c-Jun associates with the oncoprotein Ski and suppresses Smad2 transcriptional activity*

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Running title: association of c-Jun and Ski
SUMMARY

The Smad proteins are key intracellular effectors of transforming growth factor-β (TGF-β) cytokines. The ability of Smads to modulate transcription results from a functional cooperativity with the coactivators p300/CBP, or the corepressors TGIF and Ski. The c-Jun N-terminal Kinase (JNK) pathway, another downstream target activated by TGF-β receptors, has also been suggested to inhibit TGF-β signaling through interaction of c-Jun with Smad2 and Smad3. Here we show that c-Jun directly interacts with Ski to enhance the association of Ski with Smad2 in the basal state. Interestingly, TGF-β signaling induces dissociation of c-Jun from Ski, thereby relieving active repression by c-Jun. Moreover, activation of JNK pathway suppressed the ability of TGF-β to induce dissociation of c-Jun from ski. Thus, the formation of c-Jun/Ski complex maintains the repressed state of Smad2-responsive genes in the absence of ligand and participates in negative feedback regulation of TGF-β signaling by the JNK cascade.
INTRODUCTION

Transforming growth factor-β (TGF-β) is a member of a large family of cytokines that regulates a broad range of cellular functions, including cell growth regulation, production of extracellular matrix components, differentiation, and apoptosis (1). Extensive analysis of TGF-β signaling pathways has demonstrated that the transduction of TGF-β signals to the nucleus is accomplished by the sequential association of type II and type I receptors and Smad protein cascades (2-4). Binding of ligands to the type II receptor causes phosphorylation of the cytoplasmic domain of the type I receptor, leading to phosphorylation of Smad2 or Smad3 on C-terminal serine residues (1, 5). Once phosphorylated, Smad2 or Smad3 associates with Smad4, and the resulting complexes move into the nucleus to regulate expression of a large number of target genes, most of which remain to be identified (1, 5).

By interacting directly with promoter sequences or with DNA-binding proteins, Smad complexes positively or negatively regulate the transcription of target genes depending on physiological context (1, 6). Transcription activation by Smad proteins has been shown to occur, in part at least, by their ability to recruit the general coactivators p300 and CBP (7-10). p300 and CBP act as coactivators by bringing Smad2 or Smad3 within the proximity of the general transcription machinery and by modifying the chromatin structure through histone acetylation. By contrast, Smad2 or Smad3 can also interact with the corepressor TGIF in response to TGF-β, thereby forming a transcriptional repressor complex (11). Furthermore, the repression of Smad-dependent
transcription by TGIF correlates with the recruitment of a histone deacetylase (HDAC) instead of the coactivators p300/CBP into the Smad complex (11). A similar mechanism has also been proposed to explain the action of the corepressors Ski, which interacts with Smads on TGF-β-responsive promoters and represses their ability to activate TGF-β target genes by assembling a repressor complex containing N-CoR, Sin3, and a HDAC (12-14).

The nuclear function of Smad proteins can also be limited by c-Jun, a transcriptional factor activated by TGF-β through the c-Jun N-terminal Kinase (JNK) pathway (15-19). Analogous to the mitogen-activated protein kinase (MAPK) cascade, the prototypical JNK pathway involves the sequential activation of the protein kinases MAPK kinase kinase1 (MEKK1), MAPK kinase4 (MKK4), and JNK (20). Once activated, JNK phosphorylates the transactivating domain of c-Jun protein at serine residues 63 and 73, and, in turn, phosphorylated c-Jun upregulates the expression of a number of genes containing AP1 sites at their promoter, including c-Jun itself (21, 22).

In addition to activating transcription of target genes, the phosphorylated c-Jun can interact with the corepressor TGIF to repress Smad2 transcriptional activity (19). Interaction of activated c-Jun with TGIF may prevent the recruitment of p300/CBP to TGF-β-activated Smad2, thus reducing the ability of the Smad complex to activate transcription (19). Given the established role of Ski in interfering with the assembly of Smad2 and the coactivators p300/CBP in response to TGF-β signaling (12), we postulated that c-Jun may be able to recruit different transcriptional corepressors depending on cell type or physiological context. In this study, we provide evidence that c-Jun interacts with Ski in the absence of TGF-β signaling, thereby stabilizing the Ski/Smad2 corepressor
complex. Interestingly, the interaction between c-Jun and Ski was increased on activation of JNK signaling pathway, suggesting that the association of c-Jun with Ski may have important role in the JNK-dependent repression of Smad2 transcriptional activity.
EXPERIMENTAL PROCEDURES

Cell Culture and Gene Expression Analysis

The immortalized mouse embryonic cells (MEF3T3) were a generous gift from Dr. E. Böttinger. COS-7, HepG2, and 293 cells were obtained from the American Type Culture Collection. All cells were propagated in Dulbecco’s modified Eagle’s medium supplemented with 10 % heat-inactivated fetal calf serum (FCS), 5 mM glutamine, and antibiotics.

