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tumors called hamartomas develop. Germline mutations in either *TSC1* or *TSC2* cau this syndrome, and hamartomas typically display second hit events with loss of t remaining normal allele. Studies initiated in Drosophila have identified a role for the *Ts* and *Tsc2* genes in the regulation of cell and organ size, and genetic interaction studi have placed them in the PI3K-Akt-mTOR-S6K pathway. Biochemical studies have shov that activated Akt phosphorylates TSC2 in the TSC1/TSC2 protein complex, inactivatir it; while TSC1/TSC2 has GAP activity for the Rheb GTPase (a member of the ras fa ily), and activated Rheb-GTP activates mTOR. Thus, in cells lacking TSC1 or TSC2 the are increased levels of Rheb-GTP which leads to activation of mTOR, leading to cell si increase and growth. These developments provide enhanced understanding of th signaling pathway and fundamental insights into the pathogenesis of tuberous scleros and open the possibility of treatment for hamartomas by several pharmacolog approaches.

TUBEROUS SCLEROSIS—THE CLINICAL SYNDROME

Tuberous sclerosis (TSC) is an autosomal dominant genetic disorder characterized l development of unusual tumors in several organ systems.[1] The tumors of TSC are terme hamartomas to indicate that there is overgrowth of relatively mature appearing cells, in di tinct contrast to common malignancies. TSC hamartomas involve the skin, brain, and ki neys in most patients. The vast majority of TSC hamartomas display limited growt potential and do not require intervention. A small fraction display persistent growt necessitating surgical control. However, progression to malignancy is very rare in TSC, an has been seen only in TSC renal hamartomas, termed angiomyoliopoma (AML), at a fr quency of about 1.5–2% of all TSC patients.[2]

Another remarkable feature of TSC hamartomas is that they first appear at differer ages during the patient's life, and occasionally spontaneously resolve.[1] For example, cardi rhabdomyomas are often present at birth, but then typically disappear during childhoo The major morbidity of TSC is due to the hallmark cortical tubers, from which is derive the name tuberous sclerosis (Fig. 1). Cortical tubers are focal regions of disorganized co tical lamination that contain both giant cells (80 to 150 microns in diameter) and dy plastic neurons, both of which have disrupted radial orientation.[3] A TSC patient may hav as many as 50 cortical tubers. Although they are thought to be relatively static lesions, the lead to seizure disorders in the vast majority of TSC patients, and commonly contribut to developmental delay, mental retardation, and autism.

Another unusual feature of TSC is the occurrence of a unique proliferative lung diseas termed lymphangioleiomyomatosis (LAM) in which there is both cystic change an smooth muscle cell (SMC) proliferation.[1] This lesion is seen nearly exclusively in adu female TSC patients, suggesting that estrogens contribute to its development.

TSC GENES, MUTATIONS AND THE TWO HIT MECHANISM

TSC occurs due to inactivating mutations in either of *TSC1* or *TSC2*.[4-6] These gene are both relatively large (23 and 41 exons, respectively), and encode the proteins hamarti (130kDa) and tuberin (200kDa), respectively. Due to the frequent severe clinical effects o TSC, large TSC families are rare, and about 2/3 of TSC patients are sporadic cases due t new mutational events. Comprehensive mutational analyses indicate that about 85% o TSC patients will have a mutation in either *TSC1* or *TSC2* identified.[7,8] *TSC2* mutation are about 4.2 times as common as *TSC1* mutations, reflecting a higher germline mutatio

Figure 1. Brain lesions in tuberous sclerosis. A. Brain MRI of an 8-year-old boy using the FLAIR technique demonstrates several cortical tubers (white, cortical lesions, indicated by arrows) and subependymal nodules lining the ventricles (arrowheads). Courtesy of J. Egelhoff, Cincinnati, OH. B. High power view of a cortical tuber shows giant cells (white diamonds). Cortical surface is to the right. Courtesy of J. Chan, Boston, MA.

rate.[6-8] Clinical surveys also indicate that patients with *TSC1* mutations have symptoms and clinical features that are milder on average than patients with *TSC2* mutations, although there is considerable overlap in the two sets of patients.[8] Linkage studies provide evidence against a third TSC gene.[6] Patients in whom mutations cannot be found are likely partially explained by mosaicism, which compromises efforts at mutation detection.[8]

