Mutations and Deletions of the CBP Gene in Human Lung Cancer

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ABSTRACT

Purpose: Microarray-based comparative genomic hybridization analysis led us to detect a homozygous deletion at the cyclic AMP response element binding protein-binding protein (CBP) locus in a lung cancer cell line. Oncogenic roles of CBP had been suggested by functional and genetic studies; thus, involvement of CBP gene alterations in lung carcinogenesis was investigated by undertaking comprehensive analysis of genetic CBP alterations in human lung cancer.

Experimental Design: Fifty-nine cell lines and 95 surgical specimens of lung cancer were analyzed for mutations, homozygous and hemizygous deletions, and expression of the CBP gene.

Results: Homozygous CBP deletions, including two intragenic deletions, were detected in three (5.1%) lung cancer cell lines. CBP mutations, including missense, nonsense, and frame-shift mutations, were detected in six (10.2%) cell lines and five (5.3%) surgical specimens of lung cancer. The wild-type CBP allele was retained in 9 of 11 cases with CBP mutations, and both the wild-type and mutant alleles were expressed in all the six cases with heterozygous CBP mutations examined. Three mutations with amino acid substitutions in the histone acetyltransferase domain caused significant reduction in transcription activation activity of CBP protein in vivo.

Conclusions: A fraction of lung cancers carried mutations and/or deletions of the CBP gene, suggesting that genetic CBP alterations are involved in the genesis and/or progression of a subset of lung cancers.

INTRODUCTION

Gene deletion is one of the mechanisms that inactivates tumor suppressor genes in human cancer. In lung cancer, loss of heterozygosity (LOH) has been frequently detected in diverse chromosomal regions (1–3). However, up to the present, only a few target tumor suppressor genes, including p53, RB, p16, RASSF1A, and FHIT have been identified from the regions showing frequent LOH in lung cancer cells. Identification of chromosomal regions with homozygous deletions in cancer cells is an effective approach in identifying novel tumor suppressor genes involved in human carcinogenesis (1). In the course of microarray-based comparative genomic hybridization (array CGH) analysis of lung cancer cell lines, we detected a homozygous deletion at the cyclic AMP response element binding protein-binding protein (CBP) locus on chromosome 16p13.3 in a small cell carcinoma (SCC) cell line, NCI-H209.

CBP is a transcriptional coactivator and possesses acetyltransferase activity, which acetylates lysine residues of histones and nonhistone proteins, such as p53. CBP participates in basic cellular functions, including growth, differentiation, DNA repair, and apoptosis (4, 5). Oncogenic roles of the CBP gene have been indicated by the finding that the gene is fused to MOZ (6, 7). In addition, disruption of one copy of the CBP gene causes Rubinstein-Taybi syndrome, with increased susceptibility to some tumors, particularly those of neural and developmental origin (7, 8), and mice with mononucleolar inactivation of the Cbp gene develop multilineage defects in hematopoietic differentiation and an increased incidence of hematologic malignancies (9). Whether the wild-type CBP allele is lost in tumors of Rubinstein-Taybi syndrome patients or not has not been examined. However, loss of the wild-type Cbp allele shown in tumors of Cbp+/− mice indicated that Cbp/Cbp functions as a tumor suppressor. This idea is consistent with the fact that viral oncoproteins, adenovirus E1A and SV40 large T antigen, disturb CBP functions (4, 5). Recently, it was shown that two colon cancer cell lines with the microsatellite instability phenotype have insertion/deletion mutations in a (C)5 repeat in the coding region of the CBP gene, leading to the production of truncated CBP proteins (10). In addition, internal tandem duplications in the coding region of the CBP gene were detected in a subset of esophageal cancers (11). However, it was also reported that intragenic CBP mutations are rare in breast, colon, ovarian, and several other cancers (12), including those with LOH at the CBP locus (13). Thus, it still remains largely unclear in what types and fractions of human cancer the CBP gene is
genetically altered, and whether CBP acts as a tumor suppressor in human cancer development.

The presence of homozygous CBP deletion in a SCC cell line prompted us to examine a large number of lung cancers for genetic CBP alterations. Intragenic homozygous deletions in the CBP gene were detected in two other lung cancer cell lines. In addition, somatic CBP mutations, including nonsense and frameshift mutations, were detected in a fraction of lung cancers. Thus, CBP gene alterations were suggested to be involved in the development and/or progression of a subset of lung cancers.

