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Vinculin negatively regulates transcription of MT1-MMP through MEK/ERK pathway

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ABSTRACT

Vinculin regulates a variety of cellular functions partly through stabilization of tumor suppressor PTEN. In order to study the role of vinculin in tumor progression other than PTEN stabilization, vinculin was knocked down in PTEN-deficient squamous cell carcinoma HSC-4 cells. Knockdown of vinculin induced phenotypical change by reducing cell–cell and cell–extracellular matrix adhesions, and enhanced MT1-MMP expression at transcription level and subsequent cell migration. Up-regulation of MT1-MMP transcription by vinculin knockdown was abrogated by ERK inhibition. These results suggest that vinculin negatively regulates malignant phenotype of tumor cells including MT1-MMP transcription through MEK/ERK pathway.

1. Introduction

A cellular adhesiveness, including cell–cell and cell–extracellular matrix (ECM) adhesions, correlates with invasive and metastatic phenotype of cancer. Vinculin has been implicated as an inhibitor of cell migration, as it enhanced cellular adhesiveness [1–4]. Loss or reduced expression of vinculin is related to the metastatic potential of squamous cell carcinomas (SCC), although in situ SCC exhibits high level expression of vinculin [5]. The role of vinculin in suppression of the malignant phenotype of SCC still remains to be solved.

Vinculin is a well-known component of cell-ECM adhesions by interacting with several focal adhesion proteins such as talin, paxillin, tensin, VASP, zyxin, vinexin, filamin, α-actinin, actin and focal adhesion kinase (FAK) either directly or indirectly [6]. It possesses tumor suppressive functions. Studies using F9 embryonic mouse carcinoma cells showed that vinculin knockout cells exhibit more rounded phenotype, less spreading, smaller focal adhesions and increased motility compared with wild type [1,2]. Mouse embryonic fibroblasts from vinculin knockout mouse also showed decreased cell–matrix adhesion, increased motility and FAK phosphorylation compared with wild-type cells [7]. Vinculin competes with paxillin for binding to FAK, which modulates extracellular signal-regulated kinase (ERK) activation in the downstream of FAK/paxillin [8].

Vinculin also localizes at and modulates cell–cell adhesions [6,9]. It binds to α- and β-catenin, which bind to one another and regulate cell–cell adhesion by interacting with cadherin adhesion receptors. The interaction between vinculin and catenins facilitates the stability of E-cadherin adhesions [10,11]. This enhanced cadherin adhesion stabilize a well-known tumor suppressor phosphatase and tensin homolog deleted on chromosomal 10 (PTEN) by interfering with ubiquitin-mediated proteolytic degradation of PTEN, which results from the binding of vinculin with membrane-associated guanylate-kinase inverted 2/β–catenin complex [12]. Loss of vinculin attenuates the protein level of PTEN, which is recovered by ectopic expression of vinculin. PTEN stabilizes E-cadherin adhesions, and is often deleted in cancers [13]. Consequently, vinculin may facilitate tumor suppressive function of E-cadherin and PTEN.

Membrane-type 1 matrix metalloproteinase (MT1-MMP) is considered to play a significant role in tumor progression, as its expression correlates most closely with the invasive phenotype of human tumors among MMPs and the inhibition of MT1-MMP suppresses tumor cell invasion both *in vitro* and *in vivo* [14]. MT1-MMP was originally identified as a tumor-specific MMP-2 activator [15]. It activates MMP-2 and -13 and degrades a wide range of ECM components, including type I, II, III collagen, laminins, and fibronectin. This enzyme also processes and interacts with membrane-tethered proteins such as integrins and CD44

Abbreviations: DMEM, Dulbecco's modified Eagle's medium; ECM, extracellular matrix; ERK, extracellular signal-regulated kinase; FAK, focal adhesion kinase; MT1-MMP, membrane-type 1 matrix metalloproteinase; PBS, phosphate-buffered saline; PTEN, phosphatase and tensin homolog deleted on chromosomal 10; SCC, squamous cell carcinoma; siRNA, small interfering RNA.

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[14]. The activity of MT1-MMP is up-regulated by ERK [16,17] and down-regulated by PTEN [18].

The aim of this study is to understand how vinculin regulates motility and invasiveness of SCC cells that lacks PTEN. We analyzed the effect of vinculin knockdown on MT1-MMP activity and invasion of SCC cells deficient in PTEN.

2. Materials and methods

2.1. Cell culture and reagents

Human oral SCC HSC-3 and HSC-4 cells were obtained from the Health Science Research Bank (Osaka, Japan). Cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS). The reagents used were type I collagen (Nitta Gelatin, Osaka, Japan); MEK inhibitor (PD98059) (Merck Millipore, Temecula, CA, USA). A synthetic MMP inhibitor (BB94) was a kind gift from the Kotobuki Pharmaceutical Co., Ltd. (Nagano, Japan). An anti-MT1-MMP antibody was gifted by Dai-ichi fine chemicals (Toyama, Japan). The immunological reagents used were anti-E-cadherin, anti-β-catenin, anti-ERK2, and anti-paxillin antibodies (BD Biosciences, Bedford, MA, USA); anti-phospho-p44/42 MAPK and anti-PTEN antibodies (Cell Signaling Technology, Danvers, MA, USA); anti-α-tubulin and anti-vinculin antibodies (Sigma–Aldrich, St Louis, MO, USA); an anti-vinculin antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA); an anti-p53 antibody (Merck Millipore); Rhodamine-labeled phalloidin, Hoechst 33,342, and Alexa Fluor-labeled secondary antibodies (Molecular Probes, Eugene, OR, USA).

