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LETTER TO THE EDITOR

LATS1 tumor suppressor is a novel actin-binding protein and negative regulator of actin polymerization

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Dear Editor,

The LATS tumor suppressor, conserved from *Drosophila* (*dlats*) to humans (*LATS1*, *LATS2*), plays a vital role in maintaining cellular homeostasis in humans since loss of either *LATS1* or *LATS2* leads to the development of numerous cancer types such as breast cancer and leukemia [1]. Apart from its roles as a Ser/Thr kinase within the emerging Hippo pathway regulating cell proliferation and apoptosis, ultimately leading to the control of organ size and tumorigenesis [2], LATS is also implicated in a broad range of functions including regulation of genetic stability, transcription, and protein stability [1]. Recently, tumor suppressors have also been shown to affect the later stages of tumorigenesis, including metastasis. Among this group of metastasis regulators are genes that can directly affect actin dynamics by binding to F-actin, such as the tumor suppressors p53 [3], NF2 [4] and APC [5]. In this study we show that the LATS1 tumor suppressor is also a novel actin-binding protein that can modulate actin polymerization, cell migration, and cell spreading.

Combined with mass spectrometry, SILAC (Stable Isotope Labelled by Amino acids in Cell culture) is a powerful tool for the identification of specific protein-protein interactions. Applying this approach to identifying novel LATS1 interaction partners, LATS1-KO MEFs (LATS1 knockout mouse embryonic fibroblasts) and LATS1-WT-myc MEFs (LATS1 knockout MEFs infected with wild-type myc-tagged LATS1 lentivirus) were labeled with “light” (L) or “heavy” (H) amino acids, respectively, and cell lysate was extracted, immunoprecipitated for LATS1 and mixed elutes subjected to mass spectrometry analysis. From this screen, several novel LATS1-interacting partners were identified (unpublished results). Importantly, we identified β-actin as a binding partner of LATS1 (Supplementary information Figure S1). Compared to 40S ribosomal protein S3, a housekeeping gene, with a relative abundance ratio of 1.02, β-actin has a H/L ratio of 1.91, suggesting that β-actin is a novel LATS1 binding partner.

To confirm that LATS1 indeed binds β-actin, we immunoprecipitated LATS1 as before and show that β-actin is also pulled down (Figure 1A). Furthermore, co-sedimentation assays using 1.0 μM F-actin demonstrate that with increasing amounts of LATS1-GST (0.125, 0.25, 0.5, and 1.0 μM), the LATS1/actin ratios in the pellet (P) after ultracentrifuguation gradually increased (Figure 1B). These results indicate that full-length LATS1 directly and specifically interacts with F-actin in a concentration-dependent manner.

We next examined whether LATS1 co-localizes with F-actin in NIH3T3 fibroblasts using immunofluorescent analysis. Importantly, endogenous LATS1 is found on both stress fibers and the leading edge of migrating cells, regions where F-actin is also localized (Figure 1C). Dramatically, after treatment of cells with Cytochalasin D, an actin-depolymerization reagent which severs F-actin into small F-actin fragments and causes actin foci in cells, LATS1 aggregates and co-localizes perfectly with these F-actin foci (Figure 1D). Together, these findings strongly suggest that LATS1 is a novel actin-binding protein.

The identification of actin as a LATS1-binding partner poses the possibility that LATS1 may also regulate actin dynamics. Complex actin dynamics are important for many cellular functions including cell structure, division, adhesion, and motility. At the core of actin dynamics is its polymerization, which itself can involve multiple processes regulated by various actin-binding proteins. Because LATS1 binds actin, we examined how LATS1 may also affect F-actin polymerization and thus be added to the growing list of regulators. We first examined actin polymerization *in vitro* using pyrene-actin mixed with 1× PBS (control), 10 μM GST, or 10 or 25 μM LATS1-GST. Compared to the control, while addition of GST had no effect on actin polymerization, addition of increasing concentrations of LATS1-GST (from 10 μM to 25 μM) significantly inhibited actin polymerization (Figure 1E). Because LATS1 is a Ser/Thr kinase, we assessed how N-terminal LATS1, which lacks the kinase domain, or how the C-terminal kinase domain LATS1 modulates actin polymerization. Whereas 25 μM N-terminal LATS1-

