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REVIEW

Cofilin Acts as a Booster for Progression of Malignant Tumors Represented by Glioma

Shihong Lv[1,*], Zhiye Chen[2,3,*], Hailong Mi[3], Xingjiang Yu[3]

[1]Department of Gastroenterology, The Second Affiliated Hospital of Mudanjiang Medical College, Mudanjiang Medical College, Mudanjiang, 157011, People's Republic of China; [2]Department of Neurosurgery, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, 430030, People's Republic of China; [3]Department of Histology and Embryology, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, 430030, People's Republic of China

*These authors contributed equally to this work

Correspondence: Xingjiang Yu, Department of Histology and Embryology, College of Basic Medicine, Tongji Medical College, Huazhong University of Science and Technology, #13 Hangkong Road, Baofeng Street, Qiaokou District, Wuhan, Hubei Province, 430000, People's Republic of China, Tel/Fax +86-13146677984, Email yuxingjiang@hust.edu.cn

Abstract: Cofilin, as a depolymerization factor of actin filaments, has been widely studied. Evidences show that cofilin has a role in actin structural reorganization and dynamic regulation. In recent years, several studies have demonstrated a regulatory role for cofilin in the migration and invasion mediated by cell dynamics and epithelial to mesenchymal transition (EMT)/EMT-like process, apoptosis, radiotherapy resistance, immune escape, and transcriptional dysregulation of malignant tumor cells, particularly glioma cells. On this basis, it is practical to evaluate cofilin as a biomarker for predicting tumor metastasis and prognosis. Targeting cofilin regulating kinases, Lin11, Isl-1 and Mec-3 kinases (LIM kinases/LIMKs) and their major upstream molecules inhibits tumor cell migration and invasion and targeting cofilin-mediated mitochondrial pathway induces apoptosis of tumor cells represent effective options for the development of novel anti-malignant tumor drug, especially anti-glioma drugs. This review explores the structure, general biological function, and regulation of cofilin, with an emphasis on the critical functions and prospects for clinical therapeutic applications of cofilin in malignant tumors represented by glioma.

Keywords: cofilin, glioma, malignant tumor, migration, invasion, apoptosis

Introduction

Cofilin belongs to the actin-depolymerizing factor family, attached to the actin-binding protein in eukaryotic cells.[1] Researcher first isolated cofilin from the chicken embryo brain.[2] Emerging reports show that, except for red blood cells and sperm cells, cofilin is expressed in nearly all eukaryotic cells.[3–5] Cofilin is preferentially distributed in cell regions characterized by a high turnover rate of actin filaments, including the edge of dividing cells and the front part of migrating cells.[6,7]

Compelling pieces of evidence have demonstrated an indispensable role for cofilin in regulating the actin-cytoskeleton dynamics. Cofilin mainly promotes depolymerization and polymerization of actin filaments by cleaving actin filaments, accelerating the depolymerization of actin monomers from the pointed end of actin filaments and stimulating actin nucleation.[8–10] Evidence indicates that the above process is contingent on the pH of the microenvironment and the concentration ratio of cofilin relative to actin and other actin-binding proteins.[11] The phosphorylation/dephosphorylation of the N-terminal Serine3 (Ser3) site of cofilin functions as a switch in the assembly and disassembly of actin. Cofilin can be inactivated through phosphorylation driven by LIMKs and testicular protein kinases (TESKs). However, slingshot (SSH) and chronophin (CIN) can dephosphorylate cofilin to restore its activity.[12–14]

For decades, since the discovery of cofilin, there is increasing interest in finding the potential application of cofilin-driven regulation of cell movement to malignant tumor cells. Recent reports have demonstrated high cofilin expression in various malignant tumor cells, including glioma.[15–17]

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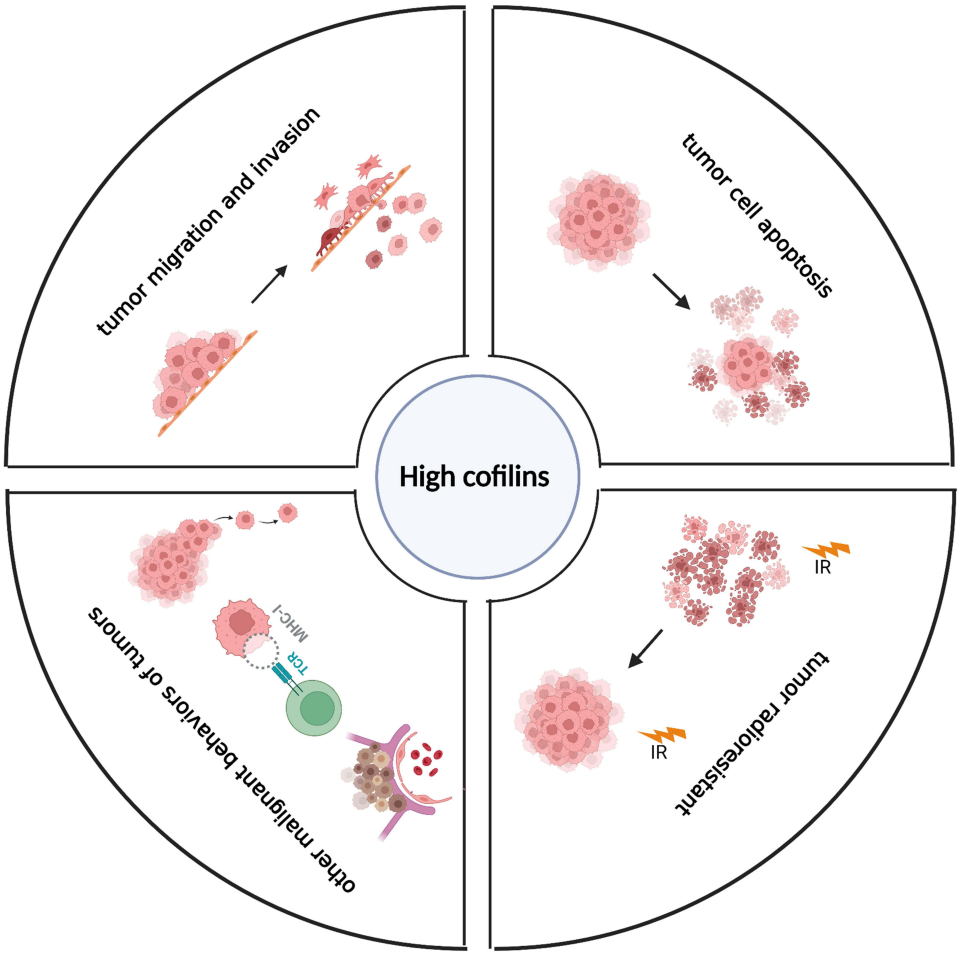
Graphical Abstract



Existing studies provide concrete evidence that cofilin is associated with regulating the migration, invasion, EMT/EMT-like, and apoptosis of malignant tumor cells and other important physiological functions both in vivo and in vitro. As the "driver" of tumor cell migration and invasion, the formation and maintenance of cell pseudopodia (including filopodia, lamellipodia, and invadopodia) are contingent on actin polymerization and depolymerization. Based on the precise adjustment of actin homeostasis, cofilin potentially regulates the direction of tumor cell migration by inducing the formation of lamellar pseudopods.[18] Additionally, studies indicate that the special spatial structure of the pseudopodia and enzymes secreted by pseudopodia which degrade the extracellular matrix can effectively promote the invasion and metastasis of tumor cells.[19–21]All in all, existing researches suggest activated cofilin has four different functions in gliomas: (I) promote tumor migration and invasion by regulating cell dynamics and EMT/EMT-like process; (II) promote tumor cell apoptosis via the mitochondrial pathway; (III) enhance tumor radioresistance through activating Ras-related C3 botulinum toxin substrate 1 (Rac1)/Wiskott-Aldrich syndrome protein (WASP)-family verprolin-homologous protein2 (WAVE2)/Actin-related protein2/3 (Arp2/3) signaling pathway. (IV) is Involved in chemoresistance, immune escape and transcriptional dysregulation. Based on existing research results and reasonable inductive reasoning, it is practical to evaluate cofilin as a biomarker for predicting tumor metastasis and prognosis. Targeting cofilin regulating kinases, LIMKs and their major upstream molecules inhibits tumor cell migration and invasion and targeting cofilin-mediated mitochondrial pathway induces apoptosis of tumor cells represent effective options for the development of novel anti-tumor drugs.

Here, to explore the precise functions of cofilin in malignant tumors, including glioma, this review clarifies the structure, general biological function, and regulation of cofilin, with an emphasis on the critical functions and prospects for clinical therapeutic applications of cofilin in malignant tumors represented by glioma.

Methods

Relevant articles from PubMed/Medline and Embase (1980–2022) were searched and collected using the phrases

Cofilin, Glioma, Malignant tumor, Migration, Invasion, EMT/epithelial to mesenchymal transformation, Apoptosis, Radiotherapy resistance, Immune escape, Transcriptional dysregulation, Prognosis biomarker.

The Gene Characteristics and Protein Structure of Cofilin

The exon-intron junction sequence of the cofilin gene is quite conservative among different species. Most mammals, including human, are characterized by two cofilin variants, non-muscular type (cofilin-1) and muscle type (cofilin-2). At the genetic level, cofilin-1 gene and cofilin-2 gene are located on chromosome 11q13 and chromosome 14, respectively.[22,23] Although cofilin-2 can transcribe two different messenger RNAs (mRNAs), the same polypeptides are produced via selective splicing. It is speculated that the location and/or stability are different. Moreover, the introns may confer a change in the tissue specificity of cofilin gene expression, thereby increasing its expression level.[24]

The structure of cofilin protein is highly conserved in both non-functional and functional region, with a common fold comprising five internal β-chains surrounded by four α-helices. It is a sequence in which cofilin binds to actin, promoting the depolymerization of filamentous actin, referred to as actin depolymerization factor homology (ADF-H) domain.[25,26] Studies have demonstrated the existence of this homologous domain in other actin-binding protein families, including Abp1p, drebrins,[27] twinfilin,[28] and coactosin,[29] with a size range between 113 amino acid (AA) and 168AA.[27] The differences in chemical properties and small structural changes of the surface exposure residues are ascribed to the diversity of ADF-H domains of different actin-binding proteins in binding to globular actin (G-actin) and filamentous actin (F-actin).[28] Different from actin depolymerization factor (ADF), two types of cofilin have a short β chain which binds to the β_3/β_4 chains at the C-terminal, changes the environment adjacent to Lysine96 (Lys96) and exerts a direct impact on the binding of cofilin to F-actin. Also, the presence of a short sequence of Tryptophan100 (Trp100) ~ Methionine115 in cofilin protein, which binds to phosphatidylinositol 4-diphosphate, contributes to the interaction of cofilin with actin. In vivo and in vitro experiments have shown that human cofilin is a pH-sensitive actin-depolymerizing protein. Structural analysis reveals that cofilin may be related to a unique salt bridge comprising Aspartic acid98 and Histidine133 at positions 98 and 133, respectively.[30,31]

Basic Functions of Cofilin

The most critical role of cofilin is to mediate the structural recombination and enhance the dynamic balance of actin. Actin filaments maintain a certain length in a steady-state through "Treadmilling", an adenosine triphosphate (ATP)-dependent process[32,33](Figure 1).

