**The tuberous sclerosis complex: balancing proliferation and survival**

Romana Tomasoni and Anna Mondino

Abstract

Mutations in genes encoding either hamartin (TSC1 (tuberous sclerosis complex 1)) or tuberin (TSC2) result in a multisystem disorder characterized by the development of benign tumours and hamartomas in several organs. The TSC1 and TSC2 proteins form a complex that lies at the crossroad of many signalling pathways integrating the energy status of the cell with signals induced by nutrients and growth factors. The TSC1/2 complex is a critical negative regulator of mTORC1 [mTOR (mammalian target of rapamycin) complex 1], and by that controls anabolic processes to promote cell growth, proliferation and survival. In the present paper, we review recent evidence highlighting the notion that the TSC1/2 complex simultaneously controls mTOR-dependent and mTOR-independent signals critical for the balancing of cell proliferation and cell death.

The TSC1/2 (tuberous sclerosis complex 1/2) complex in health and disease

Tuberous sclerosis is an autosomal-dominant genetic disease arising from mutations in either hamartin (TSC1) or tuberin (TSC2), with the formation of hamartomas in multiple organ systems with an incidence of 1 in 6000 births. Mutations in both TSC1 and TSC2 include nonsense, missense, insertion and deletion mutations, whereas large genomic deletions are more common for the TSC2 gene. TSC1 and TSC2 form a complex in cells, which is positioned at the crossroad of many different signalling pathways integrating the energy status of the cell with nutritional availability and extracellular growth factor signalling and controls different cellular mechanisms such as cell size, cell cycle, cellular proliferation and cell death [1].

Although functions for the individual TSC1 and TSC2 proteins have been proposed, whether either protein acts independent of the complex is still debated. Loss of TSC1 eliminates formation of the TSC1/2 complex and leads to biochemical consequences in terms of mTOR (mammalian target of rapamycin) activity towards Rheb [Ras homologue enriched in brain] [10]. When GTP-bound, Rheb activates mTOR by preventing its association with the endogenous inhibitor FKBP38 (FK506-binding protein 38) [11,12]. By stimulating the conversion of active Rheb-GTP into the inactive form, Rheb-GDP, the TSC1/2 complex inhibits mTOR and signalling downstream to mTORC1 (mTOR complex 1) [13]. The TSC1/2 complex can also physically associate with and activate mTORC2 in a manner independent of its GAP activity towards Rheb [14]. mTORC1 and mTORC2 in turn regulate a huge number of cellular processes, including cell growth, cell proliferation and protein translation (mTORC1) and actin cytoskeleton remodelling (mTORC2) [15]. In addition to the mTOR pathway, the TSC1/2 complex also regulates β-catenin and E2F activity [16,17] thus affecting proliferation and survival [18].

A critical role for the TSC1/2 complex is underlined by the consequences of its loss, causing multisystem disorder, characterized by the development of benign tumours and hamartomas in several organs, including renal angiomyolipomas,
cardiac rhabdomyomas and subependymal giant cell astrocytomas, and of dermatological abnormalities, such as hypomelanotic macules, in paediatric patients [1]. Mutations in either TSC1 or TSC2 also cause cortical tubers and subependymal nodules in the nervous system with cognitive defects, epilepsy, autism and attention-deficit hyperactive disorder [19]. Understanding the function of this complex and its regulation is therefore critical in physiology and pathology.

