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REVIEW

Biochemical characteristics, functions and clinical significance of cofilin

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Abstract

Cofilin is a low molecular weight actin-binding protein widely found in eukaryotic cells in ADF/cofilin family proteins, which has a wide range of biological effects and has gradually become a research hotspot in recent years. We review the structural characteristics of cofilin, the mechanism of regulating actin, biological functions, and the relationship with clinical diseases to explore its role in the development of cancer, nervous system diseases, osteoarthritis, heart and kidney diseases. The use of cofilin phosphorylation pathways as major action-targeting sites in the treatment of these diseases could provide a basis for the development of new drugs, such as Rho related protein kinases inhibitors netarsudil to prevent cancer metastasis, neuroligin C-terminal domain (NLG1-CTD) peptides with high affinity to inhibit LIM kinase thereby preventing nervous system diseases, grape seed proanthocyanidins to inhibit cofilin related oxidative stress in the treatment of heart and kidney diseases.

Key words: ADF/cofilin family protein, Actin binding protein, Actin regulation, Clinical significance

***Abbreviations*:**

PIP2, phosphatidylinositol-4,5-diphosphate; NLS, nuclear localization signal; LIMK, LIM kinase; TESK, TES kinase; ROCK, Rho-associated protein kinase; p-cofilin, phosphorylated cofilin; EGF, epidermal growth factor; PLC, phospholipase C.

***Competing interests*:**

The authors declare that they have no conflict of interest.

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Background

Actin Depolymerizing Factor (ADF)/cofilin family proteins were first discovered and purified from chicken blastocyst cells in 1980. ADF/cofilin family proteins exist in most vertebrates, especially exist in all mammals, including ADF, non-muscle microfilament severing protein cofilin-1 and muscle type cofilin-2, but Xenopus laevis only expresses two kinds of cofilin, chicken only expressed one kind of cofilin [1, 2]. ADF and cofilin have overlapping functions in the cellular process [3, 4], in which deletion of one protein causes abnormal cell division and cell movement can be compensated by over expression of another protein. This is mainly because they are in the same family and their phosphorylation regulation has the same regulatory factors. The basic function of the ADF/cofilin is to dynamically regulate the depolymerization of actin filaments in cells to maintain the functions of cell morphology, polarity, migration, etc. The main role of ADF and cofilin is to bind the actin to sever the actin filaments [5], which make them essential factors in the structure, growth, development and differentiation of cells. With the further developments of the ADF/cofilin family studies, it is found that they also play an important role in activating phospholipase, carrying actin into the nucleus, inducing apoptosis, and regulating immunity, etc. These cell functions have also produced new uses for the ADF/cofilin family in molecular and cellular processes, then caused a new research boom.

For the ADF/cofilin family clinical significance, it also provides a new field of research. A number of clinical studies of the ADF/cofilin family now focus on neurological diseases and the development of cancers [6]. For example, many studies now suggest that ADF/cofilin family plays a role in the rearrangement of nerve cells, and this function also makes it have a wide research prospect in nervous system diseases. Cofilin is also involved in the formation of the malignant phenotype and that this is likely due to an overall misregulation of the cofilin pathway. But a few clinical studies suggest that because ADF/cofilin families are widely distributed in cells, their effects on the progression of clinical diseases are much more than that. New studies suggest that ADF/cofilin family plays an important role in heart and kidney disease, a study had shown that over phosphorylation of cofilin-2 can lead to the "stress fiber-like" accumulation of actin in cardiomyocytes, which seriously impairs the contractility of cardiomyocytes [7]. In osteoarthropathy, a study suggests that when chondrocytes are subjected to certain physical or chemical stimuli, actin assembles into filaments, the actin cytoskeleton is involved in the stress conduction in chondrocytes through its own helix to form microfilaments, and the mechanical signal may be converted into biochemical in which cofilin phosphorylation/dephosphorylation signaling pathways play an important role [8].

We will review the structural characteristics of cofilin, the mechanism of regulating actin, biological functions, and the relationship with clinical diseases, and summarize its role in the development of cancer, nervous system diseases then explore new functions in osteoarthritis, heart and kidney diseases.

Cofilin coding genes, structure, expression and biochemical characteristics

ADF/cofilin family proteins, including ADF, cofilin-1 and cofilin-2, are encoded by the Destrin, cofilin-1, cofilin-2, respectively locate in 20p12.1, 11q13.1, 14q13.1 (Table1). The ADF is mainly expressed in nerve cells, epithelial cells and endothelial cells; cofilin-1 can be widely expressed in all kinds of tissues of the whole body, while cofilin-2 is highly expressed in heart and prostate tissues [9]. All three are encoded from different genes, and ADF shares 70% amino acid identity with cofilins, whereas the latter share 80% identity at the amino acid level [10]. The exon-intron junction sequence of ADF/cofilin genes is quite conserved among different species, the tertiary structure of their posttranscriptional products is very similar. For example, the human cofilin-2 transcribes two different mRNA but ends up producing the same polypeptide by alternative splicing [11]. Although the amino acid sequence difference between cofilin-1 and cofilin-2 is close to 20%, the difference undoubtedly affects the final three-dimensional structure of the two isomers of protein. The cofilin differences are due to the helical α as well as the length and direction of the β folding. These structural differences determine the specific functions of isomers, also explain why cofilin-1 and cofilin-2 have different affinities for actin [12]. The current study also shows that although ADF/cofilin family proteins can bind to filamentous F-actin and globular G-actin, ADF and cofilin-1 bind more stably to the F-actin and promote the depolymerization of actin, but cofilin-2 lack the efficiency of this combination [10, 13]. Moreover, compared with cofilin-1, ADF and cofilin-2 can sever actin filaments more effectively.

The core of the cofilin molecule is composed of six twisted hybrid single β-chains characterized by two opposite and parallel chains. And the core is surrounded by α-spirals on both sides (Figure1). The spiral α5 is characterized by bending at Ser120, which plays an important role in the combination of cofilin and G-actin. The long α-helixes are conserved, which are sequences that ADF/cofilin bind to actin and promote the depolymerization of actin filaments, which are called the ADF/cofilin homologous region. The cofilin homologous region contains two specific sites that interact with actin, the former called the F site, which

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can bind to actin monomers and actin filaments, and the latter called the G site, which is located on the opposite side of the F site and can interact with actin filaments. And G site is therefore commonly referred to as the G/F site. Moreover, there is a Trp100~Met115 sequence on the ADF/cofilin protein used to bind phosphatidylinositol-4,5-diphosphate (PIP2), which is also critical for ADF interaction with actin [14]. The presence of a nuclear localization signal (NLS) sequence was detected in loop 1 prior to the helical α2, which allows the transfer of cofilin to the nucleus through the nuclear pore.

