Published in final edited form as:

Mol Cancer Res. 2015 January ; 13(1): 3–8. doi:10.1158/1541-7786.MCR-14-0343.

Therapeutic targeting of cellular metabolism in cells with hyperactive mTORC1: A paradigm shift

Doug Medvetz¹, Carmen Priolo^{1,*}, and Elizabeth P. Henske^{1,*}

¹Division of Pulmonary and Critical Care Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA

Abstract

mTORC1 is an established master regulator of cellular metabolic homeostasis, via multiple mechanisms that include altered glucose and glutamine metabolism, and decreased autophagy. mTORC1 is hyperactive in the human disease tuberous sclerosis complex (TSC), an autosomal dominant disorder caused by germline mutations in the TSC1 or TSC2 gene. In TSC-deficient cells, metabolic wiring is extensively disrupted and rerouted as a consequence of mTORC1 hyperactivation, leading to multiple vulnerabilities, including “addiction” to glutamine, glucose, and autophagy. There is synergy between two rapidly evolving trajectories: elucidating the metabolic vulnerabilities of TSC-associated tumor cells, and the development of therapeutic agents that selectively target cancer-associated metabolic defects.

The current review focuses on recent work supporting the targeting of cellular metabolic dysregulation for the treatment of tumors in TSC, with relevance to the many other human neoplasms with mTORC1 hyperactivation. These data expose a fundamental paradox in the therapeutic targeting of tumor cells with hyperactive mTORC1: inhibition of mTORC1 may not represent the optimal therapeutic strategy. Inhibiting mTORC1 “fixes” the metabolic vulnerabilities, results in a cytostatic response, and closes the door to metabolic targeting. In contrast, leaving mTORC1 active allows the metabolic vulnerabilities to be targeted with the potential for a cytotoxic cellular response. The insights provided here suggest that therapeutic strategies for TSC and other tumors with activation of mTORC1 are at the verge of a major paradigm shift, in which optimal clinical responses will be accomplished by targeting mTORC1-associated metabolic vulnerabilities without inhibiting mTORC1 itself.

Keywords

tuberous sclerosis complex; cellular metabolism; mTORC1; therapeutic targets

*Corresponding authors: Elizabeth P. Henske, Brigham and Women's Hospital and Harvard Medical School, One Blackfan Circle, 6th Floor, Boston, MA 02115, phone: 617-355-9049, fax: 617-355-9016, ehenske@partners.org; Carmen Priolo, Brigham and Women's Hospital and Harvard Medical School, One Blackfan Circle, 6th Floor, Boston, MA 02115, phone: 617-355-9012, fax: 617-355-9016, cpriolo@partners.org.

Conflict of Interest: The authors disclose no potential conflicts of interest.

Introduction

Tuberous sclerosis complex (TSC) is an autosomal dominant tumor suppressor gene (TSG) syndrome caused by inactivating germline mutations in the TSC1 or TSC2 gene. Among TSG syndromes, TSC is arguably the most phenotypically and pathologically diverse, with clinical manifestations that impact the central nervous system (seizures, autism, and cognitive impairment), hamartomatous tumors that can begin in fetal life and ultimately involve multiple organ systems (the brain, skin, kidney, heart, and lung), and gender-specific manifestations. The tumors in TSC are pathologically quite distinctive, including subependymal giant-cell astrocytomas, facial angiofibromas, renal angiomyolipomas, and pulmonary lymphangioleiomyomatosis (LAM). Studies initially in *Drosophila* and later in mammalian cells demonstrated that the TSC protein complex inhibits the mechanistic Target of Rapamycin (mTOR) complex 1 (mTORC1) (1-4). mTORC1 is a master regulator of nutrient and growth factor-induced signaling (3, 5, 6). Pivotal clinical trials of allosteric inhibitors of mTORC1 (Rapalogs) for the treatment of angiomyolipomas and subependymal giant cell astrocytomas (SEGAs) have revealed tumors partially regress during therapy, and regrow when therapy is discontinued (7-9). In sporadic LAM, which is caused by TSC2 gene mutations (10), lung function tends to stabilize while on sirolimus and then declines when therapy is discontinued (8). These partial clinical responses are consistent with a cytostatic rather than cytotoxic impact on the TSC-deficient tumor cells. Similar results have been observed in clinical trials of Rapalogs for many human malignancies in which complete and durable clinical responses are uncommon. There are two critical caveats to the interpretation of the clinical response of TSC tumors to Rapalogs: first, Rapalogs do not completely repress mTORC1 in most cellular models (11) and second, the TSC proteins are believed to have mTORC1-independent or “non-canonical” effects that may contribute to tumor response during Rapalog therapy (12).

