Multifunctional Roles of Gelsolin in Health and Diseases

Guo Hua Li, Pamela D. Arora, Yu Chen, Christopher A. McCulloch, and Peter Liu

1Toronto General Hospital, University Health Network, Toronto, Ontario, Canada
2Canadian Institute of Health Research Matrix Dynamics Group, Faculty of Dentistry, University of Toronto, Ontario, Canada
3Department of Laboratory Medicine, Dr. Everett Chalmers Regional Hospital, Horizon Health Network, Fredericton, New Brunswick, Canada
4Canadian Institute of Health Research Group, University of Toronto, Toronto, Ontario, Canada

Abstract: Gelsolin, a Ca$^{2+}$-regulated actin filament severing, capping, and nucleating protein, is an ubiquitous, multifunctional regulator of cell structure and metabolism. More recent data show that gelsolin can act as a transcriptional cofactor in signal transduction and its own expression and function can be influenced by epigenetic changes. Here, we review the functions of the plasma and cytoplasmic forms of gelsolin, and their manifold impacts on cancer, apoptosis, infection and inflammation, cardiac injury, pulmonary diseases, and aging. An improved understanding of the functions and regulatory mechanisms of gelsolin may lead to new considerations of this protein as a potential biomarker and/or therapeutic target.

Key words: gelsolin; actin; apoptosis; cancer; cardiac remodeling

1. INTRODUCTION

The actin cytoskeleton plays a central role in many fundamental cellular processes involving the generation of force and facilitation of movement, which are enabled by the assembly of actin monomers into filaments and cooperation with a wide variety of actin binding proteins (ABPs), including members of the myosin family. Actin is involved also in the formation of stable structures, which are often linked to the plasma membrane and indirectly to the extracellular matrix through membrane-associated adhesion proteins, such as integrins.

Actin monomers (G-actin) spontaneously associate to form microfilaments (F-actin) under physiological conditions. Because each actin subunit in F-actin is oriented with its cleft...
toward the same end of the filament, F‐actin has a polarity with discernibly different ends. One end is called the barbed (or “plus”) end and the other is called the pointed (or “minus”) end. F‐actin, under certain conditions, undergoes a process called treadmilling, which refers to the addition of an actin monomer to its barbed end and, at the same time, subtracts an actin monomer from its pointed end. Monomers can add on to the ends of filaments, form nuclei with another two monomers to create new filaments, as well detach from filaments to maintain actin filaments in equilibrium with actin monomers. The assembly and three‐dimensional organization of actin filaments in cells is controlled through interactions with a large number of functionally distinct ABPs, which are classified according to their functions of cross‐linking, severing, capping, and nucleating actin filaments. One particularly high abundance ABP is gelsolin, a Ca²⁺‐dependent actin filament severing and capping protein. Since its discovery 30 years ago, much of the research on gelsolin has focused on its role in actin assembly and filament remodeling. There is now increasing evidence that gelsolin is a multifunctional regulator of cell metabolism that involves multiple mechanisms independent of its actin regulatory functions (reviewed in 3–6).

2. STRUCTURE OF GELSONIN AND ITS RELATION WITH ACTIN DYNAMIC REGULATION

The gelsolin gene likely evolved from triplication followed by duplication of an ancestral gene encoding a single domain protein of about 15 kDa. Both the intracellular (cytoplasmic, cGSN) and extracellular (secreted, pGSN) forms of gelsolin are encoded by genes on chromosome 9 in humans and on chromosome 2 in mouse, and are under the control of different promoters. The gene that codes both gelsolin isoforms is made up of at least 14 distinct exons which span a region of ~70 kb. The arrangement of cGSN at the 5′‐end is exon 1‐intron‐exon 2‐intron‐exon 4. Collectively, exons 1 and 2, which are 13 kb apart in the gene, make up the unique 5′ untranslated region of cGSN. The 5′‐end of the pGSN differs from the cGSN, as it is made up of exon3‐intron‐exon 4. Exon 3, which is found 2.3 kb upstream from exon 4, comprises a small region of untranslated sequence and codes for both the signal peptide and the first 21 residues of the pGSN.

Initiation of transcription occurs at two distinct sites in the gelsolin gene, separated by 32 kb. The mRNA of cGSN is produced when transcription is initiated at exon 1, while the plasma initiation site is found in exon 3, which is separated from the large adjacent intervening sequence during RNA splicing (Fig. 1A). Consequently, the human plasma isoform, with an actual molecular mass of 83 kDa (93 kDa by SDS‐PAGE), is slightly larger than the intracellular isoform (80 kDa). The mass difference is attributable to a 23 amino acid residue extension found at the N‐terminal of pGSN. Gelsolin is composed of six domains, designated (from the N‐terminus) as G1–G6 (Fig. 1B). Each domain contains Ca²⁺‐binding site. The helical tail is in close contact with the actin binding helix of G2 and may act as a latch to inhibit actin binding. Recent studies show that residues in the Ca²⁺‐binding sites of G2 and G6 are involved in regulating gelsolin structure, including stabilizing the Ca²⁺‐free state, promoting transitions through intermediate states and stabilizing the Ca²⁺‐bound state. Ca²⁺‐binding by G2 is critical in the activation and stabilization of gelsolin. The disulfide bond in G2 is involved in Ca²⁺ activation of gelsolin. Cys‐188, residue which forms the juxtaposition of the disulfide‐bond, and Asp‐187, Ca²⁺‐binding residue, mediate this effect.

