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TSC1/TSC2 inactivation inhibits AKT through mTORC1-dependent up-regulation of STAT3-PTEN cascade

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ABSTRACT

Aberrant activation of mammalian target of rapamycin complex 1 (mTORC1), caused by loss or inactivation of TSC1/TSC2 protein complex, leads to negative feedback inhibition of Akt. The exact mechanisms of this process are still not fully understood. Here we present evidence for the involvement of STAT3, a known mTORC1 regulated transcription factor, in this process. We demonstrate that STAT3 promotes the transcription of PTEN by directly binding on the PTEN promoter. Elevated PTEN then inhibits the proliferation of $Tsc1^{-/-}$ or $Tsc2^{-/-}$ cells through down-regulation of Akt signaling. Activation of PTEN in this pathway may thus serve as a protective mechanism against hyper-activated mTORC1 mediated tumorigenesis and contribute to the benign nature of tumors caused by loss of either TSC1 or TSC2.

1. Introduction

Tuberous sclerosis complex (TSC) is an autosomal dominant disorder with benign tumors in multiple organs such as kidney, liver, brain, and skin. This disease is caused by inactivating mutations of one of the two tumor suppressor genes: *TSC1* and *TSC2* [1,2]. Within cells, the protein products of these two genes form a functional complex, which negatively regulates a small GTPase, Ras homolog enriched in brain (Rheb), through the GTPase-activating (GAP) activity of TSC2 [3]. Inactivation of this complex by activated upstream signaling, in particular by the PI3K/Akt pathway, results in the accumulation of GTP-bound Rheb, which in turn leads to the activation of mammalian target of rapamycin (mTOR). These events together lead to uncontrolled cell growth and tumor development [1,4].

Tumor suppressor phosphatase and tensin homolog (PTEN) functions as a lipid phosphatase in dephosphorylating the three-position of PtdIns(3,4,5)P3 (PIP3) to generate PtdIns(4,5)P2 (PIP2) and therefore opposes PI3K activity and Akt signaling [5–7]. Loss of PTEN results in dysregulated Akt activity, which increases cell survival and proliferation and eventually leads to tumorigenesis [8]. While individuals with PTEN deficiency are prone to develop malignant tumors [9], TSC patients are rarely seen with malignant lesions [1]. mTOR can assemble with other proteins into rapamycin-sensitive complex 1 (mTORC1) and rapamycin-insensitive complex 2 (mTORC2) [4]. Inactivation of PTEN constitutively activates Akt, which then

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causes phosphorylation and inactivation of TSC2 and subsequently mTORC1 activation [10]. However, loss of TSC1/TSC2 complex contributes to hyper-activation of mTORC1 and in turn suppresses Akt activity [11–14]. The negative feedback inhibition of Akt by mTORC1 is believed to be the major reason of why tumors in TSC are benign [12], but the exact mechanisms are still not fully understood.

In this report, we demonstrate that loss of TSC1/TSC2 complex leads to enhanced expression of PTEN through activation of mTORC1. We further show that a mTORC1 downstream effector, signal transducer and activator of transcription 3 (STAT3), positively regulates the transcription of PTEN. Moreover, we find that elevated PTEN attenuates Akt signaling and inhibits the proliferation of $Tsc1^{-/-}$ or $Tsc2^{-/-}$ MEFs. Thus up-regulated PTEN may serve as a brake in the diseases caused by oncogenic mTORC1 signaling.

2. Materials and methods

2.1. Materials

Rapamycin and AG490 were acquired from Sigma; 4–12% Bis–Tris Nu-PAGE gels and Lipofectamine 2000 were purchased from Invitrogen. Anti-phospho-S6(Ser235/236) and S6 antibody have been described previously [12]; TSC2, TSC1 and β-actin antibodies were from Santa Cruz Biotechnology; STAT3, phospho-STAT3 (Tyr705), Rheb, mTOR, Raptor, Akt, phospho-Akt (Ser473) and PTEN antibodies were from Cell Signaling. pBabe-puro and pBabe-STAT3C (control vector and the vector expressing a constitutively activated STAT3, STAT3C, respectively) have been reported previously [15]. pSTAT3-TA-Luc was obtained from Beyotime Institute of Biotechnology. pPTEN-Luc was created by replacing the cis-acting STAT3 enhancer element in pSTAT3-TA-Luc with the STAT3 response element (TTCCGAAGAA × 4) from mouse PTEN promoter. The sequences of the two oligonucleotides for STAT3 response element are: forward: 5′-TCGAGTTCCGAAGAATTCCGAAGAATTCCGAAGAAA-3′ and reverse: 5′-GATCTTTCTTCGGAATTCTTCGGAATTCTTCGGAATTCTTCGGAAC-3′. After annealing of these two oligonucleotides, the double-stranded DNA fragments were cloned into *Xho*I and *Bgl*II digested pSTAT3-TA-Luc to generate the pPTEN-Luc. The control plasmid pRL-TK (renila luciferase reporter) was obtained from Promega. A retroviral expression construct containing a patient-derived TSC2 missense mutation (1651N → S), pLXIN-hyg-hTSC2(N1651S) [16], was generated from pLXIN-Hyg-TSC2 by replacement of A with G at TSC2 cDNA position 4952 using forward primer: 5′-CCATTGTCTACAGTGACTCCGGTGA-3′ and reverse primer: 5′-TCACCGGAGTCACTGTAGACAATGG-3′ with QuickChange site-directed mutagenesis kit (Stratagene).

