Cancer and Metastasis Reviews **24:** 265–272, 2005.

SKI pathways inducing progression of human melanoma

Jon A. Reed[1,2], Qiushi Lin[3], Dahu Chen[3], I. Saira Mian[5] and Estela E. Medrano[2,3,4*]
[1]*Department of Pathology,* [2]*Department of Dermatology,* [3]*Huffington Center on Aging,* [4]*Departments of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX, USA;* [5]*Life Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA*

Key words: SKI, TGF-β, FHL2, β-catenin

Summary

The proteins SKI and SnoN are implicated in processes as diverse as differentiation, transformation and tumor progression. Until recently, SKI was solely viewed as a nuclear protein with a principal function of inhibiting TGF-β signaling through its association with the Smad proteins. However, new studies suggest that SKI plays additional roles not only inside but also outside the nucleus. In normal melanocytes and primary non-invasive melanomas, SKI localizes predominantly in the nucleus, whereas in primary invasive melanomas SKI displays both nuclear and cytoplasmic localization. Intriguingly, metastatic melanoma tumors display nuclear and cytoplasmic or predominantly cytoplasmic SKI distribution. Cytoplasmic SKI is functional, as it associates with Smad3 and prevents its nuclear localization mediated by TGF-β. SKI can also function as a transcriptional activator, targeting the β-catenin pathway and activating MITF and NrCAM, two proteins involved in survival, migration and invasion. Intriguingly, SKI appears to live a dual life, one as a tumor suppressor and another as a transforming protein. Loss of one copy of mouse *ski* increases susceptibility to tumorigenesis in mice, whereas its overexpression is associated with cancer progression of human melanoma, esophageal, breast and colon. The molecular reasons for such dramatic change in SKI function appear to result from new acquired activities. In this review, we discuss the mechanisms by which SKI regulates crucial pathways involved in the progression of human malignant melanoma.

1. The dual life of SKI as tumor suppressor and tumor promoter

The *ski* oncogene was originally identified as the transforming gene, v-ski, of the defective SKV avian carcinoma viruses (Reviewed in [1]). *ski* encodes a transcriptional co-regulator that induces both transformation and muscle differentiation in avian fibroblasts. Ski's biological duality is mirrored by its transcriptional activities: it coactivates or corepresses transcription depending on its interactions with other transcription factors and the cellular context [2]. The human SKI protein consists of 728 aminoacids (Figure 1). Structural domains include from the amino-terminus, a proline rich area (amino acids 61–89), helix-loop-helix motifs, a cysteine/histidine rich area, a region of basic amino acids, and a leucine zipper-like domain. SKI interacts with several proteins including Smad 2, 3 and 4, MeCP2, RB, the SKI-associated protein SKIP, mSin3, and N-CoR (reviewed in [3]. Additional protein partners include the homeodomain-interacting protein kinase 2 (HIPK-2), which represses BMP-induced transcriptional activation in association with Ski [4], and FHL2, a transcriptional regulator of the β-catenin signaling pathway [5,6].

The N-terminal region of SKI is common with v-ski and SnoN proteins. In particular, the Dachshund homology domain (DACH) consists of 100 amino acids that contain a conserved CLPQ motif. Crystallographic analysis of the DACH domain of SKI suggests that it is utilized for protein-protein interactions [7]. This region is involved in N-CoR, SKIP and FHL2 binding (Figure 1). Thus, SKI could potentially form alternative complexes with these proteins, and thus, multiply its repressor and activator activities.

