SCL/TAL1 Interrupting Locus Derepresses GLI1 from the Negative Control of Suppressor-of-Fused in Pancreatic Cancer Cell

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Abstract

As a physically binding protein of GLI1 transcription factor, Suppressor-of-Fused (SUFU) has been placed in the center of negative regulation of Hedgehog (Hh) signaling. SUFU tethers GLI1 in cytoplasm, and in some circumstances, it moves into the nucleus in association with GLI1, leading to the suppression of GLI1 target gene expression by recruiting a corepressor complex. The activated transcriptional function of GLI1 is important for cellular proliferation in a variety of human cancers. However, it has not been revealed how GLI1 is derepressed from SUFU-mediated suppression. Here, we show SCL/TAL1 interrupting locus (SIL) product, a cytoplasmic protein overexpressed in pancreatic ductal adenocarcinoma (PDA), is responsible for the derepression of GLI1. We found SIL associated with the carboxyl terminus of SUFU, one of two distinct GLI1-binding domains, and this association was responsible for cytoplasmic tethering of SUFU. Overexpressed SIL attenuated SUFU-mediated cytoplasmic tethering and target gene suppression of GLI1. Knockdown of SIL in PDA cells conversely induced the nuclear accumulation of SUFU in association with GLI1 and the transcriptional suppression of GLI1 target genes. Importantly, we also showed that oncogenic K-RAS, and not Sonic hedgehog, enhanced the SIL association with the amino-terminus of SUFU, the other GLI1-binding domain that led to further increase of nuclear translocation of GLI1. These results uncover the role of SIL in derepressing GLI1 from the negative control of SUFU, which is a crucial step for activating Hh signaling in cancer cells. [Cancer Res 2008;68(19):7723–9]

Introduction

The activity of GLI1 has been shown to be under the control of Suppressor-of-Fused (SUFU) by two distinct mechanisms. First, SUFU binds GLI1 and tethers it in the cytoplasm (1): the amino- and carboxyl termini of SUFU bind the carboxyl and amino-termini of GLI1, respectively (2). Second, in some circumstances, SUFU moves into the nucleus in association with GLI1 and recruits the SAP18-mSin3 corepressor complex (3). Either mechanism results in GLI target gene suppression. However, it has still been a mystery how GLI1-negative regulation of SUFU is abrogated, which is crucial for the cellular proliferation of many types of human tumors (4–6).

Recently, mouse genetic models have revealed that the cooperative activation of K-RAS with GLI2 in pancreatic duct epithelium induced pancreatic intraepithelial neoplasia (PanIN; ref. 7), a precursor lesion of pancreatic ductal adenocarcinoma (PDA), and the early onset of PDA (8). Furthermore, oncogenic RAS signaling was shown to increase the transcriptional activity of GLI1 by enhancing its nuclear translocation in melanoma and NIH3T3 cells (9, 10), the molecular mechanism of which is not clear.

Cytoplasmic protein SCL/TAL1 interrupting locus (SIL) is implicated in the mitotic activity and cellular proliferation of cancer cells (11–13). *Sil* null mouse embryos are lethal in the early stage of development with abnormal left-right specification (14), which are also found in *sonic hedgehog* (*shh*) null embryos (14–16), and double null embryos of *sil* and *patched1*, in which the intracellular Hedgehog (Hh) signaling should be constitutively activated, were phenotypically identical with *sil* null embryos (17). This suggests SIL is indispensable for the intracellular Hh signaling.

Using PDA cells, we have elucidated the molecular mechanisms of SIL to regulate Hh signaling: SIL associates with SUFU and attenuates its suppressive role for GLI1, which is enhanced by oncogenic K-RAS but not Hh signaling itself.

Materials and Methods

Cells, antibodies, and reagents. Human PDA cell lines were obtained from the Cell Resource Center for Biomedical Research, Tohoku University (Sendai, Japan). Affinity-purified, rabbit anti-SIL antibody was raised against synthetic polypeptides (SVGTFLDVKRLRQLPKLFC) corresponding to the carboxyl terminus of human SIL protein. The other antibodies, PCR primers, and 21-nucleotide duplex small interfering RNAs (siRNA) for human SIL (siSIL), SUFU (siSUFU), and a control (siControl) are listed in Supplementary information. Transfection was carried out by using LipofectAMINE2000 (Invitrogen) for siRNAs and LipofectAMINE and PLUS reagent (Invitrogen) for vectors. Luciferase reporter assay was carried out by using Dual-luciferase system (Promega). Semiquantative reverse transcribed–PCR (RT-PCR) reaction with a mixture of oligo(dT)15 and random primers was done using *β-ACTIN* as an internal control to normalize gene expression.

Vectors. The expression vectors for mouse SIL and FLAG-tagged dominant-negative Erk (FLAG-dnERK) were gifts from Dr. Shai Izraeli (Sheba Medical Center) and Dr. Jiig-Dwan Lee (The Scripps Research Institute), respectively. GLI1-responsive *firefly* lucifarese reporter vector (8×3′) and its mutant (8×m3′) were obtained from Dr. Hiroshi Sasaki (RIKEN). The expression vectors for truncated SIL were constructed by introducing PCR-amplified cDNA fragments into pCMV-TnT vector (Promega), in which FLAG-tag sequence was sequentially introduced. PCR-amplified truncated cDNA fragments of mouse SUFU were introduced into pcDNA3.1myc-His(+) vector (Invitrogen). The expression vectors for the amino- and carboxyl termini of SUFU fused with EGFP sequence were made by sequential cloning of SUFU fragments and EGFP into pcDNA3.1(+)myc-His(+) vector. The other vectors were previously described (18).

