

c-Ski in health and disease

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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 TGF β 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; Suttrave 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; Suttrave 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.

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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

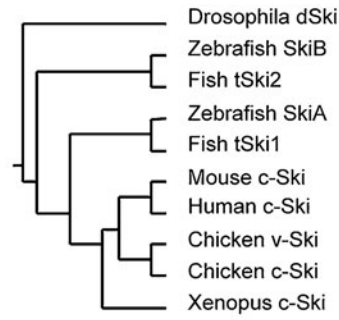
Purified, bacterially synthesized, human c-Ski has been shown only to bind DNA cellulose when mixed with nuclear extracts of mammalian cultured cells (Nagase et al. 1990), suggesting that c-Ski binds DNA only in association with other proteins. The search for the unknown nuclear factors has led to the 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),

Fig. 1 Phylogenetic 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 alignment 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 coiled-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)

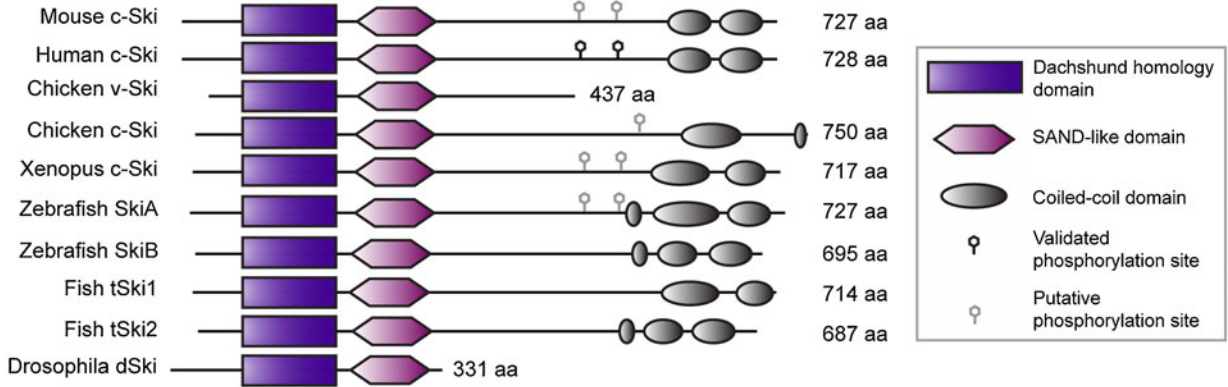
SMRT (silencing mediator for retinoid and thyroid hormone receptors), and mSin3A and forms a complex with histone deacetylase 1 (HDAC1; Nomura et al. 1999). A number of subsequent studies from the same laboratory have revealed that c-Ski also directly interacts with retinoblastoma protein (Rb; Tokitou 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 (RAR α ; 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

