory diseases [78]. Figure 3 shows the distinct patterns of intracellular and extracellular vimentin in

cultured fibroblasts, a cell type that increases exposure of cell surface vimentin during senescence [79]. Neutrophils [80] and T lymphocytes [81] undergoing apoptosis release vimentin to their extracellular surface, where in the case of thymocytes, it recruits extracellular phospholipase II and is thought to promote production of arachidonic acid. Activated platelets and platelet-released micro-particles also expose vimentin on their surface, where vimentin binds vitronectin, which stabilizes plasminogen activator inhibitor I in its active form, suggesting a role for surface vimentin in fibrinolysis [82]. Macrophages are simulated by the cytokine TNF-alpha to secrete vimentin into the medium in a process that can be blocked by inhibition of protein kinase C [83]. Oxidized low-density lipoproteins can also stimulate macrophages to secrete vimentin, and extracellular vimentin can induce macrophages to release inflammatory cytokines. These effects might be related to the finding of increased serum levels of vimentin in patients with atherosclerotic coronary artery disease and in an Apo-E null mouse model of atherosclerosis, suggesting that serum vimentin might be a useful biomarker [84].

Endothelial cells also secrete vimentin, and a subset of endothelial cells express it on their surface. Here it can interact with von Willebrand factor to form strings that mediate platelet adhesion in the vascular lumen and contribute to stroke pathology [85]. Extracellular vimentin can also bind P-selectin and lower the adhesivity of neutrophils to platelets and the endothelium [86]. A remarkable aspect of the vimentin secreted by endothelial cells is that it is a target of the PAL-E antibody, which has been used to detect blood capillaries and small veins [87]. Under non-reducing conditions, the antigen recognized by the PAL-E antibody has an apparent molecular weight of 120 kDa, or twice the size of vimentin, which drops to 55 kDa in reducing conditions, suggesting that this form of extracellular vimentin is composed of disulfide bonded dimers. The highly localized expression of this surface epitope suggests that vascular surface vimentin might be enriched at sites where circulating cells adhere most to the vessel surfaces [87].

Secretion by both macrophages and endothelial cells requires post-translational modification of vimentin, and antibodies specific to cell surface vimentin can be generated [88,89]. An important finding is that cell surface vimentin appears to be mainly in the form of oligomers with 4 – 12 monomers, but is not filamentous, a difference that is likely to relate to the much higher affinity of the oligomers to lipid bilayers compared with VIFs [90]. Other mechanisms by which extracellular vimentin can be generated are illustrated in some of the examples listed below and summarized in Table 1. The functional aspects of extracellular vimentin, which have been identified, are illustrated in Figure 4.

4.2. Wound repair, regeneration, and senescence

One of the clearest beneficial effects of extracellular vimentin is seen in the mechanism by which mesenchymal cells at the edge of a wound in an *ex vivo* mock cataract surgery model transition to a myofibroblast phenotype. Following injury, vimentin is released into the extracellular space, where it binds to mesenchymal leader cells located at the wound edge and thereby supports the contractile cell phenotype that enables wound closure. In profibrotic environments, extracellular vimentin might contribute to deleteriously high conversion or maintenance of myofibroblasts [91]. Astrocytes, activated by injury, can

produce extracellular vimentin in the form of exosomes that are delivered to neurons, which then can bind this protein on their surface even though they do not express it endogenously. Extracellular vimentin binds to neuronal insulin-like growth factor 1 receptor to promote axonal growth in vitro and improves recovering after spinal cord injury in mice [92,93]. Cell surface bound vimentin can promote entry of *Clostridium botulinum* C3 transferase (C3bot) and thereby might mediate the axonotrophic effects of C3bot after spinal cord injury [94]. Citrullinated vimentin has also been proposed as a marker for astrocyte activation [95]. Senescent human fibroblasts secrete a modified vimentin that is post-translationally modified on cysteine 328 by the oxidative adduct malondialdehyde (MDA) and MDA-modified vimentin plasma levels increase in a murine model of accelerated aging. This finding raises the possibility that innate immunity might recognize senescent cells by the presence of membrane-bound MDA-vimentin [79]. Extracellular vimentin can also have anti-inflammatory effects, as suggested by a study showing that extracellular vimentin blocked secretion of pro-inflammatory cytokines by dendritic cells through modulation of their response to lipopolysaccharide [96].

