Cell Tissue Res
DOI 10.1007/s00441-011-1180-z

REVIEW

c-Ski in health and disease

Carine Bonnon · Suzana Atanasoski

Received: 31 March 2011 / Accepted: 15 April 2011

Abstract c-Ski is an evolutionary conserved protein that is involved in diverse cellular processes such as proliferation, differentiation, transformation, and tumor progression. A large range of cellular partners of c-Ski, including transcription factors, chromatin-remodeling molecules, tumor suppressors, and nuclear hormone receptors, has been identified. Moreover, numerous mechanisms have been described by which c-Ski regulates essential signaling pathways, e.g., the TGFβ pathway. In this review, we summarize the diverse roles attributed to c-Ski during normal development and in cancer progression and discuss future strategies to unravel further the complex nature of c-Ski actions in a context-dependent manner.

Keywords c-Ski · c-Ski-interacting partners · Signaling pathways · Cancer progression · Normal development

Introduction

The Ski oncogene was first identified as the transforming protein (v-Ski) of the avian Sloan-Kettering viruses that induce transformation of chicken embryo fibroblasts (Li et al. 1986; Stavnezer et al. 1986). The retroviral *v-Ski* was subsequently shown to be a truncated version of the coding exons of the chicken cellular homolog *c-Ski* (Grimes et al. 1992; Stavnezer et al. 1989; Sutrave and Hughes 1989). To date, orthologs of this evolutionarily conserved gene have been identified in human (Nomura et al. 1989), *Xenopus* (Amaravadi et al. 1997; Sleeman and Laskey 1993), mouse (Lyons et al. 1994; Namciu et al. 1995), axolotl (Ludolph et al. 1995), tilapia (Huang et al. 1999), zebrafish (Kaufman et al. 2000), and *Drosophila* (Barrio et al. 2007) (Fig. 1a). Northern blot analyses have revealed that *c-Ski* is expressed as two major mRNAs of different size but constant ratio in all examined species, such as chicken (Li et al. 1986), various human cell lines (Nomura et al. 1989), *Xenopus* (Sleeman and Laskey 1993), and mouse (Lyons et al. 1994). The basis for the difference between the two mRNAs is unknown, but studies on chicken (Grimes et al. 1993) and mouse (Lyons et al. 1994) mRNAs indicate that it is not the result of alternative splicing within the protein coding region.

Structure of c-Ski

The c-Ski protein contains several structural domains that mediate protein-protein interactions (Fig. 1b). The amino (N)-terminal half of c-Ski is a highly conserved segment comprising two distinct domains (Fig. 1c). The more conserved of these, the Dachshund homology domain (DHD; Kim et al. 2002), defines the *Ski* gene family, which includes *Ski* (Stavnezer et al. 1989; Sutrave and Hughes 1989; Wilson et al. 2004), *SnoN* (Nomura et al. 1989; Nyman et al. 2010; Pearson-White and Crittenden 1997), *Dach* (Davis et al. 1999; Hammond et al. 1998; Kozmik et al. 1999; Wu et al. 2003), *Fussel-15* (Arndt et al. 2007), *Fussel-18* (Arndt et al. 2005), and *Corl* (Mizuhara et al.

The authors thank the Swiss National Science Foundation, the Novartis Foundation (Novartis Stiftung für medizinisch-biologische Forschung), the Botnar Foundation, and Swiss Life (Jubiläumsstiftung für Medizinische Forschung) for financial support of their research projects.

C. Bonnon · S. Atanasoski (✉)
Institute of Physiology, Department of Biomedicine,
University of Basel,
Pestalozzistrasse 20,
CH-4056 Basel, Switzerland
e-mail: suzana.atanasoski@unibas.ch

Published online: 07 June 2011

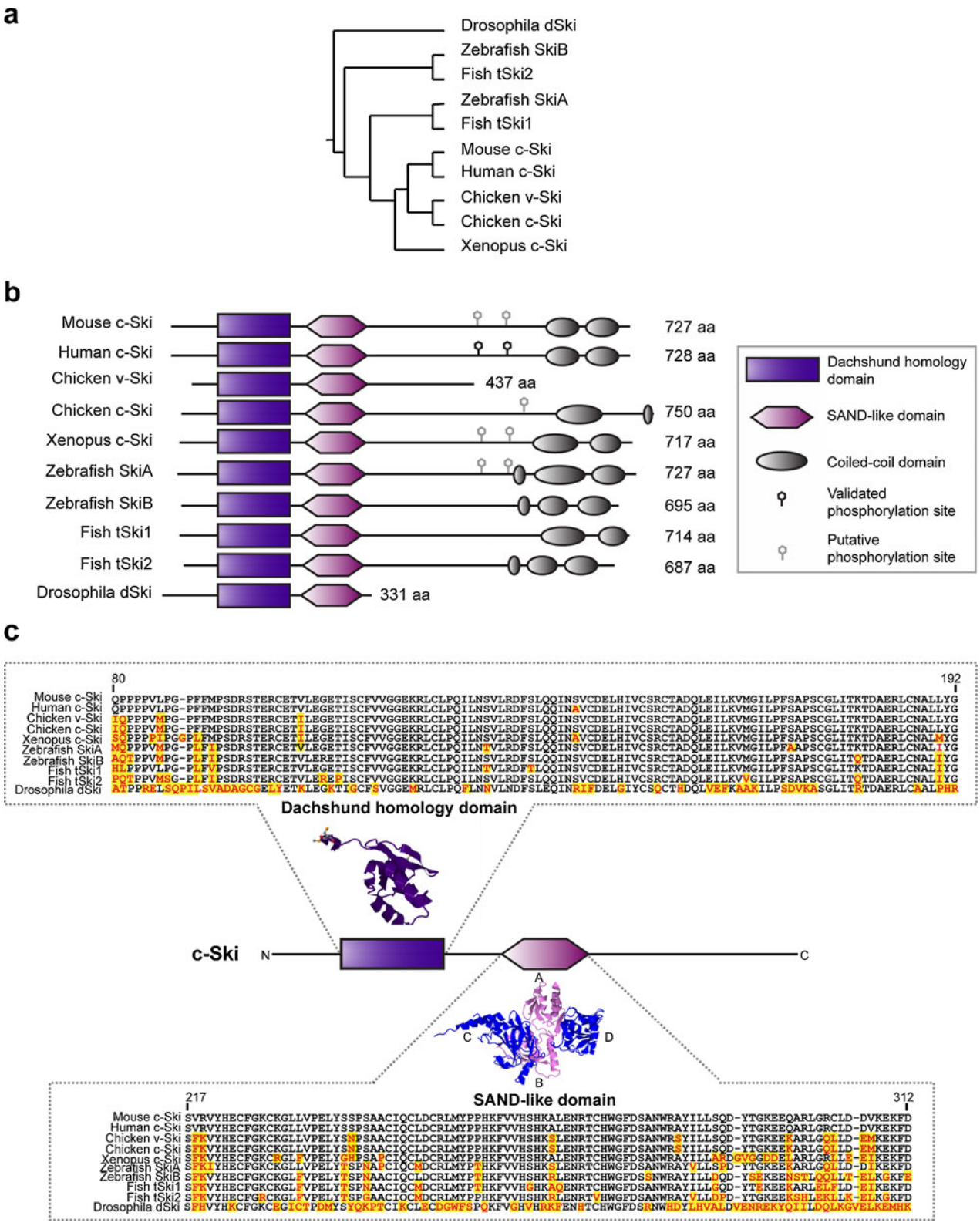
2005). Based on the analysis of the crystal structure, the DHD of human c-Ski adopts a mixed α/β structure (Wilson et al. 2004) that resembles features found in the forkhead/winged-helix family of DNA-binding proteins (Kim et al. 2002). However, striking differences are present in the surface properties between Ski-DHD and the DNA-binding surface described for the Dachshund protein, suggesting that c-Ski is unlikely to contact DNA via its DHD (Wilson et al. 2004). The second conserved region comprises the SAND domain (named after Sp100, AIRE-1, NucP41/75, and DEAF-1), which is an evolutionarily conserved sequence motif found in a number of nuclear proteins involved in chromatin-dependent transcriptional regulation (Wu et al. 2002). The SAND domain uses an interaction loop (I-loop) to interact with both protein and DNA. However, the lack of the critical DNA-binding motif in the Ski-SAND domain suggests that the I-loop in c-Ski is primarily a protein-binding segment (Wu et al. 2002). The carboxyl (C)-terminal half of c-Ski shows less sequence similarity among the family members (Fig. 1b). This region is predicted to contain an extended alpha-helical segment (coiled-coil domain) made up of a tandem repeat and a leucine zipper-like motif that cooperatively mediate both homodimerization of c-Ski and the formation of heterodimers with the related protein SnoN (Cohen et al. 1999; Heyman and Stavnezer 1994; Nagase et al. 1993; Sleeman and Laskey 1993; Zheng et al. 1997; Fig. 2). Ski and its closest family member SnoN share extensive sequence homology with similar domain structures. However, together with a certain degree of functional similarity, these closely related family members also display substantial functional and regulatory differences (for detailed reports on SnoN, see Jahchan and Luo 2010; Pot et al. 2010; structural and functional similarities/differences of c-Ski and SnoN have been reviewed by Deheuninck and Luo 2009; Luo 2004).

