S-adenosylhomocysteine hydrolase downregulation contributes to tumorigenesis

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With the idea to discover novel genes involved in proliferation, we have performed a genome-wide loss-of-function genetic screen to identify additional putative tumor suppressor genes. We have previously identified five genes belonging to different biochemical families. In this report, we focused on the study of one of these genes designated S-adenosylhomocysteine hydrolase (SAHH), which has also been previously identified in an independent short hairpin RNA screening. SAHH inactivation confers resistance to both p53 and p16INK4a-induced proliferation arrest. Interestingly, SAHH inactivation inhibits p53 transcriptional activity and impairs DNA damage-induced transcription of p21Cip1. Given that SAHH downregulation modulates senescence in primary cells, we also studied SAHH expression in human tumors at the messenger RNA (mRNA) and protein levels. SAHH mRNA was lost in 50% of tumor tissues from 206 patients with different kinds of tumors in comparison with normal tissue counterparts. Moreover, SAHH protein was also affected in some colon cancers. Such findings may be of relevance to cancer research, suggesting that SAHH might be a largely unexplored tumor suppressor.

Introduction

Primary cells must acquire limitless replicative potential to form full grown malignant tumors (1,2). This tumorigenic property may result from a collection of genetic alterations that allow bypass of cellular senescence barriers (3,4). Therefore, genes whose ectopic expression bypasses the normal replicative potential are candidate oncogenes because the proteins encoded by such genes are detected in highly immortalized cells and overexpressed in some types of human cancer (5–7). Genes whose loss-of-function bypasses replicative senescence, however, are candidate tumor suppressor genes and are found to be downregulated in tumor tissues (8). Besides telomerase activation, the most common alteration contributing to immortalization of primary human cells are those that inactivate the p53 and Rb tumor suppressor pathways. Interestingly, mutations in both pathways are very frequent in human BJ fibroblasts (8). With the aim to determine regulators of the senescence program, we performed a genome-wide loss-of-function genetic screen. For this purpose, a complementary DNA (cDNA) library from senescent mouse embryonic fibroblast (MEFs), thereby expressing antiproliferative genes, was cloned in the antisense orientation in MaRx retrovector vectors. MEFs infected with the cDNA library were screened for proliferative clones that contain information regarding those genes which inhibit proliferation (11). In such a study, we have recently reported the identification of three genes: Csn2 (a subunit of the COP9 signalosome), BRF1 (a subunit of the RNA polymerase III) and aldose reductase B1 (an enzyme that participates in glucose metabolism). In the present study, we focused on another gene identified in the same screening designated S-adenosylhomocysteine hydrolase (SAHH). SAHH catalyzes the hydrolysis of S-adenosylhomocysteine to adenosine and homocysteine. In eukaryotes, this is the major route for disposal of the S-adenosylhomocysteine formed as a common product of each of the many S-adenosylmethionine-dependent methyltransferases. The reaction is reversible, but, under normal conditions, the removal of both adenosine and homocysteine is sufficiently rapid to maintain the flux in the direction of hydrolysis. Physiologically, not only S-adenosylhomocysteine hydrolysis sustains the flux of methionine sulfur toward cysteine but also it is believed to play a critical role in the regulation of methylation reactions (Figure 1A). Therefore, SAHH is a strong inhibitor of transcription (12). Modulation of methylation is a very important biochemical mechanism that regulates gene expression in many physiological processes (13–15) and alterations in methylation patterns have been observed in malignant lesions. It is thought that epigenetic silencing of tumor suppressor genes through DNA methylation and histone modification is an early event in tumorigenesis (16,17). One of the best known inactivators of tumor suppressor genes in malignant cells is the transcriptional repression of their promoter by hypermethylation at CpG islands, a process in which methyltransferases, histone demethylases and polycomb proteins are involved (18). To date, several oncogenes that code for methyltransferases have an important role in hematopoietic malignancies, such as the MLL oncogene. A correlation of global DNA hypomethylation with poor prognosis has been reported for ovarian cancer (19), squamous cell lung cancer (20) and hepatocellular carcinoma (21).

