Resemble and Inhibit: When RLR Meets TGF-β

Shu Zhu,[1,3] Hua-Bing Li,[1,3] and Richard A. Flavell[1,2,*]
[1]Department of Immunobiology, Yale University School of Medicine, New Haven, CT 06520, USA
[2]Howard Hughes Medical Institute, Yale University, New Haven, CT 06520, USA
[3]Co-first authors
*Correspondence: richard.flavell@yale.edu
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In this issue, Xu et al. (2014) show that innate antiviral RIG-I-like receptors (RLR) signaling represses TGF-β-induced growth inhibition, epithelial-mesenchyme transition (EMT), and regulatory T cell (Treg) differentiation via IRF3-mediated Smads function.

TGF-β is a critical regulator of cell proliferation, differentiation, and development. In general, TGF-β signaling is initiated by ligand-induced oligomerization of serine/threonine receptor kinases TGFRI/II. This is followed by the phosphorylation of the cytoplasmic signaling molecules Smad2 and Smad3, which results in their binding to the common signaling transducer Smad4, and translocation to the nucleus. Activated Smads regulate diverse gene expression and biological effects by partnering with transcription factors such as CBP/p300, GRIP1, and AP-1, resulting in cell-state specific modulation of transcription (Flavell et al., 2010; Li and Flavell, 2008) (Figure 1). The recognition of microbial-derived nucleic acids and the activation of the molecular machinery governing the mammalian immune response are paramount to host survival during viral infection. Viral RNA represents a key trigger to the activation and mobilization of a series of pattern recognition receptors (PRRs) such as the Toll-like receptor (TLR) and RLR families. Upon viral dsRNA ligation, RIG-I propagates signal transduction via interactions with MAVS. Such functional aggregates are capable of recruiting key downstream signaling components, resulting in the activation of the mitogen and stress-activated protein kinases (MAPKs), the NF-κB pathway, and interferon regulatory factor 3/7 (IRF3/7), which culminates in the upregulation of protective type I interferons (IFNs) and pro-inflammatory cytokines (Kawai and Akira, 2008; Loo and Gale, 2011) (Figure 1). It is interesting to consider how innate antiviral signaling like RLR or TLR could control TGF-β signaling, allowing cells to respond to a variety of pro-inflammatory stimuli more efficiently. In this issue, Xu et al., (2014) show that RLR-activated IRF3 both prevents association of Smad3 with TGFβR and prevents functional Smad transcriptonal complex formation (Xu et al., 2014) (Figure 1).

Transfection of poly (I:C) RNA (TpIC) can activate IRF3 by C-terminal phosphorylation through RIG-I-like receptors (RLRs) (Fitzgerald et al., 2003; Sharma et al., 2003). Surprisingly, Xu and colleagues found this activation inhibits TGF-β-induced transcription of a Smad3 luciferase reporter, as well as endogenous Samd7 and c-Myc expression (Xu et al., 2014). In addition, they show that inhibition of IRF3 phosphorylation, or knockdown of IRF3, can reverse the RLR-mediated suppression of the TGF-β pathway. They further found that a constitutive active form of IRF3 (IRF3 5SD), but not wild-type unphosphorylated IRF3, mediated a striking repression of TGF-β/Smad3-activated transcription of reporters and endogenous target genes.

The authors then investigated how activated IRF3 inhibits TGF-β/Smad3 pathway. They first excluded the possibility of indirect suppression through IRF3 target genes. They then considered whether activated IRF3 either directly inhibits Smad3 activation in the cytoplasm or interferes with Smad3 binding to its target genes in the nucleus. Interestingly, IRF3 and Smad3 have strikingly similar protein structures, and both are activated via C-terminal phosphorylation, which initiates multimerization, suggesting that IRF3 may compete with Smad3 (Chacko et al., 2004; Qin et al., 2003; Takahasi et al., 2003). Indeed, Xu et al. (2014) found that wild-type IRF3 interacts with Smad4, but once activated by TpIC, the phosphorylated IRF3 associates with non-activated Smad3 and prevents its interaction with TGF-β receptor.

Using an activated form of Smad3 to overcome the inhibition by IRF3 5SD, the authors still observe strong transcription repression by IRF3 5SD, which can be only partially rescued by overexpressed Sam4, p300, or GRIP1. They further show that IRF3 5SD, not wild-type IRF3, disrupts the association between Smad3 and p300 and decreases the TGF-β-induced Smad3 binding at its target gene promoters. IRF3 5SD has higher binding affinity with p300 and thus completes and replaces Smad3 from its transcription complexes. Mutation of IRF 5SD interaction surface restores Smad3 transcription complex but not Smad3 activation. The authors conclude that activated IRF3 associates with Smad3 in the cytoplasm to prevent its activation by TGF-β and competes with activated Smad3 for co-activators in the nucleus to displace Smad3 from its transcription complex.

