SND1, a Component of RNA-Induced Silencing Complex, Is Up-regulated in Human Colon Cancers and Implicated in Early Stage Colon Carcinogenesis

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Abstract

Colon cancers have been shown to develop after accumulation of multiple genetic and epigenetic alterations with changes in global gene expression profiles, contributing to the establishment of widely diverse phenotypes. Transcriptional and posttranscriptional regulation of gene expression by small RNA species, such as the small interfering RNA and microRNA and the RNA-induced silencing complex (RISC), is currently a major focus of research with regard to cancer development. SND1, also called Tudor-SN and p100 and recently reported to be a component of RISC, is among the list of highly expressed genes in human colon cancers. In the present study, we showed remarkable up-regulation of SND1 mRNA in human colon cancer tissues, even in early-stage lesions, and also in colon cancer cell lines. When mouse Snd1 was stably overexpressed in IEC6 rat intestinal epithelial cells, contact inhibition was lost and cell growth was promoted, even after the cells became confluent. Intriguingly, IEC6 cells with high levels of Snd1 also showed an altered distribution of E-cadherin from the cell membrane to the cytoplasm, suggesting loss of cellular polarity. Furthermore, the adenomatous polyposis coli (Apc) protein was coincidentally down-regulated, with no significant changes in the Apc mRNA level. Immunohistochemical analysis using chemically induced colon cancer models clearly showed up-regulation of SND1 in human colon cancer tissues, even in early-stage lesions, and also in colon cancer cell lines. When mouse Snd1 was stably overexpressed in IEC6 rat intestinal epithelial cells, contact inhibition was lost and cell growth was promoted, even after the cells became confluent. Intriguingly, IEC6 cells with high levels of Snd1 also showed an altered distribution of E-cadherin from the cell membrane to the cytoplasm, suggesting loss of cellular polarity. Furthermore, the adenomatous polyposis coli (Apc) protein was coincidentally down-regulated, with no significant changes in the Apc mRNA level.

Introduction

Altered gene expression, as a consequence of genetic alterations and/or epigenetic changes in the methylation status at promoter CpG sites, is a characteristic of cancers (1, 2). Expression of genes is regulated at both transcriptional and posttranscriptional levels, and differential gene expression profiles confer a wide range of diverse phenotypes in cancer cells. Dysregulation of various transcription factors is well known to be a causative event involved in malignant transformation, for example, with functional inactivation or intragenic mutation of p53 (3), and gene amplification or overexpression of c-myc (4).

Recently, posttranscriptional regulation, especially translational control, of gene expression has become a major focus of research, because accumulating evidence strongly suggests its biological relevance for cancer development and progression (5). Some oncogenes and tumor suppressor genes, including c-myc (6), Mdm-2 (7), and p53 (8), have been shown to be regulated at the translational level. Overexpression of the translation initiation factor eIF4E promotes malignant transformation of normal fibroblasts (9) and cooperates with c-Myc to drive B-cell lymphomatogenesis in eIF4E transgenic mice (10). Very recently, microRNA, another important factor for both transcriptional and translational gene expression regulation (11, 12), has been revealed to have a substantial effect on human carcinogenesis (13), and there are many examples of genetic alterations in microRNA gene loci, including deletions, amplifications, and mutations (14–16).

SND1, also known as Tudor-SN and p100, is a highly conserved protein from yeasts to humans, but its biological functions remain to be fully elucidated. The protein has five repeated staphylococcal nuclease homology domains and a Tudor-homology domain suggested to act as interaction platforms for nucleic acids and proteins, respectively (17). The protein was first identified as a coactivator of the EBV nuclear antigen 2 (18) and was later shown to interact with the oncogene product c-Myb (19). It is thus a transcriptional coactivator (19–21).