MATERIALS AND METHODS

Materials. Fifty-nine lung cancer cell lines, consisting of 18 SCCs and 41 non–small cell lung cancers (NSCLC), were used in this study. The 18 SCC cell lines were H209, H1184, H1963, H2107, H2141, H2171, H2195, H128, Lu134, Lu135, Lu139, H69, H82, N417, SBC-5, H526, H841, and HCC33; whereas the 41 NSCLCs consisted of 24 adenocarcinomas (H1395, H1437, H1648, H2009, H2087, H2122, H2126, H2347, A549, RERF-LCMS, RERF-LCOK, VMRC-LCD, A427, PC3, PC7, PC9, PC14, ABC-1, H23, H322, H441, Ma-17, Ma-29, and HCC515), 10 squamous cell carcinomas (H1703, EBC-1, LK-2, Sq-1, PC10, LC1-Sq, H157, H520, SQ-5 and SK-MES-1), five large cell carcinomas (Lu99, PC13, Lu65, H1155, and H1299) and two adenosquamous carcinomas (H596 and HCC366). In four SCCs (H209, H2107, H2141, and H2171) and six adenocarcinomas (H1395, H1437, H2009, H2087, H2122, and H2347), corresponding lymphoblast cell lines were available for analysis. H- and HCC-series cell lines were provided by Drs. Y. Hayata (Tokyo Medical University, Tokyo, Japan), T. Terasaki (Kanagawa Institute of Technology, Kanagawa, Japan), and S. Hirohashi (National Cancer Center Research Institute, Tokyo, Japan) and M. Takada (National Hospital Organization Kinki-chuo Chest Medical Center, Osaka, Japan), respectively. Cell lines were also obtained from the American Type Culture Collection (Manassas, VA), the Japanese Collection of Research Bioreresources (Tokyo, Japan), and the RIKEN BioResource Center (Tsukuba, Japan). Ninety-five surgical specimens (15 SCCs, 50 adenocarcinomas, 24 squamous cell carcinomas, and 6 large cell carcinomas) of lung tumor and adjacent noncancerous tissue pairs were obtained from lung cancer patients who were treated at the National Cancer Center Hospital, Tokyo. The materials to be analyzed were selected by a pathologist to ensure that the samples were macroscopically entirely tumorous and chosen from an area devoid of necrotic tissue. High-molecular-weight DNA was prepared by the method previously described from the cell lines, tumors and adjacent noncancerous tissues (15). Polyadenylated RNA was extracted from cultured cells with a Fast Track mRNA isolation kit (Invitrogen, San Diego, CA) and total RNA was extracted from surgical specimens with a RNeasy kit (Qiagen, Valencia, CA).

Array CGH Analysis. The genome copy number was assessed using a DNA array, Genosensor Array 300 (Visis, IL), according to the procedures previously reported (16). Equal amounts of DNAs from 40 normal esophageal tissues were mixed and used for reference DNA in DNA array CGH analysis.

Deletion and Mutation Analyses. Thirty-one coding exons of the CBP gene, exons 2 to 32, were amplified from 50 ng of DNAs of 59 cell lines and 95 surgical specimens of lung cancer by PCR using 44 sets of primers (primers and conditions are available on request). If no PCR products were detected, the corresponding locus was deduced as being homozygously deleted. Homozygous deletions were confirmed by multiplex PCR using the IRF1 locus on chromosome 5q as a reference. PCR products from the cell lines and the surgical specimens were directly sequenced to detect mutations using a Big Dye Terminator Sequencing kit and an ABI Prism 3700 Genetic Analyzer (Applied Biosystems, Foster City, CA). PCR products from the surgical specimens were also subjected to mutation search by WAVE analysis as described previously (15). PCR products with different mobilities in the WAVE analysis were purified and directly sequenced.

Reverse Transcription-PCR Analysis. RNA was reverse-transcribed to cDNA by using the SuperScript First-Strand Synthesis System (Invitrogen). cDNA conversion mixtures were subjected to PCR amplification for 30 cycles (for CBP) and for 25 cycles (for glyceraldehyde-3-phosphate dehydrogenase; primer sequences and PCR conditions are available on request). In several lung cancer cases, cDNA fragments encompassing mutation sites were also amplified and directly sequenced to examine whether mutant CBP alleles were expressed.

