Kindlin-1 Regulates Keratinocyte Electrotaxis

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Kindler syndrome (KS) is an autosomal recessive blistering skin disease resulting from pathogenic mutations in *FERMT1*. This gene encodes kindlin-1, a focal adhesion protein involved in activation of the integrin family of extracellular matrix receptors. Most cases of KS show a marked reduction or complete absence of the kindlin-1 protein in keratinocytes, resulting in defective cell adhesion and migration. Electric fields also act as intrinsic regulators of adhesion and migration in the skin, but the molecular mechanisms by which this occurs are poorly understood. Here we show that keratinocytes derived from KS patients are unable to undergo electrotaxis, and this defect is restored by overexpression of wild-type kindlin-1 but not a W612A mutation that prevents kindlin-integrin binding. Moreover, deletion of the pleckstrin homology domain of kindlin-1 also failed to rescue electrotaxis in KS cells, indicating that both integrin and lipid binding are required for this function. Kindlin-1 was also required for the maintenance of lamellipodial protrusions during electrotaxis via electric field-activated β1 integrin. Indeed, inhibition of β1 integrins also leads to loss of electrotaxis in keratinocytes. Our data suggest that loss of kindlin-1 function may therefore result in epithelial insensitivity to electric fields and contribute to KS disease pathology.

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INTRODUCTION

Kindlin-1 is predominantly expressed in epithelial tissues such as skin and intestine. Loss-of-function mutations in kindlin-1 causes Kindler syndrome (KS), which is characterized by skin blistering, fragility, and photosensitivity (Jobard et al., 2003; Kindler, 1954; Siegel et al., 2003). Keratinocytes from KS patients show defects in cell migration, adhesion, and proliferation (Has et al., 2009, 2015; Lai-Cheong et al., 2009). In addition, KS keratinocytes show loss of polarized migration because of reduced cell adhesion and as a result of kindlin-1 dysfunction (Herz et al., 2006).

Kindlin-1 has been shown to bind to β1, β3, and β6 integrin cytoplasmic domains and enhance focal adhesion formation (Kloeker et al., 2004). The integrin family mediates cell adhesion to the underlying basement membrane that is critical for skin integrity. β1 integrins are the predominant receptor in basal keratinocyte focal adhesions and, through connections to the actin cytoskeleton, are key to controlling protrusion formation during cell migration (Cox et al., 2003; Saravanan et al., 2009). Physiological electric fields (EFs) are important to skin function and wound repair. Breaches of the epithelial layer generate an endogenous electric current, which is crucial in mediating cell migration, division, and polarization; angiogenesis; and nerve regeneration during wound healing (McCaig et al., 2005). Keratinocytes can sense and respond to physiological EFs and migrate specifically toward a cathode in vitro (Nishimura et al., 1996). However, the mechanisms controlling EF-induced directional migration of keratinocytes remain poorly understood. A recent report showed a role for β1 integrins in mediating fibroblast sensing and response to electric stimulation (Tsai et al., 2013). Extracellular calcium (Fang et al., 1998), EGFR (Pu et al., 2007), cAMP (Pullar and Isseroff, 2005), and the phosphoinositide 3-kinase (PI[3]K)–phosphatase and tensin homolog signaling pathway (Zhao et al., 2006) have also been implicated, but the receptor signals initiating EF-induced polarity are not defined.

In this study, we describe a mechanism by which kindlin-1 mediates efficient keratinocyte electrotaxis through activation of β1 integrins. Our data show that kindlin-1 regulates the distribution and maintenance of lamellipodial protrusions and integrin activation through association with phospholipids and that this is required for polarization and directed electrotaxis. These data provide the evidence that specific focal adhesion proteins are required for optimal cell sensing of electric gradients and further suggest that a potential defect in this pathway in KS patients may contribute to disease pathology.

RESULTS

KS keratinocytes are defective in electrotaxis

Kindlin-1 has previously been shown to be required for efficient migration of keratinocytes and loss or reduced levels of

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Abbreviations: EF, electric field; KS, Kindler syndrome; KS_MT, kindlin-1–mCherryW612A point mutation; KS_WT, Kindler syndrome cells infected with wild-type kindlin-1–mCherry; NHK, normal human keratinocyte; PH, pleckstrin homology; PI(3)K, phosphoinositide 3-kinase; PTEN, phosphatase and tensin homolog

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this protein in KS leads to defective adhesion assembly and migration (Herz et al., 2006). To determine whether kindlin-1 was also involved in response to EFs, a physiological EF range of 0−200 mV/mm were used to examine the abnormalities of KS keratinocytes compared with normal human keratinocytes (NHK). Migration directedness, trajectory, and displacement speed are used to describe cell responses to EF stimulation. Directedness indicates migration direction, where 0 is random migration and 1 is directional movement toward the cathode (Guo et al., 2010; Song et al., 2007). The trajectory and displacement speeds describe how fast cells migrate from the start to the endpoint. NHK exhibited dose-dependent responses in directedness, trajectory, and displacement speed to increasing EFs up to 200 mV/mm (Figure 1a−c). By contrast, KS cells did not show significant electrotactic responses to any conditions tested with the exception of 200 mV/mm EF (Figure 1a−c). No further increase in directional migration was observed over longer imaging periods (up to 4 hours; data not shown). Detailed analysis of the time-lapse movies showed that NHK responded immediately to EF stimulation and migrated directionally toward the cathode, whereas KS cells showed significantly lower electrotactic response (Figure 1d and e, and see Supplementary Video S1 online). Thus, we conclude that loss of kindlin-1 results in significantly impaired keratinocyte electrotaxis.

