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14-3-3 Interacts with LKB1 via recognizing phosphorylated threonine 336 residue and suppresses LKB1 kinase function

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ABSTRACT

Here we report a regulatory mechanism by which LKB1 is controlled by 14-3-3 proteins through phorsphorylation of Thr336. The results from the current study indicate that 14-3-3 ζ inhibits LKB1 from phosphorylating its substrate, AMPK (AMP-dependent protein kinase) and attenuates LKB1-mediated G1 cell cycle arrest and apoptosis by interfering with the interaction between LKB1 and its substrates. This regulation does not change either the LKB1 catalytic activity or subcellular localization of LKB1. Moreover, we demonstrate that serum starvation enhances LKB1 activity and increases the phosphorylation of Thr336. Taken together, our results suggest that autophosphorylation of Thr336 acts as an activating signal for LKB1 to recruit 14-3-3, which in turn attenuates the activation of LKB1 to keep the activity of LKB1 in check.

Structured summary of protein interactions:
LKB1 binds to **14-3-3 eta** by pull down (View interaction)
LKB1 physically interacts with **14-3-3 zeta** and **AMPKalpha1** by pull down (View interaction)
LKB1 physically interacts with **14-3-3 zeta** by pull down (View Interaction: 1, 2, 3)
LKB1 binds to **14-3-3 zeta** by pull down (View interaction)
LKB1 binds to **14-3-3 tau** by pull down (View interaction)
LKB1 binds to **14-3-3 gamma** by pull down (View interaction)
LKB1 and **14-3-3 zeta** colocalize by fluorescence microscopy (View interaction).

1. Introduction

LKB1 (STK11) is a serine/threonine kinase that was first identified in the researches on Peutz–Jeghers syndrome (PJS) patients [1,2]. It is a famous tumor suppressor which can induce cell apoptosis and inhibit cell proliferation by regulating the activity of p53 and G1 cyclin-dependent kinase (CDK)–cyclin complexes [3–6].

Recent years, LKB1 has been further investigated as a major upstream kinase of AMP-dependent protein kinase (AMPK). It directly phosphorylates AMPK within the kinase domain at Thr172 to activate AMPK [7,8] and the LKB1–AMPK pathway have been revealed to regulate the activities of some well-established tumor development related proteins such as mammalian target of rapamycin complex 1 (mTORC1), ribosomal S6 kinase (p70S6K1) and the CDK inhibitor protein p27 to suppress cell growth, cell proliferation and tumorigenesis [9–13].

While LKB1 has been extensively studied, the mechanisms which regulate the function of this enzyme remain to be further investigated. Previous research mainly focused on two aspects: (1) The subcellular localization of LKB1. Although LKB1 is mainly localized in the nucleus, it is the small fraction of LKB1 in cytoplasm that plays a important role in mediating G1 cell cycle arrest [14–16]; (2) STRAD, an accessory protein of LKB1, significantly activates the catalytic activity of LKB1 [17]. In addition, changes in the phosphorylation residues located in the C-terminal region of LKB1may play a certain role in mediating LKB1signaling [18–20]. Thr336 is a potential regulatory phosphorylation site, which was identified as a major autophosphorylation site on LKB1 [21]. It has been previously shown that the mutation of Thr336 to Glu (mimic phosphorylation) prevented LKB1 from inhibiting G361 cell growth without affecting LKB1 catalytic activity. This implies that there may be some regulatory proteins involved in the negative regulation of LKB1 function initiated by the phosphorylation of Thr336. We speculated that 14-3-3 protein family is the most potential candidate.

The 14-3-3 family proteins, including seven isoforms (β, ε, η, γ, τ, ζ, and σ) in mammals, can bind to target proteins depending on

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phosphorylated Ser or Thr residues mainly within the two 14-3-3 binding motifs, RSXpSXP (mode I), RXXXpSXP (mode II) of the target protein (pS represents phosphoserine) [22,23]. Members of the 14-3-3 family have been found to associate with a number of important protein targets [22]. The function of 14-3-3 proteins can be generally classified into 3 main types: (1) change the conformation of its binding partner; (2) physically occlude sequence specific or structural features of its binding partner; (3) act as a scaffold to anchor proteins to one another [24].

In the current study, we demonstrate that 14-3-3 interacts with LKB1 by binding to phosphorylated Thr336. Through this interaction, 14-3-3 inhibits the binding of LKB1 to its substrates, such as AMPK, to suppress the kinase function of LKB1. At the cellular level, the effect of 14-3-3 on LKB1 alleviates the G1 cell cycle arrest and apoptosis of Hela cells induced by LKB1.