For gene expression analysis, cells were plated to semiconfluency and 24 h later transfected with expression vectors by the LipofectAMINE method as described previously (23). To induce the luciferase reporter, cells were treated with human TGF-β1 (Sigma) at 2 ng/ml for 16 hr. Extracts were then prepared and assayed for luciferase activity using the luciferase assay system described by the manufacturer (PROMEGA). The luciferase activities were normalized for transfection efficiency using a β-galactosidase expressing vector (pCMV5.LacZ) and the Galacto-Star system (Perkin Elmer).

Immunoprecipitation and Immunoblotting

For immunoprecipitation, cells were lysed at 4°C in RIPA buffer (20 mM Tris-HCl [pH 8], 150 mM NaCl, 5 mM MgCl₂, 10% glycerol, 0.5% NP40, 1 mM sodium vanadate, 1 mM phenylmethylsulfonyl fluoride, 20 µg/ml aprotinin, and 20 µg/ml leupeptin). Lysates were subjected to immunoprecipitation with the appropriate antibody for 2 hr, followed by adsorption to sepharose
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coupled protein G for 1 hr. Immunoprecipitates and aliquots of cell lysates before immunoprecipitation were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a nitrocellulose membrane. The membranes were immunoblotted with various antibodies, and the bound antibodies were visualized with horseradish peroxidase-conjugated antibodies to rabbit, goat, or mouse IgG using the Enhanced Chemiluminescence (ECL) Western blotting system (Amersham).

**In vitro Binding Assays**

GST pulldown experiments were performed as described (19). GST fusion proteins coupled to glutathion-sepharose were incubated with *in vitro* translated c-Jun in RIPA buffer for 2 hr at 4 °C and washed four times with the same buffer. Samples were resolved by SDS/PAGE and bound c-Jun was visualized by autoradiography.

**Immunofluorescence**

Twenty-four hours after transfection, COS-7 cells, transfected with Flag-Ski and c-Jun together with wild-type or constitutively activated TGF-β type I receptors, were transferred into medium containing 0.1% fetal calf serum (FCS). After 48 hr, the slides were washed twice in PBS, fixed in 4% paraformaldehyde for 30 min at room temperature and permeabilized in 0.1% triton X-100. Cells were incubated overnight at 4 °C with a mixture of monoclonal anti-Flag M2 and rabbit polyclonal anti-c-Jun antibodies. Cells were washed with PBS, incubated with a mixture of FITC-
conjugated goat anti-mouse and Texas Red-conjugated goat anti-rabbit antibodies, and examined on a Leica confocal microscope.
RESULTS AND DISCUSSION

We have previously reported that c-Jun specifically interacts with the Smad corepressor TGIF in response to TGF-β signaling (19). To determine whether the TGF-β-dependent interaction of c-Jun with TGIF is physiologically relevant, we compared the effects of ectopically expressed TGIF or c-Jun on TGF-β/Smad2-dependent transcription responses using HepG2 cells or immortalized mouse embryonic fibroblasts (MEF3T3). We chose to focus our analysis on Xenopus mix.2 promoter as a target of Smad2 because activation of mix.2 by TGF-β requires the formation of a Smad2-Smad4-FAST1 complex that binds to a sequence promoter known as the TGF-β/activin response element (24). In mouse MEF3T3 cells, significantly increased activation of the ARE-Lux reporter in response to TGF-β were detected in cells transfected with FAST1. Unexpectedly, while cotransfection of c-Jun with FAST1 resulted in a marked inhibition of TGF-β-induced ARE-Lux activity, cotransfection of TGIF did not even at the highest amounts of transfected cDNA (Fig. 1A and data not shown). Under the same experimental conditions, transfection of HepG2 cells with the expression vector encoding TGIF blocked the ligand-dependent induction of luciferase activity with an efficiency approaching that elicited by transfection of c-Jun (Fig. 1B), consistent with published results (19).

