YAP enters the mTOR pathway to promote tuberous sclerosis complex

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Mutations in tuberous sclerosis complex 1 (TSC1) or TSC2 predispose to angiomyolipomas and lymphangioleiomyomatosis in a mTOR-dependent manner. In these mesenchymal lesions, mTOR suppresses macroautophagy-mediated lysosomal degradation of YAP, which is a transcriptional coactivator of the Hippo pathway and is required for the tumorigenesis of TSC. Therapeutic applications for TSC and other diseases with dysregulated mTOR activity can be envisaged.

We recently generated a mosaic Tsc1 mutant mouse model that develops renal mesenchymal lesions with similarities to the perivascular epithelioid cell tumors (PEComas) observed in human patients with tuberous sclerosis complex (TSC). To gain insights into the pathogenesis of these lesions, we screened the transcriptional outputs of several signaling pathways governing proliferation, differentiation, and maintenance of multipotency during development, including Notch, Wnt, Hedgehog, and Hippo pathways. Strikingly, we revealed a transcriptional signature of the Hippo pathway in Tsc1 mutant kidneys. We further demonstrated that Yes-associated protein (YAP1, best known as YAP), a transcriptional coactivator of the Hippo pathway, is upregulated by mammalian target of rapamycin (mTOR) kinase activity in mouse and human TSC1/2-null cells. Genetic or pharmacologic inhibition of YAP greatly attenuates the abnormal proliferation and induces apoptosis of TSC1/2 null cells, both in vitro and in PEComas of mosaic Tsc1 mutant mice. YAP accumulation in TSC1/2 deficient cells is the result of impaired degradation of the protein by autophagy in an mTOR-dependent manner. These results suggest YAP as a potential therapeutic target for TSC and other diseases with dysregulated mTOR activity. Here we discuss these findings in the context of 3 outstanding issues in the field.

The Cell of Origin of TSC-Associated PEComas

Angiomyolipomas and lymphangioleiomyomatosis contain a common cell type named perivascular epithelioid cells (PECs) that express both myogenic and melanocytic lineage markers. It is likely that PECs are precursor cells giving rise to the endothelial cells, adipocytes, and smooth muscle cells commonly found in these mesenchymal lesions. By inducing whole body mosaic deletion of Tsc1 during late embryogenesis in mice (mosaic Tsc1-KO), it is possible to observe PECs in an animal model. In the future, lineage tracing experiments and cell-type specific inactivation could be performed in mice to address the physiologic counterpart of PECs during mammalian development.

Over the past years several origins of PECs have been put forward. Derivation from the neural crest has been proposed based on the expression of melanocyte markers in PECs. Among the mesenchymal cell types that may derive from the neural crest in at least some tissues, pericytes are interesting candidates. Pericytes are the mural cells of the capillaries that closely associate with endothelial cells, contributing to the integrity of these small blood vessels. Intriguingly, pericytes have been shown to be able to differentiate in vitro into adipocytes and smooth muscle cells, 2 major components commonly observed in angiomyolipomas. In mouse renal PEComas of the Tsc1 mutant, we
observed a small proportion of degenerated pericytes that were labeled by both PDGFRβ and αSMA.

It is also possible that PECs are derived from uncharacterized mesenchymal precursor cells that develop in situ or in the circulation upon TSC1/TSC2 mutation from the bone marrow. The possibility that PECs also travel from distant organs along the vascular walls cannot be excluded. Interestingly, there is some evidence of extravascular migratory metastasis of melanomas and gliomas, by which these cells can reach distant tissues. In human biopsies, small hamartomas can be observed close to large angiomyolipomas. Whether these small lesions are newly formed hamartomas or derive from cells migrating out of the large angiomyolipoma cannot be established at present. This perivascular invasion should also be considered for lymphangioleiomyomatosis cells in the lung that might derive from PEComas in distant tissues such as the kidney. Clearly, investigation of these distinct and non-exclusive possibilities would benefit from animal models in which PECs can be observed, such as the mosaic Tsc1-KO mice.