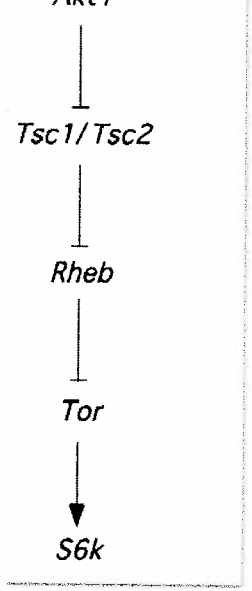
TSC hamartomas, particularly renal AMLs, often display loss of heterozygosity (LOH) for the wild type allele of either *TSC1* or *TSC2*, consistent with a two hit mechanism for complete inactivation of either *TSC1* or *TSC2*.[6,9,10] LAM lesions require microdissection of the SMCs to demonstrate LOH events and such methods have been used to show that patients with LAM but without other features of TSC also have two hit inactivation of TSC2.[11] Brain lesions have failed to showed evidence of LOH,[9] even with microdissection,[12] and alternative mechanisms of gene inactivation or cell admixture likely explain this finding. LOH is also seen in the tumors (kidney cystadenomas, liver hemangiomas, and extremity angiosarcomas) that develop in *Tsc1+/-* and *Tsc2+/-* mice.[13-15]

Consistent with a relative lack of malignancy in TSC patients, limited surveys of human cancer specimens have failed to show evidence of either *TSC1* or *TSC2* mutation, with the possible exception of bladder carcinoma.[16-18]

TSC1/TSC2, PI3K AND mTOR SIGNALLING

Major progress has occurred in recent years in our understanding of the biochemical function of the *TSC1* and *TSC2* gene products, tuberin and hamartin. These advances were initiated by seminal studies in Drosophila, which led to placement of TSC1 and TSC2 in the PI3K-Akt-mTOR-S6K signaling pathway. Here we review this work, emphasizing the role of the *TSC1* and *TSC2* genes in this pathway, and indicating the complementary nature of investigation carried out in Drosophila and in mammalian systems.

The initial studies used FRT/FLP recombination to identify *Tsc1* and *Tsc2* as targets for recessive mutations that affect fly eye size.[19-22] Cells homozygous null for either *Tsc1* or *Tsc2* develop and differentiate normally, but are increased in size with a shift from G_1 to the S and G_2 phases of the cell cycle.[19-21] Consistent with earlier work in



each of the genes in this pathway ho
functions other than controlling the ge
shown immediately below it, and there
feedback compensation throughout
pathway when one element is perturbe

mammalian cells indicating that Ts
and Tsc2 occur as a complex,[23]
overexpression of both proteins b
not either alone led to an oppos
phenotype in which cell and org
size is reduced.

These observations led to gene
interaction studies between *Tsc1* a
Tsc2, and elements of the PI3K-Al
mTOR-S6K pathway which had pr
viously been recognized to have
important role in the control of c
size in Drosophila.[19-21,25,26] Ablatic
of *Tsc1* or *Tsc2* had effects that we
dominant (epistatic) to those
homozygous loss of the insulin rece
tor, PI3K, and Akt1. Loss of *Tsc1* or *Tsc2* was synergistic with loss
Pten, leading to further enlargement of the eye. On the other han
loss of $S6k^{20}$ or Tor^{25} was dominant in effect to loss of *Tsc1* or *Tsc*
$Tsc1^{-/-}Tor^{+/-}$ embryos survived longer than $Tsc1^{-/-}$ embryos, ar
organs with $Tsc1^{-/-}Tor^{+/-}$ cells were more normal than those wi
$Tsc1^{-/-}$ cells.[25] $Tsc1^{-/-}Tor^{+/-}S6k^{+/-}$ embryos survived to adulthood ar
were semifertile.[27] These studies positioned *Tsc1/Tsc2* just above *T*
in the PI3K-Akt-Tor-S6K signalling pathway (Fig. 2).