The binding affinity of cofilin to actin depends on the type of nucleotide-binding in the gap between protein domains. That is, cofilin has the strongest affinity for ADP-actin and the weakest for ATP-actin, resulting in downstream hydrolysis and phosphatase release of filamentous areas of cofilin accumulation. Cofilin influences the "maturation" of filaments by accelerating the separation of phosphate from ADP-pi-actin subunits [15]. Through microscopic imaging and 3D model construction, it can be found that actin filaments produce additional torsion under the action of cofilin, which is caused by the torsion of 4–5° of the subunit binding to the related actin [16]. And this cofilin-induced actin conformation change can be transmitted to the distal end, which is an important link in actin depolymerization.

Cofilin mediated mechanism of actin filament dynamics control

The ADF/cofilin protein family promotes both actin polymerization and F-actin depolymerization (Figure2), depending on the molar ratio of ADF/cofilin to actin [17]. The concentrations of other actin-binding proteins also have a certain effect on this process. A small amount of binding cofilin (submicromolar concentration) leads to fragmentation of actin filaments and increased free ends. For example, when the ratio of cofilin to actin in the F-actin is less than 1:100, the F-actin is cut continuously [18]. When its ratio is 1:10 to 1:2, the cofilin briefly cuts F-actin, stabilizes the F-actin in a distorted state due to the synergistic combination of the cofilin on the F-actin [19]. When cofilin is overexpressed or cells are subjected to oxidative stress, the ratio of cofilin to actin exceeds a certain critical value and cofilin-ADP-actin polymerizes into bundles, preventing cofilin from promoting F-actin depolymerization and severing [20].

Cofilin depolymerize or polymerize actin mainly by

Table 1 Physical and biochemical characteristics of human ADF/cofilin

Isoforms	ADF	Cofilin-1	Cofilin-2
Ensembl ID	ENSG00000125868	ENSG00000172757	ENSG00000165410
Chromosome location	20p12.1	11q13.1	14q13.1
Exons	6	4	6
Transcripts	2	11	4
Length (aa)	165	166	149
Isoelectric points	7.97	8.29	8.17
Tissue expression	Nervous system	Intestinal and systemic tissue	Heart, muscle, prostate

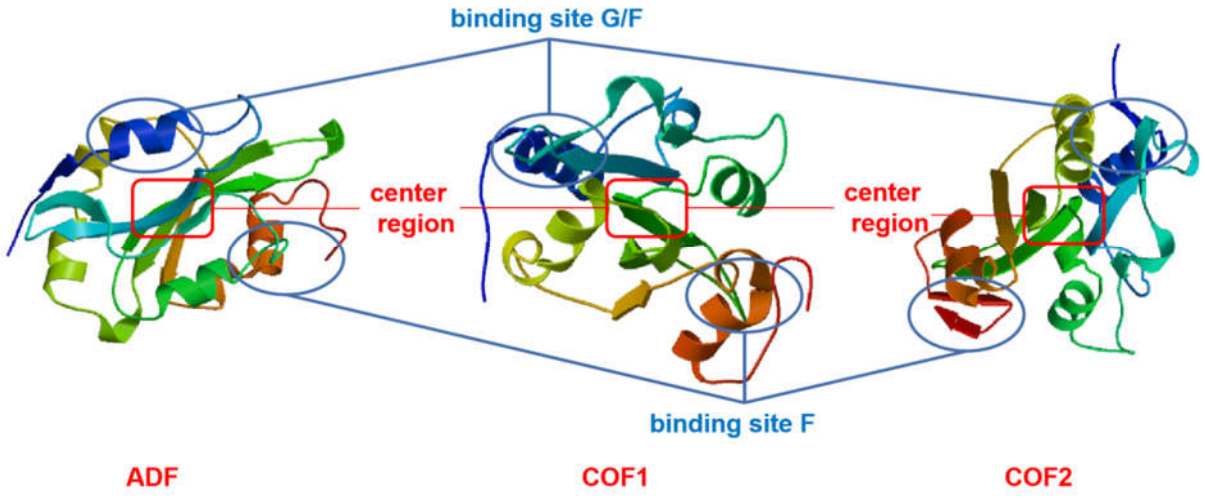

Figure 1 Morphological structure of ADF/cofilin proteins. ADF, actin-depolymerizing factor; COF1, cofilin-1; COF2, cofilin-2.

binding the G-actin and F-actin, and there are two main dynamic mechanisms at present. The first way is cofilin accelerate the depolymerization of actin and increase the concentration of G-actin in cells by cutting the F-actin at the pointed ends, which also makes the F-actin turnover rate increase. The second way is cofilin to create free barbed ends, by cutting F-actin, which allows for further polymerization [5]. The kinetic model established by Roland shows that ADF/cofilin depolymerization activity occurs by cutting off actin filaments and increasing the rate of actin monomers leaving the ends of actin filaments [21]. On the other hand, cofilin binding to dissociated ADP-actin inhibits its nucleotide exchange. The dissociation of actin filament head-end monomers is the rate-limiting step for the dynamic cycling of the cytoskeleton. As a result, ADF/cofilin promotes actin dynamics overall. New research also suggests that cofilin clusters disrupt protofilaments, consistent with a higher severing activity at boundaries compared to single cofilin. Comparison of these structures indicates that this disruption is substantially greater at pointed end sides of cofilin-actin clusters than at the barbed end. These structures, with the distribution of bound cofilin clusters, suggest that maximum binding cooperativity is achieved when two cofilins occupy adjacent sites [22].

Cofilin can change actin's spatial structure and produce certain torque when binding to actin filaments, which also accelerates the depolymerization of actin filaments. The study shows that cofilin combines on the ADP-actin of the F-actin head end to make it rotate at a small angle and increase the depolymerization rate of the F-actin head end by more than 30 times. When the two anchoring points of the actin filament are fixed, this limits the distortion of the actin filament, and cofilin binding enables the actin filament to produce a certain torque. This torque, after further observation, does not hinder the binding of cofilin to actin, but can increase the cutting efficiency of cofilin by 100 times [4, 23]. Studies also indicate that buckled actin filaments are easier to sever, while a twisting filament would mostly favor the dissociation by cofilin. And external application of mechanical stress, seemingly passive mechanical constraints such as filament and choring may also play a role [4]. The actin filaments are polar and can be polymerized and depolymerized at both ends. In cells, the fast-growing end of actin filaments faces the cell membrane, and the cofilin in some high mobility cells (embryonic cells, epithelial cells, etc.) can promote the formation of microtubule and microfilament which is necessary for prominent and targeted movement by using the leading edge of the cell. Under the stimulation of cofilin, the fast depolymerization of actin tip takes place under the cell membrane, while the continuous polymerization takes place inside the cell, which requires continuous supply of actin monomer [12].