*Corresponding author. E-mail: medrano@bcm.tmc.edu

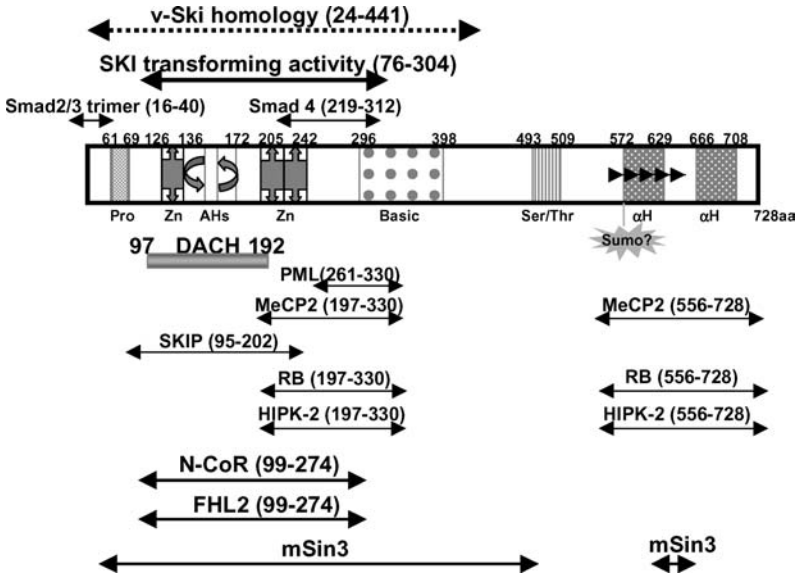

Figure 1. The SKI protein. Cartoon depicting motifs and domains required for different SKI protein-protein associations. Pro: a proline rich domain; Zn: a leucine zipper-like domain; AHs: helix-loop-helix motifs; Basic: a region of basic amino acids; αH: a unique tandem repeats of alpha helical domains which is involved in the dimerization of the SKI family through coiled-coil interactions. Arrowheads indicate three tandem repeats of 25 amino acids located at residues 572–645. SKI-HD: SKI homodimerization domain. SKI domains required for association with multiple proteins are indicated by a double arrow line. Lysine 572 localizes within a SUMOylation consensus site.

SKI protein levels correlate with human melanoma [8], esophageal squamous cell carcinoma [9] and cervical carcinoma [10] tumor progression. In addition, SKI levels are a prognostic marker in early colorectal cancer [11], whereas levels of the Ski-related novel protein N (SnoN) are a prognostic marker in estrogen receptor-positive breast carcinomas [12]. These results contradict recent findings proposing that SKI functions as a tumor suppressor. Loss of one copy of *ski* increases susceptibility to tumorigenesis in mice. In addition, mouse embryonic fibroblasts derived from $ski^{-/-}$ mice (MEFs) display increased proliferative capacity, whereas overexpression of Ski suppressed proliferation. The human *SKI* gene maps to a region close to the p73 tumor suppressor gene at the 1p36.3 locus, which is already known to contain multiple uncharacterized tumor suppressor genes. Recent evidence suggests that human SKI could also function as a tumor suppressor. For example, SKI represses the oncogenic activation of c-Myb [13], a transcription factor associated with myeloid leukemia [14] and cysplatin resistance in colon cancer [15]. In addition, Ski acts as a co-repressor of RB, a critical cell cycle regulator. However, high levels of SKI inactivate the repressor function of RB, an event strongly associated with human cancers. Together, these results suggest that high levels of SKI could result in the displacement of other proteins and/or association with proteins to which it normally does not bind, and in doing so, it changes its activity from tumor suppressor to a transforming protein.

But how are SKI levels regulated? SKI appears to be transcriptionally upregulated in human melanoma [16]. Considering that the subtelomeric localization of human SKI is conserved in chimpanzee, mouse, and rat homologues (data not shown), it is likely that this gene may be epigenetically regulated. Alternatively, or in addition, other transcriptional events and SKI protein stability may also be differentially regulated in normal versus tumor cells.

Subcellular localization, association with different protein partners and post-translational modifications can dramatically change protein function. A second level of regulation of SKI activity is its subcellular distribution. Until recently, SKI and the highly homologous protein snoN were solely viewed as nuclear proteins with a principal function of inhibiting TGF-β signaling through their association with the Smad proteins. In melanocytes and primary non-invasive melanomas, SKI localizes predominantly in the nucleus, whereas in primary invasive melanomas

SKI displays both nuclear and cytoplasmic localization. Furthermore, in metastatic melanoma tumors SKI localizes to both nuclear and cytoplasmic, or predominantly to the cytoplasmic compartment (Figure 2). Cytoplasmic SKI associates with Smad3 and prevents its nuclear localization mediated by TGF-β [8]. Apparently, the protein C184M is responsible for retaining SKI in the cytoplasm through the formation of complexes containing Smad proteins [17]. Thus, SKI plays additional roles outside the nucleus, most likely, through the formation of novel protein-protein associations.