From these results, it can be suggested that c-Jun may repress Smad2 transcriptional activity by recruiting different transcriptional corepressors depending on cell type or physiological context.
Within the nucleus, the transcriptional activity of nuclear Smad complexes can also be repressed by Ski, which, like TGIF, interacts with Smad2/3 on TGF-β responsive promoters, resulting in the recruitment of N-CoR and its associated HDAC (12-14). As a first step towards understanding the mechanism underlying the inhibitory function of c-Jun, we cotransfected MEF3T3 cells with an expression vector encoding Ski together with the ARE-Lux reporter and FAST1. As shown in Fig. 1A, expression of Ski blocked the TGF-β-dependent activation of the reporter gene with activity similar to c-Jun. Because c–Jun has been shown to interact with Smad2 (19, 25), and because the interaction of Smad2 with Ski is required for the repression of TGF-β-mediated transcriptional responses (12-14), we next sought to determine whether c-Jun could stabilize the Smad2/Ski complex. For this, 293 cells were transfected with Myc-Smad2 and Flag-Ski in the presence or absence of HA-c-Jun. Similar to previous observations (12), association of Smad2 with Ski was strongly increased following exposure of cells to TGF-β (Fig. 1C). Surprisingly, cotransfection of c-Jun resulted in a ligand-independent increase in the amount of Smad2 present in Flag-Ski immunocomplexes, suggesting that c-Jun can stabilize the Smad2/Ski complex in the absence of TGF-β signaling (Fig. 1C).

To approach the question of how c-Jun stabilizes the Smad2/Ski complex, we looked for possible interactions between c-Jun and Ski through transient transfections using COS-7 cells. Immunoprecipitation of cell lysates from transfected cells with the anti-HA antibody directed against HA-c-Jun revealed the presence of Flag-Ski, which was absent in a control transfection in which only Flag-Ski was expressed (Fig. 2A). Next, we looked for a direct interaction between Ski
Incubation of the in vitro-transcribed and translated product of c-Jun with Ski linked to GST and expressed in bacteria showed that Ski was able to interact directly with c-Jun (Fig. 2B).

To begin to understand the interaction of Ski and c-Jun, various Flag epitope Ski constructs were transfected into COS-7 cells and tested for interaction with cotransfeted HA-c-Jun. As shown in Fig. 3A, a Ski fragment between residues 338 and 490 was sufficient for interaction with c-Jun. However, inclusion of the C-terminus region precludes this interaction, indicating that this element may be altering the conformation of the truncated protein in such a way as to interfere with the ability of Ski to interact with c-Jun. Further analysis using a fragment of c-Jun (c-Jun-b-Zip) in this assay showed that the Ski-interacting region of c-Jun maps to the C-terminus domain of c-Jun, including the DNA binding domain and the leucine zipper region (Fig. 3B). It should be noted that the c-Jun-b-Zip mutant was obtained by removing the NH2-terminal transactivation domain of c-Jun that includes the binding site for JNK (19), indicating that the phosphorylation of c-Jun by JNK is not required for the assembly of c-Jun/Ski complex. In the course of these analyses, we also characterized the domains in Ski that mediate binding to Smad2 protein, using our panel of Ski mutants with Myc-Smad2. As previously described (12, 13), the amino terminal portion of Ski (residues 1-490) is responsible for binding to Smad2 (Fig. 3C). Interestingly, the c-Jun-interacting region of c-Jun (338-490) failed to bind Smad2 despite efficient expression of the truncated protein (Fig. 3C), demonstrating that Ski contains discrete binding sites for Smad2 and c-Jun.

To ascertain for the physiological relevance of the interaction of Ski with c-Jun, we examined the effect of Ski on c-Jun-mediated transcriptional activation of an AP1-Lux reporter,
which contains AP1 sites, is c-Jun responsive, and drives expression of a luciferase gene (18). This reporter had minimal basal activity in HepG2 cells but when it was cotransfected with c-Jun, we observed a strong induction of the reporter gene (Fig. 4). In cells cotransfected with Ski, we observed a significant repression of c-Jun-mediated transcriptional responses (Fig. 4), providing support for a functional interaction between c-Jun and Ski.

