Transcriptional cofactors Ski and SnoN are major regulators of the TGF-β/Smad signaling pathway in health and disease

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The transforming growth factor-β (TGF-β) family plays major pleiotropic roles by regulating many physiological processes in development and tissue homeostasis. The TGF-β signaling pathway outcome relies on the control of the spatial and temporal expression of >500 genes, which depend on the functions of the Smad protein along with those of diverse modulators of this signaling pathway, such as transcriptional factors and cofactors. Ski (Sloan-Kettering Institute) and SnoN (Ski novel) are Smad-interacting proteins that negatively regulate the TGF-β signaling pathway by disrupting the formation of R-Smad/Smad4 complexes, as well as by inhibiting Smad association with the p300/CBP coactivators. The Ski and SnoN transcriptional cofactors recruit diverse corepressors and histone deacetylases to repress gene transcription. The TGF-β/Smad pathway and coregulators Ski and SnoN clearly regulate each other through several positive and negative feedback mechanisms. Thus, these cross-regulatory processes finely modify the TGF-β signaling outcome as they control the magnitude and duration of the TGF-β signals. As a result, any alteration in these regulatory mechanisms may lead to disease development. Therefore, the design of targeted therapies to exert tight control of the levels of negative regulators of the TGF-β pathway, such as Ski and SnoN, is critical to restore cell homeostasis under the specific pathological conditions in which these cofactors are deregulated, such as fibrosis and cancer.

INTRODUCTION

The transforming growth factor-beta (TGF-β) superfamily comprises a large group of related growth factors, such as TGF-βs, activins, inhibins, bone morphogenetic proteins (BMPs), myostatin, nodal, lefty, anti-Müllerian hormone/Müllerian inhibiting substance (AMH/MIS), and growth and differentiation factors (GDFs). This family of cytokines and differentiation factors has major pleiotropic activities in development and tissue homeostasis and exerts altered functions in diverse pathologies. Upon ligand binding to type II and type I receptors, the active ligand-heterotetrameric receptor complex signals through downstream transcriptional factors named Smads (Fig. 1). The Smad protein family is divided into three groups: R-Smad (Smad1, Smad2, Smad3, Smad5, and Smad8), co-Smad (Smad4), and I-Smad (Smad6 and Smad7). Receptor-regulated Smads (R-Smad) have an SSXS motif in their C-terminal region that is phosphorylated by type I receptors; R-Smad phosphorylation (p-R-Smad) allows their association with Smad4. After translocation of the p-R-Smad/Smad4 heterotrimeric complex into the nucleus, Smads associate with other transcriptional factors and coregulators to regulate the expression of specific target genes. TGF-β signaling regulates the transcription of >500 genes, which may contain one or more Smad binding elements (SBEs) in their promoter region (Fig. 1). Multiple Smad-interacting transcription factors may cooperate with Smads to modulate-specific target gene expression, depending on the cellular type, in both physiological and pathological conditions.

The luminescence-based mammalian interactome mapping (LUMIER) dataset comprises >100 proteins associated with the TGF-β receptor complex or with Smad proteins; although, the biological relevance of each interaction remains to be elucidated. The LUMIER dataset is available to support the investigation of TGF-β signaling networks in different cell types. Furthermore, the activity and stability of Smad proteins are regulated by diverse post-translational modifications (PTMs), such as phosphorylation, acetylation, ubiquitination, sumoylation, and parylation; thus, the variety of Smad isoforms generated by PTMs increases the complexity of TGF-β signal transduction networks. The TGF-β signaling pathway is also modulated by multiple mechanisms, including the actions of diverse positive and negative regulators, which orchestrate temporal and spatial actions of TGF-β signaling. The TGF-β signals may also regulate the expression of their own regulators, generating negative feedback loops to control the magnitude and duration of TGF-β signaling. Among the negative regulators of TGF-β signals are TGIF, Ski, and SnoN transcriptional cofactors, which can inhibit Smad transcriptional activity to exert differential regulation of TGF-β-targeted gene expression (Fig. 1).

SKI AND SNON ARE MAJOR NEGATIVE REGULATORS OF THE TGF-β SIGNALING PATHWAY

The homologous Ski and SnoN proteins belong to the Ski protein family; they are mainly localized in the nucleus and may induce cell transformation when overexpressed. The Ski protein family includes several members, such as Ski, SnoN, SnoN2, Snol, SnoA, CORL-1, DACH1/Dach1, DACH2/Dach2, Fussel-15 (SKOR1; LBXCOR1), Fussel-18 (SKOR2; CORL-2), DAF-5, Dachshund, and...
Transcriptional cofactors Ski and SnoN are major regulators of gene expression through various mechanisms. These proteins act as transcriptional corepressors of Smad proteins and exhibit vital biological functions by controlling the TGF-β/Smad signaling pathway during embryogenesis and tissue homeostasis.

**Fig. 1** Regulation of the TGF-β/Smad signaling pathway by Ski and SnoN. In the absence of TGF-β, the Ski and SnoN proteins interact with Smad4 to inhibit the expression of TGF-β target genes, such as Smad7 and Skil, by recruiting other corepressors and HDACs to their promoters. Then, TGF-β induces the phosphorylation of Smad2/3 proteins to form the R-Smad/Smad4 complex, which associates with specific transcription factors and cofactors to modulate the expression of its target genes.

Ski and SnoN act as Smad corepressors by indirect binding to the consensus sequence 5′-GTCTAGAC-3′ (known as SBE) through their association with Smad proteins. According to the current model, Ski and SnoN block TGF-β signaling by forming an inhibitory complex with Smad proteins on SBEs of the TGF-β target gene promoters, and such complexes recruit histone deacetylases (HDACs) and additional corepressors to inhibit gene expression (Fig. 1).

**Fig. 2** Regulation of Ski and SnoN expression by different mechanisms. Several signaling pathways and factors induce Ski and Skil gene expression, whereas, the TGF-β signaling pathway enhances or inhibits Skil gene expression via Smad2/4 or Smad3/4, respectively. Moreover, some miRNAs control the mRNA levels of these genes. The Ski and SnoN protein levels are also regulated by different signals.

Transcriptional gene silencing is also mediated by the interactions of Ski with other factors, such as PRMT5, HDAC3, Rb, MeCP2, and Mad, as well as with the thyroid hormone receptor β (TRβ).