Kindlin-1 mediates keratinocyte electrotaxis through binding to β1 integrins

Kindlin-1 has been shown to associate directly with integrin cytoplasmic domains and is required for full activation of β1 integrins and, subsequently, cell adhesion and migration (Herz et al., 2006; Lai-Cheong et al., 2009; McMillan et al., 2007). Recent studies have shown that the C-terminal F3 domain of kindlin-1 containing W612 is required for kindlin-integrin binding. A W612A point mutation in kindlin-1 blocks the binding of kindlin-1 to the tail of integrin β1, thus abolishing its ability to activate integrins (Harburger et al., 2009). To explore whether the deficient response of KS cells to EF is due to the loss of kindlin-1 binding to integrins, we stably infected KS cells with wild-type kindlin-1−mCherry (KS_WT) or kindlin-1−mCherryW612A point mutation (KS_MT) and analyzed their responses to EF. KS_WT cells showed rescue responses similar to those of NHK, suggesting that loss of kindlin-1 was responsible for the defects in KS cells (Figure 2a−c). However, KS_MT cells failed to show the rescue effects as KS_WT cells did, with the reduced electrotactic response in all cases (Figure 2a−c). Cell trajectories (Figure 2d) and time-lapse images (Figure 2e, and see Supplementary Video S2 online) confirmed these observations. These data suggest that kindlin-1 is required for EF-induced directional migration of keratinocytes, and interaction with β1 integrins is one of the requirements for EF sensing.

Kindlin-1 is required for protrusion polarization in electrotaxis

The formation of F-actin containing lamellipodia and filopodia is critical for directional migration and requires the interaction of external guidance cues, adhesion receptors, and cytoplasmic adaptors (Fukata et al., 2003; Petrie et al., 2009; Watanabe et al., 2005). Analysis of movies showed that during 2 hours of EF stimulation, NHK displayed a persistent lamellipodia formation toward the cathode (Figure 3a, and see Supplementary Video S3 online). However, KS cells formed filopodia-like protrusions in random directions, resulting in a random migration pattern (Figure 3b, and see Supplementary Video S3). KS_WT cells exhibited formation of a single polarized lamellipodia (Figure 3c, and see Supplementary Video S3), whereas KS_MT cells failed to form stable polarized protrusions similar to KS cells (Figure 3d, and see Supplementary Video S3). Similarly, KS_WT cells showed a ratio of cathode-facing protrusions similar to NHK, whereas KS_MT cells exhibited significantly lower cathode-facing protrusions (Figure 3f). These data show that kindlin-1−integrin binding is required for the formation of protrusions that lead to efficient keratinocyte electrotaxis.

Kindlin-1 is important for the maintenance of EF-induced protrusions

The higher probability and longer maintenance of protrusion in a certain direction, the higher the possibility that cells migrate in that direction persistently (Petrie et al., 2009). We used the Quimp pseudopod analysis algorithm to further explore the effects of kindlin-1 on pseudopod maintenance under EF stimulation (Bosgraaf and Van Haastert, 2010b). Cell boundaries were masked and assigned different colors according to their dynamic behavior (red indicated protrusion, and blue indicated retraction) (Figure 4a). NHK and KS_WT cells showed persistent maintenance of the pseudopod toward the cathode, whereas KS and KS_MT failed to do so, with short-lived pseudopod generation in random directions (Figure 4a, and see Supplementary Video S4 online). We further calculated the pseudopod maintenance between consecutive time points throughout the time-lapse sequence. To classify the morphological behavior, we defined cathodal protrusions as those occurring between 135° and 225° and anodal protrusion as between −45° and 45° with respect to the EF source, because these regions provide the most representative directions with respect to cathode or anode. Data showed that KS cells had a lower pseudopod maintenance score compared with NHK cells, and this was rescued in KS_WT but not KS_MT cells (Figure 4b). The summarized distribution of pseudopod maintenance (horizontal EF vector, cathode facing left; Figure 4c and d) showed the pseudopod of KS and KS_MT cells distributed randomly, whereas NHK and KS_WT cell protrusions were concentrated specifically in the region 135−225° facing toward the cathode (Figure 4c and d).

Kindlin-1−mediated integrin β1 activation promotes keratinocyte electrotaxis

Integrins have been reported to be important in mediating EF-induced migration of keratinocytes (Pullar et al., 2006). To determine whether EF induces β1 integrin activation in a kindlin-1−dependent manner, FACS analysis was performed on all cell lines after EF stimulation by using the 12G10 antibody that specifically recognizes the active conformation of human β1 integrins (Mould et al., 1995). EF stimulation led to an increase in integrin activation in NHK and KS_WT cells but not in KS or KS_MT cells (Figure 5a). Immunostaining and intensity analysis of cells fixed immediately after exposure to