2. SKI is necessary and sufficient for repressing TGF-β signaling in human melanomas

The TGF-β pathway usually functions to suppress cellular proliferation, and transformation. We and others have recently found that SKI associates with Smad2 and Smad3 and counteracts their activation of gene expression in response to TGF-β (reviewed in [3]). The SKI-interacting domains of Smad2 and Smad3 reside within the MH2 regions [18,19], which also contain their interaction with TGF-β receptors, oligomerization, interaction with histone acetyltransferases and transcription factors (Figure 3) [20,21]. SKI represses TGF-βgignaling by modifying Smad activity through the use of apparently redundant mechanisms. SKI displaces p300/CBP coactivators from Smad complexes [22], it prevents the nuclear translocation of Smad3 after TGF-β treatment [8], it prevents ligand-dependent phosphorylation of Smad2 and Smad3 [23], and stabilizes inactive Smad complexes on Smad-binding elements [24].

Importantly, SKI is necessary and sufficient for repressing TGF-β signaling in human melanomas. Down-regulation of SKI protein levels by antisense SKI vectors [8] or RNAi technology (Chen and Medrano, unpublished) inhibits melanoma cell growth and clonogenicity and restores TGF-β-mediated growth inhibition. These results highlight the critical role SKI plays in melanoma proliferation and suggest that cessation of melanoma cell proliferation can be readily achieved by targeting SKI. Furthermore, such approach can be clinically relevant, since growth inhibition occurs in spite of likely BRAF mutations, and event associated with melanoma genesis and progression [25].

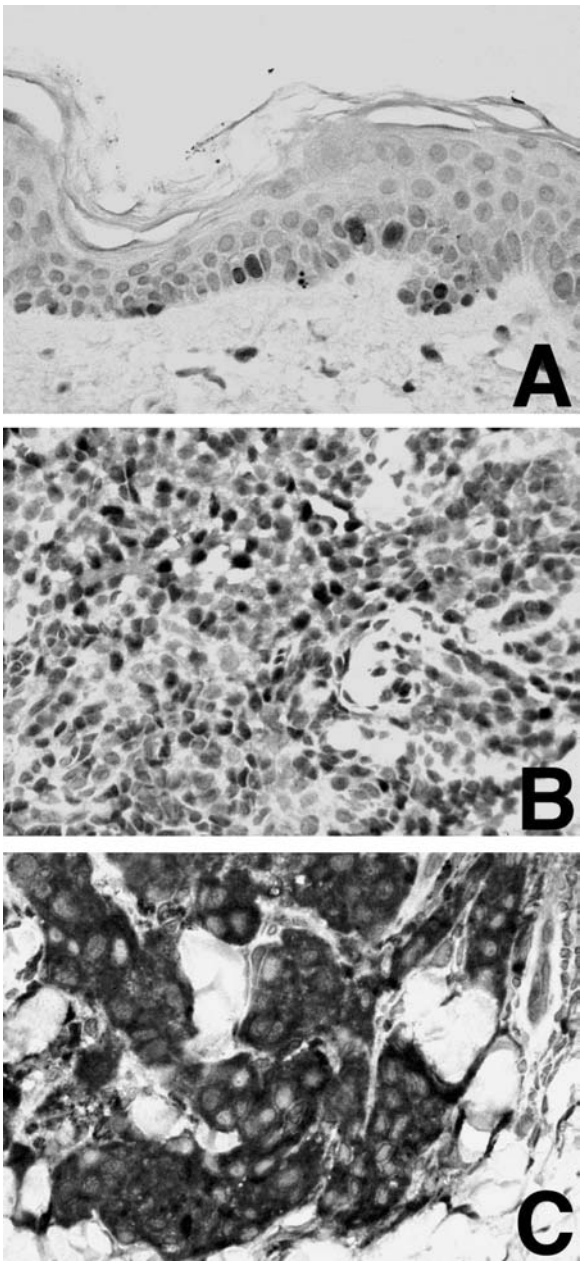

Figure 2. Overexpression and variable intracellular localization of SKI during melanoma progression *in vivo*. (A) Malignant melanoma *in situ* (intraepidermal melanoma) demonstrating strong nuclear labeling of the melanoma cells. (B) Nodular primary invasive melanoma with many, but not all, of the tumor cells labeled in a nuclear pattern. (C) Metastatic melanoma with strong, predominantly cytoplasmic labeling. All panels immunolabeled for SKI, Fast Red chromogen, brief hematoxylin counterstain, original magnification $\times$436 (A and C), $\times$218 (B).

