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AKAP12α is Associated with Promoter Methylation in Lung Cancer

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Purpose: **Promoter methylation is an important mechanism for silencing tumor-suppressor genes in cancer and it is a promising tool for the development of molecular biomarkers. The purpose of the present study was to investigate whether inactivation of the A Kinase Anchoring Protein 12 (AKAP12) gene is associated with promoter methylation in lung cancer.**

Materials and Methods: **The AKAP12 expression was examined by reverse transcription-polymerase chain reaction (RT-PCR) in ten lung cancer cell lines. The methylation status of the AKAP12α promoter was analyzed by performing bisulfite sequencing analysis in ten lung cancer cell lines, twenty four lung tissues and matched normal tissues.**

Results: **The AKAP12α expression was reduced in 6 of 10 (60%) lung cancer cell lines, whereas the AKAP12β expression was absent in 1 of 10 (10%) lung cancer cell lines. The AKAP12α expression was restored after treatment with the demethylating agent 5-aza-2'-deoxycytidine in three lung cancer cell lines. Methylation of CpG island 1 in the AKAP12α promoter was detected in 30% of the lung cancer cell lines, whereas methylation of CpG island 2 in the AKAP12α promoter was observed in the immortalized bronchial cell line and in all the lung cancer cell lines. In lung tumors, the CpG island 1 in the AKAP12α promoter was infrequently methylated. However, CpG island 2 in the AKAP12α promoter was highly methylated in lung tumors compared with the surrounding normal tissues, and this was statistically significant (p=0.0001).**

Conclusion: **Our results suggest that inactivation of the AKAP12α expression is associated with DNA methylation of the promoter region in lung cancer, and that AKAP12α may play an important role in lung cancer carcinogenesis.** ***(Cancer Res Treat. 2006;38:144-151)***

Key Words: **Promoter methylation, Lung neoplasms, AKAP12α**

INTRODUCTION

Lung cancer is a major cause of cancer death all over the world (1,2). For the early diagnosis of lung cancer to be histologically or cytologically examined and confirmed, small samples of lesion tissue must be obtained by invasive procedures. However, samples of tumor tissues are rather difficult to obtain. Other approaches for developing DNA-based assays have recently emerged in order to aid the early detection of cancer. These approaches include the detection of aberrantly hypermethylated gene-promoter regions in cancer (3,4).

Methylation of cytosine is a known endogeneous modification of DNA and it occurs by the enzymatic addition of a methyl group to the carbon-5 position of cytosine within 5'-CpG-3' dinucleotides (5). Hypermethylation of CpG islands in the promoter regions of growth controlling genes (cell cycle regulators, DNA repair enzymes and other potential tumor suppressor genes), can significantly contribute to cancer development (6,7). DNA methylation status analysis for these genes could become a powerful tool for accurate and early lung cancer diagnosis, prevention and treatment, and for making the prognosis (8).

A Kinase Anchoring Protein 12 (AKAP12; also known as src-suppressed C kinase substrate (SSeCKS) and Gravin) is a multivalent anchoring protein and it was initially recognized from the serum of myasthenia gravis patients (9,10). The AKAP12 gene encodes three transcriptionally different AKAP12 isoforms (AKAP12α, -β and -γ) that are regulated by unique promoters and they are differentially expressed in various organs (11). The AKAP12α expression is regulated by serum

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response factor (SRF) and it's inactivated by hypermethylation of the promoter CpG islands in human cancer (12,13). The AKAP12 expression is also strongly suppressed in lung cancer (14,15). The AKAP12α gene is located in chromosome 6q24-q25, which is known as a major susceptibility locus that influences the risk of lung cancer (16).

Thus, we examined whether the AKAP12α expression is associated with promoter methylation of the AKAP12α gene. To study the relation between the promoter methylation of the AKAP12α gene and lung cancer, the methylation status of the AKAP12α gene was examined in different lung cancer cell lines, and this was compared with the methylation status of two CpG islands of the AKAP12α gene in lung tumor tissues and the paired nonmalignant tissues with performing bisulfite sequencing analysis.

MATERIALS AND METHODS

1) Cells and tissues

Ten NSCLC cell lines (NCI-H460, NCI-H1666, NCI-H1703, NCI-H2009, NCI-H358, NCI-H1573, NCI-H1299, A549, Calu-1 and Calu-3) and the human bronchial epithelial cell, BEAS2B were purchased from the American Type Culture Collection (Manassas, VA). The human gastric cell line SNU-638 was obtained from the Korean Cell Line Bank (Seoul, Korea). The cell cultures were grown in RPMI-1640, F12, DMEM/F12 and Keratinocyte serum free media (Gibco/BRL, Grand Island, NY). The ACL4 medium was supplemented with 10% fetal bovine serum, and incubated in 5% CO_2 at 37°C according to the manufacturer's instructions.

