

Signal Transducer and Activator of Transcription 3 (STAT3) Protein Suppresses Adenoma-to-carcinoma Transition in *Apc^{min/+}* Mice via Regulation of Snail-1 (SNAI) Protein Stability*[§]

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Jongdae Lee¹, Joanna C. K. Kim, Shee-Eun Lee², Christine Quinley, HyeRi Kim, Scott Herdman, Maripat Corr, and Eyal Raz³

From the Department of Medicine, University of California at San Diego, La Jolla, California 92093-0663

Background: STAT3 suppresses carcinogenesis of intestinal tumors in *Apc^{min}* mice.

Results: STAT3 suppresses expression of SNAI in intestinal epithelium by regulating GSK3 β activity.

Conclusion: STAT3 induces degradation of SNAI by promoting GSK3 β activity and thereby suppresses adenoma-to-adenocarcinoma transition in *Apc^{min}* mice.

Significance: Our data provide a new insight into the role of STAT3 in colorectal cancer biology.

STAT3 was recently reported to suppress tumor invasion in *Apc^{min/+}* mice. We investigated the mechanisms by which STAT3 inhibits intestinal epithelial tumors using *Apc^{min/+}/Stat3^{IEC-KO}* mice (intestinal epithelial cell (IEC)-specific deletion of STAT3 in the *Apc^{min/+}* background) to determine the role of STAT3 in carcinogenesis *in vivo* as well as colorectal cancer cell lines *in vitro*. To inhibit invasion of IEC tumors, STAT3 functions as a molecular adaptor rather than a transcription factor. Accordingly, the tumors in *Apc^{min/+}/Stat3^{IEC-KO}* mice undergo adenoma-to-carcinoma transition and acquire an invasive phenotype. Similarly, STAT3 knockdown in a colorectal cell line enhances IEC invasion. We demonstrate that STAT3 down-regulates SNAI (Snail-1) expression levels and hence suppresses epithelial-mesenchymal transition of colorectal cancer cells. Mechanistically, STAT3 facilitates glycogen synthase kinase (GSK) 3 β -mediated degradation of SNAI by regulating phosphorylation of GSK3 β . Our data identified a new role for STAT3 in the adenoma-to-carcinoma sequence of intestinal tumors.

STAT3 is a multifunctional transcription factor that is activated by various growth factors (*e.g.* epidermal growth factor and hepatocyte growth factor) and cytokines (*e.g.* IL-6 and IL-10). Consequently, STAT3 plays a key role in many biological processes such as cell growth, apoptosis, and inflammation (1). STAT3 is phosphorylated on a tyrosine residue (Tyr-705) by an upstream kinase JAK2 (Janus kinase 2). The phosphorylated protein forms either a homo- or heterodimer with other

STAT proteins, then translocates to the nucleus and transcribes target genes. Rare nontranscriptional activities of STAT3 have also been reported. For example, STAT3 functions as an adaptor protein connecting IFNAR1 (interferon α receptor 1) and the p85 regulatory subunit of PI3K (phosphoinositide 3-kinase) (2), and it inhibits stathmin that depolymerizes microtubules (3). Recently, STAT3 was shown to inhibit intestinal epithelial cell (IEC)⁴ tumor invasion in *Apc^{min/+}* mice (4), but the underlying mechanisms remain unclear.

The development of human colon adenomas is initially induced by mutations in APC or β -catenin, and its transition to carcinoma is followed by sequential genetic mutations in K-Ras, SMAD2 or SMAD4, and p53. Whereas deletion of p53 does not provoke malignant transformation in *Apc^{min/+}* mice (5), at least two genes were found critical for adenoma-to-adenocarcinoma transition. Tumors in *Smad4^{+/-}/Apc^{min/+}* mice become malignant, showing an extensive stromal cell proliferation and submucosal invasion (6), and *Ephb3^{-/-}/Apc^{min/+}* mice develop carcinomas that invade the muscle layer (7). However, the mechanisms by which these genes suppress IEC carcinogenesis are not fully understood.

The invasion of epithelial tumors to the surrounding tissues is associated with epithelial-mesenchymal transition (EMT), a process in which the epithelial tumor cells lose their epithelial phenotype and acquire a mesenchymal phenotype. Epithelial cells that have undergone EMT display reduced intercellular interactions but increased motility and invasiveness. Several transcriptional repressors were identified as inducers of EMT, including SNAI, Slug (Snail-2), E47, Twist, and the Zeb factors (8). Main targets of these proteins are cell adhesion molecules such as E-cadherin and claudins (CLDNs) that link epithelial cells together (8–11).

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¹ To whom correspondence may be addressed: 9500 Gilman Dr., La Jolla, CA 92093-0663. Tel.: 858-822-5762; E-mail: j142lee@ucsd.edu.

² Present address: Dental Science Research Institute, Chonnam National University, 500-757 Gwangju, Korea.

³ To whom correspondence may be addressed: 9500 Gilman Dr., La Jolla, CA 92093-0663. Tel.: 858-822-5762; E-mail: eraz@ucsd.edu.

