Experimental Cell Research xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Experimental Cell Research

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

Talin is a substrate for SUMOylation in migrating cancer cells

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ARTICLE INFO

Keywords:
Talin
SUMOylation
Focal adhesions
Cancer cell migration

ABSTRACT

Focal adhesions (FAs) play an important role in cancer cell migration and metastasis by linking the actin cytoskeleton to the extracellular matrix, allowing the cell to generate traction. SUMOylation is a post-translational modification of proteins on lysine residues that can affect protein localisation, turnover and protein-protein interactions. In this study, we demonstrate that talin, a key component of FAs, can be post-translationally modified by SUMOylation in MDA-MB-231 breast cancer cells and U2OS osteosarcoma cells. Furthermore we demonstrate that SUMOylation regulates the dynamic activities of FAs including their number, size and turnover rate. Inhibiting SUMOylation significantly reduced the speed of cell migration. The identification of talin as a SUMO target provides insight into the mechanisms regulating focal adhesion formation and turnover and potentially identifies a novel mechanism underlying cell migration.

1. Introduction

SUMO (small ubiquitin-related modifier) family proteins are ubiquitin-related small proteins, which are ~15 kDa and can be conjugated to cellular substrates on lysine residues in an analogous way to ubiquitin [1]. This type of post-translational modification, known as SUMOylation, is implicated in the control of a wide variety of cellular processes, such as cell signalling, cell cycle and nuclear modification [2]. The SUMO family consists of at least three isoforms – SUMO 1 is mainly found in the nucleus while SUMO 2 and 3 share 95% homology and are generally considered together as SUMO 2/3, and are mainly located in the cytoplasm [3]. SUMO 1 and SUMO 2/3 proteins work closely with SUMO proteases and conjugating enzymes in the SUMOylation cycle [4]. Protein substrate modification with SUMO relies on a single E2 ubiquitin conjugating enzyme, Ubc9, in the SUMOylation pathway, where Ubc9 is unique among E2 enzymes in its capability to specifically recognize and conjugate SUMO1 or SUMO 2/3 to their substrates [5,6].

Focal adhesions (FAs) are large multi-protein complexes that play a central role in cell migration by linking the extracellular matrix (ECM) bound to transmembrane integrin molecules with the actin cytoskeleton, allowing the cell to generate traction [7]. Cell migration on and through ECM requires the turnover of focal adhesions [8] so that FAs form when the cell attaches to ECM, generate traction allowing the cell to move forward and then disassemble to allow new FAs to form at the leading edge of the cell [9–11]. Rapid and dynamic FA assembly and disassembly processes are controlled and regulated spatiotemporally at the leading edge and the rear end of the migrating cell and are required for successful cell migration [12,13]. More than 150 FA or FA-associated proteins have been identified [14] with some of the most important FA proteins for cell migration including focal adhesion kinase (FAK), talin, vinculin, paxillin and zyxin [15,16]. Previous studies have shown that FAK interacts with PIAS1, which promotes the FERM domain of FAK to be covalently modified by SUMO-1 at the ε-amino position of lysine 152 enhancing its autophosphorylation [17]. SUMOylation of FAK occurs mostly in the nucleus and possibly independently of cell adhesion as PIAS1 is predominantly a nuclear protein, suggesting that cytoplasmic FAK may undergo nucleocytoplasmic cycling. Inhibiting protein SUMOylation after 6 h of ginkgolic acid (GA) treatment (an inhibitor of SUMOylation) in HEK293T cells resulted in a significant decrease in SUMO1 and SUMO 2/3 conjugation and a reduction in Tyr-397 phosphorylation in the SUMOylated form of FAK; FAK was therefore identified as a substrate for SUMOylation [17–19] but no other focal adhesion proteins have subsequently been shown to be modified by SUMOylation. In addition, no studies have shown SUMO modification of proteins within focal adhesions.

