Received: 19 November 2018 | Revised: 19 December 2018 | Accepted: 23 December 2018

DOI: 10.1111/cas.13922

REVIEW ARTICLE

WILEY Cancer Science

# Phosphorylation status at Smad3 linker region modulates transforming growth factor-β-induced epithelial-mesenchymal transition and cancer progression

Akira Ooshima[1] | Jinah Park[1] | Seong-Jin Kim[1,2]

[1]Precision Medicine Research Center, Advanced Institutes of Convergence Technology, Suwon, Korea

[2]Graduate School of Convergence Science and Technology, Seoul National University, Suwon, Korea

**Correspondence**
Akira Ooshima, Precision Medicine Research Center, Advanced Institutes of Convergence Technology, Suwon, Korea.
Email: aooshima@snu.ac.kr

Seong-Jin Kim, Graduate School of Convergence Science and Technology, Seoul National University, Suwon, Korea
Email: jasonsjkim@snu.ac.kr

**Funding information**
This work was supported by a Korea Health Industry Development Institute (KHIDI) grant (HI14C2640) funded by the Korea government.

Smad3, a major transcription factor in transforming growth factor-β (TGF-β) signaling, plays critical roles in both tumor-suppressive and pro-oncogenic functions. Upon TGF-β stimulation, the C-terminal tail of Smad3 undergoes phosphorylation that is essential for canonical TGF-β signaling. The Smad3 linker region contains serine/threonine phosphorylation sites and can be phosphorylated by intracellular kinases, such as the MAPK family, cyclin-dependent kinase (CDK) family and glycogen synthase kinase-3β (GSK-3β). Previous reports based on cell culture studies by us and others showed that mutation of Smad3 linker phosphorylation sites dramatically intensifies TGF-β responses as well as growth-inhibitory function and epithelial-mesenchymal transition (EMT), suggesting that Smad3 linker phosphorylation suppresses TGF-β transcriptional activities. However, recent discoveries of Smad3-interacting molecules that preferentially bind phosphorylated Smad3 linker serine/threonine residues have shown a multitude of signal transductions that either enhance or suppress TGF-β responses associated with Smad3 turnover or cancer progression. This review aims at providing new insight into the perplexing mechanisms of TGF-β signaling affected by Smad3 linker phosphorylation and further attempts to gain insight into elimination and protection of TGF-β-mediated oncogenic and growth-suppressive signals, respectively.

**KEYWORDS**
breast cancer, EMT, metastasis, Smad3, TGF-β

## 1 | INTRODUCTION

Transforming growth factor-β (TGF-β)/Smad3 signaling plays critical roles in a wide variety of biological processes and carries out multiple functions such as growth arrest, apoptosis, differentiation, and epithelial-mesenchymal transition (EMT).[1-4] Studies using mouse embryonic fibroblasts with targeted disruption of either Smad2 or Smad3 showed that Smad3 plays significant roles in TGF-β-mediated transcriptional responses.[5,6] A critical part of Smad3 in TGF-β-induced EMT also stems from observations that Smad3 knockout mice abrogate EMT in response to epithelial injuries in vivo or by exposure to TGF-β in cell culture.[7,8] TGF-β has been reported to be suppressive in the early development of cancer, while acquiring cancer-promoting propensities in the later stages. TGF-β is abundantly expressed in most cancer tissues, and high levels of TGF-β often bode for adverse clinical outcomes.[1,2] Biological activities of TGF-β initiate its binding to a heteromeric complex of two types of transmembrane receptors consisting of type I and type II

receptors. TGF-β occupancy induces an association between the type I and types II receptors, resulting in phosphorylation of the type I receptor by the constitutively active type II receptor. The phosphorylated type I receptor then triggers activation of Smad2 and Smad3 by phosphorylating their C-terminal serine residues. Activated Smad2 and Smad3 form heteromeric complexes with Smad4 and cofactors, and translocate into the nucleus, participating in the expression of target genes. Smad2 and Smad3 contain a proline-rich region, termed the linker region, which connects the Mad homology 1 (MH1) domain that binds DNA, and the MH2 domain that interacts with the type I receptor, Smad4, and cofactors.[3,4] The linker region is divergent in sequence and function between Smad2 and Smad3. The Smad3 linker region contains phosphorylation sites at threonine 179 (T179), serine 204 (S204), serine 208 (S208) and serine 213 (Ser213) for proline-directed kinases, such as ERK, JNK, p38 MAPK, and CDK family (Figure 1A).[9-16] TGF-β signals, therefore, are mediated through a wide array of intracellular pathways through cross-talks, including a predominant Smad pathway as well as non-Smad, non-canonical pathways.[18-20] TGF-β signaling is tightly controlled and plays critical roles in embryogenesis and organogenesis together with the maintenance of cellular homeostasis. Perturbation of TGF-β signaling, therefore, causes life-threatening disorders such as cancer, organ fibrosis, and congenital malformations.[4,21]