⁴ The abbreviations used are: IEC, intestinal epithelial cell; CA, constitutively active; CLDN, claudin; CRC, colorectal cancer; EMT, epithelial-mesenchymal transition; GSK3 β , glycogen synthase kinase 3 β ; min, multiple intestinal neoplasia; MMP, matrix metalloproteinase; qPCR, quantitative PCR; SNAI, Snail-1; APC, adenomatous polyposis coli.

TABLE 1
PCR primers

Gene names	Forward primer	Reverse primer
h-GAPDH	5'-CAT GTT CGT CAT GGG TGT GAA CCA-3'	5'-AGT GAT GGC ATG GAC TGT GGT CAT-3'
h-SNAI	5'-TAC AGC GAG CTG CAG GAC TCT AAT-3'	5'-AGG ACA GAG TCC CAG ATG AGC ATT-3'

Glycogen synthase kinase 3 β (GSK3 β) performs multiple cellular functions (12). As an important regulator of metastasis, it suppresses the level of SNAI by being phosphorylated on two different sites: phosphorylation on the first site leads to ubiquitination and proteasomal degradation of SNAI whereas that on the second site controls the subcellular localization of SNAI (13). GSK3 β is inactivated by phosphorylation by several different kinases at the Ser-9 residue (12, 14) but also can be regulated by phosphorylation at other residues: it is inactivated by phosphorylation at Thr-390 (15) and is activated by phosphorylation at Tyr-216 (16, 17). In addition, GSK3 β activity is modulated by protein complex formation mediated by GSK3 β -binding proteins and its subcellular localization (18). GSK3 β is also a key regulator of β -catenin (19, 20), an effector molecule for the Wnt signaling pathway. Constitutive activation of β -catenin due to a mutation in the APC gene is the major cause of colorectal cancers (CRCs) (21) and induces the multiple intestinal neoplasia (min) phenotype in *Apc^{min/+}* mice (21–23)

Here, we identified a novel adaptor function for STAT3 in IECs, which has a broad implication to CRC. Our data show that in a nontranscriptional fashion, STAT3 suppresses expression of SNAI, a major driving force in EMT and in the invasion of CRC cells. STAT3 interacts with GSK3 β and negatively regulates phosphorylation of GSK3 β , thereby inducing degradation of SNAI.

EXPERIMENTAL PROCEDURES

Reagents and Antibodies—Anti-STAT3, anti- β -catenin, anti-phospho- β -catenin, anti-GSK3 β , anti-phospho-GSK3 β (Ser-9), anti-SNAI, anti-Slug, anti-Zeb-1, anti-matrix metalloproteinase 7 (MMP7), anti-MMP9, and anti-E-cadherin antibodies were obtained from Cell Signaling Technologies (Danvers, MA). Anti- β -actin came from Sigma. Anti-ubiquitin, anti-MMP14, and anti-MMP15 antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-claudin and anti-occludin antibodies were obtained from Lab Vision (Fremont, CA). Anti-vimentin and anti-fibronectin antibodies were obtained from BD Biosciences. Anti-axin antibody is from Invitrogen. The GFP-STAT3 plasmid was generously provided by Nancy C. Reich (24). Myc-GSK3 β -CA plasmid (13) was obtained from Addgene (Cambridge MA). LiCl was obtained from Sigma and SB216763 from EMD Biosciences (Darmstadt, Germany).

Isolation of IECs from Mouse Intestines—Isolation was performed as described previously (25, 26).

Cell Culture—Culture conditions of IEC cells have been described previously (25). HCA-7 cells were cultured in low glucose DMEM, and HCT116 and RKO cells in high glucose DMEM (Mediatech, Manassas, VA).

Cell Invasion Assay—The cell invasion assay was performed with a basement membrane-coated CytoSelect™ 24-well cell invasion assay kit according to the manufacturer's instructions

(Cell Biolabs, San Diego, CA). IECs (1×10^5 cells/well) were plated into each well 3 days after siRNA transfection and cultured for 2 days. The number of invaded cells per 4 high powered fields/well was visually counted using a microscope. The results are the average of two independent experiments, and the statistical analysis was performed by one-way ANOVA.

Quantitative PCR (qPCR), Immunoblotting, Immunohistochemistry, and Immunoprecipitation—These procedures were performed as described previously (25, 26). PCR primers are shown in Table 1.

Cell Proliferation Assay in Mice—Mice were administered BrdU intraperitoneally (2 mg/mouse) and killed 2 h after the injection. BrdU incorporation (proliferation) in paraffinized colonic tissues and the TUNEL assay in paraffinized colonic tissues were performed and analyzed according to the manufacturer's instructions (BD Biosciences).

siRNA-mediated Knockdown—Knockdown in IECs was performed with Nucleofector (Amaxa, Germany). Nontargeting siRNA #2 (luciferase targeting siRNA) from Dharmacon was used as a control. STAT3 siRNA (5'-CAACATGTCATTTGCTGAA-3'), GSK3 β siRNA (5'-TCCGAGGAGAACC-CCAATGTTTCGTATA-3'), and SNAI siRNA (Applied Biosystems, Cat. No. s13186) were used.