Cellular partners of c-Ski

Purified, bacterially synthesized, human c-Ski has been shown only to bind DNA cellulose when mixed with nuclear extracts of mammalian cultured cells (Nagase et al. 1990), suggesting that c-Ski binds DNA only in association with other proteins. The search for the unknown nuclear factors has led to the identification of a large number of transcription factors and transcriptional co-regulators that interact with distinct structural domains of c-Ski (Fig. 2). In yeast two-hybrid screens with *v-Ski* as bait, the Ski-interacting protein (Skip) has been identified and subsequently found to interact with the N-terminal region of c-Ski (Dahl et al. 1998b; Prathapam et al. 2001). Ishii and co-workers have used human *c-Ski* sequences as bait and found that c-Ski directly binds to the co-repressors N-CoR (nuclear receptor co-repressor), SMRT (silencing mediator for retinoid and thyroid hormone receptors), and mSin3A and forms a complex with histone deacetylase 1 (HDAC1; Nomura et al. 1999). A number of subsequent studies from the same laboratory have revealed that c-Ski also directly interacts with retinoblastoma protein (Rb; Tokitou et al. 1999), methyl-CpG-binding protein (MeCP2; Kokura et al. 2001), promyelocytic leukemia protein (PML; Khan et al. 2001), glioblastoma proteins (Gli; Dai et al. 2002), C184M protein (Harada et al. 2003), and co-repressor homeodomain-interacting protein kinase 2 (HIPK2; Harada et al. 2003). Moreover, work from other groups have established the direct binding of c-Ski to members of the nuclear factor I (NFI) family (Tarapore et al. 1997) and to the transcription factors c-Jun (Pessah et al. 2002) and PU.1 (Ueki et al. 2008). In addition, c-Ski has been shown to form protein complexes with p53 and the histone deacetylase SIRT1 (Inoue et al. 2011) and with the retinoic acid receptor (RARα; Dahl et al. 1998a; Ritter et al. 2006) and the vitamin D receptor (VDR; Ueki and Hayman 2003b). The most extensively investigated interactions, however, concern the association of c-Ski with Smad proteins (Akiyoshi et al. 1999; Luo et al. 1999; Sun et al. 1999a; Ueki and Hayman 2003a; Xu et al. 2000).

Taken together, most of the described interactions have been detected with purified, bacterially expressed proteins and have been further confirmed in cell lines under

Fig. 1 Phylogenic relationship and domain organization of the Ski protein. **a** Ski protein sequences from various species were aligned by using ClustalW (http://align.genome.jp/). Accession numbers used in this multiple aligment are as follows: *Drosophila* dSki (*D. melanogaster*): A0PVD0, zebrafish SkiB (*S. rerio*): Q9YI02, fish tSki2 (*I. tilapia*): Q9PU32, zebrafish SkiA (*S. rerio*): Q9YI03, fish tSki1 (*I. tilapia*): Q9PU31, mouse c-Ski (*M. musculus*): B1AUF1, human C-Ski (*H. sapiens*): P12755, chicken v-ski (*A. erythr. virus*): P17863, chicken c-ski (*G. gallus*): P49140, *Xenopus* c-Ski (*X. laevis*): Q02225. A dendrogram tree was then constructed from the aligned protein sequences. **b** Representations showing domain organization of the Ski proteins in various species. The Dachshund homology, the SAND-like, and the coil-coiled domains of the Ski proteins are shown. The amino-acid boundaries of each domain were retrieved from Pfam (http://pfam.sanger.ac.uk/). Phosphorylation sites of the human ski protein (described in Band et al. 2009; Nagata et al. 2010) and putative phosphorylation sites (predicted by using NetPhos 2.0 server) are indicated. Note that the short isoform of the *Drosophila* dSki is depicted. The length in amino-acids of each sequence is indicated *right*. **c** Ski sequences encoding the Dachshund homology domain and the SAND-like domain of each species were aligned by using ClustalW. *Highlighted* amino-acids indicate a difference compared with the amino-acids in the mouse c-Ski sequence. The amino-acid boundaries are indicated for the mouse c-Ski sequence. The crystal structure of the Dachshund homology domain of human Ski (Wilson et al. 2004) is indicated in *purple*. The crystal structure of a human Smad4-Ski complex (Wu et al. 2002) is also shown in which the Smad4 tumor suppressor C-terminal domain (chains C and D) is in *blue*, and the Smad4-binding domain of Ski (chains A and B) is in *pink*. Crystal structures generated by using the Jmol application were retrieved from the RCSB protein databank (http://www.pdb.org/pdb/home/home.do) by using the following PDB IDs: 1sbx (Dachshund homology domain of human Ski) and 1mr1 (Smad4-Ski complex)


a
Drosophila dSki
Zebrafish SkiB
Fish tSki2
Zebrafish SkiA
Fish tSki1
Mouse c-Ski
Human c-Ski
Chicken v-Ski
Chicken c-Ski
Xenopus c-Ski
b
Mouse c-Ski
Human c-Ski
Chicken v-Ski
Chicken c-Ski
Xenopus c-Ski
Zebrafish SkiA
Zebrafish SkiB
Fish tSki1
Fish tSki2
Drosophila dSki
727 aa
728 aa
437 aa
750 aa
717 aa
727 aa
695 aa
714 aa
687 aa
331 aa
Dachshund homology domain
SAND-like domain
Coiled-coil domain
Validated phosphorylation site
Putative phosphorylation site
c
80
192
Mouse c-Ski QPPPPVLPG-PFFMPSDRSTERCETVLEGETISCFVVGGEKRLCLPQILNSVLRDFSLQQINSVCDELHIVCSRCTADQLEILKVMGILPFSAPSCGLITKTDAERLCNALLYG
Human c-Ski QPPPPVLPG-PFFMPSDRSTERCETVLEGETISCFVVGGEKRLCLPQILNSVLRDFSLQQINAVCDELHIVCSRCTADQLEILKVMGILPFSAPSCGLITKTDAERLCNALLYG
Chicken v-Ski IQPPPVMPG-PFFMPSDRSTERCETILEGETISCFVVGGEKRLCLPQILNSVLRDFSLQQINSVCDELHIVCSRCTADQLEILKVMGILPFSAPSCGLITKTDAERLCNALLYG
Chicken c-Ski IQPPPVMPG-PFFMPSDRSTERCETILEGETISCFVVGGEKRLCLPQILNSVLRDFSLQQINSVCDELHIVCSRCTADQLEILKVMGILPFSAPSCGLITKTDAERLCNALLYG
Xenopus c-Ski SQPPPPIPGGPLFMPSDRSTERCETILEGETISCFVVGGEKRLCLPQILNSVLRDFSLQQINAVCDELHIVCSRCTADQLEILKVMGILPFSAPSCGLITKTDAERLCNALMYG
Zebrafish SkiA MQPPPVMPG-PLFIPSDRSTERCETVLEGETISCFVVGGEKRLCLPQILNTVLRDFSLQQINSVCDELHIVCSRCTADQLEILKVMGILPFAAPSCGLITKTDAERLCNALIYG
Zebrafish SkiB AQTPPVMPG-PLFIPSDRSTERCETVLERETISCFVVGGEKRLCLPQILNSVLRDFSLQQINSVCDELHIVCSRCTADQLEILKVMGILPFSAPSCGLITQTDAERLCNALIYG
Fish tSki1 HLPPPVLPG-PLFVPSDRSTERCETVLEGETISCFVVGGEKRLCLPQILNTVLRDFTLQQINSVCDELHIVCSRCTADQLEILKVMGILPFSAPSCGLITKTDAERLCNALIYG
Fish tSki2 PQTPPVMSG-PLFIPSDRSTERCETVLERETISCFVVGGEKRLCLPQILNSVLRDFSLQQINSVCDELHIVCSRCTADQLEILKVVGILPFSAPSCGLITQTDAERLCNALIYG
Drosophila dSki ATPPRELSQPILSVADAGCGELYETKLEGKTIGCFSVGGEMRLCLPQFLNNVLNDFSLEQINRIFDELGIYCSQCTHDQLVEFKAAKILPSDVKASGLITRTDAERLCAALPHR
Dachshund homology domain
c-Ski
N
C
A
B
C
D
217
312
SAND-like domain
Mouse c-Ski SVRVYHECFGKCKGLLVPELYSSPSAACIQCLDCRLMYPPHKFVVHSHKALENRTCHWGFDSANWRAYILLSQD-YTGKEEQARLGRCLD-DVKEKFD
Human c-Ski SVRVYHECFGKCKGLLVPELYSSPSAACIQCLDCRLMYPPHKFVVHSHKALENRTCHWGFDSANWRAYILLSQD-YTGKEEQARLGRCLD-DVKEKFD
Chicken v-Ski SFKVYHECFGKCKGLLVPELYSNPSAACIQCLDCRLMYPPHKFVVHSHKSLENRTCHWGFDSANWRSYILLSQD-YTGKEEKARLGQLLD-EMKEKFD
Chicken c-Ski SFKVYHECFGKCKGLLVPELYSNPSAACIQCLDCRLMYPPHKFVVHSHKSLENRTCHWGFDSANWRSYILLSQD-YTGKEEKARLGQLLD-EMKEKFD
Xenopus c-Ski SFKVYHECFGKCPGLFVPELYGHPSAPCIQCLDCRLMYPPHKFVVHSHKALENRTCHWGFDSANWRAYILLARDGVGGDDELARLGRLLE-EIKEKFD
Zebrafish SkiA SFKIYHECFGKCKGLLVPELYTSPNAPCIQCMDCRLMYPTHKFVVHSHKSLENRTCHWGFDSANWRAYVLLSPD-YTGKEEKARLGQLLD-DIKEKFD
Zebrafish SkiB SFKVYHECFGKCKGLFVPELYTSPNAACIQCLDCRLMYPTHKFVVHSHKRLENRTCHWGFDSSNWRAYILLDQD-YSEKEENSTLQQLLT-ELKGKFE
Fish tSki1 SFKVYHECFGKCKGLFVPELYTSPNAACIQCMDCRLMYPTHKFVVHGHKAQENRTCHWGFDSANWRAYILLGQD-YTEKEEKARLELFLD-EIKEKFD
Fish tSki2 SFKVYHECFGRCKGLFVPELYTSPGAACIQCMDCRLMYPPHKFVVHSHKRLENRTVHWGFDSANWRAYVLLDPD-YTGKEEKSHLEKLLK-ELKGKFD
Drosophila dSki SFHVYHKCFGKCEGICTPDMYSYQKPTCIKCLECDGWFSPQKFVGHVHRKFENTTCHWGFDSRNWHDYLHVALDVENREKYQIILDQLKGVELKEMHK

overexpression conditions (Fig. 2). The challenging task in the future will be to dissect which interactions with known and not yet identified cellular partners take place in vivo under physiological conditions, in distinct cell-types, tissues, and organs, both during embryonic development and in adult organisms.

c-Ski function in signaling pathways

c-Ski acts in combination with a number of cellular partners and, thus, has been shown to be involved in many signaling pathways, including those mediated by transforming growth factor β (TGFβ), nuclear hormone receptors, and Sonic hedgehog (Shh).