Metabolic rewiring in TSC-deficient cells

It is now evident that cells with hyperactive mTORC1, including TSC-deficient cells, have extensive metabolic rewiring. Over the past decade, the pathways and biochemical mechanisms through which the TSC proteins regulate cellular bioenergetic homeostasis and anabolic metabolism have been elegantly defined and are reviewed elsewhere (3, 5, 13, 14). These defects include a Warburg-like switch to aerobic glycolysis, enhanced glucose flux through the pentose phosphate pathway, glutamine “addiction,” and inhibition of autophagy, which limits cell survival particularly under nutrient deprivation conditions (15).

These data reveal a fundamental paradox in the pathogenesis of TSC: TSC-deficient cells proliferate excessively, leading to tumors of the brain, skin, heart, kidneys, and lung, yet at the same time, these cells are highly vulnerable to bioenergetic stress-induced growth arrest and apoptosis. This short review will focus primarily on metabolic pathways that can be specifically targeted in TSC2-deficient cells, including autophagy, glycolysis, and glutaminolysis.

Glucose addiction in mTORC1 hyperactive cells

One of the earliest clues to the metabolic defects in mammalian cells deficient in the TSC1 or TSC2 genes arose in 2003 from work by Ken Inoki and Kun-Liang Guan, who discovered that TSC2 protects cells from energy deprivation-induced apoptosis (16). Subsequently, Choo et al. found that in the setting of glucose deprivation, TSC-deficient cells had approximately 60% less ATP than control TSC2-expressing cells (17). mTORC1 inhibition with Rapamycin restored ATP levels in the TSC-deficient cells, thereby preventing energetic stress and cell death in these conditions.

Multiple cellular mechanisms contribute to aberrant glucose metabolism in TSC-deficient cells. It has been recognized for many years that TSC-deficient cells have high levels of HIF1- α (18-21), which is regulated at both transcriptional and translational levels by mTORC1. HIF1- α induces glycolytic gene expression, enhances glycolysis, and promotes aerobic glycolysis or the “Warburg effect” and is likely to play a central role in the metabolic consequences of TSC-deficiency. A transcriptomic analysis of TSC-deficient cells linked TSC-dependent transcription to cellular metabolism including aerobic glycolysis (the Warburg effect) via HIF1- α , and the pentose phosphate pathway and fatty acid synthesis via SREBP (22).

Another factor contributing to the glucose metabolic effects of TSC2-deficiency is the regulation of glucose uptake. Both increased glucose consumption (23) and altered localization of the glucose transporter proteins to the cytoplasm instead of the plasma membrane (24) have been reported in Tsc2-deficient cells compared to TSC2 expressing controls. As will be discussed below, glucose uptake is autophagy-dependent in Tsc2-deficient cells (15). Additional insights regarding the metabolic impact of TSC deficiency on glucose homeostasis were reported by Sun et al. who found that pyruvate kinase M2 (PKM2) is upregulated in Tsc2-null cells, both in vitro and in vivo, as a consequence of mTORC1 hyperactivation, leading to hypersensitivity of TSC2-deficient cells to the glycolytic inhibitor 3-bromopyruvate (3-BrPA) in vitro (23). Other glycolytic enzymes, as illustrated in Figure 1, may also have roles as therapeutic targets in TSC and mTORC1-dependent diseases. Most recently, the Ragulator complex, which serves as a guanine nucleotide exchange factor (GEF) for the RAG GTPases and is essential to amino acid-induced activation of mTORC1 (25) was found to act as a “sensor” under glucose starvation conditions leading to activation of AMPK and recruitment of AMPK to the lysosome (26). This work highlights the intricate connections between mTORC1 and energy sensing programs as well as the critical role of the lysosome in coordinating metabolic homeostatic programs.

Glycolytic rewiring and mitochondrial alterations may contribute to the elevated levels of reactive oxygen species (ROS) that have been identified in many TSC2-deficient cellular models. Recently, a high-throughput screen of more than 6,000 compounds identified chelerythrine, which is best known as a protein kinase C (PKC) inhibitor, as a compound that can selectively induce apoptosis in TSC2-deficient cells via an mTORC1 and ROS-dependent mechanism (27). Chelerythrine provides another critical proof-of-concept that

TSC2-deficient cells can be selectively killed via metabolic mechanisms without the use of an mTORC1 inhibitor.

These findings and others have led to optimism that glucose metabolism can be targeted for the therapy of TSC associated tumors. It is interesting to note that metabolic targeting has been used for many years in the management of epilepsy in TSC. A “ketogenic diet” that severely restricts glucose intake has been demonstrated to have efficacy for the management of refractory seizures in TSC (28). More recently, a low glycemic index treatment (LGIT) was shown to reduce seizure frequency within 6 months in about 50% of children with TSC, including all 4 children in this study who were younger than 3 years of age (29).