Twenty four paired lung tumor tissues and the matched nonmalignant lung tissues were obtained from the Korea Lung Tissue Bank (Seoul, Korea). The tissue samples were acquired after receiving approval from the Institutional Review Board, and we obtained written consents from all the patients.

2) Reverse transcription-polymerase chain reaction (RT-PCR) assay

The total RNA was extracted from the samples with using TRIzol reagent (Life Technologies, Inc., Gaithersburg, MD) according to the manufacturer's instructions. cDNA was synthesized from 1ug of total RNA using Moloney Murine Leukemia Virus reverse transcriptase (Life Technolgies, Gaithersburg, MD) and Oligo-d(T)15primer (Roche, Indianapolis, IN).

PCR amplification was carried out under the following conditions: AKAP12α (5 min at 94°C, followed by 30 cycles of 1 min at 94°C, 30 s at 61.5°C and 40 s at 72°C and this was followed by a final elongation step for 10 min at 72°C), AKAP12β (5 min at 94°C, followed by 30 cycles of 1 min at 94°C, 30 s at 56°C and 40 s at 72°C, and this was followed by a final elongation step for 5 min at 72°C) and β-actin (5 min at 94°C, followed by 30 cycles of 1 min at 94°C, 30 s at 53°C and 30 s at 72°C, and this followed by a final elongation for 5 min at 72°C). The housekeeping gene, β-actin, was used as an internal control to confirm the success of the PCR reaction. The sequences of the primers for the PCR reactions of AKAP12 and β-actin are listed in Table 1. These primer sequences were identical to the human target genes, as was confirmed by a BLAST search. The PCR products were analyzed on 2% agarose gels. The associated product sizes for AKAP12α, AKAP12β and β-actin were 165, 564 and 468 bp, respectively.

3) Treatment of cells with 5-Aza-2'-deoxycytidine

The cells (SNU-638, NCI-H460, NCI-H358, Calu-1 and NCI-H1299) were seeded at a density of 5×10^5/60 mm plate and then they were treated with 10μM 5-Aza-2'-deoxycytidine after 24 hours. The cells were then incubated for 48 and 72 hours. The total RNA was isolated from the treated cells with using Trizol reagent.

4) Analysis of the CpG islands in the AKAP12α promoter

Two CpG islands, named CpG island 1 and CpG island 2, were identified in the AKAP12α promoter (GenBank: NC_000006.10), by a CpG Island Searcher program. Detailed information about this program can be found at http://www.cpgislands.com.

5) Nucleic acid extraction

Genomic DNA from all the lung cancer cell lines was extracted by using a QIAmp DNA Blood Mini Kit (QIAGEN Inc., Valencia, CA), and the genomic DNA was extracted by a PUREGENE Kit (Gentra, Minneapolis, MN) from all the lung tissue samples. The concentrations of DNA were determined with a spectrophotometer and these were assessed by gel electrophoresis.

Table 1. Summary of the primer sequences for RT-PCR and the bisulfite sequencing analysis

		Forward primer (5'→3')	Reverse pimer (5'→3')	Product size (bp)
RT-PCR*	AKAP12α	GTCTCCTTCATTCGCAGGCT	CATGGCTCCTCCGCACTTCTC	165
	AKAP12β	AGGGCACCTCCGGTTCTC	GGTTCGCTTTCTTTGGATGC	564
Bisulfite	CpG island 1	TGTTTTTTGAGGTTTTGGGT	AACCACCTCTTAACCTCC	228
sequencing	CpG island 2	GAGGTGGTTTGGATGGGTAATTTTTAG	ACCTCCTCCCCATTAACAAAAAAAA	304
β-actin	-	GGAGTTGAAGGTAGTTTCGT	ACCCAGATCATGTTTGAGAC	468

*reverse transcription-polymerase chain reaction.