The most extensively examined roles of cGSN relate to its ability to sever, cap, uncap, and nucleate actin filaments. Gelsolin can bind to actin monomers and filaments, and is regulated by pH, phosphoinositides, lysophosphatidic acid, and high micromolar
There are three different actin binding domains which are distributed within the six repeating segments (G1–G6) in the amino acid sequence of gelsolin. There are monomer actin binding sites present in segment G1 and G4–G6, while the highest affinity for actin binding occurs in segment G2. After activation of gelsolin, the binding of domain G2 to actin enhances the binding of actin to the G1 domain and facilitates F-actin severing. Gelsolin can bind to actin monomers and filaments, a process which is regulated by micromolar concentrations of Ca^{2+}, alterations of pH, localized increases of phosphoinositide concentrations (particularly PIP2), and lysophosphatidic acid. Gelsolin contains a caspase-3 site. When activated caspase cleaves gelsolin, the N-terminal gelsolin fragment is pro-apoptotic and the C-terminal gelsolin fragment is anti-apoptotic. In the FAF a point mutation in plasma gelsolin enhances susceptibility to furin cleavage. Furin cleavage leads to the production of a C-terminal, 68 kDa gelsolin fragment, which is further cleaved by membrane type 1 matrix metalloproteinase, or possibly other related MMPs in the extracellular matrix, resulting in the formation of 8 and 5 kDa amyloidogenic fragments.
F-actin binding site is located in G2–3. These three binding sites play an important role in F-actin severing and nucleation. Several previous studies have shown that the structural activation of gelsolin is a two-step, three-state process. The first transition, from an inactive to an intermediate state, occurs at approximately 0.1–5 μM Ca^{2+}. The second transition to an activated state, occurs at approximately 10 μM–1 mM Ca^{2+}. Specifically, F-actin severing involves segment G1–3 (N-terminal half of the molecule) independent of Ca^{2+}, whereas the carboxyl-terminal segment G4–6 (C-terminal half) is regulated by Ca^{2+}. Biochemical and physical studies indicate that Ca^{2+} opens up gelsolin by inducing a conformational change in the C-terminal half to expose actin-binding sites on the N-terminal half. The proposed mechanism of F-actin severing by gelsolin involves binding of G2 to F-actin that positions G1 for strong binding to actin filaments. This disrupts the actin–actin hydrophobic bond interactions between two actin monomers, which result in severing. Subsequent to severing, gelsolin remains attached to the barbed ends of actin filaments and acts as a cap. New actin polymerization from the barbed end requires uncapping of gelsolin, which is mediated by polyphosphoinositides (PPIs) (particularly PIP_{2}), and enables the addition of free monomers on the barbed ends and actin assembly.

The conditions of ionic strength and composition, temperature, as well as the pH in the extracellular environment (such as blood plasma) favor the polymerized form of actin. The formation of F-actin may be fatal after actin is released from dying cells into the bloodstream. An extracellular actin scavenging system (EASS) has evolved to rapidly clear actin from the circulation. Notably, the plasma form of gelsolin, one of the two proteins of the EASS, may depolymerize and sequester actin released into the vasculature after cell damage and death. In addition, the scavenging role of gelsolin involves the binding and inactivation of bioactive inflammatory mediators (discussed below).

A third isoform of gelsolin (gelsolin-3) has been identified as cytoplasmic and characterized by 11 additional residues at the N-terminus. It is expressed in oligodendrocytes in the brain, lungs, and testis. Gelsolin-3 has been shown to be involved in myelin formation and CNS development.

### 3. GELSOLIN SUPERFAMILY OF PROTEINS

Lack of gelsolin expression in mice is not lethal, although gelsolin knockout mice (GSN−/−) display impaired function of platelets, defects in mammary gland morphogenesis subsequent to the onset of puberty, disruption of the organization of actin-based domains in osteoclasts, alterations in the formation of growth cones of neurites, and reduced migratory capacity of neutrophils during inflammation. These findings may be attributed to functional redundancy and compensatory expression by other gelsolin superfamily proteins, which includes at least seven members. Proteins in this conserved family are widely expressed in mammalian and non-mammalian organisms.

Gelsolin family proteins contain the core structure of gelsolin-like domains and are mainly involved in the regulation of actin dynamics. However, like gelsolin, some proteins of the gelsolin family, such as CapG, flightless-1, villin, and supervillin, are multifunctional (Table I). Compensatory functions that are exhibited by proteins of the gelsolin superfamily may add to overall biological complexity when considering gelsolin as a potential therapeutic target. Notably, an absence of effect does not necessarily mean an absence of function in vivo, but may enable improved safety in therapeutic targeting. Conceivably, the complexity and functional redundancy of these proteins may explain why development of actin cytoskeleton-based strategies to treat or prevent human disease has been relatively untapped.
**Table I.** Structure, Expression and Function of Gelsolin Superfamily Proteins

<table>
<thead>
<tr>
<th>Structures</th>
<th>Number of GRD</th>
<th>Other domains</th>
<th>Expression in mammalian</th>
<th>Cellular functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gelsolin</td>
<td>6</td>
<td></td>
<td>Ubiquitous (brain, heart, lung, kidney, liver, spleen, stomach, smooth muscle, blood, and different cells)</td>
<td>+ (Actin dynamics regulation) + Exocytosis + Cell motility + Phagocytes + Apoptosis regulation + Modulation of platelet + Anti-inflammatory + Signal transduction + Transcriptional coactivator</td>
</tr>
<tr>
<td>Adseverin</td>
<td>6</td>
<td></td>
<td>Kidney and adrenal gland intestine</td>
<td>+ (Fact and integrand-mediated) + (Complement mediated) +</td>
</tr>
<tr>
<td>CapG</td>
<td>3</td>
<td></td>
<td>Squamous epithelia, kidney, adrenal gland, spleen, and developing brain cordtex</td>
<td>+ + +</td>
</tr>
<tr>
<td>Severin</td>
<td>3</td>
<td></td>
<td>Lung (minute amount)</td>
<td>+</td>
</tr>
<tr>
<td>Fragmin</td>
<td>3</td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Flightless-1 (UniGene Hs 513984)</td>
<td>5</td>
<td>LRR</td>
<td>Actin based structures (Filopodia, neurites, and growth cones)</td>
<td>+ + + (Downregulation of the IL-1/TRA signaling)</td>
</tr>
<tr>
<td>Villin/Advillin</td>
<td>6</td>
<td>HP</td>
<td>Epithelial cell-based structures (gastrointestinal, renal, and urogenital)</td>
<td>+ + + (LPA binding)</td>
</tr>
<tr>
<td>Supervillrin</td>
<td>5</td>
<td>NLS, HP</td>
<td>muscle, bone marrow, thyroid gland, and salivary gland</td>
<td>+ + +</td>
</tr>
<tr>
<td>Protovillin</td>
<td>6</td>
<td>Neck, HP</td>
<td>Vegetative and developing cells</td>
<td>+</td>
</tr>
</tbody>
</table>
4. GELSOLIN EXPRESSION AND EPIGENETIC CHANGES