To determine whether inhibiting glucose metabolism could similarly impact tumorigenesis in TSC, Jiang et al. used a TSC2-null xenograft model and compared a low-carbohydrate “ketogenic diet” vs. a “western diet” vs. 2-deoxyglucose. The carbohydrate-free diet was designed to decrease glucose levels and increase ketones. Surprisingly, the mice on the ketogenic diet had markedly enhanced tumor growth, nearly two fold greater than the western diet (30). The molecular mechanisms underlying the unexpected promotion of tumorigenesis remain incompletely understood, and whether this is somehow specific to a xenograft model of TSC vs. endogenously arising tumors is not known. This result highlights a key complexity of metabolically targeted therapy for TSC: the neurologic manifestations of TSC may also have a metabolic underpinning and that agents that systemically target mTOR hyperactive tumor cells may not have benefit to the neurologic manifestations and vice versa – metabolic strategies to control the neurologic manifestations such as the ketogenic diet may not have benefit in tumor control.

Glutamine addiction in mTORC1 hyperactive cells

In normal proliferating cells, both glucose and glutamine can feed the TCA cycle. In glutaminolysis, glutamine is converted to alpha-ketoglutarate, which can enter the TCA cycle. Work by Choo et al. in 2010 demonstrated that the decreased viability of TSC-deficient cells under glucose deprivation conditions (as discussed above) was strongly glutamine dependent, leading to the notion of “glutamine addiction.” Downregulation of glutamate dehydrogenase (GDH) decreased the viability of TSC2-deficient cells by about 50% after 60 hrs of glucose deprivation (17). This work positioned EGCG, a green-tea component that inhibits GDH, as a potential therapeutic agent for TSC (See Figure 2). Previously, using a TSC2-deficient rat leiomyoma cell line (ELT3), Zhang et al. found that EGCG could inhibit cell growth, both in vitro and in vivo (31). However, the contribution of TSC2 or mTORC1 to this phenotype was not addressed, therefore “off target” effects of EGCG that are not TSC or mTORC1 specific cannot be excluded. Choo et al. found that inhibition of GDH decreased the viability of TSC2-null cells only in glucose-deprivation conditions. These results pose a dilemma: How can the equivalent of 60 hrs glucose deprivation be achieved, selectively, in TSC-deficient tumors in vivo? Choo et al. combined EGCG with an alkylating agent, mechlorethamine (Mechlo), which inhibits glycolysis via GAPDH by blocking poly-ADP ribose polymerase (PARP)-dependent consumption of NAD. The ultimate consequence is glycolytic inhibition, thus mimicking glucose deprivation (32). In vitro, EGCG synergized with Mechlo to kill Tsc2-null MEFs with the

greatest effect observed at 30 mM, a dose level which would be difficult to achieve in vivo. These findings, which suggest that co-targeting of glucose metabolism and glutaminolysis may have a selective cytotoxic effect in cells with hyperactive mTORC1, may represent a path forward for metabolic targeting, but clearly need to be validated in vivo.

More recently, Kim et al. have shown that glucose and glutamine positively influence mTORC1 signaling in an AMPK, TSC1/2, and Rag-independent manner (33). Similarly to Choo et al. (17), Kim et al. found that withdrawal of both glucose and glutamine strongly decreases mTORC1 activity regardless of AMPK or TSC1/2 status. Interestingly, they identified the Tel2-Tti1-Tti2 (TTT)-RUVBL1/2 complex in an siRNA screen for genes whose loss blocked mTORC1 signaling in TSC2-deficient cells. TTT-RUVBL1/2 is an ATPase containing complex that is known to regulate the stability of PI3K-related proteins, including mTOR (34-36). Kim et al. discovered that the stability of the TTT-RUVBL1/2 complex is inhibited by glucose or glutamine deprivation, and that TTT-RUVBL1/2 is required for lysosomal localization of mTORC1. The energetic stress which results from the removal of glucose and glutamine leads to a decrease in the interaction between Tel2 and mTOR, along with the TTT components and RUVBL1/2. Most importantly, the lack of these interactions led to the disruption of mTOR's localization to the lysosome and subsequent activation. This is intriguing because it suggests that inhibition of the energy-dependent functions of the TTT-RUVBL1/2 complex could provide a mechanism for metabolic targeting of cells with hyperactive mTORC1.

