MINI-REVIEW

Cortactin function in invadopodia

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ABSTRACT
Actin remodeling plays an essential role in diverse cellular processes such as cell motility, vesicle trafficking or cytokinesis. The scaffold protein and actin nucleation promoting factor Cortactin is present in virtually all actin-based structures, participating in the formation of branched actin networks. It has been involved in the control of endocytosis, and vesicle trafficking, axon guidance and organization, as well as adhesion, migration and invasion. To migrate and invade through three-dimensional environments, cells have developed specialized actin-based structures called invadosomes, a generic term to designate invadopodia and podosomes. Cortactin has emerged as a critical regulator of invadosome formation, function and disassembly. Underscoring this role, Cortactin is frequently overexpressed in several types of invasive cancers. Herein we will review the roles played by Cortactin in these specific invasive structures.

Introduction
The actin cytoskeleton is involved in multiple cellular processes such as cell division, migration, or exocytosis. Monomeric globular actin (G-actin) is a 42kDa protein that polymerizes into filaments to form actin stress fibers or branched actin networks, which are required for the formation of various cellular structures including lamellipodia and invadopodia. G-actin polymerization in filaments is initiated by the association of three actin monomers forming a nucleus. This process, called actin nucleation, is highly unfavorable and requires actin nucleators such as formins or the ARP2/3 complex to allow the formation of unbranched or branched actin filaments, respectively. Conversely, actin nucleation is inhibited by proteins like Profilin or Thymosin-β4. The intrinsic nucleation activity of ARP2/3 is very low and the complex requires binding to other proteins, the Nucleation Promoting Factors (NPF), for activation. NPFs have been subdivided in two classes: class I NPFs include WASP (Wiskott-Aldrich Syndrome Protein) and SCAR/WAVE, they bind to both monomeric actin and to the Arp2/3 complex. On the other hand, class II NPFs such as Cortactin bind to actin filaments and are thought to recruit ARP2/3 to these filaments allowing branched network assembly. Cortactin only weakly promotes the nucleation activity of ARP2/3 but stabilizes newly generated actin branching points, preventing disassembly of the network. Cortactin (p80/p85) was identified in 1991 as a new Src substrate colocalizing with F-actin in cellular protrusions and podosomes. p80 and p85 are encoded by a single mRNA and the presence of two bands is caused by post-translational modifications, likely multiple phosphorylations, with phosphorylated S418 only found in p80 and S405 phosphorylation only found in p85. The conversion of p80 to p85 is associated with the relocalization of Cortactin from the cytoplasm to the cell cortex and sites of cell/matrix contacts. The associated gene (originally called EMS1, now CTTN) was cloned in 1992 and is located on chromosome 11q13. This region is frequently amplified in human breast cancer and in head and neck squamous cell carcinomas (HNSCC) and is associated with unfavorable clinical outcome. The interaction between Cortactin and F-actin was confirmed in 1993, with the identification of F-actin-binding repeats in the amino-terminal half of the protein and of an SH3 domain in the carboxyl-terminal part of Cortactin. Given its enrichment in cortical structures such as membrane ruffles and lamellipodia, and its binding to F-actin, the name of Cortactin was coined. The same year came the realization that EMS1, amplified in several cancers, and Cortactin are in fact the same protein that may...
promote the invasive potential of tumors, owing to Cortactin localization in cellular structures dedicated to invasion and migration. Since this work, Cortactin function in cell motility and invasion, as well as in other cellular processes, has been intensely investigated and Cortactin is widely used as a marker of invadosomes. Given the domain structure of Cortactin (Fig. 1) and the many interactors and regulators identified, Cortactin is considered as a scaffold protein.

**Regulation of Cortactin expression and stability**

Human Cortactin is encoded by the CTTN gene, located on the long arm of chromosome 11 and is expressed ubiquitously, except in hematopoietic cells, which instead express the Cortactin paralog HS1. Only few studies have investigated the regulation of Cortactin expression. As far as we know, Cortactin mRNA level is increased after activation of CD44 hyaluronan receptor activation via NFkB signaling and after binding of phospho-STAT3 to the Cortactin promoter. Conversely, Cortactin expression is decreased by miR-542-3p and miR-326. Thus, Cortactin might be mostly regulated by post-translational modifications and interactions with others proteins.

