Diminished Expression of S100A2, a Putative Tumor Suppressor, at Early Stage of Human Lung Carcinogenesis

Gang Feng, Xiao-chun Xu, Emile M. Youssef, and Reuben Lotan

Departments of Thoracic/Head & Neck Medical Oncology [G. F., E. M. Y., R. L.] and Clinical Cancer Prevention [X-C. X.], The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030

ABSTRACT

To identify and understand early events in lung carcinogenesis, we used a cDNA array to screen for genes that are expressed differentially in normal human bronchial epithelial (NHBE) cells and a tumorigenic cell line (1170-I) derived from immortalized HBE cells after exposure to cigarette smoke condensate in vitro. Among these genes, we have identified the S100A2 gene, which encodes a nuclear calcium-binding protein, as being down-regulated in the 1170-I cells. Because this gene has been implicated as a tumor suppressor in breast cancer, we examined its role as a potential tumor suppressor in lung carcinogenesis. Levels of S100A2 transcript and protein, which were high in NHBE cells, decreased by up to 50% in immortalized HBE cells (BEAS-2B and 1799) and to low levels in tumorigenic (1170-I) HBE cells. Furthermore, S100A2 mRNA and protein were undetectable in 8 and expressed at a reduced level in 3 of 11 non-small cell lung cancer (NSCLC) cell lines. Positive immunohistochemical staining of S100A2 was detected in the majority (75–83%) of normal and hyperplastic lung tissues, whereas it was detected in ≤10% of metastatic lung tissues, squamous cell carcinoma, and adenocarcinoma. Treatment of 1170-I HBE and NSCLC cells with 5-aza-2'-deoxycytidine resulted in partial restoration of S100A2 expression in seven of eight cell lines. Indeed, CpG methylation was detected in the promoter region of the S100A2 gene. Our results suggest that S100A2 expression is suppressed early during lung carcinogenesis, possibly by hypermethylation of its promoter, and that its loss may be a contributing factor in lung cancer development or a biomarker of early changes in this process.

INTRODUCTION

Lung cancer is the leading cause of cancer death in the United States, accounting for 31% of all cancer deaths in men and 25% in women (1). It has been estimated that there were 164,100 new cases and 156,900 deaths from lung cancers in the United States in 2000 (1). Overall 5-year survival rates for lung cancer patients are poor and remain <15% (1), despite advances in surgery, radiotherapy, and chemotherapy. This is largely because of ineffective detection of lung cancer at its early stages (2). If diagnosed successfully, stage I lung cancer patients have a favorable 5-year survival rate of ~60% after surgical resection. However, because of the detection limits of current standard diagnostic methods, most patients are first diagnosed beyond stage I. Furthermore, sputum cytology and chest X-rays as population-screening tools have failed to show reduced lung cancer mortality after surgical resection (2). Therefore, better tools are urgently needed for the earlier detection of lung cancer. Molecular testing of preneoplastic lesions and histologically normal tissues is drawing increasing attention in this regard. In addition, clinical chemoprevention trials of lung cancer also call for the use of molecular biomarkers as intermediate end points (3).

The approach that we used for the discovery of potential biomarkers was to identify genes that are expressed differentially in normal and malignant lung epithelial cells and then determine how early in the lung carcinogenesis process they are modulated. For this purpose, we used the cDNA microarray technology that has made it possible to probe simultaneously the expression of thousands of genes and to screen for gene expression alterations between normal and cancer cells and tissues (4). We used cDNA array to compare mRNA expression in NHBE (3) cells and tumorigenic 1170-I HBE cells (5). We found that ~30 genes are down-regulated and ~20 genes are up-regulated in the 1170-I cells relative to the normal cells. Among the genes that were suppressed in the tumorigenic cells was the gene that encodes for the calcium-binding protein S100A2, which has been suggested to function as a tumor suppressor gene in breast cancer (6); its role as a potential tumor suppressor in lung carcinogenesis, however, has not been investigated thoroughly.

To investigate further how S100A2 expression is modulated during lung carcinogenesis and to determine whether its loss of expression can serve as a biomarker for early detection of lung cancer, we analyzed S100A2 gene expression at different stages of malignant transformation in an in vitro model of lung carcinogenesis, as well as in NSCLC cell lines and tissue specimens. We also began to explore the mechanism of regulation of S100A2 gene expression.