Likewise, the SnoN protein also functions as a transcriptional coregulator; it associates with Smads to inhibit gene expression, including the silencing of its own gene Skil (Fig. 1). To date, a few genes have been identified as targets of the SnoN/Smad complex, including those encoding Smad7, SnoN, FGF8, GSC, MIXL1, and AFP. Other genes that are regulated by SnoN encode miR720, miR274A, miR1274B, ADAM12, PLSCR1, Ccd1, and pS2.

Ski function as a coactivator became evident after the demonstration of its association with the nuclear factor 1 (NF1) family of transcription factors. Ski favors β-catenin signaling to promote the expression of Mifit and Nr-CAM genes in melanoma. Ski also favors myogenin gene expression and myogenesis by forming a complex with specific transcription factors, such as MyoD, as well as with Ski1 and Eya3. SnoN can also act as a coactivator for Smads and for other transcriptional factors, but this ability depends on specific target genes.

The viral form v-Ski was identified as a gene inserted into the avian Sloan-Kettering retroviruses (SKVs) genome. Later, a homologous proto-oncogene was identified in chicken and denoted c-Ski (cellular gene). Ski homologs have been detected in several vertebrate species from fish to humans. The human Ski gene is localized at chromosome 1 and generates at least two transcripts that encode a 728-amino acid (aa) protein. The cellular role of Ski protein has been studied extensively; however, some factors or signals have been identified as potential regulators of Ski expression, such as serum-response factor (SRF), peroxisome proliferator-activated receptor δ (PPARδ), and RA signaling (Fig. 2).

**Structure and regulation of Ski and Skil genes**

The viral form v-Ski was identified as a gene inserted into the avian Sloan-Kettering retroviruses (SKVs) genome. Later, a homologous proto-oncogene was identified in chicken and denoted c-Ski (cellular gene). Ski homologs have been detected in several vertebrate species from fish to humans. The human Ski gene is localized at chromosome 1 and generates at least two transcripts that encode a 728-amino acid (aa) protein. The cellular role of Ski protein has been studied extensively; however, some factors or signals have been identified as potential regulators of Ski expression, such as serum-response factor (SRF), peroxisome proliferator-activated receptor δ (PPARδ), and RA signaling (Fig. 2). Recently, it has been reported that the Ski gene is hypermethylated and silenced in human primary lung cancer tissues, supporting Ski function as a tumor suppressor. Ski mRNA levels are also modulated by different miRNAs, such as miR-21, miR-29a, miR-155, and miR-127-3p.
Human and mouse Skil genes are localized on chromosome 3 and encode for all SnoN isoforms. There is a limited characterization of the 5′ end regulatory sequences of both human and mouse Skil genes, including promoters and enhancers, due to the complex organization and modulation of these genes (Fig. 3). Both human and mouse Skil genes harbor two TGF-β-responsive elements (TRE) localized ~2 kb upstream of the translation starting site (ATG). TRE1 is localized in the core promoter and formed by three SBE sequences: SBE1, SBE2, and SBE3, whereas TRE2 is localized at exon 1 and contains the SBE4 and a Smad inhibitory element (SIE); all the SBEs are recognized by the Smad2/Smad4 complex to induce Skil gene expression, whereas SIE is recognized by the Smad3/Smad4 complex, which inhibits Skil gene expression (Fig. 3).73,108 Regarding the 5′-UTR of Skil mRNA, E1 (exon 1 with 170 bp) and a fragment of E2 (exon 2 with 633 bp) encode for the 5′-UTR of Skil mRNA and are separated by a large intron (1.9 kb).73

The TGF-β signaling pathway controls the expression of the Skil gene through diverse mechanisms: under basal conditions, the SnoN/Ski/Smad4 complex binds and represses the Skil gene promoter; then, after a short period of TGF-β stimulation, SnoN and Ski protein degradation occurs via the proteasome; whereas, the activated Smad2/Smad4 complex replaces the SnoN/Ski complex on the Skil gene promoter and promotes its induction. Both SnoN mRNA and protein levels are increased after TGF-β treatment for ~1.5 h. Subsequently, a negative feedback loop is generated, in which the SnoN protein binds the Skil gene promoter to inhibit its own expression.73 Interestingly, human Skil gene also contains a super-enhancer localized ~3.6 kb upstream from the ATG (+1) and ~1 kb upstream from the TRE1; the coordinated regulation of Skil super-enhancer by Oct4/Sox2/Nanog (OSN) transcriptional factors and Ski promoter by activated Smads seems to be essential for the maintenance of stem cell pluripotency (Figs. 2 and 3).74

TGF-β/Smad, activin, nodal, BMP7, HGF/CREB, and prolactin/STAT5 are the best-known signals that regulate Skil gene expression at the transcriptional level (Fig. 2).73,109–113 Other less studied pathways also appear to regulate Skil gene expression, such stimulation of the PI3K/Akt pathway with arsenic trioxide (As2O3), and inhibition of Smad3 phosphorylation with endocrine disrupting chemicals (EDC), such as 4-nonylphenol (NP) and bisphenol A (BPA).114,115 Furthermore, Skil expression is also regulated by miRNAs: miR-17 and miR-23a are potential regulators of the 3′-UTR of human Skil mRNA (Fig. 2);116,117 interestingly, miR-17 is a member of the miR-17-19-130 superfamily targeting tumor suppressors, such as the TGF-β, PI3K, and p53 pathways.

Molecular structure and PTMs of the Ski and SnoN proteins
The human Ski protein has 728 aa and three main domains (Fig. 4). The N-terminal Ski-dachshund homology domain (Ski-DHD) comprises a region of residues 91–192 with a folding pattern of mixed α/β structures; this domain has lost the ability to bind directly to DNA, but instead behaves as an interacting domain that binds R-Smads and other transcriptional regulators, such as NCoR and Sin3.51,56,58,71,118–121 The Ski protein also has a SAND-like domain (residues 219–312) that includes an extended I-loop motif that is responsible for Smad4 binding (Fig. 4).122 Two other N-terminal regions in the Ski protein include a proline-enriched stretch (between residues 61 and 89) and the transformation domain that is localized in the first 304 residues; this last domain is responsible for some Ski activities, such as cell transformation and transcriptional gene repression.49,50,123,124 The Ski- and SnoN-DHD crystal structure reveals that the transformation domain is important for recruiting multiple partners (Fig. 4).121,122 The Ski protein differs at its C-terminus sequence, where it has a helical domain that forms a coiled-coil (CC) region constituting two motifs: a region of five tandem repeat (TR) motifs with 25 residues in each, and a leucine zipper (LZ) motif of six heptad repeats; both structural motifs are implicated in the formation of Ski homodimers and Ski/SnoN heterodimers.124,127,128