To examine whether activation of TGF-β signaling might influence the interaction of c-Jun with Ski, COS-7 cells were transfected with Flag-Ski, HA-c-Jun and either wild-type or activated TβRI (TβRI.act), which contains a substitution of threonine residue 204 to aspartate and signals TGF-β responses in the absence of ligand and the type II receptor (26). Complexes were precipitated with anti-Flag antibody followed by immunoblotting with anti-HA antibody for the presence of c-Jun. In the absence of TGF-β signaling, constitutive interaction between Ski and c-Jun was detected. In contrast, in cells coexpressing the activated type I receptor, we observed a significant decrease in the interaction of Ski with c-Jun (Fig. 5A). A similar dissociation was obtained with transfected 293 cells (Fig. 5B) or HepG2 cells (see Fig. 6) upon TGF-β treatment, indicating that the TGF-β-dependent dissociation of Ski from c-Jun can occur in multiple cell lines.

We also tested for interaction between endogenous Ski and c-Jun. For this, c-Jun was immunoprecipitated from Hela cells using a rabbit polyclonal c-Jun antibody, and Ski was visualized by immunoblotting with a monoclonal anti-Ski antibody. In immunoprecipitated prepared with normal antiserum, no Ski was detectable (Fig. 5C). However, in the anti-c-Jun immunoprecipitates, we could clearly detect Ski coprecipitating with c-Jun (Fig. 5C). More
importantly, TGF-β treatment prior to lysis revealed decreased association of c-Jun with Ski, consistent with our previous results in transfected cells (Fig. 5C). Taken together, these results demonstrate that c-Jun can physiologically interact with Ski and further suggest that TGF-β signaling induces dissociation of c-Jun/Ski complexes.

To further confirm the *in vivo* association between Ski and c-Jun, we then examined the subcellular localization of Ski and c-Jun in transfected COS-7 cells using laser confocal microscopy. In cells expressing c-Ski alone, the Ski immunoreactivity was dispersed throughout the cell (Fig. 5D), consistent with published results (27). Surprisingly, when c-Ski was coexpressed together with c-Jun, Ski was found predominantly in the nucleus and was extensively colocalized with c-Jun, suggesting that c-Jun expression leads to the import of Ski to the nucleus (Fig. 5D). As expected, c-Jun was detected predominantly in the nucleus of the transfected cells in the presence of absence of cotransfected Ski (Fig. 5D and data not shown).

Our previous analysis showed that activation of TGF-β signaling induces the dissociation of Ski from c-Jun. To explore in more detail how the subcellular distribution of Ski and c-Jun is controlled, we examined how TGF-β signaling regulates Ski and c-Jun localization. For this, we expressed in COS-7 cells Ski and c-Jun in the presence of the constitutively activated type I receptor. Analysis of c-Jun revealed that coexpression of the activated type I receptor did not appreciably affect the staining pattern. In contrast, a substantial proportion of Ski redistributed to the cytoplasm despite efficient expression of cotransfected c-Jun (Fig. 5D). This could be nicely explained by the failure of complex formation between c-Jun and Ski when these proteins are
coexpressed together with the activated type I receptor (Fig. 5A). A quantitation of these results indicated that over 60% COS-7 cells displayed prominent nuclear staining of c-Ski upon coexpression of c-Jun, while less than 30% exhibited similar staining in the corresponding cells that have been cotransfected with the activated type I receptor (Fig. 5E). Taken together with our biochemical analysis, these results strongly suggest that Ski interacts with c-Jun and that activation of TGF-β signaling induces Ski to dissociate from c-Jun.

We have previously shown that activation of the JNK cascade blocked the ability of Smad2 to mediate TGF-β-dependent transcription (19). This observation is based on the ability of the constitutively-activated or dominant-interfering forms of MEKK1 and MKK4 to abolish or enhance the TGF-β-dependent activation of the ARE-Lux reporter, respectively. The phosphorylation of Smad2 by the activated type I TGF-β receptor and its subsequent heterodimerization with Smad4 and translocation to the nucleus form the basis for a model how Smad proteins work to transmit TGF-β signals from the plasma membrane to the nucleus (1, 5, 6). In initial experiments, we observed that activation of JNK does not interfere with any of these signaling events (data not shown). However, we have found that this inhibition takes place in the nucleus and involves a physical interaction between c-Jun and Smad2 (19). To determine the basis of the inhibitory function of JNK cascade, we tested whether activation of JNK cascade might modulate the TGF-β-dependent dissociation of Ski from c-Jun. To test this possibility, HepG2 cells were transfected with Flag-Ski and HA-c-Jun in the presence or absence of dominant-negative or constitutively-activated mutants of MEKK1 and MKK4. In control cells, a strong interaction between Ski and c-Jun could
be detected (Fig. 6). However, in cells treated with TGF-β, we observed a decrease in the amount of Ski that coprecipitated with c-Jun, consistent with our previous results (Fig. 5A, 5B, 5C). Interestingly, in cells coexpressing the constitutively activated mutants of MEKK1 and MKK4, we detected a significant increase in the interaction of c-Jun with Ski even in the presence of TGF-β (Fig. 6). Under these experimental conditions, we found that coexpression of the dominant-negative mutants of MEKK1 and MKK4 enhances the ability of TGF-β to induce the dissociation of Ski from c-Jun (Fig. 6). Together, these data suggest that activation of JNK cascade can stabilize the Ski/c-Jun complexes and that c-Jun may play a critical role in the JNK-dependent repression of Smad2 signaling.

