Biochimica et Biophysica Acta 1853 (2015) 409–421

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Role of the focal adhesion protein TRIM15 in colon cancer development

Ok-Hee Lee [a,b], Jinkyoung Lee [b], Keun Ho Lee [b], Yun Mi Woo [a,b], Ju-Hee Kang [c,d], Ho-Geun Yoon [e], Soo-Kyung Bae [f], Zhou Songyang [g], Seung Hyun Oh [c,d], Youngsok Choi [a,*]

[a] Department of Biomedical Science, CHA University, Seongnam-si, Gyeonggi-do, Republic of Korea
[b] Severance Integrative Research Institute for Cerebral and Cardiovascular Diseases, Yonsei University Health System, Seoul, Republic of Korea
[c] Research Institute, National Cancer Center, Goyang-si, Gyeonggi-do, Republic of Korea
[d] Laboratory of Preventive Pharmacy, College of Pharmacy, Gachon University, Incheon, Republic of Korea
[e] Department of Biochemistry and Molecular Biology, Center for Chronic Metabolic Disease Research, Yonsei University College of Medicine, Seoul, Republic of Korea
[f] Department of Dental Pharmacology, School of Dentistry, Pusan National University, Yangsan, Republic of Korea
[g] Verna and Marrs McLean Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, TX, USA

ARTICLE INFO

Article history:
Received 4 August 2014
Received in revised form 3 November 2014
Accepted 6 November 2014
Available online 15 November 2014

Keywords:
TRIM15
Colon cancer
Focal adhesion
Cell migration

ABSTRACT

The tripartite motif containing (TRIM) proteins are a large family of proteins that have been implicated in many biological processes including cell differentiation, apoptosis, transcriptional regulation, and signaling pathways. Here, we show that TRIM15 co-localized to focal adhesions through homo-dimerization and significantly suppressed cell migration. Domain mapping analysis indicated that B-box2 and PRY domains were essential for TRIM15 localization to focal adhesions and inhibition of cell migration. Our protein-protein interaction screen of TRIM15 with the integrin adhesome identified several TRIM15 interacting proteins including coronin 1B, cortactin, filamin binding LIM protein1, and vasodilator-stimulated phosphoprotein, which are involved in actin cytoskeleton dynamics. TRIM15 expression was tissue-restricted and downregulated in colon cancer. Level of TRIM15 expression was associated with colon cancer cell migration, as well as both *in vitro* and *in vivo* tumor growth. These data provide novel insights into the role of TRIM15 as an additional component of the integrin adhesome, regulating cell migration, and suggest that TRIM15 may function as a tumor suppressor of colon cancer.

1. Introduction

The tripartite motif containing (TRIM) protein family is characterized by the presence of three distinct motifs in the N-terminal region: a really interesting new gene (RING) domain, and one or two B-box domains, and a coiled-coil domain [1]. Given the high conservation of the order of these domains in the TRIM protein family members, the TRIM proteins are also known as RBCC proteins. Humans have more than 60 TRIM proteins, which are classified into 11 subgroups based on the domain composition of the C-terminal region [2–4]. Ten different types of C-terminal domains exist in TRIM family members, namely the COS box, fibronectin type 3, PRY/SPRY, plant homeodomain, bromodomain, NHL, filamin-type immunoglobulin, ADP ribosylation factor-like, MATH, and transmembrane domains. The most common C-terminal domain of TRIM proteins is the PRY/SPRY domain. Among the identified TRIM proteins, 34 members of the TRIM protein family contain a SPRY domain or a fusion of a PRY and SPRY domain [3,4]. Although many TRIM proteins play well-known roles in innate immunity by interfering with the viral replication cycle and regulating immune signaling pathways, TRIM proteins have also been implicated in diverse cellular processes, including cell differentiation, apoptosis, and transcriptional regulation [3–6].

TRIM15, also known as RNF93 or ZNFB7, contains a PRY/SPRY domain in the C-terminal region. A recent study proposed *TRIM15* as a candidate gene for diagnosing gastric cancer, because DNA methylation levels of *TRIM15* were higher in gastric cancer tissues than in normal gastric tissues [7]. TRIM15 mRNA expression was downregulated in gastric cancer, suggesting that lower *TRIM15* expression may be involved in development of gastric cancer. In other studies, TRIM15 was reported as a restriction factor for the murine leukemia virus and human immunodeficiency virus 1 by interacting with the retroviral Gag protein and inhibiting viral release [6]. Despite these interesting findings, little is known about the functional roles and tissue expression patterns of TRIM15. In addition, TRIM15 may be associated with other disease states. In this study, we focused on deciphering the molecular and