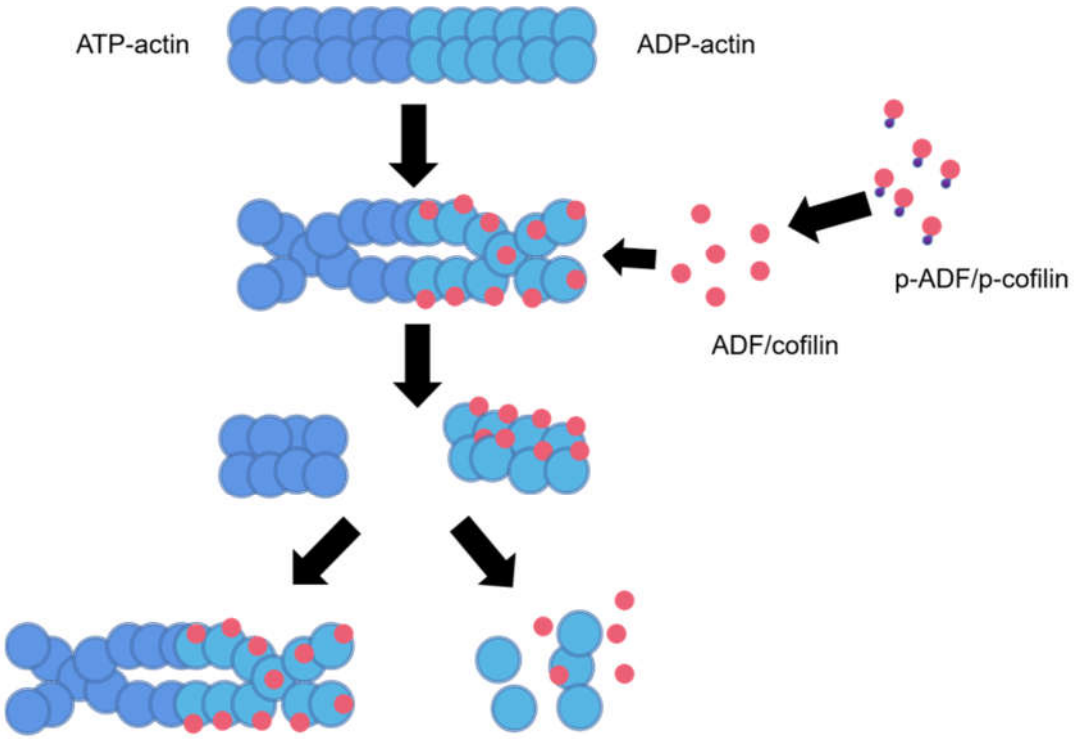

Figure 2 ADF/cofilin depolymerization and combinatorial actin processes

There are many molecules that can affect the efficiency of actin depolymerization and polymerization. Because this process is mainly affected by actin-binding protein, and it certainly includes cofilin itself. The relationship between actin polymerization efficiency with cofilin concentration has been mentioned before and it will not be discussed here. Since cofilin mainly binds to ADP-g-actin and inhibits the spontaneous exchange of nucleotides with ATP, the concentration of ATP-g-actin molecules is reduced, thus the elongation of actin filaments decreases obviously. The ADP-G-actin is also bound by cofilin competing protein twinfilin, which contains two ADF-H domains connected by an elastic segment [15]. Twinfilin can also bind to ADP-g-actin competitively, and there are two main mechanisms to inhibit actin polymerization. The first is binding directly to the spinous end of actin filaments so that it is not elongated. Second, twinfilin can bind to the capping protein and covering the barbed end such that the polymerization is further inhibited [24]. The opposite one is another protein profilin, which can transport ATP-G-actin to rapidly growing filaments to accelerate polymerization [24].

Capping protein is also a protein that increases cofilin and actin cycling efficiency. Capping protein can promote the separation of cofilin from actin, thereby enhancing actin filament dynamics. The ADF/cofilin and capping protein act independently on actin, respectively, so as to realize the precise regulation of actin filaments "treadmill movement". An interesting mechanism for regulating cofilin dependent actin kinetics is the involvement of the actin interacting protein AIP1, which binds to cofilin modified actin filaments to help the polymerization and separation of actin fragments [24].

The direct interaction between cofilin and filamentous actin is controlled by tropomyosin, which is a double-stranded super helix protein polymerized along actin filament [26]. Tropomyosin stabilize actin filaments, in cells, they exist in the areas of cells that need to grow, and can inhibit the binding of cofilin to actin. It is pointed out that protomyosin limits the torsional flexibility of actin, while cofilin increases the flexibility of actin with strong synergy [27]. However, TMBr3, as an isomer of tropomyosin, increases the binding of cofilin to actin filaments [26]. In addition to directly regulating actin dynamics, cofilin also regulates the activity of the Arp2/3 protein complex, which binds laterally to existing actin filaments. Cofilin directly competes with Arp2/3 for actin binding sites or indirectly influences Arp2/3 binding through conformational changes of actin filaments [19, 28].

Cofilin mechanism of activity regulation

Phosphorylation and Dephosphorylation

The regulation of ADF/cofilin protein activity is affected by phosphorylation and dephosphorylation (Figure3). Functionally, cofilin regulation of actin dynamics is controlled by reversible phosphorylation [7, 29]. Phosphorylation of the Ser3 (N-terminal serine) site of the cofilin makes it lose its depolymerization activity and cannot be bound to the F-actin to improve its stability, but dephosphorylation restores its depolymerization function and acts as a switch during actin assembly and cleavage [30]. Cofilin phosphorylases include LIM kinase (LIMK) and TES kinase (TESK). There are also reports of integrin-linked kinase, spleen tyrosine kinase induced cofilin phosphorylation, but there are few studies on this [31–33]. LIMK including LIMK1 and LIMK2 and TESK including TESK1 and TESK2 have a specific affinity for Ser3 sites and can phosphorylate cofilin. LIMK1 is a serine/threonine-protein kinase consisting of two LIM domains at the N-end, an intermediate PDZ domain, and a protein kinase domain at the C-end. LIMK1 activation is controlled by Rho-related protein kinases (ROCK), p21 activation kinases (PAK1, PAK2 and PAK4), MK2 and Cdc42 related protein kinases (MRCKα) [34, 35]. These Rho family downstream effector molecules, including small molecule GTPases, RhoA, Rac1, Cdc42, and CaMKIV (calcium-regulated protein kinase 4) [36]. Rho/ROCK1-2 activated LIMK1 and LIMK2 by phosphorylation at position 508 threonine and position 505 threonine, respectively negative regulation of LIMK activity: polar protein (Par-3), tumor suppressor (LATS1), nischarin and β suppressor (β-arrestin), and Slingshot family of dephosphatase (protein phosphatase Slingshot homolog, SSH) mediated dephosphorylation [37]. There are also reports that ubiquitin ligase Rnf6, miR-134, bone morphogenetic protein receptor II are also involved in negative regulation of LIMK. A research showed that SRSF1 knockout could significantly inhibit PAK/LIMK phosphorylation and reduce the phosphorylation level of cofilin [38]. TESKs have only one C- terminal proline protein kinase domain but can work with LIMKs kinases. Their activity inhibition is affected by 14-3-3 proteins β subtypes, Sprouty-4, Spred1, and actopaxin, etc. Chinmo/Bach2 are also reported to inhibit TESK activity and thus enhance cofilin severing [39].