MATERIALS AND METHODS

Cells and Cell Culture. An in vitro lung carcinogenesis model that included normal immortalized (BEAS-2B and 1799), transformed (1198), and tumorigenic (1170-I) human HBE cells was used in this study. NHBE cells were purchased from Clonetics (San Diego, CA) and used at the second passage. BEAS-2B is a cell line derived by immortalization of NHBE cells by use of a hybrid adenovirus/SV40 (7). To derive cells that were more progressed than the immortalized cells, Klein-Szanto et al. (5) used a xenotransplantation system in which the immortalized BEAS-2B cells were grown in deepithelialized rat tracheas that were transplanted s.c. into athymic nude mice. The cells that had been exposed to beeswax alone as control or to slow-release beeswax pellets containing CSC. After 6 months, immortalized cells were derived from BEAS-2B cells exposed in vivo to beeswax (cell line 1799). Transformed nonmalignant cells (cell line 1198) were derived from xenografts exposed in vivo to CSC, and transformed and tumorigenic cells (1170-I) were derived from tumors that arose in vivo from CSC-exposed BEAS-2B cells (5). The CSC induced in vivo phenotypic changes in BEAS-2B cells similar to the progressive changes that occur during human lung carcinogenesis (5). The BEAS-2B, 1799, 1198, and 1170-I cell lines were obtained from Dr. Klein-Szanto (Fox Chase Cancer Center, Philadelphia, PA; Ref. 5). All of the HBE cells were grown in Keratinocyte Serum-Free Medium (Life Technologies, Inc., Gaithersburg, MD) containing epidermal growth factor and bovine pituitary extract.

Human NSCLC cell lines H460, H1792, SK-MES-1, Calu, H292, H157, H1944, H596, H522, A549, and H226 were obtained from the American Type...
Culture Collection (Rockville, MD) or from Dr. Adi Gazdar (University of Texas Southwest Medical Center, Dallas, TX). The cells were grown in monolayer culture in a 1:1 (v/v) mixture of DMEM and Ham’s F12 medium containing 5% fetal bovine serum at 37°C in a humidified atmosphere of 95% air and 5% CO₂. In some experiments, the cells were treated with aza-dCyd (Sigma Chemical Co., St. Louis, MO).

cDNA Array Analysis of Gene Expression in NHBE and 1170-I Cells. Human GeneFilters Microarray GF211 was purchased from Research Genetics (Huntsville, AL). The filters are 5 × 7 cm charged nylon membranes on which are spotted 0.5 ng of insert DNA obtained by PCR amplification of I.M.A.G.E./LLNL cDNA clones (average size, 1 kb, including 3’ untranslated region) from ~4000 functionally known genes and total human genomic DNA and a series of housekeeping genes, which serve as references points for the analysis of images of phosphorimagher using Research Genetics software called Pathway.

cDNA microarray analysis of gene expression was performed using a protocol described by Research Genetics with some modifications. Briefly, total cellular RNA was extracted from exponentially growing NHBE and 1170-I cells by the TriReagent method (Molecular Research Center Inc., Cincinnati, OH). Total RNA (2.5 μg) was used as the template to synthesize cDNA probe for microarray hybridization in a reverse transcription reaction in which poly(dT) was used as primer and [α-3P]dCTP was used as label. After the reverse transcription, cDNA probe was purified by passage through a Bio-Spin 6 Chromatography Column (Bio-Rad, Hercules, CA). Purified probe was then denatured by boiling and placed on ice until use.

The array membrane was prehybridized for at least 2 h at 42°C in MicroHyb hybridization solution containing Cot-1 DNA and poly(dA). Denatured cDNA probe prepared from NHBE total RNA was added, and hybridization was performed overnight at 42°C. The following day, the array was washed twice at 50°C in 2× SSC-1% SDS [1× SSC = 8.765 g of sodium chloride and 4.41 g of sodium citrate in 1000 ml of H₂O (pH 7.0)] for 20 min each and once at room temperature at 5× SSC-1% SDS for 15 min. Radioisotopic signals from the array were collected using a phosphoroscope and scanned using a Storm 840 phosphorimagher (Molecular Dynamics, Sunnyvalle, CA).

