

Smad Anchor for Receptor Activation (SARA) in TGF-beta signaling

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1. ABSTRACT

Smad anchor for receptor activation (SARA) is known as Smad cofactor that interacts directly with Smad2/3 and functions to recruit Smad2/3 to the TGF-beta receptor. SARA plays an essential role in TGF-beta-induced Smad2 activation and it can modulate TGF-beta signaling through verify the balances of Smad2 and Smad3. SARA also functions as an anchor for catalytic subunit of protein phosphatase 1 (PP1c) and maybe involved in the dephosphorylation of TGF-beta type I receptor (TbetaR-I) mediated by Smad7. The expression of SARA changes as the development of epithelial to mesenchymal transition (EMT) and fibrosis and it plays a critical role in the maintenance of epithelial cell phenotype. Modulation of SARA may provide a new therapeutic approach to TGF-beta-mediated EMT and fibrosis.

2. INTRODUCTION

Transforming growth factor-beta (TGF-beta) is a multifunctional cytokine which regulates cell proliferation, differentiation, apoptosis, and extracellular matrix accumulation. It is established that TGF-beta plays a key role in the process of epithelial to mesenchymal transition (EMT) and fibrosis (1, 2). TGF-beta signaling initiates when TGF-beta binds to TGF-beta type II receptor (TbetaR-II) and this triggers transphosphorylation of TGF-beta type I receptor TbetaR-I, resulting in activation of the receptor-regulated Smads (R-Smads), Smad2 and Smad3. Once phosphorylated, the R-Smads form a heteromultimeric complex with the common mediator (Co)-Smad (Smad4) and accumulate in the nucleus to regulate transcriptional responses. On the other hands, Smad6 and Smad7 act as inhibitory Smads (I-Smads) by

antagonizing the activity of R-Smads (3, 4). Recent study found a number of additional factors that interact with the Smads and can regulate the signaling outcome, one of them are Smad anchor for receptor activation (SARA). SARA is a large protein of 1323 amino acids containing four important function domains: FYVE motif, Smad binding domain (SBD), PP1c-binding domain (PBD) and C-terminal which is TGF-beta receptor complex-interacting region. SARA is proposed to modulate TGF-beta signaling transduction and it is also related to EMT and fibrosis. This review is focus on the recent developments in the role of SARA in TGF-beta signaling.

3. SARA MODULATE TGF-beta SIGNALING

3.1. The subcellular localization of SARA and TGF-beta signaling

The FYVE motif is a zinc finger-like structure that binds phosphatidylinositol-3-phosphate (PtdIns(3)P) and directs SARA to early endosomal compartments where it can interact with both the TGF-beta receptors and Smads. It was found that Wild-type SARA and the FYVE domain of SARA (FYVE(SARA)) revealed a punctate staining pattern and co-localized with the early endosomal markers, early endosomal antigen-1 (EEA1) and hepatic growth factor-regulated tyrosine kinase substrate (Hrs). The FYVE domain is sufficient and necessary for the early endosomal localization of SARA, through its interaction with PtdIns(3)P. Disruption of the SARA endocytic localization through either expression of a mutant SARA lacking the FYVE domain (SARA/Δ1-664) or inhibition of PtdIns(3)P generation by wortmannin treatment can cause a redistribution of SARA from punctate endocytic structures to the cytosol, and attenuate or prevent TGF-beta-mediated transcriptional responses (5, 6). Ectopic expression of the FYVE domain of SARA also induces the redistribution of wild-type SARA and inhibits TGF-beta/Smad-induced transcriptional responses (5). However, Lu *et al.* found that disruption of the interaction between PtdIns(3)P and the SARA FYVE domain had no effect on TGF-beta-mediated signaling. In addition, they provided evidence that Smad2 can be recruited directly to cell surface TGF-beta receptors (7). SARA endocytic localization may play a role in TGF-beta signaling but the controversy still exists. Further more, given that SARA is mainly localized in early endosomes, whether SARA plays a role in membrane trafficking has been investigated. Yang and his colleague found that SARA played an important functional role downstream of Rab5-regulated endosomal trafficking (8). Like many other cell surface receptors, TGF-beta receptors are internalized upon ligand stimulation. Smad2 or Smad3 nuclear translocation and downstream signaling only occur after endocytic vesicle formation (9, 10). These results raise the possibility that SARA is associated with the activated TGF-beta receptors complex internalization.

3.2. SARA regulates Smad2 and Smad3 activation

The SBD domain of SARA, amino acids 665–750, is sufficient to bind Smad2 or Smad3 *in vitro* and was cocrystallized with the MH2 domain of Smad2 (11, 12). SARA interacts specifically with Smad2 and Smad3, but not Smad1 or Smad4. It preferentially interacts with the

unphosphorylated form of Smad2/3. Phosphorylation of Smad2/3 induces dissociation from SARA and promotes formation of heteromeric complexes with Smad4 by altering the affinity of Smad2 for the SBD. The C-terminal domain of SARA associates with TbetaR-I, bridging the receptor with Smad2. SARA interacts with the TGF-beta receptor independently of Smad2 binding and Smad2 cooperates to enhance association (13). SARA regulates the subcellular distribution of Smad2 and Smad3, and presents these R-Smads for phosphorylation to the activated TGF-beta receptor complex.