## 2 | SMAD3 PHOSPHORYLATION AT SERINE/THREONINE RESIDUES IN THE C-TERMINAL AND THE LINKER REGIONS

Smad3 linker serine/threonine phosphorylation sites except S213 are subject to phosphorylation in response to TGF-β (Figure 1B). Although phosphorylation of Smad3 C-terminal SXS motifs by the TGF-β receptor is required for linker phosphorylation, the receptor itself does not phosphorylate the linker region of Smad3.[12] Intracellular kinases such as the CDK family, p38 and other kinases except ERK are now known to be responsible for TGF-β-induced linker phosphorylation.[10,11,22] Even in the absence of TGF-β, most cancer cells show certain levels of constitutive phosphorylation in the Smad3 linker region because they often maintain high activities of the CDK family, MAPK family, and other kinases that are usually activated by oncogenic mutation of Ras or receptors of growth factors such as epidermal growth factor (EGF) and fibroblast growth factor (FGF).

To see the effect of Smad3 linker phosphorylation on TGF-β responses and Smad3 transcriptional activity, a high-grade human breast cancer cell line (MCF-10CA1a.cl1, hereafter known as CA1a) in culture was first treated with a pan-CDK inhibitor, flavopiridol. TGF-β transcriptional activity was significantly upregulated by treatment with flavopiridol concomitant with reduction of Smad3 linker phosphorylation at T179, S204, and S208, suggesting that Smad3

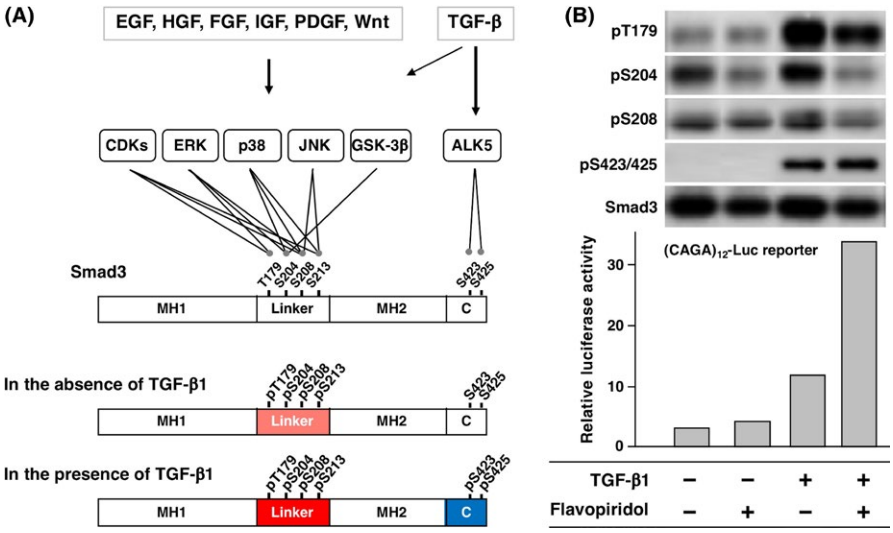


**FIGURE 1** Smad3 linker phosphorylation by intracellular kinases attenuates transforming growth factor-β (TGF-β)-induced Smad3 transcriptional activity. A, Serine/threonine residues at Smad3 C-terminal and linker region are depicted together with responsible kinases, including CDK family, ERK, JNK, p38 MAPK, and glycogen synthase kinase-3β (GSK-3β). TGF-β induces Smad3 phosphorylation at three sites in the linker region, threonine 179 (T179), serine 204 (S204), and serine 208 (S208), along with the two C-terminal residues, serine 423 (S423) and serine 425 (S425). Even in the absence of TGF-β, the linker region in cancer cells often becomes phosphorylated at a certain level (pink) by intracellular kinases that are constitutively activated, but less weakly as compared to that in the presence of TGF-β (red). B, In the breast cancer cell line (MCF-10CA1a.cl1, hereafter CA1a), treatment with a pan-cyclin-dependent kinase (CDK) inhibitor flavopiridol lowered levels of TGF-β-induced Smad3 linker phosphorylation at T179, S204, and S208. Although CDK family does not directly phosphorylate S204, it can provide a priming site (pS208) for phosphorylation by GSK-3. GSK-3 is responsible for phosphorylation at S204. Flavopiridol, therefore, can reduce the level of GSK-3β-catalyzed phosphorylation at S204 together with that of CDK-catalyzed phosphorylation at T179, S208, and S213 (not shown). Level of Smad3 linker phosphorylation is inversely correlated with TGF-β-induced Smad3 transcriptional activity. EGF, epidermal growth factor; FGF, fibroblast growth factor; HGF, hepatocyte growth factor; IGF, insulin-like growth factor; PDGF, platelet-derived growth factor