Cortactin has three isoforms generated by alternative splicing. The SV-1 and SV-2 splice variants are deleted for exon 11 or exon 10 and 11, respectively, corresponding to the 6th or 5th and 6th actin binding repeats (Fig. 1). The SV-1 variant is co-expressed with full-length Cortactin in all tissues whereas the SV-2 variant is absent from several tissues and has a decreased ability to bind F-actin and to induce actin filament polymerization. Alternative splicing of Cortactin mRNA is regulated by the RNA binding protein Rhbox2, which induces exon 11 exclusion after induction of epithelial-

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**Figure 1.** Domain structure of Cortactin and interacting partners. Cortactin is composed of an N-terminal acidic domain that allows the interaction with Arp2/3, followed by six and a half F-actin binding repeats of 32 amino acids that mediate binding to F-actin and can be acetylated on lysines or phosphorylated on serines. On its C-terminal part, Cortactin has a helical domain, a proline-rich domain that is extensively regulated by phosphorylation and whose tyrosines are targeted, among others, by Src-family kinases. Finally, Cortactin has an SH3 domain at its C-terminal end, which binds many different proteins mostly involved in the regulation of actin cytoskeleton dynamics, including N-WASP. The known binding partners of Cortactin are indicated close to the domain to which they bind to or target, when known, or in the lower part of the figure when unknown. Lysines targeted for acetylation are indicated by @ (amino acids 87, 124, 144, 161, 181, 198, 235, 272, 295, 304, 309, 346). The amino acid numbering refers to the mouse protein (NP_031829.2).
mesenchymal transition (EMT) by TGF-β.\textsuperscript{18} PTBP1, another RNA-binding protein, is also involved in Cortactin mRNA splicing. Indeed, PTBP1 induces the inclusion of exon 11, favoring the expression of full-length Cortactin and in this way tumor cell migration and invasion.\textsuperscript{19} However, the specific contribution of each Cortactin isoform to the different functions of Cortactin remains to be elucidated.

Cortactin degradation was reported to be induced by its phosphorylation by ERK on Ser405 and Ser418, which induces the interaction between Cortactin and β-TrCP, an E3 ubiquitin-ligase, and its degradation by the proteasome.\textsuperscript{20} The E3 ubiquitin-ligase Hakai binds tyrosine-phosphorylated Cortactin via a novel phosphorytysine-binding domain.\textsuperscript{21} It remains unclear however whether Hakai targets Cortactin for destruction by the proteasome. The sites phosphorylated by ERK and Src have been shown to promote Cortactin activity towards actin polymerization and cell migration/invasion\textsuperscript{22,23} and targeting Cortactin phosphorylated on these sites for degradation could constitute a means to downregulate its activity. Another means to repress Cortactin activity is via cleavage by Calpain.\textsuperscript{24}

**Functions and post-translational modifications of Cortactin**

As far as we know, Cortactin is mainly regulated by post-translational modifications. Cortactin is a substrate of Src family tyrosine kinases, which phosphorylate Cortactin on tyrosines located in its proline-rich region (Fig. 1),\textsuperscript{25-27} notably after homophilic interactions between E-cadherin or N-cadherin.\textsuperscript{28,29} Tyrosines in the proline-rich domain are also targeted by Arg and Abl, two Abl-family tyrosine kinases, after PDGF\textsuperscript{30,31} or EGFR\textsuperscript{22} stimulation or β1 integrin activation.\textsuperscript{32} Cortactin can also be phosphorylated on serines and threonines mainly located in the F-actin binding repeats and proline-rich region, regulating Cortactin function, including actin polymerization.\textsuperscript{33} Acetyltransferases and deacetylases control acetylation on several lysines within the F-actin binding repeats, also regulating Cortactin localization and activity.\textsuperscript{34-41}

