Cellular Signalling 20 (2008) 2002–2012

Contents lists available at ScienceDirect

Cellular Signalling

journal homepage: www.elsevier.com/locate/cellsig

JNK regulates cell migration through promotion of tyrosine phosphorylation of paxillin

Zhi Huang [a,b], Da-Peng Yan [a,b], Bao-Xue Ge [a,*]

[a] Institute of Health Sciences, Shanghai Institutes for Biological Sciences (SIBS), Chinese Academy of Sciences (CAS) & Shanghai Jiao Tong University School of Medicine (SJTUSM), China
[b] Graduate School of the Chinese Academy of Sciences, China

ARTICLE INFO

Article history:
Received 28 May 2008
Received in revised form 11 July 2008
Accepted 13 July 2008
Available online 31 July 2008

Keywords:
Cell migration
JNK
Paxillin
FAK

ABSTRACT

The adaptor protein paxillin plays an important role in cell migration. Although the c-Jun amino-terminal kinase (JNK) phosphorylation of paxillin on Ser 178 has been found to be critical for cell migration, the precise mechanism by which JNK regulates cell migration is still not very clear. Here, the migration of human corneal epithelial (HCE) cells was used to determine which signaling pathways are involved in EGF-induced paxillin phosphorylation. Paxillin was phosphorylated on Tyr 31 and Tyr 118 after induction of migration by EGF in HCE cells. Specific inhibition of JNK activation by inhibitor SP600125 or overexpression of a dominant-negative JNK mutant not only blocked EGF-induced cell migration, but also eliminated tyrosine phosphorylation of paxillin on Tyr 31 and Tyr 118. HCE cells overexpressing paxillin-S178A mutant also exhibited lower mobility, and reduced phosphorylation of Tyr 31 and Tyr 118. However, paxillin-S178A-inhibited cell migration can be rescued by overexpression of paxillin-Y31E/Y118E mutant. Importantly, inhibition of JNK by SP600125 or overexpression of paxillin-S178A mutant prevented the association of FAK with paxillin. Taken together, these results suggest that phosphorylation of paxillin on Ser 178 by JNK is required for the association of paxillin with FAK, and subsequent tyrosine phosphorylation of paxillin.
© 2008 Elsevier Inc. All rights reserved.

1. Introduction

Corneal epithelial cells form a protective barrier on the surface of the cornea and are often subjected to physical, chemical and biological injuries. Reaction of corneal epithelial sheets to these injures occurs via a wound healing process [1]. Successful wound healing involves a number of phenomena including cell migration, cell proliferation, matrix deposition and tissue remodeling [1–5]. Many of these biological processes are regulated by growth factors, cytokines and cellular matrix molecules [6–8]. Epithelial growth factor (EGF) is found to have stimulatory effects on corneal epithelial wound repair by increasing migration and mitosis of the epithelial cells [9,10]. Although the exact mechanism underlying EGF-induced migration of corneal epithelial cells is still not very clear, it is known that EGF signaling occurs through the EGF receptor (EGFR) that belongs to a family of tyrosine kinase receptors [11]. Ligation of EGF to EGFR increases receptor tyrosine kinase activity and triggers receptor autophosphorylation [12], which in turn recruits and activates a number of signal transducers including growth factor receptor bound-2(GRB-2) [13], phospholipase C-γ (PLC-γ) [14], the p85 subunit of phosphatidylinositol 3-kinase (PI 3-kinase) [15], the Src family of non-receptor tyrosine kinases [14,16] and Nck [17]. Activation of these signaling pathways is linked to the p21 Ras family of GTPases and Mitogen-activated protein kinases (MAPKs) cascades [6,18].