Cofilin dephosphatase has Slingshot family of dephosphatase SSH and HAD family of chronophin. SSH is widely found in various tissues of mammals, chronophin mainly in human brain tissues, both of which can induce dephosphorylation of already phosphorylated Ser3, thus activating cofilin, binding it to cleavage and promoting the depolymerization of actin filaments. SSH, as a cofilin monophosphatase plays an important role in regulating the changes of actin cytoskeleton. There are three highly conserved domains in the amino-terminal region of SSH protein:

the A, B, P domain. B, P domain is primarily responsible for identifying phosphorylated cofilin (p-cofilin) and cofilin phosphorylation processes. The main substrates of action for SSH are that ADF/cofilin and LIMKs, act on key sites to dephosphorylate already phosphorylated ADF and p-cofilin, thereby inhibiting the polymerization of actin filaments mediated by LIMK or TESK1, as the reactivation process. Soosairajah et al. found that SSH-1L can cause LIMK1 dephosphorylation and down-regulation of activity and form a complex that leads to dephosphorylation of LIMK1 sites and important position 505 threonine residues that are automatically phosphorylated in the active circuit, thereby inactivating their ability to phosphorylate cofilin [40]. Hence SSH phosphatase can dephosphorylate two different proteins in the same signaling pathway and play an important role in the regulation of actin cytoskeleton changes. SSH phosphatase activating proteins include F-actin, 14-3-3 protein, PKD family protein, PLCβ/PI3Kγ-GSK3β signaling pathway protein, calmodulin protein phosphatase and so on. Phosphatase chronophin is a specific phosphatase of cofilin except for slingshot, however, more effects of this protein have not been studied. Now a study reported that arrestin 2 and 3 can activate the phosphatases slingshot or chronophin, and control actin cytoskeleton formation [41].

PIP2 binding

Since PIP2 has a structural affinity with cofilin (Figure 4), it can prevent cofilin from binding to actin, which in turn affects the turnover efficiency of cofilin in the depolymerization of actin [42, 43]. PIP2 molecules are mainly distributed on the cell membrane, the PIP2 molecular density on the cell membrane determines the activity of cofilin molecules. Some chemoattractants

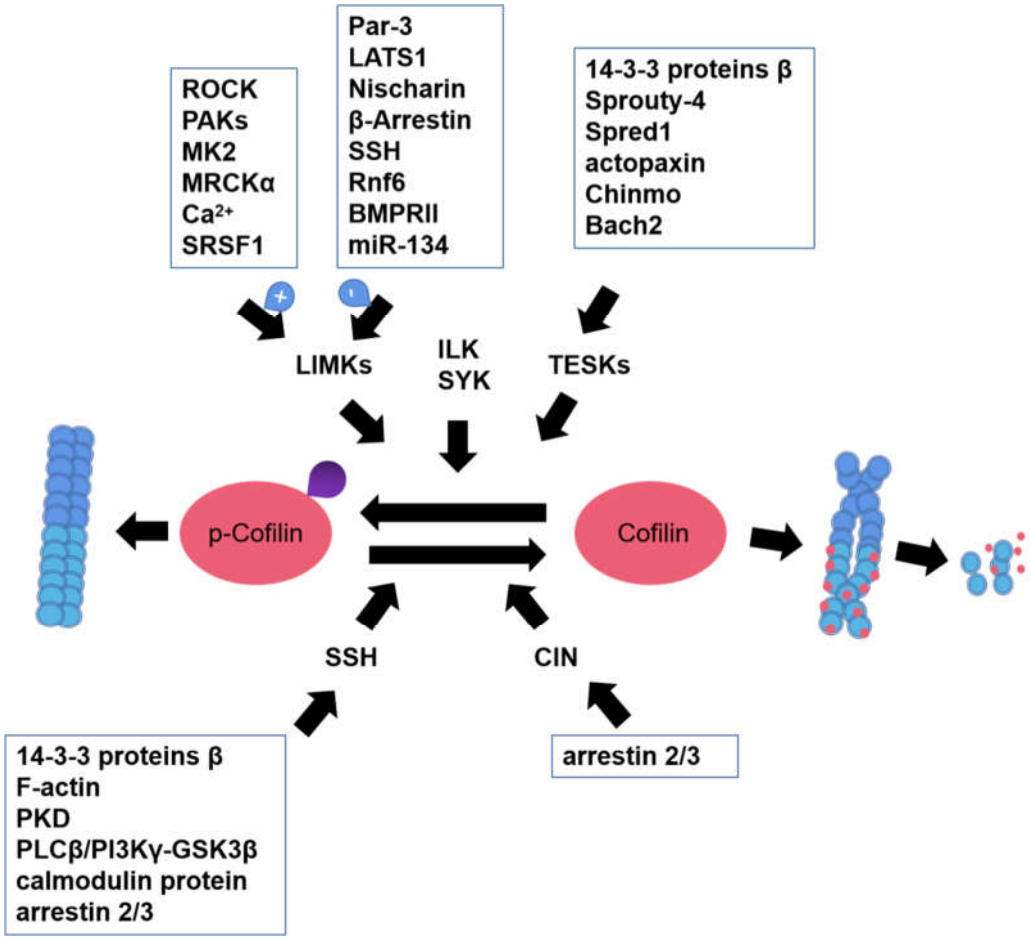

Figure 3 Phosphorylation and Dephosphorylation of cofilin. ROCK, Rho-associated protein kinase; PAKs, serine/threonine-protein kinase; MK2, MAP kinase-activated protein kinase 2; MRCKα, serine/threonine-protein kinase MRCK alpha; SRSF1, serine/arginine-rich splicing factor 1; LATS1, serine/threonine-protein kinase; Rnf6, ring finger protein 6; BMPRII, bone morphogenetic protein receptor type-2; PKD, autosomal dominant polycystic kidney disease 1 protein; PLCβ, 1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase beta; PI3Kγ, phosphatidylinositol 3-kinase regulatory subunit gamma; GSK3β, glycogen synthase kinase-3 beta.