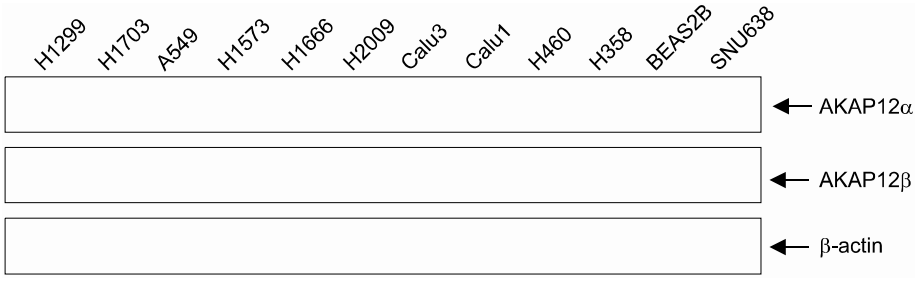

Fig. 1. Expression profiles of the AKAP12 gene in lung cancer cell lines.

6) Bisulfite DNA sequencing analysis

Geonomic DNA was first denatured to create single-stranded DNA and this was then modified with sodium bisulfite and followed by PCR amplification with using the primers listed in Table 1. The primers are specific for the modified DNA, but they do not contain any CpG sites in their sequence. Most of the primers were used as described previously (13) and others were designed using a MethPrimer website (http://www.urogene.org/methprimer). For bisulfite sequencing analysis, two PCR amplifications were carried out in the AKAP12α promoter. The first PCR amplified a 228 bp region that included 17 CpG sites and the PCR reaction was done as follows: 94°C for 5 min; then 45 cycles at 94°C for 30 s, 59°C for 30 s and 72°C for 30 s; and a final extension step for 10 min at 72°C. The second PCR amplified a 304 bp region that included 15 CpG sites and the PCR reactions were done as follows: 94°C for 15 min; then 40 cycles at 94°C for 30 s, 58.9°C for 1 min and 72°C for 1 min; and a final extension step was done for 5 min at 72°C. The amplified PCR products were resolved on 2% agarose gels, purified with using a Gel Extraction Kit (Qiagen, Valencia, CA) and then they were cloned into the pGemT Easy vector (Promega, Madison, WI). Ten individual clones were isolated from each PCR and they underwent DNA sequencing.

7) Statistical analysis

The statistical difference between groups was analyzed by paired sample *t*-tests with using SPSS version 10. All p values are two-sided, and p values less than 0.05 were defined as being statistically significant.

RESULTS

1) The AKAP12 mRNA expression was down-regulated in the lung cancer cell lines

AKAP12 encodes three isoforms: α, β and γ. The AKAP12α and β isoforms are expressed from two different transcripts, whereas the γ isoform is a proteolytic cleavage product of these two major isoforms (11,13). Thus, this work focused on AKAP12α and β, and we examined the expression pattern of AKAP12α and β from 10 lung cancer cell lines. RNA was isolated from each of the cell lines and reverse transcriptase-polymerase chain reaction (RT-PCR) was performed. We confirmed equal amounts of RNA in each lane by the density of β-actin band.

We found that the expression of AKAP12α was reduced in 6 of 10 (60%) lung cancer cell lines, whereas the expression of AKAP12β was absent in 1 of 10 (10%) lung cancer cell lines. As shown in Fig. 1, the AKAP12α expression was down-regulated in the H1573, H2009, Calu-3, Calu-1 and H460 cell lines, or it was absent from the H358 cell line as compared with the H1299, H1703, A549 and H1666 cell lines. BEAS2B and SNU-638 were used as positive and negative controls, respectively.

2) Reactivation of the AKAP12α expression after treatment with 5-Aza-2'-deoxycytidine in lung cancer cells

To determine whether a low AKAP12α expression in lung cancer cell lines could be modulated by DNA demethylation, we treated five cell lines (SNU-638, H358, H460, Calu-1 and H1299) with a DNA methyl-transferase inhibitor, 5-aza-2'-deoxycytidine, in a time-dependent manner for 48 and 72 hours. DNA methyl-transferase inhibitors inhibit de novo methylation and they maintenance methylation, and so they can be used to detect the presence of DNA methylation (17).

We isolated the RNA from the treated and untreated cells with using 5-aza-2'-deoxycytidine, and we then performed RT-PCR. As shown in Fig. 2, the AKAP12α expression was restored in the SNU-638, H358, H460 and Calu-1 cells, but not in the H1299 cells. In the H358, H460 and Calu-1 lung cancer cell lines, which show an absent or low expression level of AKAP12α, an AKAP12α expression was induced in a time-dependent manner after treatment with 5-aza-2'-deoxycytidine and the AKAP12α expression was increased up to 72 hours. Conversely, treatment with 5-aza-2'-deoxycytidine had no effect on the AKAP12α expression in the H1299 cell line, which has a high expression level of AKAP12α. The SNU-638 cell line was used as a positive-control in this experiment (13).