2.2. Cell cultures

All mouse embryonic fibroblasts (MEFs) were described previously [12,17]. Retroviral packaging PT67 cells were from Clontech. HEK293T, human hepatocarcinoma SK-Hep1 cells were from Shanghai Institutes for Biological Sciences and human lung adenocarcinoma A549 cells were from ATCC. Cells were cultured in DMEM with 10% FBS in 5% CO_2 at 37 °C. Production of retroviruses and subsequent generation of stable gene-expressing cell lines have been described previously [17].

2.3. Quantitative real time PCR (qRT-PCR)

Total RNA was harvested for qRT-PCR analysis as described previously [17]. The primer sequences are as

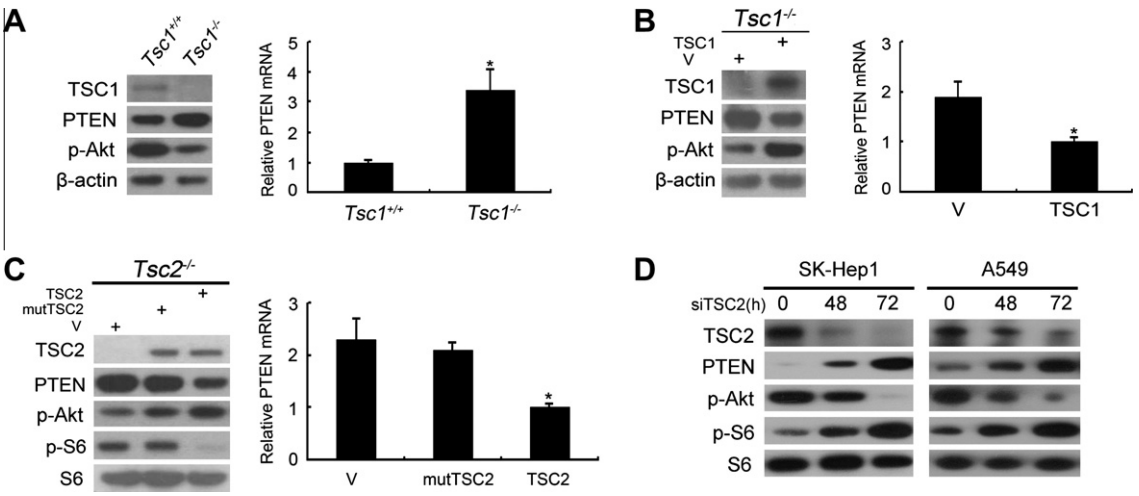

Fig. 1. TSC1/TSC2 complex negatively regulates PTEN. (A) $Tsc1^{+/+}$ and $Tsc1^{-/-}$ MEFs were harvested for immuno-detection of protein (left) and real-time quantification (qRT-PCR) of mRNA (right). (B) $Tsc1^{-/-}$ MEFs were infected with pLXIN-hygro or pLXIN-hTSC1 retroviruses. Cells were then collected for immuno-detection of protein (left) and qRT-PCR of mRNA (right). (C) $Tsc2^{-/-}$ MEFs were infected with pLXIN-hyg, pLXIN-hyg-hTSC2 or pLXIN-hyg-hTSC2 (N1651S) retroviruses. Cells were then collected for immuno-detection of protein (left) and qRT-PCR of mRNA (right). $^{*}P < 0.05$. (D) SK-Hep1 and A549 cells were transfected with TSC2 siRNAs for 48 h or 72 h. Cell lysates were collected for immunoblotting.