The Smad proteins mediate many of the gene responses to TGF-β, and these genes responses differ depending on the cell type and crosstalk between Smad signaling and other signaling systems (1, 2, 6). For example, the nuclear function of Smad proteins can be limited by c-Jun, a transcriptional factor activated by TGF-β through the JNK pathway (15, 18, 19, 28). We believed that this antagonistic crosstalk between two signaling pathways activated by TGF-β is very interesting because this mechanism would allow cells to display diverse patterns of transcriptional responses to TGF-β depending upon the relative activation of Smad proteins versus JNK signaling. The findings outlined in the present study provide strong evidence that c-Jun can function by directly associating with the corepressor Ski to stabilize the Smad2/Ski repressor complex. In the absence of TGF-β signaling, constitutive interaction between c-Jun and ski was detected. However, once signaling has commenced, c-Jun dissociates from Ski, suggesting that
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this interaction could serve a regulatory purpose either in the suppression of Smad2 that may leak into the nucleus in the basal state, or in imposing limits to positive genes responses induced by ligand-activated Smad2. Evidence of both these roles could be proposed in our model, since TGF-β is known to induce a delayed and persistent increase in JNK activity (15), which in turns may increase the interaction of c-Jun and Ski, thereby resetting the Smad2 pathway for interpretation of subsequent TGF-β signals.
The abbreviations used are: ARE, activin responsive element; JNK, c-Jun–N-terminal kinase; MKK4, mitogen-activated protein kinase kinase 4; MEKK1, mitogen-activated protein kinase kinase kinase 1; TGF-β, transforming growth factor β.

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REFERENCES

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LEGENDS TO FIGURES

Fig. 1. c-Jun stabilizes the Smad2/Ski complex

(A, B) MEF3T3 (A) or HepG2 cells (B) were transfected with ARE-Lux and FAST-1 together with the indicated expression vectors and then treated with TGF-β. After 16 h, cells were harvested and assayed for luciferase activity. The data are the mean and standard deviation of three independent experiments.

(C) 293 T cells were transfected with Flag-Ski or with Myc-Smad2 in the absence or presence of HA-c-Jun. Prior to lysis, cells were treated with or without TGF-β for 1 h. The association of Ski with Smad2 was analyzed by blotting the Myc immunoprecipitates with the anti-Flag antibody. Expression levels of proteins were determined by immunoblotting aliquots of total cell lysates.

Fig. 2. c-Jun interacts with Ski

(A) Cell lysates from transiently transfected COS-7 cells were subjected to immunoprecipitation with anti-HA antibody directed towards c-Jun and then immunoblotted using anti-Flag antibody that recognizes Flag-Ski. Expression levels of proteins were determined by immunoblotting aliquots of total cell lysates.

(B) GST pulldown assays of [35S]methionine-labeled, in vitro translated c-Jun were performed using
sepharose-bound bacterially expressed GST or GST-Ski. SDS-PAGE and autoradiography visualized bound material. Ponceau staining of the membrane showed that similar amounts of GST and GST-Ski were used in this assay (data not shown).

Fig. 3. Mapping of c-Jun/Ski interaction domains

(A) Expression vectors encoding wild-type Ski or the indicated deletion mutants fused to the Flag epitope were transfected into COS-7 cells with a HA-c-Jun expression vector. Cell lysates were subjected to immunoprecipitation with anti-Flag antibody and then immunoblotted with anti-HA antibody.

(B) COS-7 cells were transfected with Flag-Ski and HA-c-Jun or HA-c-Jun-b-Zip. Cell lysates were subjected to anti-Flag immunoprecipitation and then immunoblotted with anti-HA antibody.