2. Materials and methods

2.1. Plasmids

pDEST17-14-3-3 isoforms (β, ε, η, γ, τ, ζ, σ, and ζ K49E), pDEST26-14-3-3 ζ, pDEST26-14-3-3 ζ K49E and pDEST27-LKB1 plasmids were prepared as previously described [25] (details shown in Supplementary data).

2.2. Cell culture, transfection and lysis

Details are shown in Supplementary data.

2.3. Protein expression and purification

Details are shown in Supplementary data.

2.4. Antibodies

Details are shown in Supplementary data.

2.5. Immunoprecipitation and immunoblotting

Details are shown in Supplementary data.

2.6. LKB1 kinase assay

Details are shown in Supplementary data.

2.7. Flow cytometry

Details are shown in Supplementary data.

2.8. Subcellular localization of LKB1 and 14-3-3 in Hela cells

Details are shown in Supplementary data.

3. Results

3.1. LKB1 interacts with 14-3-3 family members

To examine whether endogenous LKB1 interacts with members of the 14-3-3 family, co-immunoprecipitation experiments were performed .Our result indicates that 14-3-3 is co-precipitated with an anti-LKB1 antibody, but not control IgG (Fig. 1a). The result of the GST pull down assay shows that four 14-3-3 isoforms, η, γ, τ and ζ can interact with recombinant GST–LKB1 protein (Fig. 1b), and they do not bind to control GST.

LKB1 plays its function in a manner of complex with STRAD and MO25. STRAD and MO25 both have two isoforms-STRAD α/β and MO25 α/β [26]. In order to make clear whether the GST–LKB1 purified from COS7 cells is associated with STRAD and MO25 and whether the STRAD and MO25 affect the interaction between LKB1 and 14-3-3, we purified GST–LKB1 from COS7 respectively using regular salt (150 mM) and high salt (600 mM) washing buffer

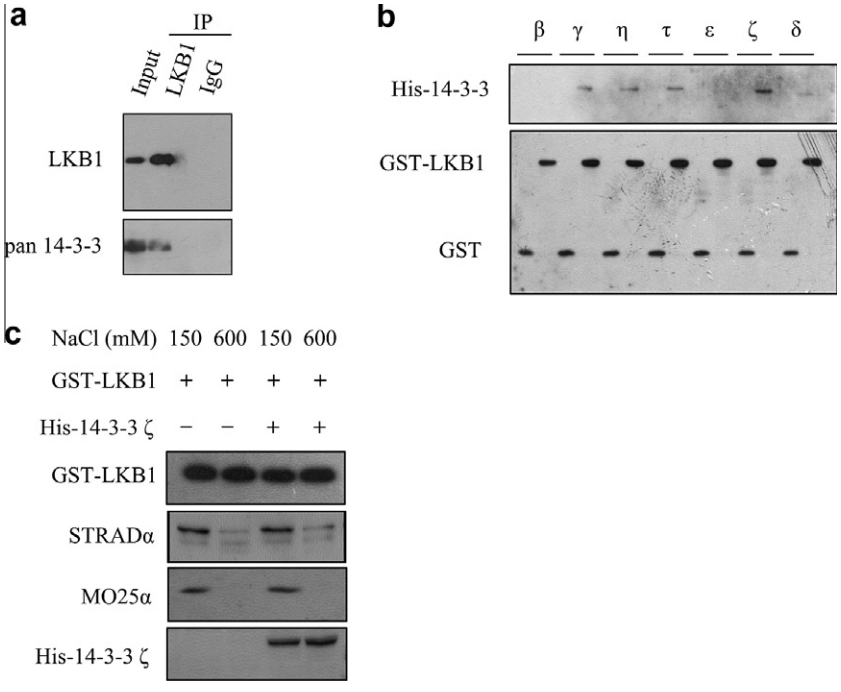

Fig. 1. Interaction of LKB1 with members of the 14-3-3 family (a) COS7 cell lysate was subjected to co-immunoprecipitation (IP) with anti-LKB1 antibody or control antibody (IgG). Immunoprecipitated proteins and 10% input were analyzed by western blot using LKB1 and pan 14-3-3 antibodies. (b) An equal quantity of each bacterially-expressed His-14-3-3 isoform (β, ε, η, γ, τ, ζ, and σ) was incubated with GST or GST–LKB1 purified from COS7 cells using GST-affinity chromatography. After GST pull down, the protein complexes were analyzed by western blot using His and GST antibodies. (c) GST–LKB1 were purified from COS7 cells using regular salt (NaCl 150 mM) and high salt (NaCl 600 mM) washing buffer respectively. The protein complexes were analyzed with STRADα, MO25α and GST antibodies. These two kinds of purified GST–LKB1 were incubated with an equal quantity of His-14-3-3 ζ respectively. After GST pull down, the protein complexes were analyzed with STRADα, MO25α, His and GST antibodies.