Three different isoenzymes of SAHH have been identified in rat and mouse livers based on different kinetic properties. A specific isoenzyme named SAHH-LT appears to play an important role in perpetuating malignant growth (22). Importantly, biochemical effects on transmethylation reactions affecting decreased activities of SAHH may be implicated in the pathogenesis of alcoholic liver disease, which is associated with many cases of liver cancer (23,24).

Interestingly, SAHH has also been previously identified in an short hairpin RNA (shRNA) screen to bypass p53-induced growth arrest (8). SAHH inhibition has been reported to induce proliferation in human BJ fibroblasts partially immortalized with human catalytic subunit of telomerase. These authors demonstrated that SAHH shRNA conferred resistance to both p53-dependent and p19ARF-dependent proliferation arrest while also abolishing DNA damage-induced G1 cell cycle arrest. The presence of genetic modifications in BJ cells that convert them to a partially immortalized phenotype may, however, mask the singular contribution of SAHH in a wild-type background. The results that we report in this study agree with the results from Berns et al. (8) and provide a molecular mechanism for the biological role of SAHH in replicative senescence of primary cells. Despite significant progress toward understanding the function of SAHH, full characterization of this gene remains incomplete. This report addresses several mechanisms of SAHH downregulation for...
bypassing senescence and highlights its role as a novel tumor suppressor gene in cancer.

Materials and methods

Cell culture and infections
MEFs were isolated from a strain of pregnant mice (CD1) at 13.5 days of gestation. The LinXE packaging cell line was used to produce the retrovirus. Details of the cDNA library construction in the MaRx vectors have been described previously (25). MEFs, NIH3T3 cells, HEK293 and IMR90 cells were grown in Dulbecco’s modified Eagle’s medium (Lonza, Basel, Switzerland) with 10% fetal calf serum (Lonza). MEFs at fourth passage (P4) were infected with the cDNA library reported previously (11). In parallel, two dominant-negative mutants of p53 cDNA, designated p53(273H) or p53(175H) and vector-infected cells (V) were used as positive and negative controls for proliferation, respectively. After selection of MEFs with 4 μg/ml of puromycin, cells were seeded at a low density to allow individual cells to form colonies. After 30 days, control plates were stained with crystal violet to validate the experimental conditions of the genetic screen. NIH3T3 cells expressing SAHH-α or vector control (V) were seeded at a density of 1 × 10^6 cells/10 cm dish. On the following day, cells were treated with DNA-damaging agents: doxorubicin at 0.2 μg/ml and cisplatin at 100 μM. In all the experiments performed to characterize SAHH function, a heterogenous population of cells, expressing the indicated genes and SAHH-α and selected with the appropriate antibiotics, was studied. SAHH cDNA was cloned into an HA-tag vector (Roche, Basel, Switzerland) and was expressed stably in NIH3T3 cells. The SAHH-α or empty vector was transduced into these cells to validate the effectiveness of the SAHH-α.

Growth curves
Vector-, SAHH- and p53(273H)-infected MEFs were seeded at 1 × 10^5 cells/10 cm plate after infection and selection. Every 3 days, cells were counted and seeded at the same density as indicated by the 3T3 protocol. Growth curves were also performed in duplicate by seeding and selection of cells infected with the indicated genes and SAHH-α at a density of 1 × 10^6 cells/10 cm dish, along with two spare plates to stain with crystal violet at each passage. The latter were destained with 10% acetic acid and quantified at 595 nm for relative cell numbers.

To validate cell proliferation by another method, colony formation assays were performed. Once MEFs had been infected and selected with the appropriate antibiotics, cells were seeded at a density of 1 × 10^4 cells/10 cm plate. Cells were continuously cultured, and growth medium was changed every 3 days. After 20 days, plates were stained with crystal violet, and cell proliferation was assessed by observing the appearance of growing colonies.

