Published in final edited form as:

Curr Mol Med. 2010 October ; 10(7): 667–673.

SnoN: Bridging Neurobiology and Cancer Biology

Isabelle Pot¹, Yoshiho Ikeuchi², Azad Bonni², and Shirin Bonni^{1,*}

¹ Department of Biochemistry and Molecular Biology, Southern Alberta Cancer Research Institute, University of Calgary, Alberta, T2N 4N1 Canada

² Department of Pathology, Harvard Medical School, Boston, MA 02115 USA

Abstract

The transcriptional regulator SnoN has been the subject of growing interest due to its diverse functions in normal and pathological settings. A large body of evidence has established a fundamental role for SnoN as a modulator of signaling and responses by the transforming growth beta (TGF β) family of cytokines, though how SnoN regulates TGF β responses remains incompletely understood. In accordance with the critical and complex roles of TGF β in tumorigenesis and metastasis, SnoN may act as a tumor promoter or suppressor depending on the stage and type of cancer. Beyond its role in cancer, SnoN has also been implicated in the control of axon morphogenesis in postmitotic neurons in the mammalian brain. Remarkably, signaling pathways that control SnoN functions in the divergent cycling cells and postmitotic neurons appear to be conserved. Identification of novel SnoN regulatory and effector mechanisms holds the promise of advances at the interface of cancer biology and neurobiology.

Keywords

SnoN; ING2; Ccd1; TGF- β ; Smad; signaling; transcription control; cell cycle; axonal growth; cancer

Role of SnoN in TGF β Signaling

SnoN has an established role as a critical regulator of signaling by the transforming growth factor beta (TGF β) family of secreted factors [1]. The TGF β s control a wide array of biological responses that are essential for normal development and homeostasis [2,3]. TGF β acts via specific cell surface serine/threonine kinase receptors that activate the TGF β -regulated-Smad (R-Smad) signaling proteins [4]. Activated R-Smads form a heteromeric complex with the common partner Smad4 [5]. The R-Smad/Smad4 complex accumulates in the nucleus, where it associates with distinct transcriptional regulators to modulate the expression of diverse TGF β -responsive genes. Together with transcriptional coactivators such as the histone acetyltransferase p300 and with corepressors such as the histone deacetylase HDAC1, the Smad complex stimulates or represses, respectively, the expression of TGF β -target genes [6–8].

SnoN function has been intimately linked in the control of TGF β -regulated transcription. Initial studies suggested that SnoN antagonizes TGF β -induced transcription [9–11]. SnoN associates with the R-Smad/Smad4 complex and is recruited to TGF β -responsive genes

*Address correspondence to this author at the Department of Biochemistry and Molecular Biology, Southern Alberta Cancer Research Institute, University of Calgary, 3330 Hospital Drive NW, Calgary, AB, T2N 4N1, Canada; sbonni@ucalgary.ca.

[1,10,12]. In turn, SnoN is thought to recruit HDACs which deacetylate histones and thereby repress transcription.

Accumulating evidence suggest that in addition to inhibiting TGF β -induced transcription and responses, SnoN may also remarkably positively regulate TGF β -signaling and responses [1]. Knockdown of SnoN by RNAi impairs TGF β -induced transcription in distinct cell types, including epithelial cells and postmitotic neurons suggesting that endogenous SnoN may mediate TGF β -dependent responses [13,14]. Consistent with these observations, expression of SnoN at low levels in ovarian epithelial cells enhances expression of the TGF β -responsive genes cyclin-dependent kinase inhibitor p21 and plasminogen activator inhibitor 1 (PAI-1), and promotes cell cycle arrest [15]. Genetic studies in *drosophila* have also indicated that *dSno* is required for the ability of the TGF β pathway to signal in the nervous system, suggesting that the positive role of SnoN in TGF β signaling is evolutionarily conserved [16]. Collectively, these data support the idea that SnoN facilitates TGF β -dependent responses in specific biological contexts.

How does SnoN stimulate TGF β -induced transcription?

A recent study has begun to shed light on this interesting feature of SnoN function. Shirin Bonni and colleagues have identified the inhibitor of growth (ING) family of proteins as playing a critical role in the ability of SnoN to promote TGF β -induced transcription and responses [13]. The ING family comprises a group of chromatin remodeling proteins that share a highly conserved plant homeodomain (PHD) zinc finger domain at their C-terminus [17–19]. The PHD domain binds with high affinity to the chromatin protein histone H3 specifically when it is trimethylated at Lysine 4 [20–22]. The trimethylated histone H3 Lysine 4 marks active transcription start sites in genes [23]. The ING proteins are thus recruited to sites of active transcription in the genome, and through their association with transcriptional coactivators, such as p300, they can stimulate transcription of responsive genes [17,24–28]. The ING family member ING2 was recently found to promote TGF β -induced transcription and cell cycle arrest in epithelial cells [13]. ING2 upregulates TGF β -induced transcription by enhancing the transcriptional activity of the R-Smads in a PHD-dependent manner [13]. Importantly, SnoN also forms a complex with ING2 in a PHD-dependent manner [13]. Further, SnoN promotes the formation a tripartite complex composed of ING2, R-Smads, and SnoN suggesting that SnoN may mediate the ability of ING2 to stimulate TGF β -dependent transcription. Consistent with this idea, loss and gain of function studies suggest co-dependency of SnoN and ING2 for their abilities to upregulate TGF β -mediated transcription [13]. Collectively, these results define a Smad-SnoN-ING2 signaling axis that mediates TGF β -induced transcription [PART A and 13]. In future studies, it will be important to investigate the downstream mechanisms by which SnoN/ING2 complex controls TGF β -dependent responses.