The main function of Cortactin is to promote the formation of branched actin networks. Several studies have shown that the Arp2/3 complex binds Cortactin on its N-terminal acidic (NTA) domain and this interaction promotes actin nucleation, supporting a NPF function for Cortactin.\textsuperscript{4,42} The interaction between Cortactin, Arp2/3 and F-actin takes place at the cell periphery and induces branched actin generation, thereby promoting migration and invasion.\textsuperscript{42,43} On the other hand, the protein MIM (Missing-in-Metastasis, MTSS1), which binds to the SH3 domain of Cortactin, inhibits actin polymerization and cell migration, possibly by opposing N-WASP activity.\textsuperscript{44} This antagonistic relation was found to play a critical role during ciliogenesis, where MIM inhibited Src-mediated Cortactin phosphorylation, thereby promoting cilia formation.\textsuperscript{45}

Cortactin binds to N-WASP and WIP (WASP-interacting protein) via its SH3 domain and since Cortactin is an Arp2/3 complex activator, it was first believed that Cortactin activated Arp2/3 via its interaction with N-WASP,\textsuperscript{46,47} but it appears that the mechanism is more complex than initially thought.\textsuperscript{1,44,48,49} Indeed, Cortactin can promote the interaction of Arp2/3 with F-actin,\textsuperscript{4,42} which activates Arp2/3. In addition, Cortactin directly stabilizes branched actin networks by remaining at newly formed branch points with Arp2/3, unlike N-WASP that is released, preventing the dissociation of branched actin.\textsuperscript{4} Cortactin also binds WAVE2, another WASP-family protein member, and this interaction induces actin polymerization.\textsuperscript{50} The Cortactin/WAVE2 interaction is inhibited by SKAP2 (Src Kinase-Associated Phosphoprotein 2), a Src substrate, and promoted by Cortactin phosphorylation on Ser405 and Ser418 by PKC.\textsuperscript{51,52} Phosphorylation of these serines by ERK also promotes N-WASP activation and actin polymerization.\textsuperscript{53} Due to its role in branched actin polymerization, Cortactin has a major function in invadopodia regulation which is described below.

Numerous studies have shown that Cortactin promotes cell migration in different cell types and by different mechanisms,\textsuperscript{16,42,54,55} often following phosphorylation by various kinases. Cortactin phosphorylation on Tyr421 and Tyr466 by Src, Fyn and Fer kinases,\textsuperscript{25,27,56} as well as on Thr401/Ser405 and Ser417/Ser418 by Akt or ERK,\textsuperscript{43,57} is pro-migratory. Although Cortactin is used as a lamellipodia marker and regulates actin nucleation in lamellipodia, it is not required for lamellipodia formation.\textsuperscript{54,55,58,59} As a matter of fact, Cortactin, whose localization in lamellipodia is regulated by several proteins (p120 catenin,\textsuperscript{60} BPGAP1,\textsuperscript{61} NEDD9,\textsuperscript{39} Rac1,\textsuperscript{62} Arp2/3\textsuperscript{43} and GIT-1\textsuperscript{63}), is required for lamellipodia persistence\textsuperscript{54,58,60} and for the regulation of actin dynamics in lamellipodia downstream of Rho GTPases and Dynamin.\textsuperscript{54,59,64-66} This regulation could be mediated by the interaction of Cortactin with different Guanine-nucleotide Exchange Factors (GEFs) and GTPase-Activating Proteins (GAPs) such as Fgd1 and BPGAP1.\textsuperscript{61,67}

Recent work supports an important role for lysine acetylation of Cortactin in the regulation of cell migration. The histone deacetylases HDAC6 (class II) and Sir2uin1 (class III) modulate the acetylation levels of Cortactin within its actin-binding repeats and consequently promote the interaction between Cortactin and...
Furthermore, the scaffold protein NEDD9 recruits HDAC6 on Cortactin, promoting its deacetylation and localization at lamellipodia. This effect could be explained by a recent study providing evidence that deacetylated Cortactin binds KEAP1 (Kelch-Like ECH-Associated Protein 1) to be exported from the nucleus, allowing its transport to lamellipodia. In endothelial cells, HDAC6-mediated Cortactin deacetylation increases cell migration and is required for angiogenesis. Cortactin can also be deacetylated by KDAC8 (HDAC8) in vascular smooth muscle cells but the specific effects of this deacetylation remain to be investigated. Cortactin acetylation is mediated by several acetyltransferases, including p300, CBP when Cortactin is nuclear, ATAT1, as well as Tip60, which is recruited on Cortactin by Fe65. Most studies support an inhibition of cell migration by Cortactin acetylation.

