Sno/Ski Proto-Oncogene Family

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This article is a revision of the previous edition article by M Frame, volume 4, p 1846, © 2001, Elsevier Inc.

Glossary

Multigene family A group of genes encoding proteins with sufficient sequence similarity to suggest that they derive from a common ancestral gene.
Proto-oncogene A gene that when mutated to overactivity contributes to tumor formation.
Signal transduction A cascade of molecular events that transmit information from the cell surface to proteins within the cell that induce a stereotypical response.
Smad A multigene family of signal transducers dedicated to TGF-β signaling proteins.
TGF-β A multigene family of signaling proteins present in all multicellular animals, which during embryonic development and adult homeostasis induce an array of cellular responses.
Tumor suppressor A gene that when mutated to inactivity contributes to tumor formation.

Family Tree and Conserved Domains

Sno and Ski have similar amino acid sequences and are part of a conserved multigene family (Figure 1(a)). Both Ski and Sno are present in mammals, but only Sno is present in flies (dSno or Snoo). Neither is present in nematodes but the Daf-5 gene may resemble their common ancestor. The mammalian and fly Sno genes are encoded as multiple protein isoforms, but all isoforms contain a well-defined Sno homology domain (Figure 1(b)). This domain is near the amino terminus and includes the following functionally defined motifs: Smad2/3 interaction site, Dac box, Corl domain, SAND domain, and APC recognition site. The I-loop is a structurally defined feature of the Sno homology domain containing the amino acids that bind to Smad4. The central region contains lysines targeted for ubiquitination as well as binding sites for other proteins. The carboxy terminal region contains a coiled-coil domain important for dimerization and protein–protein interactions.

Sno Function in Loss-of-Function Experiments

Phenotypes generated when gene function is lost, for example, by mutation, engineered knockout at the DNA level or when transcripts are knocked down with a silencing RNA are important clues to that gene's function. The first Sno knockout mouse developed spontaneous lymphomas, suggesting that Sno could function as a tumor suppressor gene. Two other Sno knockout mice, generated with a similar strategy, did not develop lymphoma but rather displayed developmental defects in T-cell proliferation. Studies utilizing small interfering RNA (siRNA) in cell lines found that Sno was necessary for TGF-β responses in mink lung cells, but not in human cervical cancer cells or fibroblasts. Taken together, loss-of-function studies in mammals indicate that Sno activity is important during development and adult homeostasis.

Studies of dSno mutations in the fly have also shown that this gene is connected to TGF-β signaling. For example, dSno mutants develop optic lobe defects in the larval brain that are similar to those with Activin (a member of the TGF-β family) mutations. Overexpression studies (similar to those discussed below) of dSno interactions with the fly Smad proteins, Mad, Medea, and dSmad2, showed that dSno acts as a pathway switch: dSno facilitates signaling for Activin via a mechanism that simultaneously antagonizes signaling by Dpp (another TGF-β family member). dSno performs this role by switching the binding affinity of Medea from Mad to dSmad2. Overall, the loss-of-function studies suggest that Sno proteins are TGF-β agonists (Figure 2(a)).

Sno Function in Gain-of-Function Experiments

Studies examining the effect of overexpressing Sno and Ski predate all loss-of-function studies and were the first to connect these proteins to TGF-β signaling. In mammalian cells, these studies showed that in the absence of TGF-β, Sno and Ski physically bind to Smad2 and Smad4 repressing their transcriptional ability. Repression is accomplished by the recruitment of co-repressors such as histone deacetylase to Sno–Smad complexes. If TGF-β proteins are present, then Sno is rapidly ubiquitinated and degraded permitting these Smads to activate target gene expression, including the transcription of Sno. This subsequent round of Sno expression leads to renewed interactions with Smads and the attenuation of Smad signaling.

Analyses of Sno regulation have identified several ubiquitin ligases capable of targeting it for destruction such as the Anaphase-Promoting Complex and Arkadia. Both ligases bind within the Sno homology domain but they ubiquitinate lysine residues elsewhere. Assays manipulating Arkadia expression levels revealed that this protein's ability to regulate Sno degradation clearly regulates TGF-β signal transduction. Overall, the gain-of-function studies suggest that Sno proteins are TGF-β antagonists (Figure 2(b)).

Studies of cancer can also be viewed as analyses of Sno overexpression since the chromosomal region containing Sno is frequently amplified in breast, esophagus, lung, ovary, cervix, head and neck, and prostate cancer. In addition, artificial overexpression of Sno in the mammary gland accelerates tumor