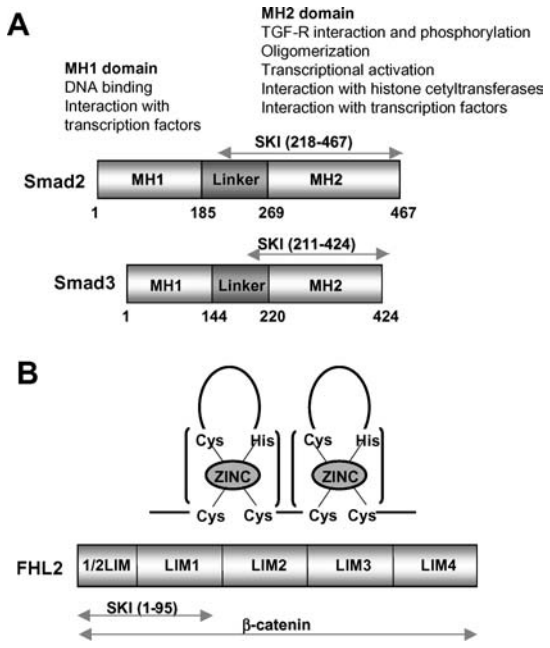

Figure 3. SKI associates with Smad proteins and FHL2 in melanoma cells. (A) SKI disrupts TGF-β signaling by associating with the Smad proteins (modified from [19,58]. (B) Association of SKI with FHL2 links SKI with the β-catenin pathway (modified from [32,34]).

3. SKI is a potent inhibitor of the retinoblastoma (RB) protein

Ski directly interacts with the retinoblastoma protein (RB), forming complexes containing mSin3 and HDAC1. Overexpression of SKI can partially represses RB activity [26]. However, SKI in association with the Ski-interacting protein 1 (Skip1) can completely overcome the G1 arrest and senescent morphology induced by RB [27]. These results suggest that high levels of SKI can also alter cellular senescence induced by RB complexes containing the PML protein [28]. Thus, high levels of SKI can cause lesions in the RB pathway similar to deletions or mutations of $p16^{INK4a}$, an event associated with mouse and human melanoma genesis and/or progression (reviewed in [29–31]).

4. SKI is a potent activator of the β-catenin-signaling pathway in human melanoma

4.1. FHL2: A SKI enigmatic partner

A yeast two hybrid screen identified FHL2/DRAL, as a SKI-binding protein [32]. The acronym LIM derives from *L*in-11, *I*sl-1, and *M*ec-3, three transcription factors in which a specific motif, LIM, was originally described [18,33]. LIM domains are characterized by the cysteine-rich consensus $CX2CX_{16-23}HX_2CX_2CX_2CX_{16-21}CX_{2-3}$(C/H/D) [34], and function as adapters and modifiers in protein interactions [35]. FHL2 also functions as a co-repressor or co-activator depending on the promoter and/or cellular context [5] FHL2 has been found to interact with many proteins including the androgen receptor [17,36], hCDC47, a member of the minichromosome maintenance protein family regulating DNA replication [37], presenilin 2 [17,36,38], RNA recognition motifs [39], DNA-binding nuclear protein hNP220 [40], TUCAN-CARDINAL [41], the promyelocytic leukemia zinc finger protein [42], β-catenin [5,6,17,36], titin, and various metabolic enzymes [43], and BRCA1 [44] among others. Transfected FHL2/DRAL displays intriguing apoptotic functions in several cell lines [45] including melanoma [32]. SKI blocks FHL2-mediated apoptosis, by turning FHL2 into a growth-stimulator for mouse melanocytes and human melanoma cells [32].

4.2. SKI activates MITF and Nr-CAM, inducing proliferation and migration of mouse melanocytes and human melanoma cells

The *mitf* gene, originally cloned from a transgenic insertional mutation at the mouse microphthalmia locus, encodes a basic-helix-loop-helix/leucine zipper protein critical for melanocyte cell-fate choice during commitment from pluripotent precursor cells in the neural crest (Reviewed in [46]). MITF cooperates with LEF-1, a key transducer of the Wnt signal, to transactivate the *dopachrome tautomerase* (DCT) gene promoter [47,48]. The DCT protein is an early melanoblast marker and a mediator of the melanoma-specific resistance to cis-diaminedichloroplatinum [47,49]. Thus, activation of the β-catenin pathway and MITF expression appears to be essential for growth and survival of melanoma cells. We demonstrated that SKI is a more potent activator of MITF than β-catenin. SKI also targets Nr-CAM, a protein involved in proliferation, motility and tumorigenicity [50].

