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Proteins interacting with the tuberous sclerosis gene products

Review Article

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Summary. Tuberous sclerosis (TSC) is an autosomal dominant tumor suppressor gene syndrome affecting about 1 in 6000 to 10000 individuals. The genes, TSC1, encoding hamartin, and TSC2, encoding tuberin are responsible for TSC. Since their identification 1997 and 1993 respectively, a variety of different functions have been described for the TSC gene products. Hamartin and tuberin form a complex, providing a tentative explanation for the similar disease phenotype in TSC patients with mutations in either of these genes. In addition, associations of hamartin or tuberin with several different proteins have been demonstrated. In this review, we summarize the current knowledge on hamartin- and tuberin-interacting proteins and discuss their role for the understanding of the functions of the TSC gene products.

Keywords: Tuberous sclerosis – Hamartin – Tuberin – TSC1 – TSC2 – Protein interaction

Tuberous sclerosis

Tuberous sclerosis (TSC) is an autosomal dominant disease occurring in about 1 in 6000 to 10000 live births. It is characterized by the development of tumor-like growths, named hamartomas, in the kidneys, heart, skin and brain. Primary diagnostic criteria for TSC are e.g. peringual fibromas, calcified retinal hamartomas, cortical tubers or renal angiomyolipomas. In addition, nearly all patients exhibit skin signs, such as hypomelanotic macules and forehead fibrous plaques, facial angiofibromas, shagreen patches and others. A hamartoma is a disorganized yet differentiated benign growth containing giant cells. Hamartomas occurring in the different organs have no common features, but often consist of multiple cell types.

The severity of TSC and its impact on the quality of life is extremely variable. The greatest source of morbidity is brain tumors, named cortical tubers. These tubers are regions of focal cerebral cortical dysplasia, which exhibit disorganized or absent cortical lamination and dysmorphic neurons with abnormal dendritic arborisation and spine density. Tubers cause seizures in 80–90% of affected individuals, and behavioural abnormalities (mostly autism) in over half of affected individuals. Additionally, complications from kidney hamartomas often result in significant morbidity and mortality. Progression of TSC hamartomas to malignancy is very rare. Malignant angiomyolipomas or malignant renal cell carcinomas occur in the kidneys in about 2% of TSC patients (Gomez et al., 1999; Kwiatkowski, 2003).

The TSC genes

Two genes have been shown to be responsible for TSC. TSC1 on chromosome 9q34 encodes a 130 kDa protein named hamartin spanning 1164 amino acids (The TSC1 Consortium, 1997) (Fig. 1) and TSC2 on chromosome 16p13.3 encodes a 200 kDa protein, named tuberin spanning 1807 amino acids (The European Chromosome 16 Tuberous Sclerosis Consortium, 1993) (Fig. 2). The human TSC1 gene occupies 55 kb and consists of 23 exons, of which 21 encode hamartin with an 8.6 kb mRNA. The human TSC2 gene consists of 42 exons, of which 41 encode tuberin with a 5.4 kb mRNA. Tuberin derived from alternatively spliced mRNA isoforms missing exon 25 and 31 is relatively common and is assumed to exhibit normal function (Cheadle et al., 2000). Sporadic new mutations are detected in about two-thirds of all patients