In previous research, the microfilaments were artificially divided into two ports (barbed end and pointed end) according to the arrow-shaped decorative morphology of myosin combined with F-actin. The assembly speed of the barbed end is faster than the pointed end, which is why it is called the plus end, whereas the pointed end is called the minus end.[34] Under physiological conditions, the polymerization of actin monomers, including G-actin occurs via four processes: actin activation, nucleation, elongation, and steady-state.[35] The nucleation period is notably a key step in actin polymerization.[36] Actin can be polymerized into dimer, trimer, or tetramer. However, actin dimer is extremely unstable. An actin dimer can form an actin polymer core, and continue to polymerize which is mediated specifically by some nucleating proteins such as ARP2/3 complex, formins, spirel, etc.[37,38] Actin monomers binding to ATP exhibit a high affinity to the plus end of F-actin. In this view, actin monomers consecutively bind to the plus end to allow the continued growth of F-actin.[39] As the F-actin growing, the bound ATP is potentially hydrolyzed into adenosine diphosphate (ADP) and phosphoric acid. In consequence, the affinity decreases between ADP-actin and the minus end of F-actin. Driven by energy from hydrolysis of ATP, ADP-actin can readily fall off from the minus end.[40] Subsequently, the dissociated ADP-

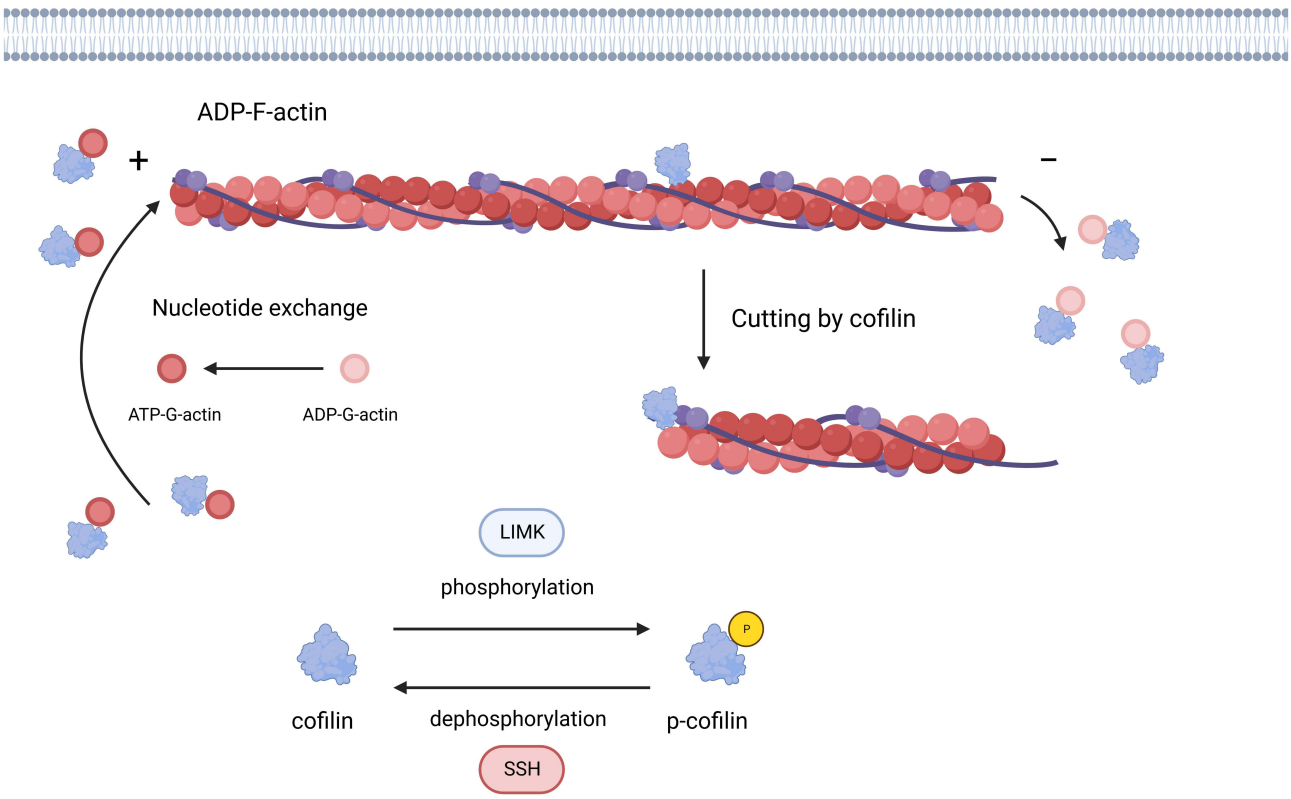


Figure 1 Cofilin participates in the "treadmilling" model of actin filaments. The combination of the abundant G-actin monomers provided by the G-actin pool with ATP can continuously bind to the barbed end (plus end) of F-actin to maintain the continuous growth of actin filaments. On the one hand, activated cofilin binds to ADP-actin on F-actin, cleaving and disaggregating actin filaments. Then, the dissociated ADP-G-actin quickly exchanges ATP and ADP to supplement G-actin monomer pool. On the other hand, cofilin can also bind to the dissociated ADP-G-actin, thereby preventing the exchange of nucleotides in the ADP-G-actin complex and inhibiting the polymerization of actin. LIMK1 can inhibit this process by phosphorylating cofilin. Conversely, SSH1 can dephosphorylate cofilin and activate it. Created with BioRender.com.

G-actin rapidly substitutes ADP with ATP in which profilin promotes the exchange of nucleotides.[41] In a steady-state, the growth rate of the plus end of the actin filament is equal to the dissociation rate of the minus end. As a result, the polymerization and depolymerization of actin attain a balanced state, and the actin filament maintains a relatively constant length.[42,43] Cofilin and capping protein independently act on actin to realize the precise regulation of "Treadmilling" of actin filaments.[44]

The kinetic model of Roland et al demonstrates that in order to exert the depolymerization activity, cofilin cleaves actin filaments and increases the rate of that actin monomers leave the ends of actin filaments.[45] Cofilin binds to ADP-actin on F-actin in a 1:1 molar ratio, destroys and cuts actin filaments by rotating each actin subunit to approximately 4–5° and twisting the actin filaments to reduce the crossover length. The cleaving of actin filaments results in several short actin fragments, which avails more ADP-actin to bind to cofilin, and accelerate actin depolymerization under sufficient cofilin level.[34,46] Cofilin also effectively increases the depolymerization rate of actin monomers from the end of actin filaments. Fluorescence-labeled ADP-binding actin subunits have been utilized in vitro experiments. Reports indicate that, according to fluorescence intensity changes before and after cofilin incubation, the nucleotide exchange rate increases in a concentration-dependent manner after cofilin incubation. This indirectly effectuates an increase in the actin monomer depolymerization rate.[8,47] On the other hand, the binding of cofilin to dissociated ADP-G-actin is a potential hindrance to the exchange of nucleotides in the ADP-G-actin complex, delaying the transition to ATP-G-actin. Consequently, this blocks the reassembly of actin monomers into F-actin and indirectly contributes to depolymerization.[48]

The cofilin with actin cleaving activity can promote the polymerization of actin monomers. Cofilin depolymerization generates more free actin monomers which can be used for polymerization, supplement the monomer pool and increase the effective concentration of actin monomers. The resultant ATP-binding actin monomers can be added to the plus end

of F-actin when needed, thereby maintaining the high elongation of actin filaments and enhancing the dynamics of the actin cytoskeleton. Cofilin also drives actin polymerization directly or indirectly by producing several free plus ends.[35,49]

In addition to the above, cofilin also can stimulate nucleation by stabilizing the "long-distance" actin dimer (the first intermediate in spontaneous assembly).[50] Previous studies have demonstrated two mutants of cofilin that affect cofilin binding to F-actin: one involves two basic amino acid residues at the beginning of the β4 chains, whereas the other involves three amino acid residues (two basic and one acidic) in the C-terminal helix.[51] Pope et al constructed two mutants K96Q and S3D via site-directed mutagenesis and confirmed the existence of two different actin-binding sites on cofilin. In the state of actin-binding, the interaction of the two sites with different subdomains of the two subunits in the double initiation helix promotes dimerization of actin monomers, consequently inducing their nucleation and assembly.[52] In the co-cultured experiment of cofilin and actin, the nucleation activity of actin decreased at the cofilin to actin ratio exceeding 2:1. This observation could be ascribed to the excessive cofilin binding to two cofilin-binding sites on the actin monomer, which blocked the dimerization of the double-bound actin monomer. The experiment further revealed that appropriate cofilin concentration could promote the dimerization and polymerization of actin monomers, and accelerate nucleation.[53]

Collectively, in order to promote the dynamics of the actin cytoskeleton, cofilin induces the depolymerization and cleaving of actin filaments as well as the polymerization and nucleation of actin monomers.