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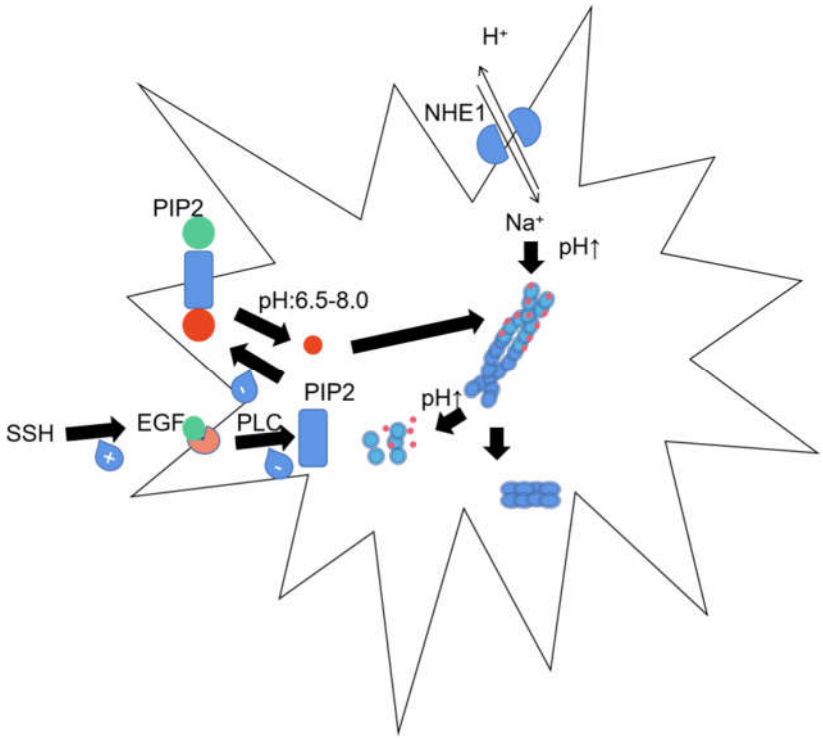

Figure 4 PIP2 and PH regulation of cofilin. PIP2, PH

have implications for the combination of PIP2 and cofilin, at present, the most studied molecule is epidermal growth factor (EGF). The study suggests that EGF can promote the hydrolysis of PIP2 by phospholipase C (PLC), other studies suggest that EGF bind to their respective probable serine/threonine-protein kinase (RTK) and activate PI3K, to turn PIP2 into phosphoinositide 3 kinase (PIP3), so that PIP2 lose the activity of binding cofilin [44]. Because of this mechanism, cofilin can induce the formation of cell membrane protrusions by localizing at the cell membrane, thus mediating the movement of cells [44]. But even the binding of phosphorylated inactive forms of cofilin on the surface of the cell membrane promotes directed cell migration, suggesting the importance of PIP2 in regulating cofilin activity [46]. The current study has not found PIP2 specific relationship with the LIMK regulatory system while regulating the cofilin activity [47, 48]. Studies have shown, however, that EGF-RTK downstream pathway may be activated by SSH1 mediated dephosphorylation and intracellular pH changes, which release the combination of cortisol and PIP2 cofilin, and may also lead to cofilin inactivation through LIMK activation [49].

Regulation through pH

Sodium hydrogen exchanger 1 (NHE1) plays an important role in the activation of cofilin, that is, the pH value in cells is important to regulate the actin depolymerization process. The results showed that when the pH value in cells was kept at 6.5–8.0, the efficiency of cofilin depolymerization actin was the best [49]. NHE1 also regulates invasiveness by promoting ECM digestion (due to its acidic optimum pH) and increasing cofilin dynamics in the invasive study of poliomyelitis [50]. Studies have shown that cofilin-induced PIP2 clustering is inhibited in higher pH environments, which reduces the density of the PIP2 above the cell membrane and increases the activity of the cofilin. This provides a factual basis for cell membrane processes and cell movements under pH regulation, this process is mainly caused by PLC hydrolysis of the PIP2 [50]. pH also affects the cleavage activity of cofilin by affecting the binding of cofilin and cortactin in breast cancer cells. When the pH increases, the binding effect of the two is weakened, which makes actin filaments more likely to produce free barbed ends for filament elongation [52]. We generally believe that ADPs are more sensitive to pH changes in cofilin families, so the influence of pH on different family members is different [52].

Regulations by miRNAs

The miRNA regulation of cofilin activity is the latest research topic in recent years. The activities of cofilin and other actin-binding proteins in cells are regulated by many miRNAs, which is carried out in many ways. Mir-17-92 cluster, for example, is highly expressed in metastatic tumors, and if it weakens its expression in

tumor cells, it can be detected that docosahexaenoic acid increases the distributions of cofilin/VASPS239 from the cell edge to the nucleus [52]. Mir-222 knockout can reduce the activity of cofilin, which is considered to be achieved by Plexin C1 upregulation [55]. Breast cancer-derived extracellular vesicles which contain miR-181c can promote BBB-TJs destruction through actin recombination, downregulation of 3-phosphate inositol-dependent protein kinase-1, and downregulation of phosphorylated coenzyme (i.e., cofilin activation) [56]. Mir-29a/b/c in Mir-29a/b/c overexpressed glioma cells inhibit tumor metastasis by inhibiting CDC42 and then reducing PAK1/2/3, LIMK1/2 and cofilin phosphorylation [57]. Loss of miR-182-5p in bladder cancer induces high levels of cofilin-1, which promote tumor cell proliferation, migration, invasion and tumorigenesis. Therefore, it can be seen that miRNA regulation of cofilin activity is carried out in different ways.

Other regulatory mechanisms

Cofilin can also be phosphorylated by viral (v)-Src at Tyr68 sites, but this phosphorylation cannot directly alter cofilin severing activity, this can change its ubiquitination and degradation through the proteasome pathway [58, 59]. At present, it has been found that the oxidation of the cysteine residue in the cofilin can regulate its binding activity with actin and thus affect cell movement [58, 59]. Oxidation-dependent activation of PKG1 followed by cofilin phosphorylation likely represents a complimentary path regulating actin dynamics [61]. And this oxidation also occurs during apoptosis in which cofilin is involved. The regulation of mechanical tension in the binding process of cofilin and actin is also very important. At present, it is believed that actin filaments under tension are protected and will not be cut off directly by cofilin [62, 63].

Cytological function of cofilin

Effects on cell movement

Cell directed motion is closely related to the dynamic changes of the cytoskeleton. The “elastic Brownian ratchet” model shows that the resilience generated by the elongation of a unit of microfilaments will squeeze the inner surface of the membrane, resulting in a push force [64]. Therefore, the contractile properties of actin filaments in cells are very important for cell movement. In addition, moving cells push the cell membrane forward through F-actin polymerization at their forward through F-actin polymerization, while at the base they rely on F-actin continuous depolymerization to drag cells forward. ADF/cofilin is enriched in the front edge of motor cells, make actin filaments extend in a fixed direction to promote cell movement, and cell movement is the basis of nerve growth cone migration, embryogenesis and tumor cell migration. During cell movement and division, a study has shown that NudC can regulate actin cytoskeletal and ciliogenesis by stabilizing cofilin-1 [65]. Researches show that localized cofilin oxidation contributes to maintaining persistent cell movement, under relaxed cellular control, cofilin facilitates tumor cell movement and dissemination [60, 66, 67]. Myosin can also regulate cofilin mediated cell movement in a specific way. When myosin competitive binding actin weakens, the consumption of ADF and cofilin leads to a large amount of accumulation [68]. But the study also points out that when only ADF is exhausted, cofilin can be compensated, and the effect can be weakened, thus regulating the motor activity of cells.