linker phosphorylation negatively regulates Smad3 transcriptional activity as determined by $(CAGA)_{12}$-luciferase ($[CAGA]_{12}$-Luc) reporter assay (Figure 1B).[23] Likewise, inhibition of Smad3 linker phosphorylation by a specific phosphatase (SCP1) acting on the Smad2/3 linker region has resulted in upregulation of TGF-β signaling.[24,25]

## 3 | MUTATION AT SMAD3 LINKER PHOSPHORYLATION SITES GREATLY ENHANCES TGF-β SIGNALING

It was initially reported that Ras-induced Smad2/3 linker phosphorylation inhibits TGF-β-induced responses by interrupting nuclear translocation of Smad2/3 and subsequent Smad-dependent transcription.[9] Direct evidence that Smad3 linker phosphorylation suppresses TGF-β responses comes from studies using a Smad3 mutant at linker phosphorylation sites.[9,10,12,13,23] We have adopted recombinant adenoviruses constitutively expressing wild-type Smad3 (Ad-Smad3) or its mutant at the linker region (Ad-EPSM), the C-terminal (Ad-3SA) or both the linker and the C-terminal (Ad-EPSM/3SA), in which all serine and threonine phosphorylation sites are replaced with alanine (A) and valine (V), respectively (Figure 2A).[23,26] When Ad-EPSM was infected with the breast cancer cell line (CA1a), TGF-β-induced Smad3 transcriptional activity as evaluated by $(CAGA)_{12}$-Luc reporter assay was greatly enhanced in Ad-EPSM-infected cells as compared to Ad-Smad3-infected counterparts. Infection with either Ad-3SA or Ad-EPSM/3SA, in contrast, completely abolished Smad3 transcriptional activity, confirming that Smad3 linker phosphorylation downregulates TGF-β-induced Smad3 transcriptional responses (Figure 2B).[23]

Although both Ad-Smad3- and Ad-EPSM-infected cells undergo TGF-β-induced EMT as characterized by spindle-shaped phenotype and loss of polarity, Ad-EPSM-infected cells show a far more prominent EMT as compared to Ad-Smad3-infected counterparts. Even in the absence of TGF-β, Ad-EPSP-infected cells showed phenotypic changes of EMT at the edge of a cell colony, suggesting that cells become highly sensitive to TGF-β so that they are capable of responding to even a trace amount of endogenous TGF-β (Figure 2C). Use of Ad-EPSM, therefore, showed that phosphorylation of the Smad3 linker region suppresses TGF-β signaling leading to EMT (Figure 3).

Remarkable changes induced by TGF-β other than EMT in Ad-EPSM-infected cells are noted by prominent apoptosis and growth arrest along with the reduction of stem cell population in breast cancer cell lines (CA1a and 4T1).[23] The magnitude of these changes is much