The array was then striped in boiling 0.5% SDS and hybridized with cDNA probe prepared from the total RNA isolated from the tumorigenic cell line 1170-I, following the same procedure as described above. The scanned filters containing the microarrays from NHBE and 1170-I were then compared and contrasted using Pathway 2.0 software from Research Genetics. Positive clones were identified as those with intensity ratio >2.

Northern Blot Analysis. Total cellular RNA was purified using the TriReagent method. Total RNA (20 μg) was electrophoresed in a 0.66 x formaldehyde-1.2% agarose gel and then transferred to a Duralon-UV membrane (Stratagene, La Jolla, CA) and UV cross-linked. The cDNA probe for S100A2 was a ~350-bp PCR-amplified fragment using IMAGE clone #1810813 (Research Genetics) as a template and T3, T7 primer (GENOSYS, Woodland, TX) as primers. The cDNA probe for GAPDH was a 340-bp EcoRI/XhoI-cut fragment of cDNA isolated from chicken muscle. The probes were labeled with [32P]dCTP (ICN, Costa Mesa, CA) to a specific activity of ~2 × 10⁶ cpm/μg (~100 cpm/μl) by a Prime-it II Random Primer labeling Kit (Stratagene). After hybridization overnight at 67°C in Rapid-Hyb Buffer (Amer sham, Arlington Heights, IL), the blots were washed once with 1× SSC-0.1% SDS at room temperature for 20 min. The blots were then washed twice in 0.2× SSC-0.1% SDS at 67°C for 20 min each and placed against an X-ray film (Hyperfilm-MP; Amersham) at ~70°C for autoradiography. S100A2 transcript was identified as an ~0.6-kb band.

Western Blot Analysis. Cells were washed twice in PBS and resuspended in an appropriate volume of PBS. A portion of the cell suspension was diluted 20-fold in 0.5% (w/v) SDS and boiled for 10 min. The total protein concentration was determined by the Bio-Rad D₀₂ protein assay kit with BSA in 0.5% SDS as the standard. The remaining cell suspension was lysed by adding an equal volume of 2× SDS-containing sample buffer and boiling for 10 min. The resulting extract was used for Western blotting.

Denatured total proteins (100 μg/lane) were fractionated by electrophoresis on 12% polyacrylamide gels in the presence of SDS and electrotransferred to PROTRAN nitrocellulose membrane (Schleicher & Schuell, Keene, NH) with a pore size of 0.1 μm. The blots were then probed with mouse anti-S100L antibody (Transduction Laboratories, Lexington, KY) or anti-β-actin antibody (Sigma). Antibody binding was detected by the enhanced chemiluminescence system (Amersham). S100A2 protein was identified as an ~10-kDa band relative to the migration of commercial molecular mass markers.

Immunohistochemistry. The immunohistochemical localization of S100A2 protein was performed by a modified avidin-biotin complex technique. Tissue sections were deparaffinized in xylene and rehydrated in a series of diluted ethanol solutions (100–50%). The endogenous peroxidase activity was blocked for 30 min by incubation in 1% methanolic hydrogen peroxide solution. This was followed by incubation with 20% normal horse serum to minimize nonspecific binding of the secondary antibody. The sections were incubated at 23°C for 4 h with the primary anti-S100A2 antibody (Transduction Laboratories) diluted 1:50. After the sections were washed three times in PBS, they were incubated for 30 min at 23°C with biotinylated horse anti-mouse IgG(H+L) (Vector Laboratories, Burlingame, CA) and then incubated with the avidin-biotin-peroxidase complex (ABC kit; Vector) for 30 min in the dark. A subsequent incubation with 3-amin-9-ethylcarbazole (Sigma) solution for 20 min served to visualize the peroxidase complex. The sections were then mounted with Aqua mounting medium (Fisher, Houston, TX) and covered with glass coverslips. Control sections were incubated with normal mouse IgG instead of primary antibodies or with second antibody only. These controls were negative. The sections were reviewed by a pathologist (X-C. X.), using an Olympus microscope, and assigned only to positive or negative staining categories where positive was any specimen with >10% positive cells.