TGFβ signaling

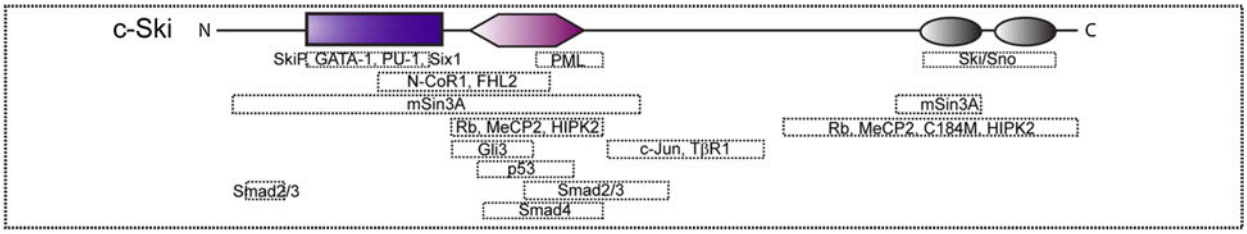
The best-characterized function of c-Ski is to regulate TGFβ signaling negatively via interaction with Smad proteins. Binding of TGFβ to its receptor serine/threonine kinases results in the phosphorylation of the receptor-regulated Smad2 and Smad3 (R-Smad) proteins. The phosphorylated R-Smads then form heteromeric complexes with a common mediator Smad4 (Co-Smad). Together, they translocate into the nucleus, where they bind to DNA and activate the transcription of target genes. The inhibitory Smad6 and Smad7 (I-Smad) proteins block R-Smad phosphorylation, and eventually TGFβ signaling, through a negative feedback loop. A number of mechanisms have been described that may contribute to the repression of Smad function by c-Ski (Fig. 3). Initially, concomitant studies converged on the conclusion that c-Ski binds to activated Smad2/3/4 heteromeric complexes and represses their ability to activate TGFβ target genes (Akiyoshi et al. 1999; Luo et al. 1999; Sun et al. 1999a; Suzuki et al. 2004; Ueki and Hayman 2003a; Xu et al. 2000). R-Smads and Co-Smad proteins have been demonstrated to bind to different regions in c-Ski (Fig. 2a; Akiyoshi et al. 1999; Luo et al. 1999; Qin et al. 2002), and mutations of both regions markedly impair the ability of c-Ski to repress TGFβ-induced transcriptional activation (He et al. 2003). Akiyoshi et al. (1999) have reported that c-Ski exclusively associates with TGFβ/activin-specific regulatory Smad2 and Smad3, but not with bone morphogenetic protein (BMP)-specific regulatory Smad1 and Smad5 proteins. These results differ from those of other groups demonstrating that c-Ski also interacts weakly with the BMP-Smads and represses BMP signaling (Barrio et al. 2007; Harada et al. 2003; Wang et al. 2000). Subsequent studies have shown that the interaction of Ski with Smad4 is indispensable for the suppression of BMP but not TGFβ signaling (Takeda et al. 2004). Analysis of the crystal structure of a Ski fragment bound to Smad4 has identified the Smad4-binding domain of c-Ski as a structural homolog of the SAND-like domain (Wu et al. 2002; Fig. 1b, c). This study has led to the proposal that c-Ski and the R-Smads compete for mutually exclusive binding to Smad4/R-Smad complexes (Wu et al. 2002), suggesting that the Smad complex is disrupted or inactivated in the presence of c-Ski (Ueki and Hayman 2003a; Wu et al. 2002). In these first reports, c-Ski was proposed to recruit co-repressors, such as HDAC1 (Akiyoshi et al. 1999) and N-CoR (Luo et al. 1999) to Smad complexes to mediate transcriptional repression of TGFβ-induced signaling. However, subsequent studies with a Ski mutant lacking N-CoR binding instead suggest that the c-Ski/N-CoR interaction is not essential for the repression of TGFβ signaling (Ueki and Hayman 2003b). Further findings have revealed that c-Ski competes with co-activators, such as CREB-binding protein (CBP) and p300 for binding to Smad3, supporting the evidence that c-Ski plays an essential role in influencing co-repressor and co-activator activities on target promoters (Akiyoshi et al. 1999; Chen et al. 2007). A number of reports have described additional modes of c-Ski actions. For example, the C184M protein induces the cytoplasmic accumulation of c-Ski, and the C184M/c-Ski complex negatively regulates TGFβ signaling by inhibiting the nuclear translocation of Smad2 (Kokura et al. 2003). Other regulatory roles assigned to c-Ski are the prevention of the phosphorylation of Smad2/3, thereby silencing Smad signaling (Prunier et al. 2003), or the direct binding to TGFβ receptor I (TβRI) and possibly interference with nuclear translocation of the R-Smad/Smad4 complex (Ferrand et al. 2010). Moreover, c-Ski/c-Jun protein complexes have been reported to suppress Smad2 transcriptional activity, thereby participating in negative feedback regulation of TGFβ signaling by the c-Jun N-terminal kinase (JNK) cascade (Pessah et al. 2002). In gastric cancer cells, the association of c-Ski with another co-repressor, MEL1 (MDS1/EVI1-like gene 1), has been demonstrated to inhibit TGFβ signaling by stabilizing the inactive c-Ski/Smad3 complex on target genes (Takahata et al. 2009). Finally, c-Ski has been shown to inhibit the expression of the inhibitory *Smad7* by associating with Smad proteins, HDAC3, and protein arginine methyltransferase 5 (PRMT5) on the *Smad7* gene promoter (Band et al. 2009; Denissova and Liu 2004; Tabata et al. 2009).

Fig. 2 Cellular partners of c-Ski. **a** Ski domains required for association with the indicated partner proteins are depicted. **b** Summary of the binding partners of the Ski protein. Ski-binding domains implicated in direct interaction are indicated but are otherwise mentioned as "complex" when no direct binding has been established

Nuclear hormone receptor signaling

In addition to modulating the TGFβ pathway, c-Ski appears to play a major role in nuclear hormone receptor signaling

a



b

Partners	Description	Binding to Ski	Reference
Homo/hetero-dimers			
Sno/Ski	Ski related novel/proto-oncogene c-Ski	Coiled-coil region	Sleeman et al. 1993; Nagase et al. 1993; Heyman et al. 1994; Zheng et al. 1997 Cohen et al. 1999
Co-repressors/Chromatin remodeling factors			
HDAC1	Histone deacetylase 1	Complex	Nomura et al. 1999
N-CoR1	Nuclear hormone receptor corepressor 1	99-274; L110P	Nomura et al. 1999; Ueki and Hayman 2003b
N-CoR2=SMRT	Nuclear receptor corepressor 2	FL	Nomura et al. 1999
mSin3A	Histone deacetylase complex subunit	N-terminal half + coiled-coil region	Nomura et al. 1999
HDAC3	Histone deacetylase 3	Complex	Zhao et al. 2009
MeCP2	Methyl CpG binding protein 2	197-330, 556-728	Kokura et al. 2001
PRMT5	Protein arginine methyl transferase 5	Complex	Tabata et al. 2009
SIRT-1	NAD-dependent deacetylase sirtuin 1	Complex	Inoue et al. 2011
HIPK2	Homeodomain interacting protein kinase 2	197-330, 556-728	Harada et al. 2003
Eya3	Eyes absent homolog 3	Complex	Zhang and Stavnezer 2009
Transcription factors			
Smad2/3	Mothers against decapentaplegic homolog 2/3	241-441; 338-490; 17-45; 17-212 L19A, L110P	Luo et al. 1999; Akiyoshi et al. 1999; Qin et al. 2002; Wu et al. 2002 Ueki and Hayman 2003a
Smad4	Mothers against decapentaplegic homolog 4	203-239; 1-309; 200-323; W255E	Luo et al. 1999; Akiyoshi et al. 1999; Wu et al. 2002; Ueki and Hayman 2003a
NF1	Nuclear factor 1	FL	Tarapore et al. 1997
PML	Promyelocytic leukemia protein	261-330	Khan et al. 2001
Gli2/Gli3	Glioblastoma protein 2/3	FL/197-261	Dai et al. 2002
c-Jun=AP-1	Activator protein 1	338-490	Pessah et al. 2002
GATA-1	GATA binding factor 1	Complex	Ueki et al. 2004
PU-1	31 kDa-transforming protein	22-186, L110P	Ueki et al. 2008
Six1	Sine oculis homeobox homolog 1	Complex	Zhang and Stavnezer 2009
MEL1	MDS1/EVI1-like gene 1	Complex	Takahata et al. 2009
Tumor suppressors			
p53	Tumor suppressor p53	Complex	Inoue et al. 2011
Rb	Retinoblastoma protein	197-330, 556-728	Tokitou et al. 1999
Mad	Max dimerization protein	Complex	Nomura et al. 1999
Hormone receptors			
RARα	Retinoid acid receptor α	FL; L110P	Dahl et al. 1998a; Ritter et al. 2006
TRβ	Thyroid hormone receptor β	Complex	Nomura et al. 1999
VDR	Vitamin D receptor	Complex	Ueki and Hayman 2003b
Others			
Skip	Ski interacting protein	79-185	Dahl et al. 1998b
FHL2	Four and a half LIM domains protein 2	Complex	Chen et al. 2003
C184M	C184M	556-728	Kokura et al. 2003
α-tubulin	α-tubulin	Complex	Marcelain and Hayman 2005
Arkadia	E3 ubiquitin ligase	Complex	Nagano et al. 2007
TβR1	Transforming growth factor β receptor 1	338-490	Ferrand et al. 2010
Siah2	Seven in absentia homolog 2	Complex	Zhao et al. 2010

via its established direct interaction with the co-repressors N-CoR/SMRT and mSin3A and by its association with HDAC1 (Nomura et al. 1999). Members of the nuclear hormone receptor family, such as thyroid hormone receptor (TR), RAR, and VDR, regulate gene expression in a ligand-dependent fashion, thereby actively repressing the transcription of target genes in the absence of ligand. The repressor function of TR is mediated by protein complexes including N-CoR/SMRT, mSin3A, HDAC, and another repressor, Mad, which bind to the ligand-binding domain of TR to enable transcriptional repression. Nomura et al. (1999) have discovered that the presence of c-Ski is required for the negative transcriptional activity of Mad and TRβ on target sequences, such as the gene for ornithine decarboxylase. Similarly, c-Ski has been shown to associate with the RAR and VDR complex, including N-CoR/SMRT and HDAC, and represses transcription from a retinoic acid response element (Dahl et al. 1998a; Khan et al. 2001; Zhao et al. 2009, 2010) and a VDR-responsive promoter (Ueki and Hayman 2003b), respectively. In support of this finding, a mutant c-Ski unable to bind N-CoR was no longer capable of repressing RARα (Ritter et al. 2006) or vitamin D (Ueki and Hayman 2003b) function. Notably, c-Ski/N-CoR interaction appears to be essential for the repression of nuclear hormone receptor signaling but not TGFβ signaling (Ueki and Hayman 2003b). Thus, depending on the signaling pathway, c-Ski appears to use distinct modes of repression, through N-CoR-dependent and -independent mechanisms.

