Increased mTORC1 Signaling UPRegulates Stress

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In this issue of *Molecular Cell*, Ozcan et al. (2008) show that the loss of the tuberous sclerosis tumor suppressor complex induces endoplasmic reticulum stress, leading to attenuation of insulin receptor signaling activity via the unfolded protein response.

The evolutionarily conserved serine/threonine kinase mammalian target of rapamycin (mTOR) coordinates nutritional and other environmental cues with the cellular growth machinery. mTOR functions in at least two multiprotein complexes (mTORC1 and mTORC2), of which mTORC1 can be acutely inhibited by the antifungal drug rapamycin. Known mTORC1 targets include the ribosomal p70 S6 kinase (S6K) and eukaryotic initiation factor 4E-binding protein (4EBP), which both affect translation. Among other functions, mTORC1 triggers increased protein synthesis and ribosomal biogenesis, thereby promoting growth (increase in cell mass). The tuberous sclerosis (TSC) tumor suppressor complex, composed of TSC1 and TSC2, negatively regulates mTORC1. TSC2 possesses GTPase activating protein (GAP) activity toward the small GTPase Rheb acting upstream of mTORC1 (Figure 1). The growth factor-responsive insulin receptor (InR) signaling cascade is wired to the mTOR pathway through Akt/PKB-mediated inhibitory phosphorylation of TSC2. Loss of TSC triggers constitutive mTORC1/mTORC2 signaling, promoting hyperplasia and hypertrophy in a variety of clinical and experimental settings. In humans, mutations in *TSC1/2* give rise to hamartomas throughout the body and disabling neurological disorders such as mental retardation, epilepsy, and autistic behavior.

Some of the conditions known to affect mTORC1/2 signaling, such as hypoxia, glucose deprivation, perturbations of intralumenal Ca^{2+} levels, changes in cellular redox state, or viral infection, also challenge the integrity of the endoplasmic reticulum (ER), whose functions include the folding and modification of secretory and transmembrane proteins. The resulting imbalance between the folding capacity and entry of new client proteins into the ER lumen causes ER stress and leads to the accumulation of mis-/unfolded proteins. This process evokes a coordinated adaptive signaling network—the so-called unfolded protein response (UPR). The UPR is largely cytoprotective in scope; however, prolonged ER stress tips the cell in favor of undergoing apoptosis. The UPR is comprised of three intertwined signaling branches, including the three proximal ER stress sensors PERK, IRE1, and ATF6 (Figure 1). UPR activation leads to general inhibition of protein synthesis and upregulation of chaperones and factors that improve protein folding and reduce the ER's protein loading stress.

Previous research in the Hotamisligil laboratory showed that obesity in mouse and cell culture models causes ER stress and promotes the development of insulin resistance. The downregulation of InR signaling in obese mice was attributed to inositol-requiring enzyme 1 (IRE1) signaling via TNF receptor-associated factor 2 (TRAF2) and c-Jun N-terminal protein kinase (JNK) to promote increased inhibitory serine 307 IRS1 phosphorylation and IRS1 degradation (Ozcan et al., 2004). These results demonstrated that the UPR contributes to a dampening of InR signaling, a situation reminiscent of the reported negative feedback loop in TSC-deficient cells, which likewise have decreased InR activity owing to S6K-mediated IRS1 inhibition (reviewed in Guertin and Sabatini, 2007). This similarity prompted the authors of the current study to investigate whether TSC loss induces ER stress. Indeed, both $TSC1^{-/-}$ and $TSC2^{-/-}$ MEFs showed augmentation of several markers of ER stress, including PERK phosphorylation, splicing of *XBP1* mRNA (s*XBP1*), and increased *GRP78* and *CHOP* mRNA levels. Restoration of TSC function in the *TSC* knockout MEFs reversed this effect, demonstrating that upregulation of UPR parameters is due to TSC deficiency. These findings were corroborated in vivo with both a *TSC* mutant mouse model and human TSC patient samples (Ozcan et al., 2008). Whether the ATF6 branch is also activated in *TSC* mutant MEFs was not determined.

Cycloheximide or rapamycin treatment, as well as a chemical chaperone that stabilizes proteins in their native conformation (4-phenyl butyric acid, PBA), largely suppressed the upregulation of UPR markers (Ozcan et al., 2008). This finding indicates that increased translation is likely the underlying cause for UPR activation in *TSC* mutant cells and that mTORC1 genetically functions upstream of ER stress (Figure 1). An interesting question is whether ER stress induction is specifically encountered in cells that have lost TSC or if it more generally applies to mutant contexts where protein synthesis is increased, such as in *PTEN* null tumors or in tumors with Ras/MAPK pathway hyperactivation.