Autophagy addiction in mTORC1 hyperactive cells

It is believed that mTORC1 mainly repressed autophagy via direct phosphorylation of ULK1 (37, 38), although parallel mechanisms are known, including regulation of the ubiquitination and stability of ULK1 via TRAF6 (39). Under nutrient sufficiency, ULK1 activity is downregulated via the phosphorylation of Ser757 by mTORC1 which disrupts the ULK1-AMPK interaction. TSC-deficient tumor cells have low autophagy levels as a consequence of hyperactivation of mTORC1 (40), although in neuronal cells higher levels of AMPK signaling appear to compensate by activating autophagy (41) and thus autophagy is not necessarily low in all TSC-deficient cells. Parkhitko et al. found that TSC2-deficient cells are strongly dependent on autophagy for survival. Downregulation of Atg5 causes central necrosis in xenografts of Tsc2-deficient MEFs (40). This is intriguing because the central areas of tumors are typically the regions experiencing the highest levels of bioenergetic stress. Additionally, the number and size of tumors in Tsc2^{+/-} mice is decreased by crossing them with Beclin1^{+/-} mice, further supporting the hypothesis that low levels of autophagy represent an Achilles' heel for TSC2-deficient cells. Thus, TSC2-deficiency results in chronically low levels of autophagy, creating a dependence on autophagy induction for survival under stress conditions. This vulnerability of "autophagy addiction" can be therapeutically targeted by co-treatment with Rapamycin and Chloroquine (CQ). Chloroquine inhibits the final step of autophagy by increasing the pH of the lysosome, which prevents fusion with the autophagosome (See Figure 3). The combination of CQ and Rapamycin for 4 weeks inhibits renal tumorigenesis in Tsc2^{+/-} mice and the combination of hydroxychloroquine and sirolimus is currently being tested clinically in a Phase I clinical trial for LAM, the sirolimus and autophagy inhibition in LAM (SAIL) trial

([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01687179) #NCT01687179). Interestingly, treatment with CQ alone for 4 months also suppresses tumorigenesis in Tsc2^{+/-} mice (15), suggesting that autophagy inhibition alone could be sufficient to reduce the growth of tumors in TSC.

Inhibition of autophagy with CQ leads to increased glucose uptake, increased glucose utilization, and increased levels of pentose phosphate pathway (PPP) intermediates, including ribose-5-phosphate, sedoheptulose-1,7-biphosphate, and fructose-1,6-biphosphate (15). Combined inhibition of autophagy with either CQ or Spautin-1 (42) and the PPP with 6-aminonicotinamide (6-AN), which inhibits glucose-6-phosphate dehydrogenase (G6PD), the rate-limiting enzyme of the PPP, selectively inhibits the growth of TSC2-deficient cells vs. TSC2-expressing cells. Interestingly, apoptosis does not appear to be induced in this setting.

It is increasingly clear that autophagy plays a major role in the survival of mTOR-hyperactive cells, and other therapeutic strategies to utilize autophagy-dependent mechanisms are emerging. Ng et al. recently found that Tsc2-deficient MEFs are hypersensitive to amino acid starvation and hypoxia, which is Atg7 dependent and likely due to the inability of the cells to fully induce autophagy (43). They found that treatment of Tsc1 or Tsc2 deficient MEFs with 24 hours of low amino acid media or 16 hours of hypoxia leads to a selective increase in cell death of the Tsc1 and Tsc2 deficient MEFs compared to their Tsc1/2 expressing control cells. Downregulation of Atg7 in Tsc2^{+/+} MEFs sensitized them to low amino acid conditions.

Qin et al. have shown that ER stress leads to cell death via the induction of autophagy (44). As expected, they observed that mTOR hyperactive cells are more resistant to ER stress. Their data indicate that the combination of an mTOR inhibitor with an ER stress inducer such as tunicamycin is a potential therapeutic strategy for TSC and other mTOR dependent diseases. Bray et al. have shown that human renal cell carcinomas are resistant to mTOR inhibitors via upregulation of autophagy (45). Interestingly, the authors show that co-inhibition of mTOR and autophagy upregulates RIP kinase activity leading to necroptosis in RCC cell lines in vitro and in xenograft tumors. Wang et al. have elucidated a mechanisms through which rapamycin-dependent upregulation of autophagy can be potentiated (46): activation of CREB1 (which is negatively regulated by autophagy) with forskolin leads to cell death when combined with sirolimus. Xie et al. showed that mTOR-dependent melanoma cells can be driven to apoptosis when treated with the mTOR inhibitor temsirolimus and the autophagy inhibitor hydroxychloroquine (47). Lastly, Alayev et al. used the naturally occurring polyphenol Resveratrol in combination with rapamycin to selectively induce death in mTOR hyperactive cells (48).

In summary, targeting autophagy in the setting of mTOR hyperactivity with either single agents or in combination with mTOR inhibitors appears to be a promising therapeutic avenue for TSC, LAM, and other mTOR-dependent tumors. The SAIL trial for women with LAM is testing the combination of sirolimus and hydroxychloroquine. As discussed earlier, optimal strategies for TSC and LAM may be independent of mTORC1 inhibition. Preclinical data in Tsc2^{+/-} mice supports the use of CQ alone, although the kinetics are different than sirolimus plus CQ, with suppression of tumorigenesis being observed only at

longer time points (4 months for CQ alone vs. 1 month for sirolimus plus CQ). Autophagy inhibitors that are potent and selective would be predicted to be active as single agents in TSC and LAM. In vitro data supports the use of autophagy inhibition plus PPP inhibitors to selectively inhibit the growth of TSC2-deficient cells. Several FDA-approved agents have been shown to inhibit the PPP, including Imatinib and DHEA.