These genetic studies were complemented by biochemical studie
which provided a partial mechanistic basis for the observations. Tsc
is phosphorylated by Akt1, and this event leads to the disruption
the Tsc1-Tsc2 complex in vivo.[26] In addition, expression of a mutar
Tsc2 which cannot be phosphorylated by Akt1 along with wild typ
Tsc1 completely suppressed the effects of Akt overexpression o
cell/organ size whereas wild type Tsc2 did not have this effect.[26]

Studies utilizing mammalian cells were performed in paralle
after the initial Drosophila findings. To understand these results w
review the PI3K-Akt-mTOR-S6K pathway in mammals (Fig. 3).[28-
When recruited to the cell membrane by binding to activated, phos
phorylated growth factor receptors or through interaction with ra
PI3K catalyzes the synthesis of 3' phosphoinositides. Those lipic
then recruit Akt to the membrane where it is phosphorylated on tw
sites, one site by either autophosphorylation or another kinas
(S473), and the second by the PDK1 kinase (T308). Akt the
phosphorylates multiple substrates involved in regulation of apop
tosis, cell energy metabolism, and the cell cycle, including Bad
procaspase-9, IKK, CREB, FKHR/AFX/FOX transcription factors
GSK-3, p21Cip1, and Raf. Akt had also been thought to regulat
mTOR activity by some means, with at least one direct phosphory

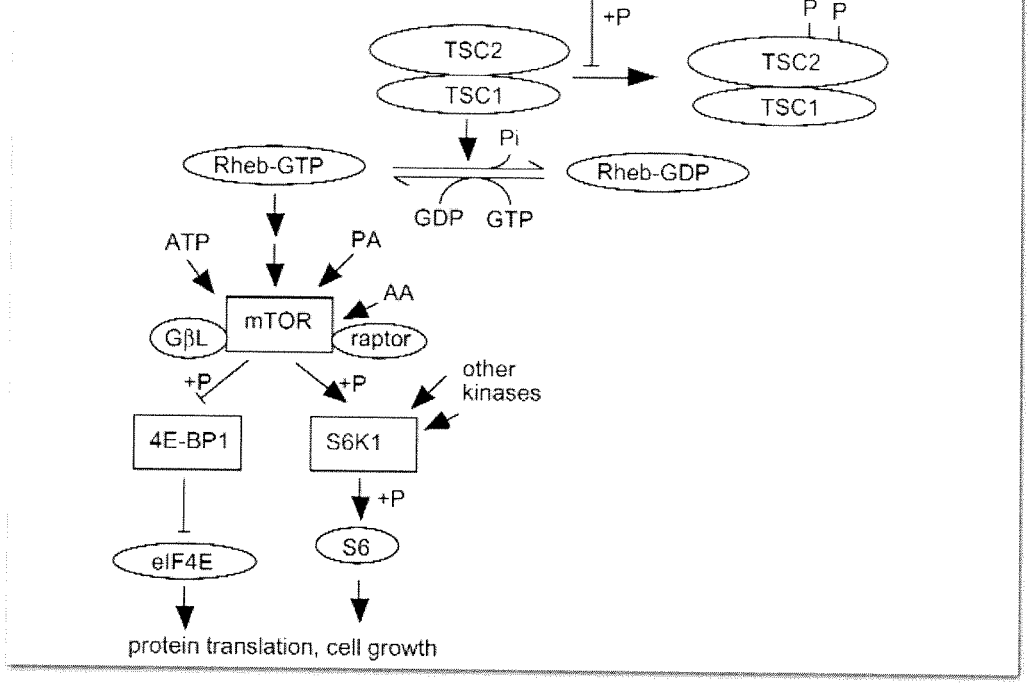


Figure 3. Signalling pathway model for the function of TSC1 and TSC2 in mammalian cells. A phosphorylated growth factor receptor is shown at upp left, to which a PI3K molecule is binding. This leads to conversion of the indicated phosphoinositides to 3′-phosphoinositides, which leads to recruitment Akt to the membrane in a position where it can be phosphorylated and activated by PDK1 and a second kinase. PTEN functions to terminate this signalir pathway by acting as a 3′ phosphatase on these phosphoinositides. Activated pAkt phosphorylates TSC2 which inactivates its GAP activity. When activ TSC1/TSC2 complex serves as a GAP for Rheb, reducing levels of Rheb-GTP. Rheb-GTP activates mTOR by an uncertain mechanism (thus 2 arrows). AT phosphatidic acid (PA), and amino acids (AA) all influence mTOR activity, although the sensing mechanisms are unknown and likely indirect. Active mTC phosphorylates 4E-BP1 and S6K1. p4E-BP1 releases from eIF4E, permitting formation of the eIF4F translation complex. pS6K1 phosphorylates S6, an together they activate the translational machinery. For simplicity only the main pathway involving TSC1, TSC2, and mTOR is shown. Arrows indicate pos tive actions and bars represent negative actions.