Cytoplasmic gelsolin is ubiquitously expressed in cells and tissues, although there are variations of expression levels during cell differentiation and in carcinogenesis,\textsuperscript{34–38} and at different stages of the life span of the organism.\textsuperscript{39,40} In contrast to cGSN which is present in most types of cells, pGSN is expressed mainly by muscle cells. In humans, normal plasma levels of gelsolin are approximately 190–300 mg/l (average \(\approx 250\) mg/l), although differences are reported depending on different methods of assaying pGSN (ELISA or functional nucleation).\textsuperscript{41–46} Gelsolin is also found in mid-trimester amniotic fluid\textsuperscript{47} and in cerebrospinal fluid.\textsuperscript{48,49}

Various data show that epigenetic regulation of gelsolin may play a role in disease, although no naturally occurring mutations of gelsolin have been identified in the context of cancer risk or the regulation of apoptosis. Epigenetic modulation involves modifications of the transcriptional activation of certain genes. For example, DNA methylation and histone deacetylation (HDAC) are associated with transcriptional repression.\textsuperscript{50,51} Gelsolin expression is increased in different types of cells after treatment with the HDAC inhibitors, trichostatin A or apicidin, or after inhibition of DNA methylation (e.g. 5-aza-2'deoxytidine) or with the heat shock protein 90-associated signal inhibitor, radicicol, which exhibits growth-inhibiting and apoptosis-inducing activities.\textsuperscript{52–57} These data suggest that epigenetic changes can alter gelsolin expression. The transcriptional activation of the gelsolin gene by apicidin may be mediated by protein kinase C (mainly through PKC\textsubscript{e}) via binding to Sp1.\textsuperscript{57} Altered expression of PKC isoforms has also been observed in experimental mouse myocardial infarction models, with and/or without gelsolin (our unpublished data). In an earlier in vivo study, pretreatment with trichostatin A increases the expression of gelsolin and protects against ischemic brain in wild-type mice but not in mice that are deficient in gelsolin.\textsuperscript{58} Furthermore, epigenetic changes influence not only the expression levels, but also the functions of gelsolin. For example, posttranslational N-myristoylation of the C-terminal of gelsolin in COS-1 cells is a requirement for its anti-apoptotic activity.\textsuperscript{59}

5. GELSOLIN AND APOPTOSIS

Apoptosis is a fundamental physiological process required for the development and homeostasis of tissues in multicellular organisms. Gelsolin can enhance or inhibit apoptosis depending on the nature of the pathological conditions, the identity of the cell types, and the specific tissues that are involved.\textsuperscript{60–64} Caspase-3, which is an important effector of apoptosis, cleaves gelsolin between residues Asp352 and Gly 353, resulting in generation of fragments with molecular masses of 39 kDa (N-terminal half) and 41 kDa (C-terminal half).\textsuperscript{60} The N-terminal gelsolin fragment not only severs actin in a Ca\textsuperscript{2+}-independent manner, but also contributes to morphological changes of apoptosis.\textsuperscript{60,61} While caspases-3, -7, and -9 cleave gelsolin in a similar manner,\textsuperscript{65} caspase-8 may also cleave gelsolin but at a different site.\textsuperscript{66} Cleavage of gelsolin is contemporaneous with the time course of poly-adenosine diphosphate ribose polymerase (PARP) cleavage, and N-benzyloxy carbonyl-Val-Ala-Asp-fmk (zVAD-fmk), a cell-permeable inhibitor of caspase-3, can block cleavage of gelsolin and PARP.\textsuperscript{60} Generation of the N-terminal gelsolin fragment during the same time as PARP cleavage and increased apoptosis has been also reported in studies in vivo.\textsuperscript{67–69}

Deoxyribonuclease (DNase I) is a key enzyme responsible for DNA degradation in apoptosis.\textsuperscript{70} Both full-length gelsolin and DNase I bind to actin in a noncompetitive manner to form a gelsolin/actin/DNase I ternary complex. In contrast, the N-terminal gelsolin fragment produced by caspase cleavage can competitively bind to actin, disrupt the actin-DNase I interaction, and release DNase I from actin to enhance apoptotic activity.\textsuperscript{71}
Furthermore, gelsolin can also enhance apoptotic activity (Fig. 2) through the gelsolin-HIF1-a-DNase I pathway. Notably, caspase-mediated gelsolin cleavage is dispensable for TNF-induced apoptosis in MCF-7 cells. In contrast to the N-terminal half of gelsolin, full-length gelsolin, especially human gelsolin (hGSN), the C-terminal half of gelsolin, and gelsolin complexed with phosphatidylinositol 4,5-bisphosphate are generally anti-apoptotic (Fig. 3). Support for this notion comes from studies showing that gelsolin overexpression inhibits loss of mitochondrial membrane potential as well as cytochrome C release from mitochondria, which inhibit activation of caspase-3, -8, and -9. Further studies have confirmed that gelsolin blocks actin-dependent VDAC, which is thought to be an essential mediator of mitochondrial-dependent cell death. This process may involve VDAC acting as a channel-forming unit within the MPT pore and as a target of Bcl-2 family members, similar to the anti-apoptotic Bcl-XL. Notably, using genetic deletion of VDAC, it has been demonstrated that loss of VDAC does not disrupt MPT function, and provides no protection from multiple forms of necrotic or apoptotic cell death. Moreover, premature closure of VDAC has been shown to promote premature death due to defects in metabolite homeostasis and coupled respiration, suggesting that loss of VDAC1/2/3 predisposes to cell death for reasons that are independent of MPT and likely related to the metabolic function of mitochondria. Further studies are needed to elucidate the role of gelsolin as an anti-apoptotic protein in mitochondrial-dependent cell death. The inositol lipids PI(4,5)P2 and PI(3,4)P2 prevent caspase-3 cleavage of gelsolin in vitro. Through the formation of a stable PI(4,5)P2-gelsolin-caspase-3 complex, PI(4,5)P2-gelsolin strongly inhibits caspase-3 and -9 activity. In addition, after posttranslational