a



b



c

80 192

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    Mouse c-Ski QPPPPVLPG-PFFMPSDRSTERCETVLEGETISCFVVGGEKRLCLPQILNSVLRDFSLQOINAVCDELHIVCSRCTADOLEILKVMGILPFSAPSGLITKTAERLCNALLYG
    Human c-Ski QPPPPVLPG-PFFMPSDRSTERCETVLEGETISCFVVGGEKRLCLPQILNSVLRDFSLQOINAVCDELHIVCSRCTADOLEILKVMGILPFSAPSGLITKTAERLCNALLYG
    Chicken v-Ski QPPPPVMPG-PFFMPSDRSTERCETVLEGETISCFVVGGEKRLCLPQILNSVLRDFSLQOINAVCDELHIVCSRCTADOLEILKVMGILPFSAPSGLITKTAERLCNALLYG
    Chicken c-Ski QPPPPVMPG-PFFMPSDRSTERCETVLEGETISCFVVGGEKRLCLPQILNSVLRDFSLQOINAVCDELHIVCSRCTADOLEILKVMGILPFSAPSGLITKTAERLCNALLYG
    Xenopus c-Ski QPPPPVMPG-PFFMPSDRSTERCETVLEGETISCFVVGGEKRLCLPQILNSVLRDFSLQOINAVCDELHIVCSRCTADOLEILKVMGILPFSAPSGLITKTAERLCNALLYG
    Zebrafish SkiA QPPPPVMPG-PFFMPSDRSTERCETVLEGETISCFVVGGEKRLCLPQILNSVLRDFSLQOINAVCDELHIVCSRCTADOLEILKVMGILPFSAPSGLITKTAERLCNALLYG
    Zebrafish SkiB QPPPPVMPG-PFFMPSDRSTERCETVLEGETISCFVVGGEKRLCLPQILNSVLRDFSLQOINAVCDELHIVCSRCTADOLEILKVMGILPFSAPSGLITKTAERLCNALLYG
    Fish tSki1 QPPPPVMPG-PFFMPSDRSTERCETVLEGETISCFVVGGEKRLCLPQILNSVLRDFSLQOINAVCDELHIVCSRCTADOLEILKVMGILPFSAPSGLITKTAERLCNALLYG
    Fish tSki2 QPPPPVMPG-PFFMPSDRSTERCETVLEGETISCFVVGGEKRLCLPQILNSVLRDFSLQOINAVCDELHIVCSRCTADOLEILKVMGILPFSAPSGLITKTAERLCNALLYG
    Drosophila dSki AIPPEKLSQPIILSVADAGCGELYETKLEGTTCGFSVGGKRLCLPQILNSVLRDFSLQOINAVCDELHIVCSRCTADOLEILKVMGILPFSAPSGLITKTAERLCNALLYG
    
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Dachshund homology domain

c-Ski N C

SAND-like domain

217 312

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    Mouse c-Ski SVRVYHECFGRCKGLVPELYSSPSAACIQCLDCLRMYPHFKFVVHSHKALENRTCHWGFDSANWRAYILLSDQ-YTGKEEQARLGRCLD-DVKEKFD
    Human c-Ski SVRVYHECFGRCKGLVPELYSSPSAACIQCLDCLRMYPHFKFVVHSHKALENRTCHWGFDSANWRAYILLSDQ-YTGKEEQARLGRCLD-DVKEKFD
    Chicken v-Ski SVRVYHECFGRCKGLVPELYSSPSAACIQCLDCLRMYPHFKFVVHSHKALENRTCHWGFDSANWRAYILLSDQ-YTGKEEQARLGRCLD-DVKEKFD
    Chicken c-Ski SVRVYHECFGRCKGLVPELYSSPSAACIQCLDCLRMYPHFKFVVHSHKALENRTCHWGFDSANWRAYILLSDQ-YTGKEEQARLGRCLD-DVKEKFD
    Xenopus c-Ski SVRVYHECFGRCKGLVPELYSSPSAACIQCLDCLRMYPHFKFVVHSHKALENRTCHWGFDSANWRAYILLSDQ-YTGKEEQARLGRCLD-DVKEKFD
    Zebrafish SkiA SVRVYHECFGRCKGLVPELYSSPSAACIQCLDCLRMYPHFKFVVHSHKALENRTCHWGFDSANWRAYILLSDQ-YTGKEEQARLGRCLD-DVKEKFD
    Zebrafish SkiB SVRVYHECFGRCKGLVPELYSSPSAACIQCLDCLRMYPHFKFVVHSHKALENRTCHWGFDSANWRAYILLSDQ-YTGKEEQARLGRCLD-DVKEKFD
    Fish tSki1 SVRVYHECFGRCKGLVPELYSSPSAACIQCLDCLRMYPHFKFVVHSHKALENRTCHWGFDSANWRAYILLSDQ-YTGKEEQARLGRCLD-DVKEKFD
    Fish tSki2 SVRVYHECFGRCKGLVPELYSSPSAACIQCLDCLRMYPHFKFVVHSHKALENRTCHWGFDSANWRAYILLSDQ-YTGKEEQARLGRCLD-DVKEKFD
    Drosophila dSki SVRVYHECFGRCKGLVPELYSSPSAACIQCLDCLRMYPHFKFVVHSHKALENRTCHWGFDSANWRAYILLSDQ-YTGKEEQARLGRCLD-DVKEKFD
    
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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 (TGF β), nuclear hormone receptors, and Sonic hedgehog (Shh).