We have identified a mouse homologue of SND1 as a single-stranded cytosine-rich DNA binding protein, which binds to the d(CGCG)n sequence derived from mouse minisatellite Pc-1 with high affinity (22), but not to a complementary guanine-rich repetitive sequence d(GGGAG)n and its double-stranded form (23). Caudy et al. recently reported that SND1 is one of the components of the RNA-induced silencing complex (RISC), and disruption of SND1 in C. elegans was shown to cause disruption of small interfering RNA-induced gene silencing (24). In addition, SND1 is among the top five genes up-regulated in human colon adenocarcinomas, as assessed using oligonucleotide microarrays (25). Although biological functions of SND1 still remain largely elusive, the recent intriguing findings suggest an involvement of SND1 as a key player in the regulation of gene expression at both transcriptional and posttranscriptional levels through direct binding to RNA in some sequence-specific manner. Our working hypothesis is that overexpression of SND1, therefore, has a substantial effect on carcinogenesis.

In the present study, we investigated the possible involvement of SND1, a component of RISC, in colon carcinogenesis. We first conducted expression analysis of SND1 mRNA in human colon...
cancer tissues and several colon cancer cell lines and found marked up-regulation of SND1 mRNA in colon cancer cells. Immunohistochemical analysis using various stages of colonic lesions induced in rats by chemical carcinogens revealed overexpression of Snd1 in precancerous lesions of the colon even before the appearance of β-catenin accumulation. Intriguingly, introducing a recombinant HA-tagged mouse Snd1 protein caused down-regulation of the adenomatous polyposis coli (Apc) protein and translocation of E-cadherin from the cell membrane to the cytoplasm, leading to loss of contact inhibition and cellular polarity. A possible involvement of SND1 in early stages of colon carcinogenesis through its novel function as a posttranscriptional regulator is proposed.

Materials and Methods

Surgical tissue specimens, cell lines, and antibodies. Paired surgical specimens of primary human colon cancers and surrounding noncancerous counterparts of the colon were obtained from 32 patients treated at the National Cancer Center Hospital, Tokyo, Japan, with documented informed consent in each case. Samples were frozen in liquid nitrogen and stored at −80°C until use. Clinicopathologic staging of cancers was done according to the criteria approved by the American Joint Committee on Cancer. Nine cases were diagnosed as stage II, 12 cases as stage III, and 11 cases as stage IV. Ten human colon cancer cell lines were maintained in the complete growth medium recommended by the American Type Culture Collection. Antibodies used in this study were rabbit anti-HA and anti-α-tubulin antibodies purchased from Sigma, rat monoclonal anti-HA antibody from Roche, anti-E-cadherin, anti-β-catenin, p120 catenin antibodies from BD Bioscience, anti-APC antibodies, C-20 and Ab-1, from Santa Cruz Biotech, Inc. and Chemicon, respectively. For secondary antibodies, Alexa-labeled anti-rabbit and mouse antibodies were from Invitrogen Corp., horseradish peroxidase-conjugated anti-rabbit and mouse IgG is from GE Health Bio-Science, and HRP-conjugated anti-rat IgG is from Sigma. A rabbit polyclonal anti-Snd1 antibody was raised by immunizing rabbits using purified recombinant thioredoxin–tagged Snd1 protein expressed in Escherichia coli. Anti-Snd1 antisera was purified by affinity chromatography using CNBr-sepharose (GE Healthcare Bio-Science) cross-linked with glutathione-S-transferase–tagged Snd1 protein.

Quantitative real-time reverse transcription-PCR analysis. Total RNA was isolated from ~30 mg of surgical samples and 5 × 10⁶ cultured cells using Trizol reagent (Invitrogen). After treatment with DNase I, 2 μg of aliquots of RNA were subjected to cDNA synthesis using SuperScript II reverse transcriptase (Invitrogen) and oligo(dT)₁₂₋₁₈ as a primer. Primer sequences for reverse transcription-PCR (RT-PCR) amplification of the human SND1 mRNA were 5′-GGACACGGGTAGTTCCGGAG-3′ (forward) and 5′-CCCAGCAGACATTTCCACACAC-3′ (reverse) designed from nucleotide sequences for reverse transcription-PCR (RT-PCR) amplification of the human SND1 mRNA. Amplification was carried out in a total of 25 μL of reaction mixture by a SmartCycler (Takara). Aliquots of RNA were subjected to RT-PCR analysis of the manufacturer’s instructions. For reference, mRNA levels of β-actin were amplified using a primer set, 5′-AGGAAAAAGGTGGAAGAG-3′ (forward) and 5′-GGACACGGGTAGTTCCGGAG-3′ (reverse), as described previously (26). Relative amounts of SND1 mRNA were calculated by the division of the copy number of SND1 transcripts with those of β-actin. Statistical analyses were done using JMP 6.0 software (JMP Japan).

