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AIMP1/p43 downregulates TGF-β signaling via stabilization of smurf2

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ABSTRACT

AIMP1 (also known as p43) is a factor associated with a macromolecular aminoacyl-tRNA synthetase (ARS) complex but also plays diverse regulatory roles in various physiological processes. Here, we report that AIMP1 negatively regulates TGF-β signaling via stabilization of Smurf2. TGF-β-dependent phosphorylation and nuclear localization of R-Smads, induction of target genes, and growth arrest were increased in AIMP1-deficient or -suppressed cells. In AIMP1-deficient or suppressed cells, the Smurf2 level was decreased. Various binding assays demonstrated the direction interaction of the C-terminal region of AIMP1 directly with the Smad7-binding region of Smurf2. The association of Smurf2 with Smad7 and its ubiquitination were inhibited by AIMP1, thereby protecting its autocatalytic degradation stimulated by Smad7. Thus, this work suggests the novel activity of AIMP1 as a component of negative feedback loop of TGF-β signaling.

AIMP1/p43 was first identified as one of the three factors associated with aminoacyl-tRNA synthetase (ARS) complex consisting of nine different enzymes and three non-enzymatic factors [1,2]. Within the complex, AIMP1 makes specific interaction with arginyl-tRNA synthetase, being critical for the activity and stability of the bound enzyme [3,4]. As many of the components for the complex are associated with various signal pathways [5,6], AIMP1 is located in different extra- and intracellular locations for the regulation of diverse processes. For instance, AIMP1 is secreted to control inflammation [7,8], angiogenesis [9] and wound healing process [10]. It is also highly enriched in pancreatic cells and works as glucagon-like hormone for glucose homeostasis [11]. Within the cells, AIMP1 was also found to be critical for the suppression of lupus-type autoimmune disease through the interaction with ER-resident chaperone, gp96 [12]. For these diverse activities of AIMP1, AIMP1-deficient mice suffer from multiple symptoms at various tissues and organs, and show high pre- and post-natal lethality.

Since AIMP1 is ubiquitously found in almost all the cellular compartments and different tissues, more functions are expected to be discovered. Within the multi-ARS complex, AIMP1 is in close proximity with another factor, AIMP2/p38 [13,14] and both are structurally interdependent and critical for the stability of other components [4]. AIMP2/p38 was previously found to mediate TGF-β signaling that is required for lung cell differentiation during development. Thus, AIMP2-deficiency cripples TGF-β signaling and AIMP2$^{-/-}$ mice were neo-natal lethal due to overproliferation of epithelial alveolar cells in lung [15]. Considering the close proximity of AIMP1 and AIMP2 within the multi-ARS complex [16], it is conceivable that AIMP1 may somehow play a role in TGF-β signal pathway as well. Here we investigated the functional significance and working mechanism of AIMP1 in the regulation of TGF-β signal pathway.

Materials and methods

Antibodies and immunoblotting. Anti-c-Myc (9E10), anti-HA, anti-TGF-beta receptor II, anti-p27, anti-p15, anti-PAI-1, anti-ERK1/2, and anti-YY1 antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-Smad2/3, anti-phospho-Smad2, and anti-phospho-Smad3 antibodies were from Cell Signaling Technology (Cell Signaling, MA). Anti-Smurf2 antibody was from Upstate. Anti-AIMP1 antibodies was from Imagene (Seoul, Korea). Proteins were extracted from the indicated cells with RIPA solution containing protease inhibitor cocktail, separated by SDS–PAGE and blotted on PDVF membrane.

Abbreviations: ARS, aminoacyl-tRNA synthetase; AIMP, ARS-interacting multifunctional protein; siRNA, small interfering RNA.

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Measurement of cell proliferation. We treated TGF-β for the indicated times with 2 ng/ml or indicating conc. for 12 h to MEF cell, which is isolated from 12.5d embryo, under the serum free condition. Before 4 h of harvest, we added 1 μCi/ml of [^{3}H]thymidine and measured the incorporated isotope through liquid scintillation counter (Wallac).

Co-immunoprecipitation. For immunoprecipitation, cells were solubilized with RIPA lysis buffer. Extracted proteins were mixed with anti-AIMP1, anti-FLAG, or anti-Myc antibody-precoupled protein A/G and subjected to immunoblot analysis.

RT-PCR. Total RNAs were isolated using Trizol following the protocol of the manufacturer (Invitrogen). One microgram of the isolated RNA was used in reverse transcription reaction using M-MLV reverse transcriptase. After the reaction, the mixture was diluted threefold with DW used in PCR with gene specific primers: PAI-1-R, 5′-AGATGTCTCCAGCCCTCACCT-3′; PAI-1-F, 5′-TTGCTTGAC CGTGCTCCGGAA-3′; GAPDH-R, 5′-CCATGA CGAACATGGGGGCAT-3′; GAPDH-F, 5′-TTTGG TCGTATTGGGCGCCTG-3′.

In vitro binding assay. For GST-pull down, we incubated with A549 cell lysates with bead-conjugated GST or GST-AIMP1 proteins for 1 h. After collection of bead-associated proteins, we performed immunoblot analysis.