SnoN is a protein of 684 aa with four conserved domains in its N-terminus: minimal transformation domain, SAND-like domain, DHD domain, and Smad-binding domain. The C-terminus of the SnoN protein is variable and contains a domain for SnoN homodimerization and Ski/SnoN heterodimerization (Fig. 4).122,127,129–131 Four SnoN isoforms have been identified: SnoN (non-alu-containing), SnoN2, SnoA (alu-containing), and Sno

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**Fig. 3** Regulatory regions of human and mouse Skil genes. The Skil gene promoter includes two TGF-β-responsive elements (TRE) containing several Smad binding elements (SBEs and SIE). The human Skil gene superenhancer region contains binding sites for Oct4, Sox2, and Nanog proteins. The mouse Skil gene promoter includes the SBEs and SIE, as well as a binding site for STAT5 that is controlled by prolactin. The positions of response elements and transcription start site (TSS) are indicated with respect to the ATG (+1).
All SnoN isoforms result from alternative splicing and almost all are ubiquitously expressed, except SnoI, which is mainly expressed in skeletal muscle. SnoN2 differs from SnoN by a deletion of 138 nucleotides; whereas, SnoI and SnoA encode truncated proteins of 399 and 415 aa, respectively. Although both SnoI and SnoA proteins lack the dimerization domain, only SnoA has a unique domain in its C-terminal region.91,132,133 The four SnoN isoforms have been identified in Drosophila and humans; whereas, SnoN and SnoN2 are the only isoforms expressed in mice.133–135 Nevertheless, the localization, regulation, and function of all SnoN isoforms have not yet been completely revealed.

Ski was initially described as a phosphorylated protein in serine residues.32 Growth factors and hormones, such as hepatocyte growth factor (HGF), insulin-like growth factor-1 (IGF-1), and insulin promote Ski protein degradation by inducing its phosphorylation at the Thr458 residue by AKT kinase.136 The Ski protein residue Ser515 is also phosphorylated, but the kinase involved has not been identified; however, mutation of Ser515 does not affect Ski activity as a transcriptional corepressor.137 Ski also interacts with Aurora A kinase (Aurka) through its C-terminal region; Aurka phosphorylates Ski at Ser326 and Ser383 to decrease Ski stability (Fig. 4).138,139 By contrast, TAK1 kinase promotes SnoN protein phosphorylation at residues Ser115, Ser117, and Thr119 after TGF-β treatment, which causes polyubiquitination and posterior degradation of the SnoN protein (Fig. 4).140

Ski and SnoN protein polyubiquitination causes their degradation via the ubiquitin-proteasome system (UPS). To date, three main E3 ubiquitin-ligases interacting with SnoN have been identified: the anaphase-promoting complex (APC), Smad-ubiquitination-related-factor 2 (Smurf2), and Arkadia all catalyze SnoN polyubiquitination (Figs. 4 and 5).141–143 The Smurf2 WW domains interact with the Smad2 PY motif, and this Smurf2/Smad2 complex recruits the SnoN protein.141 SnoN polyubiquitination by Smurf2 requires two segments of the SnoN protein:
of Smad7, Ski, and SnoN negative regulators. Arkadia interacts with Ski (at residues 211–490) and SnoN (at residues 263–355) and catalyzes their polyubiquitination in a manner dependent on the recruitment of activated Smad2/Smad3.\(^{144}\) Recently, it was shown that SmurF2 induces Smad4 monoubiquitination to inhibit its function as mediator of TGF-β signaling, whereas formation of the Ski/Smad4 complex blocks Smad4 monoubiquitination.\(^{151}\)

SnoN protein sumoylation requires the participation of Ubc9 (SUMO E2-conjugating enzyme) and PIAS1 or TIF1γ (SUMO E3 ligases); the addition of SUMO occurs at the K50 and K383 residues of the SnoN protein (Fig. 4).\(^{152,153}\) This SnoN sumoylation is important for SnoN-mediated repression of key genes, such as myogenin, in the C2C12 myoblast cell line and for the negative regulation of myogenesis.\(^{52,153}\) Sumoylation of SnoN does not appear to affect its function as a transcriptional co-regulator of Smad since the sumoylated SnoN protein can still inhibit the TGF-β-dependent repression of E-cadherin during the epithelial-mesenchymal transition (EMT) process. TGF-β signaling inhibits SnoN sumoylation by decreasing PIAS1 levels during EMT of the mammary epithelial NMuMG cell line.\(^{54}\) By contrast, sumoylation of SnoN1 by TIF1γ is necessary during acinar morphogenesis of NMuMG cells to inhibit EMT induced by TGF-β.\(^{155}\) TIF1γ catalyzes only the sumoylation of SnoN1, but not of SnoN2. Regarding Ski, there is lack of evidence about its sumoylation, although Ski interacts with the Ubc9 enzyme to enhance its activity. Thus, Ubc9 promotes MDM2 mono-sumoylation, which in turn promotes p53 protein polyubiquitination and degradation.\(^{156}\)

**REGULATION OF SUBCELLULAR LOCALIZATION AND STABILITY OF THE SKI AND SNO N PROTEINS**

The Ski and SnoN proteins can be localized in both the cytoplasm and nucleus; intriguingly, Ski and SnoN appear to have an exclusive nuclear localization in most cancer cell lines. Ski and SnoN have non-homologous nuclear localization signals (NLS); the SnoN NLS is localized at its N-terminus and depends on residues K30 and K31,\(^{157}\) whereas residues 452–458 (sequence PRKKRKL) comprise the Ski NLS.\(^{158}\) Ski and SnoN mutants in their respective NLSs exhibit high stability and may sequester Smad proteins in the cytoplasm.\(^{157,158}\)

Some proteins retain Ski and SnoN in the cytoplasm; for instance, the interaction of Ski with the C184M protein in the cytoplasm may block Smad2 translocation to the nucleus despite the activation of TGF-β signaling, in both the liver and lenses.\(^{159,160}\) The Ski and SnoN proteins can also interact with the TβRII receptor (or ALK5) in the cytoplasm, inducing failure of the R-Smad/Smad4 complex to translocate to the nucleus.\(^{161}\) The Ski protein was found first in both nuclear and cytoplasmic fractions of melanoma cells.\(^{162,163}\) SnoN levels are modified during mouse mammary gland development; however, the localization of SnoN remains cytoplasmic in normal human breast cells, but not in breast cancer tissues.\(^{157,164,165}\) Vitamin C treatment decreases cytosolic Ski protein levels in rat kidney mesangial cells.\(^{166}\)