**Figure 2.** Gelsolin regulates the apoptotic pathway. Gelsolin is a substrate of caspase-3, -6, -9, and/or -8. The resulting N-terminal gelsolin fragment not only severs actin in a Ca2+ independent manner but is also pro-apoptotic. N-terminal gelsolin competes with full-length gelsolin binding to actin, which disrupts the actin-DNase I interaction and releases DNase I from actin to enhance apoptotic activity. Furthermore, under hypoxic conditions, HIF1-a regulates the transcription of the gelsolin gene in fibroblasts. Gelsolin can enhance the expression of DNase I and can also enhance apoptotic activity through the gelsolin-HIF1-a-DNase I pathway. Moreover, gelsolin may downregulate survival factors in myocytic apoptosis through the cleavage of PARP disabling DNA repair.
N-myristoylation, the C-terminal, caspase-3-cleaved fragment of gelsolin is anti-apoptotic in COS-1 cells.\textsuperscript{59} In essence, gelsolin can be both an effector and an inhibitor of apoptosis, which underlines its important roles in cancer, cardiac disease, pulmonary injuries, and aging.

6. GELSOLIN AND SIGNAL TRANSDUCTION

Gelsolin can function upstream in a variety of signaling processes and participates in the coordinated regulation of several signal transduction pathways.\textsuperscript{75–79} As noted above, caspase-3 cleavage products of gelsolin, full-length gelsolin, and phosphatidylinositol 4,5-bisphosphate complexes with gelsolin can act as inhibitory or stimulatory factors in apoptosis.\textsuperscript{61,62,65} New insights in gelsolin-mediated signal transduction have been discovered in cancer research. The data showed that it might be the increased motility and/or invasiveness that lead to the poor prognosis of patients with erbB-2/EGFR tumor-positive condition.\textsuperscript{80,81} EGFR/erbB-2 can activate PLC\textsubscript{γ}, which in turn evokes a motility pathway depending on gelsolin expression to modulate the actin cytoskeleton dynamics.\textsuperscript{78} This interaction between erbB-2/EGFR and gelsolin activation has been confirmed in clinical studies and several cell culture systems.\textsuperscript{81,82} Moreover, activation of the small GTPases Ras and Rac plays a critical role in mediating downstream motility events, such as membrane ruffling and cell protrusion.\textsuperscript{83} Gelsolin levels regulate Rac expression\textsuperscript{84} and gelsolin is evidently a downstream effector of the Ras-PI3K signaling pathway in gelsolin-induced cellular invasion.\textsuperscript{85} Furthermore, gelsolin can affect lipid metabolism and lipid signaling pathways via regulation of phospholipase C activity.\textsuperscript{77}

Gelsolin is enriched in matrix protein adhesion complexes where it associates with integrins, focal adhesion kinase, and Src. For example, gelsolin is functionally integrated into the signaling pathway by which c-Src, osteopontin, and PI3K modulate osteoclast
function. Phosphoinositides, which are phosphorylated at the 3-hydroxyl position by PI3K, associate with gelsolin. At these adhesion sites, gelsolin also participates in actin filament reorganization and podosome formation by osteoclasts. Downregulation of c-Src by antisense oligodeoxynucleotides prevents osteopontin/αvβ3 activation of the gelsolin-PI3K complex.86 The cytoplasmic protein tyrosine phosphatase (PTP)-proline-glutamic acid-serine-threonine amino acid sequences (PEST) associates with gelsolin in the adhesions formed by osteoclast podosomes and after αvβ3-regulated phosphorylation of PTP-PEST.86

In phagocytosis of collagen, gelsolin functionally associates with β1 integrins and Rac.87,88 In summary, gelsolin is involved in many aspects of signal transduction (Fig. 4) which are quite separate from its roles in actin remodeling.

7. GELSOLIN AND PHAGOCYTOSIS

Phagocytosis is a highly conserved, complex cellular process that is characterized by receptor-mediated actin-driven steps. Phagocytosis is essential for the uptake and degradation of
microorganisms and damaged cells. This critical process plays also an important role in the remodeling of connective tissue matrices.

Three types of phagocytosis, based on unique receptor usage, have been identified to date. Complement- and IgG-opsonized phagocytosis are mediated by complement receptors and Fcγ receptors, respectively. Another type of phagocytosis in fibroblasts involves integrins. Gelsolin is present in enriched fashion in nascent phagosomes. Of the three types of phagocytosis, which are mediated by separate sets of cell surface receptors, gelsolin is known to play a role in Fc-receptor and integrin-mediated phagocytosis, but not in complement-mediated phagocytes. This observation may be because gelsolin is not known to play an important regulatory role in complement binding. Mouse neutrophils that are null for GSN have a specific deficit in Fcγ receptor-mediated phagocytosis. GSN−/− fibroblasts also exhibit defective binding and phagocytosis of collagen beads, which depends on integrin α2β1 receptors. Notably, Rac activation is a key step in Fc receptor- and integrin-mediated phagocytosis, and is strongly affected in the phagocytic functions of GSN−/− fibroblasts.