Accordingly, these results demonstrated that DNA methylation of the AKAP12α gene is related with its downregulation in lung cancer cells and it can be reversed by 5-aza-2'-deoxycytidine.

3) Identification of the AKAP12α promoter encompassed in two CpG islands

The promoter region of the AKAP12α gene was inspected in order to define whether the promoter region is associated with DNA methylation. Using the CpG Island Searcher Website (http://www.cpgislands.com), multiple potential CpG sites that could be methylated by DNA methyl-transferases (DNMTs) (18), were identified in the AKAP12α promoter. We also examined the transcription binding sites in the AKAP12α promoter with using the TRANSFAC database (http://www.

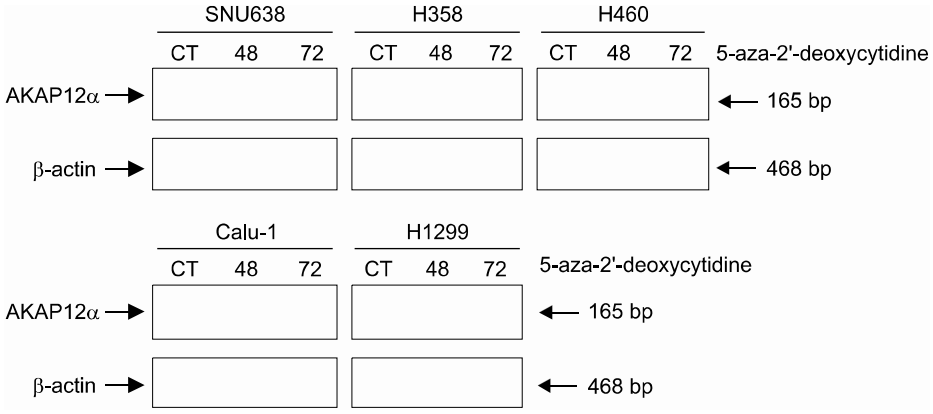

Fig. 2. The restoration of the AKAP12α expression by 5-aza-2'-deoxycytidin.

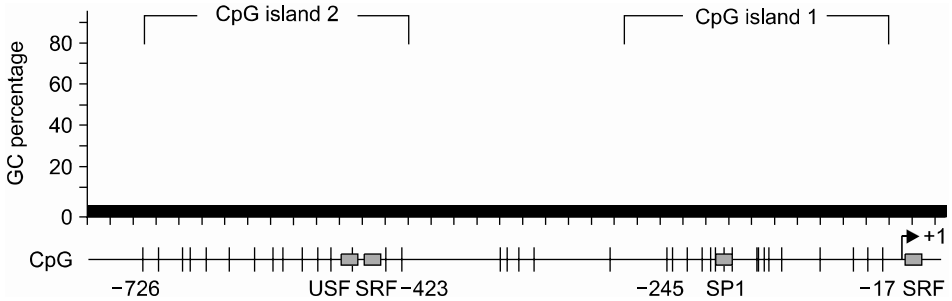

Fig. 3. A map of the CpG islands of the AKAP12α gene spanning 1 kb upstream of the transcription start site. The 5' region of the AKAP12 α gene contains two large CpG islands 1 and 2 (gray area), which encompass a putative promoter region.

gene-regulation.com). The immediate 5' region of the AKAP12α pomoter lacks a true TATA-box, but it contains multiple GC boxes that are recognition sites for stimulating the protein family of transcription factors. This could partially explain the strong activity of this promoter (11). One kb of the genomic sequence of the AKAP12α promoter that encompassed the transcription start site was examined, and two CpG islands having multiple CpG sites were identified: CpG island 1 (-245 to -17) and CpG island 2 (-726 to -423) (Fig. 3). CpG island 1 has already been described in a previous report (13). We found that these CpG islands 1 and 2 contained major transcription factors like specificity protein-1 (SP1), upstream stimulatory factor (USF) and serum response factor (SRF) binding sites, which could regulate target genes (Fig. 3).

4) Methylation status of CpG islands 1 and 2 in the AKAP12α promoter in the lung cancer cell lines

Next, to determine the methylation status of the two CpG islands of the AKAP12α promoter, bisulfite-sequencing analysis was carried out in the lung cancer cell lines. It was found that the CpG island 1, which contains 17 CpG sites in the AKAP12α promoter, was methylated in 3 of 10 (30%) lung cancer cell lines, whereas 7 of 10 (70%) lung cancer cell lines were unmethylated. Among the lung cancer cell lines that have an absent or low expression level of AKAP12α, the H358 and Calu-1 cells were methylated in the CpG island 1 of the AKAP12α promoter, but Calu-3, H1573, H2009 and H460 cells were not. Two AKAP12α expressing cell lines (BEAS2B and H1299) were unmethylated in the CpG island 1 of the AKAP12α promoter. Also, although the H1666 cells have a high expression level of AKAP12α, its CpG island 1 of the AKAP12α promoter was methylated (Fig. 4A).