Immunoblotting
Cells were washed twice with ice-cold 1 × phosphate-buffered saline and lysed by sonication in lysis buffer [50 mM Tris–HCl, pH 7.5, 1% NP-40, 10% glycerol, 150 mM NaCl and 2 mM complete protease inhibitor cocktail (Roche Diagnostics, Barcelona, Spain)]. Proteins were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, transferred onto Immobilon-P membranes (Millipore, Iberica, SA, Madrid, Spain) and immunostained with the following specific antibodies: anti-HA clone HA7 (Sigma–Aldrich, St Louis, MO), PAb FL-393 anti-p53 sc-6243 (Santa Cruz Biotechnology, Santa Cruz, CA), MAb anti-p21<sup>Wp1</sup> OP64 (Oncogene Research Products, EMD Biosciences, San Diego, CA), MAb anti-tubulin T9026 (Sigma–Aldrich), β-actin (Sigma–Aldrich), SAHH ab11165 (Abcam, Cambridge, UK), p53 DO-7 (Dako, Glostrup, Denmark) and p16<sup>INK4A</sup> DB018 (AntibodyBcn, Barcelona, Spain).

Luciferase assays
HEK293 and IMR90 cells were seeded in triplicate in 12-well plates at a density of 50 × 10^3 cells per well. The following day, cells were at 70% confluency and were transfected by the calcium phosphate method (HEK292) or lipofectamine method (IMR90) using the following plasmids: pGL3basic, pGL3basic containing the p21<sup>Wp1</sup> promoter luciferase reporter (designated pGL3basics21–Luc), p53WT, mdm2 or empty vector (V). The p53WT gene was used in all transfections as a positive control stimulating the activation of the p21<sup>Wp1</sup> promoter. Mdm2 was used as a negative control for p53 transcriptional activity. The following plasmids were used for the E2F1-luciferase assay: pGL3basic, E2F1-WT and pGL3basic containing the E2F1 promoter luciferase reporter (designated pGL3basicsE2F1–Pm–Luc). The E2F1 promoter sequence used here has been described previously (26). Given the fact that the E2F1 promoter can be activated by the E2F1 protein, we used an E2F1-WT cDNA as a positive control for E2F1 promoter luciferase reporter stimulation. Cells were lysed 48 h after transfection, and one-fifth of each sample was assayed following the manufacturer’s protocol (Promega, Madison, WI).

siRNA experiments
MEFs, IMR90 and IMR90-TERT cells were seeded at a density of 24 × 10^3 – 1 × 10^5 cells per well in six-well plates. Cells were transfected with 4–10 μl of Hiperfect reagent (Qiagen, Hilden, Germany) per well, depending on the cell line, in order to achieve optimal transfection efficiency with the lowest level of toxicity possible. The final concentration of each small interference RNA (siRNA) was 30 nM. The following siRNAs were used (Ambion, Foster City, CA): murine SAHH (ID7239, ID72248, ID152470), human SAHH (ID119089, ID119090, ID9068), glyceraldehyde-3-phosphate dehydrogenase (ID6649) and histidin (cyclophilin B) (Cultek, Madrid, Spain) as a labeled control to determine transfection efficiency. For each cell type, three independent SAHH siRNAs and a pooled sample containing all three SAHH siRNAs were used. Cell media were changed 2 days after transfection. After an additional 3 days, cells were counted. Samples were run in triplicate for each siRNA in at least two independent experiments.

