Biochimica et Biophysica Acta 1853 (2015) 3258–3265

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Tensin1 positively regulates RhoA activity through its interaction with DLC1☆

Yi-Ping Shih *, Peng Sun, Aifeng Wang, Su Hao Lo *

Department of Biochemistry and Molecular Medicine, University of California-Davis, Sacramento, CA 95817, USA

ARTICLE INFO

Article history:
Received 30 June 2015
Received in revised form 24 August 2015
Accepted 21 September 2015
Available online 28 September 2015

Keywords:
Tensin
DLC1
RhoA
Focal adhesion
Angiogenesis

ABSTRACT

DLC1 is a RhoGAP-containing tumor suppressor and many of DLC1's functions are absolutely dependent on its RhoGAP activity. Through its RhoGAP domain, DLC1 inhibits the activity of RhoA GTPase, which regulates actin cytoskeleton networks and dis/assembly of focal adhesions. Tensin1 (TNS1) is a focal adhesion molecule that links the actin cytoskeleton to integrins and forms signaling complexes through its multiple binding domains. Here, we report that TNS1 enhances RhoA activity in a DLC1-dependent manner. This is accomplished by binding to DLC1 through TNS1's C2, SH2, and PTB domains. Point mutations at these three sites disrupt TNS1's interaction with DLC1 as well as its effect on RhoA activity. The biological relevance of this TNS1–DLC1–RhoA signaling axis is investigated in TNS1 knockout (KO) cells and mice. Endothelial cells isolated from TNS1 KO mice or those silenced with TNS1 siRNA show significant reduction in proliferation, migration, and tube formation activities. Concomitantly, the RhoA activity is down-regulated in TNS1 KO cells and this reduction is restored by further silencing of DLC1. Furthermore, the angiogenic process is compromised in TNS1 KO mice. These studies demonstrate that TNS1 binds to DLC1 and fine-tunes its RhoGAP activity toward RhoA and that the TNS1–DLC1–RhoA signaling axis is critical in regulating cellular functions that lead to angiogenesis.

1. Introduction

Deleted in liver cancer 1 (DLC1, also known as ARHGAP7 and STARD12 in human and p122RhoGAP in rat) was first isolated from rat brain as a phospholipase C delta 1 binding protein [1] and then was independently cloned by subtractive hybridization as a gene homozygously deleted in a human hepatocellular carcinoma [2]. Together with its gene locus at human chromosome 8p22, a region frequently deleted in liver cancer, DLC1 was considered as a potential tumor suppressor in liver [3–5]. Further studies showed that DLC1 expression was lost or down-regulated in various cancers including liver, breast, lung, brain, stomach, colon, and prostate cancers due to either genomic deletion or aberrant DNA methylation [3,4]. Mutations that altered the expression or function of DLC1 were detected in pancreas [6], colon, and prostate cancers [7]. These results suggest that DLC1 may function as a tumor suppressor in tissues other than liver [3,4]. DLC1 contains a SAM, a RhoGAP, and a START domain. Its RhoGAP domain suppresses the activity of RhoA GTPase by converting the GTP-bound active RhoA to GDP-bound inactive form. RhoA mediates cell adhesion, shape, and migration through its roles in modulating actin cytoskeleton networks and focal adhesion turnover [8]. The essential role of the RhoGAP in DLC1's tumor suppression and other activities is well established. Without a functional RhoGAP domain, DLC1 is not able to regulate cell shape, proliferation, adhesion, and migration [4]. Therefore, tight control of its RhoGAP activity is a critical task of DLC1.

Tensin is a focal adhesion family with four members: tensin1 (TNS1), tensin2 (TNS2), tensin3 (TNS3), and C-terminal tensin-like (cten) [9]. TNS1 is the founding member of the family. It interacts with actin filaments and regulates actin polymerization [10]. Tensin contains a PTB (phosphotyrosine binding) domain that binds to the NPXY motif in β-integrin and a SH2 (Src homology 2) domain that binds to tyrosine phosphorylated proteins, including Axl, EGFR, Src, Fak, p130cas, and paxillin [9,11–13]. TNS2 (160 kD) and TNS3 (180 kD) have very similar domain structures and molecular masses with those of TNS1 (220 kD) [11,14,15]. Cten, on the other hand, is a much smaller protein (80 kD) and only shares the SH2 and PTB domains with other tensins [16]. Previously, we have discovered that the SH2 domains of all tensins bind to DLC1 in a phosphorylation-independent fashion and that this interaction is essential for recruiting DLC1 to focal adhesion sites and for DLC1's tumor suppression activity [17]. However, the effect of this interaction on DLC1's RhoGAP activity is not well understood.

In this report, we have established that TNS1 negatively regulates DLC1's RhoGAP activity toward RhoA through its multiple interactions. The biological significance of this TNS1–DLC1–RhoA signaling axis is demonstrated by showing abnormal cellular function and angiogenic response in TNS1 knockout (KO) endothelial cells and mice.

☆ The authors disclose no potential conflicts of interest.