The many functions of Cortactin

Aside from its role in migration and invasion, Cortactin is involved in the regulation of a wide variety of cellular processes that will be briefly described here. Another function of Cortactin is its involvement in vesicle trafficking. First, Cortactin regulates clathrin-dependent endocytosis and Golgi transport in association with Dynamin2. Cortactin/Dynamin2 interaction is promoted by Cortactin phosphorylation on Ser261 by aPKC and allows MT1-MMP trafficking. Cortactin function in clathrin-dependent endocytosis involves branched actin polymerization, which was negatively regulated by Hip1R (Huntingtin Interacting Protein 1 Related) and PIP2 and positively by Ack1 (Tyrosine Kinase Non Receptor 2, TNK2) and CD2AP during EGFR endocytosis. Cortactin also regulates clathrin-independent endocytosis and has been involved in IL-2 receptor endocytosis via a Rac1/PAK1-2/P-Ser405-Ser418-Cortactin pathway, which increased Cortactin/N-WASP binding and actin polymerization required for endocytosis. A recent study has shown that Cortactin promotes exosome secretion by controlling both trafficking and plasma membrane docking of multivesicular late endosomes with the Arp2/3 complex. This role of Cortactin in promoting vesicle trafficking is important in the context of invadosome function, which, when mature, secretes proteases in the intercellular space to digest matrix. By controlling vesicle trafficking, Cortactin also regulates autophagy under control of HDAC6 and INPP5E by inducing actin remodeling and stabilization, allowing autophagosome-lysosome fusion and substrate degradation.

Cortactin also plays a role in the formation of new focal adhesions. Cortactin binds to and is phosphorylated by FAK, and this interaction promotes focal adhesion turnover and cell motility.

Cortactin is involved in the response against different stresses that induce cytoskeletal remodeling to protect cells. For example, shear stress in vascular endothelial cells activates AMPK, which phosphorylates Cortactin on Thr401, priming Cortactin for deacetylation by Sir2ultin1 and allowing actin remodeling. During hyperosmotic stress inducing cell shrinkage, Cortactin is phosphorylated by Fyn and Fer kinases to mediate cytoskeletal rearrangements needed for osmoprotection. The phosphatase PTP1B also controls Cortactin tyrosine phosphorylation to prevent apoptosis induced by hyperosmotic stress.

Finally, Cortactin also regulates membrane excitability by linking the actin cytoskeleton and several ion channels, such as calcium-activated (BK) channels, potassium channels Kv1.5, Kv10.1 and sodium channels ENaC, or by regulating channel endocytosis, as reported for potassium channel Kv1.2.

During neurogenesis Cortactin appears to plays several roles: it regulates the balance between stable neurite shaft and the formation of new growth cone, the collateral branching of axons via the control of filopodia formation, dendritic spine formation and traction force generation in axonal growth cones via an interaction with Shootin.

Cortactin in cancer

Overexpression of Cortactin is frequently observed in many types of cancers, including head and neck squamous cell carcinoma (HNSCC), hepatocellular carcinoma, breast cancer, bladder cancer, renal cell carcinoma, esophageal squamous cell carcinoma, colorectal adenocarcinoma, melanoma, osteosarcoma, prostate cancer, non-small cell lung cancer, glioma, epithelial ovarian cancer, thyroid cancer and B-cell chronic lymphocytic leukemia. This overexpression is partially caused by the amplification of chromosome 11q13 where
the *CTTN* gene is located. For instance, *CTTN* amplification is found in 60–68% of esophageal cancer,\(^{101,111}\) 20–37% of HNSCC\(^{112,113}\) and almost 60% in oral squamous cell carcinoma, a HNSCC subtype.\(^{114}\) 14% of hepatocellular carcinoma,\(^{115}\) 15–26% of breast cancer\(^{116,117}\) and 11% of bladder cancer.\(^{99}\) Nevertheless, several studies showed that *CTTN* or 11q13 amplification does not always explain Cortactin overexpression, suggesting that it may be caused by other mechanisms. For example, in esophageal cancer, Cortactin expression is induced by VEGF-C, which decreases Dicer-mediated maturation of miR-326, thereby relieving the suppressive effect of miR-326 on Cortactin expression.\(^{15}\) Furthermore, in a wide variety of human tumors, the frequent constitutive activation of STAT3, which targets the *CTTN* promoter,\(^{11}\) may underlie Cortactin overexpression.\(^{118}\)

