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Invasion by actin-driven membrane protrusions: Cortactin in focus

Cortactin's multiple signaling domains

The actin binding protein cortactin plays an important role in several cellular functions involving plasma membrane changes that are dependent on a dendritic (i.e., branched) actin network: cell motility employing lamellipodia, clathrin dependent and independent endocytosis, host-pathogen interactions, maintenance of endothelial barrier integrity, and invadopodia-mediated cell invasion[1]. Cortactin is a monomeric ~80 kDa protein that derives its name from its intracellular colocalization with cortical actin at the periphery of the cell[2]. The amino terminus of cortactin harbors a domain rich in acidic amino acids (N-terminal acidic domain; NTA) that interacts with the seven subunit Arp2/3 complex that promotes dynamic actin filament branching[3]. Cortactin's NTA domain contains a DDW (Asp-Asp-Trp) motif that has been observed in the Arp2/3 binding regions of other known actin nucleation promoting factors (NPFs) as either DDW or DEW (e.g., WASP, N-WASP, Myo3, ActA)[4] (Fig. 1). NPFs are divided into two classes based on their role in promoting Arp2/3-mediated filamentous actin (F-actin) branching[5]. Class I NPFs like those mentioned above have a primary role in promoting Arp2/3 activation to drive filament branching, whereas Class II NPFs like cortactin function in branch formation and stabilization of the dynamic branched actin assembly[6,7]. Notably, while cortactin can directly activate Arp2/3 mediated F-actin branching, this activity is much weaker than is observed for Class I NPFs[6]. This is due in part to cortactin lacking the monomeric globular actin (G-actin) binding domain of class I NPFs[5]. Consistent with cortactin's role in stabilizing branched filaments, it has a central domain with 6.5 repeats of a 37-residue long sequence that binds F-actin[2,4] (Fig. 1). This domain is followed by a helical region and a proline-rich region, containing multiple sites of post-translational modifications (PTMs), and an SH3 domain that is used to recruit other proteins, including the Class I NPF N-WASP to the Arp2/3 complex at the filament branch point (for a list of binding partners, see ref. 8).

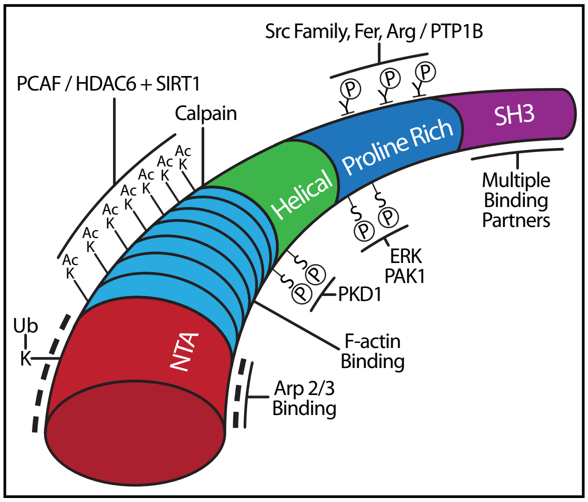

Figure 1: Schematic diagram of cortactin's primary structure with key post-translational modifications and binding domains highlighted.

The association of cortactin with aggressive cancers

The F-actin rich cellular protrusions known as invadopodia were originally identified in human cancer cell lines and were named for their invasive nature[9]. Invadopodia are 0.8-1 µm diameter membrane extensions that are 2-5 µm in length and are found on basal membranes that face the extracellular matrix (ECM)[10]. Cortactin is essential for invadopodia formation and for their focused delivery of matrix metalloproteases to promote ECM degradation and promote cellular invasion into neighboring tissues[10-12]. Several human cancers have been shown to exhibit elevated expression of cortactin including breast, colorectal, ovarian, hepatic, gastric, esophageal, melanomas, and glioblastomas[8,13]. Consequently, cortactin has become an important biomarker for malignant metastatic cancers and its elevated expression is frequently associated with a poor patient prognosis.

Continued from Page 1

The importance of cortactin post-translational modifications

The regulation of cortactin's activity through PTMs is very complex and growing evidence points to a high degree of cross-talk between different PTMs (Fig. 1). Phosphorylation of four serine residues (S405, S418, S298 and S348) by one or more kinases (ERK, PAK1, or PKD1) regulates cortactin's activity[8]. ERK phosphorylation on S405/S418 in the proline-rich domain of cortactin is thought to induce a conformational change that exposes the SH3 domain, allowing the binding and activation of class I NPFs N-WASP or WASP[14]. In contrast, phosphorylation of tyrosine residues Y421, Y466, and Y482 by Src family kinases, and presumably other tyrosine kinases that target these residues[15,16], results in the loss of cortactin's ability to promote Arp2/3-mediated F-actin branching either directly or through indirect activation and recruitment of N-WASP or WASP[14,17]. This reciprocal relationship between serine and tyrosine phosphorylation has been termed the "S-Y switch"[18]. It is interesting that both serine and tyrosine phosphorylation of cortactin act as signals for its degradation. ERK phosphorylation of the aforementioned serines acts as a trigger for K79 ubiquitination through cortactin interactions with the β-Trcp subunit of the E3 ligase complex, which ultimately leads to its proteosomal degradation[19]. In a similar manner, tyrosine phosphorylation of cortactin is a signal for its cleavage by the calcium-dependent protease calpain[20]. Cortactin's ability to interact with F-actin, and consequently activate Arp2/3, is also modulated by lysine acetylation/deacetylation mediated in part by PCAF and HDAC6, respectively[21]. Interestingly, recent evidence suggests a competition exists between tyrosine phosphorylation and lysine acetylation even though both PTMs negatively affect the activity of cortactin[22]. This alludes to yet another layer of complexity in the cross-talk of cortactin PTMs and it will be exciting to see how the details of these interconnected signaling pathways converge to regulate cortactin at the actin filament branchpoint.

As new actin research tools, including those to study the PTMs of actin and its associated proteins, become available, the physiological functions of cortactin will be further elucidated using purified actin and actin binding proteins from Cytoskeleton, Inc.

Actin Related Research Tools

Protein	Source	Purity	Cat. #	Amount
Actin Protein	Rabbit skeletal muscle	>99%	AKL99-A AKL99-B	4 x 250 ug 2 x 1 mg
Actin Protein	Human platelet, non-muscle	>99%	APHL99-A APHL99-B	2 x 250 ug 1 x 1 mg
Pre-formed Actin Filaments	Rabbit skeletal muscle	>99%	AKF99-A AKF99-B	1 x 1 mg 5 x 1 mg
Pyrene Actin Protein	Rabbit skeletal muscle	>99%	AP05-A AP05-B	1 x 1 mg 5 x 1 mg
Biotinylated Actin Protein	Rabbit skeletal muscle	>99%	AB07-A AB07-C	5 x 20 ug 20 x 20 ug

Kit	Cat. #	Amount
G-actin/F-actin In Vivo Biochem Kit™	BK037	30-100 assays
Actin Binding Protein Spin-Down Assay Biochem Kit™	BK013	30-100 assays
Actin Polymerization Biochem Kit™	BK003	30-100 assays

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