Schlafen 3, a novel gene, regulates colonic mucosal growth during aging

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Aging has been shown to be associated with increased incidence of premalignant lesions in the colon and stomach (14). In fact, the occurrence of both nonmalignant and malignant colorectal neoplasms increases with advancing age (14). An improved understanding of age-related changes of the structural and functional properties of different organ systems, including the gastrointestinal tract, is essential to counteract various diseases, particularly cancer, whose incidence increases sharply with aging.

Aging has been shown to be associated with increased incidence of premalignant lesions in the colon and stomach (14). In fact, the occurrence of both nonmalignant and malignant colorectal neoplasms increases with advancing age (14). To better understand the biochemical events associated with the age-related rise in premalignant lesions in the gastrointestinal tract, we and others have examined changes in mucosal proliferation and apoptosis, the events that maintain the homeostasis of the gastrointestinal mucosa in healthy adults. Morphological and biochemical studies from this and other laboratories have demonstrated increased proliferation and decreased apoptosis in the colonic mucosa of Fischer 344 rats, events that are seen during the development and progression of carcinogenesis (1, 10, 14–16). Moreover, in colonic mucosa, the age-related decrease in apoptosis (increase in cell survival) is evident throughout the entire length of the crypt (15). Morphological studies of the colonic mucosa of human volunteers have further revealed that, whereas cell proliferation in young is confined to the lower two-thirds of the crypt, with aging there is a major shift from the base to the middle and upper third of the gland (6, 18), a pattern commonly observed in colorectal cancer. These and other relevant observations led us to postulate that aging may predispose the gastrointestinal tract to cancer (14). In support of this postulation, we have observed that the susceptibility of the colon to carcinogens increases with advancing age. We reported that the formation of aberrant crypt foci (considered to be precursors of adenoma and carcinoma) in response to the colonic carcinogen dimethylhydrazine in the colon of aged Fischer 344 rats is higher than in young rats (20). Furthermore, our recent observation that in humans the number of polyps, the most frequent premalignant colorectal lesion, increases linearly with advancing age provides further support for the contention that aging predisposes the gastrointestinal tract to neoplasia (17). Additional support for this postulation comes from the observation that the age-related increase in colonic polyps is associated with increased expression of CD44, CD166, and epithelial specific antigen, the markers of colon cancer stemlike cells in macroscopically normal mucosa (17).

Although the regulatory mechanisms for the age-related increase in proliferation and decrease in apoptosis in the colonic mucosa remain to be fully elucidated, we have observed that these changes are associated with increased expression and activation of EGF receptor and some of its family members, particularly ErbB-2/HER-2 and ErbB-3/HER-3 (15, 16, 25). Others have reported increased activation of insulin-like growth factor receptor (9). These changes are also associated with decreased expression of proapoptotic protein Bak and increased expression antiapoptotic protein Bcl-xL, in the colonic mucosa of aged rats (15). Although these biochemical changes may explain part of the morphological changes observed in the aging colon, further studies are needed for a better and more comprehensive understanding of various age-associated changes leading to deregulated growth in the colonic mucosa. In the present investigation, we have employed microarray-based gene expression profiling to better understand the molecular changes associated with aging in the colonic mucosa of Fischer 344 rats. Although several genes involved in proliferation and apoptosis were differentially expressed in the colonic mucosa between young and aged rats, the expression of...
sclafen 3, a negative regulator of proliferation (3, 21), was found to be decreased by 8- to 10-fold in the colonic mucosa of aged than in young Fischer 344 rats.

Sclafen 3 belongs to a multigene family in mice that has 10 members. They are classified into three subgroups (short, intermediate, and long) on the basis of overall sequence homology and size of the encoded proteins. Sclafen 3 belongs to intermediate subgroup and possess the following characteristics: a unique domain, common to all members of the family, referred to as “Scln box,” which lies adjacent to a GTP/ATP binding AAA domain and the highly conserved “SWADL” domain, defined by a five-amino-acid sequence (Ser-Trp-Ala-Ser-Leu) (8). Sclafen 1 to 3 have been shown to regulate cell growth in vitro, thought to be through the inhibition of cyclin D1 (3, 8, 21). Transgenic mice expressing sclafen 1 or sclafen 8 within the T cell lineages showed an overall decrease in thymocyte number (21). In addition, inflammatory mediator proteins such as AP-1 and NF-κB regulate sclafen 2 expression, suggesting an important role of this gene in immune response (22). However, role of Sclafen family of proteins, particularly sclafen 3, in regulating gastrointestinal mucosal function is unknown. The present investigation was undertaken to examine the role that sclafen 3 might play in regulating colonic mucosal growth during aging.

