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The human tumour suppressor LATS1 is activated by human MOB1 at the membrane

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Abstract

Downregulation of the LATS1 tumour suppressor protein kinase contributes to tumour formation in mammals and flies. Strikingly, the tumour suppressor activity depends on the interaction with Dmob (*Drosphila* Mps1-One binder) in *Drosophila melanogaster*. Recently, human LATS1 was reported to interact with human MOB1 (hMOB1), but the activation of LATS1 was not addressed. Here, we identified a highly conserved hMOB1-binding motif within LATS1's primary structure. While co-expression of LATS1 with hMOB1 did not elevate LATS1 kinase activity in mammalian cells, membrane-targeting of hMOB1 resulted in a significant increase of LATS1 activity. This stimulation was dependent on intact activation segment and hydrophobic motif phosphorylation sites, and was further found to occur a few minutes after membrane association. Therefore, we suggest a potential in vivo mechanism of LATS1 activation through rapid recruitment to the plasma membrane by hMOB1 followed by multi-site phosphorylation, thereby providing insight into the molecular regulation of the LATS tumour suppressor.

Keywords: Signal transduction; LATS; Kinase; Tumour suppressor; MOB; Membrane; Activation mechanism

The conserved NDR (nuclear Dbf2-related) family represents a subclass of the AGC serine/threonine protein kinases and consists of mammalian NDR1, NDR2, LATS1 (large tumour suppressor 1) and LATS2, *Drosophila melanogaster* TRC and LATS, *Caenorhabditis elegans* SAX-1 and LATS, and a number of fungi and plant kinases [1]. Human, fly, and yeast NDR kinases have been reported to require phosphorylation on both the activation segment and the hydrophobic motif for activation [2–9]. All family members have also a conserved N-terminal regulatory (NTR) domain of different lengths, best characterized in mammalian NDR1/2 [8–12]. Human MOB1A (hMOB1A) binds to this domain, thereby probably releasing autoinhibition of activation segment autophosphorylation [10]. However, although this activation mechanism is readily recapitulated in vitro, co-expression of the various components in tissue culture cells proved inefficient to activate NDR1/2 kinases [10,11], but when NDR1/2 itself or its co-activator hMOB1A/B/2 (hMOBs) is targeted to the membrane, an increase in NDR1/2 kinase activity was readily achieved [11,13].

While the biological functions of mammalian NDR1/2 remain the subject of intense research, mammalian LATS kinases are implicated in regulating cell cycle progression and apoptosis [14–23]. Moreover, LATS1 deficient mice developed ovarian stromal cell tumours and soft tissue sarcomas [24]. Promoter inactivation and missense mutations affecting LATS1 have further been reported in human sarcomas, ovarian carcinomas, and breast cancer [25–27]. These data suggest that dysregulated LATS1 expression contributes to tumour formation in mammals. Strikingly, the *warts*/*lats* gene confers also tumour suppressor activity in invertebrates [28–30].

The signalling pathway involving Lats/Warts, Hippo, Salvador, and Yorkie proteins was previously shown to be essential for proliferation control in *D. melanogaster*

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[31–38]. However, recently it was reported that the catalytic activity of the *Drosophila* Lats protein kinase can be regulated by co-activator binding [39,40]. The Mats protein (MOB as tumour suppressor; also termed Dmob1), a member of the superfamily of MOB proteins, interacts genetically and physically with Lats. The human ortholog of Mats, hMOB1A, is able to rescue the lethality of Mats loss-of-function in flies, suggesting that the regulation of LATS by MOBs is conserved from *Drosophila* to human. In support of this notion, human LATS1 was recently found to bind to hMOB1A [14], however, this study did not address the activation of LATS1 by hMOB1.

Here, we explored further the interaction of human LATS1 with hMOB proteins. Furthermore, considering that the biochemical analysis of Lats activation by Mats was carried out solely in the presence of okadaic acid (OA) [39], a potent PP2A inhibitor proven to also activate human LATS1 kinase [2], we further investigated the activation of human LATS1 by hMOBs in a cellular context without any drug treatment. Moreover, given that human LATS1 and NDR1/2 kinases appear to be regulated in a similar manner, we also tested the role of altered subcellular localization of hMOBs on human LATS1 activation, since we reported previously that in vivo activation of human NDR1/2 kinases can be achieved by translocation to the plasma membrane [11,13].

Materials and methods

Cell culture, chemicals, drug treatments, transfections, and antibodies. COS-7 cells were cultured and transfected as described [11]. In some experiments, cells were treated for 60 min with 1 μM okadaic acid (OA; Alexis Corp.). In other experiments, cells were serum-starved for 2 h prior to transfection. The transfection mixture was removed after 4 h and cells were serum-starved overnight before stimulation with 12-*O*-tetradecanoylphorbol 13-acetate (TPA; 100 ng/ml; Amersham Biosciences). Anti-HA 12CA5 and anti-myc 9E10 antibodies were used as hybridoma supernatants. Anti-HA Y11 antibodies were from Santa Cruz.