SnoN has a dual role in tumorigenesis

Research focusing on the role of SnoN in cancer suggest that SnoN may have complex roles in cancer. SnoN involvement in TGF β signaling and responses is thought to provide a basis for its actions in cancer. One of the widely studied and important effect of TGF β is its ability to inhibit cell proliferation [2,29–31]. Another important response of TGF β is epithelial-mesenchymal transition (EMT), a fundamental process whereby epithelial cells dedifferentiate to acquire a mesenchymal and more motile phenotype [30,32–35]. Cellular hallmarks of cancer include the evasion of cell cycle arrest and induction of EMT [30,32]. TGF β is thought to play negative or positive roles in neoplastic disease depending on the stage of tumor development [36,37]. At early stages, loss of cell responsiveness to the anti-proliferative effect of TGF β may contribute to the pathogenesis of several epithelial tumors including colorectal, pancreatic, and mammary carcinomas [29,30,32]. Conversely, TGF β -

induced EMT is believed to contribute to tumor progression observed at later stages of many malignancies, including mammary, prostate, and colorectal carcinomas [30,32,35,38–40]. Interestingly, similar to TGF β , SnoN may have oncogenic or tumor suppressor roles as described below.

SnoN as an oncogene

Several lines of evidence strongly support the idea that high levels of SnoN may be oncogenic. Early studies showed that overexpression of SnoN in chicken embryo fibroblast triggers transformation [41,42]. The negative regulation of the TGF- β pathway by overexpressed SnoN has been proposed to promote malignant cellular transformation at early stages of cancer [10–12]. The ability of overexpressed SnoN to negatively regulate TGF β signaling and cell cycle arrest might provide a mechanism for the loss of responsiveness to TGF β signaling [15,43]. The high levels of SnoN may inhibit TGF β repression of *c-myc*, which is critical for TGF β -induced cell cycle arrest [44]. Thus, SnoN behaves as an oncogene in this context. Consistent with these observations, SnoN is amplified in several types of cancers, including melanomas, breast, and esophageal carcinomas [43–50]. Interestingly, SnoN may be overexpressed only at early stages of some types of cancer such as the precancerous condition Barrett's esophagus and colorectal carcinomas [51,52]. On the other hand, SnoN levels correlate with depth of invasion, recurrence and poor prognosis in esophageal squamous cell carcinoma [53,54]. Collectively, these data highlight the putative oncogenic role of SnoN, linked to its overexpression and negative regulation of TGF β signaling, specifically at early stages of tumorigenesis [55].

Expression of SnoN might be induced as a result of chromosomal amplification as has been observed in esophageal squamous cell carcinoma and ovarian cancer cells [15,53]. Other mechanisms may contribute to upregulated cellular SnoN levels. SnoN is increased at the protein level in colorectal cancer without a corresponding induction of its mRNA, suggesting that overexpression could result from increase in translation rate of mRNA or in stability of the protein [51]. Since the levels of SnoN protein are tightly regulated by the ubiquitin-proteasome pathway, the failure to degrade SnoN might result from reduced expression or dysfunction of E3 ubiquitin ligases that induce the ubiquitination of SnoN [1,15,51]. Changes in the subcellular localization of SnoN may also affect its levels, since cytoplasmic SnoN appears to be more resistant to TGF- β -dependent degradation than nuclear SnoN [56]. Although SnoN resides predominately in the nucleus, SnoN may also localize in the cytoplasm in some cell types, including cancer cells [42,56–58]. Interestingly, SnoN is mostly cytoplasmic in Barrett's esophagus [52]. Cytoplasmic SnoN may sequester Smads and block their accumulation in the nucleus, leading to the inhibition of TGF β -induced transcription and cell cycle arrest [56,57].

SnoN as a tumor suppressor

Several lines of evidence support the concept that in addition to its oncogenic role, remarkably SnoN also harbors a tumor suppressive function. Heterozygous *sno*^{+/-}-mice develop spontaneous tumors at low frequency and have an increased rate of tumor formation when challenged with chemical carcinogens [59]. In addition, as compared to wild type controls, *sno*^{+/-}-derived mouse embryonic fibroblasts (MEFs) and B and T lymphocytes exhibit increased proliferation rates, and resistance to cell cycle arrest and apoptosis by specific stimuli [59]. Together with knockdown studies showing that endogenous SnoN can promote TGF- β responses including cell cycle arrest, these data suggest that at physiological levels SnoN may act as a tumor suppressor [1].