Generation of STAT3^{IEC-KO} Mice—To generate STAT3 deficiency specifically in IEC in mice, *Stat3^{fllox/fllox}* mice (a gift from Shizou Akira, Osaka University, Osaka, Japan) were crossed to villin-Cre mice (on the C57BL/6J background; The Jackson Laboratory, Bar Harbor, ME), to create *Stat3^{IEC-KO}* mice. Genotyping was performed as described (27). *Apc^{min/+}* mice were obtained from The Jackson Laboratory. *Apc^{min/+}/Stat3^{IEC-KO}* mice were generated by crossing *Apc^{min/+}* mice with *Stat3^{IEC-KO}* mice. All animal protocols received prior approval by the Institutional Animal Care and Use Committee.

RESULTS

STAT3 Suppresses Invasion of Intestinal Tumors in *Apc^{min/+}* Mice—To determine the role of STAT3 in intestinal tumorigenesis, we conditionally deleted STAT3 in IEC (*STAT3^{IEC-KO}*) in *Apc^{min/+}* mice (supplemental Fig. 1, A and B). At 20 weeks of age, the tumor count in *Apc^{min/+}/Stat3^{IEC-KO}* mice was comparable with that in *Apc^{min/+}* mice (supplemental Fig. 1C). In addition, proliferation of IECs in normal or tumor IEC was not significantly affected by STAT3 deletion (supplemental Fig. 1D).

Whereas the IEC tumors in *Apc^{min/+}* mice were strictly confined to the mucosal crypts, those in the *Apc^{min/+}/Stat3^{IEC-KO}* mice invaded the mucosal stroma, submucosa, and muscle (Fig. 1A). Such invasive tumors were found in all of the *Apc^{min/+}/Stat3^{IEC-KO}* mice examined at 20 weeks ($n = 20$) of age and were confirmed by β -catenin immunostaining (Fig. 1A). At 25 weeks of age, the IEC tumors in *Apc^{min/+}/Stat3^{IEC-KO}* mice penetrated through the intestinal serosa (Fig. 1B). Although some of the

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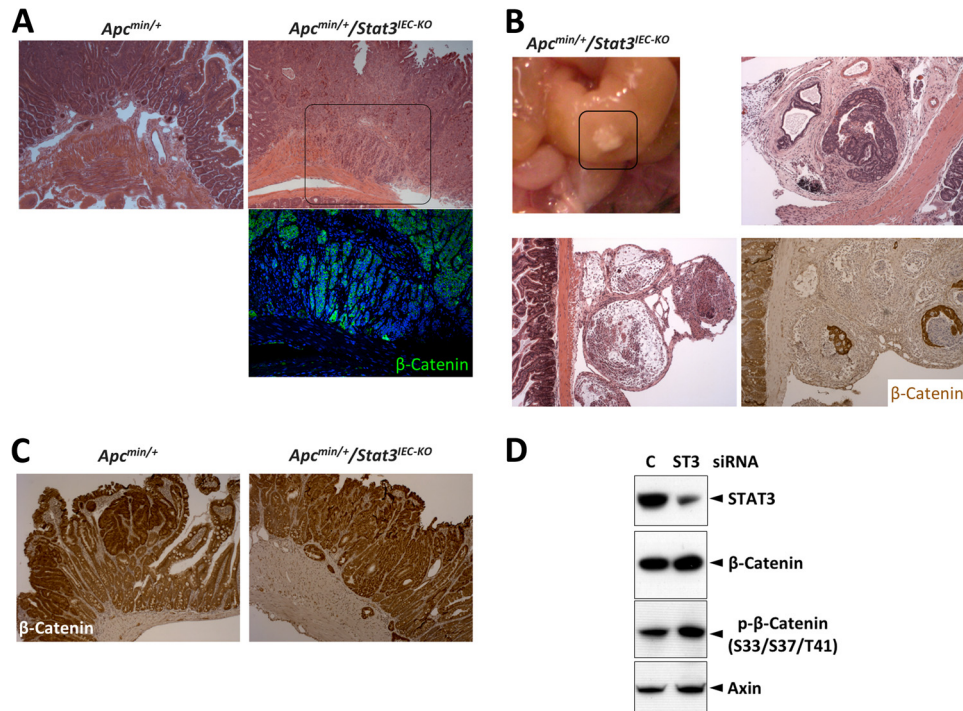


FIGURE 1. STAT3 suppresses tumor invasion in *Apc*^{min/+} mice. *A*, deletion of STAT3^{IEC} induces invasion of tumors in *Apc*^{min/+} mice. H&E (upper panel) and β -catenin (lower panel) staining of the small intestines shows the invasion of tumors in *Apc*^{min/+}/*Stat3*^{IEC-KO} mice. *B*, tumors in *Apc*^{min/+}/*Stat3*^{IEC-KO} mice penetrate the intestinal walls into the peritoneum. The boxed area in the upper left panel was analyzed by H&E and β -catenin staining. *C*, STAT3 does not regulate expression of β -catenin in *Apc*^{min/+} mice. Expression of β -catenin in the small intestines was measured by immunohistochemistry. *D*, STAT3 suppresses activation of β -catenin in a CRC cell line. HCT116 cells were transfected with GFP or GFP-STAT3-WT (ST3), and the levels of the indicated proteins were measured by immunoblotting.

tumors penetrating serosa were necrotic, live tumor cells (β -catenin-positive) were also found surrounded by stromal tissues (Fig. 1*B*). However, STAT3 deletion did not significantly affect the levels of β -catenin in tumor cells (Fig. 1*C*). In a human colorectal cell line, STAT3 knockdown slightly enhanced the levels of β -catenin, phospho- β -catenin, and axin (a target of β -catenin), indicating that it can suppress the β -catenin pathway (Fig. 1*D*). Taken together, these findings validate that STAT3 in this animal model inhibits invasion of IEC tumors without affecting tumor induction (4).