Luciferase reporter assays. Mv1Lu cells were transfected with p3TPlux (0.1 μg) and the indicated amounts of plasmid encoding AIMP1. The total amount of DNA per well was kept constant by adding empty vector. To suppress the expression of AIMP1, AIMP1 siRNA (0–60 pmol, Invitrogen), or a control siRNA (invitrogen) was introduced to Mv1Lu cells with p3TPlux (0.2 μg). TGF-β1 was treated at 80 pM for 6 h. Luciferase activity was measured using Luciferase Assay System (Promega).

Immunofluorescence microscopy. A549 cells transfected with control siRNA or AIMP1 siRNA were fixed using methanol, blocked and incubated with anti-Smurf2 antibody. Cells were stained with Alexa fluor-conjugated secondary antibodies (Molecular Probes, Eugene, OR). Nucleus was stained with DAPI.

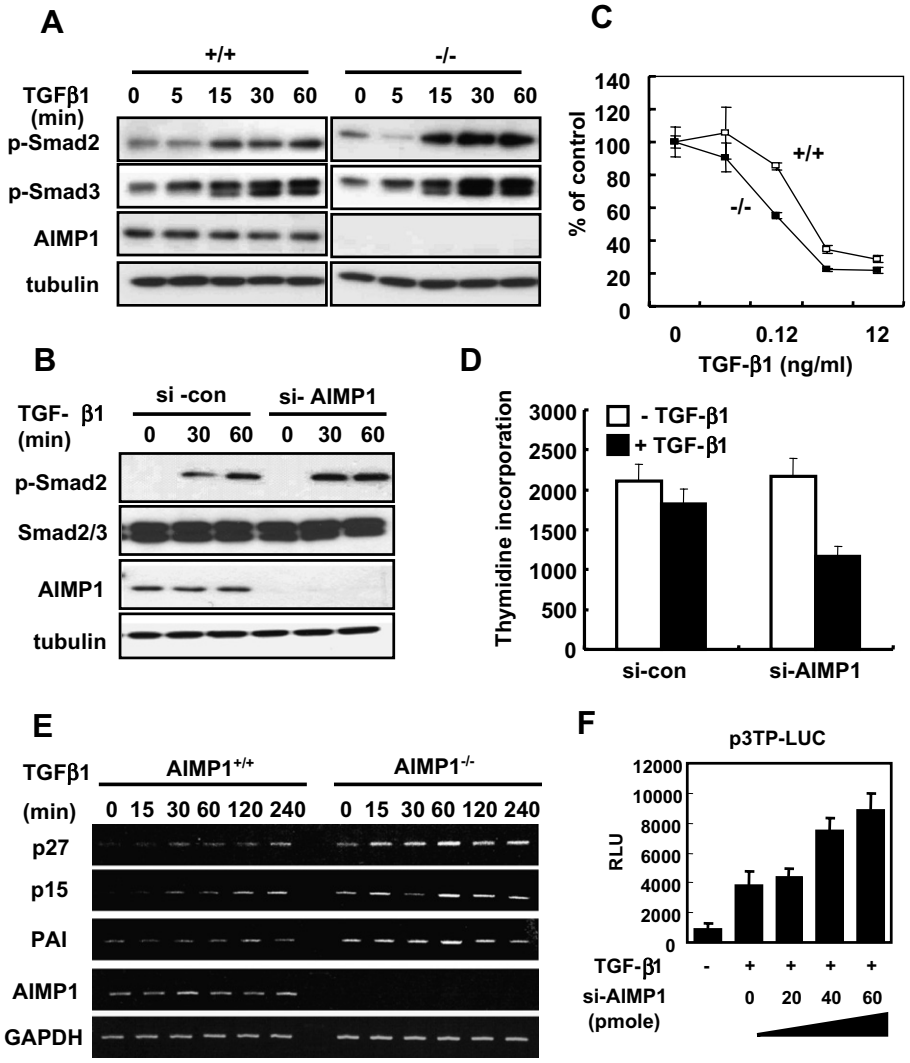

Fig. 1. Suppression of AIMP1 enhances TGF-β signaling. (A) 12.5d AIMP1$^{+/+}$ and AIMP1$^{-/-}$ MEF cells were treated with TGF-β1 (2 ng/ml) and phosphorylation of Smad2 and Smad3 was determined by their specific antibodies at time interval. Tubulin was used for loading control. (B) Suppression of AIMP1 using specific si-AIMP1 promotes phosphorylation of Smad2 in response to TGF-β in A549 cells, without alternation of total Smad expression. Tubulin was used as loading control. (C) AIMP1$^{+/+}$ and AIMP1$^{-/-}$ MEFs were treated with indicted concentration of TGF-β1 for 24 h. [^{3}H]Thymidine was then added and incubated for 4 h before of harvest and incorporated thymidine was quantified using liquid scintillation counter. The radioactivity of the control cells without TGF-b treatment was taken as 100%. The experiments were repeated three times. (D) A549 cells were transfected with si-control or si-AIMP1, and then treated with TGF-β1 (2 ng/ml) for 24 h, and cell proliferation was measured by thymidine incorporation as above. (E) Expression of p27, p15, and PAI was compared between AIMP1$^{+/+}$ and AIMP1$^{-/-}$ MEFs by RT-PCR at the indicated time interval. (F) To monitor the effect of AIMP1 knockdown on target gene expression, 3TP-lux plasmid is transfected with AIMP1 siRNA in Mv1Lu cells for 36 h and measured the luciferase activity of using luminometer. Data shown is the representative of three independent experiments.