4.3. Vimentin on the surface of cancer cells

Several cancer cell types are associated with extracellular vimentin, and this finding has led to efforts to target it as a potential therapy. For example, normal human T lymphocytes express surface vimentin only after activation, but malignant Sezary lymphocytes express it constitutively [97]. Glioblastoma multiforme stem cells express surface vimentin, which promotes spheroid formation in vitro. Treatment of these cells with an anti-vimentin antibody leads to internalization of surface vimentin, lowered cell viability due to apoptosis, and diminished tumor growth in a mouse model [88,98]. Gastric cancer cells also express cell surface vimentin, and a magnetic bead isolation assay identified circulating tumor cells (CTCs) in peripheral blood of the majority of gastric cancer patients, with high vimentin positive CTCs correlating with poor prognosis [89]. Surface vimentin positive CTCs also have potential as predictors of relapse in postremission neuroblastoma [99]. Three different human prostate cell lines express vimentin that can be detected by monoclonal antibodies recognizing the coil one rod domain and the C-terminus of vimentin [100]. Anti-cell surface vimentin antibodies have also been used to detect stem-like hepatocellular carcinoma cells with enhanced metastatic potential [101]. Magnetic particles containing anti-vimentin antibodies have been used to isolate circulating cancer cells from the blood of mice with tumors generated by the human lung cancer cell line A549 [102] and macrophage-like CTCs from the blood of patients with gastrointestinal stromal tumors [103].

4.4. CD44 signaling

Soluble CD44, a hyaluronan (HA) ligand that is overexpressed during inflammation and in some cancers also binds vimentin at the surface of endothelial cells. The vimentin binding site on CD44 overlaps with its HA binding domain and targets the N-terminus of vimentin [104]. This interaction of CD44 and vimentin might relate to the finding that both CD44 and vimentin are increased on the surface of some cancer cell lines such as those from the prostate [100] and oral squamous cell carcinoma [105]. The interactions among CD44, HA, and vimentin might affect how circulating cancer cells or other cell types interact with the endothelium.

4.5. Intracellular and extracellular vimentin are involved in pathogen infection

Vimentin is increasingly found to affect invasion of cells by pathogens. Both bacteria and viruses bind cell surface vimentin. In some cases, this binding facilitates infection of the host cell by the pathogen, and in other cases binding to vimentin appears to compete with or otherwise inhibit binding of the pathogen to the receptor that enables its entry into the host cell. An enabling role of vimentin is consistent with numerous reports that vimentin null mice are relatively resistant to some forms of infection [106–109], but a direct relation is not clear since both extracellular and cytoskeletal vimentin are important elements in the innate immune response. A recent study showed that disassembly of the vimentin cytoskeleton is an essential part of the mechanism by which cells extrude DNA-containing neutrophil extracellular traps (NETs) [110] as defense against bacterial infection. This process requires the activity of protein arginine deiminase to convert arginine to citrulline on both histones and vimentin to disassemble chromatin and VIFs, suggesting that vimentin might also be released by this mechanism. Extracellular exposure of citrullinated autoantigens including vimentin is associated with NET formation in rheumatoid arthritis [111,112]. Whether extracellular vimentin can serve a protective role against pathogens is not yet clear, but there are numerous compelling examples of vimentin enhancing the infectivity of both bacteria and viruses and several demonstrations that antibodies or other ligands for vimentin can block infection.

4.5.1. Bacterial infection—Cytoskeletal VIFs are often remodeled during bacterial infection [37], and in some cases surface vimentin is a docking site for specific bacterial surface proteins. Many of these results are discussed in a recent review [113]. Here we cite a few more recent results showing the importance of vimentin for surface binding and intracellular invasion by bacteria.