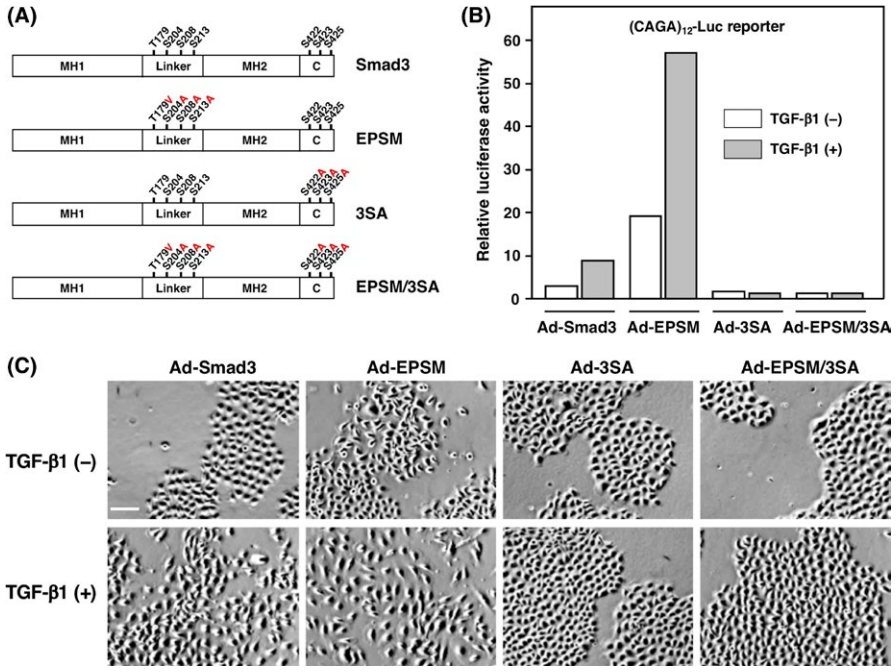


**FIGURE 2** Mutation of Smad3 linker phosphorylation sites markedly enhances transforming growth factor-β (TGF-β)-induced epithelial-mesenchymal transition (EMT) and Smad3 transcriptional activity, whereas mutation of C-terminal phosphorylation sites blocks all TGF-β responses. A, Schematic depiction of Smad3 wild-type (Smad3), Smad3 mutants at the linker phosphorylation sites (EPSM), C-terminal phosphorylation sites (3SA), and both C-terminal and linker phosphorylation sites (EPSM/3SA). For the construction of Smad3 mutants, serine and threonine residues are replaced with non-phosphorylatable amino acids, valine (V) and alanine (A), respectively. B, Adenoviruses constitutively expressing Smad3 (Ad-Smad3), EPSM (Ad-EPSM), 3SA (Ad-3SA), or EPSM/3SA (Ad-EPSM/3SA) are infected into the breast cancer cell line (CA1a). TGF-β-induced Smad3 transcriptional activity is upregulated by infection of Ad-EPSM as compared to that of Ad-Smad3, suggesting that Smad3 linker phosphorylation attenuates TGF-β responses. C, Phase-contrast pictures of the breast cancer cell line (CA1a) infected with Ad-Smad3, Ad-EPSM, Ad-3SA, and Ad-EPSM/3SA in the presence (10 ng/mL) or absence of TGF-β1. Although Ad-Smad3- and Ad-EPSM-infected breast cancer cells undergo TGF-β-induced EMT, Ad-EPSM-infected cells show a far more prominent TGF-β-induced EMT as compared to Ad-Smad3-infected counterparts. Even in the absence of TGF-β1, Ad-EPSM-infected cells show mild features of EMT. Both Ad-3SA and Ad-EPSM/3SA infection completely abrogated TGF-β-induced EMT. Scale bar, 50 μm