RNA array hybridization
Cancer Profiling Array membranes (BD Biosciences, Franklin Lanes, NJ) were prehybridized with ExpressHyb hybridization solution for 4 h at 65°C. The appropriate probe was then labeled by polymerase chain reaction (PCR) with redivue dCTP<sup>32</sup> (Amersham Biosciences, Fairfield, CT) at 50°C. The labeled probe was purified from free radiolabeled nucleotides with a Sepharose G-50 nick column (Amersham), which was denatured for 3 min at 100°C, then added to the hybridization solution. The hybridization was performed overnight at 65°C. The membrane was then washed at 65°C, twice with 2× 3 M NaCl, 0.2M NaH2PO4, 0.02M EDTA, 0.1% sodium dodecyl sulfate and twice with 1× 3 M NaCl, 0.2M NaH2PO4, 0.02M EDTA, 0.1% sodium dodecyl sulfate. The membrane was then exposed to Biomax MS film (Kodak, Rochester, New York). cDNA probes for SAHH were radioactively labeled and hybridized with the arrayed tumor or normal RNA samples. As an expression control, we hybridized the arrays with an ubiquitin-specific probe. We normalized the signals of the specific probes against the ubiquitin signal by quantitating the signals in tumor and normal samples. Finally, we identified those tumor samples in which signals were decreased by at least 50%, as compared with normal tissue.
Real-time PCR
Real-time PCRs were performed in triplicate using Assays-on-Demand Taqman Gene Expression Assays from Applied Biosystems (Foster City, CA), as described previously (27). Eight patient biopsies showing SAHH downregulation at the messenger RNA (mRNA) level were subjected to protein extraction and immunoblotting with an SAHH antibody. β-Actin was used as a loading control. Additionally, 18 patient samples with unknown SAHH mRNA levels were randomly chosen and examined for SAHH protein expression. All procedures used were approved by the Ethics Committee of the Hospital Vall d’Hebron.

Statistical analyses
Statistical analyses were performed using unpaired t-tests to compare the percentage of samples that demonstrated a 50% increase in normal versus tumor samples from the same patient. Differences in SAHH mRNA expression between normal and tumor tissues were observed at a level of P < 0.0001.

Results
SAHH inhibition bypasses replicative senescence in murine cells
Primary MEFs were infected with a retrovirus carrying an antisense senescent MEF cDNA library, as well as with p53(273H) or a vector. After infection and selection, MEFs were seeded at a low concentration, and the vector-infected cells were allowed to undergo senescence. After 30 days, spare plates of the positive and negative controls were stained with crystal violet to validate the screening experiment (Figure 1B). An SAHH antisense fragment (SAHH-αs) was recovered from two independent proliferative clones (Table 1). An SAHH-αs vector or empty vector was transduced into NIH3T3 cells previously selected for the expression of SAHH cDNA. SAHH-αs- and vector-expressing NIH3T3 cells were analyzed using HA and tubulin antibodies. SAHH protein was reduced by 50% in those cells expressing SAHH-αs, as compared with those receiving the empty vector (Figure 2A). The reduction of SAHH by the antisense fragment led to a moderate increase in MEF life span (Figure 2B). In accordance with this result, SAHH-αs also caused early immortalization of the cultures and, in 3T3 experiments, SAHH-αs increased MEF life span by approximately four population doublings (Figure 2C), demonstrating some additive effect in MEFs lacking p21Cip1 (Figure 2D). SAHH-αs was not, however, able to provide complete immortalization of MEFs in these scenarios, as the cells did eventually undergo senescence (data not shown).

In order to verify the effect provoked by SAHH-αs via another approach, three different SAHH siRNAs specific for murine cells were utilized. SAHH downregulation by siRNA provided a proliferative advantage in primary MEFs (supplementary Figure 1A is available at Carcinogenesis Online). In contrast to the effect observed in murine cells, SAHH inhibition, either via antisense or siRNA, did not delay senescence in human primary cells IMR90s (supplementary Figure 1B is available at Carcinogenesis Online and data not shown). We also examined SAHH downregulation in a ras-induced senescent model in human IMR90-TERT cells. Neither of the three different siRNAs against human SAHH nor the pool of all three SAHH siRNAs, could bypass ras-induced senescence once H-rasVal12 was induced (supplementary Figure 1C is available at Carcinogenesis Online).

p53 pathway
The p53 and pRb pathways play crucial roles in cancer (28). The ability of the antisense construct of SAHH to bypass p53-induced arrest was examined. For this purpose, SAHH-αs was expressed in a cell line derived from p53-null MEFs and expressing the temperature-sensitive mutant of p53 (Val115). Cells expressing p53 (Val115) grow actively at 39°C but arrest at 32°C when the p53 protein adopts the active, wild-type conformation (29). We observed that SAHH-αs inhibited by ~50% the cell cycle arrest induced by wild-type p53 when cells were grown at 32°C (Figure 3A). Similar results were obtained using an shRNA against SAHH (supplementary Figure 2A is available at Carcinogenesis Online).