Several tumors, including mammary and lung carcinomas, show downregulation of ING expression, suggesting that the ING proteins may act as tumor suppressors in certain types of

cancers [18,60–63]. In view of the recent findings that ING2 may mediate TGF- β -induced transcription and cell cycle arrest, it is conceivable that loss of ING2 may allow cancer cells to evade the tumor-suppressive effects of TGF β [13]. As SnoN is important for the ability of ING2 to mediate TGF β -induced transcription and responses, this may thus define a mechanism by which SnoN acts as a tumor suppressor [Part A and 13].

SnoN has also been suggested to inhibit tumor invasion at later stages of cancer. This may involve SnoN's ability to suppress TGF β -induced EMT in cancer cells [55]. The TGF β -regulated Smad pathway contributes to the ability of SnoN to inhibit EMT [55]. The SnoN-related protein Ski was recently demonstrated to block metastasis in breast and cancer cells, and TGF β -mediated degradation of Ski was suggested to enhance metastasis in late stage tumors [64]. Thus, it is conceivable that TGF β may also enhance SnoN degradation in later stages of cancer in order to overcome SnoN inhibition of EMT and thus promote metastasis.

The tumor suppressor role of SnoN may also involve other signaling pathways. A recent study using Smad-binding defective SnoN knockin mice suggested that SnoN induces cellular senescence via stabilization of p53 in PML bodies in MEFs [65]. In contrast, although transiently expressed SnoN was reported to localize in PML bodies in large T antigen immortalized human normal ovarian epithelial cells in which p53 is inactivated, stably expressed SnoN appears to promote senescence independently of PML levels or p53 status in these cells [15]. Whether the discrepancy in the findings of these two studies is due to differences in cell type or levels of SnoN remains to be investigated. Interestingly, ING1 and ING2 can promote senescence in a p53-dependent and independent manner [24,27,66,67]. That SnoN functions together with ING2 to activate TGF β -induced transcription suggests that SnoN's role in senescence may also involve its association with ING2. Further research is required to determine whether downstream targets of the ING-SnoN-Smad complex may be involved in establishing senescence [13].

Overall, these data illustrate that SnoN's role in tumorigenesis is strongly dependent on its expression level and the stage of cancer development at which SnoN is expressed. In addition, SnoN function in TGF β signaling appears to largely contribute to SnoN's roles in cancer.

Novel functions for SnoN in postmitotic neurons

In addition to the critical roles of SnoN in cycling cells, novel functions of SnoN have been uncovered in postmitotic neurons. Knockdown of SnoN in primary cerebellar granule and hippocampal neurons dramatically inhibits the growth of axons [68–72]. In addition, SnoN knockdown in postnatal rat pups profoundly impairs the morphogenesis of parallel fiber axons in the cerebellar cortex in vivo [70,72]. These observations suggest that SnoN may play a pivotal role in the development of axons in the brain.

The mechanisms that regulate SnoN function in axon morphogenesis are beginning to be elucidated. The major E3 ubiquitin ligase Cdh1-anaphase promoting complex (Cdh1-APC) stimulates the ubiquitination and consequent proteasome-dependent degradation of SnoN in neurons [72]. By inducing the degradation of SnoN, Cdh1-APC thus restricts the growth of axons in neurons [72].

The identification of the Cdh1-APC/SnoN link as a cell intrinsic pathway regulating axon morphogenesis in neurons mirrors the characterization of SnoN as a substrate of Cdh1-APC in the control of G1 phase of the cell cycle in proliferating cells. Notably, R-Smads recruit Cdh1-APC to stimulate SnoN ubiquitination in proliferating cells [9,73]. Remarkably, this mechanism is conserved in neurons. Accordingly, TGF- β -Smad signaling stimulates the Cdh1-APC/SnoN pathway and thus limits the growth of axons in neurons [71].

The steps that operate downstream of SnoN in neurons have been recently characterized [70]. Microarray analyses in SnoN knockdown neurons have been performed to identify the SnoN-regulated program of genes that promote axon growth. Strikingly, a large fraction of genes that are altered in SnoN knockdown neurons are downregulated [70]. These data suggest that endogenous SnoN may activate transcription of a subset of target genes in neurons. Consistent with this observation, SnoN interacts with the transcriptional co-activator p300. Loss of function of p300 phenocopies SnoN depletion on axon growth in neurons [70]. In addition, p300 knockdown blocks the ability of a constitutively active form of SnoN to stimulate axonal growth. These data suggest that p300 may mediate the ability of SnoN to activate the expression of genes that promote axon growth [Part B].

The actin-binding protein Ccd1 has been identified as a critical downstream gene target of SnoN in neurons [70]. SnoN and p300 are required for Ccd1 transcription in neurons [70]. Ccd1 contains coiled coil domain and a DIX (Dishevelled-Axin) domain [74,75]. Ccd1 localizes to axon terminals, suggesting a potential role in axon growth [70]. Knockdown of Ccd1 inhibits axon growth and prevents the ability of SnoN to stimulate the growth of axons. Importantly, Ccd1 is required for the development of granular neuron parallel fiber axons in the cerebellar cortex [70]. These results suggest a requirement for Ccd1 in SnoN-dependent axon morphogenesis in the brain [70]. Collectively, studies of SnoN in neurons have defined an elaborate cell-intrinsic pathway that orchestrates the growth of axons.