Analysis of CpG Methylation. The methylation status of the S100A2 gene in NSCLC cell lines was determined by PCR after bisulfite treatment of DNA followed by enzyme restriction analysis as described in detail at our Institute’s web site. In brief, genomic DNA was extracted and purified from the lung cancer cell lines by a DNA extraction kit (Stratagene), according to the manufacturer’s instructions, and 2 μg of genomic DNA were treated with sodium bisulfite for 16 h. After repurification of the DNA, a 2-μl aliquot was used as a template for PCR. To design the two oligonucleotide primers for PCR, an appropriate area of the S100A2 promoter rich in CpG islands was identified using the published human S100A2 promoter sequence (GenBank accession no. Y07755). The bisulfite-modified sense oligonucleotide used for the detection of the 198-bp bisulfite-modified nucleotide fragment originating from the human S100A2 promoter was 5’-GTTTTTATATGTAGTGGTGAGTGG-3’, which corresponds to position ~1267 to ~1244 (designating the ATG of the translation start site as site 0). The antisense oligonucleotide was 5’-AAAAACCCCTAAAATAAAATACC-3’, which corresponds to position ~1093 to ~1071. For the PCR, each primer was used at a 1 μM concentration in a 50-μl reaction volume with 1 unit of Taq polymerase (Perkin-Elmer). The first cycle of the PCR was as follows: incubation at 94°C for 3 min for denaturation, followed by incubation at 55°C for another 3 min as an annealing step. This was followed by incubation at 72°C for 1 min as an extension step. All subsequent cycles included incubations at 94°C for 40 s, 55°C for 40 s, and 72°C for 50 s. The bisulfite PCR was followed by restriction digestion as described previously (8, 9). In brief, 20 μL of the amplified products were digested with the restriction enzyme BstUI (MBI Fermentas, Hanover, MD), which recognizes and cuts the site CGCG. This would produce a band of ~106 bp if the 198-bp S100A2 sequence is methylated. The PCR product was electrophoresed on 3% agarose or 5% acrylamide gels; the bands were visualized using ethidium bromide staining and photographed using UV illumination.

RESULTS

Differential RNA Expression in NHBE and 1170-I Cells Detected by cDNA Microarray Analysis. Total RNA isolated from NHBE and 1170-I cells was used to prepare 32P-labeled cDNAs by reverse transcription. These probes were then hybridized consecutively, first NHBE then 1170-I (with a stripping step in between), to the same cDNA array membrane. The same regions of two scanned phosphorimages of the microarray are shown in Fig. 1, A and B. The scanned images demonstrate several differences, including decreased expression of transcripts that were identified as S100A2 and keratin K5 (indicated by open circle and a triangle, respectively in Fig. 1).
Because S100A2 has been reported to be a putative tumor suppressor in breast cancer (6) but its expression patterns in lung carcinogenesis have not been investigated thoroughly, we decided to focus subsequent studies on the expression of this gene in lung carcinogenesis.

**S100A2 Is Expressed in NHBE Cells, but Its Levels Decrease in Transformed and Tumorigenic HBE Cells.** To investigate changes in the level of S100A2 expression during lung carcinogenesis, we analyzed S100A2 mRNA and protein levels using Northern (Fig. 1, C and E) and Western (Fig. 1, D and F) blotting, respectively on NHBE (normal), BEAS-2B (immortalized), 1799 (immortalized), 1198 (transformed), and 1170-I (tumorigenic) cells. The results revealed that S100A2 transcripts and proteins were high in NHBE, but decreased in two stages during carcinogenesis: they first decreased 25–50% in BEAS-2B and 1799 cells, and then were nearly fully suppressed in 1198 and 1170-I cells. The results suggested that a decrease in the amount of S100A2 may play a role in early stages (immortalization) of carcinogenesis and that the nearly complete loss of expression of this gene may play a role in later stages (transformation, progression to the tumorigenic phenotype).