Next, we examined the effect of SAHH-αs on p53-dependent transcriptional activation. HEK293 cells were transected with a p21Cip1 promoter. Similar results were obtained with an shRNA against SAHH (supplementary Figure 2B is available at Carcinogenesis Online).

pRb pathway
To determine, in a more physiologically relevant setting, whether p53 transcriptional activity was compromised by SAHH-αs, we transfected NIH3T3 cells with SAHH-αs or empty vector (V). Mass cultures carrying the retroviral vectors were selected with appropriate antibiotics and treated with the DNA-damaging agents doxorubicin, at 0.2 μg/ml, or cisplatin, at 100 μM. Cells were maintained in culture for 6 or 24 h after treatment, and p53 and p21Cip1 protein expression was then examined.

In NIH3T3 cells infected with the vector and further treated with each DNA-damaging agent independently, p53 stabilization occurred after 6 and 24 h, as expected in cells with an intact p53 pathway, such as NIH3T3 cells. Such activation was accompanied by an increase in p21Cip1 protein, which was clearly visible 6 h after exposure to doxorubicin or cisplatin and which was maintained 18 h later (or 24 h after exposure) (Figure 3C). In NIH3T3 cells expressing SAHH-αs, p53 protein levels were increased 6 h after exposure to doxorubicin or cisplatin. However, upon doxorubicin treatment, p53 activation was not observed 24 h after exposure. Importantly, whether doxorubicin or cisplatin treatments were used, p21Cip1 did not increase while SAHH-αs was expressed (Figure 3C); therefore, under these conditions, p53 stabilization did not translate into an increase in p21Cip1.

Table 1. Characteristics of the SAHH antisense fragment

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<th>Length (nt)</th>
<th>cDNA Location</th>
<th>Antisense Gene</th>
<th>Times recovered</th>
<th>Location (from cDNA)</th>
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<tr>
<td>328</td>
<td>SAHH</td>
<td>2</td>
<td>139</td>
<td>1593</td>
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expression, many cells escaped growth arrest and grew colonies (Figure 3D). In order to verify the effect of SAHH downregulation in wild-type cells, where, in contrast to HEK293, the pRB pathway is intact, E2F1 activity was measured in IMR90 cells. However, SAHH-αs expression did not affect E2F1 transcriptional activity in IMR90 cells (Figure 3E). Similar results were obtained by using an shRNA against SAHH, reported previously by Berns et al. (8) (supplementary Figure 2C is available at Carcinogenesis Online).

### Human tumors

Cancer profiling array membranes (CNIO) were used to examine the expression of SAHH in paired samples of normal and tumor tissue from the same patient, for a total of 206 patients. Our data demonstrated that kidney tumors showed the greatest loss of SAHH mRNA—a 50% loss, as compared with normal tissue—and these were followed by tumors of the small intestine, pancreas and skin (Figure 4A). When all tumors were considered, SAHH mRNA levels showed a 50% reduction in tumor versus normal samples (data not shown).

To complement these studies, we performed quantitative reverse transcription–PCR (RT–PCR) analysis of SAHH in 40 patients with colon cancer (Pathology Department, Hospital Vall d’Hebron). mRNA levels were normalized in tumor and normal samples using at least two different endogenous control genes, including cyclophilin, POL2A or β-actin (Figure 4B).

To examine whether the SAHH mRNA downregulation observed by quantitative RT–PCR correlated with SAHH protein levels, eight colon cancer cases in which SAHH mRNA was known to be downregulated were selected. Interestingly, protein downregulation was observed in only four patients (50%). Two representative cases are shown (P1 and P3) in Figure 5A. In contrast, in 18 randomly chosen patients, SAHH protein was found to be upregulated in 7 of 14 colon cancer and one of 4 kidney cancer cases (Figure 5B). In six patients where SAHH was upregulated, p16INK4A and p53 were examined; one patient showed p16INK4A and p53 upregulation and four patients showed p53 upregulation. An example of four patients is shown in Figure 5B.