PERSPECTIVES

Recent studies suggest that SnoN plays important roles in both cancer biology and neurobiology. Expression analyses and functional studies indicate that SnoN may promote or suppress tumorigenesis depending on the stage and type of cancer. However, the mechanisms that regulate these opposing functions of SnoN in cancer biology have remained largely unexplored. Thus, future studies should focus on investigating potential upstream regulators and downstream mediators that might orchestrate the dual role of SnoN in cancer. Identification of novel SnoN-interacting proteins will be fruitful toward this goal. Functional studies of SnoN and SnoN-interacting proteins in cancer pathogenesis should include xenograft and transgenic in vivo models of cancer. Most of the studies thus far have focused on the tumorigenic role of SnoN as an intrinsic factor within cancer cells. Increasingly, the tumor microenvironment, immune system, and vascular system appear to also influence cancer development and metastasis. Therefore, future studies should also investigate whether SnoN, its regulators, and effector mechanisms modulate the ability of TGF β to control tumorigenesis in a non-cell autonomous manner.

The defined function of SnoN in axon growth in postmitotic neurons supports the concept that proteins with key roles in cycling cells regulate fundamental aspects of neuronal morphogenesis. The transcriptional activating function of SnoN plays a crucial role in the promotion of axon growth in neurons, and Ccd1 appears to be a critical target gene that operates downstream of SnoN in this function. Whether other SnoN-dependent genes participate in axon morphogenesis remains to be investigated. In addition, whether SnoN harbors a transcriptional repressive activity in neurons and the potential functional consequences of such an activity in brain development remain to be explored. Beyond the implications of SnoN function in axon morphogenesis in the developing brain, studies of SnoN raise the intriguing question of whether expression of exogenous SnoN might stimulate the growth of axons in the adult brain following injury.

Studies of SnoN in the context of cancer biology bear important implications for our understanding of SnoN biology in neurons. For example, the role of ING2 in SnoN-induced transcription in proliferating cells raises the question of whether ING2 forms a complex with

SnoN in neurons and thereby contributes to SnoN's ability to promote the growth of axons. Likewise, the recent characterization of the PIAS1/SnoN sumoylation pathway in proliferating cells leads to the question of whether sumoylation influences SnoN function in neurons [1,76]. Conversely, SnoN studies in neuronal morphogenesis provide interesting leads for improved understanding of SnoN functions in proliferating cells. For example, identification of Ccd1 as a physiologically relevant target of SnoN in axon growth raises the question of whether Ccd1, which harbors actin binding and signaling scaffold activities, might contribute to SnoN functions in TGF β -induced cell cycle arrest and EMT. Besides Ccd1, it will be interesting to determine whether other genes identified as SnoN targets in microarray analyses of SnoN knockdown neurons are also regulated by SnoN in proliferating cells. The TGF β -response genes thrombospondin 2 and Zeb1, which have been implicated in tumor progression and EMT respectively, were downregulated in SnoN knockdown neurons [70,77–81]. It will be interesting to determine whether thrombospondin 2 and Zeb1 operate downstream of SnoN in the control of tumorigenesis and metastasis. Studies of SnoN will likely continue to provide novel insights in cancer biology and neurobiology for years to come.

Acknowledgments

Supported by Canadian Institutes of Health Research and Alberta Cancer Research Institute (ACRI) grants to S.B., NIH grants to A.B. (NS051255 and NS064007), an Alberta Heritage for Medical Research and an ACRI Cancer Research Fellowships (I.P.), and a Human Frontier Science Program Long-term Fellowship (Y.I.).

References

1. Pot I, Bonni S. SnoN in TGF-beta signaling and cancer biology. *Curr Mol Med*. 2008 Jun; 8(4):319–28. [PubMed: 18537639]
2. Massague J, Blain SW, Lo RS. TGFbeta signaling in growth control, cancer, and heritable disorders. *Cell*. 2000 Oct 13; 103(2):295–309. [PubMed: 11057902]
3. Roberts AB, Flanders KC, Heine UI, Jakowlew S, Kondaiah P, Kim SJ, et al. Transforming growth factor-beta: multifunctional regulator of differentiation and development. *Philos Trans R Soc Lond B Biol Sci*. 1990 Mar 12; 327(1239):145–54. [PubMed: 1969655]
4. Shi Y, Massague J. Mechanisms of TGF-beta signaling from cell membrane to the nucleus. *Cell*. 2003 Jun 13; 113(6):685–700. [PubMed: 12809600]
5. Derynck R, Zhang Y, Feng XH. Smads: transcriptional activators of TGF-beta responses. *Cell*. 1998 Dec 11; 95(6):737–40. [PubMed: 9865691]
6. Miyazono K, Maeda S, Imamura T. Smad Transcriptional Co-activators and Co-repressors. In: ten Dijke P, Heldin CH, editors. *Smad Signal Transduction*. Springer; 2006. p. 277-93.
7. Attisano L, Wrana JL. Smads as transcriptional co-modulators. *Curr Opin Cell Biol*. 2000 Apr; 12(2):235–43. [PubMed: 10712925]
8. Wotton D, Massague J. Smad transcriptional corepressors in TGF beta family signaling. *Curr Top Microbiol Immunol*. 2001; 254:145–64. [PubMed: 11190572]
9. Stroschein SL, Bonni S, Wrana JL, Luo K. Smad3 recruits the anaphase-promoting complex for ubiquitination and degradation of SnoN. *Genes Dev*. 2001 Nov 1; 15(21):2822–36. [PubMed: 11691834]
10. Stroschein SL, Wang W, Zhou S, Zhou Q, Luo K. Negative feedback regulation of TGF-beta signaling by the SnoN oncoprotein. *Science*. 1999 Oct 22; 286(5440):771–4. [PubMed: 10531062]
11. Sun Y, Liu X, Ng-Eaton E, Lodish HF, Weinberg RA. SnoN and Ski protooncoproteins are rapidly degraded in response to transforming growth factor beta signaling. *Proc Natl Acad Sci U S A*. 1999 Oct 26; 96(22):12442–7. [PubMed: 10535941]
12. Liu X, Sun Y, Weinberg RA, Lodish HF. Ski/Sno and TGF-beta signaling. *Cytokine Growth Factor Rev*. 2001 Mar; 12(1):1–8. [PubMed: 11312113]