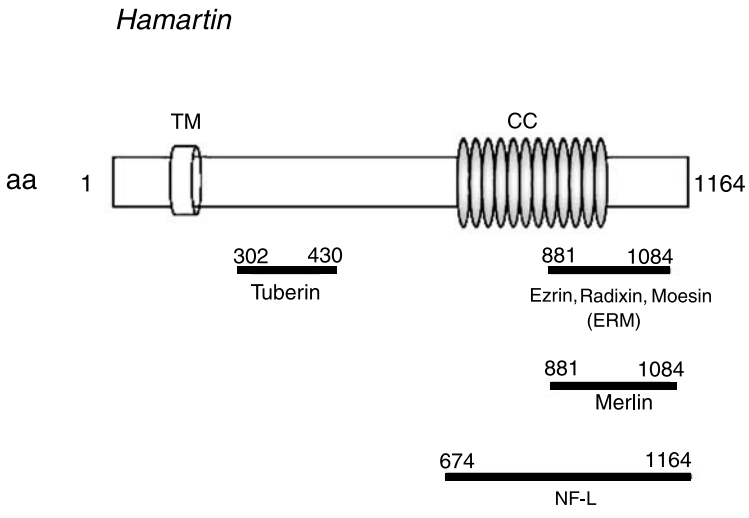

Fig. 1. Schematic representation of human hamartin. Human hamartin has a potential transmembrane domain (TM, amino acids 127–144) and a coiled-coil domain (CC, amino acids 730–996). Below the regions of hamartin known to span the interacting domains with tuberin (amino acids 302–430 of human TSC1), ezrin, radixin, moesin (amino acids 881–1084 of human TSC1), merlin (amino acids 881–1084 of human TSC1), and NF-L (neurofilament-light chain) (amino acids 674–1164 of human TSC1) are presented. For details see Table 1 and the text

