iRhom2 and TNF: partners or enemies?

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One-sentence summary: iRhom2 inhibits proinflammatory signaling that stimulates liver fibrosis (Sundaram *et al.*, in 29 October 2019 issue).

Abstract

iRhom2 is an essential cofactor for ADAM17, the metalloprotease that sheds both the proinflammatory cytokine tumor necrosis factor–α (TNF-α) and TNF receptors (TNFRs) from the cell surface. In this issue of *Science Signaling*, Sundaram *et al.* demonstrate a protective role for iRhom2 in promoting ADAM17-mediated shedding of TNFRs in hepatic stellate cells, which reduces TNFR signaling and liver fibrosis in response to injury.

ADAM17 [a disintegrin and metalloproteinase 17, also called tumor necrosis factor–α converting enzyme (TACE)] is a pleiotropic protease that cleaves many membrane-tethered substrates, including the proinflammatory cytokine tumor necrosis factor–α (TNF-α), and its receptors TNFR1 and TNFR2 (*1*). The rhomboid-family pseudoprotease iRhom2 is an essential cofactor for ADAM17 that is highly abundant in immune cells (*2*). iRhom2 is required for multiple aspects of ADAM17 biology, including the trafficking of ADAM17 into the Golgi apparatus, where it undergoes a key proteolytic maturation step; the stimulation of ADAM17 activity on the cell surface (*3*), and the control of ADAM17 proteolytic specificity (*4*). iRhom2 (*Rhbdf2*) deletion protects mice from several inflammatory diseases by blocking ADAM17-mediated TNF-α secretion in inflammatory cells (*2, 5*). The current assumption in the field, therefore, is that iRhom2 is proinflammatory. In this issue (*6*), Sundaram and co-workers demonstrate that iRhom2 plays a protective role in a mouse model of inflammatory liver injury and fibrosis by attenuating TNFR signaling.

Liver fibrosis develops as a result of chronic liver injury and can lead to liver cirrhosis and hepatocellular carcinoma. During liver injury, the release of inflammatory mediators, such as TNF-α and interleukin 1 (IL-1), activate nuclear factor κB (NF-κB) to promote the

proliferation of a normally quiescent population of cells called hepatic stellate cells (HSCs) (*7*). This triggers the proliferation and differentiation of HSCs into pro-fibrotic myofibroblasts, which secrete collagen, thus promoting liver fibrosis (*8*).

The authors of the present study found that cirrhotic human patients or mice subjected to liver fibrosis triggered by bile duct ligation (BDL), both showed increased circulating amounts of TNFR1 and TNFR2 and increased expression of *Rhbdf2* , the gene that encodes iRhom2, in the liver. Interrogating this phenomenon further, ex vivo experiments with murine HSCs showed that iRhom2 was required for the shedding of both TNFR1 and TNFR2. Consistent with this, in mice subjected to BDL, deletion of *Rhbdf2* increased TNF-α signaling in the liver, presumably by blocking the shedding of TNFRs. This, in turn, was associated with HSC proliferation and fibrosis potentially mediated by augmented TNFR1 signaling. Consistent with this hypothesis, treatment of *Rhbdf2* knockout mice with the TNF-α inhibitor Etanercept blocked HSC proliferation and ameliorated liver fibrosis. The authors conclude that following BDL, the increased expression of *Rhbdf2* in liver augmented ADAM17 activity, leading to the shedding of TNFRs, which dampened TNF-α signaling and prevented the proliferation of HSCs and the development of liver fibrosis (Fig. 1). In contrast to the conventional view of iRhom2 as proinflammatory, these findings reveal a scenario in which iRhom2 attenuates signaling through an inflammatory signaling pathway to limit over-activation of tissue repair pathways that could lead to fibrosis.