### Discussion

The ultimate aim of this investigation was to discover novel tumor suppressor genes that might be implicated in human cancer. By performing a genome-wide loss-of-function genetic screen to identify genes for which inactivation produced proliferative effects, we discovered SAHH. Here, we have shown that SAHH, a protein involved in methylation reactions, is a component of the p53 tumor suppressor pathway and also contributes, to a minor extent, to the pRB pathway.

The fact that SAHH-αs was recovered twice from our genetic screen from independent clones confirmed that its inhibition was responsible for the proliferative phenotype observed in MEFs. The proliferative effect of SAHH-αs was revealed by plotting growth curves (3T3) and comparing the results with vector-infected MEFS. To explore possible mechanisms by which SAHH may affect senescence, the involvement of SAHH-αs in the p53 and pRB checkpoints was examined. Interestingly, we observed that SAHH-αs or SAHH shRNA was able to inhibit cell cycle arrest induced by wild-type p53 in MEFS, at rates of roughly 50%, suggesting that SAHH downregulation might negatively modulate p53 activity.

Although p53 protein levels can be used as an indirect indicator of p53 function, induction of the CDK cell cycle inhibitor p21cip1, known to be a major component of the p53 antiproliferative response, represents a bona fide readout of p53 activity (32). Interestingly,
SAHH-αs and SAHH shRNA were able to significantly reduce p53 transactivation activity in HEK293 cells. This suggested that the ability of SAHH-αs to interfere with the p53 pathway is not restricted to primary cells, but represents a more generalized mechanism, also affecting human immortalized cells. These results agree with previous experiments performed using the p53(Val135) mutant (Figure 3A and supplementary Figure 2A is available at Carcinogenesis Online).

p21^{Cip1} is a critical component of G1 arrest in response to DNA damage and is required for the senescence response in murine and human fibroblasts (33). SAHH downregulation did not directly affect p53 protein stabilization upon DNA damage or any other component upstream of p53. However, in the case of doxorubicin treatment, those cells with SAHH downregulation were not able to maintain p53 stimulation to the same extent that p53 wild-type infected cells did. Additionally, NIH3T3 cells expressing SAHH-αs were not able to stabilize p21^{Cip1} following either doxorubicin or cisplatin treatment.

Collectively, we conclude that SAHH downregulation leads to a compromised p53 downstream pathway and altered p53 transcriptional activity. Apart from SAHH’s ability to interfere with p53 transcriptional activity at the p21^{Cip1} promoter, however, SAHH also appeared to affect proliferation in a p21^{Cip1}-independent manner (Figure 2D). Additionally, SAHH downregulation contributed to proliferation by bypassing the arrest imposed by p16^{INK42}, thereby also interfering with the pRB pathway. Given these results, it will be important to address in future work the question of whether SAHH-αs effects on the p53 and pRB pathways seen in this model are functionally connected.

It is worth mentioning that most of our experiments were performed with an shRNA against SAHH previously described by Berns et al. (8) and/or three independent siRNAs against SAHH (supplementary Figures 1 and 2 are available at Carcinogenesis Online and data not shown). Whether or not the effects of downregulating SAHH are directly or indirectly related to SAHH’s enzymatic ability to methylate proteins, phospholipids and/or nucleic acids remains to be determined.

Senescence represents an antitumorigenic mechanism, acting as a barrier to cancer by repressing proliferation in those cells in which mutations have occurred (34–36). Interestingly, senescence has been detected in benign human lesions but not in cancerous tissues (37,38). Thus, overcoming the obstacle of cellular senescence may be a primary step that cells must make during the process of transformation. Since SAHH reduction regulated the p53 and pRB pathways, both effectors of senescence, we have analyzed SAHH in human tumors at the RNA and protein levels.