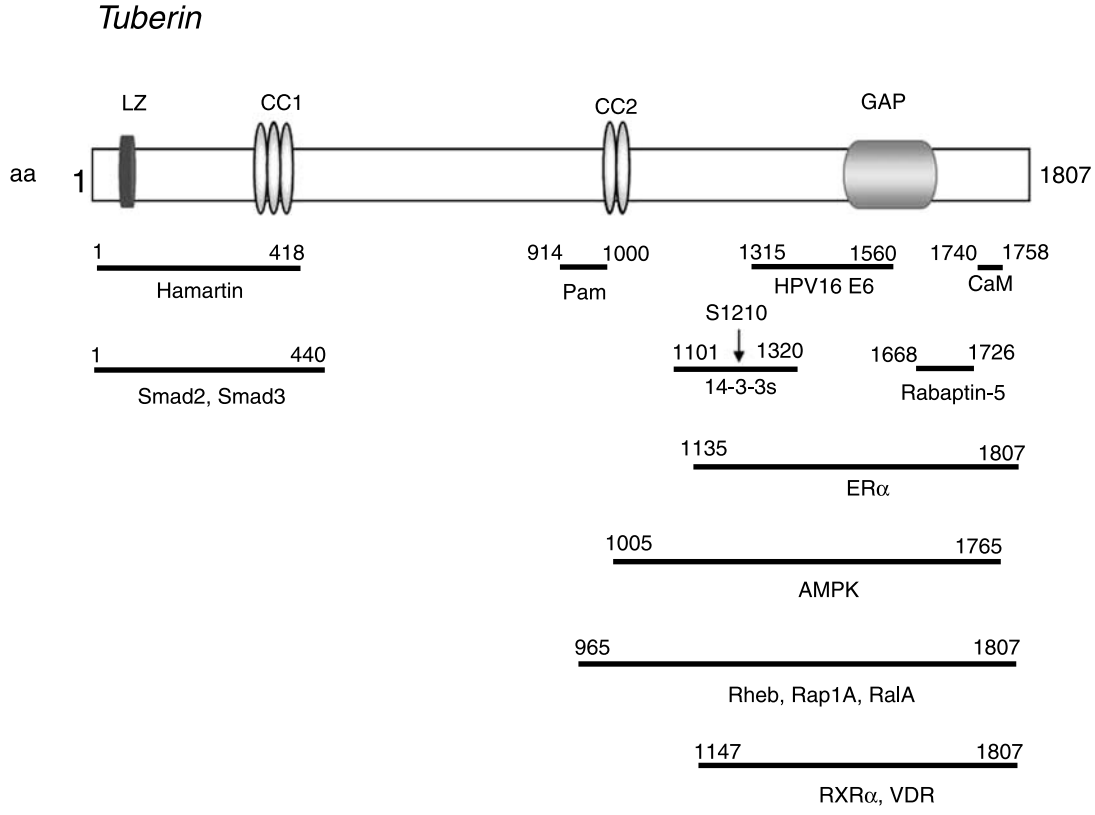

Fig. 2. Schematic representation of human tuberin. Human tuberin has a leucine zipper region (LZ, amino acids 81–98), two coiled-coil domains (CC, amino acids 346–371 and 1008–1021), and a GAP (GTPase-activating protein) homology region (GAP, amino acids 1517–1674). Below the regions of tuberin known to span the interacting domains with hamartin (amino acids 1–418 of human TSC2), Pam (protein-associated with Myc) (amino acids 914–1000 of human TSC2), HPV16 E6 (human papillomavirus 16 E6 oncoprotein) (amino acids 1315–1560 of human TSC2), CaM (calmodulin) (amino acids 1740–1758 of human/rat TSC2), Smad2, Smad3 (amino acids 1–440 of human/rat TSC2), the 14-3-3 isoforms (amino acids 1101–1320 of rat TSC2, phosphorylation of Ser^{1210} in rat tuberin is required for its association with 14-3-3), rabaptin-5 (amino acids 1668–1726 of rat TSC2), ERα (estrogen receptor α) (amino acids 1135–1807 of human/rat TSC2), AMPK (AMP-activated protein kinase) (amino acids 1005–1765 of rat TSC2), Rheb (Ras homolog enriched in brain), Rap1A, RalA (amino acids 965–1807 of human TSC2), RXRα (retinoid X receptor α), and VDR (vitamin D receptor) (amino acids 1147–1807 of human TSC2) are presented. For details see Table 1 and the text

and are more common in TSC2 than in TSC1. Linkage analysis suggests that about half of large families are linked to TSC1 and half to TSC2. The mutation spectra of the TSC genes are very heterogenous and no hotspots of mutations have been found. Large deletions and rearrangements have been described for TSC2 and insertions, deletions, nonsense, splicing and missense mutations for both TSC genes. The majority of the described mutations are inactivating. TSC patients carry a mutant TSC1 or TSC2 gene in each of their somatic cells and loss of heterozygosity has been documented in a wide variety of TSC tumors. Tumor development is assumed to be the result of somatic ''second hit'' mutations according to Knudson's tumor suppressor model. Accordingly, although TSC is a disease, which is transmitted in an autosomal dominant fashion, mutations in the TSC genes are believed to be recessive at the level of the affected cell (Cheadle et al., 2000; Kwiatkowski, 2003).

Hamartin interacts with tuberin

Mammalian hamartin and tuberin associate physically *in vivo* (van Slegtenhorst et al., 1998; Plank et al., 1998) suggesting that these two proteins function in the same complex. This interaction is regulated by tuberin phosphorylation (Aicher et al., 2001) and prevents tuberin ubiquitination (Benvenuto et al., 2000). This complex formation, which has also been demonstrated in Drosophila, provides a tentative explanation for the similar disease phenotype in TSC patients with mutations in either of these genes. The binding site for tuberin is within the region of amino acids 302–430 of human hamartin (Fig. 1). The first 418 amino acids of human tuberin contain the binding site for hamartin (Fig. 2).