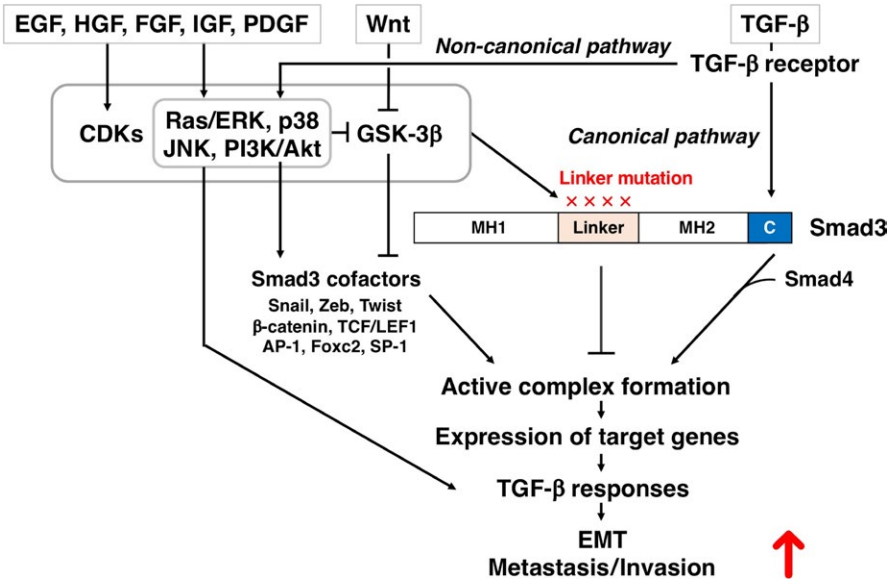


**FIGURE 3** Smad3 linker phosphorylation negatively regulates transforming growth factor-β (TGF-β)/Smad3 signaling. Blockade of Smad3 linker phosphorylation (shown as ××××) by Smad3 mutant at linker phosphorylation sites (EPSM) markedly intensifies TGF-β-induced epithelial-mesenchymal transition (EMT) as well as Smad3 transcriptional activation, indicating that Smad3 linker phosphorylation suppresses TGF-β responses (shown by a hammer-headed line under the word Linker). Although the Smad3 pathway plays a principal role in TGF-β-induced EMT, cooperation of the non-Smad3 signaling pathways is indispensable for target gene expression. Transcriptional cofactors that form complexes with Smad3 are activated through Wnt/glycogen synthase kinase-3β (GSK-3β), Ras/ERK, p38, JNK, and PI3K/Akt pathways. EMT-promoting transcriptional cofactors include Snail, Zeb, Twist, β-catenin, AP-1, Foxc2, TCF, and SP1. They are required for suppression of epithelial markers, such as E-cadherin and upregulation of mesenchymal markers, such as vimentin (not shown). Arrow- and hammer-headed lines represent activation and inhibition, respectively, in signaling pathways. Upward arrow-headed line (red) indicates augmentation of TGF-induced EMT, metastasis, and invasion by mutation of Smad3 linker phosphorylation sites; CDK, cyclin-dependent kinase; EGF, epidermal growth factor; FGF, fibroblast growth factor; HGF, hepatocyte growth factor; IGF, insulin-like growth factor; PDGF, platelet-derived growth factor

higher in Ad-EPSM-infected cells than in Ad-Smad3-infected controls. We have reported in a mouse orthotopic xenograft model with breast cancer cell lines (CA1a and 4T1) that Ad-EPSM-infected cells generate the smallest primary tumor while showing the highest frequency of lung metastasis. Conversely, Ad-3SA-infected cells create the largest primary tumor without metastatic invasion into the lung, suggesting that as a result of enhanced TGF-β signaling induced by blockade of Smad3 linker phosphorylation, Ad-EPSM-infected cells promote TGF-β-induced EMT along with apoptosis, growth arrest and even a loss of stem cell population.[23] Augmentation of TGF-β responses by Ad-EPSM was also confirmed in our separate studies.[26,27] All of these observations indicate that phosphorylation of Smad3 linker sites causes inhibitory effects on TGF-β responses, including EMT and cytostatic functions, creating part of a negative feedback loop in Smad3 transcription (Figure 3).

## 4 | LINKER REGION-PHOSPHORYLATED SMAD3 RECRUITS FUNCTIONAL MOLECULES THAT EITHER SUPPRESS TGF-β SIGNALING OR PROMOTE TGF-β-INDUCED CANCER PROGRESSION

Smad3 linker phosphorylation has critical functions, such as temporary augmentation of TGF-β function, and promotion of cancer metastasis in addition to inhibition of TGF-β responses. It was first reported that functional molecules, such as peptidyl-prolyl cis-trans isomerase NIMA-interacting 1 (Pin1) and neural precursor cell expressed developmentally downregulated gene 4-like (Nedd4L), preferentially bind to the phosphorylated Smad3 linker region and elicit either positive or negative signals in response to TGF-β in a cell context-dependent way (Figure 4). Both TGF-β and bone morphogenetic protein (BMP) receptor kinases in principle activate Smads by C-terminal phosphorylation to form active transcriptional complexes. The linker region of Smads in the complexes become phosphorylated first by nuclear CDK8/9 and later by GSK-3β. In the case of BMP signaling, Yes-associated protein (YAP), an effector of Hippo organ size control pathway, is recruited to the phosphorylated Smad1 linker sites and supports BMP/Smad1-dependent transcription as a linker coactivator. Smad3 linker phosphorylated sites are likewise recognized by Pin1, resulting in activation of TGF-β/Smad3-induced responses. It has now become evident that following activation of Smad3 signaling by Pin1, a ubiquitin ligase Nedd4L recognizes phosphorylated linker sites through its WW domains, resulting in poly-ubiquitination and proteasome-mediated degradation. Smad1 is similarly degraded but specifically by Smurf1. Interaction of Pin1 and Nedd4L with phosphorylated linker sites acts as a sequential on/off switch for activation first and degradation later in the TGF-β/Smad3 pathway, respectively (Figure 4A).[28-30]