13. Sarker KP, Kataoka H, Chan A, Netherton SJ, Pot I, Huynh MA, et al. ING2 as a novel mediator of transforming growth factor-beta-dependent responses in epithelial cells. *J Biol Chem*. 2008 May 9; 283(19):13269–79. [PubMed: 18334480]
14. Sarker KP, Wilson SM, Bonni S. SnoN is a cell type-specific mediator of transforming growth factor-beta responses. *J Biol Chem*. 2005 Apr 1; 280(13):13037–46. [PubMed: 15677458]
15. Nanjundan M, Cheng KW, Zhang F, Lahad J, Kuo WL, Schmandt R, et al. Overexpression of SnoN/SkiL, amplified at the 3q26.2 locus, in ovarian cancers: a role in ovarian pathogenesis. *Mol Oncol*. 2008 Aug; 2(2):164–81. [PubMed: 19383336]
16. Takaesu NT, Hyman-Walsh C, Ye Y, Wisotzkey RG, Stinchfield MJ, O'Connor MB, et al. dSno facilitates baboon signaling in the Drosophila brain by switching the affinity of Medea away from Mad and toward dSmad2. *Genetics*. 2006 Nov; 174(3):1299–313. [PubMed: 16951053]
17. Feng X, Hara Y, Riabowol K. Different HATS of the ING1 gene family. *Trends Cell Biol*. 2002 Nov; 12(11):532–8. [PubMed: 12446115]
18. Gong W, Suzuki K, Russell M, Riabowol K. Function of the ING family of PHD proteins in cancer. *Int J Biochem Cell Biol*. 2005 May; 37(5):1054–65. [PubMed: 15743678]
19. Soliman MA, Riabowol K. After a decade of study-ING, a PHD for a versatile family of proteins. *Trends Biochem Sci*. 2007 Nov; 32(11):509–19. [PubMed: 17949986]
20. Champagne KS, Kutateladze TG. Structural insight into histone recognition by the ING PHD fingers. *Curr Drug Targets*. 2009 May; 10(5):432–41. [PubMed: 19442115]
21. Pena PV, Davrazou F, Shi X, Walter KL, Verkhusha VV, Gozani O, et al. Molecular mechanism of histone H3K4me3 recognition by plant homeodomain of ING2. *Nature*. 2006 Jul 6; 442(7098):100–3. [PubMed: 16728977]
22. Shi X, Hong T, Walter KL, Ewalt M, Michishita E, Hung T, et al. ING2 PHD domain links histone H3 lysine 4 methylation to active gene repression. *Nature*. 2006 Jul 6; 442(7098):96–9. [PubMed: 16728974]
23. Santos-Rosa H, Schneider R, Bannister AJ, Sherriff J, Bernstein BE, Emre NC, et al. Active genes are tri-methylated at K4 of histone H3. *Nature*. 2002 Sep 26; 419(6905):407–11. [PubMed: 12353038]
24. Kataoka H, Bonnefin P, Vieyra D, Feng X, Hara Y, Miura Y, et al. ING1 represses transcription by direct DNA binding and through effects on p53. *Cancer Res*. 2003 Sep 15; 63(18):5785–92. [PubMed: 14522900]
25. Nagashima M, Shiseki M, Miura K, Hagiwara K, Linke SP, Pedoux R, et al. DNA damage-inducible gene p33ING2 negatively regulates cell proliferation through acetylation of p53. *Proc Natl Acad Sci U S A*. 2001 Aug 14; 98(17):9671–6. [PubMed: 11481424]
26. Nagashima M, Shiseki M, Pedoux RM, Okamura S, Kitahama-Shiseki M, Miura K, et al. A novel PHD-finger motif protein, p47ING3, modulates p53-mediated transcription, cell cycle control, and apoptosis. *Oncogene*. 2003 Jan 23; 22(3):343–50. [PubMed: 12545155]
27. Pedoux R, Sengupta S, Shen JC, Demidov ON, Saito S, Onogi H, et al. ING2 regulates the onset of replicative senescence by induction of p300-dependent p53 acetylation. *Mol Cell Biol*. 2005 Aug; 25(15):6639–48. [PubMed: 16024799]
28. Vieyra D, Loewith R, Scott M, Bonnefin P, Boisvert FM, Cheema P, et al. Human ING1 proteins differentially regulate histone acetylation. *J Biol Chem*. 2002 Aug 16; 277(33):29832–9. [PubMed: 12015309]
29. Chen CR, Kang Y, Siegel PM, Massague J. E2F4/5 and p107 as Smad cofactors linking the TGFbeta receptor to c-myc repression. *Cell*. 2002 Jul 12; 110(1):19–32. [PubMed: 12150994]
30. Jakowlew SB. Transforming growth factor-beta in cancer and metastasis. *Cancer Metastasis Rev*. 2006 Sep; 25(3):435–57. [PubMed: 16951986]
31. Ten Dijke P, Goumans MJ, Itoh F, Itoh S. Regulation of cell proliferation by Smad proteins. *J Cell Physiol*. 2002 Apr; 191(1):1–16. [PubMed: 11920677]
32. Massague J. TGFbeta in Cancer. *Cell*. 2008 Jul 25; 134(2):215–30. [PubMed: 18662538]
33. Thiery JP. Epithelial-mesenchymal transitions in tumour progression. *Nat Rev Cancer*. 2002 Jun; 2(6):442–54. [PubMed: 12189386]
34. Thiery JP. Epithelial-mesenchymal transitions in development and pathologies. *Curr Opin Cell Biol*. 2003 Dec; 15(6):740–6. [PubMed: 14644200]