Key questions/areas for future research

TSC and LAM elegantly illustrate how a molecular understanding of pathogenesis can catalyze remarkable clinical progress in rare diseases (49). The increasing recognition that the metabolism of TSC-deficient cells is extensively rewired provides new opportunities for selective and specific targeting of tumors with mTORC1 hyperactivity. Furthermore, emerging biology clearly connects TSC1/2, mTORC1, and nutrient sensing at the surface of the lysosome (26, 50), recently reviewed by Dunlop et al. (51), suggesting that targeting the lysosome will have distinct metabolic consequences in TSC-deficient cells. As these concepts move forward, key questions and unknowns include: 1) *What are the best metabolic targets to achieve specificity for TSC-deficient cells?* Multiple potentially targetable alterations exist in TSC-deficient cells as a consequence of mTOR activation, including glycolysis, glutaminolysis, and autophagy. Which of these targets would be predicted to have the best therapeutic window to target the metabolic defects in TSC? Ideally, metabolically targeted agents would induce cell death selectively in the TSC-deficient cells, thereby inducing a sustained and durable response; continuous therapy would not be required. 2) *What are the best preclinical models to test metabolic targeting?* TSC is a multisystem disease and the metabolic dependencies of TSC-deficient cells are likely to vary based on the tissue type, tumor size and rate of growth, and stromal factors including angiogenesis and lymphangiogenesis. Even within a tumor, there will be metabolic heterogeneity. For example, angiomyolipomas contain vascular, smooth muscle, and fat elements, each of which is likely to be metabolically distinct. In LAM, the TSC-deficient LAM cells are outnumbered by stromal cells, accompanied by distinctive lymphangiogenic network. Xenograft models, while providing many advantages in terms of efficiency and cell lineage flexibility, may have altered vascularity leading to bioenergetic dependencies that are not recapitulated by endogenously arising tumors. The Tsc2^{+/-} mouse model, which develops renal epithelial cysts and neoplasms, provides an alternative to xenograft models. Notably, autophagy inhibition with CQ has been shown to decrease renal tumorigenesis in the Tsc2^{+/-} mice (40) while Metformin had no effect (52). Proteasome inhibitors, which have in vitro efficacy in some TSC cellular models, also had no effect in the Tsc2^{+/-} mice (52). Clearly, many variables need to be carefully considered as in vitro findings are translated into preclinical and clinical studies. 3) *What are the optimal clinical scenarios in which to test metabolically-targeted therapeutic strategies in humans with TSC and LAM?* To our knowledge, the ongoing “SAIL” trial (sirolimus and autophagy inhibition in LAM, NCT01687179) which is testing the safety of rapamycin/sirolimus plus hydroxychloroquine in LAM represents one of the first initiatives to target the metabolism of TSC-deficient cells in humans. In TSC and LAM, biomarkers that identify the patients most likely to benefit from metabolically-targeted therapies and that provide early indicators of therapeutic benefit are urgently needed to streamline clinical trial design.

Conclusion

In 2010, Abraham and Eng (53) were among the first to address the balance between the pro-survival and the anti-tumor effects of mTORC1 inhibition, asking whether the “salutary effect of rapamycin-related mTORC1 inhibitors on metabolic balance under stress conditions actually compromises the antitumor activities of these compounds in the clinical setting.” They proposed that “a more complete understanding of the metabolic rewiring that allows them to survive such energy deficits may well reveal some intriguing new targets for cancer drug development.”

Four years later, the metabolic rewiring in TSC-deficient cells is further elucidated through elegant work by many laboratories. In aggregate, these data strongly support the fundamental hypothesis that inhibition of mTORC1 creates bioenergetic stability and promotes tumor viability via autophagy and other mechanisms. Most excitingly, recent work has revealed therapeutic strategies that target the metabolism of TSC-deficient cells without the use of mTORC1 inhibitors, exactly as predicted in 2010 by Abraham and Eng. TSC-deficient cells exist on a “metabolic cliff” because of the bioenergetic imbalance created by mTORC1-hyperactivation, creating exquisite sensitivity to further bioenergetic stress, such as glucose deprivation plus glutamine deprivation, or autophagy inhibition. Treatment with an mTORC1 inhibitor relieves this metabolic stress, restoring bioenergetics balance. Therefore, we believe that optimal targeting of TSC-associated tumors as well as the majority of sporadic tumors with hyperactive mTORC1 will be achieved without the use of mTORC1 inhibitors.

In conclusion, mTORC1 hyperactivation generates metabolic vulnerabilities that can be therapeutically targeted, with high specificity for the TSC-deficient cells vs. TSC-intact counterparts, and with the potential for selective induction of cell death. In striking contrast, mTORC1 inhibition relieves these metabolic vulnerabilities and induces strong cytoprotective, pro-survival pathways, including the induction of autophagy that result in cell survival, dormancy, and cytostasis. While Rapalogs generate a cytostatic response, metabolically targeted therapies offer an opportunity for a cytocidal response. We are poised to translate these key findings in preclinical models of TSC and LAM, and to identify optimal agents and strategies for clinical trials. The future of targeting mTORC1 hyperactive tumors may rely primarily on strategies that do not include mTORC1 inhibition, representing a major paradigm-shift in an active area of basic and clinical investigation.