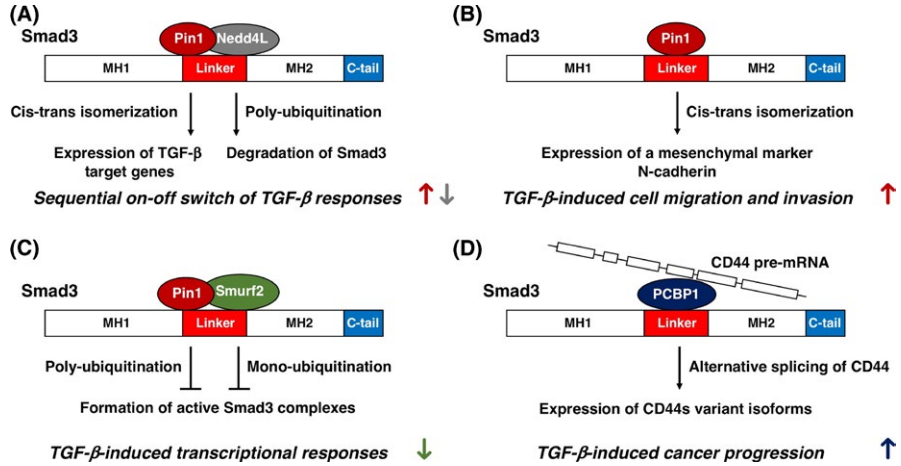


**FIGURE 4** Regulation of transforming growth factor-β (TGF-β) signaling by molecules associated with phosphorylated serine/threonine residues at the linker region of Smad3. A, Phosphorylation of Smad3 linker serine/threonine residues by cyclin-dependent kinase (CDK)8/9 first provides binding sites for peptidyl-prolyl cis-trans isomerase NIMA-interacting 1 (Pin1). Subsequent phosphorylation by glycogen synthase kinase-3β adds binding sites for neural precursor cell expressed developmentally downregulated gene 4-like (Nedd4L). Pin1 induces peak TGF-β responses in a cell context-dependent way. Nedd4L enhances proteasomal degradation of Smad3 by poly-ubiquitination. TGF-β responses, therefore, are first activated and later suppressed by a sequential on-off switch. B, Pin1 recognizes phosphorylated serine/threonine-proline motifs, catalyzes peptidyl-prolyl cis-trans isomerization of Smad3, and helps promote cell motility and migration concomitantly with N-cadherin expression in a TGF-β-dependent way. C, Pin1 facilitates Smad3 interaction with Smad ubiquitin regulatory factor 2 (Smurf2). Smurf2 induces poly-ubiquitination and leads to proteasomal degradation of Smad3. Smurf2 has also been shown to induce mono-ubiquitination at the MH2 domain of Smad3. The mono-ubiquitination blocks access of Smad3 to the TGF-β type I receptor and inhibits the formation of active Smad3-cofactor complexes required for TGF-β/Smad3 transcriptional activation. D, Poly(rC)-binding protein 1 (PCBP1) activated by epidermal growth factor (EGF) or its family member TGF-α binds to the phosphorylated Smad3 linker region. The Smad3-PCBP1 complex induces alternate exon exclusion on CD44 pre-mRNA in response to TGF-β, generating cancer-promoting CD44s isoforms. Alternative splicing by Smad3 is now known to occur in a wide variety of gene products other than CD44 protein. Arrow- and hammer-headed lines indicate acceleration and suppression, respectively. Colored upward and downward arrow-headed lines represent augmentation and suppression, respectively

Pin1 has also been shown to promote cell migration and invasion in a human PC3 prostate cancer cell line with concurrent expression of N-cadherin. Knockdown of Pin1 suppresses TGF-β-induced cell migration and invasion while causing no effect on growth arrest (Figure 4B). Although EGF phosphorylates Smad3 linker serine/threonine residues, Pin1 is unable to recognize the phosphorylated linker sites of Smad3, indicating that C-terminal phosphorylation by TGF-β is essential for interaction with Pin1.[31] In a separate report, Pin1 has been shown to promote interaction of linker-phosphorylated Smad3 with a cofactor Olig1 and accelerate TGF-β-induced cell motility but neither growth arrest nor EMT.[32]