STAT3 Suppresses EMT—Because EMT often precedes tumor invasion, we next investigated whether STAT3 deletion induces EMT in IEC tumors. Expression of E-cadherin or occludin was largely unaffected by STAT3 deletion (Fig. 2*A*), but expression of CLDN-3 and CLDN-5 was markedly decreased in tumor cells of *Apc*^{min/+}/*Stat3*^{IEC-KO} mice compared with those in *Apc*^{min/+} mice (Fig. 2*B*). Vimentin was expressed mainly in mesenchymal cells and rarely in tumor cells in *Apc*^{min/+} mice; however, all of the tumor cells in the *Apc*^{min/+}/*Stat3*^{IEC-KO} mice expressed vimentin at a high level (Fig. 2*C*). Fibronectin was also up-regulated in the tumor cells of *Apc*^{min/+}/*Stat3*^{IEC-KO} mice whereas it was not detectable in tumor from *Apc*^{min/+} mice (Fig. 2*D*). Interestingly fibronectin in *Apc*^{min/+}/*Stat3*^{IEC-KO} was observed only in IEC tumor cells around the necrotic regions (Fig. 2*D*). Collectively, these data highly suggest that STAT3 suppresses EMT in IEC tumor cells in *Apc*^{min/+} mice.

STAT3 Suppresses Expression of MMP14—We next checked the expression of MMPs, which are important for tumor inva-

sion (28, 29). Whereas MMP7 expression in tumor cells was significantly lower in *Apc*^{min/+}/*Stat3*^{IEC-KO} mice than that in *Apc*^{min/+} mice, MMP9 or MMP15 (MT2-MMP) expression was minimal and observed mostly in nonepithelial cells in both strains of mice (supplemental Fig. 2). In contrast, MMP14 (MT1-MMP) expression in tumor cells was highly induced in *Apc*^{min/+}/*Stat3*^{IEC-KO} mice but undetectable in *Apc*^{min/+} mice (Fig. 2*E*). Interestingly, MMP14, a membrane type metalloproteinase, is particularly efficient in hydrolyzing basement membranes (30, 31), and its expression can be induced by SNAI in carcinomas (30).

STAT3 Suppresses Tumor Invasion via Down-regulation of SNAI—To examine further the suppressive effect of STAT3 on EMT, known regulators of EMT were examined. Whereas expression of Slug and Zeb-1 were barely detectable, and their levels were not significantly different in IEC tumor cells from both strains of mice (supplemental Fig. 3), SNAI expression was significantly elevated in *Apc*^{min/+}/*Stat3*^{IEC-KO} tumor cells (Fig. 3*A*). To investigate further the role of STAT3 in SNAI expression and the role of SNAI in invasion, we used CRC cell lines in the subsequent studies. In three different CRC cell lines, HCT116, HCA-7, and RKO, STAT3 knockdown increased the expression of SNAI (Fig. 3*B*). Conversely, ectopic expression of STAT3-WT suppressed expression of SNAI (Fig. 3*C*).

We next tested whether the elevated SNAI was responsible for the invasive phenotype of STAT3-depleted CRC cells. STAT3, SNAI, or STAT3 with SNAI was silenced in HCT116 cells, and the transfected cells were subjected to an *in vitro* cell invasion assay. STAT3 knockdown significantly enhanced