Regulation of Cofilin

The activity of cofilin protein is primarily tuned by phosphorylation and dephosphorylation. In particularly, the phosphorylation/dephosphorylation of the most conserved N-terminal Ser3 is the key regulatory factor of the assembly and disassembly of cofilin-actin.[3,54] Phosphorylation of cofilin on the Ser3 site inhibits the activity of cleaving F-actin to produce actin monomer and decreases the intracellular concentration of G-actin. This consequently increases the number and length of F-actin and slightly improves the F-actin stability. Contrarily, dephosphorylation of the Ser3 site activates F-actin depolymerization.[48,55,56]

There are two main types of phosphorylases involved in cofilin Ser 3 phosphorylation, include LIMKs and TESKs.[57–59] LIMKs, including LIMK1 and LIMK2, are serine/threonine kinases in eukaryotes. LIMK1 is mainly expressed in brain tissue, especially in the cerebral cortex, and moderately expressed in embryonic and mature tissues, heart, and skeletal muscle. LIMK2 is widely expressed in nearly all tissues.[60,61] Structural analysis revealed two N-terminal LIM domains of LIM kinase, including a C-terminal kinase domain and a PDZ (Postsynaptic density 95, PSD-85; Discs large, Dlg; Zonula occludens-1, ZO-1) domain. The LIM domain comprises a pair of zinc finger domains containing abundant cysteine/histidine sequences. The LIM domain allows for LIMK interaction with many macromolecules, therefore contributes largely in regulating kinase activity.[62]

Various proteins and miRNA (microRNA)s are involved in regulating the activity of LIMK. LIMKs could be activated by effector kinases of Ras homology (Rho),[63] Rac,[57,58] and cell division control protein 42 (CDC42) proteins,[64] including Rho-associated kinase1/2 (ROCK1/2),[65,66] p21-activated protein kinase1/2/4 (PAK1/2/4),[67] and myotonic dystrophin-related CDC42-binding kinase-α.[68] To achieve this, these effector kinases phosphorylate the conserved threonine residues in the LIMK domain (Threonine508(Thr508) in LIMK1 or Thr505 in LIMK2), which are the primary factors tuning the activity of LIMKs.[66,69] Bone morphogenetic protein (BMP) inhibits both the activity of LIMK1 and its response to ROCK driven by the interaction of type II BMP receptor (BMPR) tail region with LIMK1.[70] However, this effect seizes with the formation of BMP-BMPRII-BMPRIA complex and LIMK detachment from the tail of BMPRII.[70] Vascular endothelial growth factor (VEGF) induces the activation of mitochondrial activated protein kinase (MAPK)-activated protein kinase-2 (MAPKAPK-2/MK2) by activating MAPK.[71] On the other hand, MK2 mediates LIMK1 phosphorylation at Ser323 site directly, and regulates cell migration via the LIMK1/cofilin signal pathway.[71] Moreover, MK2 activation induces the phosphorylation of heat shock protein27 (Hsp27). Studies show that phosphorylated Hsp27 potentially promotes actin polymerization by relieving the capping effect on actin filaments, and this drives the formation of pseudopodia and enhances cell migration ability.[71,72] Elsewhere, Pandey et al found that defatted invertase lysophosphatidic acid could regulate platelet aggregation and secretion by inducing phosphorylation/dephosphorylation of cofilin in platelets enriched with fibrinogen.[73] Particularly, the regulatory mechanism occurs in

two steps: Ca^{2+}-mediated rapid dephosphorylation of cofilin and ROCK/LIMK1-dependent cofilin re-phosphorylation. MiRNA-134 is suggested to bind to the mRNA of LIMK, blocking LIMK transcription. Related studies have revealed an excellent clinical application prospect of miRNA-134 in epileptic seizure inhibition and antidepressant.[74,75] However, TESK1 is regulated via integrin-mediated signaling pathways, which is independent of PAK1/ROCK activation. This mechanism significantly promotes integrin-mediated cofilin phosphorylation and actin recombination.[59,76]

Current research demonstrates that dephosphorylation of cofilin on Ser 3 site is potentially mediated by two specific protein phosphatases, SSH and CIN.[48,77,78] A previous exploration revealed changes in the morphology of Drosophila epithelial cells lacking the SSH gene, suggesting that SSH may influence cell extension by regulating the polymerization and depolymerization of actin filaments.[78] Through in vitro experiments, researchers have shown that SSH and its two homologs (hSSH-1/2L) promote the dissociation of F-actin via dephosphorylation and activation of cofilin. SSH also performs upstanding dephosphorylation activity of LIMK. The direct binding of the N-terminal 1–533 of SSH1 to the LIMK1 kinase domain 310–633 has an effect on the Thr508 site of LIMK1, thereby blocking the activity of LIMK1.[79,80] PAK4 and protein kinase D1 phosphorylated hSSH have been demonstrated to inhibit the phosphatase activity of SSH on LIMK1 and cofilin. Existing reports indicate that SSH can enhance phosphatase activity by binding to F-actin via multiple F-actin binding sites. In vitro co-immunoprecipitation assay demonstrates that at least three sites or domains in SSH1 are involved in binding F-actin, including Trp458 site, N-terminal leucin-His-Lys motif, and C-terminal Lys-ketoglutarate reductase motif.[81–83] Simultaneously, the N-terminal or C-terminal domain of SSH can stabilize F-actin assembly, independent of the phosphatase activity of SSH.[84]

Mounting evidence shows that cofilin-mediated depolymerization of actin filaments and the polymerization of actin monomers are contingent on the concentration of active cofilin, the relative concentration of actin monomers, and some actin-binding proteins.[10,39,85,86] Among these factors, the local concentration of active cofilin in cells may be a direct determinant of its action mechanism on actin filaments. At low cofilin concentration, some cofilin binds to actin filaments, and the number of torsional strain interfaces between twisted regions (long-range effects of cofilin binding) and non-twisted regions of actin filaments is the largest. At this time, the cleaving activity of cofilin to F-actin filament is at the maximum.[87] Within a certain concentration threshold, an increase of cofilin concentration promotes the cooperative binding of cofilin to F-actin. As a result, cofilin cuts F-actin briefly and stabilize it in a twisted state.[88] Conversely, in vitro experiments indicate that high cofilin concentrations promote actin nucleation and polymerization, with no cut-off. However, in cases where the intracellular cofilin is abnormally overexpressed or is subjected to oxidative pressure, the ratio of cofilin to actin exceeds a certain critical value. In consequence, cofilin-ADP-actin aggregates into bundles, forming cofilin–actin rods, and blocks cofilin-driven depolymerization and cleavage of F-actin.[89]

Cofilin in Malignant Tumors Represented by Glioma

Compared to normal tissues, cofilin expression is significantly increased in glioblastoma[90] and a variety of other tumors including hepatoblastoma,[91] non-small cell lung cancer,[92] prostate cancer,[93–95] breast cancer.[94,96] The analysis of clinical-pathological samples has shown the positive correlation of high expression of cofilin with the malignant degree of the tumor, metastasis risk, tumor cell dedifferentiation, and the short survival time of patients[94,95,97,98] (Table 1).

In vitro experimental evidence suggests that after cofilin knockout, the proliferation, migration, and invasion of tumor cells are inhibited in varying degrees. On the contrary, overexpression of cofilin enhanced these abilities, demonstrating a close correlation of cofilin with tumor proliferation, migration, and invasion. These facts suggest an indispensable role for cofilin in tumorigenesis and development.

Cofilin Regulates Tumor Migration and Invasion

The migration and invasion of tumor cells in the peripheral tissue promote the spread of tumor and are the main causes of death of patients with malignant tumors.

Table 1 Changes in Expression Levels of Cofilin in Tumor Cells and Their Correlated Phenotypes

Changes in Expression Levels Relative to Control Cells or Tissues	Tumor Cell Line or Tissue	Malignant Behavior of Tumor Cells or Tissues	Reference
Highly expressed	Radioresistant U251 human glioma cells	Radioresistant	[99]
Highly expressed	Glioblastoma patient tissue	Tumor proliferation and invasion	[100]
Highly expressed	Glioblastoma tissue	Tumor invasion and low survival of patients	[101]
Highly expressed	Human glioma tumor cell lines U251, U87, U373, D54, LN229, LN319, LN308, and SNB19	Tumor invasion and hypoxia tolerance	[102]
Highly expressed	Glioblastoma patient tissue	Tumor invasion	[90]
Highly expressed	Breast cancer patient tissue	Tumor malignancy or grades	[96]
Highly expressed	Human lung adenocarcinoma cells and tissues A549, and A549/DDP cells after irradiation	Multidrug resistance	[92]
Highly expressed	Typical astrocytoma patient tissue	Radioresistance	[103]
Highly expressed	Prostate cancer patient tissue	Tumor cell migration and invasion	
Highly expressed	Squamous cell, adenosquamous carcinomas and adenocarcinomas	Tumor progression, metastasis, and poor prognosis	[104]
Highly expressed	Human non-small cell lung adenocarcinoma H1299 cells	Tumor proliferation, migration and invasion	[105]
Highly expressed	Non-muscle-invasive bladder cancer, and muscle-invasive bladder cancer patient tissue	Tumor occurrence and invasiveness	[106]
Highly expressed	Lung cancer patient tissue	Tumor grades and poor prognosis	[107]
Highly expressed	Cisplatin-resistant human lung adenocarcinoma cell line, ICR-A549 cells	Chemotherapeutic	[108]
Down-regulated	Hepatocellular carcinoma cell strains with high and low metastatic potentials, MHCC97-H and MHCC97-L	Tumor metastasis	[109]

Cofilin Regulates Tumor Cell Pseudopodia Formation by Affecting the Recombination of the Actin Cytoskeleton and Regulating Cell Dynamics

Many inducers of the invasion and metastasis of tumor cells, such as WASP family proteins, Arp2/3 complexes, LIMKs, and cofilin, bind to cell surface receptors to stimulate intracellular signal transduction pathways, regulate actin cytoskeleton recombination, and induce tumor cell invasion and metastasis.[110,111] Several pieces of proof have confirmed that enhancing or inhibiting cofilin expression is potentially associated with significant differences in cell dynamics of metastatic tumors, thereby influencing the metastasis and invasion of cancer. Through transient and stable overexpression of cofilin in human glioblastoma cell line U373 MG, Yap et al found that cofilin overexpression below the threshold concentration of cofilin could ameliorate the movement rate of tumor cells and enhance their invasion and migration ability in a concentration-dependent manner.[112] In particular, the migration rate of cells in the experimental group with overexpressed cofilin levels was 4.5 times higher than that in the untreated control group. On the other hand, inhibition of cofilin expression via short hairpin RNA-mediated silencing of cofilin could reduce cell movement.[112] A variety of glioblastoma patient-derived tumor cell lines, including U87, U251, LN229 and D54 demonstrated accelerated motility to invade the normal brain tissue under hypoxic conditions.[102] Inhibition of Src family kinases by Dasatinib eliminates this phenotype. The correlation analysis identified multiple molecules including cofilin and other three molecules associated with motility in U251 cells. The findings support the idea that cofilin plays a key role in hypoxia-induced accelerated glioma cell invasion.[102]