Immunoprecipitation assay, subcellular protein fractionation, and immunoblot analysis. Whole cell lysates were prepared in lysis buffer

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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doi:10.1158/0008-5472.CAN-07-6661

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[20 mmol/L Tris-HCl (pH 7.5), 150 mmol/L NaCl, 1 mmol/L EDTA, 0.5 mmol/L sodium orthovanadate, 1% Triton X-100, and Complete protease inhibitor cocktail (Roche)] and subjected to immunoprecipitation assay using corresponding antibodies followed by purification with Protein G–Sepharose beads (GE Healthcare). Subcellular protein fractionation was carried out with ProteoExtract subcellular proteome kit (Calbiochem) according to the manufacturer's protocol. Immunoblot analysis was done as previously reported (19).

Immunohistochemical and immunofluorescence staining. Human pancreatic tissues were used for immunohistochemical staining on the approval of the Institutional Ethical Review Board. Sections from formalin-fixed, paraffin-embedded specimens were subjected to H&E staining for histologic diagnosis and also immunohistochemical staining using anti-SIL and SUFU antibodies, followed by a standard avidin-biotin horseradish peroxidase detection system (Nichirei), according to the manufacturer's protocol. Cells in culture were briefly fixed with 4% buffered paraformaldehyde and used for immunofluorescence staining.

5-Bromo-2'-deoxyuridine labeling analysis. siRNA-transfected cells were pulse-labeled with 5-bromo-2'-deoxyuridine (BrdUrd) for 6 h and fixed in 70% ethanol. After denaturation (2 N HCl for 20 min) and neurtralization