lation site identified (S2448). Regulation of mTOR kinase activity is complex, and recent advances have highlighted its occurrence in a large complex including at least two binding partners: raptor and GβL.[31-33] mTOR's kinase and autokinase activity are modulated in cells both by growth factor receptor kinase signalling and nutrient (amino acid) levels, ATP levels, and phosphatidic acid levels. mTOR has two principal downstream targets: S6K1 and 4E-BP1. Phosphorylation and activation of S6K1 occurs in a multi-step process with several kinases (PDK1, ERK) involved, with phosphorylation by mTOR as a critical step at T389. Activated S6K1 phosphorylates the ribosomal subunit S6 leading to activation of protein translation, specifically mRNAs that contain a 5' tract of oligopyrimidines, including many ribosomal proteins and translation factors. Unphosphorylated 4E-BP1 binds to and sequesters the translation initiation factor eIF4E. Phosphorylation of 4E-BP1 permits formation of the eIF4F translation complex, which enhances overall protein synthesis, as well as specific increases in translation of ornithine decarboxylase, c-myc, hypoxia-inducible fac tor 1alpha, and a number of cell cycle proteins.[30]

In cultured mammalian cells lacking either Tsc1 or Tsc2, there constitutive high level phosphorylation of S6K1, 4E-BP1, and (i neuroepithelial cells) STAT3.[34-39] (Similar results are seen i Drosophila S2 cells.[25]) Treatment of these cells with rapamycin, th highly specific inhibitor of mTOR, rapidly reverses this phosphory lation signature. Activation of S6K1 in these cells is also relativel resistant to withdrawal of amino acids from the cultur media.[25,37,39] Concordant with this activation of mTOR in th absence of either Tsc1 or Tsc2, there is an increased S phase per cen and growth rate in Tsc1 and Tsc2 null cells compared to control under some conditions.[35,37,39] Phosphorylated S6 is also prominen in these cultured cells. Immunohistochemistry analyses have con firmed that this signature of mTOR activation is present in severa types of lesions from both TSC patients and Tsc mouse models, con firming the in vivo significance of these observations.[34,35,40,41]

4E-BP1, and blocks amino acid-induced phosphorylation of S6K1.[38,44]

ENTER RHEB

Thus, the above studies determined that Tsc1/Tsc2 was a critical intermediate in the signalling pathway from PI3K to mTOR and downstream elements, serving as a brake upon mTOR activity. However, the mechanism of this effect was unknown until recently when multiple investigators discovered that Tsc1/Tsc2 functioned as a GTPase activating protein (GAP) for the heretofore relatively obscure but evolutionarily conserved member of the ras family, Rheb. Studies in yeast had shown that loss of Rheb mimicked nitrogen starvation with G_0/G_1 arrest,[45] suggesting a potential role in this pathway. Rheb is unusual in comparison to other ras family members in that it has an arginine at the third residue of the G_1 box,[46] and occurs in cells with relatively high amounts of bound GTP.[47]

Genetic screens in the fly led to identification of *Rheb* as a gene that caused eye (and other organ) enlargement when its transcription was increased and eye size reduction when it was homozygously ablated.[48-50] Rheb overexpression has effects on cell size and cell cycle that are entirely similar to the effects of Tsc1 or Tsc2 loss.[48,50] Genetic interaction studies show that loss of Tor is dominant to overexpression of Rheb, while loss of *S6k* attenuates but does not fully block eye enlargement mediated by Rheb.[48,49] Further, overexpression of Tsc1/Tsc2 blocked the cell size effects of overexpression of Rheb, while loss of Rheb was dominant in effect to loss of Tsc1 or Tsc2.[49] In addition, reduction in Rheb expression partially or completely rescued the embryonic lethality of loss of *Tsc1*.[49,51] In vitro analyses indicated that Rheb-GTP levels, Rheb stimulated S6k activation, and cell growth were independent of nutrient availability, while loss of Rheb completely blocked S6k activation.[48,49,51]