VIFs are important for infection by Chlamydia trachomatis, and vimentin is a substrate for chlamydial protease-like activity factor [37]. VIF remodeling by Chlamydia requires vimentin glycosylation [44]. Cell surface vimentin is important for several bacterial infections, such as *Listeria monocytogenes* [114,115] and meningitic *Escherichia coli* [107,116]. Vimentin-dependent infection of human microvascular endothelial cells by Listeria monocytogenes increases as substrate stiffness increases [114]. The surface adhesion factor BspC of *Streptococcus agalactiae*, which causes meningitis, enables the bacterium to enter cerebral microvascular endothelial cells by binding their surface vimentin, as well as cytoskeletal vimentin. BspC is both necessary and sufficient to infect the microvascular endothelium and to induce neutrophil chemokine expression, and vim −/− mice are protected from infection by wild type bacteria [106]. Infection of monocytes by Mycobacterium tuberculosis also leads to increased expression of cell surface vimentin which is recognized by NKp46 located on the surface of natural killer cells [117].

4.5.2. Viral infection—Both extracellular vimentin and intracellular VIFs have been identified as important mediators of multiple stages of virial infection, from initial cell invasion to intracellular viral replication and release. Cell surface vimentin is important for infection of cells by the coronaviruses SARS-CoV [118–120] and porcine reproductive and respiratory syndrome (PRRS) virus [121–124] and by enterovirus [125–127] which shares

some common targets with coronavirus. One study found that annexin 2 binds vimentin, and only when both proteins are present can PRRS virus infect its host cell [121]. Surface vimentin is also involved in infection by Dengue virus [128,129] (which can be clinically similar to covid-19 [130]) and Japanese encephalitis virus [131]. The H9N2 subtype avian influenza virus binds vimentin during infection of Madin-Darby canine kidney (MDCK) cells. Incubation of these cells with an antibody against vimentin or downregulating vimentin expression with siRNA lowered the infection rate, whereas upregulating vimentin expression increased infectivity of MDCK cells [132]. Japanese encephalitis virus (JEV) also requires vimentin for infection of porcine kidney cells. Treatment with an anti-vimentin antibody blocked infection of these cells by the virus [131]. The Chandipura virus, which causes encephalitic complications in humans, colocalizes with vimentin on the surface of murine Neuro-2a cells, and its infection of these cells can be decreased either by prior incubation of the virus with purified vimentin or of the host cell with anti-vimentin antibodies [133].

With SARS-CoV, vimentin binds the viral spike protein and enhances its delivery to the receptor angiotensin-converting enzyme 2 [120]. SARS-CoV also upregulates cytoplasmic vimentin by the TGF-β pathway to promote a fibrotic response in lung cells [118], and other reports show that intracellular VIFs can also be important for viral infection and replication. Enterovirus requires reorganization of the VIF cytoskeleton for efficient replication and export [126]. The foot-and-mouth disease virus (FMDV) also requires the VIF network for optimal replication, and this requirement has been traced to the binding of the FMDV nonstructural protein 3A to vimentin [134].Cell surface vimentin is not necessarily a viral receptor or even an augmenting factor for viral entry. Studies of human papillomavirus (HPV) infection showed that HPV16 pseudovirions bound cell surface vimentin and that pretreatment of the virus with soluble vimentin lessened its ability to infect a host cell. In this setting vimentin did not act as a receptor but bound the viral proteins to dampen the initial steps of HPV16 infection [135].

4.6. Countermeasures to cancer and infection

An exciting aspect of work related to extracellular vimentin is the identification of soluble ligands with the potential to improve identification or removal of cancer cells, and to prevent vimentin-dependent routes of infection, especially with relation to coronaviruses. A number of approaches have used antibodies to extracellular vimentin in conjunction with antibodies to other cell surface markers to detect and isolate circulating cancer cells [89,102,103,136]. Both monoclonal anti-vimentin and a vimentin-specific DNA aptamer are being used to isolate circulating cancer cells or block the effect of vimentin [137]. Similar approaches are being applied to infection by SARS-CoV [120] SARS2-CoV [138] and other viruses [131]. Citrullinated vimentin in combination with other antigens has been tested in possible vaccines to stimulate CD4-mediated anti-tumor activity and showed some efficacy in a mouse model [139].