Transporter actin into the nucleus

Actin has no NLS, but most ADF/cofilin family proteins have at least one NLS at the amino end NLS, they undertake the task of transporting actin into the nucleus. Actin is involved in chromatin remodeling, nuclear heterogeneous ribonucleoprotein complex formation and regulation of gene expression in the nucleus. When cells are subjected to external compressions, such as thermal or chemical stresses, a portion of the ADF is transferred from the cytoplasm to the nucleus, ADF may in this way move the actin [68]. Studies suggest that cofilin mediated nuclear translocation requires exposure of its NLS, and the implementation of this process requires dephosphorylation of the serine-3 domain cofilin molecule [70]. Nuclear transport is mainly related to importin-9 binding to actin, which depends on the mediation of NLS in cofilin and affects the transcriptional level of cells [70]. At the same time, importin-9-induced actin into the nucleus also weakened the extracellular actin polymerization process [72].

Apoptosis

The physiological oxidant produced by neutrophils can oxidize cofilin, and the oxidized cofilin loses affinity to actin and transfers to mitochondria, releasing cytochrome C inducing apoptosis by regulating the opening of mitochondrial permeability transition pores [73]. ADF/cofilin of phosphorylation of astrocytes can be transferred to mitochondria after persistent epileptic status and neuroblastoma induced apoptosis. The shift of cofilin depends on the mitochondrial localization sequence of its amino-terminal and the sequence of the carboxyl-terminal, but cofilin induces apoptosis is dependent on its actin-binding region. Actin and cofilin interaction proteins (adenylyl cyclase-associated protein, CAP1) also transfer into mitochondria after inducing apoptosis, but overexpression of CAP1 does not induce apoptosis and can only promote cofilin induced apoptosis, which suggests that CAP1 is a direct link between actin cytoskeleton and mitochondria.

CAP1 function depends on N-terminal localization sequence and actin-binding at C-terminal [74]. The study suggests that cofilin transport to mitochondria will also mediate amyloid-β-induced neurotoxicity, which is related to the induction of neuronal apoptosis [75]. MC-3129-mediated dephosphorylation and mitochondrial translocation of cofilin also play an important role in inducing apoptosis [76].

Participation in cytokinesis

Actin framework plays an important role in the localization and initiation of cell division. In cell mitosis, a large number of cofilin in cells are activated and F-actin disassembled, which is considered to be an important step in cell division [77]. When actin filaments are cut off cofilin also limits the accumulation of F-actin in cells, so the cofilin dephosphorylation is the key factor for the successful division of cells [77]. For example, by depolymerizing the actin filaments at both ends of the cell, the ADF1 of fission yeast aggregates a large amount of actin at the cell division, assembles the contraction ring, and maintains its structure after the formation of the contraction ring.

Lipid metabolism

The cofilin phosphorylation has always been considered inactive, but the p-cofilin can directly activate PLD1, then PLD1 increases intracellular phosphatidic acid (PtdOH) levels [78]. PLD1 is an enzyme that plays an important role in cell chemotaxis. The activation and localization of Rac are necessary for the chemotaxis of neutrophils. The accurate localization of Rac on the membrane depends on the activation of sufficient PtdOH, Rac in cells [79]. These studies further confirm that even in the inactive state of cofilin, cofilin can still make cells tend by regulating lipids and activating related signaling pathways [78, 80]. Some studies have pointed out that cofilin and moesin are also associated with lipid rafts, which are necessary to determine cell membrane distortions in the early stages of the cellular phagocytosis process, including cell membrane folds and phagocytic cup formation [81].

Transcription

We previously mentioned cofilin has the function of carrying actin into the nucleus, which also provides the basis for cofilin to affect the transcriptional activity of the nucleus. Cofilin-1 mainly affects the transcriptional activity of the nucleus by affecting the function of the RNA polymerase II, especially the elongation process of the gene coding sequence, and is not related to the promoter sequence in the untranslated region. One study showed that cell transcriptional activity was significantly reduced by silencing cofilin-1, and the correlation between actin and RNA polymerase II and gene coding regions was affected [82]. Some studies have also pointed out that active cofilin can through Src mediate transcription factor activation such as p-65, NF-κB and STAT1, and then affect cell transcriptional activity [82].

Other functions

Cofilin biological function category is gradually expanding with the deep research on cofilin in recent years. One new mechanism of cofilin is to respond to changes in environmental mechanical stress by regulating cell proliferation, a process that promotes remodeling of the actin cytoskeleton and also affects the activity of transcription coactivator Yes-associated protein 1 and Tafazzin [83]. Moreover, in addition to the NLS, cofilin also found the nuclear output signal, which also indicates that cofilin has the function of mediating the transport of substances from the nucleus. Current hotspot studies have pointed out that cofilin is also strongly associated with stress responses. Under stress conditions such as heat shock, osmotic stress, or ATP depletion, cofilin accumulate in the nucleus, where it over-binding and saturates actin filaments to form cofilin-actin rods, thus affecting the actin depolymerization process [84]. Besides the above functions, cofilin is also considered to be involved in chromatin remodeling, especially when the actin filament tension in the nucleus increases, the indentation site in the nucleus forms, which affects chromatin condensation. This regulation is generally the result of changes in contractility between actin filaments and actomyosin, a process mediated by cofilin that affects the function of chromosomes in the nucleus [84].

Clinical significance of cofilin

Cancer

For the past two decades, scientists have extensively studied the role of cofilin in tumor invasion ability, but this research has continued and has carried out new research directions in cancer metastasis. The lamellar pseudopodia play a major role in driving the migration of cancer cells, and cortical proteins regulate the formation of dendritic pseudopodia through phosphorylation and dephosphorylation, a number of studies have shown that metastasis of cancers is associated with cofilin-related mechanisms that regulate the movement of these cells [86].

Studies have been conducted to screen cofilin-1 as differentially expressed proteins in melanoma [87], ovarian cancer [88] tissues. The expression of activated cofilin-1 was significantly increased in cell lines such as breast cancer (MTLn3) [89], lung cancer (A549) [90] and prostate cancer (PC3) [91], while the expression of phosphorylated inactivated cofilin-1 was significantly decreased. Activated cofilin-1 could induce the formation of invasive pseudopodia, determine the migration direction of cancer cells, promote the

proliferation and invasion of cancer cells, and decrease the activation free cofilin-1 after knockout of cell lines, cell invasive pseudopodia production decreased or maturation disorder, cancer cell proliferation, invasion ability greatly weakened. Studies have found that cofilin-1 high expression is related to the formation of drug resistance mechanism after chemotherapy in lung adenocarcinoma and ovarian cancer, and provides a target for drug therapy [92].