This work was again complemented and extended by concurrent studies on mammalian cells. Rheb-GTP levels were increased in $Tsc2^{-/-}$ cells compared with controls.[52] In transient transfection assays, coexpression of Tsc1/Tsc2 markedly lowers the level of Rheb-GTP and S6K1 activation.[51-54] In NIH3T3 cells, insulin stimulation leads to a 1.8-fold increase in Rheb-GTP levels which is blocked by wortmannin (PI3K inhibitor) but not rapamycin.[52] Overexpression of Rheb leads to high level activation of S6K1 and phosphorylation of 4E-BP1, while reduction in Rheb levels by siRNA reduces growth factor-induced activation of S6K1.[52-56] This activity is completely independent of PI3K and MAPK activation or function, as assessed by inhibitor treatments.[53,54,56] In addition, rapamycin but not wortmannin completely inhibits the phosphorylation of S6K1 induced by Rheb overexpression,[52,53,55] as does expression of a kinase-dead mTOR,[53] and treatment with the farnesyl transferase inhibitor FTI-277.[56] Nutrient deprivation had little effect on S6K1 activation by Rheb overexpression, while ATP depletion has been variably reported to block this effect.[52-54] On the other

els of Rheb-GTP, and mTOR is inactive. In response to growth fa
tor stimulation, PI3K and Akt become activated, TSC2 is phosph
rylated by Akt and the TSC1/TSC2 complex becomes inactive as
GAP, so that Rheb-GTP levels rise, stimulating mTOR. In cells lac
ing TSC1 or TSC2, there is no GAP for Rheb, and Rheb-GTP le
els are high, leading to constitutive activation of mTOR and pho
phorylation of S6K1 and 4E-BP1. This linear model captures mo
of the reported results but simplifies many aspects of this pathwa
including the multiple targets of Akt, and the complex hierarchic
phosphorylation of S6K1 that regulates its activity, as just two exan
ples. In addition, not all the data from mammalian systems suppor
the above model.[37] Moreover, there is compelling data fro
Drosophila that S6k activation is under separate control from bot
PI3K and Akt1, suggesting that there are parallel pathways
PI3K-Akt1 and mTOR-S6k that converge and interconnect rath
than existing in a linear sequence.[49,57] However, this signaling circu
is certain to have complex feedback and compensatory mechanisn
built in, which can make interpretation of the effects of loss of or
gene on other members of the pathway difficult. For example, bot
Tsc1 and Tsc2 null cells show a profound reduction in activation
Akt in response to serum and other growth factors.[37,39] This appea
to be due to a major reduction in PDGFR expression in these cells.
These observations may explain why the TSC1 and TSC2 genes ar
rarely involved in malignancy.

OTHER FUNCTIONS OF TSC1/TSC2

The TSC1/TSC2 complex has size 330kDa and the GAP domai
of TSC2 comprises about 10 kDa of this complex. This alone sug
gests that there are other functions of the complex. The only othe
consistently reported binding partner of the TSC1/TSC2 comple
is14-3-3, through pS1210.[39,58,59] The importance of this binding
uncertain, but it may regulate the function of TSC1/TSC2.[59]

TSC1 has been reported to bind to ezrin and other ERM famil
proteins, and appears to be involved in adhesion events and rho sig
naling to the actin cytoskeleton.[60] TSC2 has been reported to hav
a role in the membrane localization of polycystin-1 in renal epithe
lial cells.[61] A role for the TSC1/TSC2 complex in beta-catenin sig
naling has also been noted.[62] Whether any of these observations ar
independent of or relate to the role of TSC1/TSC2 in the PI3K sig
naling pathway is unknown.

THERAPEUTIC PROSPECTS

Three years ago treatment for the various manifestations of TS
appeared an unreachable goal. There are now several treatmen
approaches under active consideration: rapamycin and analogues
farnesyl transferase inhibitors (FTIs), angiogenesis inhibitors, an
interferon-γ (IFNγ). Rapamycin is uniquely attractive as a therapeutic
as it targets mTOR directly. It has also been shown to be effective i
short-term treatment experiments in Tsc rat and mouse models.[63,6

therapies could also be valuable in TSC. Finally, the increased STAT expression and activation in Tsc null cell lines,[36] as well as clinical and mouse observations suggest that IFNγ may be therapeutic in TSC.[65,66] Trials of IFN-γ treatment in TSC mouse models are also underway.