Transforming growth factor-β signaling is known to be regulated by the ubiquitin-proteasome system. It has previously been reported that Pin1 binds preferentially to phosphorylated Smad2 and 3 at the linker region and enhances their interaction with Smad ubiquitination regulatory factor 2 (Smurf2), leading to proteasomal degradation of Smad2 and 3 by poly-ubiquitination (Figure 4C).[33] Generation of mice harboring targeted disruption of a Smurf2 allele has now shown that Smurf2 selectively binds to the phosphorylated Smad3 linker region and induces mono-ubiquitination instead of poly-ubiquitination in vivo under physiological conditions. Mono-ubiquitination by Smurf2 has been confirmed to inhibit the formation of homo- or hetero-trimeric Smad3 complexes required for TGF-β/Smad3 transcriptional activation. It became further apparent that mono-ubiquitination by Smurf2 interrupts access of Smad3 to the TGF-β type I receptor for C-terminal phosphorylation. Smurf2 seems highly likely to play a significant role in the suppression of TGF-β signaling by Smad3 linker phosphorylation (Figure 4C).[34]

Recently, an additional mechanism has been reported that Smad3 linker phosphorylation promotes TGF-β-mediated cancer progression by inducing alternative splicing of a stem cell marker CD44. Phosphorylated linker sites of Smad3 are first recognized by an RNA-binding protein, poly(rC)-binding protein 1 (PCBP1), and both molecules form an active complex. On the cue from TGF-β and EGF stimulation, two proteins in the complex start to interact with the variable exon region of CD44 pre-mRNA and interrupt spliceosome assembly, favorably generating the mesenchymal isoform CD44s over the epithelial isoform CD44E (Figure 4D). Alternative splicing induced by Smad3 linker phosphorylation is now extended to occur in a large number of genes required for TGF-β-induced EMT and metastasis. Upon binding of either Pin1, Nedd4L, Smurf2, or PCBP1 to phosphorylated linker sites of Smad3, phosphorylation at the T179 residue has been determined to play a pivotal role in eliciting biological activities.[35]

## 5 | MECHANISTIC INSIGHT INTO EMT AND CANCER METASTASIS

Cancer progression and acquisition of malignant properties are often associated with accumulation of genetic mutations that often lead to

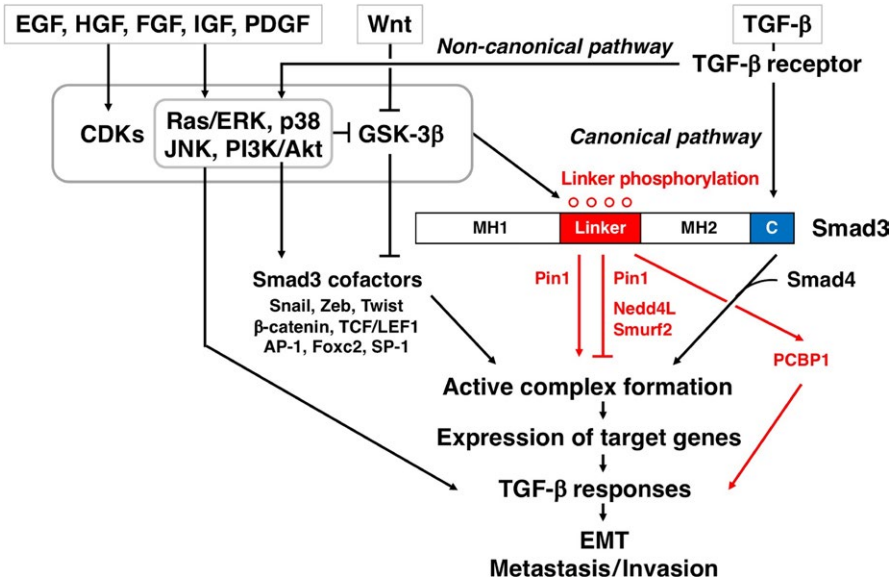


**FIGURE 5** Smad3 linker phosphorylation modulates transforming growth factor-β (TGF-β)-induced epithelial-mesenchymal transition (EMT), metastasis, and cancer progression mediated through Smad3- and non-Smad3 signaling pathways. Phosphorylated Smad3 linker serine/threonine residues (shown by oooo) provide binding sites for peptidyl-prolyl cis-trans isomerase NIMA-interacting 1 (Pin1), neural precursor cell expressed developmentally downregulated gene 4-like (Nedd4L), Smad ubiquitin regulatory factor 2 (Smurf2), and poly(rC)-binding protein 1 (PCBP1). Active complexes thus formed regulate transforming growth factor-β (TGF-β) signaling and cancer progression. Pin1 enhances TGF-β responses transiently or promotes cell migration. Nedd4L induces Smad3 proteasomal degradation by poly-ubiquitination. Pin1 further facilitates the binding of Smurf2 and Smad3, leading to poly-ubiquitination-mediated degradation. Smurf2 also suppresses formation of the Smad3 complex with Smad4 by mono-ubiquitination of Smad3, leading to suppression of TGF-β signaling. The PCBP1-Smad3 complex promotes alternative splicing and produces varied gene products, including CD44s, which favor cancer progression and metastasis. Arrow- and hammer-headed lines indicate activation and inhibition, respectively, in signaling networks. CDK, cyclin-dependent kinase; EGF, epidermal growth factor; FGF, fibroblast growth factor; GSK-3β, glycogen synthase kinase-3β; HGF, hepatocyte growth factor; IGF, insulin-like growth factor; PDGF, platelet-derived growth factor