Cortactin overexpression is consistently associated with poor prognosis and decreased patient survival in most cancers.\(^{98,100,102-105,108,110}\) Some studies suggest that phospho-Y421-Cortactin levels are also elevated in cancer and associated with poor prognosis.\(^{119,120}\) Recently, two studies focused on deciphering the involvement of each Cortactin isoform in carcinogenesis and this work suggest that full length Cortactin is overexpressed compared to the SV-1 isoform and may be responsible for the oncogenic role of Cortactin.\(^{19,110}\) This could be explained by the overexpression of PTBP1, which regulates alternative splicing of Cortactin.\(^{19}\) However, the specific contribution of each Cortactin isoform to oncogenesis is still unclear and needs to be investigated.

A major conclusion from clinical studies is that Cortactin overexpression is associated with local invasion, lymph node metastasis and/or distal metastasis in almost every cancer in which it is overexpressed.\(^{19,96,97,99,101,103,106,109,113}\) Moreover, several mouse models have provided evidence that Cortactin promotes the metastatic process.\(^{97,101,121}\) Altogether, these findings indicate that Cortactin plays an important role in promoting tumor invasion and metastasis, consistent with the major role of Cortactin in regulating lamellipodia and invadopodia formation.

**Cortactin in invadopodes**

During embryogenesis or wound healing, cells have to move. They also move during tumor invasion. Cell movement requires that they invade into their surroundings formed by a dense network of extracellular matrix (ECM) proteins. For this, the cell first attaches to the ECM, degrades it, and moves into the newly liberated space. Invadopodes, which designate invadopodia made by cancer cells and podosomes made by normal cells, are specialized cellular structures that enable all these steps.\(^{122}\) Invadopodes are very dynamic, with a half-life of a few minutes. After a stimulus, they first assemble at sites of ECM interaction with the cell, they then release proteins that degrade the matrix, such as matrix metalloproteases (MMPs), and finally disassemble again, allowing cell movement (Fig. 2A). Invadopodia often become activated in cancer cells, which allows tumor cells to invade either locally or throughout the body to form metastases. Many proteins regulating invadopodia formation have been identified, including receptors to growth factors or ECM, scaffold proteins, kinases and GTPases. Amongst them, Cortactin plays a major role in all steps of the invadopode lifecycle.

**Initiation**

Invadopodia formation is induced by different cellular signals which can be divided into 5 types: (i) growth factors such as EGF, PDGF or TGF-β\(^^{123}\) activating their receptors; (ii) oncogenic transformation induced by oncogenes like v-src or Ras\(^^{124,125}\); (iii) EMT induction\(^^{126}\); (iv) cellular environment, including matrix composition,\(^^{122}\) heterotypic cell interaction with macrophages\(^^{127}\) or hypoxia\(^^{128}\); and (v) metalloproteases activity.\(^{123}\) After one of these stimuli, several signaling pathways are activated. Among them, Src seems to play a central function by activating Cdc42 GEFs required for invadopodia formation (Vav-1, β-PIX and Fgd1,\(^^{123}\)) but also Tuba, which regulates linear invadopodes\(^^{129}\) and by phosphorylating scaffold proteins like Tks5 and Cortactin. Cortactin plays a central role by scaffolding several proteins required for invadopodia assembly at the initiation site.