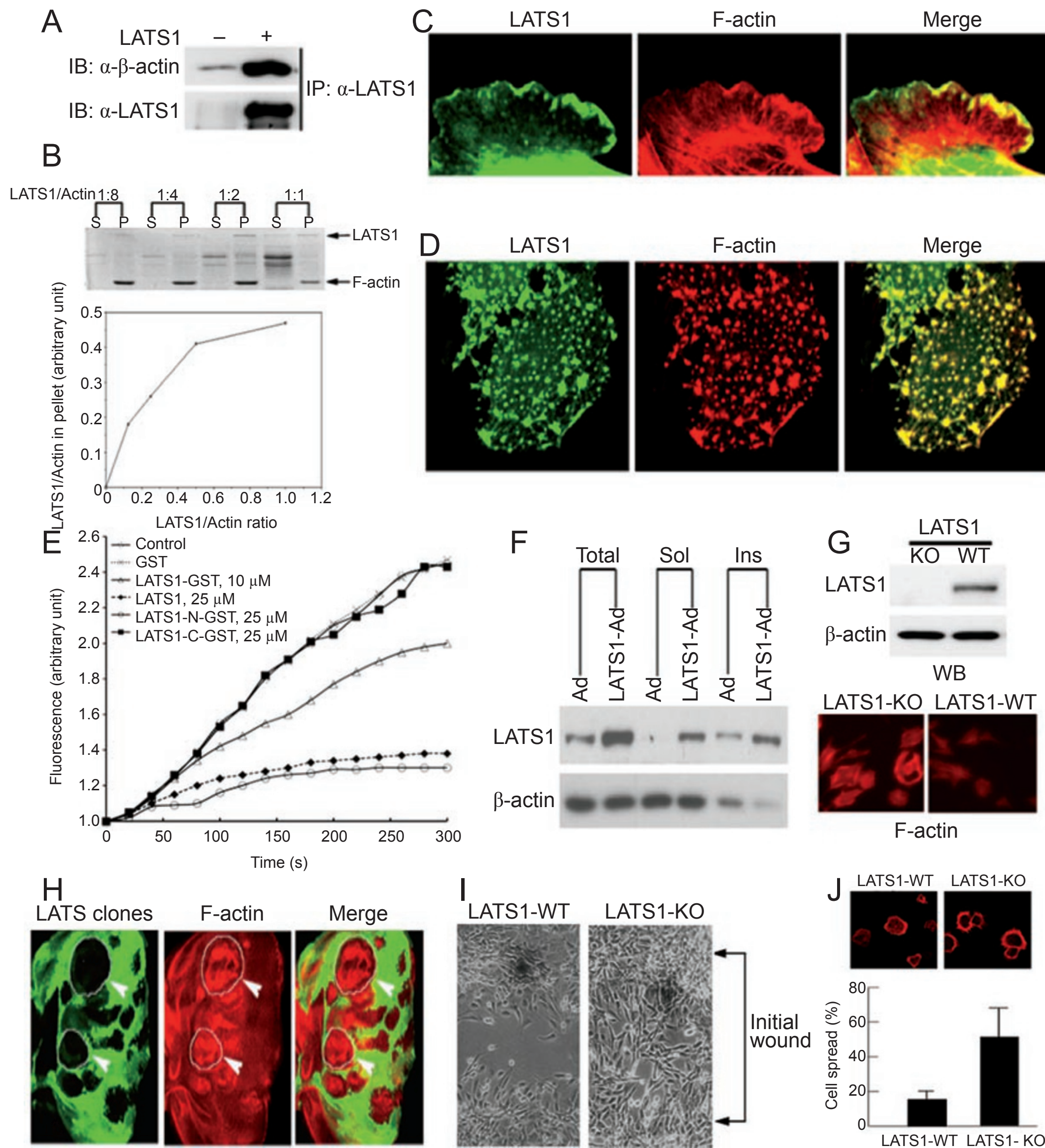

Figure 1 LATS1 interacts with β-actin and modulates actin polymerization, cell migration and cell spreading. **(A)** LATS1 was immunoprecipitated from LATS1-KO MEFs and LATS1-WT-myc MEFs with anti-LATS1 antibody. Lysates were subjected to western blot using LATS1 and β-actin antibodies. **(B)** Co-sedimentation analysis of LATS1-actin interaction *in vitro*. A fixed concentration of F-actin (1 μM) was mixed with increasing concentrations of LATS1-GST (0.125 to 1 μM). The molar ratios of LATS1 and F-actin are shown. **(C)** Migrating NIH3T3 cells were stained for LATS1 (green) using anti-LATS1 and AF488 anti-rabbit IgG antibodies and F-actin (Red) using Texas Red phalloidin. **(D)** LATS1-transfected HeLa cells were treated with Cyto D for 30 min before staining for LATS1 (green) and F-actin (red). **(E)** Regulation of actin polymerization *in vitro*. 10% pyrene-actin (10 μM) was mixed with equal volume of 1× PBS (control), 10 μM GST, and 10, 25 μM LATS1-GST, 25 μM LATS-1-N-GST (amino acids 1-585), or 25 μM LATS1-C-GST (amino acids 588-1130). Polymerization was monitored by measuring fluorescent intensity for 5 min at 365 nm emission and 407 nm excitation. **(F)** Regulation of actin polymerization *in vivo*. MDA-MB-231 cells infected with Ad or LATS1-Ad were lysed and separated into soluble (Sol) and insoluble cytoskeletal fractions (Ins) that were used for western blot. **(G)** MEFs with LATS1 knockout (–) or expressed (+) were subjected to protein extraction and western blot analysis (top panel) or stained for F-actin with TR phalloidin (bottom panel). **(H)** Loss of *dLATS* enhances actin polymerization in *Drosophila* eye imaginal discs *in vivo*. Wild-type *dLATS* cells are marked by GFP (green) and indicated by white circles and arrowheads. The disc was stained for F-actin (red) with TR phalloidin. **(I)** Cell migration. MEFs with wild-type *LATS1* (*LATS1*-WT) or *LATS1* knockout (*LATS1-KO*) were plated, wounded, and migration of cells recorded 12 h after wounding. **(J)** Cell spreading. *LATS1-WT* or *LATS1-KO* MEFs were plated onto coverslips coated with 10 μg/ml of fibronectin. Thirty minutes after plating, adherent cells were fixed by 4% formaldehyde and stained with Texas Red phalliodin. Percentage of spread cells is shown.