Cytoskeleton, especially the microfilament cytoskeleton composed of actin, has a crucial role in the movement of cell pseudopodia. Cell pseudopodia make an indispensable contribution to the migration and invasion of tumor cells, including filopodia, lamellipodia, invadopodia, podosomes, etc. Actin is the primary component of motor cell pseudopodia and the main player in the invasion and metastasis of tumor cells.[113,114] Related studies employing the immunofluorescence labeling method have demonstrated that actin microfilaments composed of actin are widely distributed in the pseudopodia of motor cells, whereas the cytoskeleton is composed of tubulin is mainly distributed in the interior of the cells. Studies using in vivo fluorescence labeling method of actin have confirmed that the microfilament cytoskeleton plays a vital role in the extension and morphological construction of the leading edge of metastatic tumor cells.[115] In this view, actin-binding proteins, including cofilin, play a critical role in maintaining the dynamic changes of actin, lamellipodia formation, and cell movement, and are indispensable regulators of cellular pseudopodia formation.[116] Ghosh et al using the cage mimic of cofilin performed experiments in tumor cells to avoid the trapped mimic being phosphorylated.[117] In the same experiment, caged cofilin increased the plus end of actin in the body and extended pseudopodia in the uncaged cofilin.[117] The findings demonstrate that dephosphorylated cofilin promotes the recombination of the actin cytoskeleton through depolymerization and re-polymerization, induces the formation and extension of pseudopodia on the leading edge of cells, and regulates the "Step forward" of cells, to ameliorate tumor metastasis. Simultaneously, the highly localized activity of cofilin is directed to produce cellular pseudopodia, which determines the direction of cell movement and is the "steering wheel" of cells.[118] Studies have also revealed that cofilin can competitively bind to actin with Arp2/3 complex.[119] Such binding greatly weakens the affinity between Arp2/3 complex and actin filament, accelerates the removal of actin filament branches, and regulates the movement of tumor cells.[119]

Previous outcomes indicate that through phosphorylation of multiple amino acid sites, the cofilin activity is regulated via the LIMK-Cofilin pathway. This consequently regulates cytoskeleton remodeling. Similarly, in tumor cells, through the Cofilin pathway, a series of biological behaviors such as invasion and migration of tumor cells can be tuned[120] (Figure 2).

Multiple studies have demonstrated high expression of LIMIK1 in malignant gliomas, prostate tumors, breast cancer, lung cancer, etc. In these cases, LIMK1-induced regulation of the balance between cofilin phosphorylation and dephosphorylation can promote the invasion and migration of tumor cells.[121–123] Cofilin is the only known enzyme substrate of LIMK1. LIMKs inactivate cofilin by phosphorylating Ser3 at the amino-terminal of cofilin, thus blocking cofilin-driven depolymerization of F-actin. These events consequently, accelerate F-actin polymerization to a lamellipodia structure, which changes the adhesion state of tumor cells to the extracellular matrix and promotes the movement and migration of tumor cells. Previous work by Guo et al indicated that in LN-229 cells, the down-regulation of protein kinase Czeta (PKCζ) expression resulted in dynamic remodeling faults of cytoskeleton in response to the epidermal growth factor (EGF) by dysregulation of LIMK/cofilin phosphorylation.[124] Similarly, down-regulation of Intersectin1-s (ITSN1-s) inhibited EGF-induced activation of PAK1/LIMK/cofilin, corresponding to their faults in G-actin polymerization.[125] LIMK is a major downstream mediator of PAK1. Active PAK1 phosphorylates LIMK by binding it at Thr508 sites in helix C and the activation loop, which results in an absolute increase in the activity of LIMK toward cofilin. Conversely, inhibiting the activity of PAK1 could suppress the LIMK-mediated F/G-actin structural remodeling.[64] The results verified that the down-regulation of ITSN1-s resulted in cytoskeleton dynamic remodeling faults in response to EGF by dysregulation of PAK1/LIMK/cofilin phosphorylation in LN229 cells.[125] The discovery by Zhu et al holds that abnormal TSG101 expression in human glioma patients potentially induces the activation of the member C of Rho (RhoC)/LIMK1/Cofilin pathway.[15] Rho GTPases activate ROCK, which in turn activates LIMK to mediate cofilin inactivation through phosphorylation of Ser3 sites. As a consequence, actin filament cleavage is inhibited, and this ultimately promotes the migration and invasion of human gliomas. Contrarily, the ability of tumor cell migration and invasion can be inhibited significantly by the interference of LIMIK1 with small hairpin RNA. In a 3D culture system, LIMK inhibition potentially reduces the level of cofilin phosphorylation, inhibits the extension of F-actin, damaging its stability. In addition, by blocking the activity of LIMK, the transcriptional activity of serum reactive factors and the contractility of collagen decreased significantly, consequently inhibiting both the degradation of the extracellular matrix and the formation of invasive pseudopodia.[126] Enzymes secreted by invasive pseudopodia can degrade and reconstruct the extracellular matrix. The action of these enzymes reduces the resistance of tumor cell migration and greatly assists tumor cells to

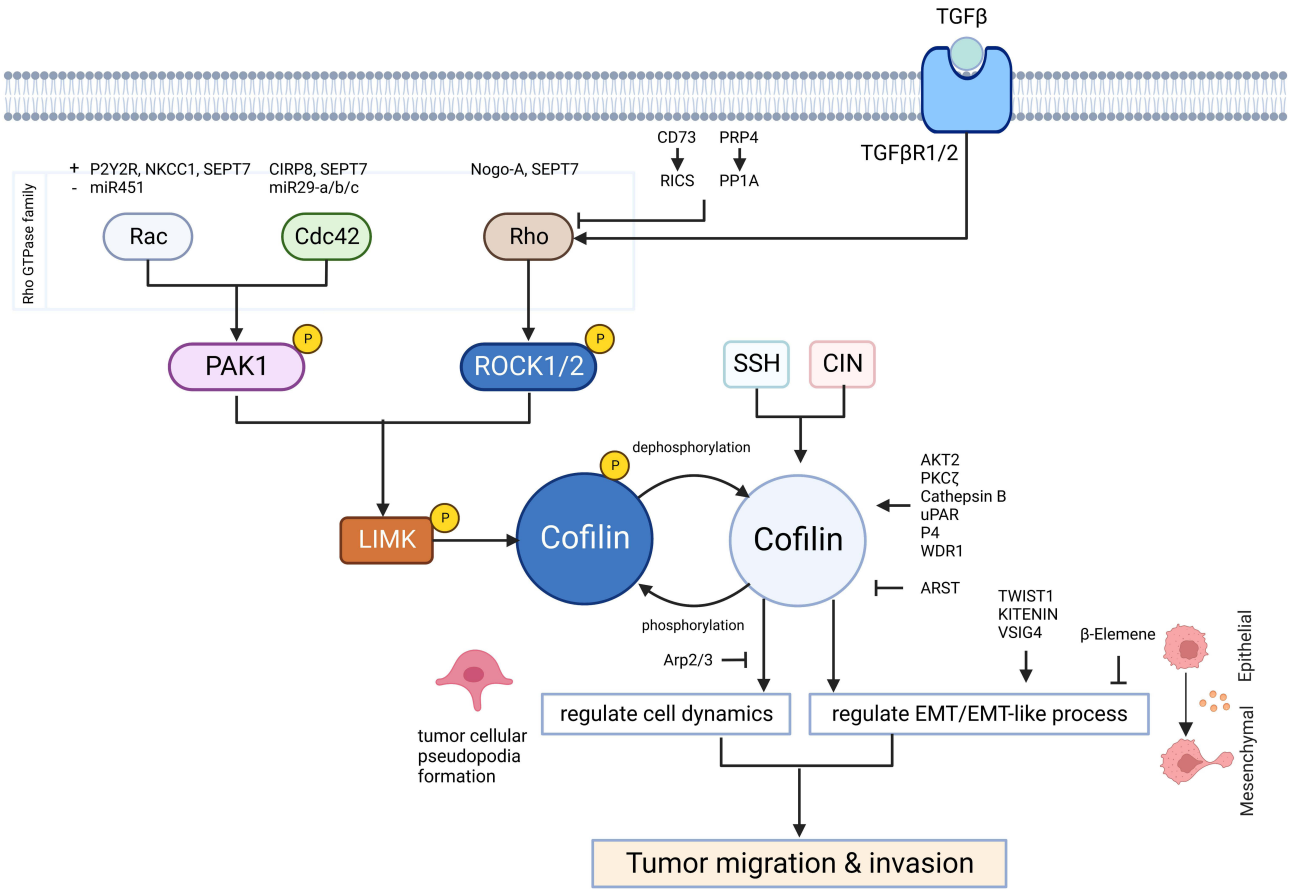


Figure 2 Cofilin regulates tumor migration and invasion represented by glioma. Dephosphorylating cofilin on Ser3 mediates its activation. Activated cofilin promotes tumor cell migration and invasion by regulating cell dynamics and EMT/EMT-like process. Cofilin can be inactivated through phosphorylation driven by LIMKs on Ser3 site, while SSH and CIN can dephosphorylate cofilin to restore its' activity. LIMK is activated by the Rho-GTPases including Rac, CDC42 and Rho through their effector kinases, PAK1 and ROCK1/2 mediating the phosphorylation of LIMK on Thr508 resulting in its activation. Multiply proteins known to regulate the activity of cofilin via Rho-GTPases /ROCK&PAK/LIMK/cofilin signaling pathway resulting in regulating the actin-cytoskeleton dynamics, are SEP17, P2Y2R, NKCC1, CIRP8, Nogo-A, CD73, PRP4, AKT2, PKCζ, Cathepsin B, uPAR, P4, WDR1, Arp2/3. Some non-coding RNAs including miR451, miR29-A/b/c and ARST are also considered to participate in the regulation of cofilin through similar process. Non-SMAD signaling of TGF-β can induce rapid activation of RhoA, followed by phosphorylation and activation of LIMK2/cofilin1 pathway, resulting in regulating EMT/EMT-like process. Furthermore, TWIST1, KITENIN, VSIG4 and β-elemene are considered to be involved in the direct regulation of EMT/EMT-like process. Created with BioRender.com.