**Materials and Methods**

**Animals and collection of tissues.** Male Fischer 344 rats, aged 4–6 (young) and 21–24 (old) mo, were used. The animals were purchased from the National Institute on Aging (Bethesda, MD) at least 2 mo before the experiment. They were housed two per cage and had access to Purina chow and water ad libitum. The reasons for using Fischer 344 rats for aging studies are because of purity of breeding, low susceptibility to spontaneous colorectal cancer, and their ability to maintain body weight. All animals were fasted overnight before being killed. The overnight-fasted animals were either used without any intervention or injected intraperitoneally with wortmannin [0.1 mg/kg body wt in 15% DMSO (15)] or vehicle 6 h before being killed. The entire colon (~18 cm) was removed, cut along the longitudinal median, and rinsed thoroughly in cold normal saline. The mucosa was obtained by scraping with glass slides. Mucosal aliquots were either processed immediately or stored at −80°C. In some experiments, the colon was used immediately to isolate cells from the mucosa, as described below.

**Isolation of colonic mucosal epithelial cells.** Cells were isolated from the entire colon by as described previously (15). Briefly, the contents of the colon were washed with PBS. The colon was everted and ligated at both ends after being filled with a 3- to 5-ml protease solution [5 mg/ml in buffer A composed of (in mM) 0.5 NaH2PO4, 1 Na2HPO4, 70 NaCl, 5 KCl, 11 glucose, 50 HEPES, 20 NaHCO3, and 110 mmol/L HCO3] and washed with PBS. The colon was used immediately to isolate cells from the mucosa, as described below.

**RT-PCR.** Two-step RT-PCR was performed by using the GeneAmp Gold RNA PCR kit (Applied Biosystems, Foster City, CA). Briefly, 1 μg of purified RNA was reverse transcribed in the presence of 2.5 mM MgCl2, 1× RT-PCR buffer, 1 mM dNTPs, 10 mM dithiothreitol, 10 units RNase inhibitor, 1.25 μM random hexamers, and 15 units Multiscribe Reverse Transcriptase in a final reaction volume of 20 μl. The mixture was incubated at 25°C for 10 min for hybridization, subsequently at 42°C for 15 min in a Gene Amp PCR system 9600 (Perkin-Elmer), and then by cooling to 4°C. The RT reactions were subjected to PCR amplification. Five microliters of cDNA products were amplified with 2.5 units of AmpliTaq Gold Polymerase (Applied Biosystems), 1× RT-PCR buffer, 1.75 mM MgCl2, 0.8 mM dNTPs, 0.15 μM of forward (5’-ATTCTGCTGTGCAGTGTTCG-3’) and reverse (5’-TGGTTTGGAGAACATGGTGCT-3’) primers for rat sclafen 3 that resulted in a 127-bp product. β-Actin forward (5’-CCAGCAACTGGAAGATCTCA-3’) and reverse (5’-ACATCT-GCTGGAAGGTCG-3’) primers (107 bp product) were used as internal controls. Reactions were carried out in the Gene Amp PCR system 9600, first hold of 10 min at 95°C for activated AmpliTaq Gold DNA Polymerase, and were followed by 20 s at 94°C, 60 s at 56°C for 40 cycles for amplification of target gene.

