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RESEARCH HIGHLIGHT

Kinases meet at TSC

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Tuberous sclerosis complex (TSC) is an autosomal dominant neurocutaneous disorder characterized by the presence of multiple hamartomas in almost every organ, most notably in the skin, brain, heart, kidneys, liver, and lungs [1]. The classic syndromes of TSC are seizures, mental retardation, and cutaneous angiofibromas [1]. Two tumor suppressor genes, *Tsc1* and *Tsc2*, have been identified for pathogenesis of TSC [2]. *Tsc1* is located on chromosome 9q34 and encodes for the protein hamartin (130 kDa). *Tsc2* is located on chromosome 16p13.3 and encodes for the protein tuberin (180 kDa). These two proteins form a tumor suppressor heterodimer and inhibit the function of mammalian target of rapamycin (mTOR) [2]. mTOR is a key serine/threonine kinase in controlling cell proliferation and organ size. It receives input from multiple signaling pathways, including growth factors and nutrients, to stimulate protein synthesis by phosphorylating two key effector molecules: p70S6K and 4E-BP1. p70S6K is responsible for increased ribosome biogenesis through phosphorylation of the ribosomal protein S6, leading to ribosome recruitment and protein translation. 4E-BP1 represses the activity of eukaryotic translation initiation factor 4E (eIF4E) and, phosphorylation of 4E-BP1 by mTOR releases eIF4E from its suppression [2]. Thus, loss of TSC1 or TSC2 results in mTOR-dependent increased phosphorylation of the ribosomal protein S6, p70S6K and 4E-BP1, leading to cell growth and proliferation. Genetic and biochemical studies indicate that the TSC1/TSC2 complex suppresses the activation of mTOR through Ras homologue enriched in brain (Rheb). Rheb belongs to the family of G proteins and has the ability to bind guanine nucleotides GTP (the active form of a G protein) and GDP (the inactive form). Rheb in its active GTP-bound state stimulates mTOR, promoting protein synthesis and cell growth. The TSC1/TSC2 heterodimer, through a critical GTPase activating protein (GAP) domain located in TSC2, facilitates the hydrolysis GTP to GDP and switches Rheb from an active GTP-bound state to an inactive GDP-bound state, and thus inhibits the function of mTOR (Figure 1). Therefore, the critical GAP

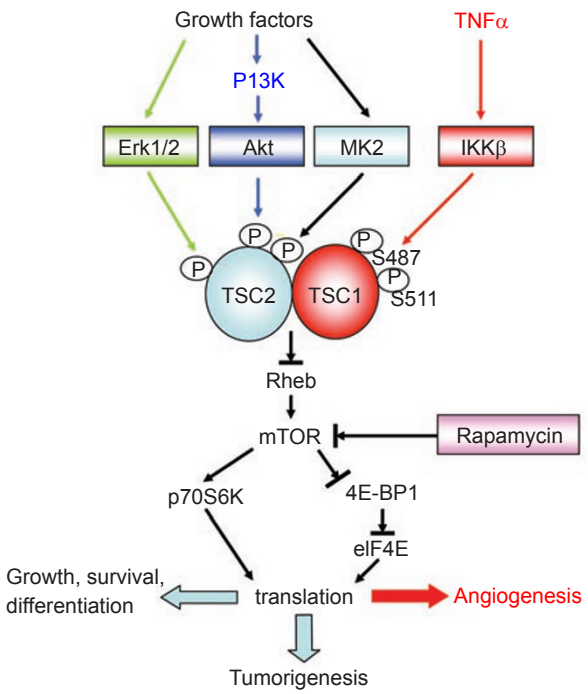

Figure 1 Kinases meet at TSC. Activation of PI3K/Akt, ERK1/2, and MK2 from growth factors leads to inactivating phosphorylation of TSC2 and decreases its GAP activity toward Rheb, which in turn activates the mTOR pathway. mTOR directly phosphorylates p70S6K and 4E-BP1, leading to the increased S6 and eIF4E activity and the enhanced protein translation. The study by Lee *et al.* identified that IKKβ phosphorylate TSC1 on Ser 487 and Ser 511, resulting in the inhibition of TSC1 and activation of mTOR. This new discovery links inflammation-mediated tumor angiogenesis to the TSC-mTOR pathway.

domain in TSC2 is a common site of multiple mutations in TSC patients. The function of TSC1 is to stabilize TSC2 and protect it from ubiquitination and degradation [3].