8. GELSOLIN AND TRANSCRIPTIONAL COACTIVATION

Sequence analyses of the gelsolin promoter have identified TBP, Sp1, AP-1, and NF-κB binding sites, which may be important for regulation of gelsolin expression. Several members of the gelsolin protein family, including gelsolin, flightless, and supervillin exhibit unexpected, potential functions in regulating transcription. For example, gelsolin can interact with hormone-bound androgen receptor, a member of the nuclear hormone receptor superfamily, to facilitate nuclear translocation. Two binding sites, identified in the C-terminal ligand-binding domain and the central DNA-binding domain of the androgen receptor, can bind gelsolin. Gelsolin, in turn, enhances the transcriptional activity of the androgen receptor in the presence of agonist. During the agonist-induced translocation of androgen receptor into the nucleus, gelsolin migrates into the peri-nuclear region. This localization is dependent on the presence of androgen receptor. The upregulation of gelsolin after depletion of androgen in prostate tumor lines indicates that gelsolin may be under negative feedback control via androgen. Reporter genes, when placed under the transcriptional control of other nuclear receptors, such as the glucocorticoid receptor, are also coactivated by gelsolin, although to a lesser degree. Moreover, gelsolin may colocalize with the thyroid hormone receptor-β1 in the nuclei of thyroid cells. The DNA binding domain of the thyroid hormone receptor is critical for binding to gelsolin, suggesting that gelsolin could affect the transcriptional activity of thyroid hormone receptors. Notably, gelsolin increases T3-dependent transcriptional activity of thyroid hormone receptor β1 in the nuclei of thyroid cells.

Recent studies showed that gelsolin interacts with hypoxia-inducible factor 1 (HIF-1). HIF-1α is a transcription factor and a key regulator of cell metabolism under hypoxic conditions. Gelsolin and HIF-1α coprecipitate and colocalize in the nuclei during hypoxia (Fig. 2). The interaction of gelsolin with HIF-1α in nuclei may play an important role in the induction of apoptosis mediated by HIF-1α-DNase I. Moreover, HIF-1α can regulate transcription of the gelsolin gene in fibroblasts under hypoxic conditions. While gelsolin expression can be regulated through the androgen receptor and/or the HIF-1-mediated pathways, it is possible that gelsolin itself directly contributes to transcriptional regulation.

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9. ALTERED EXPRESSION AND ROLE OF GELSOLIN IN VARIOUS DISEASES

Increased cGSN expression has been detected in several pathological conditions including idiopathic interstitial pneumonia,\textsuperscript{68} cardiac diseases including the failing human heart,\textsuperscript{67,105} oxidative stress,\textsuperscript{106} and in senescent cells and tissues.\textsuperscript{40} Downregulation of cGSN has been observed in various types of cancer,\textsuperscript{37,107–111} and both isoforms of gelsolin have been implicated in rheumatoid arthritis.\textsuperscript{112,113} Dramatic downregulation of pGSN levels due to a depletion of circulating gelsolin as a result of F-actin scavenging has been reported in acute liver failure, myocardial infarction, septic shock, muscle necrosis, acute respiratory distress syndrome (ARDS), experimental acute lung injury (ALI), and after allogeneic stem cell transplantation.\textsuperscript{43,44,46,114–116} Paradoxically, pGSN levels are increased in the Finnish type of familial amyloidosis\textsuperscript{41} and pediatric multiple sclerosis.\textsuperscript{117}

Enhanced expression of gelsolin may be protective and/or harmful under different conditions. For example, cGSN has five free thiol groups (Fig. 1A) that may be functionally linked to oxidative/reduction reactions.\textsuperscript{118} Conceivably, increased expression of gelsolin in response to oxidative stress may facilitate protection from cell death because of its antioxidant properties. Conversely, increased gelsolin levels can exacerbate pathological remodeling after myocardial infarction and heart failure,\textsuperscript{67} the development of pulmonary inflammation, as well as ventilator-induced lung injury.\textsuperscript{68,69} Many of these pathological changes can be mediated, at least in part, by the influence of gelsolin on apoptosis.

A. Gelsolin and Amyloidosis

Gelsolin-related amyloidosis, which is designated as familial amyloidosis of the Finnish type (FAF), is a rare, hereditary amyloid polyneuropathy characterized by corneal lattice dystrophy, progressive cranial, and peripheral neuropathy as well as skin changes.\textsuperscript{119,120} This condition is the result of a mutation in plasma gelsolin at position G654A or G654T, which results in the substitution of Asp187Asn or Asp187Tyr,\textsuperscript{121} disruption of the Cys188-Cys201 disulfide bond in gelsolin, and blockage of the binding of Ca\textsuperscript{2+} to the G2 domain and enhanced susceptibility to furin cleavage.\textsuperscript{122,123} Eventually, furin cleavage of secreted gelsolin leads to the production of a C-terminal, 68 kDa gelsolin fragment (C68). The C68 fragment is cleaved by membrane type 1 matrix metalloproteinase, or possibly other related MMPs in the extracellular matrix,\textsuperscript{124} resulting in the formation of 8 and 5 kDa amyloidogenic fragments (Fig. 1A). The 8 kDa fragment is more amyloidogenic than the 5 kDa fragment, but both gelsolin fragments aggregate by a nucleated polymerization mechanism. The C68 fragment is not amyloidogenic, even after attempted experimental seeding by 8 kDa gelsolin fragment amyloid fibrils.\textsuperscript{125} This finding is consistent with the inability to detect C68 in the amyloid extracted from a transgenic gelsolin amyloidosis mouse model.\textsuperscript{126} The toxic amyloidogenic 8 kDa and 5 kDa plasma gelsolin fragments and the subsequent process of amyloidogenesis, ultimately lead to intracellular inclusion bodies, which are composed of multiple proteins that contribute to muscle cell death, as is observed in the FAF mouse model.\textsuperscript{126} It seems that a mutation in the pGSN is the basis for the disease while the cGSN is not cleaved in the FAF patients; the cellular actin modulating function of cGSN is not affected.\textsuperscript{127}