In the peripheral nervous system, nuclear Ski regulates Schwann cell proliferation and differentiation by promoting normal myelination of axons.\(^{167}\) The TGF-β signaling may promote Ski relocalization into the cytoplasm, but without any protein degradation in Schwann cells; thus, the Ski protein is localized at early endosomes to sequester the phosphorylated retinoblastoma protein (pRb).\(^{168}\) TGF-β signaling also promotes Ski protein shuttling from the cytoplasm to the nucleus in cardiac myofibroblasts,\(^{169}\) whereas MG132 treatment causes accumulation of the Ski protein in the cytoplasm of human cervical cancer HeLa cells.\(^{158}\) Furthermore, the Ski and SnoN proteins associate with members of the Hippo pathway in the human mammary epithelial cells MDA-MB-231 and MCF10A; if these cells are grown at a high density, then Lats2 kinase promotes a decrease in the SnoN protein levels.\(^{69,170}\)

In the liver, the Ski protein has been localized in multivesicular endosomes (MVE); recently, we reported that cytoplasmic Ski is present in lipid raft-rich endosomes of normal hepatocytes and co-localizes with markers of multivesicular bodies, such as CD63 and Alix. Intriguingly, TGF-β signaling and proteasome inhibition by MG132 promote the localization of the Ski protein at these lipid raft-rich vesicles.\(^{171}\) The Ski and SnoN proteins are localized in both the cytoplasm and nucleus of C9 cells (rat hepatocyte clone 9) and primary rat hepatocytes.\(^{172}\) In this case, the stability of the Ski and SnoN proteins is regulated by actin-cytoskeleton dynamics (Fig. 2).\(^{171,172}\) The Ski protein levels are differentially modulated by GPCR (G-protein-coupled receptors) signals, i.e., GPCR signals associated with the G12/13/Rho/ROCK axis, which promote actin polymerization, downregulating the Ski protein, whereas GPCR signals associated with the Gαi/adenylate cyclase/cAMP axis, leading to the depolymerization of actin filaments, stabilizing the Ski protein. By contrast, the SnoN protein is stabilized by signals that promote actin polymerization in hepatocytes.\(^{171,172}\) Interestingly, Ski and SnoN regulation mediated by actin polymerization dynamics is lost in hepatoma cells, affecting notably the TGF-β signaling outcome.\(^{172}\) Other studies have shown that cAMP also increases the Ski protein levels, but in rat Schwann cells.\(^{168}\) Therefore, it is clear that cell density, polarity, and actin-cytoskeleton dynamics regulate Ski and SnoN protein stability, as well as their subcellular localization (Fig. 2).

The Ski and SnoN proteins stability can change during the cell cycle since protein levels decay rapidly during the transition from the G1 to S phases of the cell cycle, and both proteins are accumulated in the G2 phase and mitosis. The Ski protein is polyubiquitinated and degraded during the G1 phase, whereas it is temporally stabilized by phosphorylation during mitosis.\(^{143,174,175}\) The Ski protein may bind α- and γ-tubulin, and this interaction mediates Ski localization at the mitotic spindle and centrosomes during the cell cycle.\(^{175}\) Ski localization in the mitotic spindle is important in chromosome segregation; loss of both Ski alleles (Ski /–/–) causes aneuploidy in mouse embryonic fibroblasts (MEFs).\(^{176}\) The Ski C-terminal domain is important for its co-localization with Aurora in centrosomes; this association regulates centrosomal amplification and multipolar mitosis in MEFs.\(^{138,139}\) Intriguingly, the number of complexes between Ski and SnoN with R-Smads appears to increase during mitosis.\(^{177}\) The role of Ski and SnoN during cell proliferation is unclear, thus meriting further studies.
ROLE OF SKI AND SNO N IN DEVELOPMENT AND TISSUE HOMEOSTASIS

TGF-β family members play crucial roles in regulating embryonic development and tissue homeostasis. During these physiological processes, Ski and SnoN are strongly involved in controlling the spatio-temporal effects of the TGF-β pathway, as well as the magnitude and duration of the TGF-β signal. Ski and SnoN may also simultaneously regulate other signaling pathways during embryonic development or tissue homeostasis (Fig. 6). The role of SnoN during development has been partially determined based on the varied phenotypes exhibited by Skil knockout (KO) mice.178,179 Two Skil KO mice were generated by the deletion of exon 1: one of them resulted in embryo lethality;177 whereas, the other was viable but showed alterations in T-cell functions.178 A third Skil KO mouse was generated by deleting the Skil gene promoter, resulting in alterations in T-cell activation.179 The knock-in mouse, expressing a mutant SnoN protein that lacks the ability to interact with Smads, showed a phenotype with partial embryonic lethality due to defects in vascular system development. Studies using this transgenic mouse revealed a relevant function of SnoN to promote angiogenesis since SnoN interacts with the ALK1 receptor to increase the TGF-β/BMP9-dependent activation of Smad1 and Smad5.180 The expression of the SnoN protein is induced by TGF-β, activin, and nodal in human pluripotent embryonic stem cells, particularly to repress mesendodermal and primitive streak genes. Thus, the maintenance of embryonic stem-cell pluripotency is impaired by SnoN downregulation.176

Ski also exerts an important activity throughout embryonic development, specifically in formation of the central nervous system (CNS), skeletal muscle, and limbs.30 Endogenous Ski expression is increased during mouse embryonic development in several regions of the CNS; thus, Ski-KO mice show embryonic lethality because closure of the cranial neural tube is impaired; there are also eye malformations, abnormalities in craniofacial structures, and a decrease in skeletal muscle mass. Likewise, Ski knockdown (KD) mice show defects in eye and neural tube formation.181 Ski-KO mice mimic the same signs/symptoms of an anomaly named persistent hyperplastic primary vitreous (PHPV) observed in human and mouse models, which involves some ocular abnormalities, such as retinal malformations and microphthalmia.182 By contrast, overexpression of the Ski isoforms Skia and Skib gives rise to gastrulation alterations in zebrafish.94

