Smad7-modified alleles by various gene-targeting strategies

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Signalling of the transforming growth factor-β (TGF-β) family is tightly regulated by various mechanisms including negative feedback by Inhibitory Smad- and Mad-related proteins (Smads) (I-Smads: Smad6 and Smad7). Smad6 preferentially inhibits bone morphogenetic protein (BMP) signalling, whereas Smad7 suppresses both TGF-β and BMP signalling. To elucidate the roles of Smad7 in murine development and in TGF-β signalling, several Smad7-deficient mouse strains have been generated. Tojo et al. (Smad7-deficient mice show growth retardation with reduced viability. J. Biochem. 2012;151:621–631.) demonstrated that Smad7 null mutation caused perinatal lethality on a C57BL/6 background. However, the Smad7-deficient mice on an ICR background survived to adulthood, but showing growth retardation. Unexpectedly, phosphorylation levels of Smad2 and Smad3 were slightly reduced in murine embryonic fibroblast (MEF) cells isolated from Smad7-deficient embryos compared with wild-type MEF cells. Together with other Smad7-mutant mouse strains, these mutant mice provide useful tools to understand important roles of Smad7 in the development of murine embryos and diseases.

Keywords: cardiac dysfunction/Cre–loxP/gene-targeting/growth retardation.

Abbreviations: ALK, activin receptor-like kinase; BMP, bone morphogenetic protein; JNK, c-Jun N-terminal kinase; MEF, murine embryonic fibroblast; Smad, Sma- and Mad-related protein; TGF, transforming growth factor.

Smad6 and Smad7 were discovered as inhibitory Smad and Mad-related proteins (Smads) (I-Smads) and known to play important roles in the negative regulation of transforming growth factor-β (TGF-β) family signalling (1–6). Aberrant expression of Smad7 has been reported in patients with scleroderma and inflammatory bowel disease (7, 8). To examine in vivo functions of Smad7, several Smad7-mutant mice have been reported.

In 2006, Li et al. (9) generated Smad7-mutant (Smad7ΔExI) mice by replacing a part of the exon 1, which contains the ATG start codon, with a phosphoglycerate kinase-neomycin selection cassette. Unexpectedly, truncated protein was produced from the targeted allele, leading to a partial loss of Smad7 function (i.e., hypomorphic mutation). The truncated Smad7 protein lacks N-terminal domain (N-domain), but consists of a part of the linker domain and intact Mad homology 2 (MH2) domain. Hanyu et al. (5) have previously reported that the N-domain of Smad7 physically interacts with the MH2 domain of Smad7 and enhances the inhibitory activity of the MH2 domain through facilitating interaction with TGF-β receptors. Consistent with the findings, the truncated Smad7 protein, which can be produced in the Smad7ΔExI-mutant mice, showed a reduced inhibitory effect when over-expressed in Mv1Lu cells against p3TP-lux reporter activity induced by constitutively active ALK5, whereas wild-type Smad7 completely blocked the reporter activity. Smad7ΔExI-mutant mice were viable and fertile on a 129Sv/CD-1 mixed background and significantly smaller on a CD-1 background compared with wild-type mice. Mutant B cells also exhibited an increase in phosphorylated Smad2 compared with B cells isolated from wild-type mice (9). In agreement with the increase in TGF-β signalling, several changes (increased IgA class switch, enhanced spontaneous apoptosis and reduced proliferation after lipopolysaccharide stimulation) were observed in the mutant B cells. Recent report also showed that Smad7ΔExI-mutant mice on a CD-1 background developed more severe renal dysfunction caused by the angiotensin II infusion than the wild-type mice, which was associated with profound renal inflammation and progressive renal fibrosis (10).

In 2009, Chen et al. (11) generated Smad7-mutant mice. The exon 4, which encodes the MH2 domain of Smad7, was deleted (Smad7ΔMH2) by Cre-mediated recombination after the generation of Smad7 ‘floxed’ allele (Smad7loxP). Since the exon 4 is deleted, the Smad7ΔMH2 allele may produce truncated Smad7 protein (containing N-domain and linker domain). Compared with the previous Smad7ΔExI-mutant mice (9), Smad7ΔMH2/loxP-mutant mice had more severe phenotypes. The majority of Smad7ΔMH2/loxP-mutant mice died in utero due to multiple cardiac defects, including ventricular septal defect, non-compaction and outflow tract malformation. A small percentage of the Smad7ΔMH2/loxP-mutant mice survived to adulthood, but had impaired cardiac functions and severe arrhythmia. These results indicate important roles of Smad7 in the heart development. Furthermore, increased Smad2/3 phosphorylation levels were observed in the endocardial cushion tissue and endocardium of Smad7ΔMH2/loxP-mutant mice. Interestingly, Smad1/5/8 phosphorylation level was not altered in
Smad7fl/fl mice, Smad7 gene expression was not detectable in wild-type allele (Smad7F/C1 mice) using the Cre/loxP system. Genetic fragment containing the promoter region and exon 1 was flanked by two loxP sites to completely abolish the Smad7 expression. Smad7fl/+ mice were crossed with the mice that express the Cre-recombinase early in embryogenesis to generate Smad7 heterozygous (Smad7Δ/+ mice) (13). Smad7Δ/+ mice were intercrossed to produce Smad7 homozygous mutant (Smad7Δ/Δ) mice. However, the Smad7Δ/Δ mice were not recovered after birth, suggesting that deletion of Smad7 leads to embryonic lethality. To investigate the role of Smad7 in T cells, Smad7fl/+ mice were crossed with CD4-Cre transgenic mice (14). In the T cells isolated from CD4-Cre-Smad7fl/+ mice, Smad7 gene expression was not detectable, and TGF-β-induced Smad2 phosphorylation was augmented compared with wild-type cells. These results confirm that Smad7 negatively regulates the TGF-β signalling in T cells.