Because TNFR1 and TNFR2 can exert distinct biological outcomes, an obvious question concerns which TNFR is responsible for promoting the NF-κB–dependent signaling pathways that make HSCs pro-fibrogenic. Although Sundaram and colleagues showed that HSCs release both TNRF1 and TNFR2 in an iRhom2-dependent manner, when stimulated with TNF-α to mimic an inflammatory environment, TNFR2 was preferentially cleaved. This suggests a model whereby TNFR2 is shed as a decoy receptor, preventing TNF-α signaling through TNFR1. This is the same mechanism by which Etanercept, an engineered soluble form of TNFR2, acts. These data are consistent with previous studies that demonstrated a requirement for TNFR1, but not TNFR2, in protection from BDL-triggered fibrosis (*7*). Notably, TNFR1 activation can promote fibrogenesis by inducing the remodeling of the extracellular matrix by stimulating the production of matrix metalloproteinase-9 (MMP9) through activation of the NF-κB pathway (*7*). Therefore, the effects of iRhom2 deletion observed in the current paper can likely be attributed to TNFR1 signaling.

What this work highlights more generally is that iRhoms and ADAM17 can impinge on TNF-α signaling in multiple ways, leading to sophisticated biological scenarios. First, the shedding of soluble TNF-α (sTNF-α), which is proinflammatory and preferentially activates TNFR1, could drive inflammation—the widely reported role of iRhom2. Second, the

shedding of TNFR1 and/or TNFR2 could attenuate TNF-α signaling by blocking signal reception in cells with reduced membrane TNFRs, thus reducing signal flux. Alternatively, soluble TNFRs could act as a diffusible sink for sTNF-α, blocking signaling in a non-cell-autonomous manner. Finally, the failure to produce sTNF-α would result in the accumulation of membrane-tethered TNF-α (mTNF-α), which acts preferentially on TNFR2 (*9*), eliciting distinct biological outcomes from those elicited by TNFR1 activation (*10*). In the latter case, iRhoms, rather than tuning signaling flux, could mediate a form of mode-switching in the nature of TNF-α signaling.

Added to this already complicated scenario is the fact that mammals have a second iRhom paralog, iRhom1, with a distinct but partially overlapping tissue distribution and a shared function with iRhom2 in ADAM17 regulation. Further layered on top of this, iRhom1 and iRhom2 can differentially govern ADAM17 substrate selectivity (*4*), providing another potential basis by which iRhoms can qualify the nature of TNF-α signaling by promoting the cleavage of a specific TNFR preferentially in a given tissue. Finally, iRhoms are regulated at both the transcriptional and posttranscriptional levels, providing a sophisticated manner in which, depending on the stimulus or developmental context, the amounts of an individual iRhom in a given tissue may be modulated to control signaling in a subtle—or indeed fundamental—manner.

A full understanding of how iRhom2 regulates TNF-α signaling has been a challenge for the above noted reasons. Comparing the iRhom2 knockout mice with TNF-α, TNFR1 and TNFR2 mutants may not give sufficient clarity because of the complexity in the iRhom regulation of TNF-α signaling. In the case of the present paper, the deletion of iRhom2 or ADAM17 specifically in HSCs would clarify if, in BDL-induced liver fibrosis, the protective effect of iRhom2 is mediated by its action in HSCs, or whether other relevant cell types that also produce iRhom2 (such as hepatocytes, Kupffer cells, or monocytes), are involved. It would also be interesting to determine whether iRhom2 exerts a protective role in different models of liver fibrosis, and also in other TNF-α–mediated diseases. Nevertheless, this work reinforces the notion that iRhoms, in conjunction with ADAM17, can modulate TNF-α signaling in a highly sophisticated manner that can lead to signal attenuation, potentiation, or mode-switching.

Fig. 1. In response to BDL, iRhom2 promotes the ADAM17-dependent cleavage of TNFR2, creating a soluble form that serves as a decoy for TNF-α, attenuating HSC activation and fibrogenesis.

During liver injury, Kupffer cells or hepatocytes secrete inflammatory cytokines, including TNF-α. The resulting inflammatory environment leads to an increase in the amounts of iRhom2 in HSCs, increasing ADAM17 activity and leading to the cleavage of TNFR2. This allows the latter to act as a soluble decoy to sequester TNF-α, preventing TNFR1 signaling. In the absence of this iRhom2-dependent inhibitory mechanism, TNF-α binds to TNFR1 to promote HSC proliferation and activation, to become myofibroblasts, leading to fibrosis.