Mitogen-activated protein kinases (MAPKs) belong to a family of serine/threonine protein kinases that plays a crucial role in transmitting extracellular stimuli downstream to elicit diverse cellular responses [19–29]. Based upon its structural and biochemical features, the MAPK family can be divided into three main groups: extracellular signal-regulated kinase (ERK), stress-activated protein kinase (SAPK)/c-Jun N-terminal kinase (JNK) and p38 [30–34]. All MAPKs contain a Thr-X-Tyr (TGY) motif within the activation loop in the kinase domain. Activation of MAP kinase is achieved by phosphorylation of the TGY motif by upstream MAP kinase kinase (MAP2K), which is activated by further upstream MAP kinase kinase kinase (MAP3K). Once activated, MAP kinases phosphorylate downstream protein kinases and transcription factors, thus playing a vital role in a variety of biological activities ranging from cell migration to proliferation, oncogenesis, differentiation and inflammation [35,36].

Paxillin is a 68-kDa focal adhesion-associated protein involved in a number of biological functions including cell adhesion, cell migration and neurite outgrowth [37]. It contains five leucine-rich regions, termed LD motifs, near the N-terminus, and four LIM domains in the C-terminus. Dispersed throughout paxillin are many serine/threonine and tyrosine phosphorylation sites. Phosphorylation of paxillin on these sites in response to extracellular stimuli leads to recruitment of a

Abbreviations: JNK, c-jun N-terminal kinase; FAK, focal adhesion kinase; EGF, epithelial growth factor; THCE, SV40-immortalized human corneal epithelial; RNAi, RNA interference.

* Corresponding author.
E-mail address: gebaoxue@sibs.ac.cn (B.-X. Ge).

0898-6568/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.cellsig.2008.07.014

number of signaling molecules, thus regulates focal adhesion dynamics and cell migration. Two main tyrosine phosphorylation sites (Tyr 31 and Tyr 118) have been recently identified [38]. Tyrosine phosphorylation of paxillin on 31 and 118 by focal adhesion kinase (FAK) generates binding sites for recruitment of Src homology (SH2)-domain-containing proteins such as CrkII [39]. In addition to tyrosine phosphorylation, phosphorylation of paxillin on serine and threonine sites by MAP kinases is also well documented. JNK phosphorylates paxillin on Ser 178, regulates migration of NBT-II cells, MDA-MB-231 and Chinese hamster ovary (CHO-K1) cells [40], and mediates neurite extension in the mouse neuroblastoma cell line NIE-115 [41]. Phosphorylation of paxillin on Ser 83 by ERK or p38 regulates morphological changes of mouse epithelial mIMCD-3 cells and neurite outgrowth of rat pheochromocytoma PC12 cells [42,43], but it is still unknown whether phosphorylation of paxillin by ERK or p38 is essential for cell migration.

In this study, we have examined the phosphorylation of paxillin during EGF-stimulated migration of human corneal epithelial (HCE) cells. Interestingly, our data show that phosphorylation of paxillin on Ser 178 by JNK MAP kinase is required for the binding of paxillin with FAK and subsequent tyrosine phosphorylation of paxillin on 31 and 118.

2. Results

2.1. EGF activates MAP kinase signaling pathways during HCE cell migration

EGF has been shown to stimulate migration of a number of cell lines, including human lens epithelial cells and human prostate carcinoma cells [44,45]. To evaluate EGF signaling in corneal epithelial cell migration, THCE cells, a SV-40 immortalized HCE cell line, were stimulated by EGF in a wound healing assay. As shown in Fig. 1A and B, stimulation of HCE cells with 80 ng/ml EGF markedly increased cell migration. To examine whether MAP kinases are activated by EGF in HCE cells, cells were exposed to EGF for various time periods, and analyzed for EGF-induced changes in MAP kinase phosphorylation by Western blot analysis using dual phospho-specific MAPK antibodies. As shown in Fig. 1C, cells stimulated with EGF showed time-dependent changes in the phosphorylation status of JNK, p38 and ERK.