invade through the basement membrane and blood vessel wall.[20,127,128] In addition, the Rho/ROCK signaling pathway involved in significantly greater contractile force generation required for the assembly of stress fibers and tail retraction during cell movement. Using specific Rho kinase inhibitors targeting this pathway reduces the level of phosphorylated myosin II and cofilin, subsequently prevents tumor cell migration by inhibiting the dynamic assembly of f-actin structures.[101] P2Y2 nucleotide receptor (P2Y2R),[129] reticulon-4 isoform A (Nogo-A),[130] electroneutral Na^+-K^+-$2Cl^-$-co-transporter1 (NKCC1),[131] miR-451,[132] C1q tumor necrosis factor-related peptide8 (CTRP8),[133] miR-29a/b/c[134] etc. are considered regulating phosphorylated LIMK1/2 and cofilin through targeting different members of Rho family of GTPases, including Rac1, the member A of Rho (RhoA) and CDC42, subsequently regulating glioma cell migration and invasion. Hou et al reported that Septin 7 (SEPT7) is considered to be a cytoskeletal regulatory protein with GTPase activity, which is involved in the progression of glioma.[134] It was found that SEPT7 overexpression reduced the ratio of F-actin/G-actin and the level of total cofilin in glioma cells, while increased the level of p-cofilin, suggesting that SEPT7 inhibited the migration of glioma cells by promoting cofilin phosphorylation and accelerating F-actin depolymerization.[135] Numerous kinases have been identified as crucial participants in regulating the migration and invasion of tumor cells by directly interfering with and regulating the phosphorylation and dephosphorylation of cofilin in glioma. Zhang et al showed that Akt serine/threonine kinase 2 (Akt2) mediated the EGF-induced activation of cofilin, suggesting that Akt2 directly involved in cytoskeleton dynamic remodeling of glioma cells by regulating actin

polymerization.[136] Schulze et al observed a negative correlation between CIN expression and level of cofilin phosphorylation in glioma tissue samples.[137] In vitro verification of patient-derived glioblastoma cell lines indicated that chronophin-depleted cells showed increased phosphorylation of cofilin and polymerized actin, consistent with the cell phenotypes of enhanced cell migration orientation, invasiveness in vitro.[137] In vitro experiments further showed CIN knockdown increased the relative expression level of p-cofilin, which was consistent with decreased efficiency of colony formation in glioblastoma cell lines. Furthermore, treatment with Y-27632 (ROCK-inhibitor) resulted in the opposite phenotype compared to colony formation, supporting the hypothesis that phosphorylation regulation of cofilin leads to this phenotype.[138] These findings proved that cofilin is one of the substrates of CIN, which has been seen as a major regulator of cell migration and invasion in glioma. Cathepsin B,[139] urokinase-type plasminogen activator receptor (uPAR),[139] Progesterone (P4),[140] WD-repeat containing protein1 (WDR1)[100] have been also thought to be important activators of cofilin dephosphorylation, play key roles in glioma cell migration and invasion. In contrast, ARST (aldolase A/ALDOA-related specific transcript, a novel long non-coding RNA) can directly bind to the glycolytic enzyme ALDOA, which binds to cofilin to maintain an orderly dynamic balance of the polymerization and depolymerization of actin filaments, thereby exerting a tumor-suppressing effect[141] (Table 2).

As one of the key regulators of cofilin activity, activated SSH phosphatase in tumor cells activates cofilin. To achieve this, activated SSH phosphatase induces cofilin dephosphorylation, increases the concentration and stability of F-actin, accelerates F-actin polymerization and nucleation, induces lamellipodia formation, and promotes tumor cell metastasis and invasion. On the contrary, SSH inactivation blocks the above process[83,142,143] Furthermore, the C-terminal Trp458 site and N-terminal pleckstrin homologous domain of SSH phosphatase domain has been demonstrated to regulate cofilin phosphatase activity by binding F-actin.[84,144]

Table 2 Targets of Tumor Migration and Invasion Mediated by Cofilin

Targets	Mechanism	Malignant Behavior of Glioma	Inhibitors
PKCζ[124]	LIMK/cofilin phosphorylation	Glioma cell migration and invasion	Myristoylated pseudosubstrate
ITSN1-s(Intersectin1)[125]	EGF-induced phosphorylation of PAK1/LIMK/cofilin	Glioma cell migration and invasion	/
TSG101[15]	RhoC/LIMK1/Cofilin pathway	Glioma cell proliferation, migration, and invasion	/
P2Y2R[129]	Rac1/PAK/LIMK/ Cofilin pathway	Glioma cell migration	/
Nogo-A[130]	RhoA-cofilin pathway	Glioma cell migration and invasion	/
NKCC1[131]	Rac1/RhoA-cofilin pathway	Glioma cell dispersal and migration	/
miR-451[132]	Rac1/cofilin pathway	Inhibit glioma cell proliferation and migration	/
CTRP8[133]	CTRP8-RXFP1-JAK3-STAT3-CDC42/LIMK/Cofilin pathway	Glioma cell filopodia formation and motility, temozolomide resistance	/
miR-29a/b/c[134]	CDC42-PAK1/2/3-LIMK1/2-Cofilin pathway	Improve prognosis of patient and inhibit glioma cell invasion	/
SEPT7[135]	GTPase/Cofilin	Glioma cell migration	/
Akt2[136]	EGF-induced cofilin recycling	Glioma cell migration and invasion	/
CIN[137,138]	Cofilin phosphorylation	Glioma cell migration and invasion	/
Cathepsin B[139]	Dephosphorylation of cofilin	Glioma cell migration and invasion	/
uPAR[139]	Dephosphorylation of cofilin	Glioma cell migration and invasion	/
P4[140]	Dephosphorylation of cofilin	Astrocytoma cell migration and invasion	/
WDR1[100]	WDR1-Cofilin pathway	Poor progression-free survival and overall survival of patients	/
ARST[141]	ALDOA-Cofilin pathway	Inhibit glioma cell growth, proliferation, migration and invasion	/

It is noteworthy that tension of actin filaments might affect the activity of cofilin. Mounting evidences explore that actin filaments can directly sense the change of tension by regulating their sensitivity to cofilin-mediated cleaving. Briefly, the tension in actin filaments reduces cofilin binding, which effectuates a decrease in cleavage activity. Cofilin preferentially binds to soft and twisted F-actin. As stretching increasing the tension in the filament, the twisted rotation of the filament decreases accordingly, consequently inhibiting the interaction of cofilin with F-actin, and promoting the movement and migration of tumor cells by enhancing F-actin stability and promoting lamellipodia formation.[145,146]

Cofilin Regulates Tumor EMT/EMT-Like Process

EMT is the process by which epithelial cells acquire mesenchymal characteristics, and is also an important biological pathway via which various epithelial-derived malignant tumor cells acquire the capabilities of migration and invasion.[147] In the EMT process, the apex-basal polarity and the adhesion which directly affects the cytoskeleton and cell shape gradually disappears, leading to an increase in cell foot processes and motility, and enhanced capabilities of migration and invasion.[148] Cofilin 1 is a crucial regulator of tumor cell invasion, metastasis and EMT, which is highly correlated with tumor occurrence and development. Cofilin 1-mediated regulation of tumor cell EMT is mainly manifested in cytoskeleton changes and transformation into mesenchymal cells to reduce the dependence on the growth of external matrix adhesion.[17]

At the molecular level, activation of the EMT program is accompanied by changes in various epithelial and mesenchymal molecular markers. The expression of epithelial molecular markers E-cadherin is down-regulated, while the interstitial molecular markers N-cadherin, vimentin, fibronectin, and β-catenin are up-regulated. Among them, the E-to N-cadherin switch, also called the "cadherin switch" is considered as the most critical landmark event during EMT.[149–151]

Several studies have revealed EMT or EMT-like changes in malignant glioma cells.[152] For example, Tso[152] et al reported that a series of gene overexpression related to mature mesenchymal tissue in primary glioblastoma biopsy specimens and cell lines, including cartilage/ligament/tendon (Chitinase-3-like protein 1, collagen type IV and VI, and lysyl oxidase), endothelial/epithelial cells (Interleukin-8, Matrix metallopeptidase 9, Epidermal growth factor receptor, and VEGF and myelo-supportive stromal tissue (Pre-B cell colony enhancing factor 1 and Tenascin-C), etc. These observations provide support that primary glioblastoma has a similar stromal cell phenotype and invasiveness.[153] Lser et al found that, different from epithelial tumors, including breast cancer, thyroid cancer, and lung cancer, E-cadherin and N-cadherin are not expressed or are expressed in extremely low levels in most gliomas by analyzing the transcriptome data from the The Cancer Genome Atlas (TCGA) database.[154] Glioblastoma multiforme (GBM) might be in an intermediate state between epithelial and mesenchymal phenotypes (tend to mesenchymal state). As such, the "cadherin switch" in EMT of glioma cells may not be essential, though other pathways that mediate tumor invasion to antagonize this effect potentially exist. In this view, the occurrence of glioma EMT cannot be judged only by "cadherin conversion".[154,155] Mikheeva also reported that GBM cells with overexpression of Twist1 which has been confirmed to inhibit E-cadherin and activate N-cadherin in previous research do not undergo the typical conversion of E-cadherin to N-cadherin.[156] These pieces of evidence demonstrate that cadherin conversion is not associated with the phenotype of GBM aggressive mesenchymal cells.