Shh signaling

c-Ski has also been reported to bind directly to Gli proteins (Dai et al. 2002). Members of the Gli family of transcriptional regulators (Gli1, Gli2, and Gli3) are the intracellular mediators of Shh signaling. Shh upregulates *Gli1* transcription but downregulates Gli3 expression. Molecular analysis has revealed that Gli3 is processed into a repressor form that suppresses the *Gli1* promoter. Dai et al. (2002) have demonstrated that c-Ski interacts with the repressor domain located in the N-terminal region of Gli3, recruits the HDAC complex, and inhibits Shh-induced *Gli1* gene transcription; this study has established that the presence of c-Ski is mandatory for transcriptional repression mediated by the repressor form of Gli3.

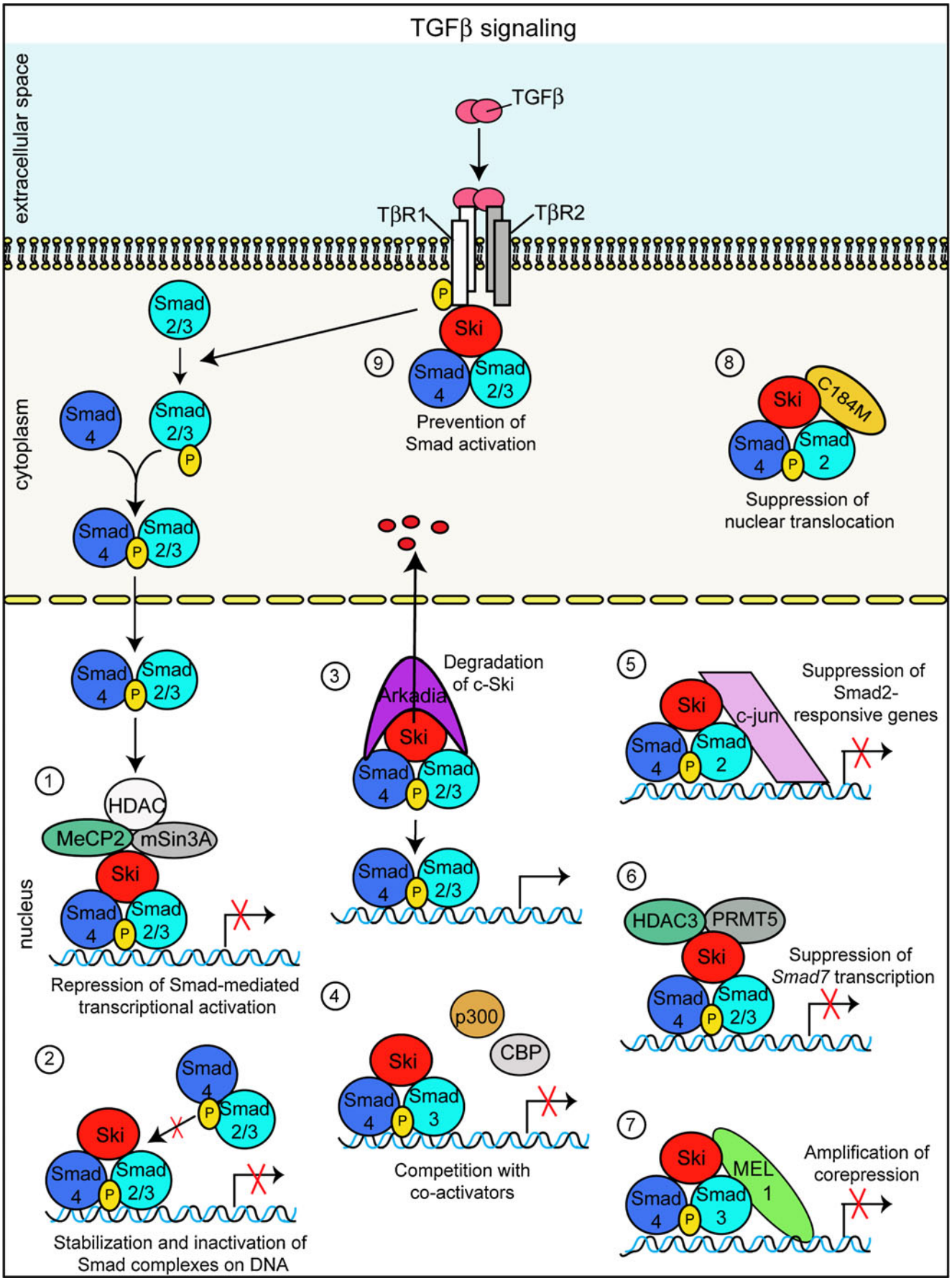
Summary of c-Ski interactions in signaling pathways

The above-described paradigms demonstrate the direct and indirect interactions of c-Ski with transcription factors (e.g., Smads, Gli proteins) including tumor suppressors (e.g., Rb, Mad), with co-repressors including chromatin remodeling factors (e.g., N-CoR/SMRT, mSin3A, HDACs), and with nuclear hormone receptors (e.g., TRβ, RARα, VDR) to regulate various signaling pathways (Fig. 2). Thus, numerous findings have established c-Ski as an essential molecule that links transcription factors and nuclear receptors to co-repressor complexes or that stabilizes and enhances the activity of repressor complexes on DNA sequences. Furthermore, c-Ski not only acts as a co-repressor, but also seems to employ additional mechanisms for transcriptional regulation, such as preventing transcription factors from binding to DNA (i.e., GATA1, p53; Inoue et al. 2011; Ueki et al. 2004) or promoting transcription of target genes as a co-activator of NFI or the four-and-a-half LIM domain protein 2 (LIM-only protein FHL2) (Chen et al. 2003; Tarapore et al. 1997). Notably, c-Ski has also been shown to localize at centrosomes and the mitotic spindle, suggesting that it might play a role during mitosis, in addition to its known role in transcription (Marcelain and Hayman 2005).

Fig. 3 Various mechanisms of modulation of TGFβ signaling by c-Ski (for an explanation of abbreviations see Fig. 2b). *1* c-Ski negatively regulates TGFβ signaling by repressing Smad-mediated transcriptional activation through its interaction with mSin3A, MeCP2, and HDAC (Akiyoshi et al. 1999; Luo et al. 1999; Sun et al. 1999a; Xu et al. 2000; Ueki and Hayman 2003a). *2* c-Ski inhibits the TGF-β signaling pathway through stabilization of inactive Smad complexes on Smad-binding elements; this in turn blocks the access of newly activated Smad complexes to DNA (Suzuki et al. 2004; Ueki and Hayman 2003a). *3* Arkadia-mediated degradation of Ski allows active phospho-Smads binding to DNA (Nagano et al. 2007). *4* c-Ski competes with the co-activators CBP and p300 proteins for binding to Smad3 (Akiyoshi et al. 1999; Chen et al. 2007). *5* c-Ski/c-jun complexes suppress Smad2 transcriptional activity (Pessah et al. 2002). *6* c-Ski suppresses the expression of the inhibitory Smad7 by associating with the Smad proteins, HDAC3 and PRMT5 (Denissova and Liu 2004; Band et al. 2009; Tabata et al. 2009). *7* MEL1 interacts with c-Ski in gastric cancer cells and cooperates to inhibit TGFβ signaling by enhancing the Smad3-Ski interaction and by promoting the binding of Smad3 to the Smad-binding DNA element (Takahata et al. 2009). *8* The C184M protein induces the cytoplasmic accumulation of c-Ski, and the C184M-Ski complex negatively regulates TGFβ signaling by inhibiting the nuclear translocation of the Smads (Kokura et al. 2003). *9* The interaction of c-Ski with TβRI suppresses nuclear accumulation of the Smads complex; this occurs as a consequence of the sequestration of this complex by TβRI (Ferrand et al. 2010)

c-Ski in cancer progression

c-Ski was originally discovered as the cellular homolog of the retroviral v-Ski and was thus classified as a proto-oncogene. Indeed, subsequent studies showed that the overexpression of c-Ski promoted the oncogenic transformation of avian fibroblasts (Colmenares et al. 1991a). Supporting this notion, protein expression of c-Ski is elevated in many human cancer cells and tissues (Fig. 4), including those obtained from patients suffering from esophageal squamous cell