Neurogenesis

Ski and SnoN are important regulators during neurogenesis and for maintaining the homeostasis of the nervous system.183–185 Ski overexpression increases neural axis formation.186 Ski is needed for normal myelination of axons in the peripheral nervous system because it regulates Schwann cell proliferation and differentiation.167 In rats with a spinal cord injury, Ski is upregulated in reactive astrocytes, but not in neurons.187 The inhibition of Smad1 and Smad2 by Ski induces the expression of some neural markers;188 whereas, Ski-KO mice exhibit reduced expression of nestin, an intermediate filament protein of neuroepithelial cells and myocytes precursors;187,189 embryos of SKi-KO mice also exhibit extra vestigial-digits.190 Ski appears to maintain the identity of collosal neurons in the developing neocortex by blocking Ctip2 gene expression through a Ski/Stab2/HDAC1 complex.65,66 By contrast, SnoN modulates neuronal branching and positioning during axonal growth and regeneration (Fig. 6).79,191,192

Hematopoiesis

Ski and SnoN are important regulators of cell differentiation; thus, they become key factors in the regulation of hematopoiesis.188 Ski induces the expression of genes involved in myeloid differentiation.194 Ski expression has been observed in megakaryocyte/erythrocyte dual-lineage progenitors, as well as in mature macrophages, mast cells, and B- and T-lineage cells.193 Ski mainly inhibits erythroid differentiation by inhibiting GATA1 activity or the RA signaling pathway in hematopoietic cells.67,199 Phorbol 12-myristate 3-acetate (PMA) can enhance Ski expression in human megakaryoblastic CHRF-288-11 cells that specifically undergo megakaryocytic differentiation;196 whereas, the process of megakaryopoiesis is stimulated by PMA, which favors activin A/Smad signaling and SnoN protein degradation (Fig. 6).197

Myogenesis

Ski promotes muscular development by enhancing the expression of muscle regulatory factors; for instance, Ski overexpression in myoblasts induces expression of genes encoding muscle creatine kinase (Mck) and myosin light chain (Mlc).198 High Ski mRNA levels are observed during the late stages of muscle differentiation of mouse embryos and in cell lines.199,200 Overexpression of v-Ski induces MyoD and myogenin expression and promotes myogenesis.87,201–203 Ski-overexpressing transgenic mice exhibit an increased skeletal muscular mass,204,205 these mice exhibit high Ski mRNA levels in hind leg muscles.206 By contrast, transgenic cattle overexpressing chicken Ski also develop muscular hypertrophy, but later show muscle degeneration.207 Ski transgene overexpression in mice produces skeletal muscle hypertrophy; whereas, Ski overexpression in osteocytes causes skeletal abnormalities.205,206 Furthermore, Ski plays an important role in muscle regeneration; thus, higher Ski levels promote the proliferation of muscle cells.208 Other studies have shown an upregulation of Ski in some stages of axolotl embryogenesis, such as limb formation, as well as during axolotl limb regeneration.92 SnoN is involved in muscle cell differentiation by regulating the formation of muscle fibers in SnoN transgenic mice.209 TGF-β decreases Ihh mRNA levels in the neonatal growth plate via Smad2/SnoN and Ski/Smad3 complexes; this study shows that TGF-β signals antagonize Ihh expression, a key regulator of the proliferation and differentiation of chondrocytes (Fig. 6).210

Metabolism

Ski is a key factor in the induction of myogenesis, but Ski may also decrease body fat mass by inducing oxidative metabolism of fatty acids. Transgenic mice overexpressing Ski show increased growth at early postnatal stages, as well as an altered body composition with decreased body fat and an enhanced lean body mass. The skeletal muscle of these transgenic mice has an enhanced fatty oxidative capacity and enhanced activity of both cytochrome C oxidase and citrate synthase; whereas, Ski represses some key genes in metabolism, such as Srebp1 and Ppary.211 Ski overexpression alters glycolysis and lactate production and enhances mitochondrial biogenesis and fatty acid oxidation; these effects may result after the upregulation of PPARy. Ski overexpression also induces the expression of some PPARγ target genes that are implicated in lipid uptake, transport, and oxidation.212 Ski inhibits AKT phosphorylation induced by insulin in Ski-transgenic mice with resistance to diet-induced obesity.213

REGULATION OF TGF-β/SMAD SIGNALING BY SKI AND SNO N

The role of Ski and SnoN in the TGF-β signaling network

The canonical TGF-β/Smad signaling pathway establishes a crosstalk with other pathways to efficiently achieve most of its biological functions, including the Wnt, Notch, Hippo, PI3K-AKT, PKC, MAPKs, and JAK-STAT signaling pathways. The regulation of Ski and SnoN protein expression is part of this crosstalk; thus, the TGF-β/R-Smad axis, activin, nodal, HGF/CREB/Sp1 axis, and prolactin/Stat5 axis are some of the known pathways that enhance SnoN expression in specific cell types (Fig. 2). Many other signals also control the SnoN protein levels, but the molecular mechanisms involved are unknown; for instance, certain EDCs increase SnoN in ovarian cancer cells, whereas
oxymatrine and MG132 treatments induce SnoN in the damaged kidney of diabetic rats (Fig. 2).\textsuperscript{115,214,215} NFAT stabilizes SnoN to control TGF-β-induced EMT in MDA-MB-231 breast cancer cells.\textsuperscript{216} By contrast, other treatments are linked to SnoN downregulation; for example, HGF treatment decreases the SnoN protein levels in proliferating renal tubular epithelial HK2 cells.\textsuperscript{217} PMA induces activin A production, which activates Smad2 and Smad3 to promote SnoN degradation in myelogenous leukemia cells.\textsuperscript{197} Smurf2 and MAD2B proteins cause SnoN downregulation in the fibrotic kidney.\textsuperscript{18,218} The antibiotics anisomycin and puromycin induce Ski and SnoN degradation via UPS only in specific human cell types, such as A549 lung cancer cells, AD293 embryonic kidney cells, and A7 melanoma cells.\textsuperscript{219,220,221} Spiroptosis induced by activin A is mediated by Smad3, whereas, the SnoN/p53 complex enhances p53-mediated transcription in MEFs.\textsuperscript{45,224} SnoN stabilizes TAZ protein by inhibiting its phosphorylation, which enhances TAZ/TEAD complex target gene expression during cell proliferation. SnoN cooperates with the Hippo pathway to induce transformation and promote EMT of breast cancer cells.\textsuperscript{170} SnoN is also induced by TGF-β and prolactin via Stat5 in late pregnancy; thus, SnoN blocks TGF-β signaling and stabilizes Stat5 protein to activate the prolactin pathway in lactogenesis.\textsuperscript{112} Ski also negatively controls the BMP, Hippo, Hedgehog, vitamin D, and RA signaling pathways. The Ski protein interacts with Lats2 and enhances its activity to destabilize the TAZ protein in mammary epithelial cells;\textsuperscript{69} whereas, in lung cancer, the Ski gene is silenced by methylation and, consequently, Ski is unable to inhibit TAZ.\textsuperscript{103} Ski and SnoN negatively regulate the expression of the Indian hedgehog (Ihh) gene in primary chondrocytes via different transcriptional complexes: SnoN/Smad2 and HDAC4 form a complex with a high affinity; whereas, the Ski/Smad3 complex has a lower affinity.\textsuperscript{210} Furthermore, gefitinib is a drug that is used to treat lung cancer; thus, the IL-6 cytokine induces Stat3 phosphorylation to repress Smad3 expression, promoting gefitinib resistance, and Stat3 interacts with Ski and SnoN to inhibit the R-Smad/co-Smad complex.\textsuperscript{225} In summary, TGF-β participates in a crosstalk with many other pathways that may regulate the expression and/or abundance of the Ski and SnoN proteins, and concomitantly, Ski and SnoN exert diverse effects on the outcome of those signaling pathways through regulatory feedback loops that control the different cellular responses.

