Cellular Signalling 27 (2015) 1110–1119

Contents lists available at ScienceDirect

Cellular Signalling

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

Sec8 regulates cytokeratin8 phosphorylation and cell migration by controlling the ERK and p38 MAPK signalling pathways

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ARTICLE INFO

Article history:
Received 1 December 2014
Received in revised form 2 February 2015
Accepted 16 February 2015
Available online 26 February 2015

Keywords:
Sec8
Cytokeratin8
p21-Activated kinases
Seven in absentia homologue 1
p53-Induced RING-H2
Cell migration

ABSTRACT

Cell migration is involved in numerous biological processes, including morphogenesis, wound healing and inflammatory responses, and is regulated by harmonic modulations of cellular cytoskeletal elements. The intermediate filament cytokeratin8 is one cytoskeletal element that has been implicated in cell migration. Sec8 is a component of an exocyst complex and is associated with various phenomena, such as cell migration, invadopodia formation, cytokinesis, glucose uptake and neural development. However, the relationship between Sec8 and cytokeratin8 remains to be elucidated. In this study, depleting Sec8 in HSC3 cells suppressed their migration by controlling the phosphorylation of cytokeratin8 at Ser73. This reduced cytokeratin8 phosphorylation at Ser73 is regulated by the activation of ERK and p38 mitogen-activated protein kinases (MAPK) signalling pathways via the downregulation of p21-activated kinases by p53-induced RING-H2 (Pirh2) and seven-in-absentia homologue 1 (Siah1) under conditions of Sec8 knockdown.

1. Introduction

Cell migration is involved in numerous biological processes, including morphogenesis, wound healing and inflammatory responses [1]. However, cell migration is also involved in pathological processes, such as metastasis and tumour invasion [1]. In general, cell migration is a kinetic process that can be divided into four stages: cell polarisation in response to external stimuli, formation of a protrusion at the leading edge of a cell, adhesion to other cells or the extracellular matrix and retraction of the cell's trailing edge, which moves the cell forward [2].

Cell migration is regulated by harmonic modulations of cytoskeletal elements, including actin, microtubules and intermediate filaments (IFs). For example, F-actin is polymerised at the front of a cell, leading to a membrane surface protrusion and forward movement of the cell [3]. The protrusion generated by F-actin is followed by contraction at the back of the cell, which is a myosin II-mediated contraction [2]. Microtubules, which comprise α- and β-tubulin heterodimers, control cell polarisation as it migrates [4]. Moreover, IFs are involved in cell migration as both effectors and suppressors [5]. Cytokeratins are epithelial specific IFs and are encoded for by type I and type II IF genes [6]. Recent studies showed that cytokeratin8, which is encoded for by a type II IF gene, was implicated in cell migration and tumour metastasis [6,7].

Mitogen-activated protein kinases (MAPKs) play essential roles in multiple cellular processes, such as proliferation, differentiation and apoptosis. MAPK pathways are regulated by three distinct kinases, MAPK kinase kinase (MAP3K), MAPK kinase (MAP2K) and MAPK. These pathways transmit signals through the sequential phosphorylation of MAP3K to MAP2K to MAPK [8,9]. Recently, three components of mammalian MAPK signalling pathways, including extracellular signal-regulated kinase (ERK), c-jun N-terminal kinase (JNK) and p38 MAPK, were found to play crucial roles in cell migration [10]. In particular, the phosphorylation and reorganisation of cytokeratin8 can be activated by ERK [11], p38 [12] or JNK [13], and they promote cell migration.

The exocyst is a protein complex comprising eight subunits, including Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70 and Exo84 [14]. Exocysts are involved in the docking and tethering of secretory vesicles, which provides spatial and temporal regulation of exocytosis [15,16], and interact either directly or indirectly with cell membranes and cytoskeletal proteins, as well as with small GTPases from the Rab, Ral and Rho subfamilies and numerous other proteins in the cell cortex [17]. During the last two decades, the interplay between exocysts and the cytoskeleton has been intensively investigated. An exocyst complex acts as an immediate effector of RalA, which is a ubiquitous GTPase from the Ras superfamily, and regulates actin organisation, cell growth and

Abbreviations: IF, intermediate filament; MAPKs, mitogen-activated protein kinases; MAP2K, MAPK kinase; MAP3K, MAPK kinase kinase; ERK, extracellular signal-regulated kinase; JNK, c-jun N-terminal kinase; KSR, kinase suppressor of Ras; MEK1/2, ERK kinase 1/2; JIP4, JNK-interacting protein 4; MKKs, mitogen-activated protein kinase kinases; PAK, p21-activated kinase; Siah1, seven-in-absentia homologue 1; Pirh2, p53-induced RING-H2.