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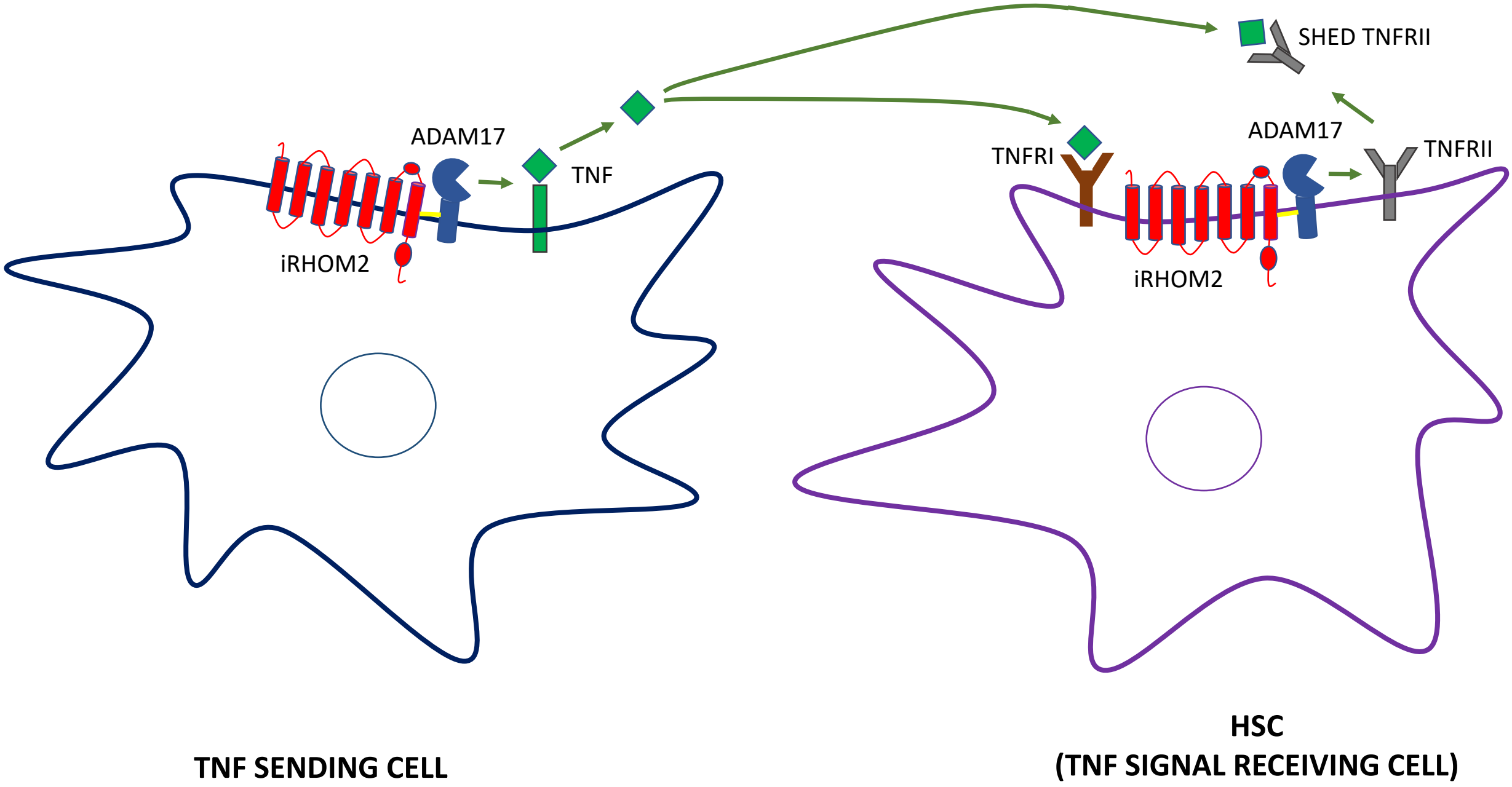
ADAM17
TNF
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ADAM17
TNFRII
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TNF SENDING CELL
HSC
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