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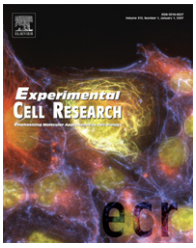

Research Article

# Cortactin involvement in the keratinocyte growth factor and fibroblast growth factor 10 promotion of migration and cortical actin assembly in human keratinocytes

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ABSTRACT

Keratinocyte growth factor (KGF/FGF7) and fibroblast growth factor 10 (FGF10/KGF2) regulate keratinocyte proliferation and differentiation by binding to the tyrosine kinase KGF receptor (KGFR). KGF induces keratinocyte motility and cytoskeletal rearrangement, whereas a direct role of FGF10 on keratinocyte migration is not clearly established. Here we analyzed the motogenic activity of FGF10 and KGF on human keratinocytes. Migration assays and immunofluorescence of actin cytoskeleton revealed that FGF10 is less efficient than KGF in promoting migration and exerts a delayed effect in inducing lamellipodia and ruffles formation. Both growth factors promoted phosphorylation and subsequent membrane translocation of cortactin, an F-actin binding protein involved in cell migration; however, FGF10-induced cortactin phosphorylation was reduced, more transient and delayed with respect to that promoted by KGF. Cortactin phosphorylation induced by both growth factors was Src-dependent, while its membrane translocation and cell migration were blocked by either Src and PI3K inhibitors, suggesting that both pathways are involved in KGF- and FGF10-dependent motility. Furthermore, siRNA-mediated downregulation of cortactin inhibited KGF- and FGF10-induced migration. These results indicate that cortactin is involved in keratinocyte migration promoted by both KGF and FGF10.



## Introduction

Cell migration is an essential process in physiological conditions, such as wound healing, as well as in pathological events, such as tumor cell invasion and metastasis. For migration, a cell needs to direct protrusions at the leading edge and retraction at the rear, accompanied by detachment from adjacent cells and the cell substratum [1]. This is achieved through dynamic reorganization of the actin cytoskeleton. In this regard, the key mediators are the Rho family of GTPases, including Rho, Rac and Cdc42 [2]. Rac is required for the formation of membrane ruffles and lamellipodia at the leading edge of the migrating cells and is thought to be the driving force for cell movement. Rho regulates stress fibers

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and focal adhesion assembly, while Cdc42 triggers filopodial extensions at the cell periphery [3,4].

Actin polymerization in lamellipodia is mediated by recruitment to the plasma membrane of several actin-associated proteins, such as the Arp (actin-related protein) 2/3 complex [5] and cortactin [6]. Cortactin is a widely expressed actin-binding protein originally identified as a substrate for Src kinase [7]. It contains an actin-binding domain, an N-terminal acidic region that binds to and activates the Arp2/3 complex, and several tyrosine phosphorylation sites. Cortactin binds to F-actin *in vitro* [8] and *in vivo* [9] and accumulates in actin-enriched peripheral structures of cells, such as lamellipodia and membrane ruffles, suggesting a role in actin network formation [10]. Cortactin translocation to the cell periphery and its ability to induce actin polymerization depend on Rac activation [6]. Cortactin has been implicated in linking transmembrane signaling to the cytoskeleton and in cell motility [11]. In fact, overexpression of cortactin in endothelial cells [11] or NIH3T3 fibroblasts [12] increases cell motility *in vitro*, and its overexpression in human tumors results in increased cell migration and metastatic potential [13]. Cortactin is phosphorylated at tyrosine residues upon various signals, including growth factors such as EGF [14], PDGF [15], HGF [16] and FGF1 [17]. Tyrosine phosphorylation mediated by Src kinase occurs on three of cortactin tyrosine residues (Y421, Y466 and Y482) [11,18]. This Src-mediated phosphorylation regulates the actin cross-linking activity of cortactin [9] and is positively correlated with cell motility. In fact, overexpression of a cortactin mutated at tyrosine phosphorylation sites impairs migration of endothelial cells [11] and metastasis formation [19].