**Suppression of S100A2 Expression in NSCLC Cells.** The carcinogenesis model described above was based on BEAS-2B cells that were immortalized with SV40 large T antigen, which causes inactivation of p53. To test whether S100A2 expression is also affected in lung cancer cell lines isolated from human lung cancers, 11 established NSCLC cell lines were assayed for the levels of S100A2 transcript and protein. Only 3 (SK-MES-1, H292, and H596) of the 11 cell lines showed detectable levels of S100A2 transcripts (Fig. 2A) and proteins (Fig. 2B). Although the SK-MES-1 cells expressed the highest level of S100A2 among the NSCLC cell lines, the levels of S100A2 in these cells were still less than one-third of those in NHBE cells (Fig. 2), indicating that S100A2 expression was suppressed in all of the NSCLC cell lines.

**Decreased Level of S100A2 in Metaplastic and Malignant Lung Tissues.** Because expression patterns of certain genes may be altered by *in vitro* culture conditions, we tested whether the lack of S100A2 expression also occurs in lung tumor tissues. A set of tissue samples, including squamous cell carcinoma and adenocarcinoma together with adjacent normal hyperplastic and metaplastic tissues, were assayed for the level of S100A2 protein by immunohistochemistry. A summary of the results is presented in Table 1. S100A2 stained positive in 83% of normal lung tissue and 75% of hyperplastic tissues. However, it stained positive in only 9% each of metaplastic tissue and squamous

---

**Table 1** Expression of S100A2 protein in bronchial and lung tissues

<table>
<thead>
<tr>
<th>Histology</th>
<th>S100A2, % (positive/total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>83.3 (5/6)</td>
</tr>
<tr>
<td>Hyperplasia</td>
<td>75 (3/4)</td>
</tr>
<tr>
<td>Metaplasia</td>
<td>9.1 (1/11)</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>9.1 (1/11)</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>0 (0/10)</td>
</tr>
</tbody>
</table>

---
cell carcinoma and was not detected in the nucleus in any of the adenocarcinoma cells. The results supported the previous conclusion obtained with cell lines that the suppression of S100A2 expression occurs in two steps, a 25–50% decrease at early stages of lung carcinogenesis and a complete loss of expression at later stages.

Partial Restoration of S100A2 Expression by aza-dCyd Treatment in Tumorigenic HBE and NSCLC Cells. In breast cancer cells, S100A2 expression has been reported to be up-regulated by treatment with the demethylating agent aza-dCyd (6). To determine whether the same holds true for immortalized, transformed, and tumorigenic HBE cells and NSCLC cells, we treated these cells with aza-dCyd and found that the S100A2 transcript was increased in the tumorigenic cell line 1170-I after aza-dCyd treatment in a dose-dependent fashion (Fig. 3A). In contrast, the level of S100A2 transcript dropped ~4-fold after aza-dCyd treatment in both BEAS-2B and 1799 cells, whereas it dropped slightly (~30%) in NHBE cells and did not change in 1198 cells [Fig. 3B, long exposure (L.E.)]. Likewise, the amount of S100A2 protein (Fig. 3C) also dropped in NHBE, BEAS-2B, and 1799 cells after aza-dCyd treatment. The levels of S100A2 protein were too low to be detected by Western blotting in the 1198 and 1170-I cells, with or without the treatment.

Among the 11 NSCLC cell lines tested, 7 (H460, H1792, Calu, H157, H1944, A549, and H226) showed increased amounts of S100A2 transcript after aza-dCyd treatment (Fig. 4A). However, S100A2 protein was not detectable in the seven cell lines even under inducing conditions (Fig. 4B), possibly because its levels were below the sensitivity of Western blotting of total cell extract.

To determine whether the silencing of S100A2 expression in NSCLC cells was the result of methylation of CpG islands in its promoter, as reported for breast carcinoma cells (10), we identified a 5' upstream sequence in the S100A2 gene that contained several CpGs (Fig. 4C). The methylation status of these CpGs was then determined by bisulfite treatment followed by restriction enzyme cleavage and PCR. Fig. 4D shows that eight of the nine NSCLC tested had meth-
ylated CpGs. Cell line SK-MES-1 had no methylated CpGs, and cell line H596 had a faint methylated band. Interestingly, these two cell lines had a high constitutive expression of the S100A2 gene. These results support the conclusion that the silencing of the S100A2 gene is caused at least in part by CpG methylation.