Table 1 represents a summary of all proteins proven to form a complex containing either tuberin or hamartin or both. Whether the formation of the hamartin/tuberin complex is necessary has not been investigated for any of the described interactions. Accordingly, it remains elusive whether hamartin is necessary for tuberin's described interactions and vice versa. Table 1 contains information on the functions of the described interactors, on the performed experiments and the cellular background of these experiments. Comments on the binding sites within the TSC gene products are included. The amino acids regions containing the binding sites are presented in Figs. 1 and 2. Not for all interactors binding sites have been described. In total, beside the hamartin/tuberin interaction, 30 proteins are known to interact with the TSC gene products. 22 proteins (for 20 proteins an interaction site has been narrowed down) have been described to associate with tuberin: the seven isoforms of 14-3-3, Akt (protein kinase B), AMPK (AMP-activated protein kinase), CaM (calmodulin), cyclin A, ERα (estrogen receptor α), HPV16 E6 (human papillomavirus 16 E6 oncoprotein), Pam (protein-associated with Myc), rabaptin-5, Rheb (Ras homolog enriched in brain), Rap1A, RalA, RXRα (retinoic X receptor α), VDR (vitamin D receptor), Smad2, and Smad3 (Table 1). 5 proteins (for all 5 an interaction site has been narrowed down) have been demonstrated to associate with hamartin: ezrin, radixin, moesin, merlin, and NF-L (neurofilament-light chain) (Table 1). For the 3 interactors, cdk1 (cyclin-dependent kinase 1), cyclin B1 and mTor (mammalian target of rapamycin), the formation of complexes containing both TSC gene products have been reported (Table 1).

The TSC proteins and the mTOR signaling network

Recently, research from several laboratories linked the TSC gene products to the insulin/mTOR signaling network, which has a central role in the regulation of cell size in response to growth factors, cellular energy and nutrient levels. In the insulin-signaling pathway following binding of IGF molecules to their receptors a signal is relayed via lipid and protein kinases that increases the translation rate of RNAs specifically regulated via the translation initiation factor eIF4E and additionally results in phosphorylation of the 40S ribosomal protein S6 by S6kinase (S6K) and increased translation rates of specific messages, mainly from genes involved in ribosome biogenesis. This cascade includes the phosphoinositide-3-kinase (PI3K), the Akt kinase and the FKBP12-rapamycin associated protein mTOR (mammalian target of rapamycin). Evidence has been provided that tuberin is phosphorylated by Akt, and that the tuberin/hamartin complex functions as GTPase activating protein against Rheb (Ras homolog enriched in brain), which in turn regulates mTOR. mTOR controls S6K and 4EBP-1 (4E-binding protein), an inhibitor of the eukaryotic initiation factor eIF4E (Fig. 3) (for recent detailed reviews on this topic see Li et al., 2003a; Pan et al., 2004). In support of this model, tuberin has been demonstrated to interact with Rheb and with Akt. Interestingly, both, tuberin and hamartin have also been reported to be in a complex with mTOR (Table 1 and Fig. 2).

Table 1. Proteins interacting with the tuberous sclerosis gene products

Protein	Involved in	Binds to		Binding site(s)	Experimental proof		Cells	References
		tuberin	hamartin					
14-3-3 isoforms $\beta, \gamma, \varepsilon, \eta, \tau, \zeta$	cell signaling/ cell cycle	+	−	Akt – phosphorylated tuberin	*in vitro*	yeast two-hybrid, bait: TSC2	COS-1, HeLa, brain cortex	Nellist et al., 2002
					in vitro	GST affinity precipitation		
					in vivo	co-IP of endogenous proteins		
14-3-3 isoforms $\beta, \gamma, \varepsilon, \eta, \sigma, \tau, \zeta$	cell signaling/ cell cycle	+	not done	Akt – phosphorylated tuberin	*in vitro*	Protein domain microarray	NIH3T3, MCF-7, TRKE-2	Liu et al., 2002
					in vivo	co-IP of endogenous proteins		
					in vitro	GST affinity precipitation		
14-3-3 isoforms $\beta, \gamma, \tau, \zeta$	cell signaling/ cell cycle	+	−	MK2 – phosphorylated tuberin, TSC2 S1210	*in vivo*	co-IP of endogenous proteins	HEK293	Li et al., 2002 Li et al., 2003b
					in vivo	co-IP of overexpressed proteins		
14-3-3 isoforms β, ζ	cell signaling/ cell cycle	+	−	phosphorylated tuberin, TSC2 S1210	*in vitro*	yeast two-hybrid, bait: TSC2	HEK293, U2OS	Shumway et al., 2003
					in vivo	co-IP of endogenous proteins		
					in vivo	co-IP of overexpressed proteins		
					in vitro	GST affinity precipitation		
Akt	cell signaling	+	−	not done	*in vivo*	co-IP of endogenous proteins	HeLa, HEK293	Dan et al., 2002
					in vivo	co-IP of overexpressed proteins		
AMPK	cell signaling	+	−	tuberin aa 1005–1765	*in vivo*	co-IP of endogenous proteins	HEK293	Inoki et al., 2003
					in vivo	co-IP of overexpressed proteins *interaction is stimulated by 2DG*		