5. Conclusions

Resistance of cancerous cells to TGF-ß has been linked to mutations in genes involved in the TGF-ß signaling

pathway, including TGF-ß receptors I and II, Smad2, or Smad4 (reviewed in [51]). However, melanoma tumors appear to have escaped the pressure for such mutations [52] by developing high levels of the SKI protein. Melanoma cells constitutively produce TGF-β1; high levels of SKI prevent the autocrine and negative effects of TGF-β, but not its paracrine effects on the tumor stroma that result in increased deposition of extracellular matrix protein in the interstitium of the tumor [53]. These observations are important, as the tumor-associated reactive stroma is beginning to be appreciated as a critical component for the tumor's evolution [54]. Furthermore, recent evidence suggests that the volume of reactive stroma in prostate tumors is a significant predictor of disease-free survival [55]. Because stroma is an integral component of all carcinoma tumors, a combination of Breslow and stromal scoring could also become a better predictor for melanoma evolution.

Compared to other transforming proteins including myc, ras and Sarc, we are only beginning to appreciate that SKI is aberrantly expressed in an ever growing number of human tumors. Because of the mechanistic link of SKI with TGF-β and β-catenin, it is becoming clear that SKI could be a powerful driver of melanoma tumor progression. β-catenin/TCF activity functions as a "master switch" and oncogene by controlling proliferation versus differentiation in epithelial cells (reviewed in [56,57]. Exploring further how SKI regulates this pathway in melanomas will be an important step for understanding the role of Wnt signaling in melanoma evolution.

Also, use of improved ChIP on Chip technologies should help define in the future a SKI-induced transcriptome in several human cancers overexpressing this protein. This will be crucial for unraveling additional pathways regulated by SKI and also for understanding how the cellular context regulates SKI function. In addition, determining whether SKI inactivation can disrupt melanoma tumor progression *in vivo* will be important for defining the pathogenesis of human melanoma and for designing new treatments for this disease.

6. Key unanswered questions

There are many intriguing questions begging for answers. We do not fully understand the biological consequences of cytoplasmic only SKI expression. Neither know we why SKI is retained in the cytoplasm of some metastatic melanoma tumors. We also know virtually nothing regarding post-translational modifications of the SKI protein. SUMO, a small ubiquitin-related modifier, is known to covalently attach to a number of nuclear regulatory proteins such as p53, IkappaB, promyelocytic leukemia protein and c-Jun. Preliminary experiments suggest that SKI can be SUMOylated in melanoma cells (Lin and Medrano, unpublished results) (Figure 1). The potential sumoylation site on Lys572 resides within the region involved in dimerization of the SKI family. Is SUMOylation required for homodimerization or heterodimerization and/or required for extending SKI protein half-life (Figure 4)? It will also be important to determine whether phosphorylation plays any role in SKI function and localization.

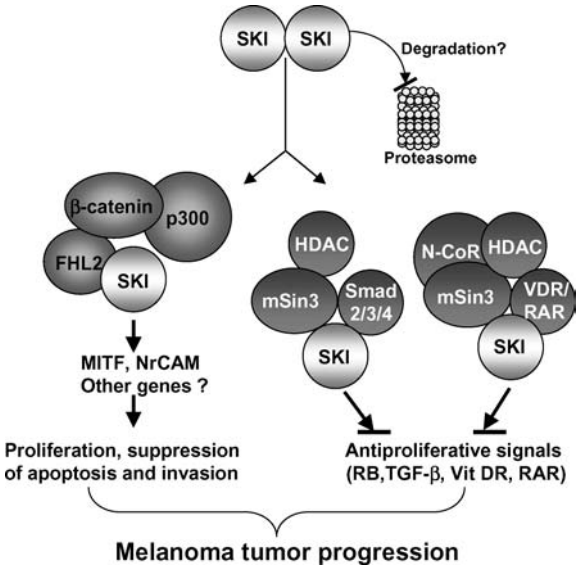

Figure 4. A model for SKI-regulated pathways involved in progression of human melanoma.