In addition to morphological changes, EMT is closely associated with stem cell-like characteristics of cancer cells. Studies exploring various human tumors have revealed that the activation of the EMT induces the initial state of the tumor, also called the cancer stem cells (CSCs) state. CSCs are a subgroup of cancer cells similar to normal stem cells, characterized by the ability to automatically regenerate, proliferate and differentiate into various cancer cell lineages through symmetric and asymmetric cell division. This consequently can induce tumor heterogeneity, drug resistance, and the emergence of specific surface markers.[157,158] A previous investigation by Mani[159] et al found that the induction of EMT in human breast epithelial cells (HMLEs) could induce the expression of mesenchymal characteristics and stem cell markers. Cells induced by Twists have been shown to exhibit an enhanced ability to form mammary glands. On the other hand, stem cells isolated from HMLEs cells also have the potential to express EMT markers.[159] Elsewhere, Gupta et al found that EMT program activation by blocking E-cadherin expression in HMLEs cells can increase the number of CSCs significantly.[160] These studies demonstrate a close interaction between EMT and CSCs.[159,160] Mounting pieces of

evidence have further confirmed the existence of similar effects in glioma cells. For instance, Lee et al investigated that the expression of the cancer-promoting factor, KAI1 C-terminal interacting tetraspanin (KITENIN), in GL261 cells significantly increased the mRNA and protein expression levels of various EMT markers (N-cadherin, Zinc finger E-box binding homeobox1/2 (ZEB1/2), Snail, and Slug), and promoted the expression of glioma stem markers (Aldehyde dehydrogenase 1, CD133, erythropoietin-producing hepatocellular receptor B1, and CD44).[161] Another investigation by Zhang et al revealed that overexpression of V-set and immunoglobulin domain-containing 4 (VSIG4) potentially induced EMT, significantly promoted the migration and invasion of glioblastoma U87MG cells, and supported the formation of glioma stem cell phenotypes in U87MG cells, simultaneously.[162] On the contrary, let-7g-5p can inhibit EMT by down-regulating VSIG4 expression in glioblastoma. This is matched with the reduced migration and invasion ability of glioma stem cell.[162] Zhu et al also demonstrated that the expression of mesenchymal markers, N-cadherin and β-catenin was down-regulated significantly after treatment with β-Elemene, a natural plant drug extracted from Curcuma wenyujin.[163] On the other hand, the expression of epithelial marker, E-calcium significantly increased in glioma U87 cells, while the expression of CD133, a stem marker of glioma, was significantly reduced. These data prove that there is a synergistic effect existing between the EMT activation and the increase of CSCs in gliomas, which jointly targets the migration and invasion of tumor cells[163] (Figure 2).

A variety of mechanisms have been revealed to play an indispensable role in EMT regulation. EMT-inducing transcription factors, including Snail, Slug, ZEB1/2, Twist1/2, Goosecoid, and Forkhead Box C, etc., can inhibit the expression of E-cadherin which plays an indispensable role in activating EMT.[164]

Evidence shows that the transforming growth factor-beta (TGF-β) is the most critical factor driving the EMT state of glioblastoma. TGF-β signaling can mediate EMT activation via the classic small mothers against decapentaplegic (SMAD) pathway or non-SMAD pathway. In the classic SMAD pathway, the TGF-β type II receptor is activated by binding to TGF-β. The activated RII recruits and binds to the TGF-β type I receptor, forming an RII-ligand-RI heterodimerization body. Subsequently, the trimer binds to intracellular SMAD family proteins and transfers to the nucleus in the form of a complex, which directly stimulates EMT-induced transcription factors. These events block the expression of epithelial cell marker proteins and promote the expression of a specific protein of mesenchymal cells, thereby promoting the activation of the EMT state. In contrast, in non-SMAD signaling pathways, TGF-β signals trigger the EMT process by activating phosphatidylinositol-3-kinase (PI3K)-AKT- mechanistic target of rapamycin or MAPK signals.[149,165] non-SMAD signaling of TGF-β induces rapid activation of RhoA, followed by phosphorylation and activation of LIMK/cofilin1 pathway.[17] Zhijun et al revealed that ecto-5′-nucleotidase/NT5E (CD73) can activate the RhoGAP involved in the β-catenin-N-cadherin and NMDA receptor signaling protein by transducing adenosinergic signals in the microenvironment, thereby inhibiting the phosphorylation of the RhoA-LIMK-cofilin pathway. They further showed that CD73 can bind to β-catenin directly and activate the EMT process.[166] Elsewhere, UlIslam et al found that pre-mRNA processing factor 4B (PRP4) overexpression potentially induce the expression of phosphoprotein phosphatase 1A, thereby blocking the Rho-ROCK-LIMK-cofilin pathway and inducing cofilin dephosphorylation. This results in actin cytoskeleton rearrangement, down-regulation of E-cadherin, and induction of EMT, which successively promote the progression of human colon cancer[167] (Figure 2).

Cofilin Regulates Tumor Cell Apoptosis

Cell death and renewal is an indispensable part of the whole life process of multicellular organisms capable of timely eliminating mechanical and damaged cells and critically contributed to the development of various tissues and organs and the immune system. Apoptosis is a crucial pathway of cell death in vivo. It is a programmed death process precisely regulated by a series of signal molecules, characterized by cell shrinkage, chromatin pyknosis, and apoptotic body shape, evident under light and electron microscope.[168] Apoptosis occurs in various stages of embryonic development, tissue remodeling, immune regulation, and tumor degeneration. Of note, unwanted, severely damaged and potentially dangerous cells can be eliminated via apoptosis.[169,170]

The molecular mechanism of apoptosis is very complex, involving multiple cellular signal pathways and a variety of cytokines. Current evidence shows that eukaryotic cells trigger apoptosis mainly via the mitochondrial pathway or internal pathway and death receptor-mediated pathway or external pathway.[171] Under normal circumstances, the

apoptosis process is strictly regulated by the body to ensure the stability of various tissues and organs in the whole life process. However, when apoptosis regulation is out of balance, excessive cell proliferation or excessive apoptosis transpires, leading to liver cancer, ovarian cancer, Alzheimer's disease, etc.[172,173]

Previous studies have shown that cofilin plays an important role during the initiation phase of apoptosis. After induction of apoptosis, cofilin, activated by dephosphorylation at Ser3 are translocated to the outer mitochondrial membrane before release of cytochrome c into the cytosol.[174] Moreover, the actin-binding activity of cofilin is crucial for its apoptosis-inducing activity. Dephosphorylated cofilin translocates to the outer mitochondrial membrane while binding to actin, leading to cytoskeletal remodeling, which in turn affects mitochondrial function. The result is mitochondrial dysfunction, cytochrome c release and apoptosis[174] (Figure 3).

It deserves a special mention that apoptosis induced by a mitochondrial pathway mediated by cofilin dephosphorylation, is closely associated with the dynamin-related protein1 (DRP1) and phosphatase and tensin homolog (PTEN)-induced kinase1/ Parkinson protein2 pathways, reported in other malignant tumors.[175] Mitochondrial mitotic protein, DRP1[175–177], locates in the cytoplasm, exerts function in the form of polymers. Following the mitochondria stimulation by various physical and chemical factors, the mitochondrial outer membrane mitochondrial adaptor fission1 summons the DRP1 and transports it to the potential cleavage site at the outer mitochondrial membrane. DRP1 polymers form a ring structure around mitochondria, which generates energy through guanosine triphosphate hydrolysis, and gradually compresses the mitochondria until it breaks. This process promotes mitochondria division.[176–178] In addition, the cofilin translocated to the mitochondria can directly bind to the potential cleavage sites in the outer mitochondrial membrane, forming a similar circular complex around the mitochondria, which interacts with DRP1 to accelerate mitochondria breakage[98] (Figure 3). Hu[179] et al transfected human breast cancer cell line MDA-MB-231 using activated plasmids cofilin (S3A) and DRP1 (S637A) that mimic cofilin and DRP1 dephosphorylation, and revealed that the two plasmids

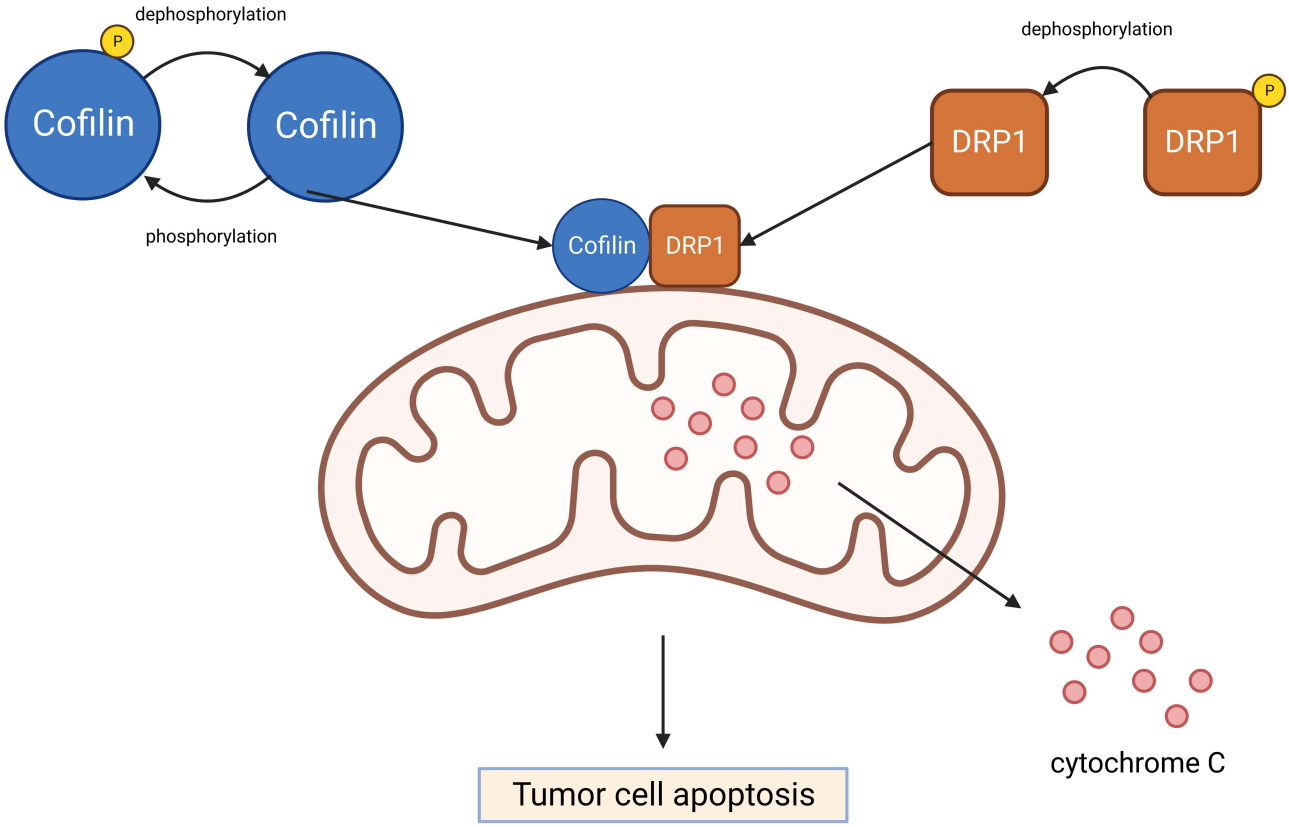


Figure 3 Cofilin regulates tumor cell apoptosis. Activated cofilin translocated to the mitochondria can directly bind to the potential cleavage sites in the outer mitochondrial membrane, which interacts with DRP1 to promote cytochrome C release and accelerate mitochondria breakage, resulting in tumor cell apoptosis. Created with BioRender.com.

induced the translocation of cofilin and DRP1 from the cytoplasm to mitochondria, inducing polymer-mediated mitochondrial cleavage. Contrarily, tumor cells transfected with cofilin (S3E) and DRP1 (S637D), which mimic cofilin and DRP1 phosphorylation, could inhibit mitochondrial division and apoptosis.[179] These results suggest that the synergistic effect of cofilin and DRP1 is critical in promoting mitochondrial pathway-mediated apoptosis. Recent data shows that mitochondrial translocation of DRP1 and cofilin is vital to this process. Silencing of DRP1 and cofilin to prevent mitochondrial translocation of DRP1 and cofilin respectively was demonstrated to significantly inhibit the mitochondrial division and fragmentation and significantly decrease apoptosis.[180,181]

Cofilin Regulates Tumor Radioresistance

Radiotherapy is currently one of the most effective non-surgical treatments for a variety of malignant tumors including glioma. Unfortunately, due to the high inherent radiation resistance of glioma cells, especially glioblastoma cells, the effectiveness of radiation therapy is greatly reduced.[182,183] Therefore, there is no doubt that it is vital to explore the causes of glioma radiotherapy resistance and enhance the radiosensitivity of glioma.