References

1. Huang J, Manning BD. The TSC1-TSC2 complex: a molecular switchboard controlling cell growth. *Biochem J.* 2008; 412(2):179–90. [PubMed: 18466115]
2. Huang J, Manning BD. A complex interplay between Akt, TSC2 and the two mTOR complexes. *Biochem Soc Trans.* 2009; 37(Pt 1):217–22. [PubMed: 19143635]
3. Dibble CC, Manning BD. Signal integration by mTORC1 coordinates nutrient input with biosynthetic output. *Nat Cell Biol.* 2013; 15(6):555–64. [PubMed: 23728461]
4. Crino PB, Nathanson KL, Henske EP. The tuberous sclerosis complex. *N Engl J Med.* 2006; 355(13):1345–56. [PubMed: 17005952]
5. Laplante M, Sabatini DM. mTOR signaling in growth control and disease. *Cell.* 2012; 149(2):274–93. [PubMed: 22500797]

6. Yecies JL, Manning BD. mTOR links oncogenic signaling to tumor cell metabolism. *J Mol Med (Berl)*. 2011; 89(3):221–8. [PubMed: 21301797]
7. Bissler JJ, McCormack FX, Young LR, Elwing JM, Chuck G, Leonard JM, et al. Sirolimus for angiomyolipoma in tuberous sclerosis complex or lymphangioleiomyomatosis. *N Engl J Med*. 2008; 358(2):140–51. [PubMed: 18184959]
8. McCormack FX, Inoue Y, Moss J, Singer LG, Strange C, Nakata K, et al. Efficacy and safety of sirolimus in lymphangioleiomyomatosis. *N Engl J Med*. 2011; 364(17):1595–606. [PubMed: 21410393]
9. Krueger DA, Care MM, Holland K, Agricola K, Tudor C, Mangeskar P, et al. Everolimus for subependymal giant-cell astrocytomas in tuberous sclerosis. *N Engl J Med*. 2010; 363(19):1801–11. [PubMed: 21047224]
10. Carsillo T, Astrinidis A, Henske EP. Mutations in the tuberous sclerosis complex gene TSC2 are a cause of sporadic pulmonary lymphangioleiomyomatosis. *Proc Natl Acad Sci U S A*. 2000; 97(11):6085–90. [PubMed: 10823953]
11. Nyfeler B, Bergman P, Triantafellow E, Wilson CJ, Zhu Y, Radetich B, et al. Relieving autophagy and 4EBP1 from rapamycin resistance. *Mol Cell Biol*. 2011; 31(14):2867–76. [PubMed: 21576371]
12. Neuman NA, Henske EP. Non-canonical functions of the tuberous sclerosis complex-Rheb signalling axis. *EMBO Mol Med*. 2011; 3(4):189–200. [PubMed: 21412983]
13. Kim SG, Buel GR, Blenis J. Nutrient regulation of the mTOR complex 1 signaling pathway. *Mol Cells*. 2013; 35(6):463–73. [PubMed: 23694989]
14. Howell JJ, Ricoult SJ, Ben-Sahra I, Manning BD. A growing role for mTOR in promoting anabolic metabolism. *Biochem Soc Trans*. 2013; 41(4):906–12. [PubMed: 23863154]
15. Parkhitko AA, Priolo C, Colloff JL, Yun J, Wu JJ, Mizumura K, et al. Autophagy-dependent metabolic reprogramming sensitizes TSC2-deficient cells to the antimetabolite 6-aminonicotinamide. *Mol Cancer Res*. 2014; 12(1):48–57. [PubMed: 24296756]
16. Inoki K, Zhu T, Guan KL. TSC2 mediates cellular energy response to control cell growth and survival. *Cell*. 2003; 115(5):577–90. [PubMed: 14651849]
17. Choo AY, Kim SG, Vander Heiden MG, Mahoney SJ, Vu H, Yoon SO, et al. Glucose addiction of TSC null cells is caused by failed mTORC1-dependent balancing of metabolic demand with supply. *Mol Cell*. 2010; 38(4):487–99. [PubMed: 20513425]
18. Liu MY, Poellinger L, Walker CL. Up-regulation of hypoxia-inducible factor 2alpha in renal cell carcinoma associated with loss of Tsc-2 tumor suppressor gene. *Cancer Res*. 2003; 63(10):2675–80. [PubMed: 12750296]
19. Brugarolas J, Lei K, Hurley RL, Manning BD, Reiling JH, Hafen E, et al. Regulation of mTOR function in response to hypoxia by REDD1 and the TSC1/TSC2 tumor suppressor complex. *Genes Dev*. 2004; 18(23):2893–904. [PubMed: 15545625]
20. Land SC, Tee AR. Hypoxia-inducible factor 1alpha is regulated by the mammalian target of rapamycin (mTOR) via an mTOR signaling motif. *J Biol Chem*. 2007; 282(28):20534–43. [PubMed: 17502379]
21. Wolff NC, Vega-Rubin-de-Celis S, Xie XJ, Castrillon DH, Kabbani W, Brugarolas J. Cell-type-dependent regulation of mTORC1 by REDD1 and the tumor suppressors TSC1/TSC2 and LKB1 in response to hypoxia. *Mol Cell Biol*. 2011; 31(9):1870–84. [PubMed: 21383064]
22. Duvel K, Yecies JL, Menon S, Raman P, Lipovsky AI, Souza AL, et al. Activation of a metabolic gene regulatory network downstream of mTOR complex 1. *Mol Cell*. 2010; 39(2):171–83. [PubMed: 20670887]
23. Sun Q, Chen X, Ma J, Peng H, Wang F, Zha X, et al. Mammalian target of rapamycin up-regulation of pyruvate kinase isoenzyme type M2 is critical for aerobic glycolysis and tumor growth. *Proc Natl Acad Sci U S A*. 2011; 108(10):4129–34. [PubMed: 21325052]
24. Jiang X, Kenerson H, Aicher L, Miyaoka R, Eary J, Bissler J, et al. The tuberous sclerosis complex regulates trafficking of glucose transporters and glucose uptake. *Am J Pathol*. 2008; 172(6):1748–56. [PubMed: 18511518]
25. Bar-Peled L, Schweitzer LD, Zoncu R, Sabatini DM. Ragulator is a GEF for the rag GTPases that signal amino acid levels to mTORC1. *Cell*. 2012; 150(6):1196–208. [PubMed: 22980980]