B. Gelsolin and Cancer

One of the most fundamental characteristics of malignant and transformed cells is the aberrant organization of the actin cytoskeleton. The mechanisms that mediate transformation and the associated disruptions of the cytoskeleton are not well defined. Increasing evidence suggests a critical role for gelsolin as a tumor suppressor. For example, decreased expression of cGSN has been observed in many cancer cells,\textsuperscript{128} including human breast,\textsuperscript{129}
colorectal, gastric, bladder, lung, prostate, kidney, ovarian, pancreatic, and oral cancers, as well as in a mouse model of follicular thyroid cancer. Transfection of the gelsolin gene into human bladder cancer cells reduces tumorigenicity and colony-forming ability, while overexpression of gelsolin causes reversion of the transformed phenotype in cells and in a mouse model. When gelsolin expression is reduced by short interfering siRNA in mammary epithelial cells, typical signs of epithelial–mesenchymal transition are frequently observed, including fibroblastic conversion, loss of contact inhibition, focus formation in monolayers, and increased motility and invasiveness. The switch from E- to N-type cadherin expression and induction of the Snail transcription factor in epithelial–mesenchymal transition indicates that gelsolin may control this conversion via Snail and also may be involved in tumor progression.

Currently, it is not clear whether dysregulated gelsolin expression mediates malignant transformation in different cancer cells and tissues because no major mutations, gross rearrangements, or deletions within the gelsolin gene have been identified in malignancies. The downregulation of gelsolin may be related to gene inactivation mediated by epigenetic modifications in breast cancer cells, as well as in human ovarian carcinomas and to the reduced promoter activity that results from negative regulation by the activating transcription factor 1. Other studies indicate that reduced gelsolin expression may be attributable to the ubiquitin-proteasome-mediated degradation of gelsolin, as is seen in pancreatic cancer cells.

In contrast to the downregulation of gelsolin observed in most cancers, high expression levels of gelsolin occur in a subset of non-small cell lung cancers and in the transition from noninvasive to invasive tumors. High levels of gelsolin expression are thought to be an independent marker for tumor recurrence and progression in urothelial tumors, particularly for high-grade variants. This observation may be explained by insights from earlier studies which showed that gelsolin is involved in modulation of several signaling pathways, including c-erb-2/EGFR, PI3K, phospholipase C, and Ras-PI3K-Rac. These perturbations result in altered actin cytoskeletal architecture and increased cell motility, both of which are important for tumor cell migration and invasive growth.

Remarkably, gelsolin has at least one consensus cleavage site for caspase-8. Activated caspase-8 cleaves wild-type gelsolin and produces an 85 kD truncated protein (GSNp85) without the C-terminus. GSNp85 has been detected frequently in the transition to more aggressive and lethal localized melanomas. A C-terminal deletion of murine gelsolin reduces its ability to suppress metastasis, suggesting that C-terminal truncations of gelsolin might modulate melanoma progression.

C. Gelsolin, Infection, Inflammation, and Injury

In a study of nonenvelope lytic paroviruses, gelsolin seems to facilitate regulated virus egress from the nucleus to the cell periphery via (virus modified) lysosomal/late endosomal vesicles. This pathway is not required for DNA replication or particle production.

As mentioned above, cGSN plays a role in Fc-receptor and integrin-mediated phagocytosis. In addition, pGSN has actin scavenging function. Plasma gelsolin scavenges and severs actin filaments released from cells during inflammation and injury. Tissue injuries in various organs and diseases leads to prolonged reductions in pGSN, which precedes and predicts the development of many of the morbidities experienced by these high risk patients, including ARDS, ALI, MODA, and sepsis. The degree of the reduction in gelsolin levels in these patients is inversely associated with the duration of assisted ventilation, the duration of intensive care unit stay, the duration of overall hospital stay, and death.
Progress in understanding the relevant biological functions of gelsolin in inflammatory diseases has been advanced through studies of mice null for gelsolin. GSN−/− mice respond more slowly to inflammatory stimuli, such as intraperitoneal thioglycollate instillation; the blunted inflammatory response in these mice is restricted to the early acute phase and increased permeability of the pulmonary vasculature. Interpretation of these results is complicated by the fact that gelsolin null mice lack both cGSN and pGSN, but the findings are consistent with the notion that pGSN plays a protective role against inflammation.

Another scavenging role of pGSN involves binding and inactivation of LPS and LTA. These molecules are bacterial wall components of Gram-negative and Gram-positive bacteria and their binding to gelsolin is through the same site to which PPIs (e.g. PIP2) interact with gelsolin. This binding site may also be the locus where other bioactive inflammatory mediators, such as LPA and PAF, bind to gelsolin. In addition, pGSN can also bind to fibronectin and (2)glycoprotein 1.

A recent report showed that gelsolin affects neutrophil infiltration and epithelial apoptosis in bleomycin- or LPS-challenged model of lung inflammation. GSN−/− mice were protected from lung inflammation and fibrosis as well as in ventilator-induced lung injury. This protection may be attributed to attenuated neutrophil infiltration, reduced chemotaxis, and reduced epithelial apoptosis.

Gelsolin is likely to play a role in chronic inflammatory diseases. For example, gelsolin expression is not detectable in rheumatoid synovial fibroblasts, which leads to severe alterations in cytoskeletal organization and induction of rheumatoid arthritis in GSN−/− mice, thereby resulting in the exacerbation of signs of disease. The reduced pGSN in combination with the presence of actin and gelsolin–actin complexes in synovial fluids suggest a local consumption of this potentially anti-inflammatory protein in the inflamed joint. Recent data have documented reduced levels of pGSN in patients that have started hemodialysis and an inverse association of pGSN levels with 1-year mortalities. The prognostic value of low pGSN levels in hemodialysis patients suggests that circulating pGSN levels may reflect the extent of systemic inflammation and muscle wasting.