Is decreased InR activity in *TSC* mutant cells solely a consequence of ER stress and concomitantly upregulated UPR components, or is it caused by other mTORC1 effectors acting in parallel, such as the previously described negative feedback

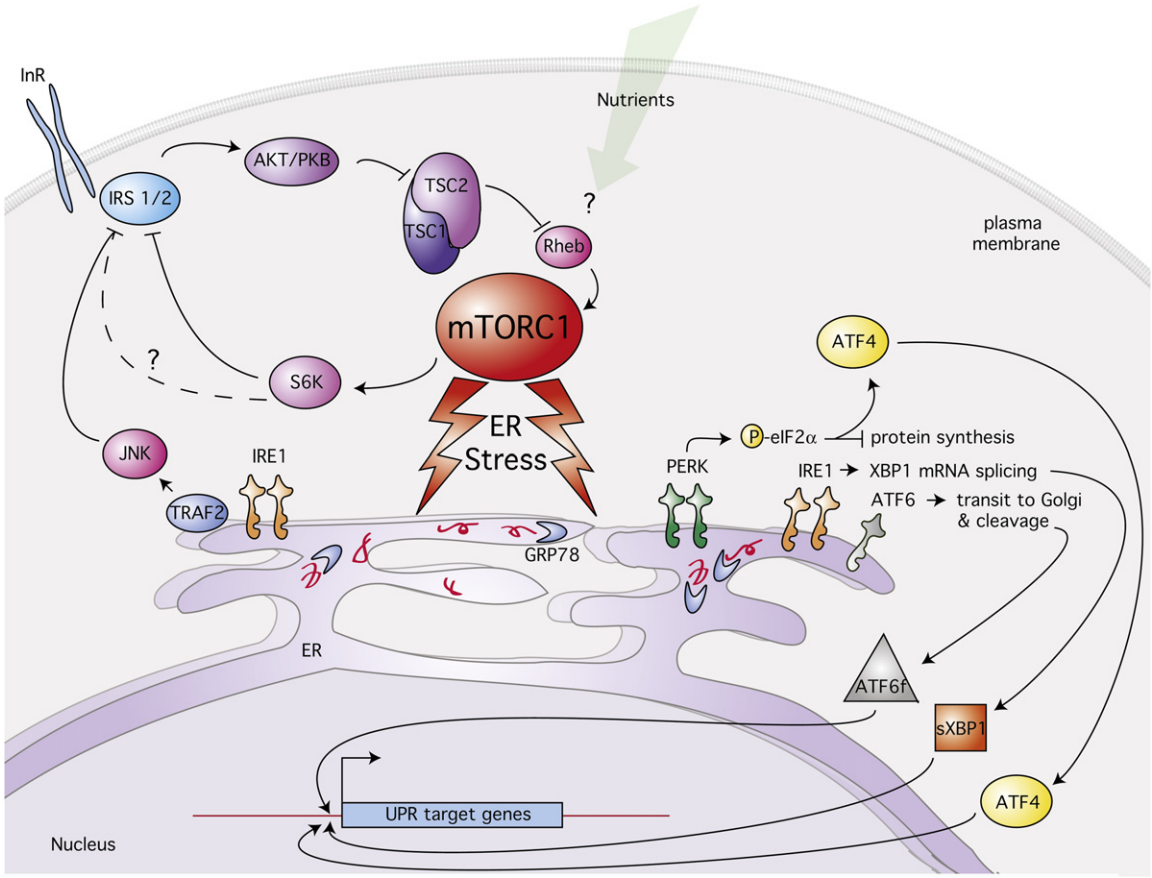

Figure 1. Loss of TSC Triggers ER Stress and the UPR

Several conditions that perturb ER homeostasis, including increased protein synthesis through mTORC1 overactivation, lead to accumulation of misfolded proteins in the ER. The most immediate response involves the ER-resident transmembrane protein PKR-like endoplasmic reticulum kinase (PERK) phosphorylating serine 51 on the eukaryotic translation initiation factor 2α (eIF2α), which leads to a general inhibition of protein synthesis. Paradoxically, eIF2α phosphorylation increases translation of the *Activating transcription factor-4* (*ATF4*) transcript, which contains two upstream open reading frames in its mRNA. ATF4 induces genes involved in amino acid metabolism and oxidative stress responses. The second branch is controlled by the basic leucine-zipper transcription factor ATF6 (α and β). ER stress liberates ATF6 from the ER membrane; it then transits to the Golgi where it is further cleaved to form an active transcription factor (ATF6f) that enters the nucleus to induce ER chaperones. Mounting of an effective UPR also includes the activation of the serine/threonine kinase IRE1, which harbors an additional endoribonuclease domain. In order to become active, IRE1—like PERK—homodimerizes and autophosphorylates upon ER stress induction. IRE1 cleaves a 26 nucleotide sequence from the *X-box binding protein-1* (*XBP1*) mRNA in an unconventional splicing event, thereby creating a translational frameshift leading to a potent transcription factor (sXBP1). sXBP1 is able to heterodimerize with ATF6f and upregulates genes assisting in protein folding and ER-associated degradation (ERAD). IRE1, through association with TRAF2, also induces JNK activity, leading to the inhibition of InR pathway activity. PERK, ATF6, and IRE1 are kept inactive under normal conditions by binding to the ER lumenal chaperone GRP78/BiP. The accumulation of unfolded peptides in response to ER stress dissociates GRP78 from binding to the three UPR transducers, leading to UPR activation. See text for further details.