The dysregulation of the TSC-mTOR pathway has been linked to several hamartoma syndromes that predispose to cancer, such as TSC, Cowden disease, Peutz-Jeghers syndrome (PJS), neurofibromatosis, familial adenomatous polyposis (FAP), and juvenile polyposis syndrome (JPS) [2, 3]. In fact, hamartomas are a histologically defined subtype of benign tumor in which the tumor cells maintain normal differentiation but are disorganized in tissue architecture. The evidence indicates that the TSC-mTOR pathway is commonly deregulated in many human cancers. Among familial and sporadic cases of TSC, mutations on either *Tsc1* or *Tsc2* have been found. Although loss of heterozygosity (LOH) of either *Tsc1* or *Tsc2* is often detected in hamartomatous tumors from patients with TSC (Knudsen's two-hit mutation model), LOH has not been observed in all tumors and patients still develop hamartomas, indicating that other signaling mechanisms exist to inactivate the TSC1/TSC2 complex. Indeed, many signaling pathways impinge on the TSC1/TSC2 complex to control the association, the degradation, and the catalytic activity of this complex [2, 3]. For example, TSC2 is phosphorylated by Akt, MK2, and ERK kinases. Akt directly phosphorylates two sites on TSC2 (S939 and T1462), which destabilizes TSC2 and disrupts its interaction with TSC1 [2, 3]. These Akt phosphorylations are concomitant with the activation of mTOR, p70S6K, and phosphorylation of 4E-BP1 for protein synthesis and account for the major mechanism for Akt-mediated cell growth [2, 3]. The phosphorylation of TSC2 at residue S1210 by MAPK-activated protein kinase 2 (MK2) promotes the binding of TSC2 with 14-3-3 proteins, which sequesters TSC2 from its physiological substrate [2, 3]. Inactivating phosphorylation of TSC2 at residue S664 by ERK2 causes the disruption of the TSC1/TSC2 complex and activates mTOR [2, 3]. A single nonphosphorylatable TSC2 mutant at S664 inhibits tumorigenicity of a cell line with constitutively activated MAPK pathway [2, 3]. All those phosphorylation events inactivate the TSC1/TSC2 complex and positively regulate the mTOR signaling pathway. Although the regulation of TSC2 is under extensive studies, the regulation of TSC1 is less known. A recent elegant study published in *Cell* by Lee *et al.* demonstrates that TSC1 is also subjected to protein phosphorylation and provides a novel insight for the dysregulation of the TSC1/TSC2 complex in cancer development [4]. The authors convincingly showed that IKKβ physically interacts with and phosphorylates TSC1 at Ser487 and Ser511 and these phosphorylations lead to the destabilization of TSC1 and the disruption of the TSC1/TSC2 complex. Their thorough and stimulating study provides a novel link between the IKKβ-mediated dysregulation of TSC1 and inflammation-mediated cancer angiogenesis [4]. This study, together with the previous works [2-4], presents a clear notion that the activity of the TSC1/TSC2 complex is subjected to delicate control by multiple extracellular signaling pathways. Alteration of the association and the stability of this complex will result in the activation of mTOR and the consequence of tumorigenesis.