Establishment of recombinant Snd1-expressing rat intestinal epithelial cells, IEC6. Snd1 cDNA derived from mouse NIH3T3 cells (23) was subcloned into a pEF6-HA vector by the standard method (27). The plasmid, pEF6-HA-Snd1 or pEF6-HA (vector control), was introduced in the IEC6 cells using LipofectAMINE 2000 (Invitrogen) according to the manufacturer’s instructions. Transfected cells were grown in the selection medium composed of complete DMEM containing 5% fetal bovine serum (FBS), 10 μg/mL insulin, and 3 μg/mL blasticidin, and stable clones were isolated. Expression of HA-Snd1 in transfected cells was confirmed by immunoblot analysis and/or immunostaining of the cells using anti-HA antibody.

Cell growth assay and immunocytochemistry. IEC6 cells stably expressing the exogenously introduced HA-Snd1, and vector-transfected IEC6 cells were seeded on 24-well plates at a density of 2.5 × 10⁴ cells/mL (0.5 mL/well) and grown in complete growth medium as described above for the indicated number of days. Cells were collected and counted at each time point. Cells, seeded on glass-bottomed plates, were fixed in cold methanol and blocked in 5% bovine serum albumin/PBS for 60 min at room temperature. Fixed cells were incubated in blocking buffer containing the first antibodies of anti-HA or anti-E-cadherin for 60 min, then incubated with the secondary antibody for 60 min. After washing with PBS containing Hoechst 33258, cells were subjected to fluorescent microscopic observation using an Axiovert 200 fluorescence microscope (Carl Zeiss).

Immunohistochemical analysis of Snd1 in colon tumors and AFc. Paraffin sections of colon cancers and AFc induced in the rat colon by azoxymethane and PhlP were stained with H&E to evaluate the histologic grade of AFc, namely nondysplastic ACF and dysplastic ACF, according to their cytologic abnormalities, as described previously (30). Animal experiments in the present study were approved by the Institutional Animal Care and Use Committee at the National Cancer Center.

Immunohistochemical analysis of SND1 in colon tumors and AFc. Immunohistochemical staining was done by the avidin-biotin complex method (ABC) using the Vectastain Elite ABC system (Vector Laboratories) for Snd1 and in 10 mmol/L citrate buffer (pH 6.0) for β-catenin. Immunohistochemical staining was done with the avidin-biotin complex method (ABC) using the Vectastain Elite ABC system (Vector Laboratories) as described previously (30). Anti-SND1 was used at a concentration of 0.2 μg/mL. Biotinylated goat anti-rabbit IgG (Vector Laboratories) was used as a secondary antibody for both at a dilution of 1:200.

Results

Up-regulation of SND1 mRNA expression in human colon cancers. As shown in Fig. 1A, quantitative RT-PCR analysis revealed marked up-regulation of SND1 mRNA in most human cancer tissues examined, and 28 of 32 colon cancer cases showed 2-fold or greater increase compared with their noncancerous counterparts. The expression levels of SND1 mRNA in colon cancer tissues normalized to that of the β-actin transcript (SND1 mRNA copy number/β-actin mRNA copy number) was 0.033 ± 0.015, which is ~5-fold higher compared with those of normal counterparts, 0.007 ± 0.003. Statistical analysis confirmed a highly significant up-regulation of SND1 mRNA in colon cancers (paired t test,
No significant differences were observed in \textit{SND1} expression levels among different clinicopathologic stages of cancer (Student’s \textit{t} test, \( P > 0.2 \)). Cytoplasmic accumulation of \textit{SND1} protein was confirmed by immunohistochemical analysis using colon cancer tissues (Fig. 1B). High expression of \textit{SND1} mRNA was also observed in all 10 human colon cancer cell lines examined (Fig. 1C). The average expression level of \textit{SND1} mRNA in colon cancer cell lines, being 0.063 \( \pm \) 0.045, was comparable with that detected in colon cancer tissues.