2.2. Activation of JNK is essential for EGF-induced migration of HCE cells

JNK activation has been shown to play an important role in many cellular functions. To examine whether the JNK signaling pathway is involved in EGF-induced migration of HCE cells, SP600125, a specific inhibitor of JNK, was employed. Specific inhibition of JNK by SP600125 (20 μM) significantly reduced EGF-induced HCE cell migration (Fig. 2A and B). To further examine whether JNK activity is required for EGF-stimulated HCE cell migration, JNK-dn (T183A, Y185F), a dominant-negative (dn) mutant of JNK [40], was transfected into HCE cells. EGF-induced migration of HCE cells was greatly reduced by the expression of the JNK-dn mutant, but not by a similarly transfected control vector (Fig. 2C and D), suggesting that EGF mediates HCE cell migration via JNK signaling pathways.

2.3. Paxillin is required for EGF-induced HCE cell migration

Paxillin, an important focal adhesion adaptor, is a scaffold for recruitment of many signaling transducers to the plasma membrane, including the tyrosine kinases FAK, Src and Abl [37,46,47]. When integrins are engaged by the extracellular matrix or when receptor tyrosine kinases are activated by growth factors, paxillin is phosphorylated and regulates the actin cytoskeleton [47,48]. To evaluate a

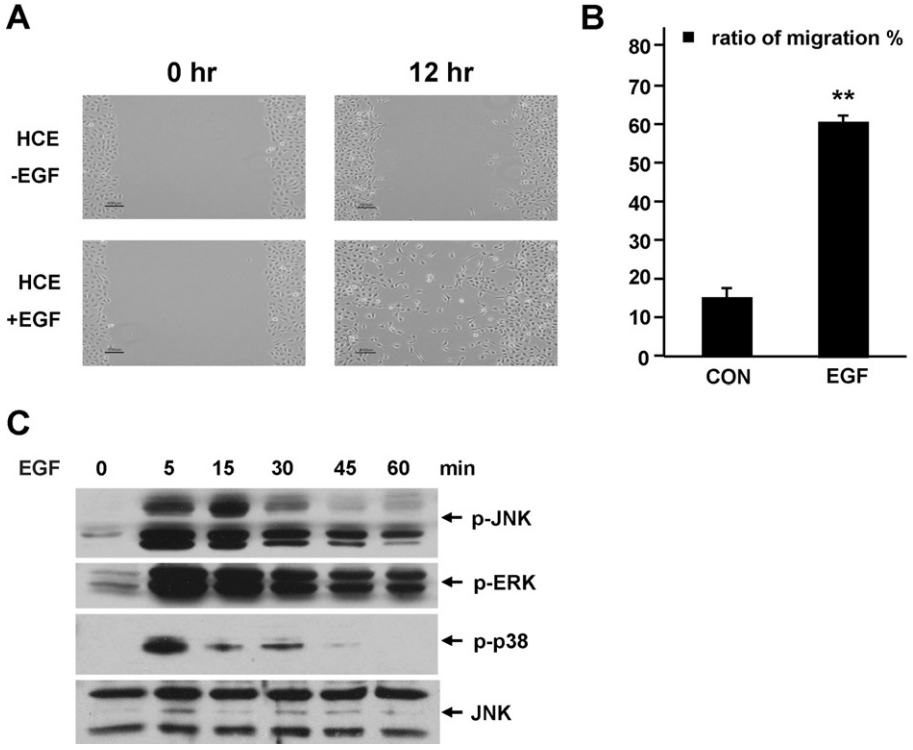

Fig. 1. EGF activates the MAP kinase signaling pathway during HCE cell migration. A) After serum-starvation for 24 h, HCE cells with 80 ng/ml EGF (top panels) or without EGF (bottom panels) were wounded and images were recorded when wounds were made (left panels) and after incubation for 12 h (right panels). B) Average migratory ratio of HCE was statistically analyzed from three independent experiments as described in panels A and C) HCE cells were serum starved for 24 h before EGF (80 ng/ml) stimulation. Cells were harvested at the indicated time, and the activation kinetics of MAP kinases was assessed using antibodies against phospho-p38, phospho-JNK and phospho-ERK. Total JNK was used as control to assure equal sample loading. Data are representative of three independent experiments.

Download English Version:

https://daneshyari.com/en/article/1964053

Download Persian Version:

https://daneshyari.com/article/1964053

Daneshyari.com