The TSC1/2 complex controls cell growth, metabolism and proliferation

TSC1/2, being upstream of mTORC1, plays a central role in cell growth and metabolism, balancing catabolic and anabolic activities. Activation of the PI3K/Akt/mTOR pathway in growth-factor-dependent cells and tumour cells enhances many of the metabolic activities that support cellular biosynthesis. It permits cells to increase the surface expression of nutrient transporters, enabling increased uptake of glucose, amino acids and other nutrients; it increases glycolysis and lactate production and it enhances the biosynthesis of macromolecules (both lipids and proteins). Activation of mTORC1 is indeed sufficient to induce a metabolic gene regulatory network [20]. When the cell senses a rich environment, growth-factor-activated kinases phosphorylate TSC2, leading to decreased GAP activity towards Rheb, which remains GTP-bound and activates mTORC1, leading to increased cell growth in part through augmented protein synthesis and nutrients uptake [21]. In contrast, during nutrient deprivation or under energy-limiting conditions, AMPK phosphorylates and activates TSC1/2 leading to conversion of Rheb-GTP into Rheb-GDP and mTORC1 inhibition. As a consequence, catabolic processes such as fatty acid oxidation or autophagy are induced to provide a constant supply of nutrients and maintain ATP production [22]. TSC1/2-deficient cells prove highly sensitive to glucose deprivation [23], due to hyperactivation of mTORC1, and its failure to balance the demand of metabolites with available resources [24]. This often leads to autophagy and, in several instances, apoptotic cell death. TSC1/2–mTORC1 also
regulates glucose transporter translocation and expression, nutrient receptor translocation and expression of metabolic enzymes, and integrate signals that induce quiescent cells into the cell cycle with the reorganization of metabolic activity allowing quiescent cells to begin to proliferate [22]. Thus the TSC1/2–mTORC1 signalling network simultaneously controls metabolic supply and demand in mammalian cells, affecting the ability of the cells to grow, proliferate and survive.

The role for TSC1/2 in cell proliferation has been proven by experiments involving the down-regulation or overexpression of the complex or its function. Original experiments performed in the Eker rat model, showed that a germline mutation in the Ts2 gene predisposed the rats to the development of a variety of neoplasias. Fibroblast and smooth muscle cells derived from these rats also revealed higher proliferation rates, and silencing Tsc2 in Rat1 fibroblasts shortened the G1-phase of the cell cycle, favouring cell-cycle entry [25,26]. Studies in Drosophila also supported a role for TSC1/2 in proliferation. Inactivating mutations in the Drosophila orthologues of Tsc1 and Tsc2 gave rise to indistinguishable phenotypes and caused the cells to endoreplicate their DNA [27]. Furthermore, although tumour cell lines derived from the Eker rats revealed a proliferative advantage, overexpression of TSC2 in tumour cell lines derived from the Eker rats lowered their proliferation rate [28,29]. Together, these results support the contribution of the TSC1/2 complex to entering into the M- or S-phase of the cell cycle. Mice with disruption of Ts2 die at early embryonic stages. Conditional deletion of Tsc genes has thus been adopted to analyse the function of TSC1/2 in vivo. For instance, conditional inactivation of Tsc1 drives haemopoietic stem cells from quiescence into rapid cycling. However, because of increased mitochondrial biogenesis and elevated levels of ROS (reactive oxygen species), both haematopoiesis and self-renewal of haemopoietic stem cells was reduced [30]. Likewise, Tsc2 inactivation in pancreatic β-cells augmented proliferation and β-cell size, causing hypoglycaemia and hyperinsulinaemia and improving glucose tolerance [31].

The molecular mechanism(s) by which the TSC1/2 complex controls cell proliferation and whether it plays a direct or indirect role remain to be fully elucidated and might imply cell-type-restricted events. Several growth factor receptors cause inhibition of the TSC1/2 complex by phosphorylation of several residues with consequent mTORC1 activation and dependent cell growth and proliferation. For instance, insulin or insulin-like growth factors inhibit the TSC1/2 complex primarily through Akt-mediated phosphorylation and inactivation of TSC2. In addition to Akt, also ERK mediates TSC2 phosphorylation. This causes the dissociation of the TSC1/2 complex, leading to diminished inhibition of mTOR and consequent proliferative advantage [8,32].

Of note, this ERK-mediated phosphorylation and inactivation of TSC2 has been proposed as a pathogenic mechanism underlying tumorigenesis of Ts2−/− cells. In Drosophila, dTSC1 mutations co-operate with rbf1, the Drosophila orthologue of retinoblastoma protein, mutations to promote both G1 cell-cycle progression and unscheduled S-phase entry (and cell death) during eye development, and this can be explained, at least in part, by de2F1 expression being post-transcriptionally increased in mutant cells through the Rheb/TOR (target of rapamycin)/S6K (S6 kinase) pathway [33].