TGF β signaling

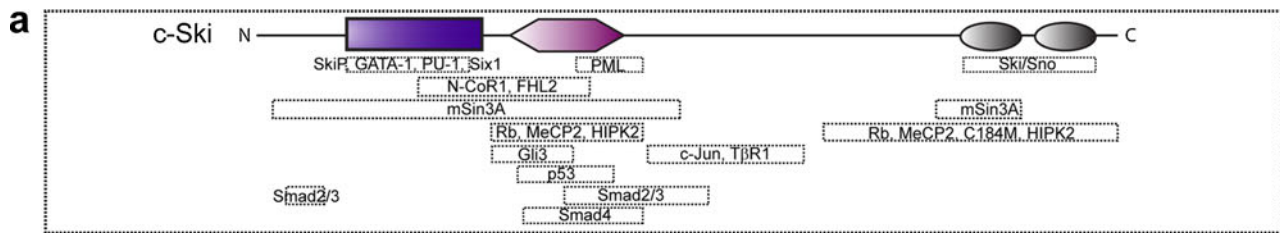
The best-characterized function of c-Ski is to regulate TGF β signaling negatively via interaction with Smad proteins. Binding of TGF β to its receptor serine/threonine kinases results in the phosphorylation of the receptor-regulated Smad2 and Smad3 (R-Smad) proteins. The phosphorylated R-Smads then 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 TGF β 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 TGF β 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 TGF β -induced transcriptional activation (He et al. 2003). Akiyoshi et al. (1999) have reported that c-Ski exclusively associates with TGF β /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 TGF β signaling (Takeda et al. 2004). Analysis of the crystal structure of a Ski fragment bound to Smad4 has identified the Smad4-binding domain of

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

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 TGF β -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 TGF β 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 TGF β 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 TGF β receptor I (T β RI) 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 TGF β 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 TGF β 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).

Nuclear hormone receptor signaling

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



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/EV11-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			
RAR α	Retinoid acid receptor α	FL; L110P	Dahl et al. 1998a; Ritter et al. 2006
TR β	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
T β R1	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 TR β 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 RAR α (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 TGF β 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