Abbreviations: CORO1B, coronin 1B; CTTN, cortactin; DAPI, 4′,6-diamidino-2-phenylindole; DMEM, Dulbecco's Modified Eagle's Medium; ECM, cell-extracellular matrix; EGF, epidermal growth factor; FBLIM1, filamin binding LIM protein 1; FBS, fetal bovine serum; GFP, green fluorescence protein; GST, glutathione s-transferase; RING, really interesting new gene; TRIM, tripartite motif-containing; VASP, vasodilator-stimulated phosphoprotein; YFP, yellow fluorescence protein

* Corresponding author at: Department of Biomedical Science, CHA University, 335 Pangyo-ro, Bundang-gu, Seongnam-si, Gyeonggi-do, 463-400, Republic of Korea. Tel.: +82 31 881 7149; fax: +82 31 881 7249.
E-mail address: youngsokchoi@cha.ac.kr (Y. Choi).

http://dx.doi.org/10.1016/j.bbamcr.2014.11.007
0167-4889/

cellular functions of TRIM15. We found TRIM15 to be a cell-extracellular matrix (ECM) adhesion component that interacted with several regulators involved in actin dynamics, ultimately suppressing cell migration. Furthermore, we demonstrated that TRIM15 was highly expressed in normal human colon tissues, and its expression was diminished in colon adenocarcinoma. Restoring expression of TRIM15 in colon cancer cells reduced tumor growth in mice, suggesting an important role for TRIM15 in suppression of cellular transformation.

2. Materials and methods

2.1. Plasmids

The pDONR223 vector that encoded TRIM15 was obtained from the human ORFeome v3.1 (Open Biosystems, Lafayette, CO, USA). Using Gateway recombination (Invitrogen, Carlsbad, CA, USA), the *TRIM15* was transferred into destination vectors to tag TRIM15 with N-terminal half yellow fluorescence protein (YFPn; pBabe-CMV-TRIM15-YFPn-neo), C-terminal half yellow fluorescence protein (YFPc; pCl-CMV-TRIM15-YFPc-puro), glutathione S-transferase (GST; pDEST27-TRIM15), or FLAG (pCl-CMV-2xFLAG-TRIM15-puro) [8]. The DNA fragments encoding TRIM15 deletion mutants were generated by PCR, cloned into the pENTR/D-TOPO vector (Invitrogen), and transferred into pCl-CMV-2xFLAG-DEST-puro vector using Gateway recombination.

2.2. Cell cultures

U2OS and 293T cells were cultured in high glucose Dulbecco's Modified Eagle's Medium (DMEM), HCT116, HT-29, and LOVO cells were cultured in RPMI medium, and CCD18Co cells were maintained in Minimum Essential Medium. The media for all the cells were supplemented with 10% fetal bovine serum (FBS), 100 units/ml penicillin, and 100 μg/ml streptomycin. To establish cells that stably expressed vector or FLAG-TRIM15, empty pCl-CMV-2xFLAG-puro vector [8] or the pCl-CMV-2xFLAG-TRIM15-puro vector was co-transfected with pCl-Ampho vector into 293T cells. After 2 days, U2OS, HCT116, or HT-29 cells were infected with the collected retroviral supernatants from the 293T cells and treated with 1 μg/ml puromycin for more than 10 days. To stimulate cells with epidermal growth factor (EGF), HCT116 cells stably expressing vector or FLAG-TRIM15 were serum-starved for 24 h and treated with 50 ng/ml of human recombinant EGF (Sigma-Aldrich, St. Louis, MO, USA) for the indicated time. For knockdown experiments, TRIM15 and CTTN siRNAs were obtained from Bioneer (Seoul, Korea) and transfected into LOVO or U2OS cells using Lipofectamine RNAiMax (Invitrogen). The sequence of CTTN siRNA was 5′-GGAACUUGAAACAGGACCA-3′. The sequences for the TRIM15 siRNAs were the following: siRNA1 (5′-AGCAGGCAGAGACU CCCAU-3′) and siRNA2 (5′-CCCAAUCCUCGGGCAAGAU-3′).