**Cloning of rat sclafen 3 cDNA and recombinant plasmid construct.** The total RNA from rat colonic mucosal cells was reverse transcribed with sclafen 3 sequence-specific oligos, 5’-TGTTAGAGCGGTGTGCTCAG-3’, and the sclafen 3 cDNA was amplified by use of primers designed against the database sequence of coding region (NM_053687): forward primer, 5’-TCTAACGCTGGATT-TCATCTGGGAAGCAG-3’, reverse primer, 5’-GTGGATCTCCAGGCTCTGGATTCCAG-3’. Briefly, 1 μg of purified RNA was reverse transcribed in the presence of 2.5 mM MgCl2, 1× RT-PCR buffer, 1.75 mM MgCl2, 0.8 mM dNTPs, 0.15 μM of forward (5’-ATTCTGCTGTGCAGTGTTCG-3’) and reverse (5’-TGGTTTGGAGAACATGGTGCT-3’) primers for rat sclafen 3 that resulted in a 127-bp product. β-Actin forward (5’-CCAGCAACTGGAAGATCTCA-3’) and reverse (5’-ACATCT-GCTGGAAGGTCG-3’) primers (107 bp product) were used as internal controls. Reactions were carried out in the Gene Amp PCR system 9600, first hold of 10 min at 95°C for activated AmpliTaq Gold DNA Polymerase, and were followed by 20 s at 94°C, 60 s at 56°C for 40 cycles for amplification of target gene.

**Microarray analysis.** Age-related changes in gene expression in mucosal cells isolated from upper and lower regions of the colon of young and aged rats were performed at the Genomic Core Facility, Karmanos Cancer Institute by utilizing Illumina Rat Sentrix-12 BeadChip Arrays essentially according to manufacturer’s instruction (Illumina). Briefly, 0.5 μg total RNA was biotinylated, hybridized with BeadChips. The signal was detected with streptova-
buffer (1 mM dNTPs, 10 mM dithiothreitol, 10 units RNase inhibitor, 1.25 μM schlafen 3) sequence-specific oligo and 15 units Multiscribe Reverse Transcriptase in a final reaction volume of 20 μl; the components were mixed, briefly spun down, and incubated at 25°C for 10 min for hybridization. Reactions were carried out at 42°C for 30 min in a Gene Amp PCR system 9600 (Perkin-Elmer) and then by cooling to 4°C. The RT reactions were subjected to PCR amplification, and 5 μl of cDNA products were amplified with 25 μl of PfuUltra hotstart 2× PCR Master Mix (Stratagene, La Jolla, CA), 0.3 μM upstream primers, and 0.3 μM downstream primers in 50 μl reaction volume. Reactions were carried out in the Gene Amp PCR system 9600, first hold of 2 min at 95°C for activated PfuUltra Hotstart DNA Polymerase, followed by 40 cycles of 95°C for 30 s, 62°C for 60 s, 72°C for 2 min, and final extension at 72°C for 10 min. The PCR products were separated by electrophoresis in a 1% agarose gel and visualized by ethidium bromide staining. The 1,816 bp of target DNA fragments were recovered, digested, and cloned into HindIII and BamHI sites of plasmid, pEGFP-N1 (Clontech, CA) to generate Slfn3-GFP protein. The constructs was sequenced and confirmed to contain *Rattus norvegicus* schlafen 3.

Cellular growth. This was assessed by 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay according to our standard protocol. Briefly, the cells were dispersed by trypsin-EDTA treatment, and 2.5 × 10^4 cells/ml, resuspended in DMEM containing 10% of FBS, were seeded into 96-well culture plates with six replicates. At the end of the 24-h incubation period, the reaction was terminated by adding 20 μl of 5 mg/ml stock MTT to each well. The reaction was allowed to proceed for 3 h at 37°C. The culture medium was then removed. The formazan crystals were then dissolved by adding 0.1 ml of DMSO. The intensity of the color developed, reflecting the number of live cells, was measured at a wavelength of 570 nm. All the values were compared with the corresponding controls.

Cell cycle analysis. Twenty-four hours after vector and schlafen 3 transfected HCT-116 cells were fixed in 70% ethanol and treated with the staining solution (10 μg/ml propidium iodide, 50 μg/ml RNAse, 0.1% Triton X-100 and 0.1 mM EDTA) for at least 30 min at 4°C before being subjected to a BD Biosciences FAC Scan cytometer (BD Biosciences, San Jose, CA) and analyzed by Modfit software.