TGFβ signaling
extracellular space
cytoplasm
nucleus
TGFβ
TβR1
TβR2
P
Smad 2/3
Smad 4
Ski
9
Prevention of Smad activation
8
C184M
Smad 2
Suppression of nuclear translocation
3
Degradation of c-Ski
Arkadia
5
c-jun
Suppression of Smad2-responsive genes
1
HDAC
MeCP2
mSin3A
Repression of Smad-mediated transcriptional activation
6
HDAC3
PRMT5
Suppression of Smad7 transcription
4
p300
CBP
Smad 3
Competition with co-activators
2
Stabilization and inactivation of Smad complexes on DNA
7
MEL 1
Amplification of corepression

carcinoma (Fukuchi et al. 2004), Barrett's esophagus (Villanacci et al. 2008), leukemia (Ritter et al. 2006), colorectal cancer (Bravou et al. 2009), gastric cancer (Takahata et al. 2009), pancreatic cancer (Heider et al. 2007; Wang et al. 2009), hemangiomas (O et al. 2009), and melanoma (Boone et al. 2009; Reed et al. 2001). Further evidence supporting the pro-oncogenic function of c-Ski in tumor progression comes from studies of various human cancer cell lines (Fig. 4b). For example, the overexpression of c-Ski in diffuse-type gastric carcinoma cells inhibits TGFβ signaling and promotes extensive growth of subcutaneous xenografts in nude mice (Kiyono et al. 2009). In agreement, the knockdown of c-Ski restores TGFβ responsiveness in these cells and reduces tumor growth in vivo (Takahata et al. 2009). Similarly, the inhibition of c-Ski through RNA interference increases TGFβ signaling and growth inhibition in pancreatic cell lines (Heider et al. 2007; Wang et al. 2009) and melanoma (Chen et al. 2009; Reed et al. 2001). TGFβ is not the only pathway that is disturbed because of aberrant c-Ski expression levels in tumors. Studies of leukemia cell lines have revealed that the overexpression of c-Ski inhibits the pro-differentiating effects of RARα signaling in acute myeloid leukemia (Ritter et al. 2006), and work with melanoma cells has demonstrated that c-Ski stimulates growth by associating with the transcriptional modulators FHL2 and β-catenin and by activating the Wnt signaling pathway (Chen et al. 2003). Further, a recent study has reported that c-Ski interacts with p53 and attenuates the biological functions of this tumor suppressor (Inoue et al. 2011). Interestingly, c-Ski has been found to bind the histone deacetylase SIRT1 and stabilize the p53-SIRT1 interaction to promote p53 deacetylation, which subsequently decreases the DNA-binding activity of p53 (Inoue et al. 2011). These results, however, contradict findings proposing that c-Ski acts as a tumor suppressor in some cancer cells (Le Scolan et al. 2008). In support of these results, the loss of one copy of *c-Ski* leads to increased susceptibility to carcinogen-induced tumors in mice (Shinagawa et al. 2001). Further, c-Ski represses the oncogenic activation of c-Myb (Nomura et al. 2004) and acts as a co-repressor of tumor suppressors such as Rb (Tokitou et al. 1999) and Mad (Nomura et al. 1999).

In conclusion, the physiological levels of c-Ski appear to protect cells from transformation, whereas high expression levels of c-Ski in cancer cells and tumors promote growth by modulating antiproliferative (e.g., TGFβ, RAR) and proliferative (e.g., Wnt) signals. Additionally, aberrant cellular localization might contribute to the pro-oncogenic function of c-Ski. Originally, c-Ski was identified as a nuclear protein (Barkas et al. 1986; Colmenares et al. 1991a; Sutrave et al. 1990a), and more recent work has identified a nuclear localization signal in human c-Ski (Nagata et al. 2006). However, in tumor cells of virtually all analyzed specimens, c-Ski is reported to localize also to the cytoplasm, which might enhance its growth-promoting properties (Bravou et al. 2009; Fukuchi et al. 2004; Heider et al. 2007; Reed et al. 2001; Villanacci et al. 2008). Mechanisms employed by cytoplasmic c-Ski, such as the promotion of cell proliferation by sequestering the tumor suppressor Rb to the cytoplasm (Jacob et al. 2008) or the inhibition of TGFβ signaling by interfering with its cytoplasmic intermediates (Ferrand et al. 2010; Kokura et al. 2003; Prunier et al. 2003), have been proposed (Fig. 3). Whether such modes of action also take place in tumor cells remains to be seen. The mechanisms leading to elevated c-Ski expression in cancerous cells, however, continue to be elusive. Indeed, no data is available on the transcriptional regulation of c-Ski. Possibly the degradation pathways affecting c-Ski expression levels (Le Scolan et al. 2008; Macdonald et al. 2004; Nagano et al. 2007; Sun et al. 1999b) are differently regulated in tumor cells, contributing to the high expression levels of c-Ski (Nagano et al. 2010). Further, an amplification of the *Ski* gene locus, as discovered in colorectal cancer cells, might also account for the higher expression levels in other types of tumors (Buess et al. 2004). Alternatively, extracellular and intracellular cues that are aberrantly active in tumor tissue might lead to a deregulation of c-Ski protein expression.

Fig. 4 c-Ski and cancer. **a** Summary showing human tumor tissues in which c-Ski expression is elevated. **b** Summary of the effects of the over-expression or knock-down (*KD*) of c-Ski on TGFβ (*yellow*), RARα (*blue*), Wnt (*pink*), and p53 (*green*) signaling in various human cancer cell lines

c-Ski function during normal development

c-Ski transcripts are widely distributed in various species and organs, and based on extensive in vivo studies, critical roles in muscle and nervous system development have been attributed to c-Ski function. In *Xenopus*, *c-Ski* transcripts are maternally regulated and present throughout early development (Sleeman and Laskey 1993). Overexpression of *Xenopus c-Ski* RNA in embryos results in cell-autonomous induction of secondary neural axis formation and neural-specific gene expression in ectodermal explants (Amaravadi et al. 1997; Wang et al. 2000). In zebrafish, overexpression of *SkiA* or *SkiB* disrupts gastrulation and results in a dorsalized phenotype. In particular, this study suggests a role of c-Ski proteins in neural patterning (Kaufman et al. 2000). In mouse, *c-Ski* transcripts are detectable in most embryonic tissues at low levels, with the highest *c-Ski* message expression being found in brain and lung (Lyons et al. 1994; Namciu et al. 1995). Accordingly, mice lacking c-Ski display highly pleiotropic defects including aberrant neural, ocular, and muscle development (Berk et al. 1997; McGannon et al. 2006; Shinagawa and Ishii 2003). Pups suffer from exencephaly attributable to

a

Elevated Ski in human tumors	Reference
Esophageal squamous cell carcinoma	Fukuchi et al. 2004
Barrett's esophagus	Villanacci et al. 2008
Leukemia	Ritter et al. 2006
Colorectal carcinoma	Bravou et al. 2009
Gastric carcinoma	Takahata et al. 2009
Pancreatic carcinoma	Heider et al. 2007; Wang et al. 2009
Hemangioma	O et al. 2009
Melanoma	Reed et al. 2001; Boone et al. 2009

b

Human cancer cell line	Ski expression	Effects	Reference
Gastric carcinoma (OCUM-2MLN)	over-expression	TGFβ signaling inhibited Increase in tumor growth and angiogenesis	Kiyono et al. 2009
Gastric carcinoma (MKN-7, MKN-28)	KD	TGFβ response restored Reduction of tumor growth	Takahata et al. 2009
Pancreatic adeno-carcinoma (PANC-1)	KD	TGFβ signaling increased Reduction of tumor growth	Heider et al. 2007
Pancreatic adeno-carcinoma (SW-1990, BxPC-3)	KD	TGFβ signaling increased Reduction of tumor growth Altered metastasis, angiogenesis & cell proliferation	Wang et al. 2009
Cutaneous melanoma (IIB-MEL-J, UCD-MEL-N, A-375)	KD	TGFβ signaling increased Inhibition of tumor growth	Reed et al. 2001
Metastatic breast carcinoma (IDA-MB-231) Lung adeno-carcinoma (A549)	KD	Enhancement of tumor metastasis TGFβ-induced degradation of Ski	Le Scolan et al. 2008
Melanoma (UCD-MEL-N, A-375)	KD	Prevention of the growth of melanoma xenografts by restoring active TGFβ growth inhibitory signals	Chen et al. 2009
Leukemic monocyte lymphoma (U937)	over-expression	Pro-differentiation effects of RARα signaling inhibited	Ritter et al. 2006
Melanoma (UCD-MEL-N, A-375)	over-expression	Stimulation of growth by FHL2/β-catenin active Wnt signaling	Chen et al. 2003
Lung carcinoma (H1299)	over-expression	Reduction of p53 activity by p53-SIRT1 interaction	Inoue et al. 2011
Breast adeno-carcinoma (MCF-7)	KD	Increase of p53 activity	Inoue et al. 2011

failed neural tube closure and die perinatally. The penetrance of the phenotypes is strain-dependent, yielding mutant fetuses that display facial clefting and other craniofacial and skeletal abnormalities of varying severity (Colmenares et al. 2002). Additionally, we have shown that myelinating Schwann cells, the main glial cells of the peripheral nervous system, express c-Ski protein in vivo, and that, in c-Ski-deficient sciatic nerves, Schwann cells fail to upregulate genes encoding myelin components (Atanasoski et al. 2004). Consistent with these findings, loss of c-Ski prevents myelination in vitro, whereas enforced c-Ski expression promotes the expression of myelin-related genes in Schwann cells (Atanasoski et al. 2004). c-Ski-deficient mice also show a strong decrease in skeletal muscle mass (Berk et al. 1997). Conversely, transgenic mice that express high levels of ectopic c-Ski in skeletal muscle display hypertrophy of fast skeletal muscle fibers (Lana et al. 1996; Leferovich et al. 1995; Sutrave et al. 1990b, 2000). Previous studies have indicated the essential function of c-Ski in myogenesis. Overexpression of c-Ski in cultured avian fibroblasts induces terminal differentiation of muscle cells (Colmenares and Stavnezer 1989), and ectopic expression of c-Ski in various muscle cell lines triggers the expression of muscle-specific genes (Colmenares et al. 1991b; Engert et al. 1995; Ichikawa et al. 1997; Kobayashi et al. 2007; Zhang and Stavnezer 2009). In addition to muscle and neuronal lineages, c-Ski modulates the differentiation of hematopoietic cells (Dahl et al. 1998a; Pearson-White et al. 1995; Ueki et al. 2004, 2008) and fibroblasts (Cunnington et al. 2011; Jinnin et al. 2007; Liu et al. 2008; Marcelain et al. 2011) in vitro. Notably, the expression of c-Ski is altered under many pathological conditions, including demyelinated or injured peripheral nerves (Atanasoski et al. 2004), wound healing (Li et al. 2011; Liu et al. 2010), liver regeneration (Macias-Silva et al. 2002), and skeletal muscle regeneration (Soeta et al. 2001). Finally, the symptoms of the human genetic disorder 1p36 syndrome mirror the phenotypes of Ski-deficient mice, suggesting that the deletion of the *c-Ski* gene located on chromosome 1p36.3 is partially responsible for the disease (Colmenares et al. 2002; Rosenfeld et al. 2010).