**Therapeutic Amelioration of TSC Lesions**

We have shown that the YAP transcriptional coactivator is upregulated in PEComas of Tsc1 mutant mouse models and human PEComas associated with TSC. Inhibition of YAP by genetic means or by treatment with verteporfin, a compound that disrupts the interaction between YAP and its transcription factor TEAD, greatly suppresses the abnormal proliferation and survival of Tsc1/Tsc2 null cells in vitro and in vivo. These results indicate that YAP is a potential target for TSC therapy and that this mouse model can be used to test the efficacy of multiple interventions that modulate YAP activity for the treatment of TSC.

The use of rapamycin derivatives that act as mTOR allosteric inhibitors has been approved for therapy against TSC. The response rate of rapamycin derivatives against angiomyolipomas is approximately 50%, although the hamartoma regrows when treatment is discontinued. The perspective of long-term treatment with rapamycin derivatives should favor the development of combinatorial therapies to limit side effects. Interesting options that may act on the YAP pathway are described below.

**G-protein coupled receptor agonist/antagonist**

A large variety of G protein coupled receptors (GPCRs) have been recently identified as positive and negative upstream regulators of the Hippo/YAP pathway. Of note, pharmacologic agents acting on the angiotensin and adrenergic GPCR are well tolerated and have interesting actions that should be considered. Angiotensin antagonists ameliorate renal injury in conditions of hypertension and fibrosis, whereas propranolol, a β adrenergic receptor antagonist, has striking efficacy against infantile hemangioma, possibly due to inhibition of angiogenesis and pericyte functions. Future studies should test whether these agents act on YAP and are effective against TSC PEComas.

**Statins**

The lipid product of the mevalonate pathway, geranylgeranyl pyrophosphate, plays an important role in the post-translational modification of small GTPases, such as Rho and Rho. Statins, inhibitors of the rate-limiting enzyme 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA)-reductase in the mevalonate pathway that lead to downregulation of mTOR complex 1 activity through Rho inhibition, can also induce YAP/TAZ phosphorylation and nuclear export by acting on Rho GTPase. Interestingly, simvastatin has already been shown to inhibit the proliferation and survival of TSC2 null cells in vitro, whereas the efficacy of atorvastatin against TSC remains controversial. Therefore, the effectiveness and molecular mechanisms underlying the roles of statins in treating TSC remain to be fully understood.

**Peptides that inhibit the interaction between YAP and TEAD**

The mammalian vestigial-like protein VGLL4 has been identified as a transcriptional repressor of YAP through its competitive binding to TEAD1–4 via the Tondo domains. Peptides mimicking the Tondo domains of VGLL4 attenuate YAP-dependent tumor growth in vitro and in vivo. This may represent an additional level of inhibitory intervention against the YAP pathway.

**Crosstalk of mTOR and YAP Pathways by Autophagy Control**

mTOR and Hippo/YAP pathways are master regulators of tissue growth, as demonstrated by genetic screening for alterations of organ size in flies. The functions of these 2 pathways are not redundant. mTOR mainly controls cell size and cell number in response to nutrient availability in a cell autonomous manner. The Hippo/YAP pathway transduces mechanical cues during development to alter cell proliferation and survival, in part through the control of humoral factors in the tissues. Crosstalk between these pathways has been predicted, yet has remained elusive for several years. In high-density conditions and under mechanical constraints, the half-life of the YAP protein is mainly controlled by the upstream kinases Lats1/2 that phosphorylate YAP and promote proteasomal degradation. However, we have shown that in low-density growing conditions the half-life of YAP is controlled by nutrients and the mTOR pathway, which impair the lysosomal degradation of YAP though autophagy (Fig. 1).

Autophagy is an evolutionally conserved catabolic pathway that recycles cellular proteins and organelles in lysosomes. Three different forms of autophagy have been described: macroautophagy, microautophagy and chaperone-mediated autophagy. Macroautophagy (hereafter called autophagy) sequesters cellular proteins and damaged organelles in double-membrane autophagosomes and delivers this cargo to lysosomes for degradation. We have revealed that accumulation of YAP protein in TSC1/2 null cells is caused by blockage of macroautophagic degradation of YAP in an mTOR-dependent manner, because YAP degradation resulting from inhibition of mTOR is...
Figure 1. Tight control of YAP half-life. Mechanotransduction through Rho proteins and the actin cytoskeleton regulates the phosphorylation of YAP by the upstream kinases Lats1/2 and its degradation by proteasomes. Nutritional cues and TSC mutations leading to mTOR activation regulate degradation of YAP by lysosomes through the autophagy pathway.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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