**D. Gelsolin and Cardiovascular Diseases**

The expression of gelsolin in human heart tissues and mouse models is increased after different types of cardiac injuries, including pressure overload, dilated and ischemic cardiomyopathy, MI, and end-stage heart failure. However, plasma gelsolin levels may be decreased temporarily in acute MI patients. The difference in the expression levels of cytoplasmic and plasma gelsolin suggests that these two different forms of gelsolin may play different roles after cardiac injury. Plasma gelsolin, as an important component of the EASS, may have evolved to rapidly clear actin filaments from the circulation that are released by injured or dead cells.

The gelsolin in cardiac cells can bind tightly to myofibrils and physiologically modulates the organization, assembly, and turnover of thin filaments within myocytes. The rearrangement of actin filaments mediated by gelsolin may contribute to calcium channel inactivation that has been observed in neonatal mouse cardiac myocytes. The increased expression of gelsolin in failing hearts could contribute to perturbation of thin filament organization and attenuation of Ca2+-induced Ca2+ release in failing heart muscle. Moreover, HIF-1α, an important mediator of the hypoxic response, can regulate gelsolin transcription in fibroblasts under hypoxic conditions. In this context, we found that increased gelsolin levels after experimental MI lead to detrimental cardiac remodeling because of caspase-cleaved-N-gelsolin fragments and gelsolin-HIF-1α-DNase I-mediated apoptosis. Gelsolin may also be involved in the downregulation of survival factors in myocytic apoptosis after MI as a result of caspase cleavage of poly(ADP)-ribose polymerase (PARP).
Gelsolin deficiency is not always associated with either reduced apoptosis or with amelioration of injury in other disease models. Compared to wild-type mice, gelsolin-deficient mice exhibit increased pulmonary vascular permeability that is worsened after ischemia. The defects in cytoskeletal remodeling in GSN\(^{-/-}\) mice were attributed to this increased permeability of the pulmonary endothelium.\(^{146}\) Worsening of ischemic brain injury occurred also in GSN\(^{-/-}\) mice.\(^{58}\) Fas-mediated liver fibrosis is exacerbated in GSN\(^{-/-}\) mice in association with selective apoptosis of sinusoidal endothelial cells.\(^{158}\)

Gelsolin is an important component of the focal adhesion kinase-dependent, force-induced upregulation of \(\alpha\)-smooth muscle actin,\(^{159}\) a marker for myofibroblast differentiation. This pathway is of crucial importance in the stiffening of the myocardium caused by the synthesis and secretion of fibrillar collagens in pressure or volume overload hypertrophic cardiomyopathy.\(^{160,161}\)

Assembly and turnover of actin filaments by gelsolin is detectable in atrial cardiac myocytes.\(^{155}\) Persistent atrial fibrillation is promoted by Ca\(^{2+}\) overload in isolated atrial cardiomyocytes.\(^{162}\) Gelsolin deficiency is associated with elevated activation of L-type Ca\(^{2+}\) channels\(^{157}\) and perpetuates atrial fibrillation in the mouse heart.\(^{163}\) The increase (albeit nonstatistically significant) of end-diastolic pressure in gelsolin null mice after experimental MI\(^{67}\) may be related to increased L-type calcium currents. Conceivably, the lack of gelsolin may increase left ventricular stiffness through the stabilization of actin filaments and enhanced L-type calcium currents.

\section*{E. Gelsolin and Alzheimer’s Disease (AD)}

AD is the most common form of dementia in the elderly. Fibrillar amyloid \(\beta\)-protein (A\(\beta\); 39–43 amino acids in size) is a major component of amyloid plaques in the brains of individuals with AD.\(^{164}\) Amyloid peptides are involved in many of the pathological and toxic alterations of AD, such as mitochondrial dysfunction, oxidative stress, caspase activation, and cell death.\(^{165–169}\) Gelsolin expression is reduced both in choroid plexus and in the cerebral spinal fluid from AD patients.\(^{170}\) In contrast to the mutated gelsolin that causes the Finnish type familial amyloidosis, both isoforms of gelsolin (cGSN and pGSN) can bind A\(\beta\) and may prevent or delay the progression of AD.\(^{106,170–172}\)

Plasma gelsolin can bind to A\(\beta\) within known physiological concentrations of gelsolin and the A\(\beta\)-gelsolin complex exists in the plasma and cytosol.\(^{106,173}\) Gelsolin perturbs the \(\beta\)-pleated sheet structure and fibril formation of A\(\beta\)\(^{173}\) and facilitates loss of preformed fibrils of A\(\beta\).\(^{174}\) In two different transgenic mouse models, peripheral administration of plasma gelsolin reduced A\(\beta\)1–40/A\(\beta\)1–42 and amyloid load. Peripheral transgene expression of plasma gelsolin also led to reduced amyloid load in brain.\(^{175,176}\) Furthermore, gelsolin can inhibit A\(\beta\)-induced neurotoxicity.\(^{64}\)

Oxidative stress plays an important role in neuronal degeneration in AD.\(^{177}\) It is produced by free radicals, including reactive oxygen species and reactive nitrogen species (nitric oxide).\(^{169,177}\) A\(\beta\), mitochondrial abnormalities, and aging are contributing factors which increase oxidative stress in AD. Under oxidative stress conditions in an in vitro model, cGSN was upregulated and this upregulation involved PKC.\(^{178}\) Cytoplasmic gelsolin can increase mitochondrial function by regulating the activity of mitochondrial respiratory chain complex IV, affecting the reduction of NO production and cell death, and reducing A\(\beta\) burden in a mouse model of AD.\(^{170}\)

\section*{10. GELSOlin AND AGING}

During aging, organisms lose the capacity to effectively handle the stresses and strains of life. For example, aging is associated with replicative senescence, loss of cell responsiveness to external signals, increased resistance to apoptosis, and reduced bone mass. Gelsolin is elevated in

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senescent cells, in contrast to other actin-related proteins, such as profilin and α-actinin.