A critical clinical issue in tuberous sclerosis is the molecular basis of epileptogenesis in TSC. Alterations in expression of several neurotransmitter receptors have been seen in tuber giant cells compared to normal neurons, and are likely to underlie this problem.[67] mTOR has been shown to regulate protein synthesis at the synapse,[68] and be involved in memory formation, so that it seems likely that aberrant mTOR activation in tuber giant cells accounts for their large size and abnormal expression patterns. Whether rapamycin and analogues might be effective in control of the CNS manifestations of TSC is an exciting though perhaps distant prospect. Nonetheless, rapamycin is effective in extending survival in Tsc mouse brain models (Onda, Meikle, Kwiatkowski, unpublished observations).

CONCLUSIONS AND FUTURE PROSPECTS

Now is a time of tremendous excitement in the field of TSC research, given major progress in the identification of the signaling pathway the TSC proteins participate in, and the discovery of several potential therapeutic approaches. The intense scrutiny brought to bear on these genes is very likely to yield significant further advances in the near future. Questions that seem particularly important or fruitful at this time include the following. What is the mechanism by which Rheb-GTP influences mTOR activity? Since rapamycin treatment rapidly dephosphorylates S6K1, S6, and 4E-BP1 in cells,[34-39] is phosphatase regulation in mammalian cells under the control of mTOR as suggested by some studies?[69] Are all of the reported cellular findings in the absence of TSC1 or TSC2 due to the activation of Rheb and mTOR, or does the complex have additional functions? Will rapamycin or its analogues, FTIs, angiogenesis inhibitors, or IFNγ prove to be of clinical benefit to TSC patients?