Acknowledgments

Work in the EEM laboratory is supported by an RO-1 grant from the National Cancer Institute.

References

1. Colmenares C, Stavnezer E: Structure and activities of the ski oncogene. Semin Cancer Biol 1: 383–387, 1990
2. Nicol R, Zheng G, Sutrave P, Foster DN, Stavnezer E: Association of specific DNA binding and transcriptional

repression with the transforming and myogenic activities of c-Ski. Cell Growth Differ 10: 243–254, 1999
3. Medrano EE: Repression of TGF-beta signaling by the oncogenic protein SKI in human melanomas: Consequences for proliferation, survival, and metastasis. Oncogene 22: 3123–3129, 2003.
4. Harada J, Kokura K, Kanei-Ishii C, Nomura T, Khan MM, Kim Y, Ishii S: 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, 2003.
5. Martin B, Schneider R, Janetzky S, Waibler Z, Pandur P, Kuhl M, Behrens J, Von Der MK, Starzinski-Powitz A, Wixler V: The LIM-only protein FHL2 interacts with {beta}-catenin promotes differentiation of mouse myoblasts. J Cell Biol 159: 113–122, 2002
6. Wei Y, Renard CA, Labalette C, Wu Y, Levy L, Neuveut C, Prieur X, Flajolet M, Prigent S, Buendia MA: Identification of the LIM protein FHL2 as a coactivator of beta-catenin. J Biol Chem 278: 5188–5194, 2003
7. Wilson JJ, Malakhova M, Zhang R, Joachimiak A, Hegde RS: Crystal structure of the dachshund homology domain of human SKI. Structure (Camb) 12: 785–792, 2004
8. Reed JA, Bales E, Xu W, Okan NA, Bandyopadhyay D, Medrano EE: 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, 2001
9. Fukuchi M, Nakajima M, Fukai Y, Miyazaki T, Masuda N, Sohda M, Manda R, Tsukada K, Kato H, Kuwano H: 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, 2004
10. Fujimoto T, Nishikawa A, Iwasaki M, Akutagawa N, Teramoto M, Kudo R: Gene expression profiling in two morphologically different uterine cervical carcinoma cell lines derived from a single donor using a human cancer cDNA array. Gynecol Oncol 93: 446–453, 2004
11. Buess M, Terracciano L, Reuter J, Ballabeni P, Boulay JL, Laffer U, Metzger U, Herrmann R, Rochlitz C: Amplification of SKI is a prognostic marker in early colorectal cancer. Neoplasia 6: 207–212, 2004
12. Zhang F, Lundin M, Ristimaki A, Heikkila P, Lundin J, Isola J, Joensuu H, Laiho M: Ski-related novel protein N (SnoN), a negative controller of transforming growth factor-beta signaling, is a prognostic marker in estrogen receptor-positive breast carcinomas. Cancer Res 63: 5005–5010, 2003
13. Nomura T, Tanikawa J, Akimaru H, Kanei-Ishii C, Ichikawa-Iwata E, Khan MM, Ito H, Ishii S: Oncogenic activation of c-Myb correlates with a loss of negative regulation by TIF1beta and Ski. J Biol Chem 279: 16715–16726, 2004
14. Jahagirdar BN, Miller JS, Shet A, Verfaillie CM: Novel therapies for chronic myelogenous leukemia. Exp Hematol 29: 543–556, 2001
15. Funato T, Satou J, Kozawa K, Fujimaki S, Miura T, Kaku M: Use of c-myb antisense oligonucleotides to increase the sensitivity of human colon cancer cells to cisplatin. Oncol Rep 8: 807–810, 2001
16. Fumagalli S, Doneda L, Nomura N, Larizza L: Expression of the c-ski proto-oncogene in human melanoma cell lines. Melanoma Res 3: 23–27, 1993
17. Kokura K, Kim H, Shinagawa T, Khan MM, Nomura T, Ishii S: 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, 2003
18. Wei Y, Renard CA, Labalette C, Wu Y, Levy L, Neuveut C, Prieur X, Flajolet M, Prigent S, Buendia, MA: Identification of the LIM protein FHL2 as a coactivator of beta-catenin. J Biol Chem 278: 5188–5194, 2003
19. Xu W, Angelis K, Danielpour D, Haddad MM, Bischof O, Campisi J, Stavnezer E, Medrano EE: 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, 2000
20. Shi Y, Massague J: Mechanisms of TGF-beta signaling from cell membrane to the nucleus. Cell 113: 685–700, 2003
21. Massague J: How cells read TGF-beta signals. Nat Rev Mol Cell Biol 1: 169–178, 2000
22. Akiyoshi S, Inoue H, Hanai J, Kusanagi K, Nemoto N, Miyazono K, Kawabata M: c-Ski acts as a transcriptional co-repressor in transforming growth factor-beta signaling through interaction with smads. J Biol Chem 274: 35269–35277, 1999
23. Prunier C, Pessah M, Ferrand N, Seo SR, Howe P, Atfi A: The oncoprotein Ski acts as an antagonist of transforming growth factor-beta signaling by suppressing Smad2 phosphorylation. J Biol Chem 278: 26249–26257, 2003
24. Suzuki H, Yagi K, Kondo M, Kato M, Miyazono K, Miyazawa K: c-Ski inhibits the TGF-beta signaling pathway through stabilization of inactive Smad complexes on Smad-binding elements. Oncogene 2004
25. Wellbrock C, Ogilvie L, Hedley D, Karasarides M, Martin J, Niculescu-Duvaz D, Springer CJ, Marais R: V599EB-RAF is an oncogene in melanocytes. Cancer Res 64: 2338–2342, 2004
26. Tokitou F, Nomura T, Khan MM, Kaul SC, Wadhwa R, Yasukawa T, Kohno I, Ishii S: Viral ski inhibits retinoblastoma protein (Rb)-mediated transcriptional repression in a dominant negative fashion. J Biol Chem 274: 4485–4488, 1999