Western blot analysis. Western blot analysis was performed essentially according to our standard protocol (15, 16). Briefly, aliquots of cell lysates containing 50 μg of protein were separated by SDS-PAGE. Following electrophoresis, proteins were transferred electrophoretically onto nitrocellulose membranes (Osmonics, Gloucester, MA) and subsequently incubated for 1 h at room temperature with blocking buffer, TBS-T (20 mM Tris, pH 7.6, 100 mM NaCl, 0.1% Tween-20), and 5% nonfat dry milk with gentle agitation. After the membranes were washed with TBS-T, they were incubated overnight.
Table 1. List of selected growth and apoptosis regulatory genes differentially expressed in colonic mucosa of old compared with young rats

<table>
<thead>
<tr>
<th>Number</th>
<th>Gene</th>
<th>ID</th>
<th>Fold Change</th>
<th>Putative Growth Regulatory Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Schlafen 3</td>
<td>ILMN_50410</td>
<td>U: −10.0</td>
<td>Growth inhibition (G1 arrest)</td>
</tr>
<tr>
<td>2</td>
<td>Secretoglobulin family 1A</td>
<td>ILMN_49298</td>
<td>U: −3.5</td>
<td>Proliferation and apoptosis</td>
</tr>
<tr>
<td>3</td>
<td>Dnase 1/3</td>
<td>ILMN_62202</td>
<td>U: −3.02</td>
<td>Inhibition of phospholipase A2</td>
</tr>
<tr>
<td>4</td>
<td>Granzyme B</td>
<td>ILMN_49667</td>
<td>U: −2.94</td>
<td>Apoptosis</td>
</tr>
<tr>
<td>5</td>
<td>Annexin A1</td>
<td>ILMN_60211</td>
<td>U: −1.78</td>
<td>Apoptosis</td>
</tr>
<tr>
<td>6</td>
<td>Granzyme G</td>
<td>ILMN_57532</td>
<td>U: −3.1</td>
<td>Inhibition of phospholipase A2</td>
</tr>
<tr>
<td>7</td>
<td>Adrenomedullin</td>
<td>ILMN_60494</td>
<td>U: −1.44</td>
<td>Growth stimulation</td>
</tr>
<tr>
<td>8</td>
<td>Granzyme A</td>
<td>ILMN_69553</td>
<td>U: −1.95</td>
<td>NF-κB and AKT activation</td>
</tr>
<tr>
<td>9</td>
<td>DOCK11</td>
<td>ILMN_54582</td>
<td>U: −1.59</td>
<td>?Stress-mediated apoptosis</td>
</tr>
</tbody>
</table>

Genes with similar change in expression in both sites

Genes with differential expression only in the upper third of the crypt

<table>
<thead>
<tr>
<th>Number</th>
<th>Gene</th>
<th>ID</th>
<th>Fold Change</th>
<th>Putative Growth Regulatory Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Melanoma antigen, family D, 1</td>
<td>ILMN_69307</td>
<td>−2</td>
<td>Apoptosis response to NGF stimulation</td>
</tr>
<tr>
<td>2</td>
<td>Rho GTPase activating protein 9</td>
<td>ILMN_69091</td>
<td>−2</td>
<td>?Inhibition of migration/proliferation</td>
</tr>
<tr>
<td>3</td>
<td>Rho, GDP disassociation inhibitor (GDI) beta</td>
<td>ILMN_66176</td>
<td>−2</td>
<td>Inhibition of Rac-1</td>
</tr>
<tr>
<td>4</td>
<td>Annexin A3</td>
<td>ILMN_57519</td>
<td>−2</td>
<td>Inhibition of Rho GTP binding proteins</td>
</tr>
</tbody>
</table>

Genes with differential expression only in the lower third of the crypt

<table>
<thead>
<tr>
<th>Number</th>
<th>Gene</th>
<th>ID</th>
<th>Fold Change</th>
<th>Putative Growth Regulatory Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PDZ binding kinase (predicted)</td>
<td>ILMN_48491</td>
<td>−1.9</td>
<td>Stress-associated apoptosis</td>
</tr>
</tbody>
</table>

U, upper third of the colonic crypt; L, lower third of the colonic crypt.

RESULTS

Unsupervised and supervised analysis of gene expression patterns in colonic mucosa. Upper and lower thirds of the colonic crypts are involved in different physiological processes, namely apoptosis and proliferation, respectively, that maintain homeostasis of the colonic mucosa. Hence, we studied gene expression in two different sites (upper and lower) of the colonic mucosa with an additional variable of age (young: 4–6 mo; old 21–24 mo). This yielded four groups with two major comparisons: namely, upper third of the crypt in young vs. old and lower third of the crypt in young vs. old.