In summary, these findings suggest a cell-type specific function for c-Ski with regard to the regulation of proliferation and differentiation processes during normal development. Based on the large set of available biochemical data, the physiological role of c-Ski might be closely related to the presence or absence of interaction partners at distinct developmental stages, controlling cell type-specific downstream target genes. In addition, the function of c-Ski within a given cell type might even vary during the course of development, because of environmental changes caused by temporally and regionally restricted expression of signaling cues, including members of the TGFβ family, retinoic acid, Wnt, or Shh.

Future directions

Numerous studies in healthy and diseased cells have made evident that the expression levels of c-Ski strongly impact the molecular and cellular machinery of distinct cell types. Thus, detailed analyses of the spatial and temporal distribution of the c-Ski protein during organogenesis and in the adult will be mandatory to discover cell-type and stage-specific expression patterns. Such information will provide a basis for elucidating the complex physiological roles of c-Ski. The low expression levels of endogenous c-Ski protein and/or the lack of suitable anti-Ski antibodies have greatly hampered such progress. Novel tools and strategies will have to be developed to improve our knowledge of endogenous c-Ski expression and of candidate partners in a context-dependent manner. Moreover, in view of the large range of proposed cell-type-specific functions for c-Ski, new animal models will have to be generated to enable conditional and cell-type-specific c-Ski ablation. Such approaches will greatly facilitate the dissection of the cell-autonomous functions of c-Ski in health and disease.

Acknowledgements The authors thank Ned Mantei for critical reading of the manuscript.

References

Akiyoshi S, Inoue H, Hanai J, Kusanagi K, Nemoto N, Miyazono K, Kawabata M (1999) c-Ski acts as a transcriptional co-repressor in transforming growth factor-beta signaling through interaction with smads. J Biol Chem 274:35269–35277

Amaravadi LS, Neff AW, Sleeman JP, Smith RC (1997) Autonomous neural axis formation by ectopic expression of the protooncogene c-ski. Dev Biol 192:392–404

Arndt S, Poser I, Schubert T, Moser M, Bosserhoff AK (2005) Cloning and functional characterization of a new Ski homolog, Fussel-18, specifically expressed in neuronal tissues. Lab Invest 85:1330–1341

Arndt S, Poser I, Moser M, Bosserhoff AK (2007) Fussel-15, a novel Ski/Sno homolog protein, antagonizes BMP signaling. Mol Cell Neurosci 34:603–611

Atanasoski S, Notterpek L, Lee HY, Castagner F, Young P, Ehrengruber MU, Meijer D, Sommer L, Stavnezer E, Colmenares C, Suter U (2004) The protooncogene Ski controls Schwann cell proliferation and myelination. Neuron 43:499–511

Band AM, Bjorklund M, Laiho M (2009) The phosphatidylinositol 3-kinase/Akt pathway regulates transforming growth factor-{beta} signaling by destabilizing ski and inducing Smad7. J Biol Chem 284:35441–35449

Barkas AE, Brodeur D, Stavnezer E (1986) Polyproteins containing a domain encoded by the V-SKI oncogene are located in the nuclei of SKV-transformed cells. Virology 151:131–138

Barrio R, Lopez-Varea A, Casado M, Celis JF de (2007) Characterization of dSnoN and its relationship to decapentaplegic signaling in *Drosophila*. Dev Biol 306:66–81

Berk M, Desai SY, Heyman HC, Colmenares C (1997) Mice lacking the ski proto-oncogene have defects in neurulation, craniofacial, patterning, and skeletal muscle development. Genes Dev 11:2029–2039

Boone B, Haspeslagh M, Brochez L (2009) Clinical significance of the expression of c-Ski and SnoN, possible mediators in TGF-beta resistance, in primary cutaneous melanoma. J Dermatol Sci 53:26–33

Bravou V, Antonacopoulou A, Papadaki H, Floratou K, Stavropoulos M, Episkopou V, Petropoulou C, Kalofonos H (2009) TGF-beta repressors SnoN and Ski are implicated in human colorectal carcinogenesis. Cell Oncol 31:41–51

Buess M, Terracciano L, Reuter J, Ballabeni P, Boulay JL, Laffer U, Metzger U, Herrmann R, Rochlitz C (2004) Amplification of SKI is a prognostic marker in early colorectal cancer. Neoplasia 6:207–212

Chen D, Xu W, Bales E, Colmenares C, Conacci-Sorrell M, Ishii S, Stavnezer E, Campisi J, Fisher DE, Ben-Ze'ev A, Medrano EE (2003) SKI activates Wnt/beta-catenin signaling in human melanoma. Cancer Res 63:6626–6634

Chen D, Lin Q, Box N, Roop D, Ishii S, Matsuzaki K, Fang T, Hornyak TJ, Reed JA, Stavnezer E, Timchenko NA, Medrano EE (2009) SKI knockdown inhibits human melanoma tumor growth in vivo. Pigment Cell Melanoma Res (in press)

Chen W, Lam SS, Srinath H, Schiffer CA, Royer WE Jr, Lin K (2007) Competition between Ski and CREB-binding protein for binding to Smad proteins in transforming growth factor-beta signaling. J Biol Chem 282:11365–11376

Cohen SB, Zheng G, Heyman HC, Stavnezer E (1999) Heterodimers of the SnoN and Ski oncoproteins form preferentially over homodimers and are more potent transforming agents. Nucleic Acids Res 27:1006–1014

Colmenares C, Stavnezer E (1989) The ski oncogene induces muscle differentiation in quail embryo cells. Cell 59:293–303

Colmenares C, Sutrave P, Hughes SH, Stavnezer E (1991a) Activation of the c-ski oncogene by overexpression. J Virol 65:4929–4935

Colmenares C, Teumer JK, Stavnezer E (1991b) Transformation-defective v-ski induces MyoD and myogenin expression but not myotube formation. Mol Cell Biol 11:1167–1170

Colmenares C, Heilstedt HA, Shaffer LG, Schwartz S, Berk M, Murray JC, Stavnezer E (2002) Loss of the SKI proto-oncogene in individuals affected with 1p36 deletion syndrome is predicted by strain-dependent defects in Ski−/− mice. Nat Genet 30:106–109

Cunnington RH, Wang B, Ghavami S, Bathe KL, Rattan SG, Dixon IM (2011) Antifibrotic properties of c-Ski and its regulation of cardiac myofibroblast phenotype and contractility. Am J Physiol Cell Physiol 300:C176–C186

Dahl R, Kieslinger M, Beug H, Hayman MJ (1998a) Transformation of hematopoietic cells by the Ski oncoprotein involves repression of retinoic acid receptor signaling. Proc Natl Acad Sci USA 95:11187–11192

Dahl R, Wani B, Hayman MJ (1998b) The Ski oncoprotein interacts with Skip, the human homolog of *Drosophila* Bx42. Oncogene 16:1579–1586

Dai P, Shinagawa T, Nomura T, Harada J, Kaul SC, Wadhwa R, Khan MM, Akimaru H, Sasaki H, Colmenares C, Ishii S (2002) Ski is involved in transcriptional regulation by the repressor and full-length forms of Gli3. Genes Dev 16:2843–2848

Davis RJ, Shen W, Heanue TA, Mardon G (1999) Mouse Dach, a homologue of *Drosophila* dachshund, is expressed in the developing retina, brain and limbs. Dev Genes Evol 209:526–536

Deheuninck J, Luo K (2009) Ski and SnoN, potent negative regulators of TGF-beta signaling. Cell Res 19:47–57

Denissova NG, Liu F (2004) Repression of endogenous Smad7 by Ski. J Biol Chem 279:28143–28148

Engert JC, Servaes S, Sutrave P, Hughes SH, Rosenthal N (1995) Activation of a muscle-specific enhancer by the Ski proto-oncogene. Nucleic Acids Res 23:2988–2994

Ferrand N, Atfi A, Prunier C (2010) The oncoprotein c-ski functions as a direct antagonist of the transforming growth factor-{beta} type I receptor. Cancer Res 70:8457–8466

Fukuchi M, Nakajima M, Fukai Y, Miyazaki T, Masuda N, Sohda M, Manda R, Tsukada K, Kato H, Kuwano H (2004) Increased expression of c-Ski as a co-repressor in transforming growth factor-beta signaling correlates with progression of esophageal squamous cell carcinoma. Int J Cancer 108:818–824

Grimes HL, Szente BE, Goodenow MM (1992) C-ski cDNAs are encoded by eight exons, six of which are closely linked within the chicken genome. Nucleic Acids Res 20:1511–1516

Grimes HL, Ambrose MR, Goodenow MM (1993) C-ski transcripts with and without exon 2 are expressed in skeletal muscle and throughout chick embryogenesis. Oncogene 8:2863–2868

Hammond KL, Hanson IM, Brown AG, Lettice LA, Hill RE (1998) Mammalian and *Drosophila* dachshund genes are related to the Ski proto-oncogene and are expressed in eye and limb. Mech Dev 74:121–131

Harada J, Kokura K, Kanei-Ishii C, Nomura T, Khan MM, Kim Y, Ishii S (2003) Requirement of the co-repressor homeodomain-interacting protein kinase 2 for ski-mediated inhibition of bone morphogenetic protein-induced transcriptional activation. J Biol Chem 278:38998–39005

He J, Tegen SB, Krawitz AR, Martin GS, Luo K (2003) The transforming activity of Ski and SnoN is dependent on their ability to repress the activity of Smad proteins. J Biol Chem 278:30540–30547

Heider TR, Lyman S, Schoonhoven R, Behrns KE (2007) Ski promotes tumor growth through abrogation of transforming growth factor-beta signaling in pancreatic cancer. Ann Surg 246:61–68

Heyman HC, Stavnezer E (1994) A carboxyl-terminal region of the ski oncoprotein mediates homodimerization as well as hetero-dimerization with the related protein SnoN. J Biol Chem 269:26996–27003

Huang CJ, Lin JY, Tsai HJ (1999) Two distinct c-ski cDNAs of fish, tilapia (*Oreochromis aurea*). Mol Reprod Dev 54:223–231