Expression of recombinant Snd1 leads to loss of contact inhibition and promotes cell proliferation in IEC6 cells. IEC6 cells were stably transfected with a construct encoding HA-tagged mouse Snd1, and five independent clones were established expressing much higher levels of total Snd1 protein (a series of IEC-Snd clones) compared with the vector-transfected cell clones (a series of IEC-Vector clones). The expression levels of HA-Snd1 and total Snd1 (endogenous Snd1 plus HA-Snd1) in IEC-Snd clones and IEC-Vector clones were confirmed by immunoblot
analysis (Fig. 2A and Supplementary Fig. S1B). Overexpression of Snd1 significantly enhanced the cell proliferation especially in the exponentially growing phase (Fig. 2B). IEC-Snd clones showed continuous cell growth even after formation of cell-cell contact, and cell densities of IEC-Snd clones at day 9 were significantly and markedly higher than those of IEC-Vector clones (P < 0.001). The morphology of IEC-Snd cells in the confluent culture led us to consider the possibility that Snd1 overexpression in IEC6 cells disrupts the monolayer growth of the cells (Supplementary Fig. S2). Thus, cells lost contact inhibition and piled up on each other. To further characterize the disruption of monolayer growth in IEC-Snd clones, subcellular localization of E-cadherin was analyzed by immunocytochemistry. As shown in Fig. 2C, vector-transfected cells showed the typical localization of E-cadherin at the cell-cell contact regions (IEC-vector Cl-1, bottom). In contrast, no localization of E-cadherin was observed at the cell-cell contact region in IEC-Snd clones 2 and 5 (Fig. 2C, top and middle), but it was dispersed in the cytoplasm, suggesting that overexpression of Snd1 leads to the translocation of cell membrane-associated E-cadherin, and consequently to the loss of contact growth inhibition in normal intestinal epithelial cells. Other IEC-Snd clones (Cl-1, Cl-3, and Cl-4) also showed the same localization pattern of E-cadherin (data not shown). In a soft agar colony formation assay, IEC-Snd clones indeed showed weak anchorage-independent growth activity (Table 1 and Supplementary Fig. S3). However, a growth assay in collagen gel, in which transformed cells rapidly form colonies, gave negative results (data not shown), indicating that Snd1 overexpression does confer a weak, but not a comprehensive, transforming activity to intestinal epithelial cells.

**Down-regulation of Apc protein by overexpression of Snd1.** To clarify the molecular mechanisms for translocation of E-cadherin by Snd1 overexpression, we carried out immunoblot analysis for several key molecules, which are implicated in cell adhesion, cell-cell interaction, and colon carcinogenesis, namely, E-cadherin, p120 catenin, β-catenin, and Apc. Among these candidates, only Apc protein levels were markedly down-regulated in IEC-Snd clones (Snd Cl-1 to Cl-5) compared with that of IEC-Vector Cl-1 (Fig. 3A) and other IEC-Vector clones (data not shown).

No significant decrease was observed in the amounts of Apc mRNA by real-time RT-PCR analysis among these clones (Fig. 3B). Furthermore, Apc mRNA was clearly detected only in the immunoprecipitated fraction from each IEC-Snd clone, but not in IEC-Vector Cl-1, indicating the association of Snd1 with Apc mRNA in cells (Fig. 3C). The interaction of Snd1 with Apc mRNA was also confirmed by transient expression of HA-Snd1 into HeLa cells (data not shown). This strongly suggests that the expression of the Apc protein could be regulated by some posttranscriptional mechanisms, neither by transcription nor by mRNA stability. Transient introduction of the human Snd1 to HCT 116 and SW 48 colon cancer cell lines and HeLa cells, all of which express wild-type Apc, also caused the down-regulation of the Apc protein (Supplementary Fig. S4A) without significant changes in Apc mRNA levels (Supplementary Fig. S4B).