Several signaling pathways like Src, PAK or Erk\(^{130}\) converge to Cortactin to regulate its function in invadopodia via phosphorylations. Nevertheless, Cortactin phosphorylation by PAK1 may have antagonistic effects depending on cell context. Indeed, Cortactin phosphorylation on Ser113 by PAK1 increases ECM degradation\(^^{130}\) but also induces invadopodia disassembly.\(^{131,132}\) Stimulation of c-Met by HGF induces invadopodia formation and cell invasion mediated by an interaction of Cortactin with Grb2 and Gab1.\(^{133,134}\) Furthermore, c-Met directly binds Cortactin and induces its phosphorylation, but whether c-Met directly phosphorylates Cortactin is still unclear.\(^{133,134}\)

**Assembly**

Invadopodia assembly is driven by branched F-actin generation and the formation of a cellular protrusion. The first step is the assembly of the invadopodial core, with the recruitment of Cortactin, which scaffolds Arp2/3, Cofilin and N-WASP, to the membrane. Even if Tyr421
and Tyr466 of Cortactin can be phosphorylated by Src, at the invadopodia, it seems that they are mainly targeted by the tyrosine kinase Arg. Arg is activated both by β1-integrin and the EGFR/Src pathway. However, Arg activation seems to have opposite roles according to the cellular context. Indeed, in mammary tumor cells, Arg promotes invadopodia formation, whereas it has an inhibitory role in squamous cell carcinoma cells.

Cortactin phosphorylation on Y421 and Y466 allows its association with Nck1, which recruits N-WASP to Cortactin, as well as Vav2, promoting the generation of free actin barbed ends and branched actin polymerization.

Inside the invadopodial core, Cofilin plays a major role. Cofilin, an actin filament-severing protein, generates new free actin barbed ends, promoting branched actin formation by the Arp2/3 complex. Cortactin inhibits Cofilin’s severing activity inside the invadopodia. This inhibition is relieved when Cortactin is phosphorylated on tyrosines (Y421, Y466 and Y482), allowing the recruitment of NHE1, which induces a local increase of pH. This increase promotes the dissociation of Cortactin and Cofilin, allowing Cofilin to sever actin filaments and to promote Arp2/3 activity. Cofilin scaffolds ARP2/3, F-actin barbed ends and N-WASP to promotes actin nucleation. After invadopodia formation, Cortactin is dephosphorylated, inhibiting again Cofilin to allow invadopodia growth and stabilization. Cofilin activity is also spatially regulated by RhoC, whose active form surrounds invadopodia and activates the ROCK/LIMK pathway, maintaining Cofilin in its Ser3-phosphorylated (a target of LIMK), inhibited form, thus concentrating Cofilin activity within the invadopodial core. The regulation of RhoC activation state is mediated by p190RhoGEF and p190RhoGAP as well as Podoplanin. Cofilin function in invadopodia is
also regulated after transient biomechanical forces via β3-integrin signaling.142

Invadopodia maturation is promoted by the Mena isoform MenaINV, an actin barbed-end capping protein antagonist. MenaINV is recruited just after invadopodia formation initiates and promotes Tyr421 phosphorylation of Cortactin, possibly by displacing PTP1B from the invadopodial core and preventing Cortactin dephosphorylation on Tyr421.143 By displacing actin barbed end capping proteins, Mena also promotes branched actin polymerization in invadopodia. The phosphatase SHIP2 is also involved in invadopodia assembly by controlling Mena recruitment as well as PI(3,4)P2 accumulation, which promotes Tks5 recruitment via its phox homology (PX) domain.144,145

Even if branched actin generation by Arp2/3, N-WASP, Cortactin and Cofilin is required for invadopodia formation, several studies found that unbranched actin is also present in invadopodia. Indeed, different F-actin bundling proteins such as α-Actinin, Fimbrin or Fascin are present in invadopodia and Fascin knockdown decreases invadopodia formation and ECM degradation.146,147 Moreover, formins from the DRF family are also involved in these two processes, confirming that unbranched F-actin is important for invadopodia formation and activity.148

Finally, Cortactin function in invadosome is also regulated by Caldesmon, which inhibits invadopodia formation by an unknown mechanism,149-151 but could involve a competition with Arp2/3 complex binding to Cortactin, since Caldesmon also binds to the NTA domain of Cortactin,149 or a direct inhibition of Arp2/3 in invadopodia.152