Ichikawa K, Nagase T, Ishii S, Asano A, Mimura N (1997) Trans-regulation of myogenin promoter/enhancer activity by c-ski during skeletal-muscle differentiation: the C-terminus of the c-Ski protein is essential for transcriptional regulatory activity in myotubes. Biochem J 328:607–613

Inoue Y, Iemura S, Natsume T, Miyazawa K, Imamura T (2011) Suppression of p53 activity through the cooperative action of Ski and histone deacetylase SIRT1. J Biol Chem 286:6311–6320

Jacob C, Grabner H, Atanasoski S, Suter U (2008) Expression and localization of Ski determine cell type-specific TGFbeta signaling effects on the cell cycle. J Cell Biol 182:519–530

Jahchan NS, Luo K (2010) SnoN in mammalian development, function and diseases. Curr Opin Pharmacol 10:670–675

Jinnin M, Ihn H, Mimura Y, Asano Y, Tamaki K (2007) Involvement of the constitutive complex formation of c-Ski/SnoN with Smads in the impaired negative feedback regulation of transforming growth factor beta signaling in scleroderma fibroblasts. Arthritis Rheum 56:1694–1705

Kaufman CD, Martinez-Rodriguez G, Hackett PB Jr (2000) Ectopic expression of c-ski disrupts gastrulation and neural patterning in zebrafish. Mech Dev 95:147–162

Khan MM, Nomura T, Kim H, Kaul SC, Wadhwa R, Shinagawa T, Ichikawa-Iwata E, Zhong S, Pandolfi PP, Ishii S (2001) Role of PML and PML-RARalpha in Mad-mediated transcriptional repression. Mol Cell 7:1233–1243

Kim SS, Zhang RG, Braunstein SE, Joachimiak A, Cvekl A, Hegde RS (2002) Structure of the retinal determination protein Dachshund reveals a DNA binding motif. Structure 10:787–795

Kiyono K, Suzuki HI, Morishita Y, Komuro A, Iwata C, Yashiro M, Hirakawa K, Kano MR, Miyazono K (2009) c-Ski overexpression promotes tumor growth and angiogenesis through inhibition of transforming growth factor-beta signaling in diffuse-type gastric carcinoma. Cancer Sci 100:1809–1816

Kobayashi N, Goto K, Horiguchi K, Nagata M, Kawata M, Miyazawa K, Saitoh M, Miyazono K (2007) c-Ski activates MyoD in the nucleus of myoblastic cells through suppression of histone deacetylases. Genes Cells 12:375–385

Kokura K, Kaul SC, Wadhwa R, Nomura T, Khan MM, Shinagawa T, Yasukawa T, Colmenares C, Ishii S (2001) The Ski protein family is required for MeCP2-mediated transcriptional repression. J Biol Chem 276:34115–34121

Kokura K, Kim H, Shinagawa T, Khan MM, Nomura T, Ishii S (2003) The Ski-binding protein C184M negatively regulates tumor growth factor-beta signaling by sequestering the Smad proteins in the cytoplasm. J Biol Chem 278:20133–20139

Kozmik Z, Pfeffer P, Kralova J, Paces J, Paces V, Kalousova A, Cvekl A (1999) Molecular cloning and expression of the human and mouse homologues of the *Drosophila* dachshund gene. Dev Genes Evol 209:537–545

Lana DP, Leferovich JM, Kelly AM, Hughes SH (1996) Selective expression of a ski transgene affects IIb fast muscles and skeletal structure. Dev Dyn 205:13–23

Le Scolan E, Zhu Q, Wang L, Bandyopadhyay A, Javelaud D, Mauviel A, Sun L, Luo K (2008) Transforming growth factor-beta suppresses the ability of Ski to inhibit tumor metastasis by inducing its degradation. Cancer Res 68:3277–3285

Leferovich JM, Lana DP, Sutrave P, Hughes SH, Kelly AM (1995) Regulation of c-ski transgene expression in developing and mature mice. J Neurosci 15:596–603

Li Y, Turck CM, Teumer JK, Stavnezer E (1986) Unique sequence, ski, in Sloan-Kettering avian retroviruses with properties of a new cell-derived oncogene. J Virol 57:1065–1072

Li P, Liu P, Xiong RP, Chen XY, Zhao Y, Lu WP, Liu X, Ning YL, Yang N, Zhou YG (2011) Ski, a modulator of wound healing and scar formation in the rat skin and rabbit ear. J Pathol 223:659–671

Liu X, Li P, Liu P, Xiong R, Zhang E, Chen X, Gu D, Zhao Y, Wang Z, Zhou Y (2008) The essential role for c-Ski in mediating TGF-beta1-induced bi-directional effects on skin fibroblast proliferation through a feedback loop. Biochem J 409:289–297

Liu X, Li P, Chen XY, Zhou YG (2010) c-Ski promotes skin fibroblast proliferation but decreases type I collagen: implications for wound healing and scar formation. Clin Exp Dermatol 35:417–424

Ludolph DC, Neff AW, Parker MA, Mescher AL, Smith RC, Malacinski GM (1995) Cloning and expression of the axolotl proto-oncogene ski. Biochim Biophys Acta 1260:102–104

Luo K (2004) Ski and SnoN: negative regulators of TGF-beta signaling. Curr Opin Genet Dev 14:65–70

Luo K, Stroschein SL, Wang W, Chen D, Martens E, Zhou S, Zhou Q (1999) The Ski oncoprotein interacts with the Smad proteins to repress TGFbeta signaling. Genes Dev 13:2196–2206

Lyons GE, Micales BK, Herr MJ, Horrigan SK, Namciu S, Shardy D, Stavnezer E (1994) Protooncogene c-ski is expressed in both proliferating and postmitotic neuronal populations. Dev Dyn 201:354–365

Macdonald M, Wan Y, Wang W, Roberts E, Cheung TH, Erickson R, Knuesel MT, Liu X (2004) Control of cell cycle-dependent degradation of c-Ski proto-oncoprotein by Cdc34. Oncogene 23:5643–5653

Macias-Silva M, Li W, Leu JI, Crissey MA, Taub R (2002) Up-regulated transcriptional repressors SnoN and Ski bind Smad proteins to antagonize transforming growth factor-beta signals during liver regeneration. J Biol Chem 277:28483–28490

Marcelain K, Hayman MJ (2005) The Ski oncoprotein is upregulated and localized at the centrosomes and mitotic spindle during mitosis. Oncogene 24:4321–4329

Marcelain K, Armisen R, Aguirre A, Ueki N, Toro J, Colmenares C, Hayman MJ (2011) Chromosomal instability in mouse embryonic fibroblasts null for the transcriptional co-repressor Ski. J Cell Physiol (in press)

McGannon P, Miyazaki Y, Gupta PC, Traboulsi EI, Colmenares C (2006) Ocular abnormalities in mice lacking the Ski proto-oncogene. Invest Ophthalmol Vis Sci 47:4231–4237

Mizuhara E, Nakatani T, Minaki Y, Sakamoto Y, Ono Y (2005) Corl1, a novel neuronal lineage-specific transcriptional corepressor for the homeodomain transcription factor Lbx1. J Biol Chem 280:3645–3655

Nagano Y, Mavrakis KJ, Lee KL, Fujii T, Koinuma D, Sase H, Yuki K, Isogaya K, Saitoh M, Imamura T, Episkopou V, Miyazono K, Miyazawa K (2007) Arkadia induces degradation of SnoN and c-Ski to enhance transforming growth factor-beta signaling. J Biol Chem 282:20492–20501

Nagano Y, Koinuma D, Miyazawa K, Miyazono K (2010) Context-dependent regulation of the expression of c-Ski protein by Arkadia in human cancer cells. J Biochem 147:545–554

Nagase T, Mizuguchi G, Nomura N, Ishizaki R, Ueno Y, Ishii S (1990) Requirement of protein co-factor for the DNA-binding function of the human ski proto-oncogene product. Nucleic Acids Res 18:337–343

Nagase T, Nomura N, Ishii S (1993) Complex formation between proteins encoded by the ski gene family. J Biol Chem 268:13710–13716

Nagata M, Goto K, Ehata S, Kobayashi N, Saitoh M, Miyoshi H, Imamura T, Miyazawa K, Miyazono K (2006) Nuclear and cytoplasmic c-Ski differently modulate cellular functions. Genes Cells 11:1267–1280

Nagata M, Nagata S, Yuki K, Isogaya K, Saitoh M, Miyazono K, Miyazawa K (2010) Identification of a phosphorylation site in c-Ski as serine 515. J Biochem 148:423–427

Namciu S, Lyons GE, Micales BK, Heyman HC, Colmenares C, Stavnezer E (1995) Enhanced expression of mouse c-ski accompanies terminal skeletal muscle differentiation in vivo and in vitro. Dev Dyn 204:291–300

Nomura N, Sasamoto S, Ishii S, Date T, Matsui M, Ishizaki R (1989) Isolation of human cDNA clones of ski and the ski-related gene, sno. Nucleic Acids Res 17:5489–5500

Nomura T, Khan MM, Kaul SC, Dong HD, Wadhwa R, Colmenares C, Kohno I, Ishii S (1999) Ski is a component of the histone deacetylase complex required for transcriptional repression by Mad and thyroid hormone receptor. Genes Dev 13:412–423

Nomura T, Tanikawa J, Akimaru H, Kanei-Ishii C, Ichikawa-Iwata E, Khan MM, Ito H, Ishii S (2004) Oncogenic activation of c-Myb correlates with a loss of negative regulation by TIF1beta and Ski. J Biol Chem 279:16715–16726

Nyman T, Tresaugues L, Welin M, Lehtio L, Flodin S, Persson C, Johansson I, Hammarstrom M, Nordlund P (2010) The crystal structure of the Dachshund domain of human SnoN reveals flexibility in the putative protein interaction surface. PLoS ONE 5:e12907

O TM, Tan M, Tarango M, Fink L, Mihm M, Ma Y, Waner M (2009) Differential expression of SKI oncogene protein in hemangiomas. Otolaryngol Head Neck Surg 141:213–218