The role of Ski and SnoN in the regulation of TGF-β signaling in disease

The physiological and pathological relevance of Ski and SnoN transcriptional cofactors has been clearly documented, but there is scarce information about their target genes and about the biological relevance of such gene regulation. Ski and SnoN may regulate gene expression in both Smad-dependent and -independent manners, and they may also depend on many other transcription factors to regulate gene transcription. This biological function of Ski and SnoN is important in tissue homeostasis, whereas alterations in Ski and SnoN expression and function have been linked to some diseases.

**Wound healing and fibrosis.** TGF-β superfamily members exert key roles during wound healing and fibrosis, regulating processes, such as myofibroblast (MFB) differentiation and EMT promotion.\textsuperscript{226–228} Ski and SnoN also exert differential effects during wound healing and tissue remodeling, depending on the cell context (Fig. 6). A few examples include the following: Ski and SnoN expression is enhanced during liver regeneration, probably to neutralize TGF-β/Smad antiproliferative actions;\textsuperscript{171,229} the regeneration of tendon-to-bone insertion is promoted by both SnoN overexpression and Tgif gene induction;\textsuperscript{230} Ski inhibits Smad3-induced apoptosis to promote cell proliferation in skin fibroblasts during wound healing;\textsuperscript{231} and Ski reduces scarring when it is used for gene therapy in rats.\textsuperscript{232} Ski also inhibits Smad3 activation but stimulates the p38 pathway to block TGF-β-induced vascular smooth muscle cell proliferation after vascular injury.\textsuperscript{233} Ski and SnoN cofactors may also mediate antiﬁbrotic responses by blocking TGF-β signals. The renal model of fibrosis induced by unilateral ureteral obstruction (UUO) exhibits an activated TGF-β pathway as a consequence of increased expression of Smurf2 and proterosomal degradation of the Ski and SnoN proteins.\textsuperscript{18,218} TGF-β profibrotic activity is inhibited after Ski and SnoN overexpression in renal cells, whereas the absence of SnoN sensitizes tubular epithelial HKC cells to TGF-β signaling.\textsuperscript{234} Smurf2 overexpression promotes SnoN downregulation in renal proximal tubule epithelial cells when they are cultured under high-glucose conditions;\textsuperscript{118} whereas, SnoN overexpression protects these tubular epithelial cells from undergoing EMT.\textsuperscript{235} Furthermore, the alkaloid oxymatrine extracted from the herb *Senhpora japonica* prevents SnoN downregulation in renal tubules during the EMT process induced by high glucose.\textsuperscript{215} By contrast, enhancement of SnoN protein stability due to treatment with MG132 decreases kidney damage in diabetic rats.\textsuperscript{214} Likewise, Smurf2 knockdown increases SnoN levels and blocks TGF-β signaling in rats with obstructive nephropathy.\textsuperscript{236} It is clear that the polyubiquitination and degradation of SnoN via Smurf2 induced by the TGF-β/Smad pathway is critical in diabetic nephropathy.\textsuperscript{237} BMP7 enhances the levels of SnoN mRNA and protein, which is important for controlling diabetic nephropathy and renal fibrosis.\textsuperscript{113} Moreover, the upregulation of SnoN inhibits EMT and renal fibrogenesis induced by high glucose. The miR-23a causes SnoN downregulation, whereas depletion of miR-23a increases the SnoN protein levels and decreases EMT and renal fibrogenesis.\textsuperscript{17} Interestingly, the expression of SnoN, TGF-β, and Arkadia can be increased in renal tubular cells treated with high glucose, whereas the SnoN protein levels are decreased; however, the SnoN protein levels can be increased if Arkadia is downregulated, leading to the inhibition of high glucose-induced EMT.\textsuperscript{238} Similarly, fibrogenesis in different tissues correlates with changes in SnoN levels, as occurs in the fibrotic lung\textsuperscript{239} and during renal fibrosis.\textsuperscript{239} Thus, regulation of SnoN protein abundance seems to be key in the implementation of therapeutic strategies for diabetic nephropathy.

TGF-β induces cellular matrix production by activating MFB during cardiac fibrogenesis; however, transient Ski overexpression in MFB decreases Smad2 phosphorylation, which results in a decrease in type I collagen synthesis and α-SMA levels.\textsuperscript{169,240} In this case, stable overexpression of Ski promotes cardiac MFB apoptosis.\textsuperscript{241} Bone marrow progenitor cell therapy modulates cardiac fibrosis in diabetic hearts by decreasing miR-155 levels, whereas overexpression of miR-155 in cardiac fibroblasts inhibits Ski and SnoN expression.\textsuperscript{242} Ski overexpression also induces Meox2 gene expression and blocks Zeb2 gene expression, affecting the cardiac MFB phenotype.\textsuperscript{243} Furthermore, TGF-β can induce endothelial-mesenchymal transition (EndMT) of human coronary artery endothelial cells (HCAECs) to generate matrix-producing fibroblasts and promote cardiac fibrosis. Importantly, Ski overexpression inhibits the TGF-β-induced EndMT of HCAECs via a mechanism that involves the regulation of miR-155.\textsuperscript{107} During liver fibrogenesis induced by TGF-β and acetaldehyde, an ethanol metabolite, increased collagen synthesis occurs in hepatic stellate cells (HSC); in these cells, the Ski/Smad4 complex is translocated to the cytoplasm, and the Ski protein is degraded via UPS.\textsuperscript{244} In systemic sclerosis, the levels of the Ski and SnoN proteins are increased in scleroderma fibroblasts, although they fail to inhibit the TGF-β pathway because they are unable to...
T-cells, B-cells, and carcinogens, revealing the anti-oncogenic activity of SnoN. The metastasis.247 apoptosis and cell cycle arrest, supporting the role of SnoN as a abundance, and function in cancer.