27. Prathapam T, Kuhne C, Banks L: Skip interacts with the retinoblastoma tumor suppressor and inhibits its transcriptional repression activity. Nucleic Acids Res 30: 5261–5268, 2002
28. Khan MM, Nomura T, Kim H, Kaul SC, Wadhwa R, Shinagawa T, Ichikawa-Iwata E, Zhong S, Pandolfi PP, Ishii S: Role of PML and PML-RARalpha in Mad-mediated transcriptional repression. Mol Cell 7: 1233–1243, 2001
29. Yang FC, Merlino G, Chin L: Genetic dissection of melanoma pathways in the mouse. Semin Cancer Biol 11: 261–268, 2001
30. Bardeesy N, Bastian BC, Hezel A, Pinkel D, DePinho RA, Chin L: Dual inactivation of RB and p53 pathways in RAS-induced melanomas. Mol Cell Biol 21: 2144–2153, 2001
31. Tietze MK, Chin L: Murine models of malignant melanoma. Mol Med Today 6: 408–410, 2000
32. 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: SKI activates Wnt/beta-catenin signaling in human melanoma. Cancer Res 63: 6626–6634, 2003
33. El Mourabit H, Muller S, Tunggal L, Paulsson M, Aumailley M: Analysis of the adaptor function of the LIM domain-containing protein FHL2 using an affinity chromatography approach. J Cell Biochem 92: 612–625, 2004
34. Schmeichel KL, Beckerle MC: Molecular dissection of a LIM domain. Mol Biol Cell 8: 219–230, 1997
35. Dawid IB, Breen JJ, Toyama R: LIM domains: multiple roles as adapters and functional modifiers in protein interactions. Trends Genet 14: 156–162, 1998
36. Muller JM, Isele U, Metzger E, Rempel A, Moser M, Pscherer A, Breyer T, Holubarsch C, Buettner R, Schule R: FHL2, a novel tissue-specific coactivator of the androgen receptor. EMBO J 19: 359–369, 2000
37. Chan KK, Tsui SK, Ngai SM, Lee SM, Kotaka M, Waye MM, Lee CY, Fung KP: Protein-protein interaction of FHL2, a LIM domain protein preferentially expressed in human heart, with hCDC47. J Cell Biochem 76: 499–508, 2000
38. Tanahashi H, Tabira T: Alzheimer's disease-associated presenilin 2 interacts with DRAL, an LIM- domain protein. Hum Mol Genet 9: 2281–2289, 2000
39. Dye BT, Patton JG: An RNA recognition motif (RRM) is required for the localization of PTB-associated splicing factor (PSF) to subnuclear speckles. Exp Cell Res 263: 131–144, 2001
40. Ng EK, Chan KK, Wong CH, Tsui SK, Ngai SM, Lee SM, Kotaka M, Lee CY, Waye MM, Fung KP: Interaction of the heart-specific LIM domain protein, FHL2, with DNA-binding nuclear protein, hNP220. J Cell Biochem 84: 556–566, 2002
41. Stilo R, Leonardi A, Formisano L, Di Jeso B, Vito P, Liguoro D: TUCAN/CARDINAL and DRAL participate in a common pathway for modulation of NF-kappaB activation. FEBS Lett 521: 165–169, 2002
42. McLoughlin P, Ehler E, Carlile G, Licht JD, Schafer BW: The LIM-only protein DRAL/FHL2 interacts with and is a corepressor for the promyelocytic leukemia zinc finger protein. J Biol Chem 277: 37045–37053, 2002