After elucidating the molecular mechanism of how IRF3 controls TGF-β pathway, Xu et al. (2014) proceeded to test whether IRF3 activation could also affect TGF-β regulated biological processes. Indeed, in the TGF-β-induced epithelia-mesenchymal transition (EMT) in vitro model, knockdown of IRF3, upregulates TGF-β-induced mesenchymal gene expression and morphology changes, while activation of IRF3 by virus infection attenuated EMT responses. Next, they confirmed that silencing IRF3 expression could enhance TGF-β-induced cell proliferation inhibition. TGF-β is well known to control T cell differentiation, especially from naive T cells to FoxP3+ regulatory T cells. The authors

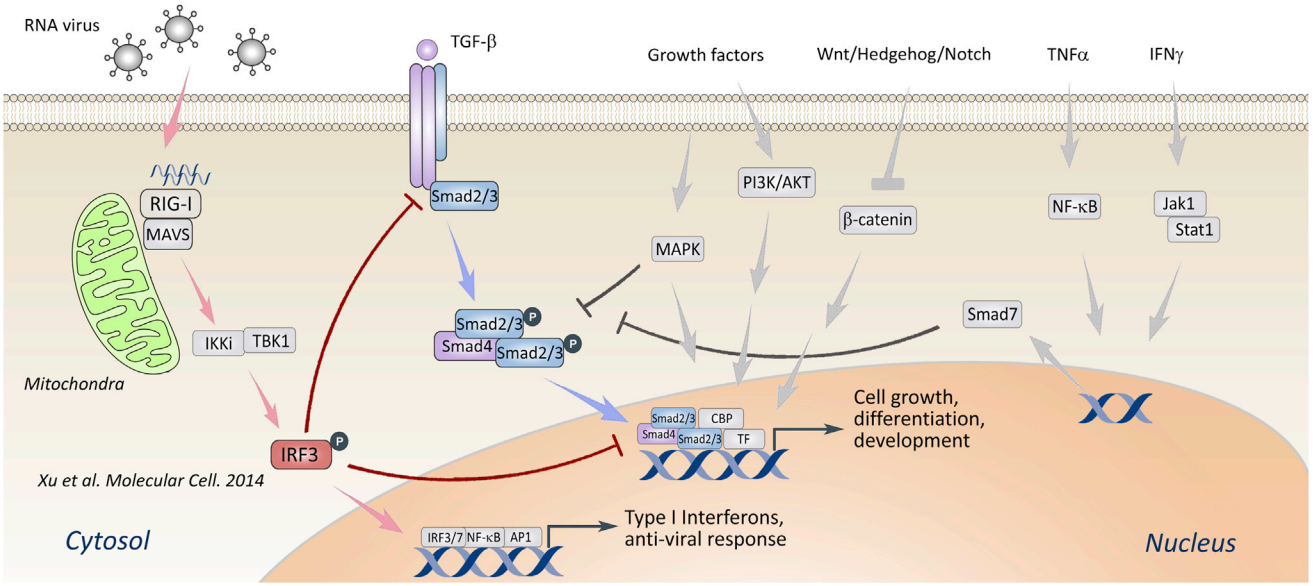

Figure 1. Crosstalk between TGF-β Signaling and Other Pathways

TGF-β signaling: ligand-induced oligomerization of TGFRI/II; phosphorylation of Smad2 and Smad3; binding of Smad2/3 to Smad4; translocation of the complex to the nucleus to activate transcription. RLR signaling: activation by viral dsRNA ligation; interaction with MAVS; activation of downstream NF-κB, MAPK pathways, and IRF3/7; induction of type I interferons. In the Xu et al. (2014) study, RLR-activated IRF3 prevents both association of Smad3 with TGFβR and Smad transcriptonal complex formation. Receptor tyrosine kinases (RTKs), such as EGFR, FGFR, IGFR, and insulin receptor, activated MAPK and PI3K/Akt signaling; Wnt/Hg/Notch signaling induce the translocation of β-catenin to nucleus; inflammatory cytokine TNFα or IFNγ mediated NF-κB or Jak-Stat activation and Smad7 induction; and their crosstalk with TGF-β signaling is also shown for completeness.

indeed observe that IRF3 activation by virus infection potently suppresses FoxP3 expression and thus the generation of iTreg cells in vitro. They also use IRF3 knockout cells to confirm that TGF-β can induce normal iTreg differentiation even in the presence of virus infection. More intriguingly, they observe more FoxP3 Treg cells in Irf3 knockout mice compared to wild-type mice. They further explore the in vivo mouse model of influenza activation of IRF3 and found that TGF-β target genes are significantly attenuated in the lungs of wild-type mice, but not of Irf3−/− mice, which is consistent with their in vitro observations that virus-activated RLR-IRF3 signaling inhibits TGF-β-Smad3-induced gene expression and function.

The unique mechanism of TGF-β activation and the plasticity of TGF-β signaling create a stage for TGF-β to integrate signals from multiple cell types and environmental cues. TGF-β signaling was previously reported to cross-talk with other signaling pathways including MAPK, phosphatidylinositol-3 kinase (PI3K)/Akt, Wnt/Hedgehog/Notch activated β-catenin, and the interferon (IFN)-γ/tumor necrosis factor (TNF)-α induced Stat and NF-κB signaling (Guo and Wang, 2009). The study by Xu and colleagues now adds RLR signaling and IRF3 to the exquisite regulation of TGF-β signaling (Xu et al., 2014) (Figure 1). The proper activation and control of such an important TGF-β signaling pathway depends on its constitutive and extensive communication with other signaling pathways like MAPK, PI3K, NF-κB, and Jak-Stat—and we must now consider RLR antiviral signaling, as well. The integration with these pathways leads to synergistic or antagonistic effects and eventually desirable biological outcomes.

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