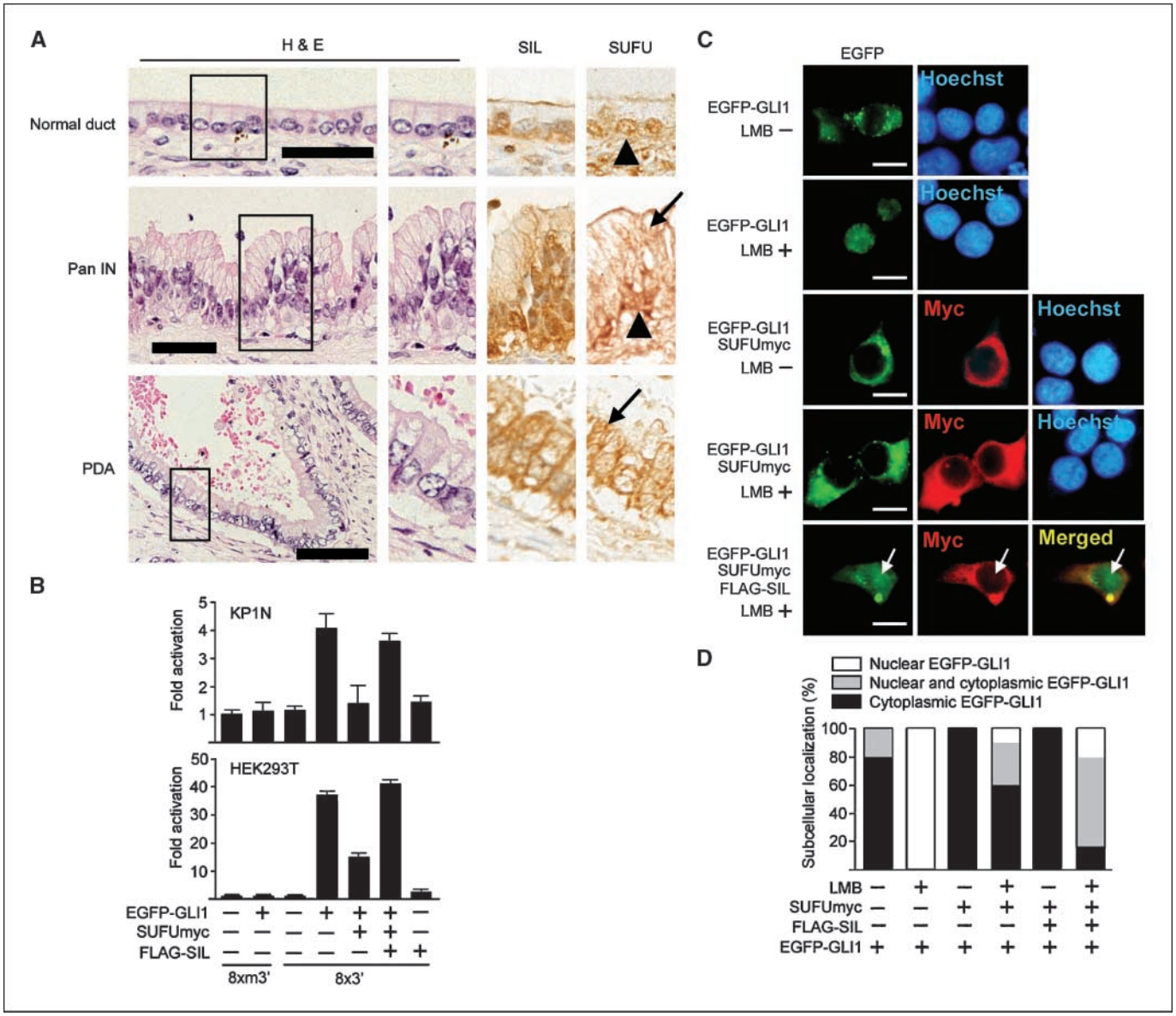

Figure 1. Overexpression of SIL in human PDA and its role in attenuating SUFU-mediated suppression of GLI1. *A,* H&E staining of human pancreatic tissue and immunohistochemical staining with anti-SIL and anti-SUFU antibodies corresponding to rectangle areas. *Arrowheads,* nuclear staining of SUFU; *arrows,* cytoplasmic staining of SUFU. Note the increased staining intensity of SIL (quantitation of SIL staining intensity in Supplementary Fig. S1) and also the cytoplasmic staining of SUFU in PDA. *Bar,* 50 μm (normal duct and PanIN) and 100 μm (PDA). *B,* luciferase reporter assay in KP1N and HEK293T cells. Cells were transiently transfected with either GLI1-responsive (8×3′) or nonresponsive mutant (8×m3′) *firefly* luciferase reporter vectors in conjunction with a control *Renilla* luciferase expression vector. EGFP-tagged GLI1 (*EGFP-GLI1*), myc-tagged SUFU (*SUFUmyc*), and FLAG-tagged SIL (*FLAG-SIL*) vectors were also used. *Columns,* means of three independent experiments; *bars,* SD. *C*, subcellular localization of EGFP-GLI1 in HEK293T cells. Cells were transiently transfected with EGFP-GLI1 in conjunction with indicated expression vectors. To block GLI1 nuclear export, LMB treatment was done in indicated transfectants. Protein expression was represented by immunofluorescence staining using anti-myc and anti-FLAG (data not shown) antibodies. Nuclear images were represented by Hoechst33342 (*Hoechst*) staining. *Arrows,* nucleus. *Bar*, 20 μm. *D,* quantitation of subcellular localization of GLI1 by calculating the ratio of transfectants having nuclear EGFP-GLI1 (*white columns*), cytoplasmic EGFP-GLI1 (*black columns*), and nuclear and cytoplasmic EGFP-GLI1 (*gray columns*). Quantitated data from one representative experiment was shown. In one experiment, >100 transfectants of each vector combination were estimated. Three independent experiments were done with similar results.

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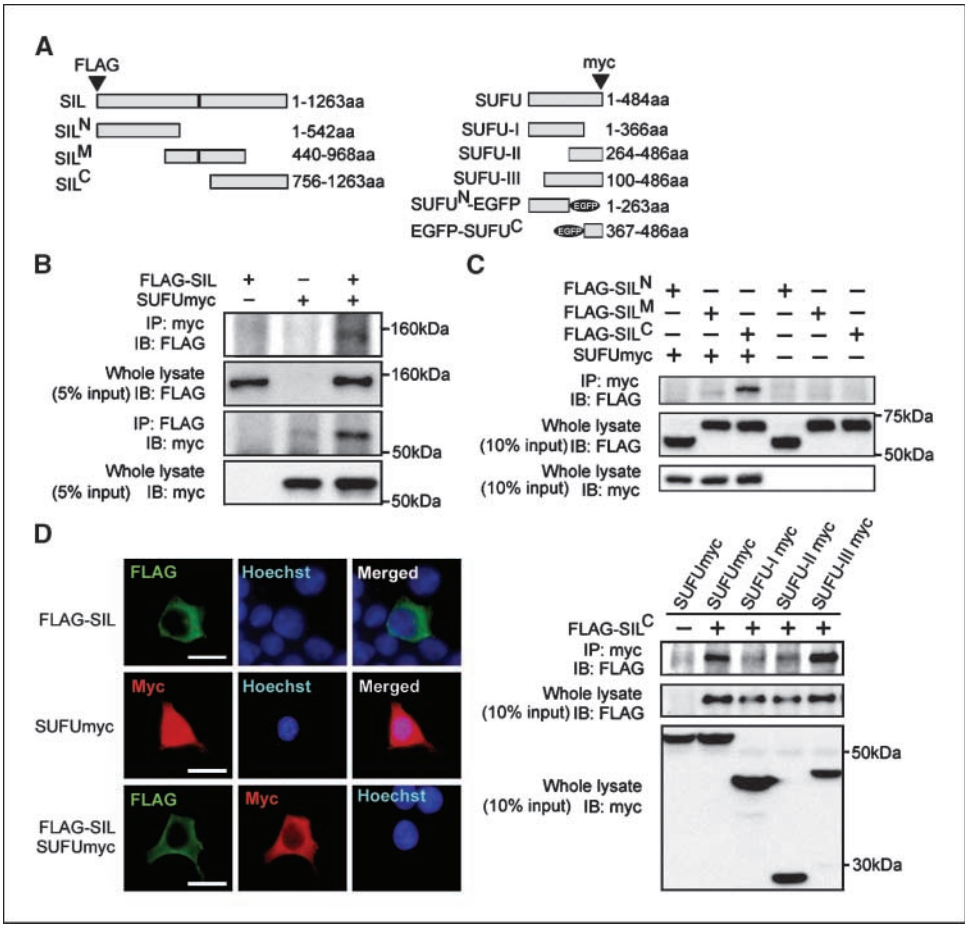