43. Lange S, Auerbach D, McLoughlin P, Perriard E, Schafer BW, Perriard JC, Ehler E: Subcellular targeting of metabolic enzymes to titin in heart muscle may be mediated by DRAL/FHL-2. J Cell Sci 115: 4925–4936, 2002
44. Yan J, Zhu J, Zhong H, Lu Q, Huang C, Ye Q: BRCA1 interacts with FHL2 and enhances FHL2 transactivation function. FEBS Lett 553: 183–189, 2003
45. Scholl FA, McLoughlin P, Ehler E, de Giovanni C, Schafer BW: DRAL is a p53-responsive gene whose four and a half LIM domain protein product induces apoptosis. J Cell Biol 151: 495–506, 2000
46. Widlund HR, Fisher DE: Microphthalamia-associated transcription factor: A critical regulator of pigment cell development and survival. Oncogene 22: 3035–3041, 2003
47. Goding CR: Mitf from neural crest to melanoma: signal transduction and transcription in the melanocyte lineage. Genes Dev 14: 1712–1728, 2000
48. Yasumoto K, Takeda K, Saito H, Watanabe K, Takahashi K, Shibahara S: Microphthalmia-associated transcription factor interacts with LEF-1, a mediator of Wnt signaling. EMBO J 21: 2703–2714, 2002
49. Chu W, Pak BJ, Bani MR, Kapoor M, Lu SJ, Tamir A, Kerbel RS, Ben David Y: Tyrosinase-related protein 2 as a mediator of melanoma specific resistance to cis-diamminedichloroplatinum(II): therapeutic implications. Oncogene 19: 395–402, 2000
50. Conacci-Sorrell ME, Ben Yedidia T, Shtutman M, Feinstein E, Einat P, Ben Ze'ev A: Nr-CAM is a target gene of the beta-catenin/LEF-1 pathway in melanoma and colon cancer and its expression enhances motility and confers tumorigenesis. Genes Dev 16: 2058–2072, 2002
51. Siegel PM, Massague J: Cytostatic and apoptotic actions of TGF-beta in homeostasis and cancer. Nat Rev Cancer 3: 807–821, 2003
52. Rodeck U, Nishiyama T, Mauviel A: Independent regulation of growth and SMAD-mediated transcription by transforming growth factor beta in human melanoma cells. Cancer Res 59: 547–550, 1999
53. Berking C, Takemoto R, Schaider H, Showe L, Satyamoorthy K, Robbins P, Herlyn M: Transforming Growth Factor-beta1 Increases Survival of Human Melanoma through Stroma Remodeling. Cancer Res 61: 8306–8316, 2001

54. De Wever O, Mareel M: Role of tissue stroma in cancer cell invasion. J Pathol 200: 429–447, 2003
55. Ayala G, Tuxhorn JA, Wheeler TM, Frolov A, Scardino PT, Ohori M, Wheeler M, Spitler J, Rowley DR: Reactive stroma as a predictor of biochemical-free recurrence in prostate cancer. Clin Cancer Res 9: 4792–4801, 2003
56. van Es JH, Barker N, Clevers H: You Wnt some, you lose some: Oncogenes in the Wnt signaling pathway. Curr Opin Genet Dev 13: 28–33, 2003
57. Smalley MJ, Dale TC: Wnt signalling in mammalian development and cancer. Cancer Metastasis Rev 18: 215–230, 1999
58. Derynck R, Zhang YE: Smad-dependent and Smad-independent pathways in TGF-beta family signalling. Nature 425: 577–584, 2003