The quality control, based on probe intensity distributions, revealed that an artifact affected one sample of old lower third group in which the intensities of most of the probes were found

Table 2. Schlafen 3 mRNA expression by real-time RT-PCR in colonic mucosa of old compared with young rats in both upper and lower thirds of the crypt

<table>
<thead>
<tr>
<th></th>
<th>Schlafen 3 Expression (Real-Time RT-PCR)</th>
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<tbody>
<tr>
<td></td>
<td>Young (4–6 mo)</td>
</tr>
<tr>
<td></td>
<td>Old (22–24 mo)</td>
</tr>
<tr>
<td>Upper (third) colon crypt</td>
<td>0.16 (12)</td>
</tr>
<tr>
<td>Lower (third) colon crypt</td>
<td>0.03 (3)</td>
</tr>
</tbody>
</table>

Data are expressed as relative fold increase over the corresponding β-actin.

at 4°C in TBS-T buffer containing 2.5% milk with goat polyclonal schlafen 3 (Santa Cruz Biotechnology, Santa Cruz, CA) antibodies at 1:500 dilution. The membranes were washed three times with TBS-T, subsequently incubated with appropriate secondary antibodies (1:5,000 dilution) in TBS-T-2.5% milk for 2 h at room temperature. The membranes were washed again with TBS-T. The protein bands were visualized by enhanced chemiluminescence detection system (Amer- sham Bioscience). The membranes were then stripped and reprobed with either β-actin or α-tubulin as a loading control.

Immunohistochemistry. For immunohistochemical staining, an immunoperoxidase method was used with a streptavidin biotinylated horseradish peroxidase complex (Dako, Carpenteria, CA). The rat colonic tissues were formalin fixed and paraffin embedded, and 5-μm serial sections were generated. The tissue sections were deparaffinized and microwaved for 15 min in citrate buffer (0.1 M citric acid and 0.1 M sodium citrate, pH 6.0) for antigen retrieval. Endogenous peroxidase activity was quenched with 3% hydrogen peroxide and subsequently incubated with 5% horse serum to block nonspecific binding. The slides were then incubated at room temperature for 2 h with polyclonal antibodies to PCNA at 1:50 dilution. All slides were slightly counterstained with Harris’s hematoxylin. At least ten well-oriented crypts on each slide and five slides from each sample were examined under high power. At least 750 cells/slide were counted for immunohistochemistry.
This sample was therefore removed from further preprocessing and analysis. The unsupervised analyses depicted in Fig. 1, B and C, show that samples in the same group produce a very similar gene expression pattern and that most of the differences are between the groups. This result is reassuring since high within-group similarity and large between-group dissimilarity is the key for reliable identification of differentially expressed genes. The supervised analysis based on a moderated t-test and fold change calculations revealed hundreds of genes as being differentially expressed in the two major comparisons we have performed.

On the basis of the supervised analysis we also found that 66 genes were differentially expressed in the same direction (up- or downregulation) in both upper and lower thirds of the colonic crypt between young and old animals. Of these genes, 44 were downregulated and 22 were upregulated in old animals compared with their young counterparts. Some of the differentially expressed genes involved in proliferation and apoptosis are shown in Table 1. Schlafen 3, a recently isolated negative regulator of proliferation (3, 21), was found to be most differentially expressed in the colonic mucosa between young and aged rats. We have observed that the expression schlafen 3 was decreased by 10- and 4-fold in mucosal cells isolated from upper and lower region of the crypt of aged rats, respectively, compared with their corresponding younger counterparts (Table 1).

Age-related changes in expression of schlafen-3. To examine age-related changes in expression of schlafen 3 gene in the colonic mucosa, we carried out real-time RT-PCR analysis. There was a 12- and 3-fold decrease, respectively, in the mucosa from upper and lower third of the colonic crypt of old, compared with young rats (Table 2). To further determine whether the age-related decrease in schlafen 3 mRNA expression would be reflected in the protein levels, Western blot analysis was also carried out. The age-related decline in schlafen 3 mRNA levels in the colonic mucosa was also reflected in protein levels of the gene in that the levels were found to be substantially lower in mucosal cells isolated from both upper and lower third of the colonic crypt of aged than in young rats (Fig. 2). In contrast to what we observed in the colonic mucosa, schlafen 3 expression remained unchanged in the relatively stable organ such as liver of young and aged rats (Fig. 2).