Construction of plasmids. All human hMOB1A, hMOB1B, and hMOB2 constructs have been described previously [11]. Human LATS1 cDNAs were subcloned into pcDNA3-derivatives containing a haemagglutination (HA) eptitope. Except for LATS1 (D846A), all mutants of LATS1 were generated by using the QuikChange procedure with pcDNA3-HA-LATS1 as template. All constructs were confirmed by sequence analysis. Experimental details of the generation of constructs are available upon request.

Fractionation of cells and immunofluorescence microscopy. Cytosolic and membrane-associated proteins were separated as described recently [11]. Processing of cells for immunofluorescence has also been described elsewhere [41].

Immunoblotting, immunoprecipitation, and HA-LATS1 kinase assay. To detect LATS1 or hMOB proteins, samples were resolved by 6% or 12% SDS–PAGE and processed as reported [11]. Harvesting and processing of samples for immunoprecipitation was also carried out as described [11]. Analysis of immunoprecipitated HA-LATS1 kinase activity was performed as reported for HA-NDR using the same buffer conditions and substrate peptide [11].

HA-LATS1 autophosphorylation assay. Cells were processed for immunoprecipitation as described elsewhere [11]. After the final wash with IP buffer, immunocomplexes were washed twice with kinase buffer (25 mM Hepes, pH 7.4, 50 mM NaCl, 5 mM $MgCl_2$, 5 mM β-glycerophosphate, 1 mM benzamidine, 4 μM leupeptin, and 1 mM DTT). Beads were resuspended in 30 μl kinase buffer containing 1 μM microcystin, 1 μM cAMP-dependent protein kinase inhibitor peptide, and 100 μM [γ-P^{32}]-ATP (~1000 cpm/pmol). After 30 min incubation at 30 °C, reactions were stopped by addition of Laemmli buffer, and samples were analysed by SDS–PAGE, followed by autoradiography.

Results

Human LATS1 kinase activity can be measured using the substrate peptide of human NDR1/2

Since LATS kinase activity has previously only been monitored by autophosphorylation [2,39], we established a kinase assay to monitor human LATS1 transphosphorylation activity. By applying the same conditions as reported for human NDR1/2 kinases [11], we could readily measure human LATS1 kinase activity towards the standard NDR1/2 substrate peptide (Fig. 1). As predicted, HA-LATS1 immunoprecipitates displayed elevated kinase activity once HA-LATS1 wild-type (wt) expressing cells had been treated with OA (Fig. 1B). The kinase activity measured by peptide phosphorylation was mirrored by increased autophosphorylation activity of LATS1 (Fig. 1A). HA-LATS1 kinase dead (D846A), and phosphorylation site mutants (S909A), (T1079A) or

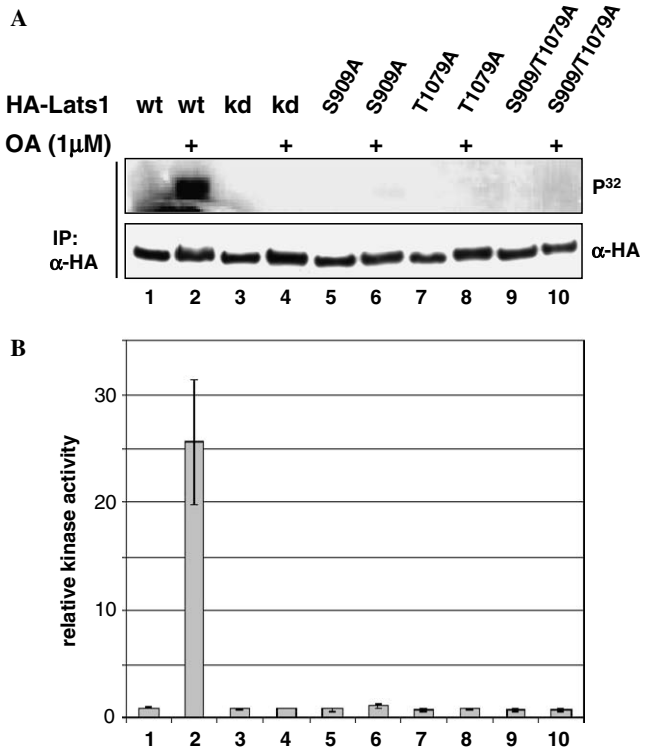

Fig. 1. Activation of LATS1 requires Ser909 and Thr1079. (A) Cells expressing HA-LATS1 (wt), (kd), (S909A), (T1079A) or (S909/T1079A) were incubated in the absence (−) or presence (+) of OA, before processing for immunoprecipitation using anti-HA 12CA5 antibody. Complexes were analysed by immunoblotting using anti-HA (bottom panel) or subjected to an autophosphorylation assay (top panel). (B) In parallel, immunocomplexes were subjected to peptide kinase assays.