Figure 2. Association between SIL and SUFU. *A,* schematic representation of SIL (*left*) and SUFU (*right*) expression vectors. Short coiled-coil structure of SIL was predicted by the COILS algorithm and highlighted in black. *Arrowheads,* either FLAG or myc-tag sequences. *B* and *C*, immunoprecipitation assays in KP1NL (*B*) and HEK293T (*C, top* and *bottom*) cells. At 24 h after transient transfection with indicated expression vectors, whole cell lysates of transfectants were subjected to immunoprecipitation (*IP*) followed by immunoblot (*IB*) analyses using anti-myc or anti-FLAG antibodies. Note that in *C*, FLAG-SIL^M and FLAG-SIL^C, which are rich in hydrophobic amino acids, show slow migration in SDSPAGE. MALDI-TOF mass spectrum of trypsin-digested peptide fragments of FLAG-SIL^N, FLAG-SIL^M, and FLAG-SIL^C revealed their consistency with the predicted polypeptides of corresponding SIL regions (Supplementary Fig. S4*A* and S4*B*). *D,* subcellular localization of FLAG-SIL and SUFUmyc in transiently transfected HEK293T cells. Protein expression was represented by immunofluorescence staining using anti-FLAG rabbit antibody and anti-myc mouse antibody. Nuclear images were represented by Hoechst 33342 (*Hoechst*) staining. More than 100 transfectants were estimated, and photos of representative transfectants were shown. *Bar,* 20μm.

(0.1 mol/L sodium borate, pH 8.5), cells were incubated with Alexa 488–labeled anti-BrdUrd monoclonal antibody (Invitrogen). Ratio of BrdUrd-positive cells was analyzed by FACSCalibur (BectonDickinson).

Results and Discussion

Overexpressed SIL attenuates SUFU-mediated GLI1 suppression. To clarify the role of SIL in Hh signaling, we started with immunohistochemical analysis of SIL and SUFU in human pancreatic tissues (Fig. 1*A*). SUFU expression was found in the nucleus of normal duct epithelium (Fig. 1*A, arrowheads*). Importantly, SUFU was detected in both the nucleus and cytoplasm of PanIN and was seen exclusively in the cytoplasm of PDA cells (Fig. 1*A, arrows*). SIL expression was weakly detected in the basal side of normal duct epithelium, but its staining intensity significantly increased in the cytoplasm of PanIN and PDA cells (quantitation of SIL staining intensity in Supplementary Fig. S1). We, therefore, presumed that increased SIL expression might tether SUFU in the cytoplasm of PanIN and PDA cells, which somehow derepresses GLI1 from SUFU-mediated control.

To prove this hypothesis, we switched to cell culture experiments using human PDA cell line KP1N and KP1NL, in which SIL mRNA and protein were expressed (Supplementary Fig. S2; Supplementary information for references about cell lines). We first examined whether SIL might modify SUFU-mediated transcriptional suppression of GLI1 by luciferase reporter assay in KP1N and HEK293T cells (Fig. 1*B*), because we previously found that overexpressed SIL enhanced the transcriptional activity and nuclear accumulation of GLI1 in HEK293T cells (Supplementary Fig. S3). As previously reported (1–3), SUFU effectively suppressed GLI1-dependent activation of 8×3′ reporter vector. We found that SIL recovered the reporter activity from SUFU-induced suppression in both cell lines. Next, we examined the effect of SIL on SUFU-mediated cytoplasmic tethering of GLI1. It was reported that GLI1 shows nuclear-cytoplasmic shuttling and its nuclear export is CRM1-dependent (1). To estimate GLI1 nuclear translocation, we used Leptomycin B (LMB), a specific inhibitor for CRM1, as indicated (Fig. 1*C* and *D*). In agreement with previous reports (1), GLI1 accumulated in the nucleus in LMB-treated cells, and this GLI1 accumulation was blocked in the presence of SUFU. We found that SIL transfection counteracted SUFU, resulting in a decrease of "cytoplasmic GLI1" and an increase of both "nuclear GLI1" and "nuclear and cytoplasmic GLI1" (photos of representative transfectants in Fig. 1*C* and quantitation of subcellular localization of EGFP-GLI1 in Fig. 1*D*). These observations indicate that increased expression of SIL attenuated the function of SUFU, leading to the nuclear accumulation and target gene activation of GLI1.

