From TOR and SMAD, why HIF-1α can be bad

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Transforming growth factor (TGF-β) is a pleiotropic cytokine that regulates cell growth, differentiation, proliferation, immune response, and extracellular matrix remodeling; it plays a pivotal role in fibrosis in multiple organs (1). TGF-β signals via type I (TβRI) and type II (TβRII) receptors. Binding of TGF-β to TβRI, a serine/threonine kinase, recruits TβRII to form a heteromeric ligand-receptor complex—activating TGF-β receptor kinase, which phosphorylates and activates SMAD2 and SMAD3 (1), ultimately leading to the formation of a heteromultimeric complex that includes SMAD4; this complex translocates to the nucleus and regulates TGF-β target genes, including type I collagen (1). TGF-β-induced fibrogenesis requires the action of other signaling pathways that include p38 kinase, Jun N-terminal kinase (JNK), extracellular regulated kinase (ERK), phosphatidylinositide 3-kinase (PI3K/AKT), and mammalian target of rapamycin (mTOR) (2, 12). mTOR is a serine/threonine protein kinase subunit found in mTOR complex 1 (mTORC1; also includes Raptor, Daptor, mLST8, and mLST8; and PRAS40) and mTOR complex 2 (mTORC2; also includes Daptor, mLST8, Rictor, and mSin) (7). mTORC1 is activated by PI3K/AKT, ERK1/2, and Wnt pathways and it is inhibited by AMP kinase (AMPK) (7). mTORC1 phosphorylates S6 kinase 1 (S6K1) and 4E-related protein (4E-RP) and promotes cell growth (hypertrophy) and proliferation; the activity of mTORC1 is tightly regulated by the availability of amino acids, growth factors, and energy stores, ensuring that conditions are optimal for growth and proliferation (7, 8). In addition, mTORC1 activates hypoxia-inducible factor-1α (HIF-1α), peroxisome proliferator-activated receptor (PPAR)-γ and its activator (PGC)-1α—to control angiogenesis, mitochondrial function, and adipogenesis (7). mTORC2 targets include PI3K, protein kinase Cα (PKCα), and serum and glucocorticoid-induced protein kinase 1 (SGK1); however, upstream activation of mTORC2 is not clearly defined (7). Activation of mTORC2 modulates cell survival and leads to changes in the actin cytoskeleton, cell polarity, and activity of the aldosterone-sensitive sodium channel (7).

mTOR plays an important role in kidney disease progression (8). Both mTORC1 and mTORC2 are activated by TGF-β, and this activation is essential for TGF-β-induced collagen production (9); inhibition of mTORC1 delays disease progression in a number of experimental models of renal disease (8). However, inhibition of mTORC1 reduces pancreatic β cell mass and insulin production and may worsen proteinuria and glomerular injury (8). While rapamycin inhibits both mTORC1 and mTORC2, inhibition of mTORC1 may promote longevity, whereas disruption of mTORC2 may induce insulin resistance (5). Thus, the availability of more specific inhibitors of mTORC1 and mTORC2 will further define the roles of mTOR in renal disease.

A recent paper by Rozen-Zvi and colleagues (10) published in the American Journal of Physiology-Renal Physiology extends the observation made by the group (3), and it demonstrates that mTORC1 is activated by TGF-β in human glomerular mesangial cells in a SMAD3-dependent manner; this activation plays a major role in HIF-1α expression and activation under normoxic conditions and contributes to collagen expression (10). Overexpression of HIF-1α overcomes the inhibitory effect of mTORC1 blockade on collagen expression downstream of TGF-β, thus establishing an important link between HIF and fibrogenesis, and it suggests a signaling cascade from TGF-β→Smad3→mTOR→HIF→collagen/fibrogenesis under normoxic conditions. These observations are consistent with recent data suggesting that overexpression of HIF-2α under the control of the kidney epithelial cell-specific promoter (KSP-cadherin) increases kidney fibrosis and cyst formation (11).

The adaptation to hypoxia in cells and tissues leads to the induction of genes that participate in angiogenesis, iron and glucose metabolism, cell proliferation and survival (4). The primary factor mediating this response is HIF-1. HIF-1 consists of a constitutively expressed HIF-1β subunit and an oxygen-regulated HIF-1α subunit (or its paralogs HIF-2α and HIF-3α). The activity of HIF is primarily regulated through posttranslational modifications (hydroxylation, ubiquitination, acetylation, and phosphorylation) of the α-subunit that alter its stability. In normoxia, hydroxylation of two proline residues (by prolyl hydroxylase) and acetylation of a lysine residue at the oxygen-dependent degradation domain of HIF-1α triggers its association with von Hippel-Lindau (pVHL) E3 ligase complex, leading to HIF-1α degradation via ubiquitin-proteasome pathway. In addition, hydroxylation of an asparagine residue in the transactivation domain inhibits the association of HIF-1α with CBP/p300, diminishing HIF-1α-induced transcriptional activity (6). Recent data, however, suggest that TGF-β increases HIF-1α levels under normoxic conditions through effects on protein translation (3). In hypoxia, the HIF-1α subunit becomes stable and interacts with coactivators to regulate target gene expression.

Fe2+ is loosely bound by two histidine residues and one aspartic acid at the active site of the prolyl hydroxylase domains (PHDs). The requirement for Fe2+ for activity of the prolyl hydroxylases is illustrated by the observation that iron chelators and metal ions (such as Co2+, Ni2+, and Mn2+) are able to stabilize HIF-1α, likely by diminishing Fe2+ availability for the enzyme or displacement of Fe2+ from its binding site, respectively (4). Inactivation of the PHD by 2-oxoglutarate analogs can increase the half-life of HIF-1α (4). Besides hypoxia, HIF-1 is regulated by cytokines, growth factors, environmental stimuli, and signaling molecules including MAPK and PI3K (4).

pVHL is ubiquitously expressed and localizes predominately in the cytoplasm. It shuttles between the cytoplasm and nucleus and provides HIF-1α degradation in both compartments (4). pVHL also interacts with other proteins involved in
HIF-1 signaling, and thus may affect HIF-1α activity independent of its effects on HIF-1 stability. In addition to pVHL, a number of proteins affect HIF-1α ubiquitination and stability; for example, the murine double minute 2 (MDM2) E3 ubiquitin ligase leads to ubiquitination of HIF-1α in a p53-dependent manner (4), whereas Jab1, a transcriptional coactivator of c-Jun and JunD, increases HIF-1α levels under hypoxia, likely by decreasing p53 binding to HIF-1α (4).

The contribution by Rozen-Zvi et al. (10) is very important, as it brings new insights into TGF-β signaling and highlights the importance of HIF-1α in renal disease progression. It is unclear at present whether the induction of HIF-1α by TGF-β under normoxic conditions is mediated through effects on pVHL, alterations in p53 expression/action, changes in the activity or expression of the prolyl hydroxylases, or other posttranslational modifications in HIF-1α (such as acetylation and phosphorylation). What is certain, these finding pave the way for some exciting research on the role of HIF-1α in fibrogenesis.

DISCLOSURES
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D.S.-H. drafted manuscript; D.S.-H. edited and reviewed manuscript; D.S.-H. approved final version of manuscript.

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