Accumulating evidence from epidemiological studies has shown that chronic inflammation contributes significantly to the pathogenesis of cancer [5]. TNFα, the key mediator of inflammation, activates the major inflammatory response nuclear factor κB (NF-κB) pathway, which contributes to tumor development by promoting tumor cell proliferation and blocking apoptosis [6]. TNFα also induces angiogenic factor upregulation which in turn promotes angiogenesis and tumor progression. However, the underlying mechanism of TNFα-induced angiogenesis was unclear. In their study, Lee *et al.* hypothesized that dysregulation of the TSC-mTOR pathway could be responsible for the inflammation induced tumor angiogenesis [4], because activation of the mTOR pathway enhances the level of VEGF through both transcriptional and translational processes [7]. In addition, patients with TSC develop renal acute myelogenous leukemia and skin angiofibromas that are characterized by the numerous abnormal blood vessels. Mice with heterozygous for *Tsc1*- and *Tsc2*- develop liver hemangiomas that associate with extensive abnormal vasculization. Furthermore, MEFs with knockout of *Tsc1* or *Tsc2* have elevated VEGF, and the elevation of VEGF is dependent on mTOR activation [8]. These evidences point to the critical function of the TSC-mTOR pathway in controlling angiogenesis. In several cleverly designed experiments, the authors found that TNFα induced the phosphorylation of p70S6K and 4E-BP1, two critical targets of mTOR, within a short period of stimulation. However, they did not find any significant activation of Akt, ERK and p38 pathways, suggesting that IKK, the major downstream kinase in the TNFα signaling pathway, is involved in the activation of the mTOR pathway. IKK complex consists of three core subunit, the catalytic subunits IKKα, IKKβ and several copies of the regulator subunit IKKγ. Genetic experiments have shown that IKKβ is the predominant IκB kinase in the canonical NF-κB pathway. Lee *et al.* demonstrated that only IKKβ, but not IKKα, was responsible for the activation of the mTOR pathway. How IKKβ activates the mTOR pathway? Is the TSC1/TSC2 complex involved in this activation and how IKKβ regulates the TSC1/TSC2 complex? Strikingly, they detected that IKKβ physically interacted with TSC1 but not TSC2 or Rheb. With an *in vitro* kinase assay and mass spectrometry analysis, they

found that two serine residues (Ser 487 and Ser 511) on TSC1 were phosphorylated by IKKβ both *in vitro* and *in vivo* and IKKβ was required for TNFα-induced phosphorylation of TSC1. Phosphorylation of TSC1 by IKKβ suppressed TSC1 function similar to the inhibition of TSC2 mediated by Akt and ERK phosphorylations. In addition, they discovered that TNFα treatment did not change the TSC1 subcellular localization but mediated the migration of membrane-associated TSC2 to the cytoplasm. How TSC2 migrates and what is the function for this migration remain to be further investigated. Most importantly, the authors demonstrated that phosphorylation of TSC1 by IKKβ correlated with the tumor angiogenesis in animal model and human tumor samples. It would be interesting to examine whether similar phosphorylation-mimic mutations also exist in tumor samples to enhance angiogenesis and whether such mutations confer angiofibromas in patients with TSC. Given that many signaling pathways merge on the TSC1/TSC2 complex, it will be important to determine how these signaling pathways (such as Akt, ERK, and IKK) cooperate or synergize with each other to control angiogenesis through the TSC1/TSC2 complex in cancer development.

The discovery of IKKβ phosphorylation on TSC1 by Lee *et al.* sheds new light on the intricacies of regulation of the TSC-mTOR pathway and suggests that mTOR may constitute a therapeutic target for cancer prevention and treatment. In fact, rapamycin and its derivatives that specifically inhibit mTOR are now being actively evaluated in clinical trials. However, caution should be paid for the single use of these agents, because two negative feedback regulations on Akt and NF-κB by mTOR have been observed [9, 10]. The inefficient activation of Akt after growth factor stimulation in *Tsc2*-null cells leads to the identification of a negative feedback loop downstream of insulin receptor substrate, IRS1 and IRS2. p70S6K directly phosphorylates IRS1/2 and destabilizes IRS proteins in *Tsc2*-null cells, leading to the inactivation of Akt [9]. Similarly, Ghosh *et al.* found the suppression of NF-κB activation in Tsc-null cells with constitutive mTOR activation [10]. Therefore, long-term rapamycin treatment may increase the risk of malignant tumors in TSC patients by reactivating the Akt and NF-κB pathways. Future studies that dissect the crosstalk between the TSC1/TSC2 complex and multiple signaling pathways will provide a comprehensive understanding for the regulation of this pathway in cancer development and thus will guide the clinical mTOR-targeted cancer therapy.

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