Through two-dimensional high-performance liquid chromatography- mass spectrometry, Wenbo Sun et al found that both phosphoglycerate kinase1 and cofilin1 are significantly highly expressed in the tumor tissue from glioma patients who are tolerant to radiotherapy.[99,103] The capacities of cell viability, migration and invasion were significantly down-regulated and the proportion of cells arrested in G2/M phase was increased in cofilin1-silenced radioresistant-U251 and U251 cells, compared with that of the untreated cells. In contrast, overexpressing cofilin1 showed progressive malignant behavior of tumor in U251 cells.[184] Similarly, when the Rac1-WAVE2-Arp2/3 signaling transduction was blocked, cell proliferation, migration and invasion abilities were decreased with the down-regulation of cofilin1.[185] These results indicated that cofilin1 plays an important role in enhancing radioresistance through activating Rac1-WAVE2-Arp2 /3-Cofilin signaling pathway in U251 human glioma cells (Figure 4).

Cofilin Regulates Other Malignant Behaviors of Tumors

The blood-brain barrier (BBB) maintains the homeostasis of the central nervous system by forming neurovascular units, which are consisted of endothelial cells, neurons, glia, smooth muscle cells, and pericytes.[186] However, the BBB also blocks effectiveness of treatments for brain tumors. The BBB is disrupted during the progression of brain tumor, resulting

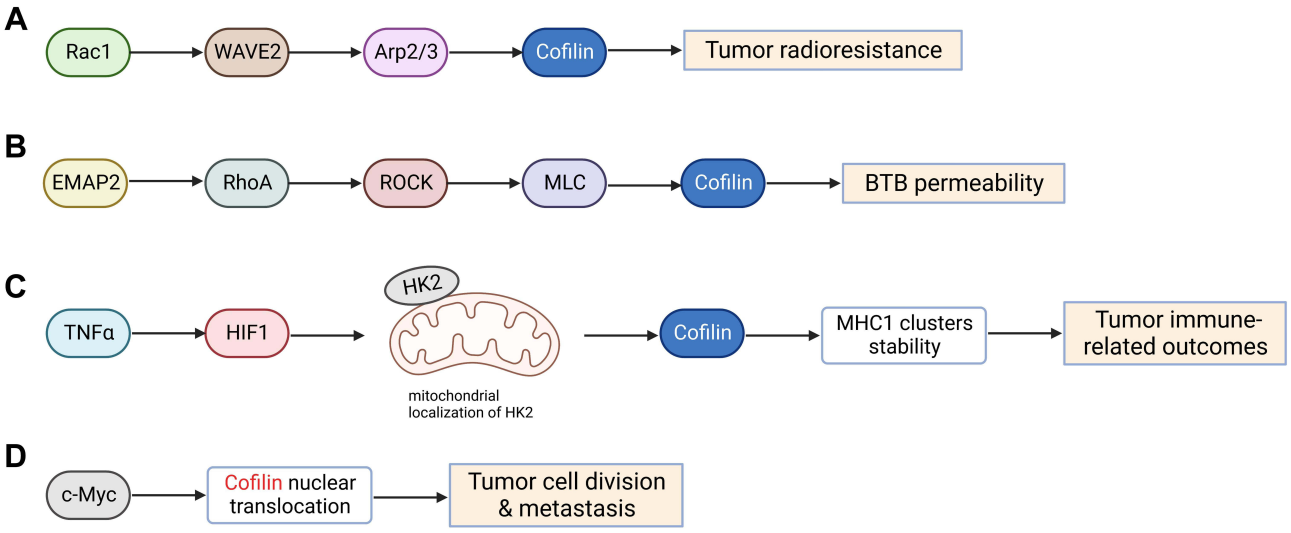


Figure 4 Cofilin regulates tumor radioresistance and other malignant behaviors. (**A**) Cofilin can enhance tumor radioresistance through activating Rac1-WAVE2-Arp2 /3-Cofilin signaling pathway. (**B**) Low-dose EMAP-II promotes increased the activation of MLC and cofilin via RhoA/ROCK/MLC/Cofilin pathway, thereby triggering actin cytoskeleton dynamic remodeling and ultimately increasing the permeability of BTB. (**C**) HIF1α induced by TNFα impulses cofilin-mediated changes in actin filament dynamics to stabilize MHC-I clusters through affecting mitochondrial localization of HKII, eventually affecting tumor immune-related outcomes. (**D**) Activation of c-Myc promotes nuclear translocation of cofilin and F-actin cytoskeleton dynamic remodeling, which may in turn affect tumor cell division and metastasis. Created with BioRender.com.

in a vasculature with high heterogeneity known as the blood–tumor barrier (BTB).[187] BTB exhibits different structural and functional characteristics than normal cerebral capillaries that form BBB. Although BTB is more permeable than BBB, its heterogeneous permeability to small and macromolecules can still hinder the entry of antitumor drugs into brain tumor tissue.[187,188] Therefore, it is crucial to explore the methods to selectively open the BTB to improve the effectiveness of chemotherapy for glioma. Low-dose endothelial monocyte-activating polypeptide-II (EMAP-II) promotes increased phosphorylation of myosin light chain (MLC) and cofilin by inducing activation of RhoA/ROCK, thereby triggering actin cytoskeleton dynamic remodeling and ultimately increasing the permeability of BTB[189,190] (Figure 4) Furthermore, inhibition of PI3K significantly suppress ed EMAP-II-induced phosphorylation of both MLC and cofilin, suggesting that the activation of PI3K was involved in regulation of MLC and cofilin phosphorylation mediated by RhoA/ROCK signaling pathway.[191]

Cofilin is involved in the immune escape process of glioma. The expression of Hexokinase-II (HKII) is regulated by hypoxic induced factor-1α (HIF-1α)[192,193] which is believed to be the basis for maintaining high metabolic requirements in malignant tumors under hypoxia. This is also conducive to promote various malignant behaviors of tumors, such as immune evasion in gliomas. Considering that HIF-1α regulates gene transcription of Major Histocompatibility Complex class I (MHC-I) induced by inflammation,[194] and as actin cytoskeleton is actively involved in enhancing the formation of stable MHC-I clusters, changes of cofilin activity closely correlated with glioma hypermetabolism and immune escape. Ghosh et al demonstrate that HIF-1α induced by tumor necrosis factor alpha both affects mitochondrial localization of HKII and impulses cofilin-mediated changes in actin filament dynamics to stabilize MHC-I clusters in glioma cells, eventually affecting immune-related outcomes[195] (Figure 4) In addition, cofilin1 has been identified as one of five key T-cell target immunogenic tumor-associated antigens (TAA) for immunity therapy in mutations in isocitrate dehydrogenase glioma patients.

Notably, one study in medulloblastoma showed cofilin is associated with RNA polymerase II -mediated transcriptional activity.[196] The protein sequence of cofilin contains nuclear localization signals, which allows the transport of depolymerized actin to the nucleus,[197] thereby regulating transcription and chromatin structure. Activation of c-myc, a transcription factor encoded by the proto-oncogene c-myc could promote nuclear translocation of cofilin and F-actin cytoskeleton dynamic remodeling in medulloblastoma cells which may in turn affect tumor cell division and metastasis[196] (Figure 4).

Prospects of Cofilin in Clinical Therapeutic Applications of Malignant Tumors Represented by Glioma

Evaluate Cofilin as a Biomarker for Predicting Tumor Metastasis and Prognosis

Previous studies in various types of tumors have been observed the positive correlation of high expression of cofilin with the malignant degree of the tumor, metastasis risk, tumor cell dedifferentiation, and the short survival time of patients.[94,95,97,98] In a retrospective cohort of non-small cell lung cancer patients, survival analysis showed that patient with high cofilin-1 had a lower overall survival rate ($P<0.05$), which be used to distinguish between good and poor prognosis. However, it is important to note that no correlation was found between age, sex or histological type and patient outcome or level of cofilin.[198] Li WP et al reported that the presence of cofilin in Juvenile nasopharyngeal angiofibroma (JNA) was correlated with tumor stage ($p=0.012$) and volume of intraoperative hemorrhage ($p<0.001$). The recurrence rate was higher in JNA patients with high cofilin expression than in those with low cofilin expression ($p = 0.012$). Similarly, high levels of cofilin indicated tumor progression and poor prognosis of patients in glioma,[99] pancreatic ductal adenocarcinoma[98] and other lethal malignancies.

In conclusion, all of these studies indicate that the expression of cofilin and its activation status are closely associated with the malignancy of tumors, which is expected to be a biomarker for predicting tumor metastasis and prognosis.

Targeting Cofilin Regulating Kinases, LIMKs and Their Major Upstream Molecules Inhibits Tumor Cell Migration and Invasion

LIMKs promote polymerization of actin via the LIMK-Cofilin pathway, which contributes to the formation and extension of actin filaments, resulting in driving membrane forward at the front part of migrating tumor cells. Thus, targeting cofilin regulating kinases, LIMKs and their major upstream molecules is significant for inhibiting tumor cell migration and invasion.