CaM	cell signaling	+	not done	tuberin aa 1740–1758	*in vitro*	Far Western screening		Noonan et al., 2002
					in vitro	IP of purified, labeled proteins *interaction is Ca^{2+}-dependent*		
Cdk1	cell cycle	complex		not done	*in vivo*	co-IP of endogenous proteins	NT2, K562, HEK293	Catania et al., 2001
					in vivo	co-localization experiments		
Cyclin B1	cell cycle	complex		not done	*in vivo*	co-IP of endogenous proteins	NT2, K562, HEK293	Catania et al., 2001
					in vivo	co-localization experiments		
Cyclin A	cell cycle	+	not done	not done	*in vivo*	co-IP of endogenous proteins	K562, HEK293	Catania et al., 2001
ERα	cell growth/ differentiation	+	not done	tuberin aa 1135–1807	*in vivo*	co-IP of overexpressed proteins	HEK293, ELT-3	Finlay et al., 2004
					in vitro	GST affinity precipitation		
Ezrin, Radixin, Moesin (ERM)	cell adhesion	not done	+	hamartin aa 881–1084	*in vitro*	yeast two-hybrid, bait: ERM	HUVEC	Lamb et al., 2000
					in vitro	slot-blot and blot-overlay assay		
					in vivo	co-IP of endogenous proteins		
					in vivo	co-localization experiments		
Ezrin, Radixin, Moesin (ERM)	cell adhesion	not done	+	not done	*in vitro*	GST affinity precipitation	HeLa, primary neurons	Haddad et al., 2002
					in vivo	co-localization experiments		
HPV16 E6	Oncoprotein/ carcinogenesis	+	not done	tuberin aa 1315–1560	*in vitro*	yeast two-hybrid, bait: HPV16 E6	NIH3T3, BGC823, COS-7	Lu et al., 2004
					in vitro	GST affinity precipitation		

(*continued*)

Table 1 (*continued*)

Protein	Involved in	Binds to		Binding site(s)	Experimental proof		Cells	References
		tuberin	hamartin					
Merlin	cell adhesion	not done	+	hamartin aa 881–1084	*in vitro*	yeast two-hybrid, bait: Merlin		Lamb et al., 2002
					in vitro	slot-blot		
Merlin	cell adhesion	not done	+	not done	*in vitro*	GST affinity precipitation	HeLa	Haddad et al., 2002
mTOR	cell signaling	complex		not done	*in vivo*	co-IP of overexpressed proteins	S2	Gao et al., 2002
NF-L	neuronal cytoskeleton	−	+	hamartin aa 674–1164	*in vitro*	yeast two-hybrid, bait: TSC1	HeLa, COS-7 primary neurons	Haddad et al., 2002
					in vitro	GST affinity precipitation		
					in vivo	co-IP of overexpressed proteins		
					in vivo	co-localization experiments		
Pam	synaptic growth/ ubiquitination	+	−	tuberin aa 914–1000	*in vitro*	yeast two-hybrid, bait: TSC2	PC12, primary neurons	Murthy et al., 2004
					in vitro	GST affinity precipitation		
					in vitro	co-IP of endogenous proteins		
					in vitro	co-localization experiments		
Rabaptin-5	cell signaling	+	not done	tuberin aa 1668–1726	*in vitro*	yeast two-hybrid, bait: TSC2	COS-7, HeLa, CCL-136	Xiao et al., 1997
					in vitro	co-IP of endogenous proteins		
					in vitro	co-IP of overexpressed proteins		
Rheb, Rap1A, RalA	cell signaling	+	not done	tuberin aa 965–1807	*in vitro*	GST affinity precipitation	HEK293	Castro et al., 2003
RXRα, VDR	transcription	+	not done	tuberin aa 1147–1807	*in vitro*	GST affinity precipitation	Sf9	Henry et al., 1998