These results suggest that methylation of the CpG island 1 of the AKAP12α promoter correlates with the transcriptional inactivation of the AKAP12α gene in lung cancer cell lines. However, based on the H460 and H1666 data, it appears that the AKAP12α expression might be influenced by other CpG islands as well as the CpG island 1.

As was mentioned above, an analysis of the AKAP12α promoter showed that two CpG islands were present. Subsequently, we studied whether methylation of CpG island 2 of the AKAP12α promoter was associated with the lung cancer cell lines. Bisulfite-sequencing analysis was performed in a 304 bp fragment of the AKAP12α promoter that contained 15 CpG sites.

It was found that most of the CpG sites in CpG island 2 of the AKAP12α promoter were highly methylated in the immortalized cell line (BEAS2B) and the lung cancer cell lines, even though they have high AKAP12α expression levels. Also, the H358, Calu-1, Calu-3, H1573, H2009 and H460 cells, which have an absent or low expression of AKAP12α, were methylated at most CpG sites of CpG island 2 (Fig. 4B).

This implied that methylation of CpG island 2 may be a common finding of immortalized cell lines regardless of the AKAP12α expression.

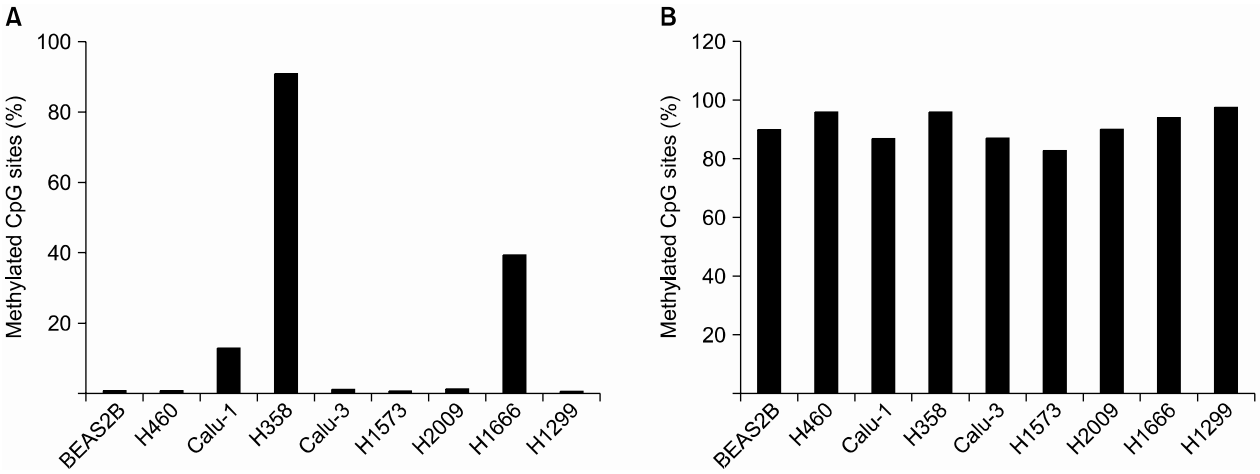

Fig. 4. Methylation status of the AKAP12α promoter in lung cancer cell lines. (A) The frequency of CpG island 1 methylation for each sample is indicated by the methylatied CpG site/total CpG sites (%). (B) The frequency of CpG island 2 methylation for each sample is indicated bymethylated CpG site/total CpG sites (%).