Several antineoplastic drugs have been shown to inhibit cell migration and invasion by interfering with and regularing the kinase activity of LIMKs in glioma. For instance, BMS-5 and Cucurbitacin I have been demonstrated directly blocking the cofilin regulating kinases, LIMK1 and LIMK2. The cell viability of glioma cells treated with BMS-5 and Cucurbitacin I was significant decreases, while no cytotoxicity was observed in normal astrocytes lacking LIMKs. BMS-5 and Cucurbitacin I promoted enhancement of GBM cell adhesion, suppressed cell migration and invasion.[90] T56-LIMKi has high specificity in inhibiting LIMK2, without almost any cross-reactivity with LIMK1. It decreases level of phosphorylated cofilin, subsequently inhibits growth of a variety of tumor cell lines, including glioma, schwannoma and pancreatic cancer.[199] Wang X et al reported that Alantolactone (ATL), a natural small molecule inhibitor, activated cofilin by specifically inhibiting the activity of LIMKs enzyme, which thus upregulated the ratio of G/F actin through actin polymerization inhibition, and blocked the migration and invasion of GBM cell.[200] Treatment with recombinant bone morphogenetic protein 9 protein triggers the activation of SMADs in patient-derived GBM cells, and strongly suppresses cell proliferation and invasion by inhibiting the activation of RhoA/Cofilin and PI3K/AKT/MAPK pathways.[201] In addition, it was reported that Eucalyptal A, a natural phloroglucinol-terpene adducts could downregulate expression of Serine/Arginine splicing factor1 (SRSF1) and rescue SRSF1-mediated unexpected alternative splicing of myosin IB mRNA, eventually exert anti-GBM effect by the PAK/Cofilin and 3-phosphoinositide-dependent kinase 1 (PDK1)/AKT/c-Myc signaling pathways.[202] As mentioned above, miRNAs, such as miR-451[132] and miR-29a/b/c,[134] inhibit glioma cell migration and invasion through regulating the cofilin activity via LIMK/cofilin pathway, acting as promising inhibitors of tumor migration and invasion.

Targeting Cofilin-Mediated Mitochondrial Pathway Induces Apoptosis of Tumor Cells

Activated cofilin translocates to the external mitochondrial membrane, inducing Mitochondrial outer membrane permeabilization (MOMP) and lysis of mitochondria, while interacting with DRP1 to cause excessive mitochondrial division and mitochondrial damage, ultimately leading to cytochrome C release and driving the apoptotic process. Therefore, targeting cofilin to induce apoptosis of glioma cells represent effective option for the development of novel anti-tumor drugs.

Various stimulants, including iso-lanolin lactone,[203] allyl isothiocyanate (AITC),[204] uric acid,[205] 4- (methylthio) butyl isothiocyanate,[206] and ATL[200] induce apoptosis of glioma cells via the cofilin-mediated mitochondrial pathway. Isoalantolactone (Iso) is a lactone compound extracted from the roots of Eucalyptus plants, which has been revealed to exert upstanding insect repellent and antibacterial effects.[207] Previous in vitro studies show that Iso has an anti-tumor effect.[208–210] A recent investigation of glioblastoma cell lines, U87MG, U251, and U118, explored that Iso inhibits cofilin phosphorylation by targeting LIMK kinase and down-regulating LIMK activity. This, in consequence, induces the transfer of cofilin and G-actin to the mitochondrial inner membrane and promotes the release of cytochrome C from the mitochondria to the cytoplasm. AITC induces cofilin dephosphorylation by activating serine/threonine protein phosphatase1/2A and activates mitochondrial pathway-mediated apoptosis by promoting G-actin translocation to mitochondria. Evidence suggests that the blockade of the ROCK1/PTEN/PI3K signal pathway plays an important role in the activation of cofilin.[211] Similarly, ATL activated cofilin by specifically inhibiting the activity of LIMKs enzyme and upregulated the proportion of G/F-actin. Activated cofilin can be jointly transferred to the mitochondria for initiating release of cytochrome c across the mitochondrial membrane, which eventually induce glioma cell apoptosis.[200]

Conclusion

Compelling evidence demonstrates that cofilin, as a depolymerization factor of actin filaments, potentially plays a crucial role in mediating actin structural reorganization and dynamic regulation via different mechanisms, including depolymerization, repolymerization, severing, and nucleation. Several active enzymes such as LIMK and SSH can achieve precise regulation of cofilin through phosphorylation or dephosphorylation. In recent years, emerging pieces of evidence have shown a regulatory role for cofilin in the migration, invasion, EMT, apoptosis, radiotherapy and chemotherapy resistance, immune escape and transcriptional dysregulation of malignant tumors represented by glioma. Given findings, it is practical to evaluate cofilin as a biomarker for predicting tumor metastasis and prognosis. Targeting cofilin regulating kinases, LIMKs and their major upstream molecules inhibits tumor cell migration and invasion and targeting cofilin-mediated mitochondrial pathway induces apoptosis of tumor cells represent effective options for the development of novel anti-malignant tumor drug, especially anti-glioma drugs.

Existing studies generally support that LIMK1/2 are significantly upregulated in glioma (especially glioblastoma) and many other infiltrative tumors, and strongly regulate tumor invasive motility and progression, which support further investigation of LIMK1/2 as options for the development of novel anti-tumor drugs.[121–123,212] However, some investigations on the final effect of LIMK reveal the opposite results, in which, LIMK overexpression inhibits tumor metastasis and invasion, whereas LIMK silencing promotes tumor cell metastasis and invasion. Zebda et al found that promotion of LIMK-regulated cofilin phosphorylation in metastatic rat breast cancer MTLn3 cells almost completely inactivated cofilin, significantly decreased actin turnover under sufficient G-actin levels, and almost completely inhibited lamellipodia formation, making the cell protrude, lengthen and move slowly, unidirectionally in a straight line.[213] These phenomena are related to the loss of consistent chemosensitivity to EGF stimulators on the surfaces of MTLn3 cells, indicating that LIMK-induced cofilin phosphorylation can not only inhibit the dynamic renewal of actin and lamellipodia formation but also impede the response of whole tumor cells to a chemotactic stimulator in any area of its surface. This eventually influences the chemotaxis and movement of tumor cells.[213] Wang et al suggested that it is the overall activity of the LIMK1/cofilin pathway and not that of LIMK1 expression alone that determines the migration and invasion status of malignant tumor cells.[214] Furthermore, different tumor cell types used in the different studies might have contributed to the inconsistency of results. Future studies are required to focus on elucidating the differential regulatory mechanisms of the effect of the LIMK/cofilin pathway in gliomas and other malignancies. Besides that, it is important to study whether cofilin has a similar promoting effect on tumor angiogenesis and tumor cell proliferation in gliomas compared to other malignancies, which will be critical for future drug development targeting cofilin.

Abbreviations

EMT, epithelial to mesenchymal transition; LIM kinases/LIMKs, Lin11, Isl-1 and Mec-3 kinases; Ser, Serine; TESKs, testicular protein kinases; SSH, slingshot; CIN, chronophin; Rac1, Ras-related C3 botulinum toxin substrate1; WASP, Wiskott-Aldrich syndrome protein; WAVE2, WASP family verprolin-homologous protein2; Arp2/3, Actin-related protein 2/3; mRNA, messenger RNA; ADF-H, actin depolymerization factor homology; AA, amino acid; G-actin, globular actin; F-actin, filamentous actin; ADF, actin depolymerization factor; Lys, Lysine; Trp, Tryptophan; ATP, adenosine triphosphate; PDZ, Postsynaptic density 95, PSD-85; Discs large, Dlg; Zonula occludens-1, ZO-1; miRNA, microRNA; Rho, Ras homology; RhoC, the member C of Rho; RhoA, the member A of Rho; CDC42, cell division control protein 42; ROCK, Rho-associated kinase; PAK, p21-activated protein kinase; Thr, Threonine; BMP, bone morphogenetic protein; BMPRII/IA, BMPR receptor II/IA; VEGF, vascular endothelial growth factor; MAPK, mitochondrial activated protein kinase; MAPKAPK-2/MK2, MAPK-activated protein kinase-2; Hsp27, heat shock protein27; PKCζ, protein kinase Czeta; EGF, epidermal growth factor; ITSN1-s, Intersectin1-s; P2Y2R, P2Y2 nucleotide receptor; Nogo-A, reticulon-4 isoform A; NKCC1, electroneutral Na^+-K^+-$2Cl^-$-co-transporter1; CTRP8, C1q tumor necrosis factor-related peptide8; SEPT7, Septin 7; Akt2, Akt serine/threonine kinase 2; uPAR, urokinase-type plasminogen activator receptor; P4, Progesterone; WDR1, WD-repeat containing protein1; ARST, aldolase A/ALDOA-related specific transcript; TCGA, The Cancer Genome Atlas; GBM, glioblastoma multiforme; CSCs, cancer

stem cells; HMLEs, human breast epithelial cells; KITENIN, KAI1 C-terminal interacting tetraspanin; ZEB1/2, Zinc finger E-box binding homeobox1/2; VSIG4, V-set and immunoglobulin domain-containing 4; TGF-β, transforming growth factor-beta; SMAD, small mothers against decapentaplegic; PI3K, phosphatidylinositol-3-kinase; CD73, ecto-5′-nucleotidase, NT5E; PRP4, pre-mRNA processing factor 4B; DRP1, dynamin-related protein1; PTEN, phosphatase and tensin homolog; BBB, blood-brain barrier; BTB, blood–tumor barrier; EMAP-II, endothelial monocyte-activating polypeptide-II; MLC, myosin light chain; HKII, Hexokinase-II; HIF-1α, hypoxic induced factor-1α; MHC-I, Major Histocompatibility Complex class I; JNA, Juvenile nasopharyngeal angiofibroma; ATL, Alantolactone; SRSF1, Serine/Arginine splicing factor 1; AITC, allyl isothiocyanate; Iso, Isoalantolactone.

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Author Contributions

S.L. and Z.C. mainly wrote the manuscript and drew all figures and graphical abstract. H.M. revised the spelling and grammar of the manuscript. X.Y. designed the overall review and proofed the manuscript. All authors gave final approval of the version to be published, have agreed on the journal to which the article has been submitted and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no conflict of interest.

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