**Accumulation of Snd1 protein in ACF and colon tumors of rats.** To gain further insight into the biological role of Snd1 in an *in vivo* setting, we investigated whether Snd1 is expressed in chemically induced colonic lesions induced in rats. Immunohistochemical analysis of Snd1 and β-catenin was carried out in colon cancers induced by azoxymethane or PhIP and in ACF. Colon cancers induced by azoxymethane (Fig. 4A) and PhIP (data not shown) showed accumulation of both β-catenin and Snd1, as expected. Surprisingly, dysplastic ACF also showed remarkable accumulation of Snd1 in the cytoplasm (Fig. 4B, a and c) along with the β-catenin accumulation in the cytoplasm (Fig. 4B, b and d) or the nucleus (data not shown). Comparison of nondysplastic and dysplastic ACF induced by PhIP, the former being considered as earlier colonic lesions, revealed 7 of 12 and 6 of 6, respectively, to have Snd1 overexpression, whereas only two and four showed β-catenin accumulation in the cytoplasm (Table 2). Snd1 accumulation was also detected in nondysplastic ACF induced by azoxymethane without β-catenin accumulation (Table 2). Taken together, cytoplasmic accumulation of Snd1 seems to occur at early stages in colon carcinogenesis induced by PhIP and azoxymethane. Positive staining was not detected with the same amount of rabbit IgG (Supplementary Fig. S5).

**Discussion**

In the present study, the incidence of SND1 mRNA up-regulation in human colon cancer tissues was extremely high, and 87.5% of cases showed an increase by 2-fold or greater compared with the corresponding noncancerous counterparts. Overexpression of SND1 may have some significant role in colon carcinogenesis, as well as other tissues. In support of this hypothesis, overexpression of Snd1 was commonly observed in colon tumors and dysplastic ACF, and surprisingly, some nondysplastic ACF also showed Snd1 overexpression even before cytoplasmic accumulation of β-catenin protein. Considering that overexpression of SND1 suppresses the levels of APC protein, which is a “gatekeeper-type” tumor suppressor gene in colon cancer development, induction of SND1 in colon epithelial cells could occur at very early stages of colon carcinogenesis. Loss of Apc leads to the stabilization of β-catenin protein by preventing proteasomal degradation (32). In IEC-Snd clones, however, a significant increase of β-catenin protein was not evident (data not shown), suggesting that down-regulation of Apc alone may not be enough for the further accumulation of β-catenin. Alternatively, an Apc-independent pathway for the degradation of β-catenin might be involved (33).

It is important to note that IEC6 cells with high expression levels of Snd1 manifested loss of cell-cell contact inhibition of growth and enhancement of cell proliferation even after the cells became confluent on culture dishes. These characteristic features seemed linked to loss of cellular polarity, as indicated by findings for APC known to maintain cell-cell adhesion and cell polarity through the regulation of localization of E-cadherin to the plasma membrane (34). Loss of cellular polarity and abnormal growth of cryptic epithelial cells are suspected to occur at the very beginning of intestinal carcinogenesis (35), as is also well represented in the case of *Apc*-disrupted mice (36). Collectively, functional activation of SND1 could be a novel molecular basis underlying the initial development of precancerous lesions in the colon.