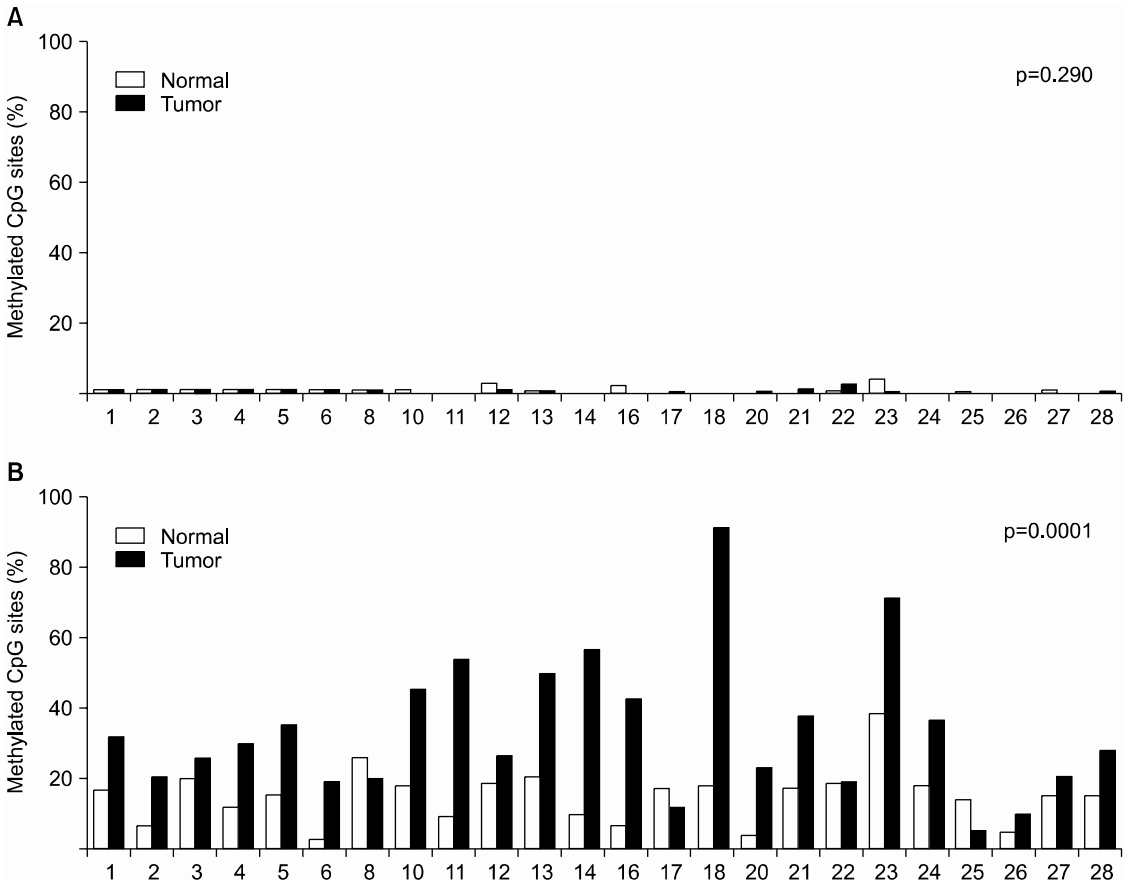

Fig. 5. Methylation status of the AKAP12α promoter in lung cancer tissues and the matched normal tissues. (A) The frequency of CpG island 1 methylation for each sample is indicated by methylated CpG site/total CpG sites (%). (B) The frequency of CpG island 1 methylation for each sample is indicated by methylated CpG site/total CpG sites (%). p-value ($p < 0.05$) computed from a paired sample *t*-test.

5) Methylation status of CpG islands 1 and 2 in the AKAP12α promoter regions in lung cancer tissues and the matched normal tissues

We further examined 24 pairs of primary lung cancer specimens and the surrounding normal lung tissues. The methylation pattern of the CpG sites in the CpG island 1 of AKAP12α by using the bisulfite-sequencing method was also investigated. Bisulfite-sequencing analysis showed a few methylated CpG sites in the lung tumor tissues and the matched normal lung tissues (Fig. 5A). Accordingly, these results suggest that the DNA methylation of CpG island 1 of AKAP12α promoter is infrequent in the lung tissues from NSCLC patients.

Next, the methylation patterns of the CpG island 2 in the AKAP12α promoter in 24 primary lung tumors were examined along with their matched normal lungs. Sequencing analysis of the bisulfite-modified DNA from the clinical specimens showed that the CpG sites of CpG island 2 were highly methylated in the lung tumors compared to the matched normal tissues (Fig. 5B), and such a finding is statistically significant (Table 2). Although methylated CpG sites existed in normal lungs, they were randomly distributed in the CpG sites of CpG island 2, and they were significantly infrequent compared with the tumor tissues (Fig. 5B). Also, the USF binding motif, which exists in CpG island 2, exhibits a higher degree of methylation as compared with the matched normal tissues (Fig. 6). The correlation between the methylation status of the CpG island 2 methylation in the AKAP12α promoter and patients' clinicopathological factors was also investigated. CpG island 2 methylation in the AKAP12α promoter is found to be associated with the male gender, an older population, smokers, advanced stage and recurrence among the patients (Table 2).