5. Conclusion.

The striking viscoelastic properties of VIF networks *in vitro* suggest cellular functions based on resistance to mechanical stress and regulation of cell shape. Indeed VIFs are essential for protection of the nucleus from potentially damaging forces and forms much of the cytoskeleton. However, many studies point to important non-mechanical functions. Vimentin, either in the form of small oligomers or VIFs, binds numerous proteins and nucleic acids, and functions to integrate with other cytoskeletal systems, regulate lipid metabolism and perform numerous other intracellular functions. In addition, the rapidly growing study of extracellular vimentin, especially in the regulated disassembly of VIF and the secretion of modified vimentin, reveals many new functions for the protein, from mediating pathogen invasion to promoting wound healing. Clearly there is much more to be learned about this abundant but understudied protein.

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Abbreviations:

AKT	Protein kinase B
dreh	down-regulated in its expression by hepatitis B
GlcNAc	N-acetylglucosamine
IF	Intermediate filament
LDL	low density lipoprotein
LINC	linker of nucleoskeleton and cytoskeleton
MDA	malondialdehyde
VIF	vimentin intermediate filament
TNF-alpha	tumor necrosis factor alpha
IGFR	insulin like growth factor receptor

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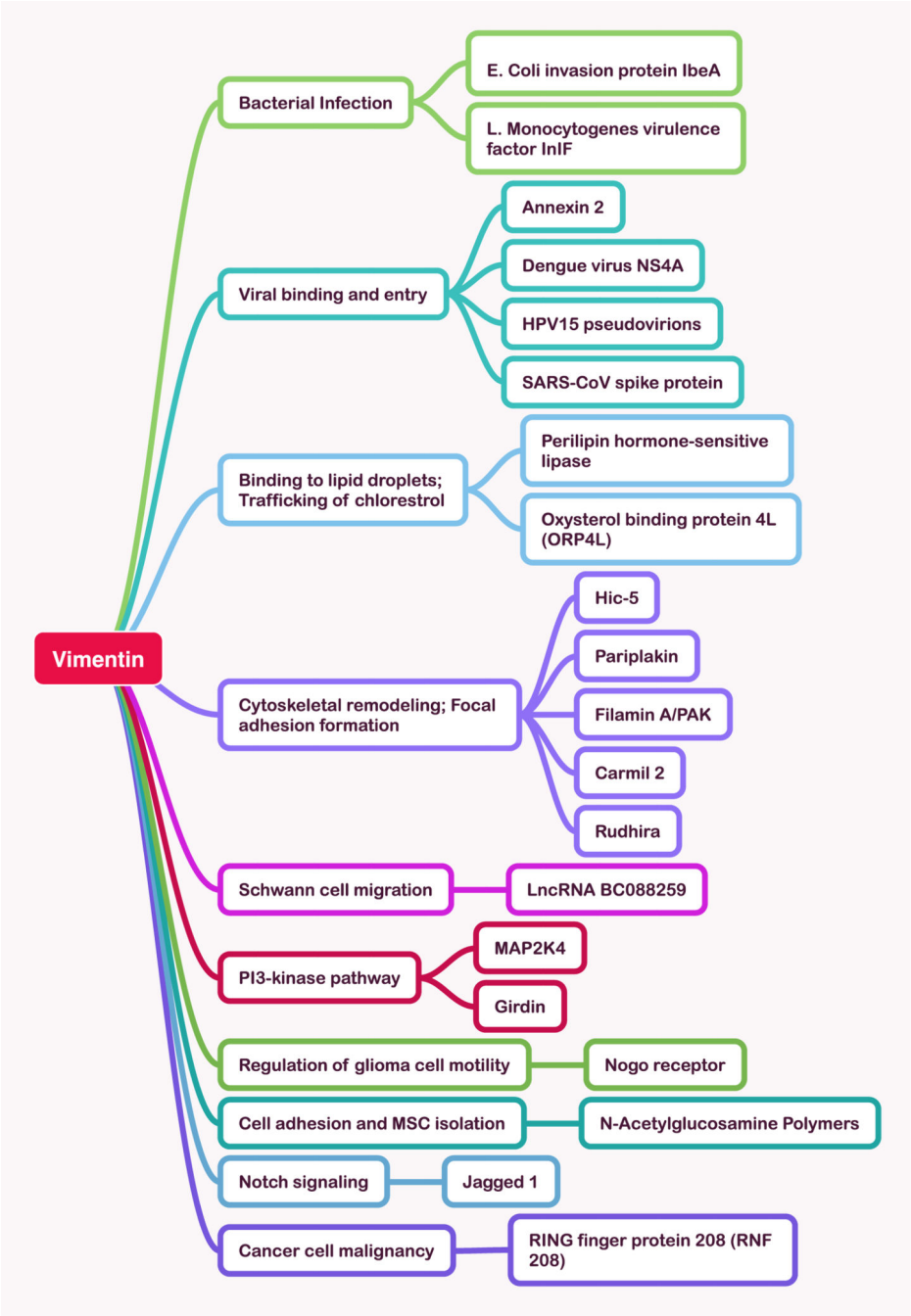