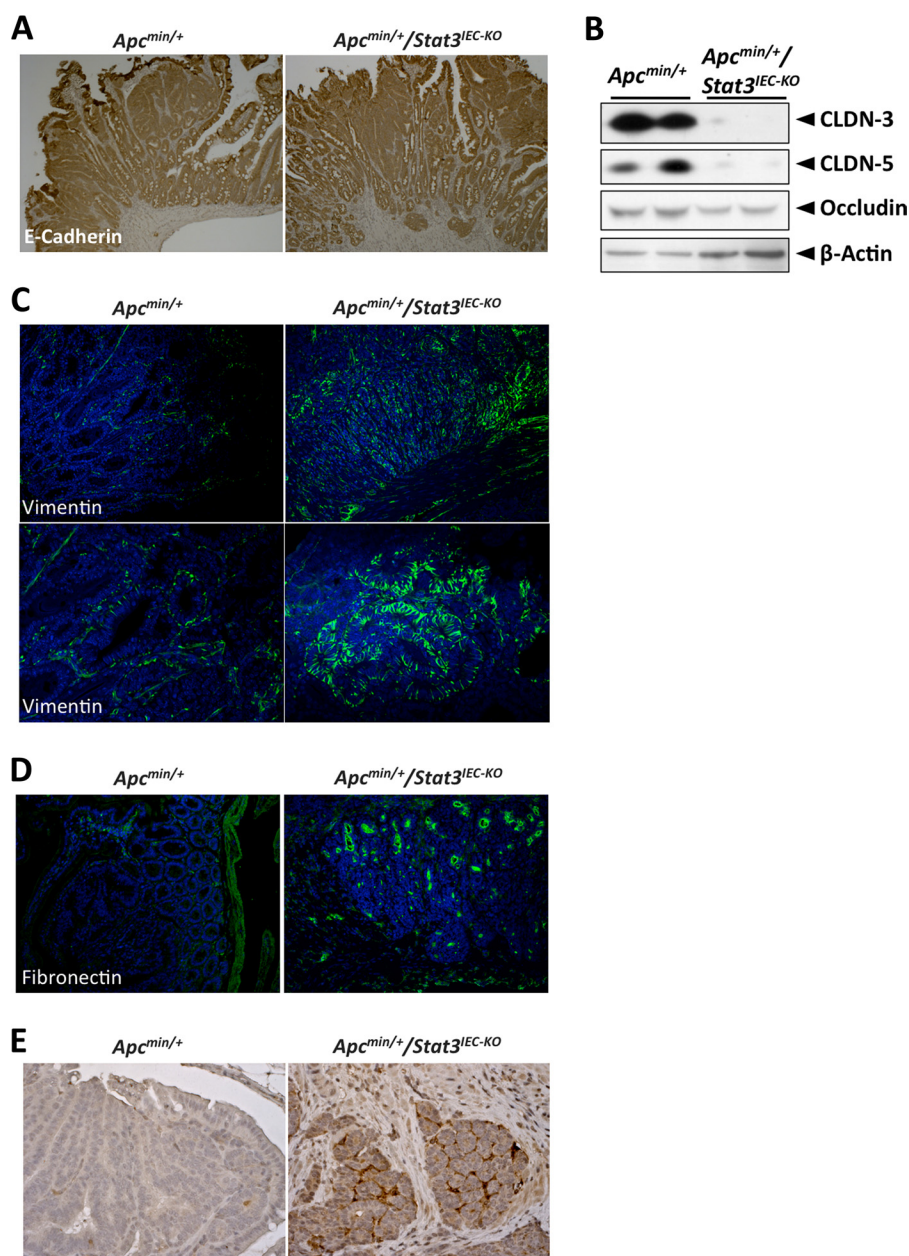


FIGURE 2. STAT3 suppresses EMT in $Apc^{min/+}$ mice. *A*, STAT3 does not regulate expression of E-cadherin in $Apc^{min/+}$ mice. Expression of E-cadherin in the small intestines was measured by immunohistochemistry. *B*, STAT3^{IEC} maintains the expression of CLDNs in $Apc^{min/+}$ mice. Expression of the indicated proteins was measured by immunoblotting with the isolated tumor cells from the small intestines. *C*, STAT3 suppresses the expression of mesenchymal marker vimentin in $Apc^{min/+}$ mice. Expression of vimentin was measured by confocal imaging in the small intestines (*lower panel* original magnification, $\times 100$). *D*, STAT3 suppresses the expression of mesenchymal marker fibronectin in $Apc^{min/+}$ mice. Expression of vimentin was measured by confocal imaging in the small intestines. Note that most of the fibronectin expression in $Apc^{min/+}/Stat3^{IEC-KO}$ mice is found in tumor cells around the necrotic regions. *E*, STAT3 suppresses the expression of MMP14 in $Apc^{min/+}$ mice. Expression of MMP14 was measured by immunohistochemistry in the small intestines.

invasiveness compared with control. In addition, SNAI knock-down alone or a combination of STAT3 and SNAI knockdown significantly curtailed the invasion (Fig. 3*D*). Thus, these data indicate that SNAI promotes invasion and that STAT3 down-regulates SNAI and thereby suppresses invasion of CRC cells.

STAT3 Promotes GSK3 β Activity to Induce Degradation of SNAI—We next studied how STAT3 regulates SNAI expression. STAT3 deletion or knockdown in IEC did not significantly affect the transcription of SNAI (Fig. 4, *A* and *B*). We therefore examined whether STAT3 regulated SNAI via post-translational modification. SNAI in HCT116 cells was constitutively

ubiquitinated and degraded by proteasomes (Fig. 4*C*). STAT3 knockdown significantly diminished the levels of SNAI ubiquitination, even though the levels of SNAI were higher than control (Fig. 4*C*). Because GSK3 β phosphorylates SNAI, which leads to ubiquitination and proteasomal degradation (13), we investigated whether STAT3 is involved in the ubiquitin-mediated proteasomal degradation of SNAI via GSK3 β . First, we found that GSK3 β also down-regulates SNAI in CRC cell lines (Fig. 5*A*) and that inhibition of GSK3 β activity with pharmacological inhibitors significantly increases SNAI (Fig. 5*B*). In addition, STAT3 forms a complex with GSK3 β (Fig. 5*C*). Because