35. Thiery JP, Acloque H, Huang RY, Nieto MA. Epithelial-mesenchymal transitions in development and disease. *Cell*. 2009 Nov 25; 139(5):871–90. [PubMed: 19945376]
36. Bachman KE, Park BH. Dual nature of TGF-beta signaling: tumor suppressor vs. tumor promoter. *Curr Opin Oncol*. 2005 Jan; 17(1):49–54. [PubMed: 15608513]
37. Roberts AB, Wakefield LM. The two faces of transforming growth factor beta in carcinogenesis. *Proc Natl Acad Sci U S A*. 2003 Jul 22; 100(15):8621–3. [PubMed: 12861075]
38. Bates RC, Mercurio AM. The epithelial-mesenchymal transition (EMT) and colorectal cancer progression. *Cancer Biol Ther*. 2005 Apr; 4(4):365–70. [PubMed: 15846061]
39. Bates RC, Pursell BM, Mercurio AM. Epithelial-mesenchymal transition and colorectal cancer: gaining insights into tumor progression using LIM 1863 cells. *Cells Tissues Organs*. 2007; 185(1–3):29–39. [PubMed: 17587805]
40. Hugo H, Ackland ML, Blick T, Lawrence MG, Clements JA, Williams ED, et al. Epithelial–mesenchymal and mesenchymal–epithelial transitions in carcinoma progression. *J Cell Physiol*. 2007 Nov; 213(2):374–83. [PubMed: 17680632]
41. Nomura N, Sasamoto S, Ishii S, Date T, Matsui M, Ishizaki R. Isolation of human cDNA clones of ski and the ski-related gene, sno. *Nucleic Acids Res*. 1989 Jul 25; 17(14):5489–500. [PubMed: 2762147]
42. Boyer PL, Colmenares C, Stavnezer E, Hughes SH. Sequence and biological activity of chicken snoN cDNA clones. *Oncogene*. 1993 Feb; 8(2):457–66. [PubMed: 8426750]
43. Boone B, Haspelslagh M, Brochez L. Clinical significance of the expression of c-Ski and SnoN, possible mediators in TGF-beta resistance, in primary cutaneous melanoma. *J Dermatol Sci*. 2009 Jan; 53(1):26–33. [PubMed: 18782659]
44. Edmiston JS, Yeudall WA, Chung TD, Lebman DA. Inability of transforming growth factor-beta to cause SnoN degradation leads to resistance to transforming growth factor-beta-induced growth arrest in esophageal cancer cells. *Cancer Res*. 2005 Jun 1; 65(11):4782–8. [PubMed: 15930298]
45. Colmenares C, Suttrave P, Hughes SH, Stavnezer E. Activation of the c-ski oncogene by overexpression. *J Virol*. 1991 Sep; 65(9):4929–35. [PubMed: 1870207]
46. Imoto I, Pimkhaokham A, Fukuda Y, Yang ZQ, Shimada Y, Nomura N, et al. SNO is a probable target for gene amplification at 3q26 in squamous-cell carcinomas of the esophagus. *Biochem Biophys Res Commun*. 2001 Aug 24; 286(3):559–65. [PubMed: 11511096]
47. Poser I, Rothhammer T, Dooley S, Weiskirchen R, Bosserhoff AK. Characterization of Sno expression in malignant melanoma. *Int J Oncol*. 2005 May; 26(5):1411–7. [PubMed: 15809735]
48. Seoane J. Escaping from the TGFbeta anti-proliferative control. *Carcinogenesis*. 2006 Nov; 27(11):2148–56. [PubMed: 16698802]
49. Zhang L, Sato E, Amagasaki K, Nakao A, Naganuma H. Participation of an abnormality in the transforming growth factor-beta signaling pathway in resistance of malignant glioma cells to growth inhibition induced by that factor. *J Neurosurg*. 2006 Jul; 105(1):119–28. [PubMed: 16871886]
50. Colmenares C, Stavnezer E. Structure and activities of the ski oncogene. *Semin Cancer Biol*. 1990 Dec; 1(6):383–7. [PubMed: 2103510]
51. Bravou V, Antonacopoulou A, Papadaki H, Floratou K, Stavropoulos M, Episkopou V, et al. TGF-beta repressors SnoN and Ski are implicated in human colorectal carcinogenesis. *Cell Oncol*. 2009; 31(1):41–51. [PubMed: 19096149]
52. Villanacci V, Bellone G, Battaglia E, Rossi E, Carbone A, Prati A, et al. Ski/SnoN expression in the sequence metaplasia-dysplasia-adenocarcinoma of Barrett's esophagus. *Hum Pathol*. 2008 Mar; 39(3):403–9. [PubMed: 18261624]
53. Akagi I, Miyashita M, Makino H, Nomura T, Hagiwara N, Takahashi K, et al. SnoN overexpression is predictive of poor survival in patients with esophageal squamous cell carcinoma. *Ann Surg Oncol*. 2008 Oct; 15(10):2965–75. [PubMed: 18612694]
54. Zhang X, Egawa K, Xie Y, Ihn H. The expression of SnoN in normal human skin and cutaneous keratinous neoplasms. *Int J Dermatol*. 2009 Jun; 48(6):579–83. [PubMed: 19538364]
55. Zhu Q, Krakowski AR, Dunham EE, Wang L, Bandyopadhyay A, Berdeaux R, et al. Dual role of SnoN in mammalian tumorigenesis. *Mol Cell Biol*. 2007 Jan; 27(1):324–39. [PubMed: 17074815]