Smad2, Smad3	Regulation of cell growth/ differentiation by TGF-β1	+	not done	tuberin aa 1–440	*in vivo* *in vivo* *in vivo* *in vivo*	co-IP of overexpressed proteins co-IP of endogenous proteins GST affinity precipitation co-localization experiments *interaction is induced by TGF-β1*	CCL64, HEK293, pZ157-4, pZ119-4	Birchenall-Roberts et al., 2004

– for full protein names see figures and text

– other abbreviations used: *2DG*, 2-deoxy-glucose; *GST*, glutathione S-transferase; *IP*, immunoprecipitation; *MK2*, MAP kinase-activated protein kinase-2; *TGF-β1*, transforming growth factor beta 1

– cell lines: *BGC823*, gastric carcinoma cell line; *CCL-136*, human sarcoma cell line; *CCL64*, mink lung epithelial cell line; *COS-1*, monkey kidney fibroblast cell line; *ELT-3*, Eker rat uterine leiomyoma-derived smooth muscle cells (TSC2$-/-$); *HEK293*, human embryonic kidney cell line; *Hela*, human cervical carcinoma cell line; *HUVEC*, primary human umbilical vein endothelial cells; *K562*, human erythroleukemia cell line; *MCF-7*, human breast cancer cell line; *NIH3T3*, mouse embryonic fibroblasts; *NT2*, human neuronal precursor-restricted cell line of teratocarcinoma origin; *PC12*, rat pheochromocytoma cell line; *pZ119-4*, rat TSC2 $-/-$; *pZ157-4*, rat TSC2 $+/-$; *S2*, drosophila Schneider cells; *Sf9*, worm ovary epithelial cell line; *TRKE-2*, rat kidney epithelial cell line; *U2OS*, human osteosarcoma cell line

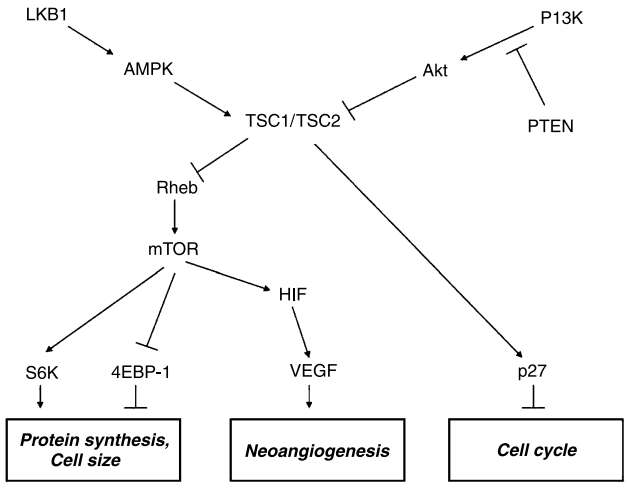

Fig. 3. Involvement of the tuberous sclerosis gene products in the regulation of cell size, neoangiogenesis and cell cycle. Pointed arrowheads indicate activation, and flat arrow heads indicate inhibition. For details see the text. *LKB1*, Peutz-Jeghers tumor suppressor gene; *AMPK*, AMP-activated protein kinase; *PI3K*, phosphoinositide-3-kinase; *Akt*, protein kinase B; *PTEN*, *p*hosphatase and *ten*sin homolog deleted from chromosome 10 – mutated in Cowden syndrome and Bannayan-Riley-Ruvalcaba syndrome; *TSC1/TSC2*, tuberous sclerosis gene product complex; *Rheb*, Ras homolog enriched in brain; *mTOR*, mammalian target of rapamycin; *S6K*, p70 S6 ribosomal kinase; *4EBP-1*, 4E-binding protein – an inhibitor of the eukaryotic initiation factor 4E; *HIF*, hypoxia-inducible transcription factor; *VEGF*, vascular endothelial growth factor; *p27*, cyclin-dependent kinase inhibitor p27