In addition to mTORC1, TSC1/2 has been linked to several other pathways leading to cell proliferation. For instance, TSC2 acts as a GAP towards the GTPase Rap1 [34], which when microinjected into NIH 3T3 cells can induce DNA synthesis [35]. Likewise, TSC1/2 has been shown to indirectly control nuclear levels of the CDK inhibitor p27. Indeed, in Tsc2-deficient cells GTP-bound Rheb leads to mTORC1-independent activation of AMPK, and this lowers nuclear representation of p27, allowing the cells to progress through the cell cycle [36]. Furthermore, via GSK3 and axin, the TSC1/2 complex promotes β-catenin degradation [16]. In TSC-related angiomyolipomas and lymphangioleiomyomatosis, β-catenin and its effectors, cyclin D1 and connexin43, are indeed found to be up-regulated [37]. The TSC/β-catenin link also appears important for nerve cells. Indeed, in collaboration with the group of J. Meldolesi, we recently showed that the proliferation of PC12 nerve cells is under the influence of TSC2 via a rapamycin-insensitive β-catenin-dependent pathway (R. Tomasoni, S. Negrini, S. Fiordaliso, A. Mondino, J. Meldolesi and R. D’Alessandro, unpublished work). Likewise, impaired TSC1/2 function during development was reported to enhance mTOR-dependent cell proliferation of cerebellar granule neuron precursors by GSK3 inactivation, and cytoplasmic localization of the CDK inhibitor p27Kip1 [38].

Recent evidence also supports a role for TSC1/2 in growth/proliferation initiated by other signalling pathways. For instance, TSC2 has been proven under the control of c-Myc, which represses TSC2 transcription and therefore influences translation initiation [39]. This might initiate a feed-forward loop whereby a gain of c-Myc would be reinforced by its translational enhancement as TSC2 decreases, and a loss of TSC2 would be reinforced by a resulting increase in Myc protein levels. This might account for the synergy of Akt and Myc transformation, as both of these molecules would affect TSC2 and further derepress mTORC1 activation of mTOR [40].

Thus, together, available results support a critical role for TSC1/2 in the control of intracellular events leading to cell growth and proliferation.

**The TSC1/2 complex controls proliferation and survival**

The TSC1/2 complex, in addition to cell growth and proliferation, also influences survival and death signals both by mTOR-dependent and mTOR-independent pathways (Figure 2).

The best characterized target of TSC1/2 involved in cell survival is Akt. Following phosphorylation by PDK1 (phosphoinositide-dependent kinase 1) and mTORC2, Akt directly phosphorylates and inhibits several downstream
targets, including members of the FOXO (forkhead box O) family of transcription factors and the pro-apoptotic Bcl-2 family member BAD, thus exerting anti-apoptotic effects. In the absence of TSC1/2 function, Akt fails to be properly phosphorylated and thus to exert its inhibitory function. As a result, cells become prone to apoptotic cell death.

TSC1/2 also controls cell death by E2F. For instance, in the Drosophila eye, TSC1/2 has been shown to regulate dE2F1 expression post-transcriptionally. In tsc1 or tsc2 mutant cells, the dE2F1 protein level is increased (and conversely decreased in Rheb or dTor mutant cells), and this results in ectopic G1–S cell-cycle transition and cell death. Thus, through E2F and the canonical Rheb/TOR/S6K pathway, TSC1 and TSC2 can control both cell-cycle progression and survival [33,41].

The TSC1/2/Rheb/mTOR pathway is also crucial in the UPR (unfolded protein response) evoked by the ER (endoplasmic reticulum) stress response, which in several instances leads to autophagy and apoptosis. Accordingly, neurons and mouse embryonic fibroblasts lacking a functional TSC1/2 complex reveal increased vulnerability to ER stress-induced cell death. The mechanism by which death is induced might be cell-specific. Indeed, death of TSC-deficient neurons appears to be due to the rapamycin-sensitive accumulation of stress markers such as CHOP (C/EBP homologous protein) and HO-1 (haem oxygenase 1) [42]. In contrast, TSC-deficient MEFs (mouse embryonic fibroblasts) show elevated eIF2α (eukaryotic initiation factor 2α) phosphorylation, but reduced ATF (activating transcription factor) 4, ATF6 and CHOP activation, and a defect in the ER stress response can be restored by raptor (regulatory associated protein of mTOR) knockdown, but not by rapamycin treatment [43].

