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Research Article

Cortactin affects cell migration by regulating intercellular adhesion and cell spreading

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ABSTRACT

Cortactin is an F-actin binding protein that stabilizes F-actin networks and promotes actin polymerization by activating the Arp2/3 complex. Overexpression of cortactin, as observed in several human cancers, stimulates cell migration, invasion, and experimental metastasis; however, the underlying mechanism is not understood. To investigate the importance of cortactin in cell migration, we downregulated its expression using RNA interference (RNAi). Stable downregulation of cortactin in HBL100 breast epithelial cells resulted in (i) decreased cell migration and invasion, (ii) enhanced cell–cell adhesion, and (iii) accelerated cell spreading. These phenotypic changes were reversed by expression of RNAi-resistant mouse cortactin. Cortactin colocalized with cadherin and β-catenin in adherens junctions, consistent with its role in intercellular adhesion. Remarkably, cortactin deficiency did not affect lamellipodia formation. Instead, downregulation of cortactin in human squamous carcinoma cells that overexpress cortactin changed the cytoskeletal organization. We conclude that increased levels of cortactin, as found in human carcinomas, promote cell migration and invasion by reducing cell spreading and intercellular adhesive strength.

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Introduction

Remodeling of the actin cytoskeleton is indispensable for cell migration, cell adhesion, and wound healing as well as for tumor cell invasion and metastasis. Cell migration requires the formation of lamellipodia at the leading edge and detachment from adjacent cells and the cell substratum [1,2]. Cell shape changes are driven by actin-related protein 2/3 (Arp2/3) complex-mediated assembly of actin filaments [3,4]. Cortactin is an F-actin binding protein that binds and activates the Arp2/3 complex and consequently promotes actin polymerization [5,6]. In addition, cortactin inhibits debranching and disassembly of dendritic actin networks [7]. Cortactin interacts via its Src homology 3 (SH3) domain with various proteins involved in (i) actin polymerization (N-WASP, MIM, WIP, Fgd1 [8–11]), (ii) endocytosis (dynamin2 [12]), (iii) activating GTPases (BPGAP1, AMAP1 [13,14]), and (iv) cell–cell interactions (ZO-1 [15,16]). Cell migration is a complex process that requires the continuous, coordinated formation and disassembly of cell–cell and cell–substratum adhesions and the assembly and disassembly of the cytoskeleton. Thus, as an activator of the Arp2/3 complex and modulator of the actin cytoskeleton, cortactin may influence cell migration and adhesion by

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coupling the actin polymerization machinery to protein complexes involved in cell migration and adhesion.

The Arp2/3 complex is recruited to cell–substratum adhesions by virtue of its direct binding to the focal adhesion protein vinculin [17]. Cell adhesion to the extracellular matrix (ECM) is mediated by integrins. Following binding to the ECM, integrins cluster to recruit F-actin, actin-binding proteins and signaling proteins, thereby promoting the formation of focal adhesions [18]. Cortactin is highly tyrosine phosphorylated in response to integrin activation [19,20]; yet, there is no evidence that cortactin localizes to focal adhesions themselves. On the other hand, cortactin colocalizes with Src, N-WASP, and Arp2/3 complex in adhesive structures called podosomes, as observed in osteoclasts [21,22], macrophages [23], and smooth muscle cells [24], as well as in Rous sarcoma virus (RSV) transformed chicken fibroblasts [25] and carcinoma cells [26].

The formation of cadherin-mediated cell–cell junctions is accompanied by a profound remodeling of the actin cytoskeleton. The Arp2/3 complex is implicated in the formation of E-cadherin-based adhesive contacts [27–29] and, as a consequence, links rearrangements of the actin cytoskeleton directly to adherens junctions. Imbalance of F-actin assembly/disassembly is assumed to be responsible for the invasive potential of human carcinomas [30,31]. Via its association with the Arp2/3 complex, cortactin might exert a functional role in cell–substratum and cell–cell adhesions [29,32] and consequently may affect migration and invasion.

Several human cancers overexpress cortactin due to DNA amplification of the chromosome 11q13 region [33–35]. The 11q13 amplification correlates with the presence of lymph node metastases and increased mortality [36–40]. Cells overexpressing cortactin show enhanced cell migration and invasion [41–43] and increased metastatic potential in mice [44]. Cortactin was originally identified as a prominent substrate for Src in RSV-transformed chicken fibroblasts [25]; it is tyrosine phosphorylated in response to various stimuli, including growth factors and integrin cross-linking (reviewed in [45,46]). Cortactin affects F-actin dynamics and cell migration not only through overexpression but also by tyrosine phosphorylation [41,44,47,48] and alternative splicing of its actin-binding domain [43]. Given the results of in vitro studies, overexpression of cortactin, as seen in human tumors, is likely to disturb the balance of Arp2/3 complex-mediated actin polymerization and F-actin stability.