SIL associates with SUFU. To clarify how SIL attenuated SUFU function, we examined whether SIL associates with SUFU by immunoprecipitation assay. As shown in Fig. 2*B*, FLAG-SIL was coprecipitated with SUFUmyc, and reciprocally, SUFUmyc was coprecipitated with FLAG-SIL, indicating their specific association. To narrow down the responsible region for the association, immunoprecipitation with expression vectors for truncated SIL

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and SUFU (Fig. 2*A*) was carried out. We found that among three truncated SIL proteins (Fig. 2*A, left*), only FLAG-SIL^{C} (756-1263aa) was coprecipitated with SUFUmyc (Fig. 2*C, top*). Next, using myc-tagged truncated SUFUs (Fig. 2*A, right*), we found that FLAG-SIL^{C} was coprecipitated with SUFU-IIImyc (100-486aa), as well as SUFUmyc (1-486aa; Fig. 2*C, bottom*). Coprecipitated FLAG-SIL^{C} was substantially reduced, but still detectable, in the SUFU-IImyc (264-484aa) precipitate, whereas it was poorly detected in the SUFU-Imyc (1-336aa) precipitate (Fig. 2*C*). As supported by Fig. 3*A* (see below), this result suggests that SUFU contains two distinct regions to associate with FLAG-SIL^{C}: the carboxyl terminus of SUFU (367-486aa) for strong association and the amino-terminus of SUFU (1-263aa, especially 100-263aa) for weak association. We also examined subcellular localization of FLAG-SIL and SUFUmyc in HEK293T cells (Fig. 2*D*). FLAG-SIL was localized in the cytoplasm, and SUFUmyc was distributed throughout the cytoplasm and nucleus as previously shown (1). We found that SUFUmyc accumulated in the cytoplasm in conjunction with FLAG-SIL coexpression, supporting the SIL-SUFU association *in vivo*.

Oncogenic K-RAS, but not SHH, enhances SIL association with SUFU. Given recent reports showing that oncogenic RAS signaling enhances the transcriptional function of GLI1 (9, 10), we examined whether oncogenic RAS signaling might affect SIL-SUFU association. For this purpose, we constructed myc-tagged EGFP-fused expression vectors of either the amino-terminus (1-263aa) or carboxyl terminus (367-486aa) of SUFU (SUFU^{N}-EGFPmyc, EGFP-SUFU^{C}myc; Fig. 2*A, right*) and myc-tagged EGFP vector as a control, because it was previously reported that either terminus