Tuberous sclerosis, Peutz-Jeghers syndrome, Cowden syndrome, Bannayan-Riley-Ruvalcaba syndrome

Peutz-Jeghers syndrome is also a dominantly inherited hamartomatous syndrome. The responsible LKB1 tumor suppressor gene was known to code for a serine/threonine kinase (Brugarolas and Kaelin, 2004). Very recently, the signaling pathways responsible for TSC and the Peutz-Jeghers syndrome have been linked. LKB1 phosphorylates and activates AMPK (AMP-activated protein kinase) and AMPK phosphorylates and activates TSC2 (Corradetti et al., 2004; Shaw et al., 2004) (Fig. 3). AMPK interacts with tuberin in the region of amino acids 1005–1765 (Table 1 and Fig. 2).

The PTEN tumor suppressor gene is a lipid phosphatase that negatively regulates cell survival also by affecting the PI3K/Akt signaling pathway. Loss of PTEN increases Akt activity, which downregulates TSC2 function (Brugarolas and Kaelin, 2004) (Fig. 3). Interestingly, two of the syndromes caused by germline PTEN mutations, the Cowden syndrome and the Bannayan-Riley-Ruvalcaba syndrome, are also characterized by hamartomas. Recently, it has been shown that the TSC proteins regulate the expression of the vascular endothelial growth factor (VEGF). TSC2 downregulates the hypoxia-inducible transcription factor (HIF), which is regulating VEGF expression, through mTOR-dependent and -independent pathways (Brugarolas et al., 2003) (Fig. 3). Since PTEN, LKB1 and TSC1/2 have been demonstrated to be involved in the regulation of HIF and VEGF, it has been speculated that increased HIF and VEGF levels may be a common feature of familial hamartoma syndromes (Brugarolas and Kaelin, 2004).

The role of the TSC proteins in cell cycle control

In the mammalian cell cycle mitogen dependent D-type cyclins are expressed first during early G1 phase, associating with the cyclin-dependent kinases cdk4 or cdk6 to form an active kinase complex that phosphorylates the retinoblastoma protein and thereby activates E2F transcriptional activity. Among the genes activated via the transcription factor family E2F are cyclin E, which complexed with cdk2 promotes the G1 to S phase transition by phosphorylation of different substrates, and cyclin A, which activates cdk2 to further initiate DNA replication. The transition from G2 to M phase is regulated by cdk1(cdc2)/cyclin B and cdk1/cyclin A complexes. In addition, two families of cdk inhibitors are known: the INK4 family (p15, p16, p18, and p19) regulating cdk4 and cdk6, and the Cip/Kip family (p21, p27, and p57) inhibiting a broader range of cdks (Sherr and Roberts, 1999).

It has been demonstrated that antisense inhibition of TSC2 expression induces quiescent fibroblasts to enter the cell cycle and TSC2-negative cells exhibit a shortened G1 phase. Overexpression of TSC1 or TSC2 negatively regulates cell cycle progression. Tuberin negatively regulates the activity of cdk2 and the p27 stability is decreased in tuberin-negative cells (Soucek et al., 1997; Soucek et al., 1998b; Miloloza et al., 2000) (Fig. 3). Although the TSC proteins have been shown to interact with cdk1, cyclin B and cyclin A (Table 1), these associations cannot explain the observed cell cycle effects of the TSC proteins on the G1 progression and p27 stability. On the other hand, the very recently discovered physical interaction of tuberin with p27 was found to mediate the regulation of p27 stability and cell cycle progression (M. Rosner and M. Hengstschläger, unpublished observation).