Wortmannin, a specific PI3K inhibitor, stimulates schlafen-3 expression in the colonic mucosa during aging. Previously, we have reported that inhibition of PI3K activity in the colonic mucosa of aged rats by wortmannin greatly stimulates apoptosis (15). To determine whether schlafen 3 might be involved in regulating colonic mucosal growth during aging, young and old rats were injected with wortmannin or vehicle (controls) and the levels of schlafen 3 were determined in the colonic mucosa after 6 h. Interestingly, we observed that wortmannin treatment produced more than threefold increase in schlafen 3 levels in the colonic mucosa of aged rats, accompanied by a

Fig. 2. Representative Western blot showing changes in protein expression of schlafen 3 in the upper and lower fraction of colonic mucosa as well as in the liver of young (4-mo-old) and old (22-mo-old) Fisher 344 rats. A small part of the liver and epithelial cells isolated from the colonic mucosa of the young and old rats and were subjected to Western blot analysis. The experiment was repeated at least 3 times. Slfn3/4, schlafen 3. The commercial antibodies for schlafen 3 used in this experiment also cross-react with schlafen 4. 4, 6, and 22 represent age in months.

Fig. 3. Changes in mucosal proliferation as measured by PCNA immunoreactivity (A) and schlafen 3 protein expression (B) in colonic mucosa of young (4-mo-old) and old (21-mo-old) Fisher 344 rats 6 h after a single injection of wortmannin (wort; 0.1 mg/kg ip in 15% DMSO or vehicle). Cont, control. *P < 0.01, compared with controls.
concomitant reduction in proliferative activity, as evidenced decreased PCNA (proliferating cell nuclear antigen) immunoreactivity, compared with their corresponding younger counterparts (Fig. 3, A and B).

Overexpression of schlafen-3 gene inhibits cellular growth. To determine the role of schlafen 3 in regulating cellular growth, human colon cancer HCT-116 cells, that are devoid of rat schlafen 3 gene, were transfected with schlafen 3 cDNA. Twenty-four hours after transfection, cellular growth was determined by MTT assay. Results revealed that transfection of schlafen 3 caused a significant 25–30% reduction in cell growth compared with the vector-transfected controls (Fig. 4A).

To determine regulatory mechanisms of schlafen 3-induced inhibition of cell growth, HCT-116 cells transfected with schlafen 3 cDNA were selected by G418 following 2 wk of transfection. Flow cytometric analysis showed that transfection of schlafen 3 gene in colon cancer HCT-116 cells caused a 57% increase in accumulation of cells at the G0/G1 phase and corresponding reduction in S phase, suggesting a block in G1 to S progression (Table 3). To further determine regulatory mechanism of blockage of G1 to S phase progression, expression of cyclin D1, a critical regulator of this process, was examined. Levels of cyclin D1 in schlafen 3 transfected cells were greatly reduced, compared with the vector transfected controls (Fig. 4B). In fact, no detectable levels of cyclin D1 were observed in schlafen 3 transfected HCT-116 cells (Fig. 4B).

Retinoblastoma protein (Rb) is known to be differentially phosphorylated during the cell cycle. In its hypophosphorylated state, Rb binds different cellular proteins, including the transcription factor E2F. The latter is, however, released when Rb is hyperphosphorylated during progression of the cell cycle through the S and G2 (19). As expected, the levels of the phosphorylated form of Rb (pRb) were found to be markedly lower in schlafen 3-transfected HCT-116 cells, compared with the vector transfected controls (Fig. 4B). These changes were also accompanied by a concomitant reduction in the levels of PCNA (Fig. 4B), an auxiliary protein for DNA polymerase β that is expressed during S phase of the cell cycle (4, 5) and has been shown its levels to correlate positively with proliferation.