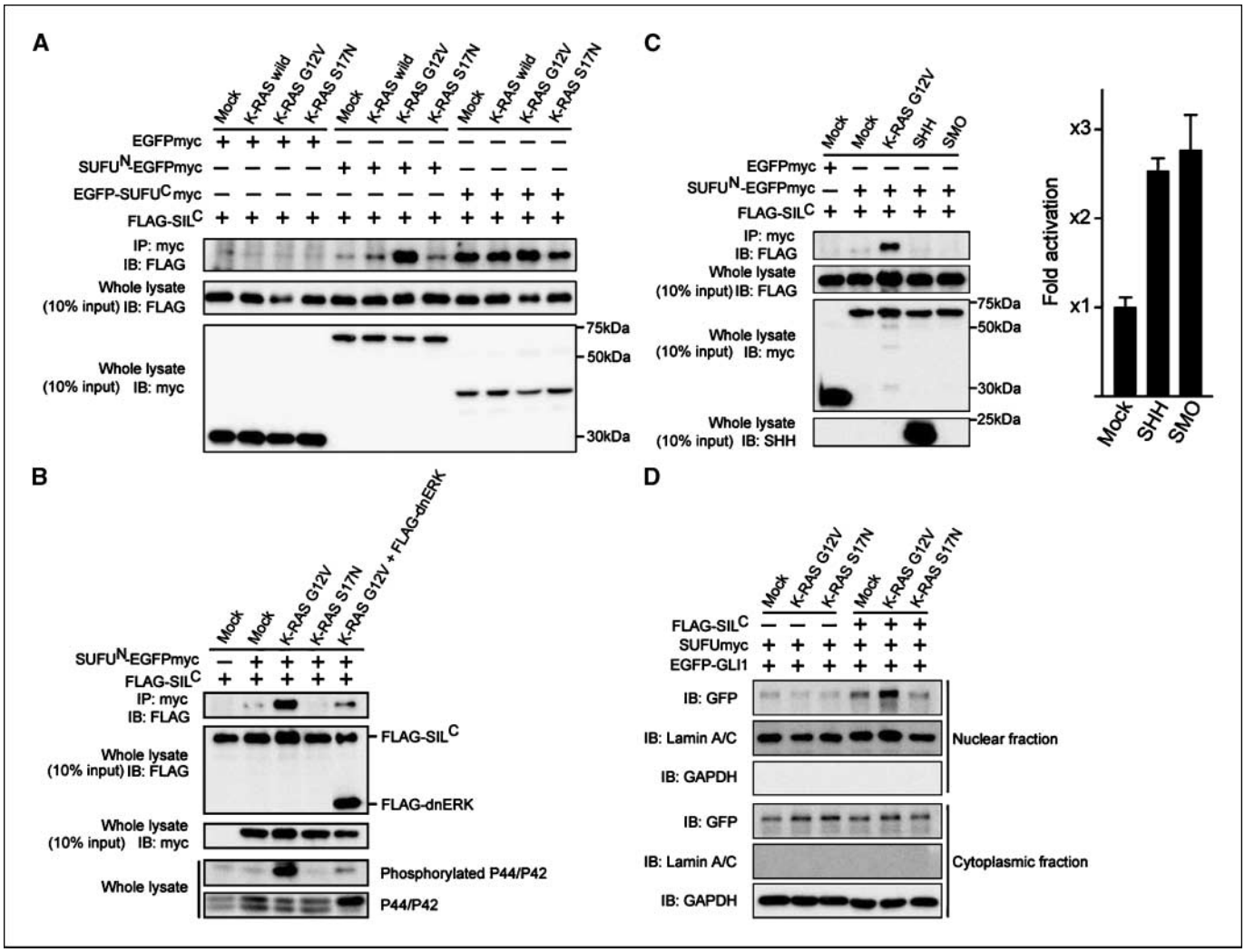

Figure 3. Oncogenic K-RAS but not Hh signaling enhanced SIL association with SUFU. *A,* immunoprecipitation assay in HEK293T cells. At 24 h after transient transfection with indicated expression vectors, whole cell lysates of transfectants were subjected to immunoprecipitation (*IP*) followed by immunoblot (*IB*) analyses using anti-myc or anti-FLAG antibodies. *B,* immunoprecipitation assay in HEK293T cells. FLAG-tagged dominant-negative Erk vector (FLAG-dnErk) was used to abrogate RAS signaling, which was monitored by the phosphorylation status of the P44/42 Erk proteins. Note that the expression of FLAG-dnERK, which was detected by anti-FLAG and anti-P44/P42 antibodies, reduced phosphorylated P44/P42 in the presence of K-RAS G12V. *C, left,* immunoprecipitation assay in HEK293T cells. *Right*, luciferase reporter assay in HEK293T cells using *GLI1*-promoter containing *firefly* luciferase reporter vector (18) in conjunction with indicated expression vectors and a control *Renilla* luciferase expression vector. *Columns,* means of three independent experiments; *bars,* SD. *D,* oncogenic K-RAS enhanced SIL-mediated nuclear accumulation of GLI1. HEK293T cells were transiently transfected with indicated expression vectors followed by LMB treatment. Nuclear and cytoplasmic protein fractions of transfectants were subjected to immunoblot analysis using anti-GFP antibody. To validate fractionation, anti-Lamin A/C and anti–glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) antibodies were also used for immunoblot.

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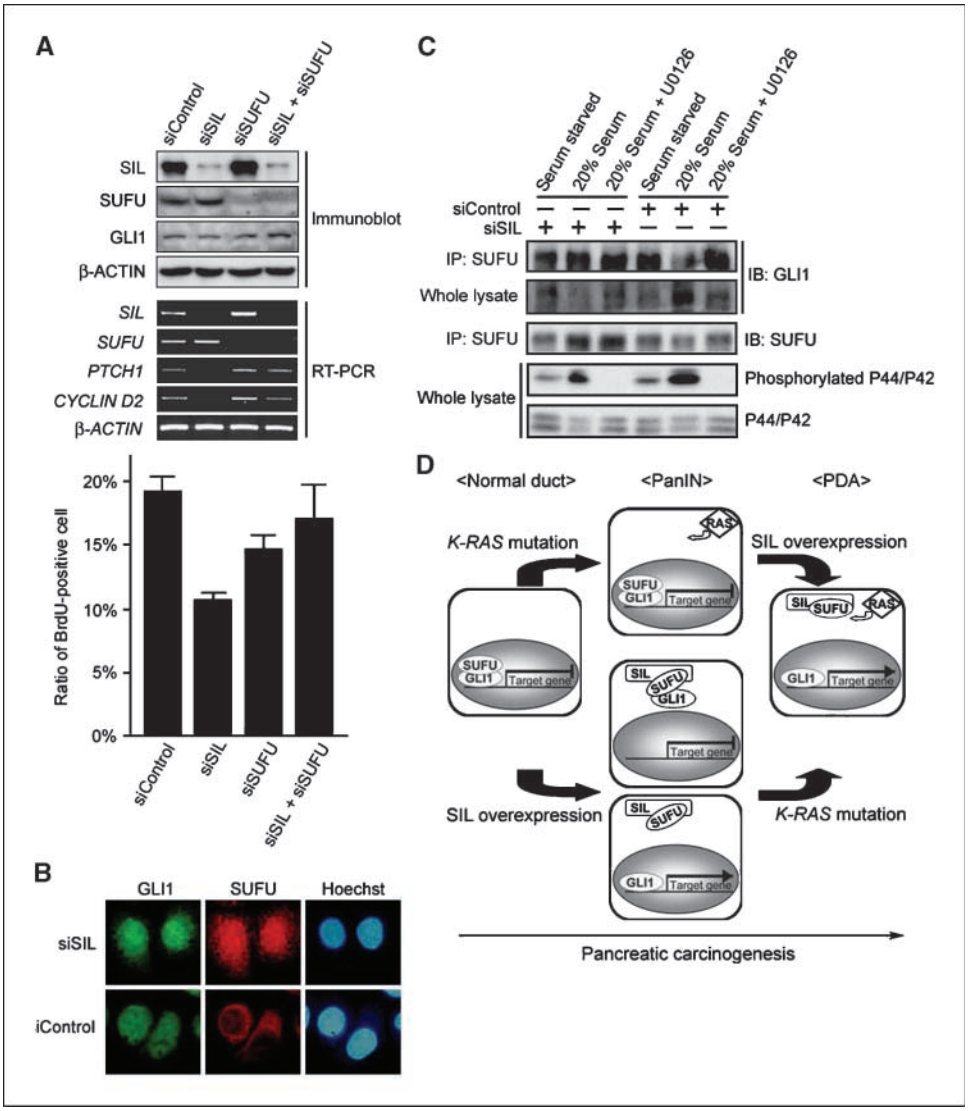