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http://dx.doi.org/10.1016/j.cellsig.2015.02.015
0898-6568/

differentiation in mammalian cells [18]. An exocyst-RalA complex also integrates the secretory pathway and actin skeleton near the plasma membrane [14,19–21]. Moreover, exocyst complexes are associated with microtubules and microtubule-organising centres and mediate vesicle targeting in mammalian cells [22].

However, the relationship between exocysts and cytokeratins during cell migration remains unclear. In this study, we demonstrate that Sec8 regulates the phosphorylation of cytokeratin8 through the control of the ERK and p38 MAPK signalling pathways during cell migration.

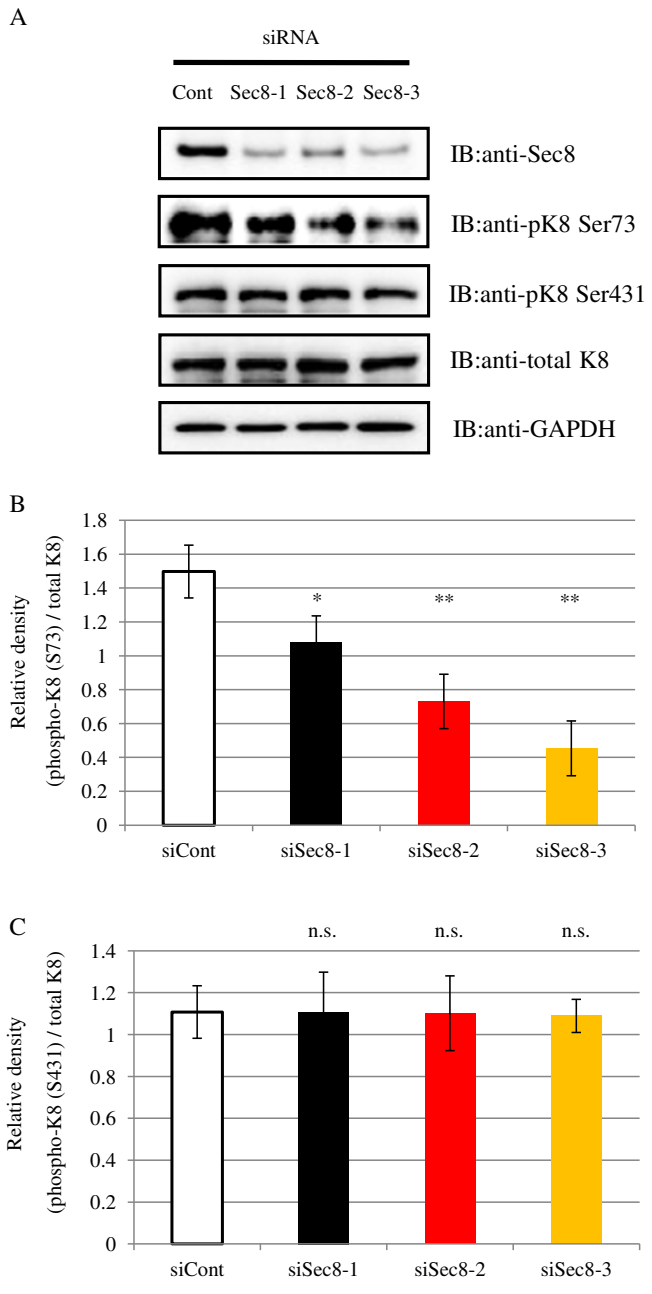

Fig. 1. Sec8 knockdown results in reduced cytokeratin8 phosphorylation at Ser73 but not at Ser431. (A) HSC3 cells were transfected with negative control or Sec8 siRNA. After 48 h, whole cell lysates were analysed by immunoblotting using the indicated antibodies. GAPDH was used as the loading control. (B, C) Histograms of relative expression of phospho-cytokeratin8 (S73)/total cytokeratin8 (B) and phospho-cytokeratin8 (S431)/total cytokeratin8 (C). Results are means ± standard deviations of three separate experiments. Asterisks indicate significant differences compared with cells transfected with negative control siRNA. n.s., not significant, $^{*}P < 0.05$, $^{**}P < 0.01$ (Student's *t*-test).