We also noted that knockdown of Snd1 by small interfering RNA did not cause significant up-regulation of APC protein level in several colon cancer cell lines, except SW48 cells, and did not inhibit cell proliferation. Down-regulation of Snd1 alone may not be sufficient to increase the APC protein level in cancer cells or to overcome the aberrant cell proliferation of fully transformed cells, which harbor complex genetic and epigenetic changes, including those implicated in the WNT signaling pathway, p53, K-RAS, and SMADs. We further failed to detect the translocation of E-cadherin in both chemically induced precancerous lesions in the rat colon and human colon cancer tissues (data not shown). This might be
Figure 2. Establishment of the IEC-Snd cells. 
A, immunoblot analysis of recombinant HA-Snd1 expressed in IEC-Snd cells. Snd1-transfected IEC6 clones (a series of IEC-Snd clones; IEC-Snd Cl-1 to Cl-5) and a Vector-transfected clone (IEC-Vector Cl-1) were lysed in Laemmli’s SDS sample buffer and subjected to immunoblot analysis. Top, HA-Snd1; middle, total Snd1 (endogenous Snd1 plus HA-Snd1); bottom, loading control tubulin. Lane 1, IEC-Vector Cl-1; Lanes 2 to 6, IEC-Snd Cl-1, Snd Cl-2, Snd Cl-3, Snd Cl-4 and Snd Cl-5, respectively. B, cell proliferation analysis. IEC-Snd and IEC-Vector cell clones were seeded on a 24-well plate at 2.5 × 10^4 cells/mL (0.5 mL/well), and the cell numbers were counted at indicated time points. The culture medium was changed at days 4 and 7. The data indicate the average with SD of a 4-well culture. Statistical analysis carried out by the Dunnett’s test. C, localization of E-cadherin in IEC-Snd cells. IEC-Snd Cl-2 and Cl-5 and IEC-Vector Cl-1 cells, seeded on the glass-bottom dishes, were fixed with methanol and subjected to immunostaining, as detailed in Materials and Methods, and subjected to fluorescence microscopic analysis. Magnification, ×400; white bars, 50 μm.
cases, and the incidence of APC mutations were detected in only 37% of human colon cancer in ACF (38, 39). In recent studies by Luchtenborg et al., however, mutations has been observed in colon adenomas and occasionally Apc down-regulation of the mutations in either Apc carcinogens, and frequent in colon cancers induced in rodents by chemical in vivo system and due to the differences in cellular context between cell culture and in vivo tissues. In other words, translocation of E-cadherin in transformed cancer cells in tissues might require additional genetic and/or epigenetic alterations after the accumulation of Snd1 and β-catenin.

It is well known that the APC gene is mutated or deleted in the majority of human colon cancers. Mutant forms of the APC protein cause activation of the Wnt-β-catenin signaling pathway and enhance the expression of cyclin D1 and c-myc oncogenes leading to development and progression of colorectal cancers (37). Activation of the Wnt-β-catenin signal pathway by Apc mutations has been observed in colon adenomas and occasionally in ACF (38, 39). In recent studies by Luchtenborg et al., however, APC mutations were detected in only 37% of human colon cancer cases, and the incidence of β-catenin mutations affecting phosphorylation sites was only 5 of 464 cases (1.1%; 40). Moreover, the mutation frequency in the Apc gene is much less frequent in colon cancers induced in rodents by chemical carcinogens, and ~30% to 40% of the cases do not harbor mutations in either Apc or β-catenin gene (41, 42). However, down-regulation of the Apc protein was frequently observed in azoxymethane- and PhIP-induced colon cancers (ref. 41 and our unpublished observation), despite the rare occurrence of Apc mutations (41, 42). Therefore, other unknown mechanisms have been considered to be involved, at least in part, in the activation of the Wnt-β-catenin signaling pathway. Down-regulation of the Apc protein by SND1 overexpression could be an alternative molecular mechanism acting in the initial stages of colon carcinogenesis.

An important subject to be addressed is how Snd1 down-regulates the Apc protein. Although we currently do not have a clear idea on this point, gene-specific translational repression of tumor suppressor protein has been shown in several human cancer cells. For example, CAAT/enhancer binding protein α, a critical factor for myeloid cell differentiation, was suppressed in BCR/ABL-positive chronic myelogenous leukemia cells by the poly r(C)-binding heterogeneous ribonucleoprotein E2 (hnRNP E2; 44), hnRNP E1, a family member of hnRNP E2, is also known to bind to the differentiation control element of the 3′-untranslated region in the lipoxigenase mRNA (45). In colon cancer cells, thymidylate synthase is reported to bind to p53 mRNA and repress translation.