* Corresponding authors at: Department of Biochemistry and Molecular Medicine, University of California-Davis, 4635 Second Ave. Research I, Room 3210, Sacramento CA 95817, USA.
E-mail address: ypshih@ucdavis.edu (Y.-P. Shih).

http://dx.doi.org/10.1016/j.bbamcr.2015.09.028
0167-4889/

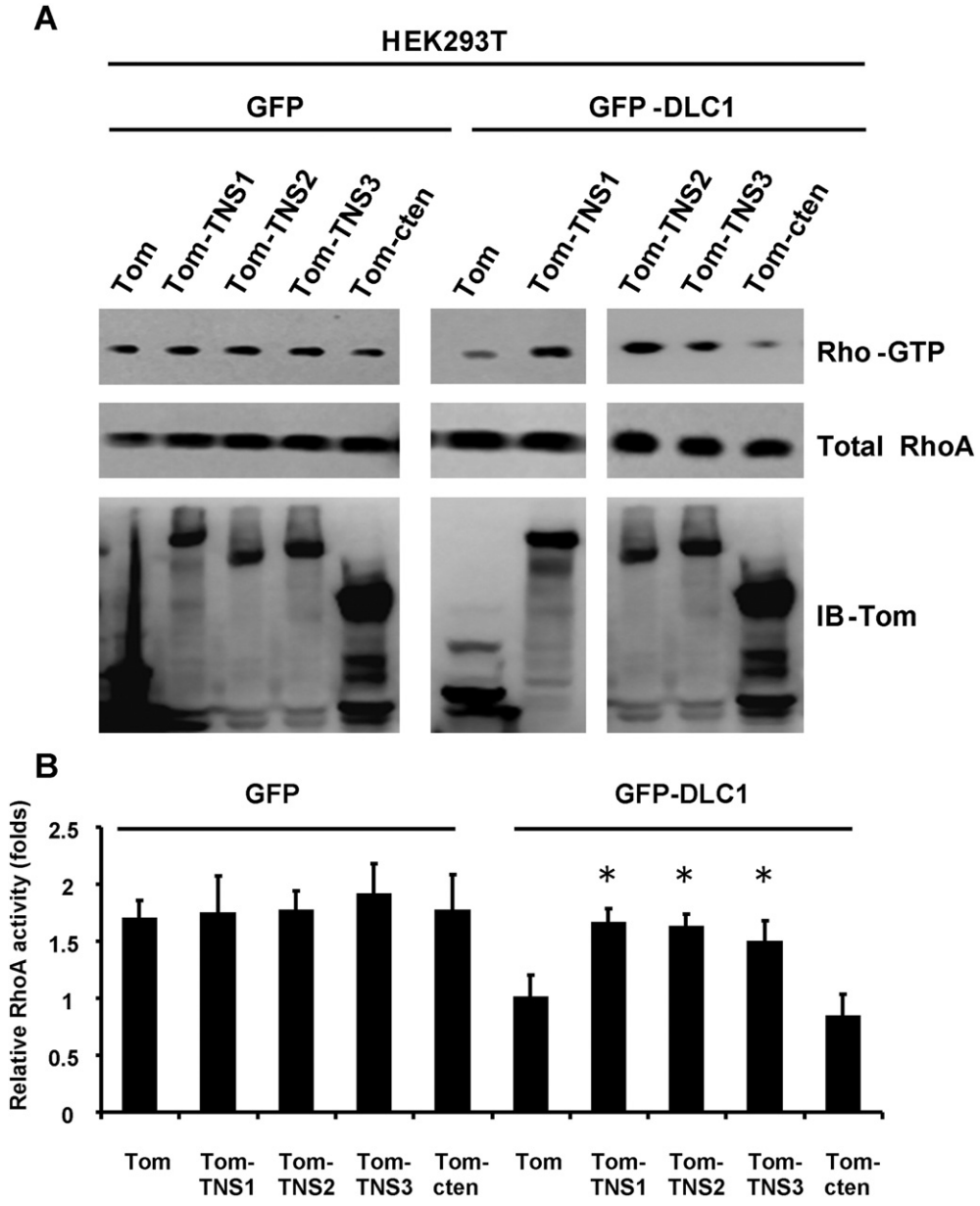

Fig. 1. TNS1, TNS2, and TNS3, but not cten, regulate RhoA activity through DLC1. (A) Cell lysates from HEK293 transfected with indicated constructs were used to determine RhoA activities by rhotekin pull down assays and shown as Rho-GTP levels. Whole cell lysates were immunoblotted (IB) with antibodies against RhoA or tomato (Tom) to show the expression levels of endogenous RhoA and recombinant fusion proteins. (B) Bar graph shows quantification of Rho-GTP level. Shown are means ± SD for a minimum of three repetitions per transfection condition. TNS1, TNS2, and TNS3 significantly reduced RhoGAP activity of DLC1 when they were individually compared to control (Tom). *P* value was calculated compared to Tom control by Student's t-test. *, $P < 0.05$.

2. Materials and methods

2.1. Mice

TNS1 KO mice were produced by our group [18]. All animal procedures were performed according to UC Davis guidelines for the care and use of laboratory animals.

2.2. Cell culture and reagents

HUVECs were cultured in endothelial cell growth medium (ScienCell). 293T cells were maintained in DMEM/high glucose with 10% fetal bovine serum. Lipofectamine-2000 (Invitrogen) was used for transfections. TNS1 siRNA and DLC1 siRNA were purchased from Sigma-Aldrich.

2.3. Plasmid constructions and mutagenesis

The full-length or truncated cDNA fragments encoding human TNS1 residues 75 to 310, 75 to 210, and 211 to 310 were subcloned in frame into mammalian expression vector pTomato or pEGFP (Clontech). The site-specific mutation of 244M (GD244AA), 252M (HK252AA), SH2M (R1488A), and PTBM (FFRR1656AAAA) was introduced into TNS1 cDNA by site-directed mutagenesis. All constructs were verified by DNA sequencing.

2.4. Isolation of endothelial cells from mouse lung tissues

Lung tissues from three 7–10 day old mice were removed aseptically and rinsed in ice-cold DMEM. After removal of the larger blood vessels,