Our understanding of the biological role of cortactin has relied on overexpression studies, which do not allow conclusions about the normal function of cortactin. A more informative and elegant approach to assess the importance of cytoskeletal proteins, like cortactin, is to knockdown their endogenous expression levels using RNA interference (RNAi). Recent reports showed that cortactin protein knockdown experiments by RNAi resulted in (i) depletion of dendritic spines in hippocampal neurons, [49]; (ii) inhibition of clathrin-dependent endocytosis in an actin polymerization-dependent manner [50]; (iii) inhibition of Arp2/3-dependent actin assembly at E-cadherin adhesive contacts [29], (iv) decreased strengthening of N-cadherin-mediated intercellular adhesions [32], and (v) a defect in the persistence of lamellipodial protrusions [51].

In the present study, we show that RNAi induced stable downregulation of cortactin in highly invasive human epithelial cell lines reduces cell migration and invasion, enhances cell–cell adhesion, and accelerates cell spreading. Furthermore, in an 11q13-amplified cortactin-overexpressing carcinoma cell line, RNAi-mediated downregulation of cortactin restored the cytoskeletal organization. We conclude that cortactin might also affect cell migration and invasion by regulating intercellular adhesions and cell spreading.

Materials and methods

Antibodies

Antibodies to the following proteins were used: monoclonal antibody (mAb) 4F11 directed against human cortactin obtained from Dr. J.T. Parsons (University of Virginia, VA); polyclonal antibody (pAb) RA444 directed against human cortactin [33]; mAb to E-cadherin (Transduction Laboratories, Becton Dickinson, Austria); pAb to β-catenin (Santa Cruz Biotechnologies, CA, USA); pAb to p34-Arc (Upstate Biotechnologies Inc., Lake Placid, NY, USA); pAb to occludin (Zymed); mAb to vinculin (Sigma, St. Louis, MO, USA), and mAb to actin (Mab1501R, Chemicon, Temecula, CA, USA). Fluorescent-labeled secondary antibodies (Texas-Red and Alexa488 conjugated goat anti-mouse or goat anti-rabbit) or -goat Alexa488-conjugated phalloidin was purchased from Molecular Probes (Leiden, The Netherlands). Horseradish peroxidase-conjugated anti-mouse and anti-rabbit secondary antibodies were purchased from Dako (Glostrup, Denmark).

Cell culture and transfection

HEK293T (Human Embryonic Kidney) and HBL100 cells [52] were purchased from American Type Cell Culture (ATCC), and UMSCC2 (University of Michigan squamous cell carcinoma) cell line was kindly provided by Dr. T. Carey. HEK293T, HBL100, and UMSCC2 cells were grown in Dulbecco's modified Eagle's medium (DMEM, GIBCO Invitrogen, Carlsbad, CA, USA) supplemented with penicillin/streptomycin plus 8% fetal calf serum (FCS) at 37°C in 5% CO_2. HBL100 and UMSCC2 cells were transfected with LipofectAMINE PLUS reagent (Invitrogen) according to the manufactures instructions and HEK293T cells with calcium phosphate precipitates [53]. Transient transfection efficiency in HEK293T and UMSCC2 cells is approximately 95% and 25% respectively, explaining the difficulty to detect downregulation of endogenous cortactin in UMSCC2 cells. For all functional cell biological assays, we generated stably transfected HBL100 cell lines containing the puromycin resistance gene were supplemented with 1 μg/ml puromycin (Sigma) and with 1.25mg/ml G418 (Invitrogen) when containing the neomycin resistance gene. Selection medium was removed a day before experiments. In UMSCC2 cells, lamellipodia were visualized after 5 min treatment with 40 ng/ml EGF and in HEK293T cells after 15-min treatment with 50 nM phorbol ester (TPA).

Cortactin silencing by RNA interference

For cortactin silencing by RNA interference, a target (19-nt) oligonucleotide (purchased from Sigma-Aldrich), 5′-GGACAAAGTGGATAAGAGC-3′, corresponding to nucleotides 777–795

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