The cytoskeletal protein paxillin, a component of the focal adhesions, is also involved in the regulation of actin cytoskeleton reorganization that is necessary for cell motility events. In fact, paxillin functions as a scaffold protein at focal adhesions [20,21] and its tyrosine phosphorylation, triggered by several growth factors such as EGF [22], HGF [23], IGF1 [24] and PDGF [25], has a promoting effect for cell migration, probably allowing the formation of a protein complex that can lead to Rac activation and to the production of lamellipodia extensions [26].

Many studies reported the effect of growth factors on cell migration. In fact, several growth factors including HGF [27], EGF [28,29], IGF1 and PDGF [30] have been shown to induce cell motility in various cell types. Some of these growth factors exert their motogenic activity specifically on epithelial cells: EGF is a chemotactic and motogenic factor for keratinocytes [31,32], and it accelerates wound healing [33]; TGFα induces cell migration in human keratinocytes [34]; HGF stimulates keratinocyte motility [35].

Among growth factors, keratinocyte growth factor (KGF or FGF7) is a known motogenic mediator for keratinocytes [36,37] and it seems to be a good candidate for playing a role in orchestrating the activation of proteins involved in cell migration. KGF, a member of the fibroblast growth factors (FGFs) family, is a powerful paracrine agent, secreted by stromal fibroblasts and targeted to epithelial cells [38]. Fibroblast growth factor 10 (FGF10 or KGF2), another member of the FGFs family, is highly similar to KGF in aminoacidic sequence and tissue expression [39,40]. Both KGF and FGF10 act by binding with high affinity to the tyrosine kinase keratinocyte growth factor receptor (KGFR), which is a splicing variant of the FGFR2 [41–43] exclusively expressed on epithelial cells [38,41,42]. Both these growth factors are potent mitogens for primary human keratinocytes [44,45] and promote their differentiation program [44,45], although they differ in sensitivity to heparin/heparan sulfate proteoglycans (HSPGs), as assessed by binding and proliferation assays [43]. In addition, it has been shown that KGF plays a role in wound healing [46–48], and its expression is strongly upregulated during the process of reepithelialization [48,49]. FGF10 expression does not seem to change in experimental wounds [50], but its topical addition induces wound repair [51,52]. Moreover, KGF stimulates migration of keratinocytes [36,37], induces reorganization of actin cytoskeleton and lamellipodia emission and promotes epithelial repair via increasing epithelial cell motility [53]. However, little is known about which signaling is essential for KGF-mediated migration.

In order to clarify the molecular mechanism involved in this process, in our study we examined keratinocyte migration in response to treatment with KGF and FGF10, showing that both the growth factors are motogenic for this cell type, although with different potencies. We also showed that KGF and FGF10 induce actin cytoskeleton reorganization at various time points by activating the protein cortactin with different kinetics. Moreover, taking advantage of the availability of potent pharmacological inhibitors for two of the major pathways commonly involved in cell motility, such as Src tyrosine kinase [54] and phosphatidylinositol 3-kinase (PI3K) [55] pathways, we showed that KGF- and FGF10-induced cortactin phosphorylation depends on Src kinase, while its translocation to the plasma membrane and subsequent migration depend on both Src and PI3K activity.

## Materials and methods

### *Materials*

Human recombinant KGF and anti-phosphotyrosine monoclonal antibody (clone 4G10) were purchased from Upstate Biotechnology (Lake Placid, NY, USA). Recombinant FGF10 was from PeproTech (London, UK). Human recombinant HGF was kindly provided by Prof. Antonella Stoppacciaro (Ospedale Sant'Andrea, Rome, Italy). Recombinant EGF was from Collaborative Research (Lexington, MA, USA). Src inhibitors PP2 and SU6656 were obtained by Calbiochem (San Diego, CA, USA). Wortmannin, LY294002 and TRITC–phalloidin were from Sigma Chemicals (St. Louis, MO, USA). Mouse anti-paxillin monoclonal antibody was purchased from BD Transduction Laboratories (San Diego, CA, USA), anti-phospho-PI3K-p85 (Tyr458)/p55 (Tyr199) and anti-PI3K-p85 antibodies were from Cell Signaling Technology, Inc. (Danvers, MA, USA), and anti-cortactin polyclonal antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA, USA). FITC-conjugated goat anti-mouse IgG and goat anti-rabbit IgG were obtained from Cappel Research Products (Durham, NC, USA).