56. Krakowski AR, Laboureau J, Mauviel A, Bissell MJ, Luo K. Cytoplasmic SnoN in normal tissues and nonmalignant cells antagonizes TGF- β signaling by sequestration of the Smad proteins. *Proc Natl Acad Sci U S A*. 2005 Aug 30; 102(35):12437–42. [PubMed: 16109768]
57. Reed JA, Lin Q, Chen D, Mian IS, Medrano EE. SKI pathways inducing progression of human melanoma. *Cancer Metastasis Rev*. 2005 Jun; 24(2):265–72. [PubMed: 15986136]
58. Zhang F, Lundin M, Ristimäki A, Heikkilä P, Lundin J, Isola J, et al. Ski-related novel protein N (SnoN), a negative controller of transforming growth factor- β signaling, is a prognostic marker in estrogen receptor-positive breast carcinomas. *Cancer Res*. 2003 Aug 15; 63(16):5005–10. [PubMed: 12941827]
59. Shinagawa T, Dong HD, Xu M, Maekawa T, Ishii S. The sno gene, which encodes a component of the histone deacetylase complex, acts as a tumor suppressor in mice. *Embo J*. 2000 May 15; 19(10):2280–91. [PubMed: 10811619]
60. Chen L, Matsubara N, Yoshino T, Nagasaka T, Hoshizima N, Shirakawa Y, et al. Genetic alterations of candidate tumor suppressor ING1 in human esophageal squamous cell cancer. *Cancer Res*. 2001 Jun 1; 61(11):4345–9. [PubMed: 11389058]
61. Kameyama K, Huang CL, Liu D, Masuya D, Nakashima T, Sumitomo S, et al. Reduced ING1b gene expression plays an important role in carcinogenesis of non-small cell lung cancer patients. *Clin Cancer Res*. 2003 Oct 15; 9(13):4926–34. [PubMed: 14581367]
62. Toyama T, Iwase H, Watson P, Muzik H, Saettler E, Magliocco A, et al. Suppression of ING1 expression in sporadic breast cancer. *Oncogene*. 1999 Sep 16; 18(37):5187–93. [PubMed: 10498868]
63. Ythier D, Larrieu D, Brambilla C, Brambilla E, Pedoux R. The new tumor suppressor genes ING: genomic structure and status in cancer. *Int J Cancer*. 2008 Oct 1; 123(7):1483–90. [PubMed: 18636562]
64. Le Scolan E, Zhu Q, Wang L, Bandyopadhyay A, Javelaud D, Mauviel A, et al. Transforming growth factor- β suppresses the ability of Ski to inhibit tumor metastasis by inducing its degradation. *Cancer Res*. 2008 May 1; 68(9):3277–85. [PubMed: 18451154]
65. Pan D, Zhu Q, Luo K. SnoN functions as a tumour suppressor by inducing premature senescence. *EMBO J*. 2009 Nov 18; 28(22):3500–13. [PubMed: 19745809]
66. Coles AH, Liang H, Zhu Z, Marfella CG, Kang J, Imbalzano AN, et al. Deletion of p37Ing1 in mice reveals a p53-independent role for Ing1 in the suppression of cell proliferation, apoptosis, and tumorigenesis. *Cancer Res*. 2007 Mar 1; 67(5):2054–61. [PubMed: 17332334]
67. Helbing CC, Veillette C, Riabowol K, Johnston RN, Garkavtsev I. A novel candidate tumor suppressor, ING1, is involved in the regulation of apoptosis. *Cancer Res*. 1997 Apr 1; 57(7):1255–8. [PubMed: 9102209]
68. Kim AH, Bonni A. Thinking within the D box: initial identification of Cdh1-APC substrates in the nervous system. *Mol Cell Neurosci*. 2007 Mar; 34(3):281–7. [PubMed: 17223572]
69. Yang Y, Kim AH, Bonni A. The dynamic ubiquitin ligase duo: Cdh1-APC and Cdc20-APC regulate neuronal morphogenesis and connectivity. *Curr Opin Neurobiol*. Feb; 20(1):92–9. [PubMed: 20060286]
70. Ikeuchi Y, Stegmüller J, Netherton S, Huynh MA, Masu M, Frank D, et al. A SnoN-Ccd1 pathway promotes axonal morphogenesis in the mammalian brain. *J Neurosci*. 2009 Apr 1; 29(13):4312–21. [PubMed: 19339625]
71. Stegmüller J, Huynh MA, Yuan Z, Konishi Y, Bonni A. TGF β -Smad2 signaling regulates the Cdh1-APC/SnoN pathway of axonal morphogenesis. *J Neurosci*. 2008 Feb 20; 28(8):1961–9. [PubMed: 18287512]
72. Stegmüller J, Konishi Y, Huynh MA, Yuan Z, Dibacco S, Bonni A. Cell-intrinsic regulation of axonal morphogenesis by the Cdh1-APC target SnoN. *Neuron*. 2006 May 4; 50(3):389–400. [PubMed: 16675394]
73. Wan Y, Liu X, Kirschner MW. The anaphase-promoting complex mediates TGF- β signaling by targeting SnoN for destruction. *Mol Cell*. 2001 Nov; 8(5):1027–39. [PubMed: 11741538]
74. Shiomi K, Uchida H, Keino-Masu K, Masu M. Ccd1, a novel protein with a DIX domain, is a positive regulator in the Wnt signaling during zebrafish neural patterning. *Curr Biol*. 2003 Jan 8; 13(1):73–7. [PubMed: 12526749]