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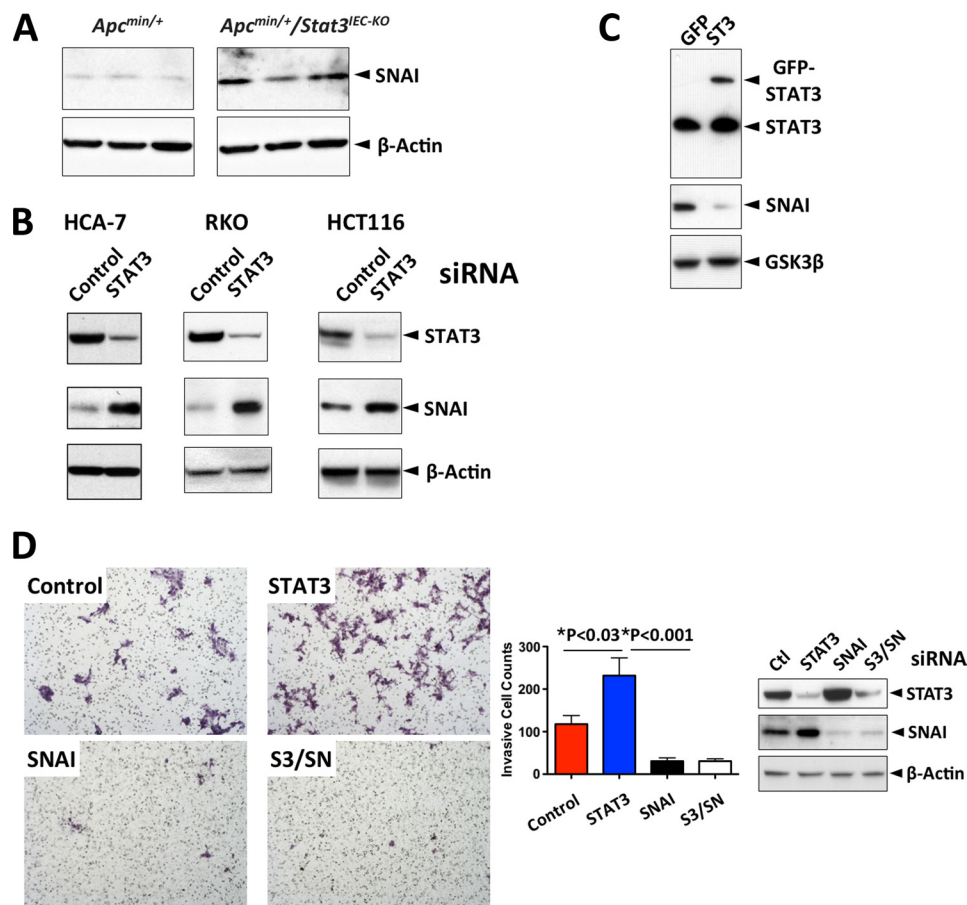


FIGURE 3. STAT3 inhibits tumor invasion by suppressing expression of SNAI. *A*, STAT3 suppresses expression of SNAI in *Apc^{min/+}* mice. Expression of the indicated proteins was measured by immunoblotting from the isolated tumor cells from the small intestines. *B*, STAT3 suppresses expression of SNAI in human CRC lines. STAT3 was silenced by transfection of siRNA, and expression of the indicated proteins in the indicated CRC lines was measured by immunoblotting 3 days after transfection. *C*, STAT3 suppresses expression of SNAI in a human CRC cell line. HCT116 cells were transfected with either GFP or GFP-STAT3-WT, and the levels of the indicated proteins were measured by immunoblotting. *D*, SNAI mediates the invasion of the STAT3-depleted HCT116 cells. Cancer cell invasion assay was performed as described under "Experimental Procedures" 3 days after transfection of the indicated siRNA (S3, STAT3; SN, SNAI). The results are the average of two independent experiments ($n = 6$). The levels of the indicated proteins on the *bottom right* were measured by immunoblotting. *Error bars*, S.D.