**DISCUSSION**

To effectively increase the rate of overall 5-year survival in lung cancer patients, continued efforts must be made to search for better detection tools for earlier stages of lung cancer (2, 3), and clearly, the key to the development of early detection tools resides at the identification and understanding of the early events in lung tumorigenesis. We have searched for potential biomarkers for early detection of NSCLC, using a cDNA array for identification of genes that are expressed differentially between normal and tumorigenic HBE cells. The present study describes the identification of S100A2 as a gene whose expression is diminished during lung carcinogenesis. We were interested in exploring the expression of this gene in lung carcinogenesis because it was originally isolated in a screen for tumor suppressor genes in breast cancer (6).

The S100A2 gene, also known as S100L and CaN19, encodes a 99-amino acid protein that is a member of the calcium-binding proteins of the EF-hand family (11). The gene is located on chromosome 1q21 (6), in a region that is frequently rearranged in a number of tumors, including NSCLC (12). Unlike >12 other S100 proteins, which are cytoplasmic proteins, S100A2 is located primarily in the cell nucleus (13). However, the mechanism of S100A2 action is not clear. In normal cells, S100A2 expression is regulated during the cell cycle; its levels increase as cells enter S phase and it is induced by growth factors in early G1 phase (6).

The role of S100A2, if any, in the cell cycle is not known. S100A2 can be regulated by the tumor suppressor p53, and because p53 is known to mediate apoptosis induced by DNA damaging agents, the loss of S100A2 may contribute to increased resistance to apoptosis (14). It has been speculated that S100A2 may transduce Ca$^{2+}$ signaling via interaction with different types of target proteins and may be involved in keratinocyte differentiation (15, 16). Irrespective of its exact mechanism of action, expression profiles of S100A2 gene indicate that it is a candidate class II tumor suppressor gene (6). Among the tissues and cell lines previously examined (breast, cervical, skin, oral), S100A2 expression is high in normal cells and low in cancer cells (6, 10, 13, 15, 17–19). Moreover, S100A2 transcription repression in breast cancer cells (6, 10) and, in our study, in NSCLC cell lines (Fig. 4D) is mediated at least in part by site-specific methylation in the promoter region, similar to a number of known and putative tumor suppressor genes.

We found that S100A2 expression decreased in HBE cells, which represent immortalized cells, and was completely suppressed in cells representing transformed and tumorigenic cells in a system of isogenic cells derived from the BEAS-2B cell line by exposure to CSC (5). Furthermore, we have shown that S100A2 was suppressed in a variety of NSCLC cell lines derived from human squamous cell carcinomas and adenocarcinomas and that S100A2 expression is suppressed in vivo in metaplastic lesions and in NSCLC cancers. The mechanism underpinning the suppression of S100A2 expression was shown to involve, at least in part, hypermethylation of the gene in tumorigenic HBE cells and in most of the NSCLC cell lines.

This study extends previous reports of similar events in breast cancer and melanoma development. For example, S100A2 mRNA was detected in all normal epithelium samples and benign breast lesions examined but in only 37% of carcinoma in situ and <15% of carcinoma specimens (20). In addition, dysplastic nevi showed moderate to high expression levels of S100A2 mRNA, whereas the expression level was low in primary melanomas and undetectable in melanoma metastases (18). Furthermore, Lauriola et al. (16) reported that S100A2 protein staining by immunohistochemical methods decreased in high-grade laryngeal squamous cell carcinoma and found a correlation between S100A2 tumor positivity and longer relapse-free and overall survival. These authors (16) also found that S100A2 expression was associated with cell commitment to squamous differentiation in laryngeal lesions. In our study with NSCLC cell lines and tissues, however, loss of S100A2 expression was not limited to squamous cell carcinomas; it was also decreased in adenocarcinomas. Therefore, the decrease in S100A2 in NSCLC may reflect suppression of a putative tumor suppressor rather than merely a loss of the squamous differentiation commitment. Although our study represents the first thorough examination of the expression of S100A2 mRNA and protein in a defined human lung carcinogenesis cell system and a collection of NSCLC cell lines, it is noteworthy that previous reports showed that one lung NSCLC cell line (Calu-I) had diminished expression of S100A2 mRNA compared with normal skin keratinocytes (15) and that another report demonstrated that S100A2 protein could be detected immunohistochemically in normal but not in cancerous lung tissue (17).