26. Zhang CS, Jiang B, Li M, Zhu M, Peng Y, Zhang YL, et al. The Lysosomal v-ATPase-Ragulator Complex Is a Common Activator for AMPK and mTORC1, Acting as a Switch between Catabolism and Anabolism. *Cell Metab.* 2014
27. Medvetz, Douglas; Sun, Yang; Li, Chenggang; Khabibullin, Damir; Balan, Murugabaskar; Parkhitko, Andrey, et al. High-throughput Drug Screen Identifies Chelerythrine as a Selective Inducer of Death in a TSC2-null Setting. *Molecular Cancer Research.* 2014
28. Kossoff EH, Thiele EA, Pfeifer HH, McGrogan JR, Freeman JM. Tuberous sclerosis complex and the ketogenic diet. *Epilepsia.* 2005; 46(10):1684–6. [PubMed: 16190943]
29. Larson AM, Pfeifer HH, Thiele EA. Low glycemic index treatment for epilepsy in tuberous sclerosis complex. *Epilepsy Res.* 2012; 99(1-2):180–2. [PubMed: 22119636]
30. Jiang X, Kenerson HL, Yeung RS. Glucose deprivation in tuberous sclerosis complex-related tumors. *Cell Biosci.* 2011; 1:34. [PubMed: 22018000]
31. Zhang D, Al-Hendy M, Richard-Davis G, Montgomery-Rice V, Sharan C, Rajaratnam V, et al. Green tea extract inhibits proliferation of uterine leiomyoma cells in vitro and in nude mice. *Am J Obstet Gynecol.* 2010; 202(3):289 e1–9. [PubMed: 20074693]
32. Zong WX, Ditsworth D, Bauer DE, Wang ZQ, Thompson CB. Alkylating DNA damage stimulates a regulated form of necrotic cell death. *Genes Dev.* 2004; 18(11):1272–82. [PubMed: 15145826]
33. Kim SG, Hoffman GR, Poulogiannis G, Buel GR, Jang YJ, Lee KW, et al. Metabolic stress controls mTORC1 lysosomal localization and dimerization by regulating the TTT-RUVBL1/2 complex. *Mol Cell.* 2013; 49(1):172–85. [PubMed: 23142078]
34. Takai H, Wang RC, Takai KK, Yang H, de Lange T. Tel2 regulates the stability of PI3K-related protein kinases. *Cell.* 2007; 131(7):1248–59. [PubMed: 18160036]
35. Horejsi Z, Takai H, Adelman CA, Collis SJ, Flynn H, Maslen S, et al. CK2 phospho-dependent binding of R2TP complex to TEL2 is essential for mTOR and SMG1 stability. *Mol Cell.* 2010; 39(6):839–50. [PubMed: 20864032]
36. Kaizuka T, Hara T, Oshiro N, Kikkawa U, Yonezawa K, Takehana K, et al. Tti1 and Tel2 are critical factors in mammalian target of rapamycin complex assembly. *J Biol Chem.* 2010; 285(26):20109–16. [PubMed: 20427287]
37. Kim J, Kundu M, Viollet B, Guan KL. AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. *Nat Cell Biol.* 2011; 13(2):132–41. [PubMed: 21258367]
38. Egan D, Kim J, Shaw RJ, Guan KL. The autophagy initiating kinase ULK1 is regulated via opposing phosphorylation by AMPK and mTOR. *Autophagy.* 2011; 7(6):643–4. [PubMed: 21460621]
39. Nazio F, Strappazzon F, Antonioli M, Bielli P, Cianfanelli V, Bordin M, et al. mTOR inhibits autophagy by controlling ULK1 ubiquitylation, self-association and function through AMBRA1 and TRAF6. *Nat Cell Biol.* 2013; 15(4):406–16. [PubMed: 23524951]
40. Parkhitko A, Myachina F, Morrison TA, Hindi KM, Auricchio N, Karbowniczek M, et al. Tumorigenesis in tuberous sclerosis complex is autophagy and p62/sequestosome 1 (SQSTM1)-dependent. *Proc Natl Acad Sci U S A.* 2011; 108(30):12455–60. [PubMed: 21746920]
41. Di Nardo A, Wertz MH, Kwiatkowski E, Tsai PT, Leech JD, Greene-Colozzi E, et al. Neuronal Tsc1/2 complex controls autophagy through AMPK-dependent regulation of ULK1. *Hum Mol Genet.* 2014; 23(14):3865–74. [PubMed: 24599401]
42. Liu J, Xia H, Kim M, Xu L, Li Y, Zhang L, et al. Beclin1 controls the levels of p53 by regulating the deubiquitination activity of USP10 and USP13. *Cell.* 2011; 147(1):223–34. [PubMed: 21962518]
43. Ng S, Wu YT, Chen B, Zhou J, Shen HM. Impaired autophagy due to constitutive mTOR activation sensitizes TSC2-null cells to cell death under stress. *Autophagy.* 2011; 7(10):1173–86. [PubMed: 21808151]
44. Qin L, Wang Z, Tao L, Wang Y. ER stress negatively regulates AKT/TSC/mTOR pathway to enhance autophagy. *Autophagy.* 2010; 6(2):239–47. [PubMed: 20104019]
45. Bray K, Mathew R, Lau A, Kamphorst JJ, Fan J, Chen J, et al. Autophagy suppresses RIP kinase-dependent necrosis enabling survival to mTOR inhibition. *PLoS One.* 2012; 7(7):e41831. [PubMed: 22848625]