The ROCK/Lin11, ISL-1 and LIMK/cofilin signaling cascades are thought to be crucial for the regulation of this link in the cofilin molecular regulatory mechanisms of cancer metastasis. More broadly, the factors that activate the signaling cascade mainly include receptor tyrosine kinases, G protein-coupled receptors, integrins and their ligands, growth factors, hormones, fibronectin, collagen, and laminin. This also provides a theoretical basis for ROCK/LIMK/cofilin signaling proteins as good candidates for cancer prevention strategies or treatment [93].

Some recent studies, for example, have shown that these molecules are associated with known signaling pathways that affect cofilin, like PLCY1, PLCβ1 which are considered another pathway for cofilin activation in breast cancer [94]. LIMK controls cancer cell migration by connecting signals from Rho families, altering cofilin activity and regulating actin dynamics. Whereas in lung cancer A549 cells and human osteoblastoma cells, there is a cofilin signaling pathway that responds to folate levels and stromal cell-derived factor-1 to regulate cell motility, respectively. A recent study implicates Pak1 is associated with the regulation of the Limk1/cofilin pathway, which is responsible for governing cell motility and morphologic changes, thereby facilitating cancer metastasis [95]. These new studies also suggest the correlation between cancer metastasis and ROCK/LIMK/cofilin signaling proteins, so some scholars have proposed therapeutic strategies to use ROCK inhibitor netarsudil to prevent cancer metastasis [93]. However, related clinical trials are still lacking, and researchers can regulate the cofilin activity of molecules as an important goal in the study of tumor metastasis prevention.

Neurological diseases

ADF/cofilin plays an important role in the improvement of the structure and function of the nervous system, such as growth cone extension, axonal transport, cell tail retraction and so on. Cofilin effects on nervous system diseases are mainly carried out in three aspects. (1) Involvement in neuronal differentiation: Talens-Visconti have found that the Rho protein family is widely distributed in the nervous system, and the RhoE molecules can promote the remodeling of actin to induce neuronal differentiation by inhibiting the signal pathway [93]. (2) Participation in neuronal movements: lee and other studies have found that the directional movement of neurons is closely related to the dynamic changes of actin [64]. The leading edge of neurons pushes the neurons forward through F-actin remodeling and pulls the neurons forward at the base through the depolymerization of the neurons. Marsick studies have found that nerve growth factors and netrin-1 are injected into temporal retinal nerve cells to activate cofilin signaling pathways in nerve cells to accelerate actin polymerization [97]. Growth cone cells (vegetative cone) that are suitable for growth in the environment eventually turn to growth. (3) The plasticity involved in synapses: actin cytoskeleton plays an important role in the structure and function of postsynaptic and synaptic plasticity [98]. Cofilin can accelerate the depolymerization of actin monomers by severing actin filaments, thus improving the turnover and deformation ability of actin. This characteristic makes cofilin have the ability to regulate the dynamic changes of dendrites and spinous processes of neurons. Zhang studies have found that when the Ser3 site of cofilin protein is in a non-phosphorylated state, the protein is activated, which causes the morphological structure of dendritic spine to deform [98].

Due to the role of these cofilin in the development of nervous system diseases, cofilin is mainly involved in the pathogenesis of epilepsy, Parkinson's disease, Alzheimer's disease, spinal cord injury, ischemic brain injury and neuropathic pain. In addition, it was also found that cofilin can play an important role in regulating the progression of spinal cord lateral bundle-related diseases [100]. Xiao and others found that the expression level of cyclin (cell division cycle 42, cdc42) in patients with epilepsy was significantly increased, which could cdc42 activate LIM1 and promote the phosphorylation of cofilin protein. The formation of neural cones and processes leads to the formation of abnormal neural circuits [101]. An LRRK2-PKA-cofilin signaling pathway is associated with Parkinson's disease. Parisiadou found out: after LRRK2 gene knockout, the activity of SPNs protein kinase A and postsynaptic density protein-95 in striatal projection neurons decreased, and the expression of the latter decreased, which led to the hyperphosphorylation of cofilin and hindered the maturation of dendrites [102]. Cofilin is associated with chronic neuroinflammation during Alzheimer's disease, a process that exacerbates the oxidative cascade of neurodegeneration by cofilin- actin complexes to accelerate mitochondrial decline and ATP depletion and destroy essential actin dynamics [103]. A model study of spinal cord injury in rats found that the Mst3b/LIMK1/cofilin system and involved in the pathogenesis and repair of spinal cord injury. When the Mst3b/LIMK1/cofilin system in the injured spinal cord cells is overactivated, the injured spinal cord cells have more growth cone branches, while in the contrast test, the pathway is inhibited by silencing Mst3b molecules.

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It was found that there were very few neural cones in this kind of spinal cord cells. The pathogenesis of ischemic brain injury is similar to that of this disease, both of which are due to the imbalance of cofilin and p-cofilin. This physiological process is related to hypoxic-ischemic brain damage, and may also be related to the occurrence of learning and memory dysfunction. Other studies have found that when various factors stimulate the sciatic nerve to cause its injury, the Rho/LIMK/cofilin system will be activated, causing neurogenic pain in the body, while in contrast tests, the use of Simvastatin to treat injured neurons can reduce the activity of the Rho/LIMK/cofilin system. Symptoms of neurogenic pain are also alleviated [104].

A number of recent studies have found it possible to treat neurological diseases by regulating cofilin activity and controlling actin. During treatment, cofilin activity-related neurological diseases can be treated by developing targeted and cell-specific peptide reagents. Current research needs to focus on developing NLG1-CTD peptides with higher affinity to bind and lead to SPAR degradation, but this peptide may provide a neuron-specific cofilin phosphorylation enhancer that works upstream of LIMK activation, and possibly long term stability through the use of D-amino acids, might be the future direction for research in this area [105, 106].

Kidney disease

The phenotypic characteristics of the inherent cells in renal tissue can be changed with different physiological and pathological states. Except for glomerular endothelial cells, all the other inherent cells have reported cytoskeletal proteins as phenotypic markers. As an important member of the cytoskeletal protein, silk fibroin also plays an important role in renal disease by regulating the actin cytoskeleton of various inherent cells of kidney. Cofilin-related renal diseases are associated with glomerular epithelial cells and renal tubular epithelial cells.