Figure 1:
Examples of newly reported ligands and targets of vimentin.

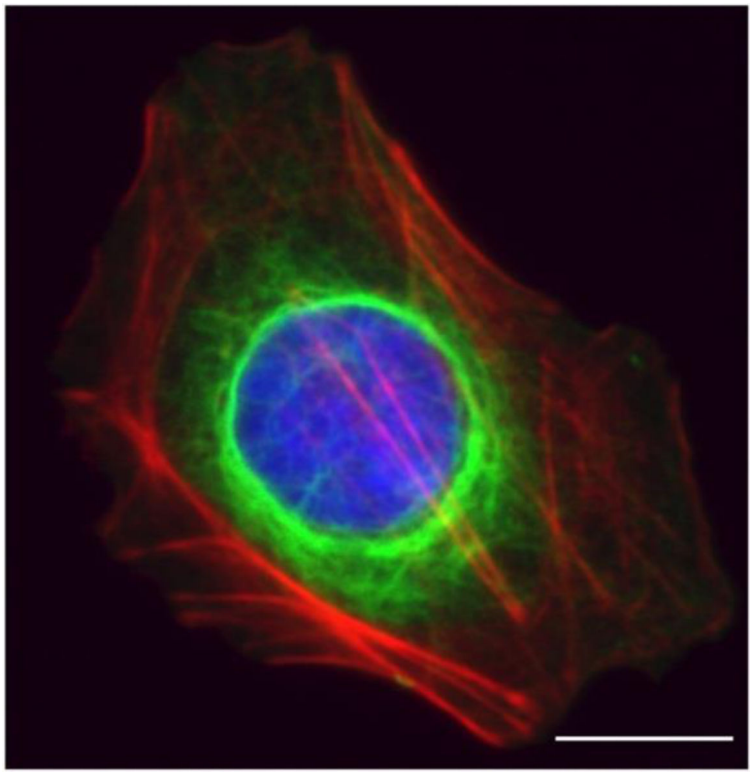

Figure 2:
Intermediate filament networks and the cell nucleus. The greater localization of VIFs juxtaposed to the nucleus is indicative of a distinct cage. Immunofluorescence image of a HUH7 cell marked for vimentin (green), actin (red), and DNA (blue). Scale bar is 15 μm.