2.3. Antibodies

The following antibodies were used for the immunoblotting experiments: anti-FLAG M2 (Sigma-Aldrich), anti-GST (GE Healthcare, Buckinghamshire, UK), anti-phospho-cortactin (Tyr421), anti-cortactin (Cell Signaling Technology Inc., Danvers, MA, USA), and anti-β-actin (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) antibodies. A rabbit polyclonal antibody against the TRIM15 was generated by Abfrontier (Seoul, Korea) by immunizing rabbits with the keyhole-limpet hemocyanin-conjugated synthetic peptides C-EQQIWKERDEYITK and C-SEDRKSVRYTRQKKS that correspond to the amino acid residues 195-208 and 310-324 of the human TRIM15, respectively. The anti-TRIM15 antibody was affinity-purified, and the specificity of the antibody was validated. Anti-TRIM15 antibody was applicable to Western blotting and immunohistochemistry, but not to immunoprecipitation (Fig. S1).

2.4. Immunofluorescence

Cells were cultured on 12 mm glass coverslips and fixed with 3% paraformaldehyde/2% sucrose in phosphate buffered saline for 10 minute (min) at room temperature. The cells permeabilized with Triton X-100 buffer (0.5% Triton X-100, 20 mM Hepes-KOH, pH7.9, 50 mM NaCl, 3 mM MgCl2, and 300 mM sucrose) were incubated with the rabbit anti-FLAG (Sigma-Aldrich) and mouse anti-vinculin (Sigma-Aldrich) antibodies for 1 hour (h) at room temperature. After washing, the cells were incubated with an Alexa Fluor 488 conjugated anti-mouse antibody (Invitrogen), a Rhodamine Red-X conjugated anti-rabbit antibody (Invitrogen), and an Alexa Fluor 633 conjugated phalloidin (Invitrogen) for 1 h at room temperature. The cells were mounted with the ProLong antifade reagent (Invitrogen) containing 4′,6-diamidino-2-phenylindole (DAPI). The images were captured with an inverted LSM700 confocal microscope (Carl Zeiss, Inc., Oberkochen, Germany).

2.5. Transwell migration assay

Migration assay was performed according to a previously described method [9]. Briefly, bottom surfaces of filters with an 8 μm pore diameter were coated with 10 μg/ml fibronectin. Cells (2×10^5) were suspended in 100 μl of DMEM containing 1 mg/ml of bovine serum albumin and added to upper chamber of a transwell (Corning Costar, Corning, NY, USA). The lower compartments of the transwell chambers were filled with 600 μl of DMEM containing 10% FBS. After an incubation at 37 °C, the migrated cells were fixed and stained with hematoxylin. Incubation time was optimized for each cell types (U2OS cells for 7 h, HCT116 cells for 1 day, HT-29 and LOVO cells for 2 days). After removing cells that did not migrate, migration was quantified by counting the cells that migrated through pores. Eight independent fields were counted under a microscope (200× magnification) (Carl Zeiss). The results were expressed as a percentage of control cells.

2.6. Fluorescent protein complementation assay

Fluorescent protein complementation assay was performed as previously described [8]. Briefly, the pBabe-CMV-TRIM15-YFPn-neo and pCl-Ampho vectors were co-transfected into 293T cells for retrovirus production and subsequent infection of HTC75 cells. The bait cells stably expressing TRIM15 conjugated with YFPn were selected by treatment with 300 μg/ml G418. To screen the TRIM15 binding proteins with integrin adhesome proteins (www.adhesome.org) [10], 127 of available integrin adhesome proteins were selected from our previous high-throughput protein complementation array screen system and reconstituted into 96 well plates (Fig. S2). Individual clone of YFPc-adhesome proteins with pCl-Ampho were then co-transfected into 293T cells to generate retroviruses. Retroviral supernatant was collected at 48 h post-transfection. The TRIM15 bait cells were seeded onto 24-well plates and infected with the retrovirus encoding a YFPc-tagged integrin adhesome protein. At 2 days following the infection, cells were selected with 1 μg/ml of puromycin for 10 days. Cells were then harvested for flow cytometry analysis using the LSRII flow cytometer (BD Biosciences, San Jose, CA, USA). According to data of our previous complementation assay, we considered the nonspecific signals for complementation assay by 6.37 ± 3.66% YFP positives [8]. The identified proteins were analyzed by the Ingenuity Pathway Analysis program (Redwood City, CA, USA) to determine network and function.

2.7. Co-precipitation and immunoblotting

The indicated genes were cloned into either pDEST27 (Invitrogen) to tag with GST or pCl-CMV-2xFLAG-DEST-puro for FLAG. The indicated plasmids were co-transfected into 293T cells using Lipofectamine 2000 (Invitrogen). The transfected cells were harvested 2 days after