The results suggest that DNA methylation of the AKAP12α promoter occurs in CpG island 2 during lung carcinogenesis, and CpG island 2 is a more important region than the CpG island 1 in lung cancer tissues.

Table 2. Comparison of CpG island 2 methylation in lung tissues with the clinicopathologic features

Characteristic	Methylated CpG sites <40%		Methyalted CpG sites ≥40%	
Patients, no	17	(%)	7	(%)
Female	9	53	1	14
Male	7	41	6	86
≥60	10	59	6	86
<60	7	41	1	14
Smoker	10	59	5	71
Nonsmoker	7	41	1	14
Drinker	7	41	3	43
Non-drinker	10	59	3	43
Stage				
1	7	41	1	14
2	4	24	3	43
3	6	35	3	43
4				
Recurrence				
Recur	10	59	1	14
Non-recur	7	41	6	86
Cell type				
Adeno carcinoma	9	53	3	43
Squamous cell carcinoma	8	47	4	57

DISCUSSION

In the present study, we tested the hypothesis that the AKAP12α expression can be reduced in human lung cancer by aberrant DNA methylation in the promoter region of the AKAP12α gene. To do this, the AKAP12α expression and the DNA methylation status of the promoter in lung tumor cell lines, primary lung tumors and adjacent normal tissues were examined. In agreement with previous reports (14,15), this study showed that the AKAP12α expression was suppressed in 6 out of 10 (60%) human lung cancer cell lines. Epigenetic analysis of the AKAP12α gene confirmed that the AKAP12α expression was restored after treatment with the DNA methyltransferase inhibitor 5-aza-2'-deoxycytidine in human lung

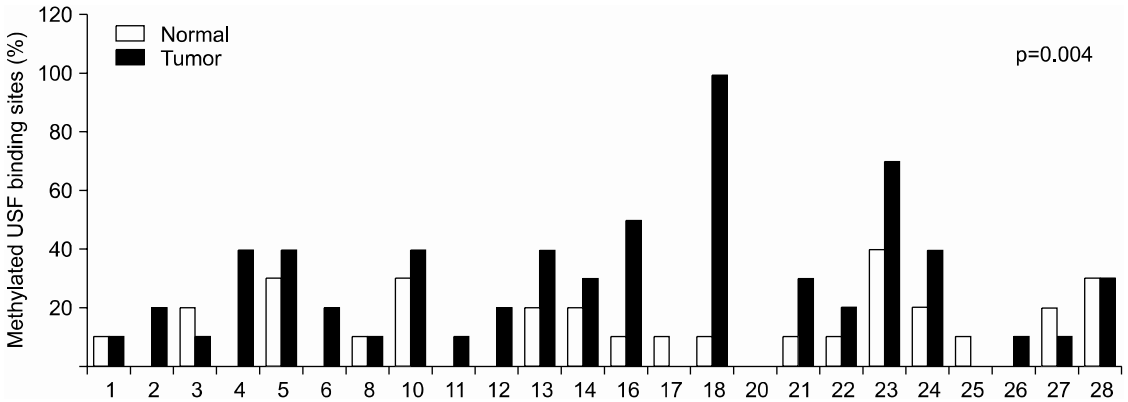

Fig. 6. Percentage of methylated USF binding sites in CpG island 2 of the AKAP12α promoter. The frequency of methylation for each sample is indicated by the methylated USF binding site/total USF binding CpG sites (%).

cancer cell lines. Moreover, two approaches, MSP and bisulfite-sequencing analysis, were used to study the methylation status of the AKAP12α CpG islands. The results showed that two CpG islands of the AKAP12α promoter were methylated in the lung cancer cell lines and tissues. The study demonstrated that DNA methylation of CpG island 1 is directly involved in the transcriptional silencing of the AKAP12α gene in NSCLC cells.

The CpG islands located in the promoter of the tumor suppressor genes undergo aberrant hypermethylation in human cancer. This process is an important mechanism that's responsible for gene silencing in human cancer (19). CpG islands can be located in different positions of genes, and differential methylation can be observed in any of these CpG islands (20). A report recently revealed that the 5'-flanking sequence from -1,034 to +1 is important in AKAP12α transcription by performing promoter analysis of the AKAP12α gene in NIH-3TS cells (21). Thus, we investigated whether the AKAP12α promoter has CpG islands that are methylated by DNMTs. We found two CpG islands, named CpG island 1 and CpG island 2, in the AKAP12α promoter. The CpG island 1 of the AKAP12α promoter is associated with DNA methylation in lung cancer cell lines, according to bisulfite-sequencing analysis. The CpG island 1 of the AKAP12α promoter was methylated in H358 and Calu-1 cells, which have absent or low levels of AKAP12α expression. Surprisingly, although H460 cells have a low AKAP12α expression level, H460 cells were not methylated in the CpG sites of CpG island 1. In addition, 24 human lung cancers and the matched normal tissues had no detectable methylated CpG sites in the CpG island 1. The results implied that the AKAP12α expression was influenced by other CpG islands' methylation as well as CpG island 1 methylation.