Figure 4. *SIL* knockdown experiments in PDA cells. *A, top* and *middle,* immunoblot (*top*) and RT-PCR (*middle*) analyses of KP1NL cells 48 h after transfection with siSIL, siSUFU, and siControl; *bottom,* BrdUrd labeling analysis of siRNA-transfected KP1NL cells. *Columns,* means of three independent experiments; *bars,* SD. *B,* subcellular localization of endogenous GLI1 and SUFU in siRNA-transfected KP1N cells. At 48 h after transfection, cells were treated 4 h with LMB in the culture medium supplemented with 10% serum, followed by immunofluorescence staining using anti-GLI1 and anti-SUFU antibodies. More than 100 transfectants were estimated, and photos of representative transfectants were shown. *C,* immunoprecipitation assay of siRNA-transfected KP1NL cells. At 24 h after transfection, cells were switched into the serum-starved medium and maintained another 12 h. After serum stimulation with or without U0126 pretreatment, whole cell lysates of transfectants were subjected to immunoprecipitation using anti-SUFU antibody. Note that coprecipitated GLI1 was reduced by the serum stimulation in siControl-transfectants, but not in siSIL-transfectants. *D,* schematic presentation of proposed mechanism for the stepwise GLI1 activation during pancreatic carcinogenesis. In normal duct epithelium of pancreas, SUFU accumulates in the nucleus and suppresses the transcriptional activity of GLI1. In the course of carcinogenesis, the increased expression of SIL tethers SUFU in cytoplasm through binding with the carboxyl terminus of SUFU. In this situation, GLI1 might bind the amino-terminus of SUFU in the cytoplasm, but this binding is not strong enough to anchor GLI1 in cytoplasm; GLI1 in part might be released from cytoplasmic SUFU and move into the nucleus. The activated RAS signaling due to oncogenic mutation in *K-RAS* gene enhances the association between SIL and the amino-terminus of SUFU, thereby enhancing the release of GLI1 from SUFU, resulting in the full activation of GLI1 transcriptional function. Hh signaling is crucial for the expression of *GLI1* (18) but does not enhance SIL-SUFU association.

of SUFU, corresponding to $SUFU^N$ and $SUFU^C$, independently bound GLI1, but binding with both termini was needed for effective suppression of GLI1 (2). And we used three types of human K-RAS expression vectors [wild type (wild), constitutive active mutant (G12V), and dominant-negative mutant (S17N)] to modulate RAS signaling. As shown in Fig. 3*A*, we found that FLAG-SIL^C was coprecipitated not with control EGFPmyc but with EGFP-$SUFU^C$ myc, and this coprecipitation was not affected by the status of K-RAS, suggesting SIL associated with the carboxyl terminus of SUFU constitutively. In contrast, the coprecipitation of FLAG-SIL^C with $SUFU^N$-EGFPmyc significantly increased in the presence of K-RAS G12V, but not K-RAS S17N (Fig. 3*A*). Furthermore, the K-RAS G12V-dependent FLAG-SIL^C coprecipitation with $SUFU^N$-EGFPmyc was reduced in the presence of dominant-negative Erk (FLAG-dnERK; Fig. 3*B*). These results indicate that SIL associates with the amino-terminus of SUFU in oncogenic K-RAS–dependent manner. Interestingly, we found that the FLAG-SIL^C coprecipitation with $SUFU^N$-EGFPmyc was not increased by cotransfection of either SHH or SMOOTHENED (SMO) expression vectors (Fig. 3*C, left*). Because transfection of either SHH or SMO expression vectors

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activated *GLI1* promoter-containing luciferase reporter vector (Fig. 3*C, right*; ref. 18), we assume that Hh signaling itself does not enhance SIL-SUFU association.

Oncogenic K-RAS enhances SIL-mediated nuclear accumulation of GLI1. Next, we examined whether oncogenic K-RAS might enhance SIL-mediated nuclear accumulation of GLI1 in the presence of SUFU. HEK293T cells were cotransfected with EGFP-GLI1, SUFUmyc, and FLAG-SIL^C in conjunction with K-RAS expression vectors and were treated with LMB. Nuclear and cytoplasmic protein fractions of transfectants were subjected to immunoblot analysis using anti-GFP antibody to reveal the amount of nuclear and cytoplasmic EGFP-GLI1 protein (Fig. 3*D*). We found an increase of nuclear EGFP-GLI1 by FLAG-SIL^C transfection, which was further increased by K-RAS G12V, but not S17N, transfection. Importantly, K-RAS G12V transfection without FLAG-SIL^C did not increase nuclear EGFP-GLI1, suggesting the role of oncogenic K-RAS might be SIL-dependent.