In this study, we show that talin, a key component of focal adhesions is a SUMO substrate. Talin is required in FAs for linking integrin to actin filaments and, together with kindlin, it is important for inside-out integrin activation, which can relay the inside-out signals to maintain an activated integrin state at the ECM-substrate surfaces [20–23]. We demonstrate that talin localised in FA's is SUMOylated and also show that inhibiting SUMOylation significantly increased the number and

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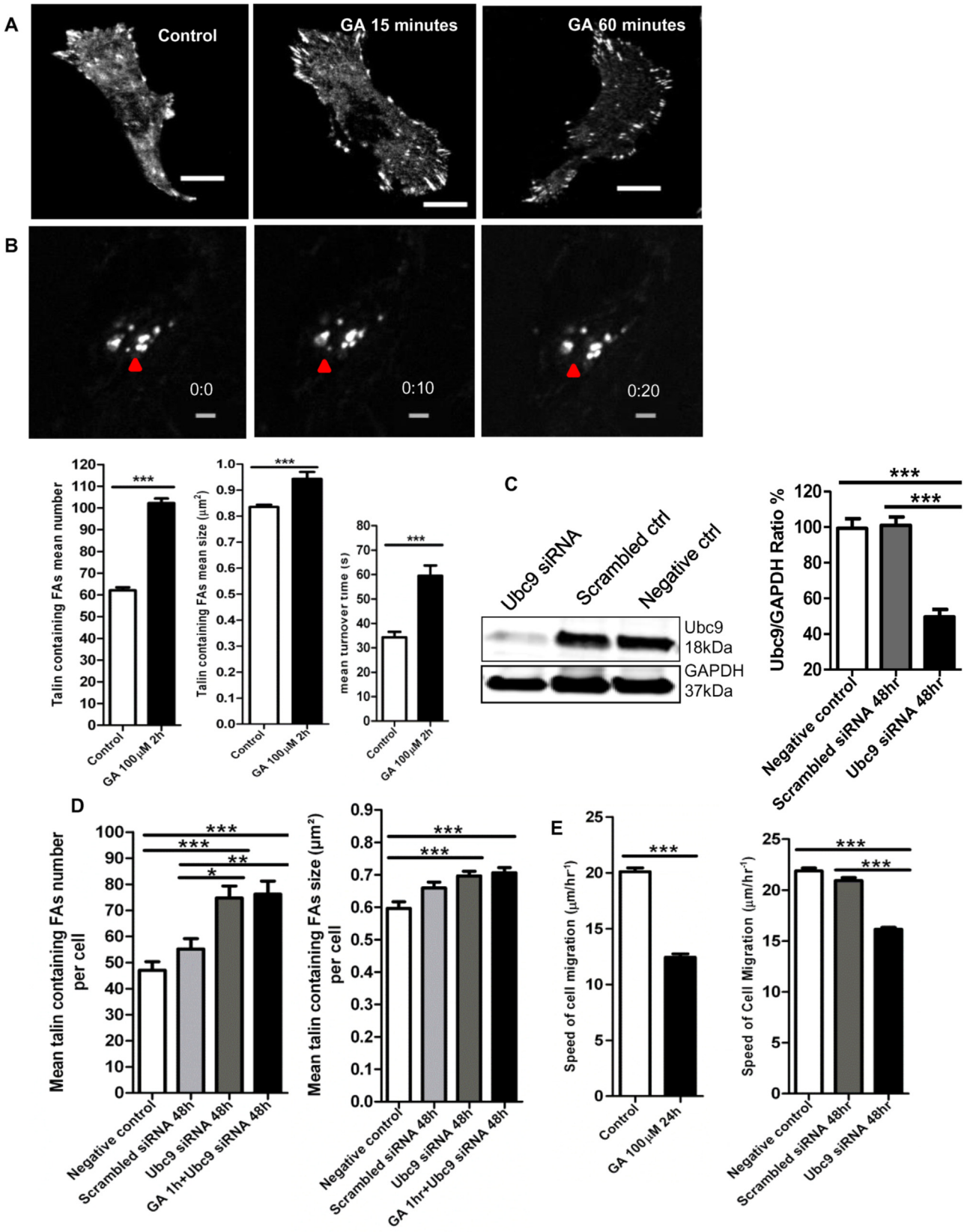
https://doi.org/10.1016/j.yexcr.2018.07.005
Received 3 January 2018; Received in revised form 25 June 2018; Accepted 3 July 2018

size of talin-containing FAs as well as reducing their turnover rate and decreasing the speed of cell migration. We also propose that SUMOylation of talin may regulate talin cleavage by calpain, an important regulator of FA disassembly and turnover. We have therefore have identified a potential new role for SUMOylation in the regulation and function of talin which could be important in cell migration.



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