Pearson-White S, Crittenden R (1997) Proto-oncogene Sno expression, alternative isoforms and immediate early serum response. Nucleic Acids Res 25:2930–2937

Pearson-White S, Deacon D, Crittenden R, Brady G, Iscove N, Quesenberry PJ (1995) The ski/sno protooncogene family in hematopoietic development. Blood 86:2146–2155

Pessah M, Marais J, Prunier C, Ferrand N, Lallemand F, Mauviel A, Atfi A (2002) c-Jun associates with the oncoprotein Ski and suppresses Smad2 transcriptional activity. J Biol Chem 277:29094–29100

Pot I, Ikeuchi Y, Bonni A, Bonni S (2010) SnoN: bridging neurobiology and cancer biology. Curr Mol Med 10:667–673

Prathapam T, Kuhne C, Hayman M, Banks L (2001) Ski interacts with the evolutionarily conserved SNW domain of Skip. Nucleic Acids Res 29:3469–3476

Prunier C, Pessah M, Ferrand N, Seo SR, Howe P, Atfi A (2003) The oncoprotein Ski acts as an antagonist of transforming growth factor-beta signaling by suppressing Smad2 phosphorylation. J Biol Chem 278:26249–26257

Qin BY, Lam SS, Correia JJ, Lin K (2002) Smad3 allostery links TGF-beta receptor kinase activation to transcriptional control. Genes Dev 16:1950–1963

Reed JA, Bales E, Xu W, Okan NA, Bandyopadhyay D, Medrano EE (2001) Cytoplasmic localization of the oncogenic protein Ski in human cutaneous melanomas in vivo: functional implications for transforming growth factor beta signaling. Cancer Res 61:8074–8078

Ritter M, Kattmann D, Teichler S, Hartmann O, Samuelsson MK, Burchert A, Bach JP, Kim TD, Berwanger B, Thiede C, Jager R, Ehninger G, Schafer H, Ueki N, Hayman MJ, Eilers M, Neubauer A (2006) Inhibition of retinoic acid receptor signaling by Ski in acute myeloid leukemia. Leukemia 20:437–443

Rosenfeld JA, Crolla JA, Tomkins S, Bader P, Morrow B, Gorski J, Troxell R, Forster-Gibson C, Cilliers D, Hislop RG, Lamb A, Torchia B, Ballif BC, Shaffer LG (2010) Refinement of causative genes in monosomy 1p36 through clinical and molecular cytogenetic characterization of small interstitial deletions. Am J Med Genet A 152A:1951–1959

Shinagawa T, Ishii S (2003) Generation of Ski-knockdown mice by expressing a long double-strand RNA from an RNA polymerase II promoter. Genes Dev 17:1340–1345

Shinagawa T, Nomura T, Colmenares C, Ohira M, Nakagawara A, Ishii S (2001) Increased susceptibility to tumorigenesis of ski-deficient heterozygous mice. Oncogene 20:8100–8108

Sleeman JP, Laskey RA (1993) *Xenopus* c-ski contains a novel coiled-coil protein domain, and is maternally expressed during development. Oncogene 8:67–77

Soeta C, Suzuki M, Suzuki S, Naito K, Tachi C, Tojo H (2001) Possible role for the c-ski gene in the proliferation of myogenic cells in regenerating skeletal muscles of rats. Dev Growth Differ 43:155–164

Stavnezer E, Barkas AE, Brennan LA, Brodeur D, Li Y (1986) Transforming Sloan-Kettering viruses generated from the cloned v-ski oncogene by in vitro and in vivo recombinations. J Virol 57:1073–1083

Stavnezer E, Brodeur D, Brennan LA (1989) The v-ski oncogene encodes a truncated set of c-ski coding exons with limited sequence and structural relatedness to v-myc. Mol Cell Biol 9:4038–4045

Sun Y, Liu X, Eaton EN, Lane WS, Lodish HF, Weinberg RA (1999a) Interaction of the Ski oncoprotein with Smad3 regulates TGF-beta signaling. Mol Cell 4:499–509

Sun Y, Liu X, Ng-Eaton E, Lodish HF, Weinberg RA (1999b) SnoN and Ski protooncoproteins are rapidly degraded in response to transforming growth factor beta signaling. Proc Natl Acad Sci USA 96:12442–12447

Sutrave P, Hughes SH (1989) Isolation and characterization of three distinct cDNAs for the chicken c-ski gene. Mol Cell Biol 9:4046–4051

Sutrave P, Copeland TD, Showalter SD, Hughes SH (1990a) Characterization of chicken c-ski oncogene products expressed by retrovirus vectors. Mol Cell Biol 10:3137–3144

Sutrave P, Kelly AM, Hughes SH (1990b) Ski can cause selective growth of skeletal muscle in transgenic mice. Genes Dev 4:1462–1472

Sutrave P, Leferovich JM, Kelly AM, Hughes SH (2000) The induction of skeletal muscle hypertrophy by a ski transgene is promoter-dependent. Gene 241:107–116

Suzuki H, Yagi K, Kondo M, Kato M, Miyazono K, Miyazawa K (2004) c-Ski inhibits the TGF-beta signaling pathway through stabilization of inactive Smad complexes on Smad-binding elements. Oncogene 23:5068–5076

Tabata T, Kokura K, Ten Dijke P, Ishii S (2009) Ski co-repressor complexes maintain the basal repressed state of the TGF-beta target gene, SMAD7, via HDAC3 and PRMT5. Genes Cells 14:17–28

Takahata M, Inoue Y, Tsuda H, Imoto I, Koinuma D, Hayashi M, Ichikura T, Yamori T, Nagasaki K, Yoshida M, Matsuoka M, Morishita K, Yuki K, Hanyu A, Miyazawa K, Inazawa J, Miyazono K, Imamura T (2009) SKI and MEL1 cooperate to inhibit transforming growth factor-beta signal in gastric cancer cells. J Biol Chem 284:3334–3344

Takeda M, Mizuide M, Oka M, Watabe T, Inoue H, Suzuki H, Fujita T, Imamura T, Miyazono K, Miyazawa K (2004) Interaction with Smad4 is indispensable for suppression of BMP signaling by c-Ski. Mol Biol Cell 15:963–972

Tarapore P, Richmond C, Zheng G, Cohen SB, Kelder B, Kopchick J, Kruse U, Sippel AE, Colmenares C, Stavnezer E (1997) DNA binding and transcriptional activation by the Ski oncoprotein mediated by interaction with NFI. Nucleic Acids Res 25:3895–3903

Tokitou F, Nomura T, Khan MM, Kaul SC, Wadhwa R, Yasukawa T, Kohno I, Ishii S (1999) Viral ski inhibits retinoblastoma protein (Rb)-mediated transcriptional repression in a dominant negative fashion. J Biol Chem 274:4485–4488

Ueki N, Hayman MJ (2003a) Direct interaction of Ski with either Smad3 or Smad4 is necessary and sufficient for Ski-mediated repression of transforming growth factor-beta signaling. J Biol Chem 278:32489–32492

Ueki N, Hayman MJ (2003b) Signal-dependent N-CoR requirement for repression by the Ski oncoprotein. J Biol Chem 278:24858–24864

Ueki N, Zhang L, Hayman MJ (2004) Ski negatively regulates erythroid differentiation through its interaction with GATA1. Mol Cell Biol 24:10118–10125

Ueki N, Zhang L, Hayman MJ (2008) Ski can negatively regulate macrophage differentiation through its interaction with PU.1. Oncogene 27:300–307

Villanacci V, Bellone G, Battaglia E, Rossi E, Carbone A, Prati A, Verna C, Niola P, Morelli A, Grassini M, Bassotti G (2008) Ski/SnoN expression in the sequence metaplasia-dysplasia-adenocarcinoma of Barrett's esophagus. Hum Pathol 39:403–409

Wang P, Chen Z, Meng ZQ, Fan J, Luo JM, Liang W, Lin JH, Zhou ZH, Chen H, Wang K, Shen YH, Xu ZD, Liu LM (2009) Dual role of Ski in pancreatic cancer cells: tumor-promoting versus metastasis-suppressive function. Carcinogenesis 30:1497–1506

Wang W, Mariani FV, Harland RM, Luo K (2000) Ski represses bone morphogenic protein signaling in *Xenopus* and mammalian cells. Proc Natl Acad Sci USA 97:14394–14399

Wilson JJ, Malakhova M, Zhang R, Joachimiak A, Hegde RS (2004) Crystal structure of the dachshund homology domain of human SKI. Structure 12:785–792

Wu JW, Krawitz AR, Chai J, Li W, Zhang F, Luo K, Shi Y (2002) Structural mechanism of Smad4 recognition by the nuclear oncoprotein Ski: insights on Ski-mediated repression of TGF-beta signaling. Cell 111:357–367

Wu K, Yang Y, Wang C, Davoli MA, D'Amico M, Li A, Cveklova K, Kozmik Z, Lisanti MP, Russell RG, Cvekl A, Pestell RG

(2003) DACH1 inhibits transforming growth factor-beta signaling through binding Smad4. J Biol Chem 278:51673–51684

Xu W, Angelis K, Danielpour D, Haddad MM, Bischof O, Campisi J, Stavnezer E, Medrano EE (2000) Ski acts as a co-repressor with Smad2 and Smad3 to regulate the response to type beta transforming growth factor. Proc Natl Acad Sci USA 97:5924–5929

Zhang H, Stavnezer E (2009) Ski regulates muscle terminal differentiation by transcriptional activation of Myog in a complex with Six1 and Eya3. J Biol Chem 284:2867–2879

Zhao HL, Ueki N, Marcelain K, Hayman MJ (2009) The Ski protein can inhibit ligand induced RARalpha and HDAC3 degradation in the retinoic acid signaling pathway. Biochem Biophys Res Commun 383:119–124

Zhao HL, Ueki N, Hayman MJ (2010) The Ski protein negatively regulates Siah2-mediated HDAC3 degradation. Biochem Biophys Res Commun 399:623–628

Zheng G, Blumenthal KM, Ji Y, Shardy DL, Cohen SB, Stavnezer E (1997) High affinity dimerization by Ski involves parallel pairing of a novel bipartite alpha-helical domain. J Biol Chem 272:31855–31864