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β TGF- immortalization, and it also promotes apoptosis in normal cells and early carcinomas. By contrast, the tumor-promoting effects of TGF-β include the promotion of EMT, cell migration, invasion, and metastasis.247–249 Thus, TGF-β cytostatic and protective effects are frequently lost as tumors develop. Loss-of-TGF-β signaling is involved in hyperproliferative disorders, inflammation, autoimmune diseases, and tumor formation; whereas, gain of TGF-β signaling promotes immunosuppression and tumor metastasis.24 In addition, most tumors may arise after mutations or deletions in genes encoding components of the TGF-β signaling pathway.2,3,250

Ski and SnoN were initially described as proto-oncoproteins because of their ability to induce cellular transformation in vitro.50,89,201,202,251,252 Interestingly, the Ski and SnoN heterodimers are better able to induce cellular transformation than their respective homodimers or monomers.50,138 Thus, SnoN and Ski are up- or downregulated in many types of cancer cells, including leukemia, lymphoma, melanoma, breast cancer, cervical cancer, esophageal squamous-cell carcinoma, colorectal carcinoma, pancreatic cancer, and gastrointestinal tumors.85,104,178,253 However, it is worth mentioning that no mutations of Ski and SnoN have been found in any of the different cancer types studied to date.

Currently, it has been observed that Ski and SnoN are differentially expressed in normal and cancerous cells, and some evidence also supports the alteration of their localization, abundance, and function in cancer. Skil heterozygous mice develop spontaneous lymphoma and are quite sensitive to carcinogens, revealing the anti-oncogenic activity of SnoN. The T-cells, B-cells, and fibroblasts of these mice exhibit resistance to apoptosis and cell cycle arrest, supporting the role of SnoN as a tumor suppressor.178 Likewise, Ski tumor suppression activity has been observed in Ski-deficient mice, which exhibit higher sensitivity to tumor formation induced with carcinogens.277 Ski loss increases MEF proliferation, whereas Ski overexpression inhibits MEF proliferation via transcriptional gene repression in association with Rb and MAD.275 Both SnoN and Ski seem to be important in some cancer types. A correlation between β-catenin activation and upregulation of the SnoN and Ski proteins has been observed in early stages of colorectal cancer.262 Another study has shown that the p53 protein is stabilized after the SnoN protein is recruited to PML nuclear bodies through PML, which induces cellular senescence in a Smad-independent manner and inhibits tumorigenesis.45,224 By contrast, Ski inhibits p53 activity and promotes p53 protein degradation.68,156 Defective control of TGF-β signaling is responsible for cancer induction in Barret’s esophagus; patients with low-grade dysplasia exhibit low Ski and SnoN protein levels in dysplastic areas, whereas these proteins are absent in patients with high-grade dysplasia/adenocarcinoma.3,275

The mechanisms linked to SnoN deregulation in cancer have been better studied. Altered Ski gene transcriptional regulation, Ski gene amplification, or higher SnoN protein stability are among the main causes of SnoN upregulation, whereas SnoN can be decreased by allelic loss in some cancer types. Hence, SnoN expression in human colorectal cancer is downregulated in <40% of tumors with high-level microsatellite instability, whereas 50% of microsatellite-stable tumors present an upregulation of SnoN.275 Furthermore, in 179 human colorectal tumor biopsies, 55.2% of tumors have either partial or complete allelic loss of the Ski gene, whereas 15.1% of tumors present amplification of the Ski gene.256 Ski gene amplification was initially observed in esophage squamous cell carcinoma,254 but it has also been observed in immortalized human mammary epithelial cells, where Skil and Tloc1 may cooperate to promote cell proliferation, transformation, invasion, and tumor growth.276 By contrast, TGF-β is unable to induce SnoN protein degradation in human esophageal cancer cell lines and in rat A5-30D hepatoma cells; indeed, high levels of the SnoN protein have been detected in both cancer types.172,260 Skil and Ski genes are often co-amplified with other genes with oncogenic activity. For instance, Skil and Tloc1 genes are contained in the 3q26 chromosome, specifically in a region that is frequently amplified in several cancer types.277 The progression of nasopharyngeal carcinoma is associated with co-amplification of Gpr160 and Ski genes, both of which are localized at 3q26.2–q26.32, along with deletion of the Adamts9 and Lrig1 genes, which are localized at 3p12.3-p14.2.277 The 3q26 region is often amplified in ovarian cancer, increasing the mRNA levels of some genes, such as Ski, Evil, and Plscr187,278,279 The Ski gene is co-amplified with the Mel1 gene in MKN28 gastric cancer cells; both genes are localized at 1p36 and cooperate to inhibit TGF-β anti-proliferative actions.267

**Carcinogenesis.** The TGF-β cytokine exerts tumor-suppressive actions that include inhibition of cellular proliferation and immortalization, and it also promotes apoptosis in normal cells and early carcinomas. By contrast, the tumor-promoting effects of TGF-β include the promotion of EMT, cell migration, invasion, and metastasis.247–249 Thus, TGF-β cytostatic and protective effects are frequently lost as tumors develop. Loss-of-TGF-β signaling is involved in hyperproliferative disorders, inflammation, autoimmune diseases, and tumor formation; whereas, gain of TGF-β signaling promotes immunosuppression and tumor metastasis.24 In addition, most tumors may arise after mutations or deletions in genes encoding components of the TGF-β signaling pathway.2,3,250