References

1. Gomez M, Sampson J, Whittemore V. The tuberous sclerosis complex. Oxford, England: Oxford University Press, 1999.
2. Al-Saleem T, Wessner LL, Scheithauer BW, Patterson K, Roach ES, Dreyer SJ et al. Malignant tumors of the kidney, brain, and soft tissues in children and young adults with the tuberous sclerosis complex. Cancer 1998; 83:2208-16.
3. Crino PB, Henske EP. New developments in the neurobiology of the tuberous sclerosis complex. Neurology 1999; 53:1384-90.
4. The European chromosome 16 tuberous sclerosis Consortium. Identification and characterization of the tuberous sclerosis gene on chromosome 16. Cell 1993; 75:1305-15.
5. van Slegtenhorst M, de Hoogt R, Hermans C, Nellist M, Janssen B, Verhoef S, et al. Identification of the tuberous sclerosis gene TSC1 on chromosome 9q34. Science 1997; 277:805-8.
6. Cheadle JP, Reeve MP, Sampson JR, Kwiatkowski DJ. Molecular genetic advances in tuberous sclerosis. Hum Genet 2000; 107:97-114.
12. Niida Y, Stemmer-Rachamimov AO, Logrip M, Tapon D, Perez R, Kwiatkowski DJ, et Survey of somatic mutations in tuberous sclerosis complex (TSC) hamartomas suggests ferent genetic mechanisms for pathogenesis of TSC lesions. Am J Hum Genet 20 69:493-503.
13. Onda H, Lueck A, Marks PW, Warren HB, Kwiatkowski DJ. Tsc2(+/-) mice deve tumors in multiple sites that express gelsolin and are influenced by genetic backgroun Clin Invest 1999; 104:687-95.
14. Kobayashi T, Minowa O, Kuno J, Mitani H, Hino O, Noda T. Renal carcinogenesis, hep ic hemangiomatosis, and embryonic lethality caused by a germ-line Tsc2 mutation in m Cancer Res 1999; 59:1206-11.
15. Kobayashi T, Minowa O, Sugitani Y, Takai S, Mitani H, Kobayashi E, et al. A germ-l Tsc1 mutation causes tumor development and embryonic lethality that are similar, but identical to, those caused by Tsc2 mutation in mice. Proc Natl Acad Sci USA 20 98:8762-7.
16. Hornigold N, Devlin J, Davies AM, Aveyard JS, Habuchi T, Knowles MA. Mutation of 9q34 gene TSC1 in sporadic bladder cancer. Oncogene 1999; 18:2657-61.
17. Parry L, Maynard JH, Patel A, Hodges AK, von Deimling A, Sampson JR, et al. Molecu analysis of the TSC1 and TSC2 tumour suppressor genes in sporadic glial and glioneuro tumours. Hum Genet 2000; 107:350-6.
18. Parry L, Maynard JH, Patel A, Clifford SC, Morrissey C, Maher ER, et al. Analysis of TSC1 and TSC2 genes in sporadic renal cell carcinomas. Br J Cancer 2001; 85:1226-3
19. Gao X, Pan D. TSC1 and TSC2 tumor suppressors antagonize insulin signaling in growth. Genes Dev 2001; 15:1383-92.
20. Potter CJ, Huang H, Xu T. Drosophila Tsc1 functions with Tsc2 to antagonize insulin s naling in regulating cell growth, cell proliferation, and organ size. Cell 2001; 105:357-
21. Tapon N, Ito N, Dickson BJ, Treisman JE, Hariharan IK. The drosophila tuberous scle sis complex gene homologs restrict cell growth and cell proliferation. Cell 20 105:345-55.
22. Ito N, Rubin GM. gigas, a Drosophila homolog of tuberous sclerosis gene product-2, r ulates the cell cycle. Cell 1999; 96:529-39.
23. Plank TL, Yeung RS, Henske EP. Hamartin, the product of the tuberous sclerosis 1 (TSC gene, interacts with tuberin and appears to be localized to cytoplasmic vesicles. Cancer 1998; 58:4766-70.
24. van Slegtenhorst M, Nellist M, Nagelkerken B, Cheadle J, Snell R, van den Ouweland et al. Interaction between hamartin and tuberin, the TSC1 and TSC2 gene produc Human Mol Genet 1998; 7:1053-1058.
25. Gao X, Zhang Y, Arrazola P, Hino O, Kobayashi T, Yeung RS, et al. Tsc tumour suppr sor proteins antagonize amino-acid-TOR signalling. Nat Cell Biol 2002; 4:699-704.
26. Potter CJ, Pedraza LG, Xu T. Akt regulates growth by directly phosphorylating Tsc2. N Cell Biol 2002; 4:658-65.
27. Radimerski T, Montagne J, Hemmings-Mieszczak M, Thomas G. Lethality of Drosoph lacking TSC tumor suppressor function rescued by reducing dS6K signaling. Genes D 2002; 16:2627-32.
28. Chang F, Lee JT, Navolanic PM, Steelman LS, Shelton JG, Blalock WL, et al. Involveme of PI3K/Akt pathway in cell cycle progression, apoptosis, and neoplastic transformation: target for cancer chemotherapy. Leukemia 2003; 17:590-603.
29. Jacinto E, Hall MN. Tor signalling in bugs, brain and brawn. Nat Rev Mol Cell Biol 200 4:117-26.
30. Huang S, Bjornsti M, Houghton PJ. Rapamycins: Mechanism of action and cellular resis ance. Cancer Biol Ther 2003; 2:222-232.
31. Hara K, Maruki Y, Long X, Yoshino K, Oshiro N, Hidayat S, et al. Raptor, a binding par ner of target of rapamycin (TOR), mediates TOR action. Cell 2002; 110:177-89.
32. Kim DH, Sarbassov dos D, Ali SM, King JE, Latek RR, Erdjument-Bromage H, et mTOR interacts with raptor to form a nutrient-sensitive complex that signals to the c growth machinery. Cell 2002; 110:163-75.
33. Kim DH, Sarbassov dos D, Ali SM, Latek RR, Guntur KV, Erdjument-Bromage H, et GbetaL, a positive regulator of the rapamycin-sensitive pathway required for the nutr ent-sensitive interaction between raptor and mTOR. Mol Cell 2003; 11:895-904.
34. Kwiatkowski DJ, Zhang H, Bandura JL, Heiberger KM, Glogauer M, el-Hashemite N, al. A mouse model of TSC1 reveals sex-dependent lethality from liver hemangiomas, an up-regulation of p70S6 kinase activity in Tsc1 null cells. Hum Mol Genet 200 11:525-34.

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