(C) Cell lysates from transiently transfected COS-7 cells were subjected to immunoprecipitation with anti-Flag antibody directed towards Ski mutants and then immunoblotted with anti-Myc antibody that recognizes Myc-Smad2. Expression levels of proteins were determined by immunoblotting aliquots of total cell lysates.

Fig. 4. Ski suppressed the c-Jun-dependent activation of AP1

HepG2 cells were cotransfected with AP1-Lux together with HA-c-Jun and increasing amounts of Ski. After 48 h, luciferase activity was determined and normalized to β-galactosidase activity. The data are the mean and standard deviation of three independent experiments.
**Fig. 5. TGF-β induced dissociation of Ski from c-Jun**

(A) COS-7 cells were transfected with HA-c-Jun and Flag-Ski together with TβRI or TβRI.act. Cell lysates were subjected to immunoprecipitation with anti-Flag antibody and then immunoblotted with anti-HA antibody.

(B) 293 cells were transfected with Flag-Ski and HA-c-Jun before being treated with or without TGF-β for 1 hr. The association of Ski with c-Jun was analyzed by blotting the Flag immunoprecipitates with the anti-HA antibody. Expression levels of proteins were determined by immunoblotting aliquots of total cell lysates.

(C) Lysates from Hela cells, either untreated or treated for 1 h with TGF-β, were immunoprecipitated with an anti-rabbit polyclonal antibody specific for c-Jun or normal rabbit antiserum. Coprecipitated Ski was analyzed by immunoblotting with a monoclonal anti-Ski antibody. For comparison, a portion of cell lysates was probed with anti-c-Jun or anti-Ski antibodies.

(D) COS-7 cells were transfected with Flag-Ski and c-Jun together with TβRI or TβRI.act. Forty-eight hours after transfection, cells were fixed and the localization of Flag-Ski (green) and c-Jun (red) were visualized by a confocal microscope. Colocalization of Ski and c-Jun (overlay) appears as yellow.
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(E) Determination of the percentage of cells with Ski stained exclusively in the nucleus. At least 100 cells from five randomly chosen fields were counted for each data point.

Fig. 6. Activation of JNK cascade stabilized the Ski/c-Jun complex

HepG2 cells were transfected with combinations of HA-c-Jun, Flag-Ski, and the constitutively activated mutants MEKK1.EE and M KK4.ED, or the dominant-negative mutants MEKK1.K432A and M KK4.Ala. Then, cells were treated with or without TGF-β for 1 h. The association of Ski with c-Jun was analyzed by blotting the Flag immunoprecipitates with the anti-HA antibody. The expression of HA-c-Jun and Flag-Ski was monitored by direct immunoblotting. The results are representative of at least three independent experiments.
Figure 2

A

| Flag-Ski | +   | +   |
| HA-c-Jun | -   | +   |

IP: α-HA
Blot: α-Flag
Blot: α-HA

Myc-Smad2
HA-c-Jun

B

| Myc-Smad2 | +   | +   |
| HA-c-Jun  | -   | +   |

IP: α-HA
Blot: α-Myc
Blot: α-Myc
Blot: α-HA

INPUT
GST
GST-Ski
Figure 3

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Figure 4

Luciferase activity (arbitrary units)
Figure 5

A

| HA-TβRI | + | - | + | - |
| HA-TβRIact | - | + | - | + |
| Flag-Ski | - | - | + | + |
| HA-c-Jun | + | + | + | + |

Blot: α-Flag
Blot: α-HA

% of control (x100) 0 0 1 0.4

B

| Flag-Ski | + | + |
| HA-c-Jun | + | + |

IP: α-Flag
Blot: α-HA

% of control (x100) 1 0.4

C

| Ski | - | + |
| Flag-Ski | - | - |
| HA-c-Jun | + | + |

IP: α-c-Jun
Blot: α-Ski

% of control (x100) 1 0.7

D

Tr: Ski
Im: Ski

Tr: Ski + c-Jun
Im: c-Jun

Tr: Ski + c-Jun
Im: Merge

Tr: Ski + c-Jun
Im: Merge

E

% of Nuclear Ski (arbitrary units)

0 10 20 30 40 50 60 70 80 90 100

Ski
Ski + c-Jun
Ski + c-Jun + TβRIact

0 0.4% of control (x100)
Figure 6

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c-Jun associates with the oncoprotein Ski and suppresses Smad2 transcriptional activity

Marcia Pessah, Jacqueline Marais, Céline Prunier, Nathalie Ferrand, François Lallemand, Alain Mauviel and Azeddine Atfi

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