46. Wang Y, Hu Z, Liu Z, Chen R, Peng H, Guo J, et al. MTOR inhibition attenuates DNA damage and apoptosis through autophagy-mediated suppression of CREB1. *Autophagy*. 2013; 9(12):2069–86. [PubMed: 24189100]
47. Xie X, White EP, Mehnert JM. Coordinate autophagy and mTOR pathway inhibition enhances cell death in melanoma. *PLoS One*. 2013; 8(1):e55096. [PubMed: 23383069]
48. Alayev A, Sun Y, Snyder RB, Berger SM, Yu JJ, Holz MK. Resveratrol prevents rapamycin-induced upregulation of autophagy and selectively induces apoptosis in TSC2-deficient cells. *Cell Cycle*. 2014; 13(3):371–82. [PubMed: 24304514]
49. Fishman MC. Power of rare diseases: found in translation. *Sci Transl Med*. 2013; 5(201):201ps11.
50. Menon S, Dibble CC, Talbott G, Hoxhaj G, Valvezan AJ, Takahashi H, et al. Spatial control of the TSC complex integrates insulin and nutrient regulation of mTORC1 at the lysosome. *Cell*. 2014; 156(4):771–85. [PubMed: 24529379]
51. Dunlop EA, Tee AR. mTOR and autophagy: A dynamic relationship governed by nutrients and energy. *Semin Cell Dev Biol*. 2014
52. Auricchio N, Malinowska I, Shaw R, Manning BD, Kwiatkowski DJ. Therapeutic trial of metformin and bortezomib in a mouse model of tuberous sclerosis complex (TSC). *PLoS One*. 2012; 7(2):e31900. [PubMed: 22363765]
53. Abraham RT, Eng CH. A metabolic (re-)balancing act. *Mol Cell*. 2010; 38(4):481–2. [PubMed: 20513422]

Glucose metabolism

Figure 1.

Selected inhibitors of glucose metabolism with potential efficacy in TSC. 3-BrPA – 3-bromopyruvic acid; 2-DG – 2-deoxyglucose; 6-AN – 6-aminonicotinamide.

Glutaminolysis

Figure 2.

Selected inhibitors of glutaminolysis with potential efficacy in TSC. DON - 6-Diazo-5-oxo-L-norleucine; EGCG - epigallocatechin-3-gallate.

Autophagy

Figure 3. Selected inhibitors of autophagy

Inhibitors highlighted in bold have shown potential efficacy in TSC. 3-MA – 3-methyladenine.