Down-regulation of p53 protein levels in thymidylate synthase overexpressing human colon cancer cell lines has also been described (46). These previous findings and our current results point to an intriguing and novel scenario, whereby gene-specific suppression of translation could serve as part of critical initial events in the development of colon cancers. To prove, however, whether the translational repression by SND1 is exerted in either a

<table>
<thead>
<tr>
<th>Clones</th>
<th>No. colonies (per well)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Snd Cl-1</td>
<td>484</td>
</tr>
<tr>
<td>Snd Cl-2</td>
<td>513</td>
</tr>
<tr>
<td>Snd Cl-3</td>
<td>151</td>
</tr>
<tr>
<td>Snd Cl-4</td>
<td>76</td>
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<tr>
<td>Snd Cl-5</td>
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<td>Vector Cl-1</td>
<td>10</td>
</tr>
<tr>
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</tr>
<tr>
<td>Vector Cl-3</td>
<td>2</td>
</tr>
<tr>
<td>Vector Cl-4</td>
<td>4</td>
</tr>
<tr>
<td>Vector Cl-5</td>
<td>13</td>
</tr>
</tbody>
</table>

Figure 3. Down-regulation of the Apc protein without a significant change of Apc mRNA level in IEC-Snd clones. A, immunoblot analysis of the Apc protein in IEC-Snd clones. IEC-Snd and IEC-Vector clones were lysed in Laemmli’s SDS sample buffer and subjected to immunoblot analysis. Immunoblot analysis of Apc (top) and control tubulin (bottom). The relative amounts of Apc were calculated by dividing the band densities of Apc by those of tubulin, which were determined by LAS3000 image analyzer and image gauge software (Fuji Film; bottom). Lanes 1 to 5, IEC-Snd Cl-1 to Cl-5, respectively; lane 6, IEC-Vector Cl-1. B, quantitative real-time PCR. Total RNAs from each IEC-Snd clones were subjected to reverse-transcriptase reaction using oligo(dT) primer and SuperScript II RT (Invitrogen). First strand of cDNA was subjected to PCR analysis using Apc and 3′-actin primer sets using PowerSYBR Green PCR Mater Mix (Applied Biosystems). The relative amounts of Apc mRNA were normalized to those of 3′-actin. C, interaction of Snd1 with Apc mRNA. HA-Snd1 was precipitated using anti-HA agarose beads, and Snd1-bound RNAs were purified and subjected to RT-PCR analysis. Top and middle, immunoblot and RT-PCR analyses of immunoprecipitates from IEC-Snd clones, respectively; bottom, RT-PCR analysis of total RNA from each IEC clone.
gene-specific or nonspecific (or global) manner requires further study. Taking into consideration the present observation that SND1 overexpression did not induce a significant decrease in the β-catenin or tubulin protein levels, it is highly plausible that SND1 exerts its biological function as a translational repressor in some gene-specific manner.

We should also emphasize that SND1 may not be a sole regulator for the cellular APC protein level because the extent of

Table 2. Immunohistologic analysis for Snd1 and β-catenin in ACF induced by PhIP and azoxymethane

<table>
<thead>
<tr>
<th>Types of lesions</th>
<th>No. lesions analyzed</th>
<th>Status of β-catenin localization*</th>
<th>No. lesions with Snd1 accumulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>PhIP Nondysplastic ACF</td>
<td>12</td>
<td>Cytoplasm 2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Membrane 10</td>
<td>5</td>
</tr>
<tr>
<td>Dysplastic ACF</td>
<td>6</td>
<td>Cytoplasm 4</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Membrane 2</td>
<td>2</td>
</tr>
<tr>
<td>Azoxymethane Nondysplastic ACF</td>
<td>5</td>
<td>Cytoplasm 0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Membrane 5</td>
<td>3</td>
</tr>
<tr>
<td>Dysplastic ACF</td>
<td>18</td>
<td>Cytoplasm 12</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Membrane 6</td>
<td>5</td>
</tr>
</tbody>
</table>