GST inhibits actin polymerization in a similar manner to wildtype LATS1, 25 μM C-terminal of LATS1-GST has no effect on polyermization (Figure 1E). Together, this shows that LATS1 inhibits actin polymerization independently of its C-terminal kinase activity.

Since actin polymerization usually leads to decreased levels of G-actin (soluble cytosolic fraction) with a concomitant increase in F-actin levels (insoluble cytoskeletal fraction), assessing the relative levels of G-actin and F-actin serves as an appropriate assay to measure *in vivo* actin polymerization. As shown in Figure 1F, overexpression of LATS1 (LATS1-Ad) through adenovirus-mediated infection in MDA-MB-231 breast carcinoma cells, compared to empty vector (Ad), causes a minor increase in the G-actin level (soluble fraction) but a significant decrease in F-actin (insoluble cytoskeletal fraction). In addition, consistent with our above experimental findings that LATS1 is a cytoskeletal protein due to its interaction with F-actin, LATS1 was mostly found in the insoluble cytoskeletal fraction. Finally, decreased levels of F-actin are visualized in actin-stained LATS1-WT-MEFs compared to LATS1-KO MEFs (Figure 1G). These results provide convincing evidence that LATS1 is negative regulator of actin polymerization both *in vitro* and *in vivo.*

Homologs of human LATS1 have also been shown to influence actin organization. For example, mutations in either d*lats* or *trc*, the Ndr homolog in *Drosophila,* result in defects in splitting or branching of many cytoskeletal structures including hairs, bristles, and denticles [6], whereas the *C. elegans* homolog, *sax-1*, has been shown to modulate actin polymerization with effects on neurite initiation, spreading, branching, and tiling [7]. In line with these studies, we also show that d*lats* affects actin polymerization in *Drosophila* eye imaginal discs (Figure 1H). A combination of wild-type cells marked by GFP and cells with loss of d*lats* (cells without GFP) were stained for F-actin. Significantly, there is noticeably more F-actin in d*lats* knockout cells. Therefore, the ability of LATS1 to interact with and inhibit actin polymerization is an evolutionarily conserved trait.

Since actin polymerization is essential for cell migration and LATS1 colocalizes with F-actin during cell migration (Figure 1C), we examined how LATS1 affects cell migration. Notably, we found that LATS1 inhibits cell migration in MEFs. Using the classic wound healing assay, 12 hrs after wound introduction, LATS1-KO MEFs rather than LATS1-WT MEFs were able to completely close the wound (Figure 1I), concurring with other studies that shows how loss of tumor suppressors enhances cell migration [3]. Similar results describing a role for LATS1 in cell migration have been observed. For example, we have shown that loss of both LATS1 and LATS2 in HeLa cells through siRNA-mediated knockdown enhances cell migration [8]. In the current study we also show that LATS1 inhibits cell spreading, an important attribute of migrating cells that is also dependent on actin polymerization. Significantly, the percentage of cells expressing LATS1 (LATS1-WT MEFs) that spread onto fibronectin-coated plates was significantly less compared to LATS1-KO MEFs (Figure 1J). Therefore, expression of LATS1 can inhibit a cell's ability to adhere, spread, and migrate.

The ability of LATS1 to bind to and inhibit actin polymerization provides a viable mechanism through which LATS1 may be able to modulate cytoskeletal dynamics and cell migration. However, other possible mechanism cannot be excluded. Since LATS1 also binds to and inhibits two positive regulators of actin polymerization, LIMK1 and Zyxin [1], enhanced motility in MEFs derived from LATS1 knockout mice may be caused by increased actin polymerization resulting from the release of either direct inhibition of actin polymerization by LATS1 or indirect inhibition of actin polymerization by LATS1 inhibition of LIMK1 or Zyxin. In addition, one study proposed that LATS1 affects cell migration through its substrate YAP [9]. Future studies will need to decipher the precise mechanisms mediating LATS1 effects on cell migration.

In summary, in the present study we discovered that LATS1, a tumor suppressor and cell cycle regulator, is also a cytoskeletal protein that directly binds to F-actin and inhibits actin polymerization both *in vitro* and *in vivo*. This adds LATS1 to a growing number of tumor suppressors that can directly modulate the cytoskeleton and thus play a crucial role in regulating various aspects of tumorigenesis. On top of this, we also show that expression of LATS1 inhibits both cell migration and cell spreading. These results provide a new mechanism for mediating LATS1 functions and implicate LATS1 as a potential regulator of metastasis.

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(**Supplementary information** is linked to the online version of the paper on the *Cell Research* website.)