It has been shown that SARA plays a significant role in TGF-beta-induced Smad2 activation. SARA regulates the subcellular localization of Smad2 and is required for TGF-beta/Smad2-mediated signaling. Mutations in SARA that cause mislocalization of Smad2 inhibit TGF-beta-dependent transcriptional responses (11, 13). It was also found that SARA expression changed in parallel with the loss of TGF-beta1-mediated Smad2 phosphorylation during the transdifferentiation process of hepatic stellate cells (HSC) induced by TGF-beta1 (14). On the other hands, SARA has recently been shown to play an additional role in TGF-beta signaling beyond the regulation of subcellular localization of Smad2. It can mask the nuclear import signal of Smad2 and prevent the inappropriate nuclear import before activation (15). Furthermore, it is established that the loss of SARA expression results in a reduction in Smad2 expression levels through enhanced Smad2-Smurf2 interaction and Smad2 ubiquitination (16). These results demonstrate that SARA plays an essential role in TGF-beta-induced Smad2 activation.

However, Goto *et al.* reported that in contrast to SARA/Smad2 interaction, SARA/Smad3 interaction was not essential for TGF-beta/Smad3-mediated signaling. In their study, they found that a mutant Smad3 (Smad3NS) that lacked the binding to SARA was phosphorylated by TGF-beta type I receptor at the similar level to that in wild-type Smad3 (Smad3WT). Smad3NS also formed complexes with Smad4 and translocated into the nucleus. Moreover, Smad3NS and Smad3WT equally enhanced TGF-beta-induced transcription (17). It was confirmed that during the process of renal tubular EMT, Smad2 and Smad3 had different activation level (16). In addition that Smad2 and Smad3 have different or even opposite functional effects (18). It suggests that the effects of SARA in TGF-beta signaling are mediated through modification of the balance between Smad2 and Smad3 activation.

3.3. SARA and the negative regulation of TGF-beta signaling

SARA has a PBD domain which recruits catalytic subunit of protein phosphatase 1 PP1c to Smad7- growth arrest and DNA damage protein (GADD34) complex and the later have the function of dephosphorylation of the TbetaR-I. It was reported that Smad7 interacted with GADD34, a regulatory subunit of the protein phosphatase 1 (PP1) holoenzyme, which subsequently recruited PP1c to dephosphorylated TbetaR-I. SARA enhances the recruitment PP1c to the Smad7-GADD34 complex by

controlling the specific subcellular localization of PP1c. SARA appears to serve as an anchor protein to enhance the availability of PP1c to GADD34. Expression of dominant-negative SARA with a mutation in the PBD domain (F678A) results in inhibition of the recruitment of PP1c to the triple complex and hyperphosphorylation of the TbetaR-I and stimulated expression of a TGF-beta signaling target (19, 20). All these study demonstrate that SARA maybe involved in the dephosphorylation of TbetaR-I mediated by Smad7.

4. SARA IN TGF-beta ASSOCIATED EMT AND FIBROSIS

During the last few years, the role of SARA in TGF-beta induced pathological process has attracted more and more scientific attention. Tao *et al.* found that during liver fibrosis developing and recovery phases, SARA was significantly negatively correlated with TGF-beta1 and alpha-SMA expressions. It was negatively correlated with liver fibrosis formation(21). SARA was expressed in quiescent HSC but was lost with *in vitro* activation (14). Recent study demonstrated that SARA decreased while renal tubular epithelial cells were exposed to TGF-beta1, and this decline was both required and sufficient for the induction of renal tubular EMT. Over-expression of SARA in NMuMg cells or HK-2 disrupted TGF-beta1-induced alpha-SMA expression and maintained the epithelial cell phenotype(16). Zhao *et al.* reported an alternative approach for more specifically disrupting Smad-dependent signaling using a peptide aptamer, Trx-SARA, which is comprised a rigid scaffold, the Escherichia coli thioredoxin A protein (Trx), displaying a constrained 56-amino acid SBD from SARA protein. Trx-SARA bound specifically to Smad2 and Smad3 and inhibited both TGF-beta-induced reporter gene expression and EMT in NMuMG murine mammary epithelial cells. The key mode of action of Trx-SARA was to reduce the level of Smad2 and Smad3 in complex with Smad4 after TGF-beta1 stimulation (22). These reports demonstrate that SARA plays an important role in TGF-beta1 induced EMT and fibrosis. These also give the possibility that prevention of EMT and fibrosis by virtue of targeting SARA.

5. SUMMARY AND PERSPECTIVE

SARA is recognized as a FYVE domain protein that interacts directly with Smad2/3 and TGF-beta receptor complex then functions to regulate the Smad2 and Smad3 activation levels. SARA also functions as an anchor for PP1c and be involved in the dephosphorylation of TbetaR-I mediated by Smad7. Thus, it plays a crucial role in downstream TGF-beta signaling. The structure and function of SARA have been well identified, which allowed us to think about the possibility of modulation of TGF-beta signaling by targeting SARA. Additional studies are required to understand the exact role of SARA in the process of TGF-beta dependent gene expression and human disorders such as EMT and fibrosis. It is hoped that better understanding of SARA through intensive investigations will ultimately translate into more effective therapies to TGF-beta induced pathological process.

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Abbreviations: TGF-beta: Transforming growth factor-beta; EMT: epithelial to mesenchymal transition; TbetaR-II:

TGF-beta type II receptor; TbetaR-I: TGF-beta type I receptor; R-Smads: receptor-regulated Smads; I-Smads: inhibitory Smads; SARA: Smad anchor for receptor activation; PI3P: PtdIns(3)P, phosphatidylinositol-3-phosphate; EEA1:early endosomal antigen-1; Hrs: hepatic growth factor-regulated tyrosine kinase substrate; HSC: hepatic stellate cells; PP1c: catalytic subunit of protein phosphatase 1; GADD34: growth arrest and DNA damage protein ; α -SMA: alpha-smooth muscle actin

Key words: Smad anchor for receptor activation, Transforming growth factor-beta, Epithelial to mesenchymal transition, Fibrosis, Review

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