high activities of intracellular kinases such as oncogenic Ras, CDK family, and MAPK family.[1,2,36] Growing evidence has shown that both canonical and non-Smad pathways collaborate and participate in TGF-β signaling to regulate a wide array of downstream cellular responses. Inhibition of any one of EGF/Ras/ERK or p38 signaling branches has been shown to abrogate TGF-β-induced EMT, suggesting the involvement of multiple signaling pathways and their cross-talks (Figure 5).[37-39]

It has previously been reported that active Ras is required for induction of TGF-β-induced EMT in keratinocytes.[40] In pancreatic cancer cells, transfection of oncogenic Ras has markedly enhanced expression of Snail1, a critical mediator for EMT. However, the synergism between TGF-β and active Ras is confined to the expression of Snail1 but not any of other TGF-β target genes such as Smad7.[41] From our previous work, it can be speculated that oncogenic Ras might raise the level of Smad3 linker phosphorylation by activating the MEK/ERK pathway, probably resulting in suppression of TGF-β-induced EMT. Activation of Ras, however, actually enhances TGF-β-induced EMT by upregulating Snail1 expression as stated above. It is, therefore, reasonable to assume that cancer cells might be well equipped with prometastatic machinery that counteracts or overrides the inhibitory effect of Smad3 linker phosphorylation. Supporting part of this notion, Pin1 and PCBP1 are now known to mediate the malignant conversion of cancer by accelerating motility and conferring tumor-promoting properties, respectively.[31,34]

Signals from the Wnt pathway promote activation of Snail in addition to Zeb, β-catenin and other EMT-promoting transcription factors. It is of note that GSK-3β works as a nodal protein in non-Smad and Wnt pathways. Activation of Wnt and Ras/ERK-PI3K/AKT pathways leads to inhibition of GSK-3β, thereby stabilizing Snail and β-catenin.[39,42] Moreover, inhibition of GSK-3β most likely reduces the phosphorylation level of the Smad3 linker region, leading to sensitization of TGF-β responses, including EMT.

## 6 | CONCLUDING REMARKS AND PERSPECTIVES

Smad3 signaling is mediated through multiple cross-talks between the canonical- and non-canonical pathways in a cell context-dependent way. A previous report by others showed that inhibition of CDK4/6 by a highly specific inhibitor induces TGF-β-induced EMT and enhances invasiveness of pancreatic cell lines, while significantly suppressing cell growth.[43] In melanoma cells, suppression of Smurf2 by RNA interference was reported to increase the cytotoxic effects

of mitogen-activated protein kinase (MAPK) inhibition by sensitizing the TGF-β signaling.[44] These findings underscore the significance of suppressive impacts on TGF-β responses by Smad3 linker phosphorylation.

Abrogation of Smad3 linker phosphorylation, therefore, acts as a double-edged sword, either enhancing TGF-β-induced EMT or increasing cytotoxicity of anticancer reagents. Although Smad3 linker phosphorylation adds another layer of complexity to TGF-β responses, it may help find a particular target to block EMT or promote cell death. A deeper understanding and clearer insight into EMT and cell growth mediated through TGF-β signaling may provide better combinatorial cancer chemotherapies in the future.

## ACKNOWLEDGMENTS

We are deeply indebted to Drs Kazuhito Naka and Eunjin Bae for their contribution in conducting research.

## CONFLICTS OF INTEREST

Authors declare no conflicts of interest for this article.

## ORCID

*Akira Ooshima* https://orcid.org/0000-0002-2637-1219

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**How to cite this article:** Ooshima A, Park J, Kim S-J. Phosphorylation status at Smad3 linker region modulates transforming growth factor-β-induced epithelial-mesenchymal transition and cancer progression. *Cancer Sci*. 2019;110:481–488. https://doi.org/10.1111/cas.13922