75. Wang X, Zheng L, Zeng Z, Zhou G, Chien J, Qian C, et al. DIXDC1 isoform, l-DIXDC1, is a novel filamentous actin-binding protein. *Biochem Biophys Res Commun*. 2006 Aug 18; 347(1): 22–30. [PubMed: 16814745]
76. Hsu YH, Sarker KP, Pot I, Chan A, Netherton SJ, Bonni S. Sumoylated SnoN represses transcription in a promoter-specific manner. *J Biol Chem*. 2006 Nov 3; 281(44):33008–18. [PubMed: 16966324]
77. Lawler J, Detmar M. Tumor progression: the effects of thrombospondin-1 and -2. *Int J Biochem Cell Biol*. 2004 Jun; 36(6):1038–45. [PubMed: 15094119]
78. Miyazono K. Transforming growth factor-beta signaling in epithelial-mesenchymal transition and progression of cancer. *Proc Jpn Acad Ser B Phys Biol Sci*. 2009; 85(8):314–23.
79. Negoescu A, Lafeuillade B, Pellerin S, Chambaz EM, Feige JJ. Transforming growth factors beta stimulate both thrombospondin-1 and CISP/thrombospondin-2 synthesis by bovine adrenocortical cells. *Exp Cell Res*. 1995 Apr; 217(2):404–9. [PubMed: 7698241]
80. Rendtlew Danielsen JM, Knudsen LM, Dahl IM, Lodahl M, Rasmussen T. Dysregulation of CD47 and the ligands thrombospondin 1 and 2 in multiple myeloma. *Br J Haematol*. 2007 Sep; 138(6): 756–60. [PubMed: 17760807]
81. Shirakihara T, Saitoh M, Miyazono K. Differential regulation of epithelial and mesenchymal markers by deltaEF1 proteins in epithelial mesenchymal transition induced by TGF-beta. *Mol Biol Cell*. 2007 Sep; 18(9):3533–44. [PubMed: 17615296]

Part A.

SnoN collaborates with ING2 to promote TGF β -induced transcription and biological responses. SnoN acts as an adaptor to induce the assembly of a R-Smad-SnoN-ING2 multiprotein complex that enhances TGF β -induced transcription and cell cycle arrest.

Part B.

SnoN stimulates axon growth in postmitotic neurons in the mammalian brain. SnoN associates with the histone acetyltransferase p300 to induce the transcription of actin-binding and signaling scaffolding gene *Ccd1*. The protein *Ccd1*, which localizes to axon terminals, plays a critical role in the growth of axons. The ability of the SnoN-p300-*Ccd1* pathway to stimulate axon growth is restricted by the TGF β -Smad pathway which recruits the E3 ubiquitin ligase Cdh1-APC to SnoN leading to its ubiquitination and consequent degradation.