First, actin dynamics determines and maintains the normal morphology of renal podocytes during development, as well as its recovery after damage, and plays an important role in the branches of ureteral buds during renal development. The loss of cofilin-1 and destrin in the epithelium of the ureter bud in mice caused normal epithelial tissue to be disturbed, cell migration to be inhibited, and branch morphogenesis of the kidney was inhibited in the early stage, resulting in renal failure [107]. Mutant mice with specific knockdown of podocyte cofilin were unable to form podocyte secondary podocytes, resulting in typical podocyte fusion and a large amount of proteinuria and renal impairment were observed at three months [108]. Lee and others confirmed by in vitro experiments: TGF-β stimulation can cause phosphorylation of cofilin in mice and human podocytes [109]. Gary and others have confirmed in vitro that cofilin and nephrin are located on the cell membrane together, which can be dephosphorylated and activated by PI3K/SSH1L pathway to maintain the normal morphology and function of podocytes [110]. The specific knockout of podocyte cofilin gene can lead to podocyte injury and proteinuria. In mouse and human podocytes, cofilin and synaptic polar proteins are distributed in the same position. The therapeutic effect of cyclosporine A on massive proteinuria in nephrotic syndrome is related to the immune regulation of T cells and the stabilization of podocyte actin cytoskeleton by acting on synaptic polar proteins. Cyclosporine A can inhibit purine mycin-induced fusion of rat podocytes and decrease the expression of synaptic polar protein, reduce proteinuria, and increase the expression of cofilin. The effect of cyclosporine A on reducing urinary protein may be to stabilize the actin cytoskeleton of podocytes by up regulating the expression of cofilin. A large amount of evidence shows that high glucose and high osmotic pressure can activate the Rho-ROCK signaling pathway and inactivate the phosphorylation of cofilin in diabetes mellitus, which affects actin dynamics and is one of the pathogenic factors of diabetic nephropathy [111].

Secondly, several studies have found that cofilin promotes actin depolymerization and plays a key role in maintaining the polarity and function of proximal tubular epithelial cells [112]. Cofilin proteins play an important role in the acidification mechanism of renal tubular epithelial cells and the assembly and transportation macromolecular substances by depolymerizing filamentous actin [113]. When porcine proximal tubular epithelial cells were cultured in high glucose environment for 6 h, the of intracellular p-cofilin and LIMK1 increased in a time-dependent manner, which indicated that high glucose glucose could increase the phosphorylation of cofilin by activating RhoA/ROCK/LIMK1 signaling pathway [114]. Actin kinetics changes damage the polarity and function of proximal tubular epithelial cells. Recent studies also indicate that cofilin may regulate the inflammatory response of renal tissue in hypertensive nephropathy by affecting the nuclear factor kappa-B activity of renal tubular epithelial cells and nuclear translocation. grape seed proanthocyanidins can regulate this inflammatory response through antioxidant mechanisms [115].

Heart disease

Cofilin and actin alterations, as well as their interactions, have been shown to be important for the study of the pathogenesis of human myocardial disease. For example, the study noted that drug-stimulated cofilin-2 phosphorylation and gene overexpressed phosphorylation protein promote accumulated “stress-like” fibers and severely damaged cardiac cell contraction [7]. Cofilin-1 also correlates with the

severity of heart failure as an atrial natriuretic peptide, and the dual role of cofilin-1 in heart and renal failure may also be the reason why cofilin-1 could be used as a biomarker among cardiorenal syndrome patients [116]. Aggregates with presenilin-1 and cofilin-2 have been identified in up to one-third of idiopathic dilated cardiomyopathy cases studied, indicating the potential predominance of misfolded proteins in heart failure [117]. But the role of cofilin in heart disease is still lacking, and many intermediate links are unclear. Some studies have pointed out that cofilin-2 is a specific target for miR-21, and hydrogen sulfide can protect ischemia and inflammatory injury after myocardial ischemia by inducing microRNA-21. And 28 days after myocardial infarction, the expression of cofilin-2 promoting apoptosis has changed significantly [118]. Recent studies have shown that cofilin-2 and Bax form a protein complex in cardiomyocytes in response to oxidative stress [101]; therefore, it suggests that cofilin-2 may function as a Bax transporter of mitochondria. But overall, cofilin studies on the direction of heart disease are lacking.

Osteoarthritis

Cytoskeleton is a protein fiber grid system in eukaryotic cells, which plays an important role in the biomechanical or biochemical pathway of cell-specific stress conduction. Actin filaments are the main components of the cytoskeleton, providing the main viscoelastic solid features of chondrocytes. Studies have shown that destruction of the chondrocyte cytoskeleton is considered to be an important factor leading to a degeneration of articular cartilage. Cytoskeleton is involved in stress conduction in chondrocytes and may convert mechanical signals into biochemical signals, in which ADF/cofilin phosphorylation/dephosphorylation signaling pathways play an important role. When chondrocytes are subjected to certain physical or chemical stimulation, the free G-actin polymerizes to form F-actin, and microfilaments are formed through their own helix to complete the rearrangement of the cytoskeleton. Studies have shown that cofilin genes and proteins are highly expressed in chondrocyte model cells induced by mechanical force in the early stage of osteoarthritis, suggesting that cofilin gene expression is related to the biological characteristics of chondrocytes in the early stage of osteoarthritis [119]. Meanwhile, the cytoskeleton of osteoarthritis chondrocytes was rearranged under low concentration of leptin, which up regulated the protein content of cofilin and LIMK and inhibited the activity of cofilin protein [120]. Moreover, cofilin-1 is associated with many inflammatory conditions, such as osteoarthritis and inflammatory pain, and some investigations indicated that overexpression of cofilin-1 can decrease glucocorticoid receptor expression and nuclear factor kappa-B activity [121]. Additional, stabilizing actin filaments by inhibiting gene expression of the two main actin depolymerizing factors: cofilin 1 and destrin in hMSCs, enhanced cell viability and differentiation into osteoblastic cells in vitro, as well as heterotopic bone formation in vivo [122]. These studies provide a theoretical basis for targeted treatment of degenerative bone disease.

Concluding remarks

Through the discussion of the latest mechanism of cofilin action, we believe that the advantages of cofilin in clinical practice are gradually reflected, and the most used at present is its research as a biomarker of tumor, nervous system disease and heart/kidney disease. But we also cannot ignore the significance of cofilin as actin-binding protein that severs actin filaments to cell movement. As a result, the presence of cofilin is very important in the rearrangement of chondrocytes and neurons, which also provides a basis for targeted clinical treatment of osteoarthropathy and nervous system diseases. Current diseases resulting from changes in activity due to phosphorylation of actin-binding proteins, are often possible to control actin-binding activity by designing targeted drugs, and its potential cannot be underestimated [123]. For example, phosphorylation of many actin-binding proteins is regulated by RhoA/ROCK and the cAMP/PKA pathways [124], among the ADF/cofilin families discussed in this paper, these signaling pathways also play an important role, and we can also intervene by designing targeted drugs for these pathways. Because cofilin protein is widely existed in human body, when using its phosphorylation inhibitor as a treatment method, it often has systemic side effects, so we can develop some drugs or treatment methods for local application. There are also relevant literature reports that warm acupuncture in rat knee osteoarthritis by down-regulating the expression of chondrocyte cytoskeleton protein ROCK, p-cofilin and phosphorylated LIMK1 to reduce arthritis injury in rats with knee osteoarthritis. This kind of treatment is worthy of further discussion [119].

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