Maturation

During the maturation stage of the invadopodia lifecycle, the structure is transiently stabilized and the surrounding ECM is degraded by proteases. Several proteases degrade ECM at invadopodia, including MMPs, ADAMs, Cathepsins and serine proteinases.153 MT1-MMP (also called MMP-14) has a preponderant function at invadopodia to degrade ECM.154 Proteases recruitment involves kinesin activity along the microtubule network to bring them via vesicles from the Golgi apparatus, which often localizes close to invadopodia, and then vesicles merge with the plasma membrane.155 ECM stiffness and rigidity also promote degradation activity at invadopodia.156-158 Several studies investigating MT1-MMP delivery to plasma membrane have shown that it is regulated by the exocyst complex, an 8-protein complex involved in vesicle trafficking regulated by IQGAP1 (under control of Cdc42 and RhoA) and the WASH complex.159,160 MT1-MMP delivery is also regulated by the v-SNARE VAMP-7 and negatively regulated by CIP4 and SNX9, two Src-substrates.161-163 Tks5 promotes Rab40b-mediated transport of MMP-2 and MMP-9 to invadopodia.164 Cortactin has a major function in matrix degradation and MMP-2, MMP-9 and MT1-MMP secretion.154,165,166 Indeed, the cytoplasmic tail of MT1-MMP is phosphorylated by LIMK, allowing its interaction with Cortactin which is required for its trafficking to invadopodia.167 A recent study shows that Cortactin phosphorylation by PKCβ allows its association with Dynamin-2, promoting trafficking of MT1-MMP containing endosomes.70 Cortactin acetylation levels also appear to regulate its role in MT1-MMP transport and ECM degradation.40 Recent evidence has shown a key role for Cortactin in late endosomal vesicle trafficking and exosome secretion.77 The function of Cortactin in trafficking of others proteases, such as MMP-2 or MMP-9 that also have an important function in invadosomes, still needs to be investigated. Together, evidence suggests that Cortactin acts as a hub for both invadopodia formation and function.

Disassembly

Invadopodia disassembly is clearly the least understood step of the invadopodia lifecycle. Nevertheless, recent studies seem to involve two different pathways in the regulation of this critical step. The first pathway begins with the activation of Rac1 by one of its GEFs, Trio, whose upstream activators are not described yet. In turn, active Rac1 activates its effector PAK1, which is recruited by p27 on Cortactin and phosphorlates Cortactin on Ser113, S150 and/or S282131,132 (Fig. 2B). Inhibiting this pathway increases invadopodia lifetime, thereby increasing invadopodia number and matrix degradation. Surprisingly, cell invasion is negatively affected by inhibition of this pathway, showing that invadopodia turnover is required for an efficient invasion.131,132 The mechanism involved in invadopodia dissolution after PAK-mediated Cortactin phosphorylation is still unclear but may be due to a decreased affinity of Cortactin phosphorylated within its actin-binding repeats for F-actin, which may destabilize invadopodia.168

A second pathway inducing invadopodia disassembly has been described recently. RhO, a Rho GTPase of the Rac subfamily, promotes the phosphorylation of Paxillin.169 Then, phosphorylated Paxillin induces invado-some dissolution by an ERK/Ca^2+ pathway.170 Calpain is a cysteine protease that promotes podosome disassembly by cleaving Talin, Pyk2 and WASP.171 However, since Talin and Pyk2 functions have mainly been described in podosomes, it is unclear if these proteins are also Calpain substrates in invadopodia. As Cortactin is a
substrate of Calpain,\textsuperscript{172} it would be interesting to investigate if Calpain function in invadopodia disassembly could be mediated by Cortactin cleavage.

The pathway involved in invadopodia disassembly seems dependent on the upstream pathway activated or on the cell type investigated. Indeed, in cells where RhoG induces invadopodia disassembly, Rac1 is involved in invadopodia initiation.\textsuperscript{169} Reciprocally, RhoG knock-down in cells where Rac1/PAK1/Cortactin promotes invadopodia disassembly does not affect invadopodia turnover.\textsuperscript{131} Further investigations are needed to fully understand the pathways that lead to invadopodia disassembly.