**Metastasis.** The role of Ski in cancer metastasis depends on the context and cellular type.278 The Ski protein levels are decreased in metastatic non-small lung cancer cells (NSCLCs) and lung cancer tissues, whereas Ski overexpression blocks the TGF-β-mediated EMT process.280 Ski downregulation by RNAi inhibits human melanoma growth in vivo,281 whereas it has a dual effect on pancreatic-cancer tumorigenesis because Ski KD inhibits tumor growth and enhances metastasis to the lung; additionally, Ski downregulation alters the expression of TGF-β target genes linked to pancreatic cancer cell metastasis.282 Ski also participates in maintaining the stemness of pancreatic cancer stem cells by promoting the expression of components of the sonic hedgehog (Shh) pathway, such as Shh, Ptc1, Smo, Gli-1, and Gli-2.282 Ski overexpression has been observed in acute myeloid leukemia (AML), where Ski inhibits retinoic acid receptor (RAR) signaling.283 Ski upregulation activates cancer-associated fibroblasts (CAFs) in breast tumors to promote breast cancer cell invasion.283 In addition, the expression of HPV16 genes is mediated by NF1 in association with Ski in cervical carcinoma.85 Ski is a negative prognostic marker in primary esophageal squamous carcinoma,257 pancreatic ductal adenocarcinoma (PDAC),258 colorectal carcinoma,259 and gastric cancer.269 Untreated patients with chronic lymphocytic leukemia (CLL) have a two-gene signature consisting of Ski and Smad1 that predicts time to treatment and survival.269 High levels of cytoplasmic Ski negatively correlate with tumor size, stage, and lymph node status in invasive breast carcinomas and positively with survival.289 Interestingly, the Ski gene is methylated in lung cancer, which correlates with the progression of this type of cancer. Under this scenario, Ski fails to block TAZ activity and the proliferation of lung cancer cells.101 Moreover, one of the treatments to regulate Ski protein levels includes the use of the TGF-β inhibitory P144 peptide, which is derived from the extracellular sequence of the human TGF-β type III receptor, and interestingly, P144 promotes Ski downregulation in human glioblastoma cell lines, such as A172 and U-87 MG.286

SnoN duality as an oncogene and tumor suppressor was confirmed through xenotransplantation in athymic mice of lung A549 and breast MDA-MB-231 cancer cells expressing either high or low SnoN protein levels. Surprisingly, xenotransplant of breast cancer cells expressing low SnoN levels exhibited high cell metastasis to the bones and lungs, but poor tumor development.287 SnoN is an important regulator of EMT induced by TGF-β in MDA-MB231 breast cancer cells. During EMT, upregulated NFAT modifies SnoN subcellular localization from the cytoplasm to the nucleus and prevents SnoN degradation by sequestering Smad3. NFAT cooperates with SnoN to promote TGF-β-induced EMT by increasing the expression of genes encoding MMP-2, MMP-9, and N-cadherin.216 Moreover, sumoylation of SnoN by Pias1 has an
Ski and SnoN as targets in therapies to regulate TGF-β signaling. The TGF-β signaling pathway is deregulated in different diseases, such as fibrosis and cancer. Multiple pharmacological compounds have been used to target the components of the TGF-β pathway, such as anti-sense oligonucleotides, antibodies, and kinase inhibitors, among others. However, it has been a real challenge to target the components of this pathway due to its pleiotropic nature and because of the side effects generated by the pharmacological agents. Intriguingly, Ski-KO mouse and Ski-KD zebrafish have similar phenotypes to those observed in SGS, i.e., craniofacial abnormalities and aortic aneurysms, among others (Fig. 6). 

Table 1. Ski and SnoN levels in cancer

| Cancer types          | Cofactor | Expression | Mechanism                                      | References |
|-----------------------|----------|------------|------------------------------------------------|------------|
| Acute myeloid leukemia| Ski      | Upregulated| Loss-of-Ski regulation by miR-29a              | 104,242    |
| Breast cancer         | SnoN     | Upregulated| N.D.                                           | 255        |
| Cervical              | Ski      | Upregulated| N.D.                                           | 85,271     |
| Chronic Myelogenous Leukemia | Ski | Upregulated| N.D.                                           | 258        |
| Colorectal            | Ski      | Downregulated| Partial or complete allelic loss               | 256,262    |
|                       | SnoN     | Downregulated| Ski gene amplification                       | 256,275    |
| Esophageal            | Ski      | Upregulated| N.D.                                           | 257,274    |
|                       | SnoN     | Upregulated| Stability of SnoN protein                     | 254,260,274|
| Gastrointestinal      | Ski/SnoN | Upregulated| N.D.                                           | 265,267,269,270 |
| Hemangioma            | Ski      | Upregulated| N.D.                                           | 266        |
| Hepatocarcinoma       | SnoN     | Upregulated| N.D.                                           | 172        |
| Lymphoma              | SnoN     | Downregulated| sno-heterozygous mice                        | 178        |
| Lung cancer           | Ski      | Downregulated| Ski gene methylation                       | 101        |
| Melanoma              | Ski/SnoN | Upregulated| N.D.                                           | 162,163,253,259,263,264 |
| Nasopharyngeal        | SnoN     | Upregulated| Skil gene amplification                      | 277        |
| Ovarian               | SnoN     | Upregulated| Skil gene amplification                      | 81,279     |
| Pancreatic            | Ski/SnoN | Upregulated| N.D.                                           | 261,268,272,281 |

N.D. Not Demonstrated

Human genetic diseases. Shprintzen-Goldberg syndrome (SGS) is a human disorder characterized by craniofacial, cardiovascular, neuromuscular, and skeletal anomalies. The specific mutation consists of an in-frame deletion in exon 1 of the human Ski gene; this mutation falls within the Ski protein region of the gene. This alteration results in a frameshift mutation that causes the deletion of a large portion of the protein. The resulting protein is predicted to be unstable and therefore unable to function. 

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tumor growth. For instance, the downregulation of SnoN with specific interfering RNAs reduces proliferation and promotes apoptosis of hepatoma and pancreatic cancer cells. Of note, there is a pitfall associated with a significant reduction of Ski or SnoN levels since this approach can lead to the promotion of tumor metastasis, as has been shown in breast, lung, and pancreatic cancer cells, and thus the use of this strategy as an anti-cancer therapy can result in a serious disadvantage.

CONCLUDING REMARKS

The Ski and SnoN proteins play critical roles in health and disease by controlling the outcome of TGF-β and other signaling pathways. Therefore, the maintenance of balanced Ski and SnoN expression levels must be tightly controlled to normalize the TGF-β signaling outcome in diverse pathological conditions in which they are deregulated. Therefore, Ski and SnoN are potential therapeutic targets under the pathological conditions in which they are deregulated. The design of therapeutic strategies must be focused on restoring the expression levels of Ski and SnoN, with the purpose of recovering the function of TGF-β signaling and, perhaps, other signaling pathways, as well as re-establishing cellular homeostasis. Notably, the Ski and SnoN proteins are downregulated in fibrosis and cancer metastasis and upregulated in tumor growth; thus, it is important to develop diverse therapeutic strategies to target Ski and SnoN to regulate the TGF-β signaling outcome by attacking only the pro-fibrotic and tumor-promoting effects of this cytokine.

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ADDITIONAL INFORMATION

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