 doi:10.1016/B978-0-12-374984-0.01436-4

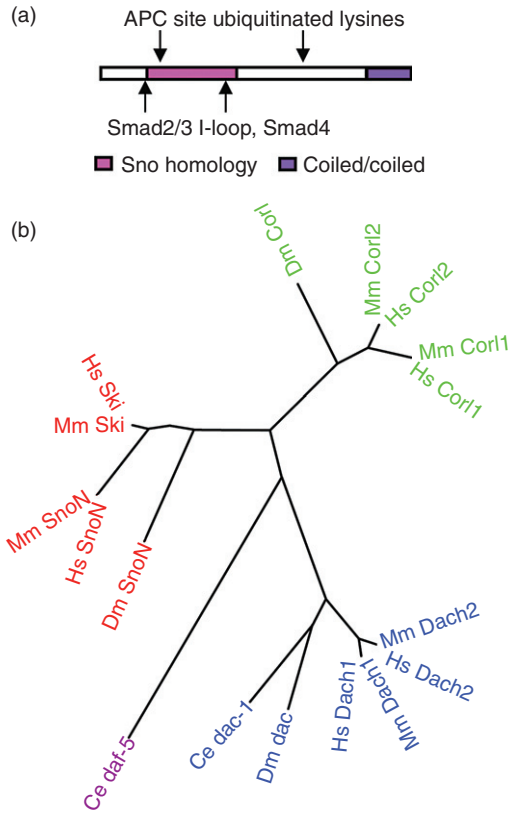

Figure 1 Sno protein and Sno/Ski family tree. (a) Sno domain organization is shown with colored boxes and arrows indicating the location of functionally defined motifs. (b) Subfamilies of closely related proteins are colored similarly. The branch lengths are drawn to scale such that longer lines represent more amino acid differences between that of the protein and the others.

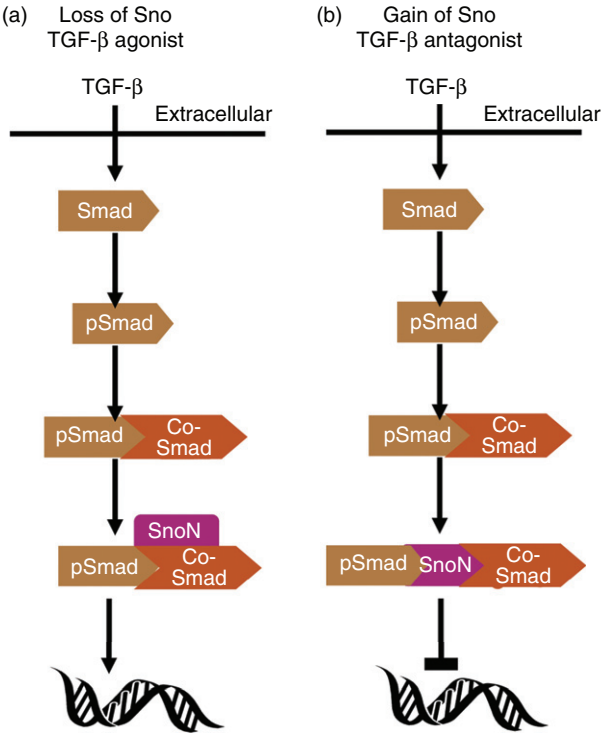

Figure 2 Sno functions revealed by loss and gain of function experiments. (a) Experiments employing mutations or silencing RNA suggest that Sno facilitates TGF-β signaling. (b) Experiments employing transfected cells or injected frog embryos suggest that Sno antagonizes TGF-β signaling.

growth and metastasis in a mouse model of breast cancer. Conversely, an analysis of invasive breast and a lung tumor cell lines showed that reduction of Sno transcript levels with a siRNA inhibited their growth, suggesting the possibility of targeting Sno as a cancer treatment.

Ski Function in Gain- and Loss-of-Function Experiments

Knockout mice with a mutation in Ski die perinatally from neural tube defects and a general reduction in skeletal muscle mass, showing that it has distinct developmental roles from Sno. The neural tube defect was caused by excessive apoptosis due to ectopic expression of ornithine decarboxylase that is normally tightly regulated by transcriptional repression. Thus, Ski also functions with co-repressors such as histone deacetylase. Unlike Sno, Ski overexpression studies are consistent with the loss-of-function data and also demonstrate that co-repressors are drawn to Smad complexes when bound by Ski, thus repressing TGF-β target genes. Ski can block signaling by both major TGF-β subfamilies: by binding to Smad3 to inhibit Activin signaling and by binding to Smad4 to inhibit Dpp/BMP signaling. Overall, loss- and gain-of-function studies suggest that Ski proteins are TGF-β antagonists.

Current Studies

Recent investigations on Sno have begun to explore interactions with other signaling pathways. A study in mice utilizing a special type of Sno mutation revealed that Sno can function as a tumor suppressor by interacting with the promyelocytic leukemia protein to stabilize p53. This inhibits oncogenic transformation induced by Ras and Myc. An analysis in flies exploiting a tissue-specific overexpression assay showed that dSno is an inhibitor of the Wingless/Wnt pathway and that pathway antagonism could possibly involve the signal transducer Armadillo (β-catenin in vertebrates). The possibility that these proteins may become therapeutic targets in cancer means they will remain at the forefront of scientific investigation.

See also: Chromatin; Gene Family; Oncogenes; Proto-Oncogene; *Ras* Gene Family; Signal Transduction; Tumor Suppressor Genes; Ubiquitin.

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