2. Materials and methods

2.1. Cell culture

HSC3 cells are a human oral squamous cell carcinoma-derived metastatic cell line with high metastatic potential. These cells were maintained in Dulbecco's modified Eagle's medium (Wako, Osaka, Japan) supplemented with 10% heat-inactivated foetal bovine serum and 100 U/ml of penicillin and streptomycin. For routine cell culture, all cultures were grown at 37 °C in a humidified atmosphere of 5% CO_2.

2.2. Reagents

U0126, a specific ERK inhibitor, was from Cell Signalling Technology (Danvers, MA, USA), and SB202190, a specific p38 inhibitor, was from Abcam (Cambridge, UK).

2.3. RNA interference

Two sets of small interfering RNA (siRNA) duplexes for each targeted protein were used to knock down human Sec8 (Sec8-1: 5′-UCGGAAACUGUGGAUUGAAGGAAUU-3′, Sec8-2: 5′-CCCUGUUUAUGGGUAUCCUCAUUAA-3′, Sec8-3: 5′-CACUGGACGAGAGUUUGCAGCCUUU-3′), human Sec6 (Sec6-1: 5′-UUUGACGUCAUUCAGGGCGUUGUGG-3′, Sec6-2: 5′-AAUUCUGGUGGUCACAGUCCUCUCC-3′, Sec6-3: 5′-ACGCUCACUUCAAACAUCAUCGCCU-3′), human p53-induced RING-H2 (Pirh2) (5′-CUUCCUUGAAGAUUCAUAGCUAGGC-3′) and human seven-in-absentia homologue 1 (Siah1) (5′-UGUAAUGGACUUAUGCUGAUGCAUC-3′). All siRNA duplexes for each targeted protein were from Invitrogen (Carlsbad, CA, USA). All Stars Negative Control siRNA (Qiagen, Hilden, Germany) was used as the control. Cells were transfected with RNAi duplexes using Lipofectamine RNAiMAX (Invitrogen). Experiments were performed at 48 h after transfection.

2.4. Immunoblot analysis

Preparations for immunoblot analysis were as previously described [23]. PVDF membranes were immunoblotted using primary antibodies against Sec8 (1:1000; 14; Santa Cruz, CA, USA), Sec6 (1:1000; 9H5; Millipore, Billerica, MA, USA), GAPDH (1:5000; ab128915; Abcam), cytokeratin8 (1:5000; ab53280; Abcam), phospho-cytokeratin (S73) (1:1000; ab32579; Abcam), phospho-cytokeratin (S431) (1:1000;

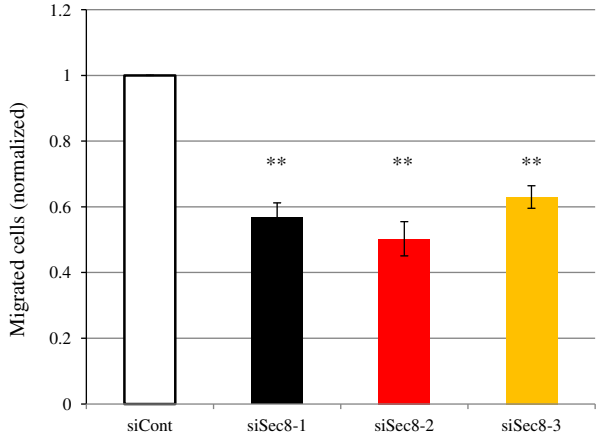

Fig. 2. Sec8 knockdown suppresses cell migration. HSC3 cells were transfected with negative control or Sec8 siRNA. After 48 h, the number of cells that had migrated to the lower filter surfaces was counted and normalised to the number of migrated cells transfected with negative control siRNA. Results are means ± standard deviations of three separate experiments. Asterisks indicate significant differences compared with cells transfected with negative control siRNA, $^{**}P < 0.01$ (Student's *t*-test).