Furthermore, TSC1/2 together with mTORC1 controls energy-deprivation-induced apoptosis. Under energy-starvation conditions, AMPK phosphorylates TSC2, enhancing its inhibitory function required for translation regulation and cell size control in response to energy deprivation, and protection from energy-deprivation-induced apoptosis [23]. Loss of TSC1 or TSC2 abrogates suppression of translation in response to stress conditions, resulting in the accumulation of p53 and consequent cell death [44]. In keeping with this, TSC−/− cells are more sensitive compared with TSC-sufficient cells to glucose deprivation [23]. Thus, although the inactivation of mTOR under nutrient stress, by way of TSC, prevents cell death, loss of TSC favours cell death by p53-mediated genomic damage, probably providing a possible explanation for the benign nature of hamartoma syndromes, including TSC.

TSC1/2 function is also under the control of DAPK (death-associated protein kinase) that functions downstream of the RAS/MEK (mitogen-activated protein kinase/ERK kinase)/ERK and PI3K/Akt growth factor signalling pathways. Phosphorylation of TSC2 by DAPK causes dissociation of the TSC1/2 complex, affecting sensitivity to apoptosis and autophagy [45]. Likewise, binding of the forkhead transcription factor FOXO1 to TSC2 inhibits the TSC1/2 complex, causing activation of the mTOR signalling cascade. In the presence of insulin or growth factors, FOXO transcription factors are degraded by Akt- and GSK-mediated phosphorylation, and this prevents expression of target genes, and FOXO-dependent cell death [46]. Upon growth factor withdrawal, FOXO family members remain in the dephosphorylated form, translocate into the nucleus to regulate cell-cycle arrest and DNA repair, and also the expression of pro-apoptotic factors [TRAIL (tumour-necrosis-factor-related apoptosis-inducing ligand) and FasL (Fas ligand)]. We recently found that TSC1-deficient T-cells reveal constitutive mTORC1 signalling and enhanced proliferative responses, but also an increased propensity to undergo cell death, probably due to defective
phosphorylation/inhibition of FOXO1/3 (R. Tomasoni, unpublished work).

Thus there is evidence to support the notion that TSC1/2 mediates signalling events critical for cell survival.

**TSC1/2 balances proliferation and survival**

Because of its critical positioning within the cells, i.e. both downstream (Figure 1) and upstream (Figure 2) of critical signalling modules, the TSC1/2 complex appears to be capable of simultaneously fine-tuning proliferative and pro-survival signals in response to growth-promoting agents, and energy and stress signals (Figure 3). In TSC-sufficient cells, growth-promoting events mediate TSC1/2 inhibition, and promote Akt phosphorylation and mTORC2 activation. mTORC2 phosphorylates Akt allowing it to phosphorylate TSC2, thus contributing to its inhibition. Akt negatively regulates death-promoting factors, thus promoting cell survival. In TSC-sufficient cells, this loop is kept under control by the mTORC1 negative-feedback loop [47,48], which contributes to the termination of growth-promoting signals. Thus co-ordinated activation of signals promoting cell growth, cell-cycle progression and cell survival leads to cell proliferation, confined in time by the negative-feedback loop. In TSC-deficient cells, TSC-dependent mTORC1 inhibition is lacking and mTORC1 constitutively promotes cell growth and proliferation, desensitizing the cells from extracellular signals and promoting cell division even under conditions of growth factor withdrawal. Persistent mTORC1 signalling thus leads to the inhibition of both mTORC2 and Akt functions. Consistent with this notion is the finding that mTORC1-dependent feedback inhibition of Akt signalling limits the growth of tumours lacking TSC2 [49,50]. This might be due to deregulated activity of death-promoting factors (such as FOXO), favouring cell death. We thus speculate that, depending on the cell type, energy state and availability of nutrients, TSC1/2 might differentially result in cell proliferation or cell death.

**Acknowledgements**

We thank our colleagues at the San Raffaele Scientific Institute for useful discussion and invaluable reagents. We apologize to all those authors whose work was not cited in this review because of space constraints.

**Funding**

We thank the Associazione Italiana Ricerca sul Cancro (AIRC) for support.

**References**


Received 11 November 2010 doi:10.1042/BSI10390466