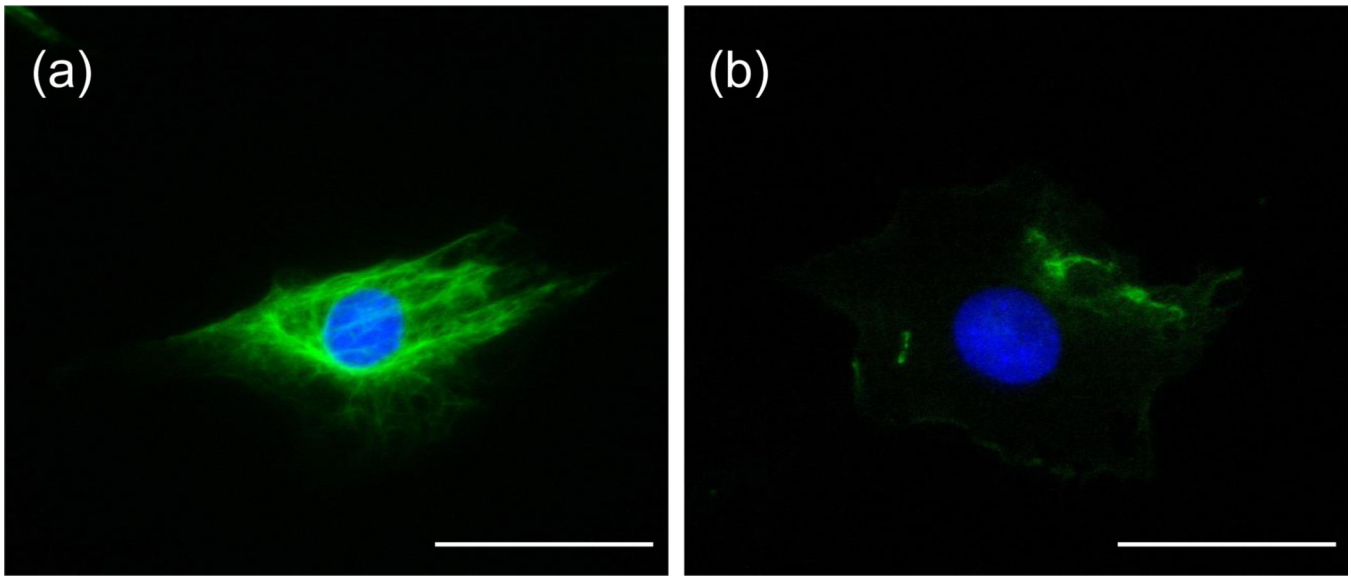

Figure 3:
Immunofluorescence images of (a) intracellular and (b) extracellular vimentin in mouse embryo fibroblasts. The cell on the left was treated with triton to permeabilize the cell membrane and the cell on the right was not. Vimentin is shown in green; DNA in blue. Scale bar is 50 μm.