SIL is required for GLI1 derepression from SUFU in PDA cells. Given genetic evidence showing that SIL is indispensable for Hh signaling during mouse development (14, 17), we examined the effect of *SIL* knockdown on GLI1 target gene expression in KP1NL cells (Fig. 4*A*). We found that in contrast to siControl, siSIL transfection suppressed the expression of GLI1 target genes (20), including *PATCHED1* (*PTCH1*) and *CYCLIN D2* (Fig. 4*A, middle*), whereas the expression of GLI1 and SUFU proteins remained unchanged (Fig. 4*A, top*). Importantly, we also found that cotransfection of siSUFU abrogated suppressive effect of siSIL on the GLI1 target gene expression. According to *CYCLIN D2* suppression, siSIL transfection reduced ratio of BrdUrd-labeled cells, which was relieved by cotransfection of siSUFU (Fig. 4*A, bottom*). We next examined subcellular localization of endogenous GLI1 and SUFU in the knockdown cells (Fig. 4*B*). Either siSIL-transfected or siControl-transfected KP1N cells showed nuclear accumulation of GLI1 upon LMB treatment. We found nuclear accumulation of SUFU in siSIL-transfected cells in contrast to the cytoplasmic retention of SUFU in siControl-transfected cells (Fig. 4*B*). These results indicate that knockdown of SIL abrogates the transcriptional activity of GLI1 by increase of nuclear SUFU, which was reported to recruit the SAP18-mSin3 corepressor complex (3). Next, we examined the interaction between endogenous GLI1 and SUFU in siRNA-transfected KP1NL cells by immunoprecipitation assay (Fig. 4*C*). To modulate RAS signaling, siRNA-transfected cells were placed in three different culture conditions: serum starvation, 20% serum stimulation after serum starvation, and 20% serum stimulation after treatment with U0126, a specific inhibitor of RAS downstream effector MEK1. In this experiment, KP1NL cells in the serum-starved medium showed quite weak phosphorylation of P44/42 Erk proteins (Fig. 4*C*), although KP1NL cells have GGT (Gly) to GAT (Asp) mutation in codon 12 of *KRAS* gene (data not shown). We found that in siControl-transfected KP1NL cells, the serum stimulation reduced the GLI1 coprecipitation with SUFU whereas U0126 treatment did not, indicating the serum-activated MEK1 signaling should enhance the dissociation of GLI1 from SUFU. However, this dissociation by the serum stimulation was not observed in siSIL-transfected KP1NL cells (Fig. 4*C*). These results indicate that SIL is required for the dissociation of GLI1 from SUFU in PDA cells, which is enhanced by the activated RAS signaling. Previous report showed that SIL protein was hyperphosphorylated, especially during mitosis; however, the carboxyl terminus of SIL, corresponding to SIL^C in our study, was also revealed to be phosphorylated by either interphase or mitotic oocyte extracts of *Xenopus* (12), suggesting a role of phosphorylated SIL^C in the interphase. SIL^C contains many serine/threonine residues that are conserved between species (12). Hence, we hypothesize that oncogenic RAS signaling participates in the phosphorylation of SIL^C, which increases the binding affinity to the amino-terminus of SUFU.

Having the evidences shown here, we propose the stepwise activation of GLI1 during pancreatic carcinogenesis (Fig. 4*D*): in normal duct epithelium that SIL is poorly expressed, SUFU accumulates in the nucleus and suppresses GLI1 transcriptional activity. In the course of carcinogenesis, increased SIL protein retains SUFU in the cytoplasm. Oncogenic mutation in *K-RAS* gene, which is thought to be the early genetic event during pancreatic carcinogenesis, further enhances SIL-SUFU association, thereby releasing GLI1 from the control of SUFU, which results in the full activation of GLI1 transcriptional function.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

Received 12/16/2007; revised 7/1/2008; accepted 7/21/2008.

Grant support: Japan Society for the Promotion of Science Scientific Research grants 19590371 (K. Kasai) and 18790283 (S. Inaguma).

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We thank Shai Izraeli, Jiig-Dwan Lee, and Hiroshi Sasaki for vectors; Yukiko Matsubara, Motoyasu Takeuchi, Naoki Igari, Minoru Fukayama, and Makoto Naruse (Aichi Medical University) for assistance with immunohistochemical staining, MALDI-TOF-MS, and FACS analyses; Heinz Arnheiter (NIH) for discussion and comments on the manuscript; and Camilo Omana Villanueva, Jr. (Aichi Medical University) for English editing.

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SCL/TAL1 Interrupting Locus Derepresses GLI1 from the Negative Control of Suppressor-of-Fused in Pancreatic Cancer Cell

Kenji Kasai, Shingo Inaguma, Akiko Yoneyama, et al.

Cancer Res 2008;68:7723-7729.

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