*Lesions demonstrating β-catenin localization at the cytoplasm were considered to be positive for β-catein accumulation and those with membrane localization were estimated to be negative.
† Among 12 nondysplastic ACF induced by PhIP, two were selected because they showed the accumulation of β-catein in the cytoplasm. The remaining 10 lesions were randomly selected and subjected to immunohistochemical analysis.
‡ Among five nondysplastic ACF induced by azoxymethane, no accumulation of β-catein in cytoplasm were detected.
down-regulation of Apc protein does not necessarily correlate with the Snd1 protein levels in IEC-Snd1 clones. Snd1 may regulate Apc mRNA translation in cooperation with other translational regulators. Furthermore, growth rates and the soft-agar colony formation activity of IEC-Snd1 clones did not completely correlate either with the total amount of Snd1 or with the Apc protein levels, indicating that Snd1 could also be involved in translational regulation of other mRNAs associated with cell proliferation. Identification of other target mRNA species and interacting partners of Snd1 protein should provide us with further insights into more precise roles of Snd1 in colon carcinogenesis. Sequence-specific binding of Snd1 to single-stranded oligonucleotides (23) may contribute to this feature.

Another intriguing aspect of Snd1 is its role as a possible component of RISC, and it indeed binds to some microRNA species (ref. 24 and our unpublished observation). Recently, gene specific component of RISC, and it indeed binds to some microRNA species may contribute to this feature.

The expression profiles of microRNA in cancer tissues provided new insight into cancer diagnosis and prognosis and also into their roles in carcinogenesis (47–50). Although abnormality of microRNA gene expression is now an accepted signature of various cancers, functional analysis of effector RISC remains to be done. Aberrant regulation of microRNAs by RISC might also induce the malignant phenotype of cells. Snd1 may thus control gene expression of APC or other cancer-related genes through control of microRNA–induced translational repression.

In summary, we report here, for the first time, that overexpression of Snd1, a component of RISC, suppresses Apc protein levels via posttranscriptional mechanisms. Functional activation of Snd1 could be a novel mechanism underlying the initial development of precancerous lesions in the colon and contribute in an important way to the development of colon cancers at their very early stages.

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References

1. Lengauer C, Kinzler KW, Vogelstein B. Genetic insta-

mality of microRNA gene expression is now an accepted signature

translational control induced by microRNA species has been

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References

1. Lengauer C, Kinzler KW, Vogelstein B. Genetic insta-

mality of microRNA gene expression is now an accepted signature

translational control induced by microRNA species has been

recently, gene specific component of RISC, and it indeed binds to some microRNA species

may contribute to this feature.

Identification of other target mRNA species and interacting

partners of SND1 protein should provide us with further insights

into more precise roles of SND1 in colon carcinogenesis. Sequence-

specific binding of Snd1 to single-stranded oligonucleotides (23)

may contribute to this feature.

Another intriguing aspect of Snd1 is its role as a possible component of RISC, and it indeed binds to some microRNA species (ref. 24 and our unpublished observation). Recently, gene specific component of RISC, and it indeed binds to some microRNA species may contribute to this feature.

The expression profiles of microRNA in cancer tissues provided new insight into cancer diagnosis and prognosis and also into their roles in carcinogenesis (47–50). Although abnormality of microRNA gene expression is now an accepted signature of various cancers, functional analysis of effector RISC remains to be done. Aberrant regulation of microRNAs by RISC might also induce the malignant phenotype of cells. Snd1 may thus control gene expression of APC or other cancer-related genes through control of microRNA–induced translational repression.

In summary, we report here, for the first time, that overexpression of Snd1, a component of RISC, suppresses Apc protein levels via posttranscriptional mechanisms. Functional activation of Snd1 could be a novel mechanism underlying the initial development of precancerous lesions in the colon and contribute in an important way to the development of colon cancers at their very early stages.

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Naoto Tsuchiya, Masako Ochiai, Katsuhiko Nakashima, et al.


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