There have been exceptions to the inverse correlation between S100A2 expression and malignancy. One study found that the expression of S100A2 decreased in malignant head and neck epithelial cells and tissues compared with normal keratinocytes (15), whereas a more recent study found this gene to be overexpressed in malignant head and neck carcinomas compared with normal oral mucosa (21). The reason for this discrepancy is not clear, but it may reflect differences in the type of cells and tissues used to represent the normal tissue and differences in the analysis methods.

S100A2 seems to be an ideal candidate for the development of early lung cancer detection because it is abundantly present in normal HBE cells and lung tissues, making it easily detectable, and because its expression dropped to a distinctively different level in transformed HBE cells, tumorigenic HBE cells, and NSCLC cells, as well as in metaplastic lung tissue and carcinomas, making it a marker of malignancy progression. Further studies with more specimens from patients with proper follow-up are planned to determine whether the loss of S100A2 is related to survival of NSCLC patients.

The reduced level of S100A2 in immortalized, transformed, and tumorigenic HBE cells and NSCLC cells cannot be attributed entirely to the hypermethylation of S100A2 gene, because aza-dCyd treatment only partially restored S100A2 expression. It has been reported that there is a p53-binding site in the promoter region of the S100A2 gene and that the S100A2 gene is transcriptionally activated by p53 in human osteogenic sarcoma cell lines (22). However, loss of p53 function does not seem likely to play a major role in the down-regulation of S100A2 because the immortalized HBE cell lines BEAS-2B and 1799, both of which have their p53 inactivated by SV40 large T antigen (7), still retain relatively high levels of S100A2 expression. In addition, no apparent correlation exists between p53 status and S100A2 expression in NSCLC cells. aza-dCyd treatment restored S100A2 expression in NSCLC cells with a wild-type p53 (23) or with a mutant p53. Therefore, in addition to hypermethylation and p53 activation, some other mechanisms mediate S100A2 gene expression. Both epidermal growth factor (24) and phorbol ester can induce S100A2 expression. The latter can act via an enhancer element in the S100A2 promoter region that harbors two activator protein 1-like binding sites (10).

It is puzzling that treatment of aza-dCyd actually decreased S100A2 expression in normal and immortalized HBE cells. One would think that the treatment should at least maintain the S100A2
level, if not increase it further. One possible explanation is that a negative regulator of S100A2 is repressed by hypermethylation in these cells and that treatment with a demethylating agent increased the expression of the negative regulator.

Not only can S100A2 be developed into a biomarker of lung cancer development, it also has the potential to be a drug target. The fact that S100A2 expression could be partially restored by aza-dCyd treatment in the tumorigenic HBE cell line 1170-I and the majority of NSCLC cells lines tested suggest that the gene has not been lost during lung tumorigenesis. Rather, it is repressed by hypermethylation and other regulatory events. This result opens the door for chemopreventive and chemotherapeutic interventions. In the future, after validation of the role of S100A2 in lung tumorigenesis, drugs that can up-regulate S100A2 expression should be sought.

ACKNOWLEDGMENTS

We thank Hong Wu for technical help in the immunohistochemistry experiments and Dafna Lotan for culturing the NHBE cells.

REFERENCES

Diminished Expression of S100A2, a Putative Tumor Suppressor, at Early Stage of Human Lung Carcinogenesis

Gang Feng, Xiao-chun Xu, Emile M. Youssef, et al.

Cancer Res 2001;61:7999-8004.

Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/61/21/7999

Cited articles
This article cites 23 articles, 8 of which you can access for free at:
http://cancerres.aacrjournals.org/content/61/21/7999.full.html#ref-list-1

Citing articles
This article has been cited by 14 HighWire-hosted articles. Access the articles at:
/content/61/21/7999.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.