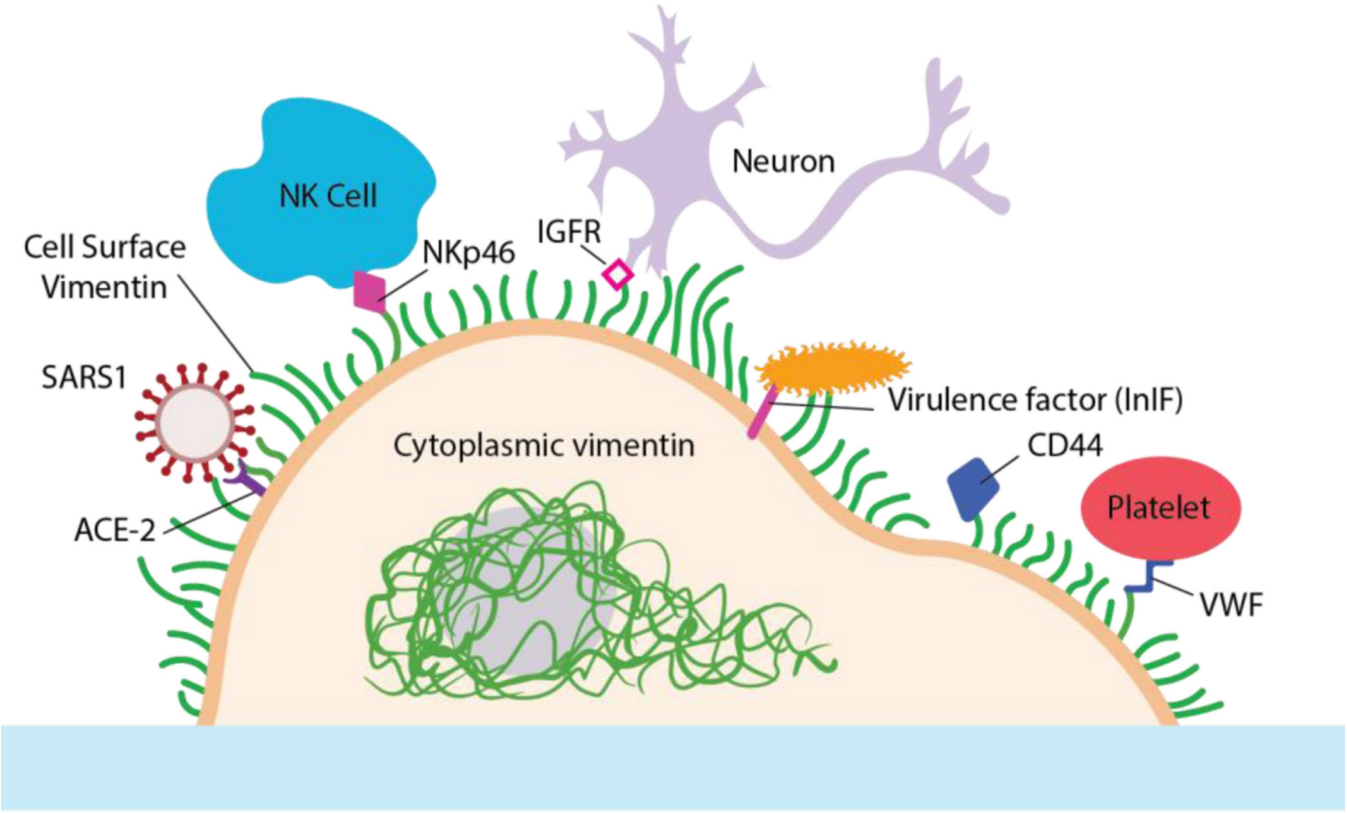

Figure 4:

Examples of extracellular vimentin functions. Viruses are bound by specific surface proteins to vimentin to facilitate their delivery to the viral receptor that mediate entry. Bacterial virulence factors or adhesions bind vimentin directly to enable their entry into the host. Cell surface vimentin binds soluble CD44 and enhances its initiation of intracellular signals. Vimentin on the surface of activated monocytes binds the natural killer cell surface protein NKp46 to initiate cell killing. Extracellular vimentin binds IGFR to enhance neuronal repair.

Table 1.

Sources of extracellular vimentin

Cell type	Modification	Function	Reference
Neutrophil	Citrullinated	Released during apoptosis or NET formation Source of serum citrullinated vimentin commonly found in rheumatoid arthritis	[80,110]
T-lymphocyte	Released on apoptosis	Binds phospholipase II to promote arachidonic acid	[81]
Monocyte/ macrophage	Phosphorylated	Secreted after simulation by TNF-alpha or oxidized LDL	[83,95]
Astrocyte	Citrullinated; Packaged in exosome	Delivery to neuron to promote wound healing	[92–95]
Platelet		Altered fibrinolysis; Binding to von Willebrand factor	[82,85]
Endothelial cells	Disulfide-dimerized	Increased platelet binding; decreased neutrophil binding. Ligand for CD44 and several pathogens	[13,85,87,104,116,118]
Senescent fibroblast	Malondialdehyde-ligated	Increased clearance by immune system	[79]
Cancer cells	Sometimes proteolyzed	Target for isolating circulating tumor cells, designing vaccines and enhanced chemotherapy	[21,33,94,97,100,103,136,140,141]