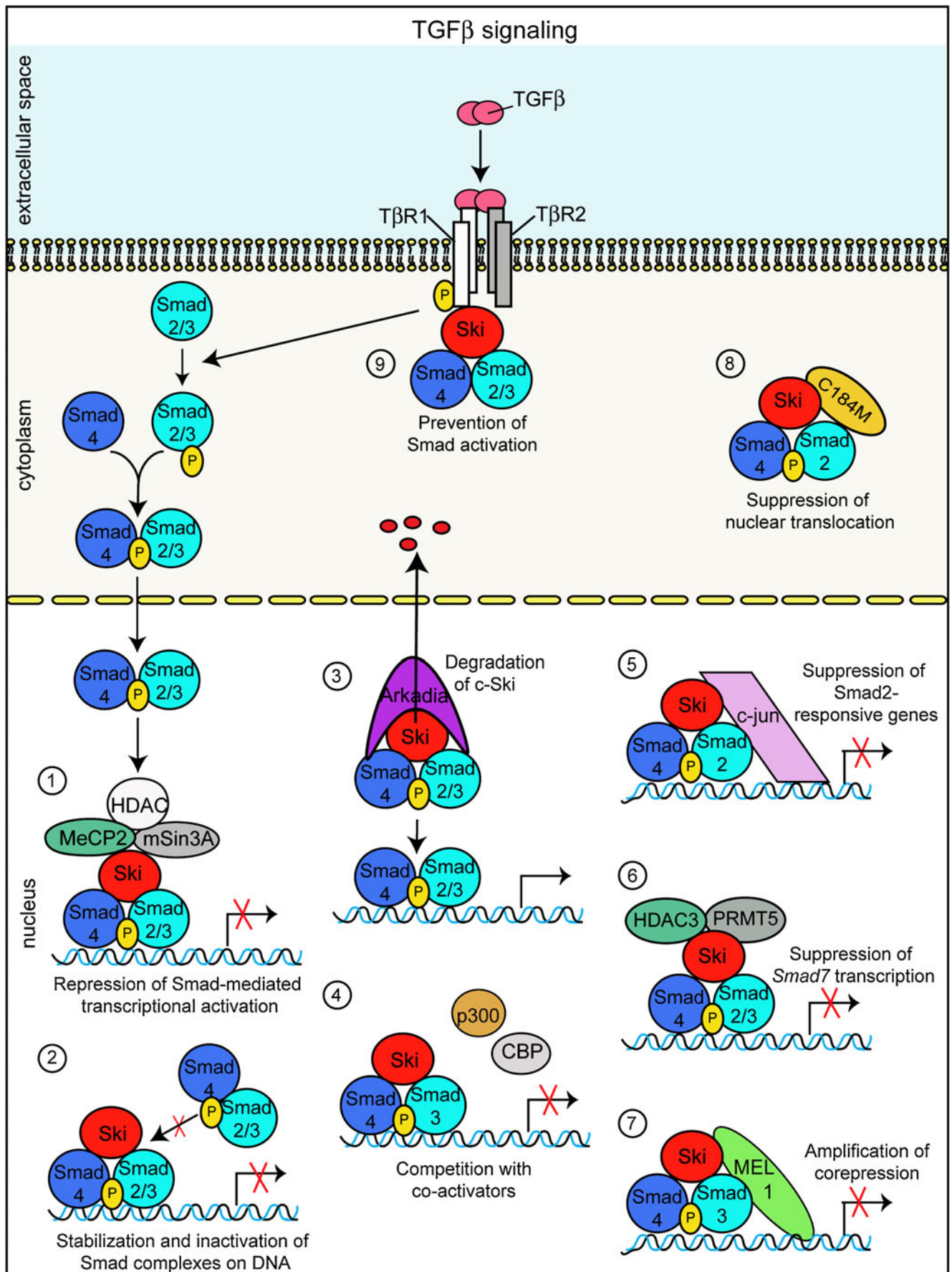
The above-described paradigms demonstrate the direct and indirect interactions of c-Ski with transcription factors (e.g., Smads, Gli proteins) including tumor suppressors (e.g., Rb, Mad), with co-repressors including chromatin remodeling factors (e.g., N-CoR/SMRT, mSin3A, HDACs), and with nuclear hormone receptors (e.g., TR β , RAR α , VDR) to

Fig. 3 Various mechanisms of modulation of TGF β signaling by c-Ski (for an explanation of abbreviations see Fig. 2b). 1 c-Ski negatively regulates TGF β 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 TGF β 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 TGF β signaling by inhibiting the nuclear translocation of the Smads (Kokura et al. 2003). 9 The interaction of c-Ski with T β RI suppresses nuclear accumulation of the Smads complex; this occurs as a consequence of the sequestration of this complex by T β RI (Ferrand et al. 2010)

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).

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



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 TGF β signaling and promotes extensive growth of subcutaneous xenografts in nude mice (Kiyono et al. 2009). In agreement, the knockdown of c-Ski restores TGF β responsiveness in these cells and reduces tumor growth in vivo (Takahata et al. 2009). Similarly, the inhibition of c-Ski through RNA interference increases TGF β 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). TGF β 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 RAR α 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., TGF β , 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

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 TGF β (*yellow*), RAR α (*blue*), Wnt (*pink*), and p53 (*green*) signaling in various human cancer cell lines

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 TGF β 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.

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	TGF β signaling inhibited Increase in tumor growth and angiogenesis	Kiyono et al. 2009
	Gastric carcinoma (MKN-7, MKN-28)	KD	TGF β response restored Reduction of tumor growth	Takahata et al. 2009
	Pancreatic adeno-carcinoma (PANC-1)	KD	TGF β signaling increased Reduction of tumor growth	Heider et al. 2007
	Pancreatic adeno-carcinoma (SW-1990, BxPC-3)	KD	TGF β 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	TGF β 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 TGF β -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 TGF β growth inhibitory signals	Chen et al. 2009
	Leukemic monocyte lymphoma (U937)	over-expression	Pro-differentiation effects of RAR α 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 TGF β 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.

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