Recently, Tojo et al. (15) generated Smad7 ‘floxed’ allele (Smad7F/+), in which the MH2 domain and poly-A signal sequence were flanked by loxP sites. By crossing the Smad7F/+ mice with Ayu-1 Cre transgenic mice (16), Smad7Δ/Δ mice were produced on an ICR or C57BL/6 background, and then intercrossed to generate Smad7Δ/Δ ‘total-null’ mice. Neither Smad7 mRNA nor Smad7 protein was detected in the murine embryonic fibroblast (MEF) cells isolated from the Smad7Δ/Δ mouse. Smad7Δ/Δ mice on a C57BL/6 background died within a few days after birth, whereas Smad7Δ/Δ mice on an ICR background developed to adulthood, but they were significantly smaller than wild-type mice. These results suggest that loss of Smad7 may be compensated for its functions by other molecules (e.g. Smad6) on different genetic background. Although Smad7 is expected to negatively regulate TGF-β signalling, there were only modest differences in Smad2/3 and Smad1/5/8 phosphorylation between wild-type and Smad7-deficient MEF cells. However, loss of Smad7 influenced the mitogen-activated protein kinase activation (p38 and JNK1 phosphorylation) in response to TGF-β signalling in T cells. In the previous studies using other Smad7-mutant mice, TGF-β signalling was augmented in cardiac tissue, B cells or T cells (9, 11, 12). Therefore, it might be possible that Smad7 is not actively involved in the regulation of TGF-β signalling in MEF cells. To date, four Smad7-mutant alleles, including ‘null’ and ‘hypomorphic’ alleles, were reported (Fig. 1). These mutant mice were generated by different targeting strategies, exhibiting various phenotypes depending on the

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<tr>
<th>Targeted alleles</th>
<th>Expressed Smad7 protein</th>
<th>Phenotypes</th>
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<tbody>
<tr>
<td>Wild-type</td>
<td>N-domain Linker MH2</td>
<td>Mutant mice were viable, but significantly smaller on the outbred CD-1 background. Mutant B cells showed an increase of phosphorylated Smad2 and exhibited increased Ig class switch to IgA. Mutant mice developed severe renal dysfunction by angiotensin II infusion. (Li et al, 2006; Liu et al, 2013)</td>
</tr>
<tr>
<td>Smad7ΔEx1/ΔEx1</td>
<td>Linker MH2</td>
<td>The majority of mutant mice died in utero due to multiple cardiac defects. Smad2/3 phosphorylation was elevated in atrioventricular cushion of the mutant hearts. (Chen et al, 2009)</td>
</tr>
<tr>
<td>Smad7ΔMH2/ΔMH2</td>
<td>N-domain Linker</td>
<td>Deletion of Smad7 leads to embryonic lethality on a C57BL/6 background. T cell-specific deletion of Smad7 showed increased Smad2 phosphorylation and led to immunosuppression. (Kleiter et al, 2010)</td>
</tr>
<tr>
<td>Smad7Δ/Δ</td>
<td>no Smad7 protein detected</td>
<td>Mutant mice on a C57BL/6 background died within a few days after birth, whereas mutant mice on an ICR background developed to adulthood, but they were significantly smaller than wild-type mice. Smad2/3 phosphorylation was slightly decreased in the mutant MEF cells. (Tojo et al, 2012)</td>
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Fig. 1 Comparison between genetically modified mouse strains for the Smad7 gene. Four mutant Smad7 mouse strains have been reported to date. Due to the different targeting strategies and genetic backgrounds, the phenotypes are variable.

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nature of the mutations and the genetic backgrounds. The genetically modified biological resources should provide useful tools to uncover developmental and/or tissue-specific functions of Smad7 in mammals.

**Conflict of Interest**
None declared.

**References**