loop mediated by S6K? Treatment with PBA or overexpression of sXBP-1, which improves protein folding, reversed the inhibition of InR signaling as assessed by insulin-stimulated phospho-IRS1/IRS2 and phospho-Akt levels, notably even under conditions with high S6K activity. In addition, these treatments alleviated the degradation of IRS1/IRS2 encountered in cells exposed to prolonged ER stress (Ozcan et al., 2008). Although without a clear quantitative readout of InR pathway activity it is not possible to draw a definitive conclusion about the S6K-dependent contribution to InR signaling inhibition, these results underscore that ER stress is an important etiologic factor in the development of insulin resistance.

Does increased ER stress in *TSC* mutant cells play a physiologic role, and what might be the functional significance? From an evolutionary standpoint, it would make sense to diminish InR pathway activity, which under normal conditions heightens protein synthesis and therefore would further exacerbate ER protein loading stress in a *TSC* mutant background. Accordingly, it was recently shown that activation of the InR pathway in two cancer cell lines enhanced ER stress and apoptosis under hypoxic conditions (Endo et al., 2007). Moreover, *TSC*-deficient cells undergo increased cell death upon glucose withdrawal (Inoki et al., 2003). Ozcan et al. (2008) show in this current work that the enhanced glucose deprivation-induced cell death observed in *TSC* mutant cells could be ameliorated by PBA treatment. Furthermore, tunicamycin or thapsigargin treatment led to increased apoptosis of cells devoid of TSC function in a rapamycin-sensitive fashion both in vitro and in vivo. Thus, loss of TSC in this context seems to render cells hypersensitive to ER stress-induced cell

death. However, the situation is likely more complex, as *TSC2* mutant MEFs subjected to hypoxia (which also induces the UPR) showed enhanced survival in a rapamycin-resistant fashion (Kaper et al., 2006). These findings indicate that survival of *TSC* mutant cells depends on the combined action of multiple downstream effectors acting in parallel and/or that viability is contingent on the strength or type of ER stress stimulus.

Malignant cells already facing enhanced ER protein trafficking due to higher protein synthesis rates than normal cells might be more prone to protein buildup and misfolding-induced apoptosis when ER function is compromised. Xenograft studies strongly suggest that a functional UPR is critical for tumorigenesis because cells with abrogated PERK, IRE1, XBP1, or GRP78 function display significantly impaired tumor formation (reviewed in Ma and Hendershot, 2004, and Moenner et al., 2007). The development of drugs targeting components of the UPR or more generally inducing ER stress might therefore be an attractive anticancer modality to specifically eradicate tumor cells. For example, proteasome inhibitors such as Bortezomib (Velcade), an FDA-approved drug for the treatment of multiple myeloma, at least partially act by induction of ER stress due to ERAD inhibition. The classic ER stress inducers tunicamycin and thapsigargin in conjunction with MG-132 or Bortezomib synergistically induced apoptosis in cancer cells (Lee et al., 2003; Nawrocki et al., 2005). Other antitumor drugs, including the alkyllysophospholipid edelfosine, cannabinoids, cisplatin, or the Hsp90 inhibitor geldanamycin, share the common property of increasing ER stress, again suggesting that interference with protein folding and chaperone homeosotasis sensitizes tumorigenic cells to apoptosis (Ma and Hendershot, 2004; Moenner et al., 2007; Nawrocki et al., 2005; Nieto-Miguel et al., 2007 and references therein).

Taken together, the increased susceptibility to ER stress of cells deficient in TSC function or with aberrantly increased mTOR activity might open up new avenues in cancer treatment, especially for those tumors that are insensitive to rapamycin.

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"Unraveling the Tail" of How SRPK1 Phosphorylates ASF/SF2

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In this issue of *Molecular Cell*, Ngo et al. (2008) describe the crystal structure of the SRPK1 protein kinase in complex with its substrate, the spliceosome factor ASF/SF2, providing an unprecedented view of multiple targeting mechanisms in action on a single substrate.

The phosphorylation of proteins by eukaryotic protein kinases controls diverse cellular functions. The unique behavior of each protein kinase is imparted by differences in catalytic switching and substrate-recognition mechanisms. Using X-ray crystallographic methods, we have gained great insight into both aspects of kinase function, and yet much remains to be learned. In this issue of *Molecular Cell*, Ngo et al. (2008) describe the X-ray crystal structure of SR protein kinase 1 (SRPK1) bound to