We then examined whether CpG island 2 in the AKAP12α promoter was correlated with DNA methylation in lung cancer. MSP analysis could not be used because specific primers for the unmethylated and methylated DNA could not be found. Only bisulfite-sequencing analysis of the CpG island 2 of the AKAP12α promoter was carried out. Interestingly, most of the CpG sites of CpG island 2 in the AKAP12α promoter were fully methylated in the immortalized cell lines and the lung cancer cell lines irrespective of the AKAP12α expression. We also examined the methylation status of the CpG island 2 in the AKAP12α promoter in 24 lung cancers and the matched normal tissues. We found that 21 of 24 lung tumor tissues had highly methylated CpG sites of the CpG island 2 in the AKAP12α promoter as compared with the paired normal tissues, and this finding was statistically significant ($p < 0.0001$). We also observed lower levels of AKAP12α methylation in all the normal lung specimens. As the normal specimens were derived from morphologically normal regions adjacent to tumors, it is possible that methylation of the CpG island 2 in the AKAP12α promoter is an early event in the development of lung cancer and that the data reflect a "field effect" (22). The timing of CpG island 2 methylation in the AKAP12α promoter during tumor development and progression remains to be investigated. Accordingly, the data we obtained demonstrated that CpG island 2 of the AKAP12α promoter was associated with DNA methylation, and we showed that CpG island 1 and 2 methylation in the AKAP12α promoter manifested differently according to the lung cancer cell lines and the lung tumor tissues.

Methylation-induced suppression of gene transcription is thought to occur either by interference with the binding of transcription factors or through the action of MBD proteins (23). We investigated whether the CpG islands 1 and 2 in the AKAP12α promoter contain these transcription factor-binding motifs via a transcription factor predicting program (http://www.gene-regulation.com). It was found that transcription factors such as SP1, USF and SRF bind to the CpG islands 1 and 2 in the AKAP12α promoter. Especially, SP1 and USF have a CpG site in their binding motif and they are frequently methylated in cancer, and these transcription factors are strong transcription regulators in cancer (24,25). According to the obtained data, although H1666 cells were methylated in both CpG island 1 and CpG island 2 in the AKAP12α promoter, H1666 cells expresses a high level of AKAP12α. As shown in Fig. 3, the SP1 element binding site is located in the 7th of the 17 CpG sites in CpG island 1 and it is unmethylated in H1666 cells. Thus, H1666 cells could express AKAP12α because the SP1 binding site in CpG island 1 was unmethylated. In the CpG island 2, the USF binding motif manifested higher methylation in tumor tissues than that of the matched normal tissues (Table 2). The methylation of the USF binding site may be influenced by the expression of the AKAP12α gene. We further investigated the correlation between the methylation status of the CpG island 2 methylation in the AKAP12α promoter and the patients' clinicopathological factors. CpG island 2 methylation in the AKAP12α promoter is associated with males, elderly patients, smokers, advanced stage and recurrence among the patients. Accordingly, DNA methylation of the AKAP12α promoter occurs in CpG island 2 in lung cancer tissues, and CpG island 2 is a more important region than CpG island 1 in lung carcinogenesis.

The AKAP12 gene encodes three transcriptionally distinct isoforms (AKAP12α, -β and -γ) that have different physical properties (11). In the present study, we have shown that the AKAP12α promoter is specifically methylated in lung cancer. The promoter of the other major AKAP12 isoform, AKAP12β, is not suppressed in lung cancer and its expression is not associated with promoter methylation in lung cancer. Because these two AKAP12 isoforms have different subcellular distributions (11), this finding highlights the importance of recognizing the isoforms as distinct entities when attempting to elucidate their functional roles in lung cancer. The specific role of the AKAP12α gene in the signaling pathway of lung cancer should be explored further.

CONCLUSIONS

Our data demonstrated that promoter methylation of the AKAP12α gene occurs in lung cancer, and this is associated with the transcriptional silencing of the AKAP12α gene. This epigenetic mechanism includes the methylation of both CpG islands 1 and 2 in the AKAP12α promoter. Further studies of the AKAP12α gene should clarify the important role that the AKAP12α gene plays in lung carcinogenesis.

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