RNA array hybridization analysis of 206 tumors showed that SAHH was lost, on average, in 50% of tumor tissues, as compared with normal tissue from the same patient. Our group has previously studied SAHH at the mRNA level in a small number of patients (27). Here, we analyzed SAHH mRNA in a larger series of 40 colon cancer patients. Using quantitative RT–PCR, we detected SAHH downregulation in 27 out of 40 cases (67%). However, a different percentage of cases demonstrated changes in SAHH mRNA levels when array methodology was used on another series of patients. Such differences might be related to the number of cases analyzed or the origin of the series studied (the series used for the arrays was from the CNIO, whereas the series used for the quantitative RT–PCR analysis was from the Hospital Vall d’Hebron).

SAHH protein levels were studied in eight patients who showed mRNA downregulation, but protein downregulation was found in only
four cases (50%). The fact that SAHH protein levels did not correlate with mRNA expression in all cases could be explained by the different genetic backgrounds of the tumors. Alternatively, the mRNA data might have been overestimated. The latter possibility seems less probable, however, because different endogenous genes were used to validate the quantitative RT–PCR results.

Based on our in vivo data, we propose two different mechanisms by which SAHH could be altered in human tumors. In the first, changes in SAHH could occur at the mRNA level and, consequently, both mRNA and protein levels of SAHH would be downregulated. A second mechanism could involve SAHH stabilization at the protein level, possibly rendering the protein functionally inactive. Point mutations in certain tumor suppressor genes have been shown to provoke stabilization of a protein that is no longer active, as occurs with specific ‘hot spots’ in the p53 gene (39). Therefore, it seems plausible that the same could occur for SAHH. As of yet, examination of the SAHH gene in human samples has revealed only polymorphisms and not mutations (27,40). However, we observed here two different and apparently opposite patterns of SAHH protein regulation, which could be explained by the two mechanisms detailed above. Indeed, both mechanisms have been described for tumor suppressor genes.

Although our series of patients was very limited, making it difficult to draw strong conclusions, the possibility that SAHH upregulation occurs in those patients with concomitant alterations in the pRB and/or p53 pathway in vivo (five out of six patients analyzed fulfilled these criteria) would be an interesting proposal. Ultimately, gaining a better understanding of how SAHH modifications affect its enzymatic properties, and of how these changes affect tumor development, will provide new insights regarding the role of metabolic enzymes in the proliferation of human cancers.

Fig. 4. SAHH mRNA in human samples. (A) Cancer profiling array membranes of tumor and normal tissues from the same patient were hybridized with 32P-labeled probes to SAHH to determine mRNA levels. The relative amount of repression for each type of tumor in relation to its corresponding normal tissue is shown. An example of an array of the small intestine (right panel) where the signal was further quantified (left panel) is shown. (B) Quantitative RT–PCR of normal (N) and tumor tissue (T) from 40 colon cancer patients is shown. Relative mRNA levels were compared with endogenous controls 18S, POL2A or β-actin.

Fig. 5. SAHH protein in human colon cancer. (A) SAHH protein expression in four patients where mRNA downregulation was previously observed. Downregulation was only observed in 50% of cases. (B) Protein analysis from four patients chosen randomly from the tumor bank of the Pathology Department. SAHH protein stabilization was observed in the tumor tissue. p16\(^{INK4A}\) and p53 proteins are shown. HeLa cells were used as a positive control for SAHH and p16\(^{INK4A}\) expression (C+). HCT116-p53\(^+/+\) cells were used as a positive control for p53 expression (C+). β-Actin is shown as internal control.
We propose here that SAHH might function independently as a tumor suppressor gene in cancer. Importantly, the fact that SAHH is downregulated in a considerable percentage of different kinds of tumors supports this notion. Examination of possible correlations between SAHH protein expression and tumor pathology in a larger series of patients will undoubtedly be very useful in understanding the multistep process of tumorigenesis that leads to neoplastic transformation.

Supplementary material

Supplementary Figures 1 and 2 can be found at http://carcin.oxfordjournals.org/

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Conflict of Interest Statement: None declared.

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