DISCUSSION

Aging is associated with changes in the structural and functional properties of the gastrointestinal mucosa as reflected by alterations in growth, differentiation, and immunity (14). However, a consistent pathological observation with aging is the increased incidence of gastrointestinal cancer, particularly in the colon, a leading cause of morbidity and mortality. Although colorectal cancer is a multistage process, increased proliferation and decrease in apoptosis are considered to be critical early events in the progression of cancer (13). Although earlier observations in the mouse and a recent report in rats suggest that the proliferative activity of the intestine either decreases or remains unchanged with aging (7, 9, 12), several morphological and biochemical studies from our and other laboratories have demonstrated that, in barrier-reared Fischer 344 rats, aging is associated with increased proliferative activity and decreased apoptosis in the colonic mucosa (1, 10, 11, 14–16, 26). On the basis of the observations that aging is associated with increased proliferation and decreased apoptosis in the colonic mucosa, it has been postulated that aging may predispose the colon to malignant transformation (1, 14).

Many of the genes that are differentially expressed in the colonic mucosa of young and old rats can be grouped into growth suppressors, growth promoters, and proapoptotic and antiapoptotic genes. Of various genes identified in such fashion, schlafen 3 was most differentially expressed in the colonic mucosa of old rats. schlafen 3 expression was found to be increased in old rats, whereas the expression of cyclin D1, a critical regulator of G1 to S progression, was decreased. schlafen 3 expression was also found to be increased in human colon cancer cells, HCT-116, that were transfected with schlafen 3 cDNA. These changes were accompanied by a concomitant reduction in proliferative activity, as evidenced by decreased PCNA immunoreactivity, compared with the vector-transfected controls. schlafen 3 expression was also found to be increased in human colon cancer cells, HCT-116, that were transfected with schlafen 3 cDNA. These changes were accompanied by a concomitant reduction in proliferative activity, as evidenced by decreased PCNA immunoreactivity, compared with the vector-transfected controls.
Schlafens are a family of proteins that includes at least eight members in mice (8, 21). They are classified into three groups on the basis of COOH-terminal length as short (schlafen 1 and 2), intermediate (schlafen 3 and 4), and long (schlafen 5, 8, 9 and 10) forms. The common NH₂-terminal domain has a GTP/ATP binding site, whereas COOH-terminal domain in long form of schlafens possesses helicase-like domain, which is thought to play a role in DNA repair or transcriptional regulation (8, 28).

Although the functional properties of murine schlafen family of proteins have not been fully elucidated, they have been suggested to play a critical role in cell proliferation as a negative regulator by downregulating cyclin D1 (3, 8). It has been demonstrated that overexpression of schlafen 3 results in growth inhibition and greatly impairs anchorage-independent growth of various cells (21). Furthermore, schlafen 1, a short-form member of the schlafen family, has been shown to be a negative regulator of cell cycle progression in response to stimulation with a variety of mitogens including EGF, PDGF, and G protein-coupled receptors (3). However, these observations were made in lymphocytes and fibroblasts. Our present data, for the first time, demonstrate a growth regulatory role of schlafen 3 in epithelial cells. The basis for this inference comes from the observation that transfection of schlafen 3 in human colon cancer HCT-116 cells, which are devoid of schlafen 3 (present only in rodents), inhibits cellular growth as determined by MTT assay, accompanied by a concomitant reduction of PCNA, an auxiliary protein for DNA polymerase 8 that is expressed during S phase of the cell cycle (4, 5) and has been shown its levels to correlate positively with proliferation.

As opposed to the murine family of schlafens, human schlafens consists of seven known members that are located on long arm of chromosome 17. Of these, schlafen 12 shows 47% sequence homology with the murine schlafen 3 gene. However, the functional significance of human family of schlafens including schlafen 12, remains to be elucidated (http://www.ncbi.nlm.nih.gov/UniGene/clust.cgi?ORG=Hs&CID=663548; http://www.ihop-net.org/Unipub/IHP?search=SCHLAFEN&field=all&ncbi_tax_id=0&organism_syn=).

In conclusion, our data show that aging is associated with downregulation of genes involved in suppression of proliferation and induction of apoptosis. In particular, the expression of schlafen 3, a negative regulator of proliferation, was decreased by 8- to 10-fold in the colonic mucosa of aged than in young rats. That schlafen 3 could be a negative regulator of growth was supported by the observation that transfection of schlafen 3 in colon cancer cells leads to inhibition of proliferation, which could be attributed to downregulation of cyclin D1 and PCNA and attenuation of phosphorylation of Rb.

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