Conclusion and perspectives

Since its identification, numerous studies have described roles for Cortactin in a wide variety of cellular processes including cell migration, vesicle trafficking or neurite outgrowth. Most of these functions appear to relate to its ability to regulate actin cytoskeleton dynamics, either directly or via the scaffolding of proteins involved in signaling pathways that impinge on the actin cytoskeleton. An outstanding question to be addressed is the contribution of each splice variants of Cortactin in its different functions. The best characterized function of Cortactin is in the regulation of cell migration and invasion, which probably underlies its frequent overexpression in several metastatic cancers, as Cortactin is involved in every step of the invadopodia lifecycle, allowing matrix degradation and tumor invasion.

Similarly, Cortactin is present in podosomes in different types of cells, such as v-Src transformed fibroblasts,\textsuperscript{173} vascular smooth muscle cells,\textsuperscript{174} osteoclasts\textsuperscript{175} and dendritic cells.\textsuperscript{176} Cortactin is recruited to podosomes via Tks5\textsuperscript{177} and acts as a scaffold to recruit several proteins required for podosome formation, including N-WASP,\textsuperscript{173} Fgd1,\textsuperscript{178} AFAP1L1\textsuperscript{179} or ZO-1.\textsuperscript{180} Unlike what is described in invadopodia, Cortactin also regulates microtubule dynamics\textsuperscript{181} and actomyosin contractility in podosomes.\textsuperscript{174} It is interesting to notice that HS1, the Cortactin homologue expressed in hematopoietic cells, has a function in the regulation of podosome organization in dendritic cells.\textsuperscript{182} Overall, Cortactin is involved in the formation,\textsuperscript{183} maturation\textsuperscript{175} and matrix degradation\textsuperscript{176} at podosomes but a potential role of Cortactin in podosome disassembly remains to be investigated.

Despite the numerous articles characterizing invadopodium function in vitro, there are much less reports studying them in vivo, undoubtedly due to the technical difficulty to observe such ephemeral structures within tissues or organisms. A first line of evidence of invadosomes’ existence in vivo is provided by the fact that in different cancers, many proteins playing a major role in invadopodia formation or maturation are associated with poor prognosis or metastasis, including Cortactin, Fascin, Fgd1, MT1-MMP or Tks5.\textsuperscript{184} In mouse models, the use of an shRNA against N-WASP dramatically reduces the ability of cells to form cellular protrusions, intravasate or metastasize.\textsuperscript{185} Similarly, mammary tumor cells in which Tks5 is knocked-down lose their ability to invade locally or to form distant metastases.\textsuperscript{126} Moreover, primary cells derived from patients with different types of cancer form invadopodia enriched in Cortactin in culture, which are structurally identical to those observed in cell lines.\textsuperscript{185}

Different studies have directly observed invadosomes in in vivo models. When they intravasate, tumor cells form protrusions which resemble invadopodia\textsuperscript{186} and this phenomenon is also observed in zebrafish.\textsuperscript{187} In the nematode C. elegans, anchor cells generate invasive protrusions to break down the basement membrane in response to inducing signals.\textsuperscript{188} In mice, Cortactin- and Tks5-enriched cellular protrusions have been observed in xenografted tumor cells by immunohisto-fluorescence, suggesting invadopodia presence in vivo.\textsuperscript{126,185} Finally, in recent work using the chorioallantoic membrane model in chicken embryos, human tumor cells injected in capillaries were observed extravasating by intravital microscopy.\textsuperscript{189} This approach has shown that, during extravasation, cells form membrane protrusions enriched in Cortactin, Tks4 and Tks5 allowing them to breakdown blood vessel walls.\textsuperscript{189} Knockdown of Cortactin with shRNA inhibited tumor cells extravasation. This study validates in vivo the presence of invadopodia in tumor cells and their role in extravasation.\textsuperscript{189} Nevertheless, further investigations are still required to fully understand the function of invadopodia in tumor cell invasion and the specific roles of Cortactin in this process.

Acknowledgments

The Besson group is supported by funds from the Fondation ARC pour la Recherche sur le Cancer and by an “FRM Equipes” grant (DEQ20170336707) from the Fondation pour la Recherche Médicale.

Funding

Fondation pour la Recherche Medicale, DEQ20170336707.

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