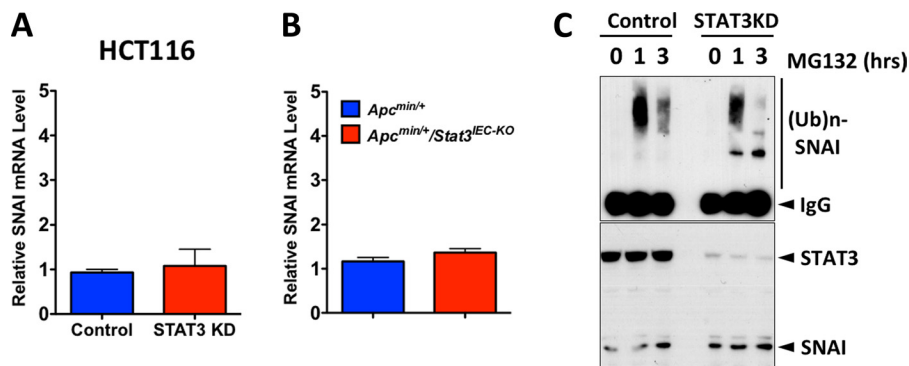


FIGURE 4. STAT3 regulates SNAI at the post-transcriptional level. *A*, STAT3 does not regulate transcription of SNAI. The mRNA levels of SNAI were measured by qPCR in HCT116 cells 3 days after siRNA transfection ($n = 4$). *B*, STAT3 does not regulate transcription of SNAI. The mRNA levels of SNAI were measured by qPCR in the indicated tumor cells ($n = 4$). *C*, STAT3 promotes ubiquitin-mediated degradation of SNAI in IECs. HCT116 cells were transfected with either control or STAT3 siRNA, treated with MG132 ($10 \mu\text{M}$) as indicated, SNAI was immunoprecipitated, and the gel was immunoblotted for ubiquitin (*upper panel* (Ub)n, polyubiquitin). The levels of STAT3 and SNAI were measured by immunoblotting (*lower panel*).

STAT3 deletion or knockdown did not significantly affect the expression of GSK3 β (Fig. 5C), we examined whether STAT3 regulates activation (or phosphorylation) of GSK3 β by Phos-tag SDS-PAGE (13, 32). Whereas there was no noticeable difference in GSK3 β expression level between control and STAT3-deleted cells in a traditional SDS-PAGE analysis, the

putative phosphorylated (retarded in mobility) GSK3 β level was much higher in STAT3-deleted cells compared with that in control cells (Fig. 5D). STAT3 knockdown significantly increased the phosphorylation level on Ser9 residue of GSK3 β (Fig. 5E). This result indicated that STAT3 negatively affects phosphorylation of GSK3 β and suggested that STAT3 regu-