Increased gelsolin levels are ubiquitous in many senescent tissues, including brain, heart, lung, liver, kidney, stomach, and spleen. These increased expression levels of gelsolin may play a role in resistance to apoptosis, particularly in view of the function of gelsolin in modulating cytochrome c release through mitochondrial porins, and the increased susceptibility to cancer in senescence.

Gelsolin may play a role in the aging process involving the skeleton, particularly in the osteoporosis that is seen most in postmenopausal females, and which is also becoming an increasingly important public health problem in elderly males. One of the primary mechanisms driving age-related bone loss is expansion of the osteoclast precursor population. In osteoporosis, bone resorption by osteoclasts is relatively greater than the amount of new bone formed by osteoblasts. Gelsolin may be involved in these processes because it is expressed in osteoblasts and is functionally linked to the bone-related protein osteopontin. As a result of interactions between osteopontin and the αβ3 integrin, bone resorptive signals are enhanced by gelsolin in osteoclasts. In addition, gelsolin can modulate bone resorption by regulating podosome assembly in osteoclasts as a result of its effect on signaling through the osteopontin-mediated signaling pathway. Notably, osteoclasts of gelsolin null mice do not respond to exogenous osteopontin as assessed by motility and bone resorption assays.

11. POTENTIAL CLINICAL APPLICATIONS OF GELSOLIN

Gelsolin may function as an assayable peripheral blood biomarker for several disease conditions. But the information provided by gelsolin concentration is likely context sensitive. For example, in the setting of amyloidosis, the direct sequencing of gelsolin can confirm the diagnosis. The levels of gelsolin in a cancer setting may give an index of metastatic potential, given its role in actin remodeling. Gelsolin may also be important in cardiovascular disease, with its abundance an indicator of chronic heart failure. In contrast, reduction in gelsolin during an acute myocardial infarction may be an index of the severity of the acute disease.

In reference to the functional role of gelsolin in disease, inhibition of gelsolin may be considered in the setting of chronic cancer treatment to inhibit metastasis, chronic cardiovascular disease to prevent remodeling, and chronic inflammatory disease to decrease immune cell activation. It may be worth infusing recombinant GSN or a small molecular mimetic in cases of acute neurodegeneration or excessive protein deposition, to take advantage of gelsolin's ability to cleave actin into fragments and/or bind to Aβ. Indeed, it was reported that appropriately dosed infusions of recombinant human gelsolin can diminish evolving injury or reduce mortality rates in animal models of hyperoxia, burns, and sepsis.

12. CONCLUSIONS AND FUTURE DIRECTIONS

Gelsolin evidently plays crucial roles in many different pathological processes. Earlier studies of gelsolin that focused on Ca2+-regulated remodeling of actin filaments are now being extended to include mechanisms by which gelsolin is involved in apoptosis, signal transduction, transcriptional regulation, and epigenetic processes. An improved understanding of the multiple effects of gelsolin in cancer, infection and inflammation, cardiac injury, pulmonary diseases, AD, and aging may facilitate a deeper appreciation of gelsolin as an important biological regulator (Fig. 5). This also opens up the opportunities for its role as a biomarker and a potential therapeutic target. New insights into the epigenetic control of gelsolin could promote an improved understanding of how gelsolin expression and
function are regulated. Furthermore, gelsolin may be regulated by microRNAs. These posttranscriptional regulators bind to complementary sequences in the three prime untranslated regions (3' UTRs) of target messenger RNA transcripts (mRNAs) and usually result in gene silencing. In addition, a α-myosin promoter-driven conditional knockout model of gelsolin would be useful for defining specifically the effects of gelsolin in cardiac function. Moreover, the role of gelsolin in aging is poorly understood. Does the normal increase in gelsolin with aging influence the structure and function of heart, lung, bone, and other systems? This interesting and multifunctional protein evidently has much to teach us.

13. ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
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<tbody>
<tr>
<td>ABPs</td>
<td>Actin binding proteins</td>
</tr>
<tr>
<td>AD</td>
<td>Alzheimer’s disease</td>
</tr>
<tr>
<td>AP-1</td>
<td>activator protein 1 (a transcription factor)</td>
</tr>
<tr>
<td>Asn</td>
<td>asparagine</td>
</tr>
<tr>
<td>Asp</td>
<td>aspartic acid</td>
</tr>
<tr>
<td>Bcl-2 family</td>
<td>proteins governing mitochondrial outer membrane permeabilization and can be either pro-apoptotic (Bax, BAD, Bak, and Bok, among others) or anti-apoptotic (including Bcl-2 proper, Bcl-xL, and Bcl-w, among an assortment of others).</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>calcium ion</td>
</tr>
<tr>
<td>CGSN</td>
<td>cytoplasmic gelsolin</td>
</tr>
<tr>
<td>CNS</td>
<td>central nervous system</td>
</tr>
<tr>
<td>COS-1 cells</td>
<td>kidney cell line that can produce large T antigen but has a defect in genomic replication</td>
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<tr>
<td>c-Src</td>
<td>cellular sarcoma</td>
</tr>
<tr>
<td>Cys</td>
<td>cysteine</td>
</tr>
<tr>
<td>DNase I</td>
<td>deoxyribonuclease I</td>
</tr>
<tr>
<td>erbB-2</td>
<td>human epidermal growth factor receptor 2</td>
</tr>
<tr>
<td>EGFR</td>
<td>epidermal growth factor receptor (ErbB-1)</td>
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