Other tuberin- or hamartin-interacting proteins

As described above, the tuberin/hamartin complex functions as GTPase activating protein against Rheb. In addi-

tion, tuberin harbours GAP activity for Rap1A (Wienecke et al., 1995) and for Rab5 (Xiao et al., 1997). These data have been supported by the found associations of tuberin with Rap1A and rabaptin-5, which is an adaptor-like molecule for the GTPase Rab5 (Table 1, Fig. 2).

In the last two years, several groups have reported the interactions of tuberin with all isoforms of 14-3-3 proteins (Table 1, Fig. 2). The 14-3-3 proteins are a family of abundant, widely expressed 28–33 kDa acidic polypeptides. They are expressed in all eukaryotic cells and are highly conserved in amino acid sequences in a wide range of organisms, including higher eukaryotes, invertebrates and plants. Seven isoforms encoded by seven distinct genes are identified in mammals. Variants of 14-3-3 proteins assemble in homo- and heterodimers. They bind to phosphoserine-containing motifs in a sequence-specific manner and function as adaptor molecules modulating interactions/functions of components involved in signal transduction and in cell cycle control. Localizations of 14-3-3 proteins to cytoplasm, nucleus, various membranes, and cytoskeletal and centrosome structures have been reported. The results of an increasing number of studies provide evidence for a pathophysiological importance of changes in 14-3-3 expression and localization in conditions such as cancer and neurodegenerative diseases (Tzivion and Avruch, 2002). Which functions of the TSC proteins are affected by their interaction with 14-3-3 proteins and whether specific functions of 14-3-3 proteins might be controlled via the association with tuberin is still under investigation.

The E6 oncoprotein of the human papillomavirus (HPV16 E6) has been found to interact with tuberin (Table 1, Fig. 2), what leads to the proteasome mediated degradation of tuberin and to activation of S6K (Lu et al., 2004). This is a new and interesting role of tuberin, as a target of other oncoproteins, independently of the disease TSC. Pam (protein-associated with Myc) was originally identified as a binding partner for Myc. Although it is expressed abundantly in brain, its function in the mammalian central nervous system remains unknown, so far. It will be of interest to once reconsider the relevance of the recently described interaction of Pam and tuberin (Table 1, Fig. 2) for tuberin's function and for the molecular development of TSC.

Tuberin was found to interact with Smad2 and Smad3 (Table 1, Fig. 2) and to augment Smad-dependent transcriptional activation by TGF-β1 (Birchenall-Roberts et al., 2004). Tuberin is also a direct modulator of transcription events mediated by steroid/nuclear receptor family members. Interactions of tuberin with the estrogen receptor α (ERα), the retinoid X receptor α (RXRα), and the vitamin D receptor (VDR) have been reported. In addition, the interaction of tuberin with calmodulin (CaM) has been shown to play an essential role in tuberin's ability to modulate steroid/nuclear receptor signaling (see the literature cited in Table 1).

In TSC the central nervous system lesions, such as cortical tubers, result in a variety of neurological manifestations, including mental retardation and seizures (Gomez et al., 1999; Kwiatkowski, 2003). Furthermore, tuberin has been reported to be involved in the regulation of neuronal differentiation (Soucek et al., 1998a). Hamartin has been demonstrated to interact with neurofilament-light chain (NF-L) (Table 1, Fig. 1) and hamartin and tuberin co-localize with NF-L preferentially in the proximal to central growth cone region of cultured cortical neurons (Haddad et al., 2002). Hamartin has been shown to interact with the ERM-family proteins, ezrin, radixin, moesin, and to weakly bind the ERM-related tumor suppressor protein merlin (Table 1, Fig. 1). The interaction between hamartin and the ERM-family proteins has been shown to be required for activation of the small GTP-binding protein Rho by serum and to be involved in the regulation of cell adhesion (Lamb et al., 2000)

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