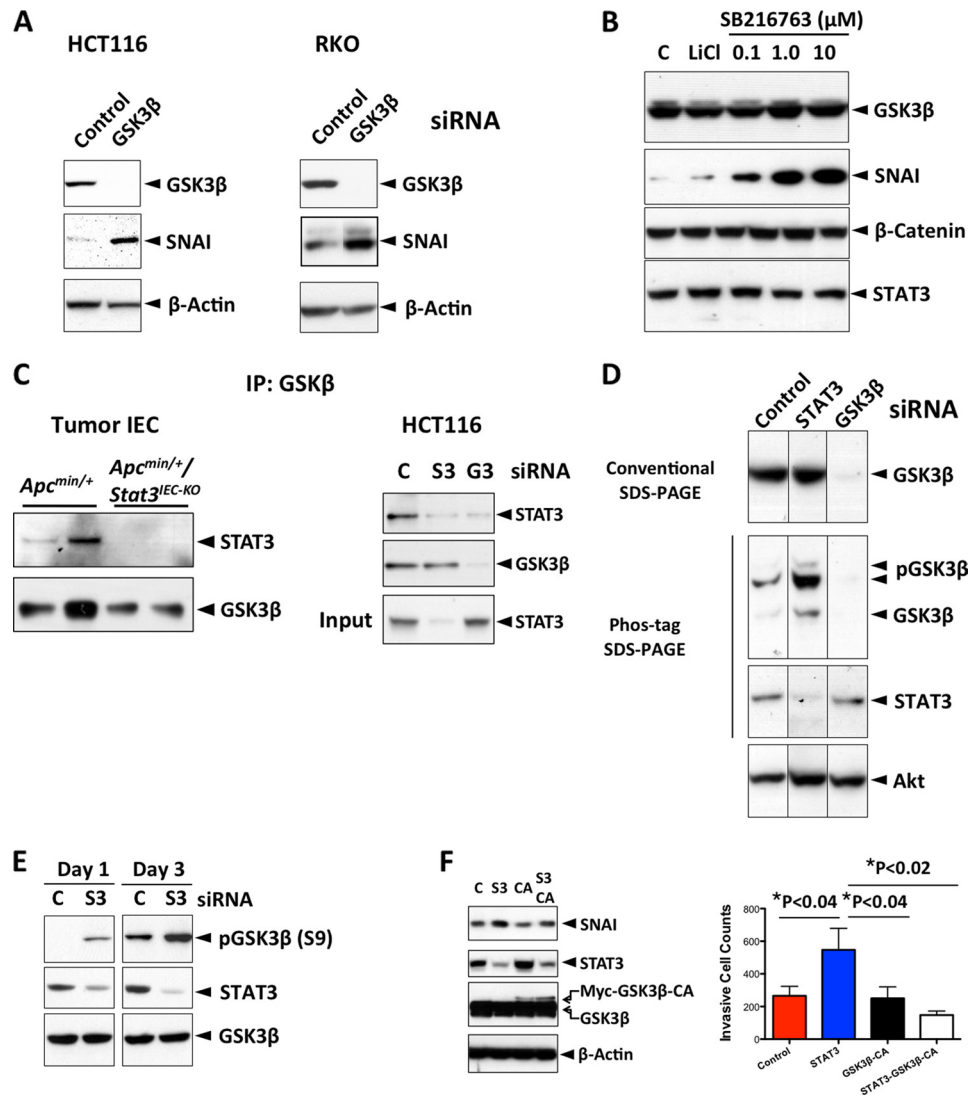


FIGURE 5. **STAT3 promotes GSK3 β activity to induce degradation of SNAI.** *A*, GSK3 β suppresses SNAI expression in CRC lines. The indicated protein levels were measured by immunoblotting 3 days after siRNA transfection. *B*, GSK3 β suppresses SNAI via its kinase activity. HCT116 cells were treated with either LiCl (4 mM) or SB216763, GSK3 β inhibitors as indicated for 4 h, and the indicated proteins were measured by immunoblotting. *C*, STAT3 interacts with GSK3 β . GSK3 β in isolated mouse tumor cells or in HCT116 cells was immunoprecipitated and immunoblotted for STAT3 (S3, STAT3; G3, GSK3 β). *D*, STAT3 regulates phosphorylation levels of GSK3 β . Lysates from HCT116 cells transfected with the indicated siRNA were divided and separated by either traditional SDS-PAGE or Phos-tag SDS-PAGE, and the indicated proteins were measured by immunoblotting. *E*, STAT3 inhibits phosphorylation at Ser-9 of GSK3 β . HCT116 cells were transfected with the indicated siRNA (C, control; S3, STAT3), total cell lysates were collected 1 or 3 days after transfection, and the levels of indicated proteins were measured by immunoblotting. *F*, GSK3 β -CA reverses the effects of STAT3 depletion. HCT116 cells were transfected with control siRNA (C), STAT3 siRNA (S3), GSK3 β -CA plasmid, or STAT3 siRNA (S3) plus GSK3 β -CA plasmid. Cell invasion assay ($n = 3$) and immunoblotting were performed 3 days after transfection.

lates SNAI expression and tumor invasion by promoting GSK3 β activity. Therefore, we tested whether a constitutively active GSK3 β (GSK3 β -CA) mutant can reverse the effects of STAT3 depletion in HCT116 cells. Indeed, expression of GSK3 β -CA reversed the increased SNAI expression and the increased invasiveness induced by STAT3 depletion (Fig. 5*F*). Taken together, these data provide evidence for a nontranscriptional and inhibitory role of STAT3 in IEC tumor invasion.

DISCUSSION

Although no naturally occurring mutations in STAT3 have been identified as a cause of any human cancer, it is considered as a strong promoter of carcinogenesis. STAT3 is activated in multiple solid and hematologic human malignancies, and its role in carcinogenesis has been validated in animal models (1,

33). For example, STAT3 was found to be crucial in initiation and progression of skin carcinoma (34), and the growth and survival of lymphomas are dependent on STAT3 (35). Recently, constitutively activating STAT3 mutations were identified in human hepatocellular adenomas, where the Tyr-640 mutant homodimerizes independently of upstream signals and is hypersensitive to IL-6 stimulation (36). In CRC, STAT3 activation (phosphorylation) was considerably up-regulated during adenoma-to-carcinoma progression (37, 38), but the precise role of STAT3 in CRC has not been yet defined.

Our data provide the mechanisms by which STAT3 suppresses tumor invasion both in an animal model and in CRC cell lines. STAT3 in IEC tumors of *Apc^{min/+}* mice inhibits expression of an EMT inducer SNAI and mesenchymal markers, such as vimentin and fibronectin. Similarly, in CRC cell line

HCT116, STAT3 suppresses invasiveness by limiting expression of SNAI. Overexpression of a dominant negative STAT3 mutant in a CRC cell line was reported to cause down-regulation of E-cadherin (39). Although SNAI is widely known as a suppressor of E-cadherin, our data and another recent report (4) show that its expression is not significantly affected by STAT3 deletion in IEC. However, overexpression of SNAI did not suppress E-cadherin in a breast cancer cell line, whereas a mutant form (6SA) was able to do so (13). Therefore, it is possible that Ser-6 of SNAI may be phosphorylated in STAT3-deficient tumor cells.

Like STAT3, GSK3 β is also a multifunctional protein involved in a wide range of physiological activities such as metabolism, cell development, and body pattern formation (12). It also plays a key role in Wnt signaling whose dysregulation is the most common cause of human CRC (2, 20). Our data show that STAT3 regulates GSK3 β activity to control SNAI expression. We also found that STAT3 inhibits activation of β -catenin in a CRC cell line, but the mechanisms behind this phenomenon are yet to be investigated.

Interestingly, STAT3 in IECs is required for tumor induction in models of colitis-associated cancer. In colitis-associated cancer, STAT3 activation in IEC regulates cell survival and cell cycle progression through its transcriptional activation of downstream targets such as Bcl-X_L, c-Myc, and cyclin D1 (40–42). This colitis-associated cancer model employs a chemical mutagen, azoxymethane, and three cycles of the chemical irritant, dextran sodium sulfate, to induce colorectal tumors. Despite the observation that *Stat3*^{IEC-KO} mice are much more susceptible to dextran sodium sulfate-induced colitis (41), it appears that STAT3 in IEC in *Apc* min model is necessary for their malignant transformation.

Overall, our data document the restraining role of STAT3 on the acquisition of an invasive phenotype of IEC tumors and explain the molecular basis that controls this process. We therefore propose that the inhibition of STAT3 in patients with CRC especially in advanced stages should be practiced with caution.

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