2.2. Small interfering RNA (siRNA)-mediated protein knockdown

A siRNA for negative control was purchased from Qiagen (Valencia, CA, USA). The siRNA sequences used here were: *mt1-mmp*, 5′-GCGAUGAAGUCUUCACUUATT; *vinculin-1*, 5′-GGC AUAGAGGAAGCUUUAATT; *vinculin-3*, 5′-GCCAAGCAGUGCACAGAU ATT. Cells were transfected with 20 nM of siRNA duplexes in Opti-MEM (Invitrogen, Carlsbad, CA, USA) using Lipofectamine RNAi MAX (Invitrogen), according to the manufacturer's instructions, and were incubated for 48 h.

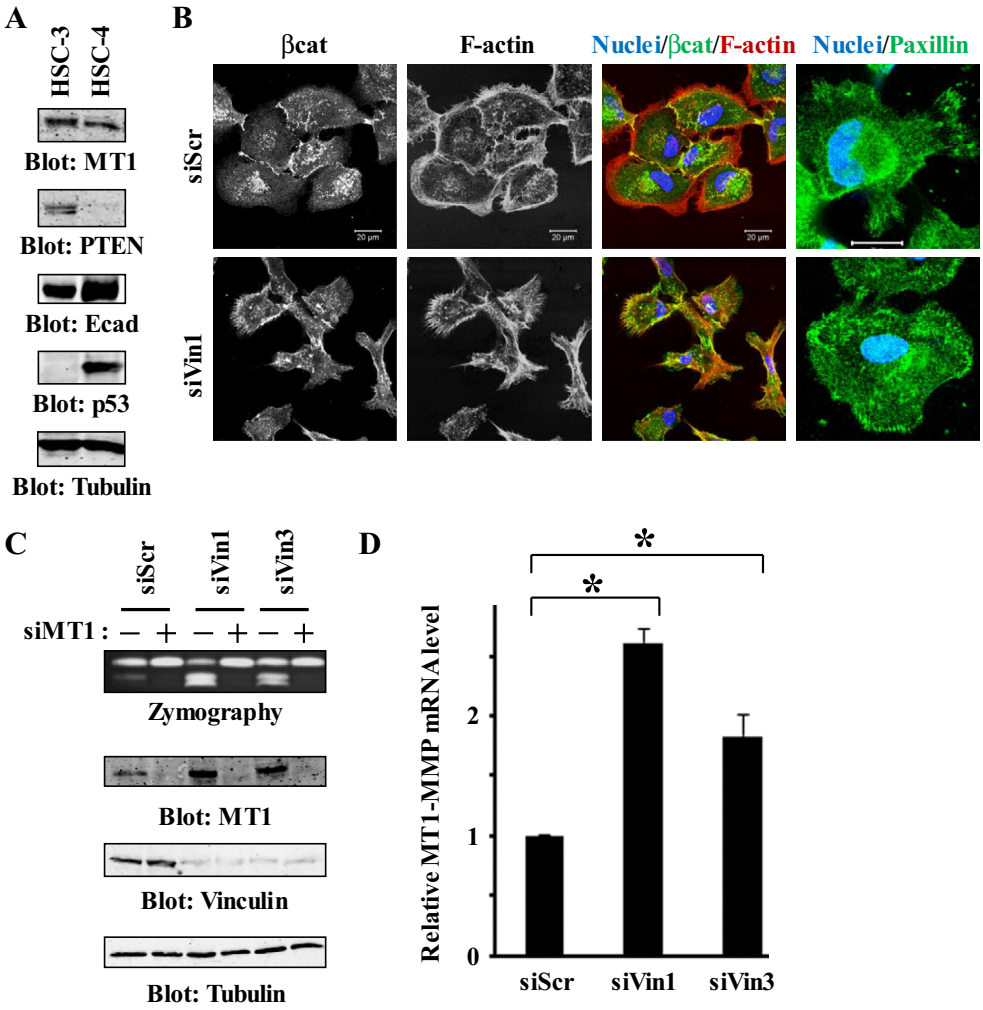

Fig. 1. Vinculin knockdown promotes MT1-MMP-mediated proMMP-2 activation. (A) Cell lysates from either HSC-3 or HSC-4 cells were analyzed by immunoblotting using MT1-MMP (MT1), anti-PTEN, anti-E-cadherin (Ecad), anti-p53 or anti-tubulin antibodies. (B) HSC-4 cells were cultured on glass coverslips, and transfected with siRNA for control (siScr) or vinculin (siVin1) for 48 h. The cells were analyzed by immunofluorescence staining using anti-β-catenin (βcat) or anti-paxillin antibodies, Rhodamine-phalloidin (F-actin), and Hoechst 33,342 (Nuclei). Scale bars are 20 μm. (C) HSC-4 cells were transfected with siRNA for either control or vinculin (siVin1 or siVin3) plus siRNA for MT1-MMP (siMT1). The conditioned media were analyzed by gelatin zymography, and cells lysates were analyzed by immunoblotting using anti-MT1-MMP, anti-vinculin or anti-tubulin antibodies. (D) HSC-4 cells were transfected with siRNA for either control or vinculin (siVin1 or siVin3) and mRNA was prepared from these cells. Quantitative RT-PCR for mRNA expression of MT1-MMP was performed as described in "Section 2". Expressions of MT1-MMP mRNA are normalized to that of cells transfected with siRNA for control. The *error bars* are standard deviations of the mean values obtained from three independent experiments. $^{*}p < 0.05$.