Western Blot Analysis. Cultured cells were lysed in 1× SDS buffer containing 0.025 mol/L Tris (pH 6.8), 1% SDS, 3% β-ME, 2.5% glycerol, and 0.0025% BPB. Fifty micrograms of the heat-denatured protein extracts were subjected to a 6% SDS-PAGE, and were transferred onto polyvinylidene difluoride membranes (Amersham, Arlington Heights, IL). The membranes were immunostained with antibodies against the N-terminal (A-22, Santa Cruz Biotechnology, Santa Cruz, CA) and C-terminal (C-20, Santa Cruz Biotechnology) peptide of CBP, and β-actin (Sigma, St. Louis, MO). In the reporter gene assay (described below), a monoclonal antibody against Gal4DBD (sc-510, Santa Cruz Biotechnology) was used to detect Gal4DBD-CBP protein. Immunoreactivities were visualized with the enhanced chemiluminescence system (Amersham).

Loss of Heterozygosity and Allelic Imbalance Analyses. LOH in cell lines and allelic imbalance (AI) in primary tumors in the CBP locus were assessed by genomic PCR of microsatellite sequences, MS2 and D16S3065, in the CBP locus (17), according to the method described previously (15, 18).

Reporter Gene Assay. A Gal4DBD-wild-type-CBP fusion construct, which expresses a Gal4DBD-human full-length CBP (amino acids 2-2,826) fusion protein, was assembled using the pFA-CMV plasmid (Stratagene, La Jolla, CA). A CBP cDNA fragment covering the HAT domain was then subcloned into the pBluescript plasmid, and mutations were introduced by using a Quickchange site-directed mutagenesis kit (Stratagene). Plasmid vectors to express Gal4DBD-mutant CBP fusion proteins were reconstructed by exchanging the wild-type cDNA fragment with cDNA fragments containing mutations. Nucleotide substitutions for these cDNA inserts were confirmed by sequencing of the plasmids.

293T cells were maintained in DMEM with 10% FCS. Cells ($n = 2.5 \times 10^5$) were transfected using the LipofectAMINE2000 reagent (Invitrogen), with 500 ng of a pFA-CMV plasmid with...
or without CBP fragments, 500 ng of a Luciferase-reporter plasmid, pFR-luc (Stratagene), containing five Gal4 binding sites inducing luciferase gene expression, and 5 ng of a control reporter plasmid, phRL-CMV (Promega, Madison, WI), containing a cDNA encoding Renilla luciferase downstream of the cytomegalovirus promoter. The cells were harvested 24 hours after transfection. Half of the cells was subjected to luciferase assays using the Dual-Luciferase Reporter Assay System (Promega) as described (19). The remaining half of the cells was subjected to Western blot analysis.

RESULTS

Homozygous Deletions of the CBP Gene. Chromosomal loci showing homozygous deletions were searched for in 30 lung cancer cell lines by an array CGH analysis covering 287 loci containing frequently amplified or deleted loci in a variety of cancers. Signal intensities of several loci on chromosome 9p were decreased (relative copy number < 0.5) in three cell lines, and this finding was consistent with the fact that homozygous deletions of the 9p21-p22 segment containing the p16 tumor suppressor locus had been found in these cell lines in our previous study (20). In addition, a decrease in signal intensity (relative copy number = 0.27) was observed at the CBP locus in a SCC cell line, H209, suggesting that this locus was homozygously deleted in this cell line. The findings to date on oncogenic roles of CBP prompted us to further investigate CBP alterations in lung cancer. Genomic PCR analysis revealed that exons 4 to 32 of the CBP gene and several exons of the neighboring gene, TRAP, were homozygously deleted in the H209 cell line (Fig. 1A). Homozygous deletions at the CBP locus were further searched for by genomic PCR analysis of all coding exons in the remaining 29 and in an additional 29 lung cancer cell lines. Intragene homozygous deletions were detected in another SCC cell line, H1963, and a squamous cell carcinoma cell line, LK-2 (Fig. 1A). Exons 1 to 3 were deleted in H1963 and exon 3 alone was deleted in LK-2; however, neighboring genes were not affected. Therefore, the CBP locus was considered to be a common target of these three homozygous deletions, and this locus was homozygously deleted in 5.1% (3 of 59) of lung cancer cell lines examined. Interstitial deletions covering the CBP locus have been detected in patients with Rubinstein-Taybi syndrome (17, 21–24). However, frequencies of the autosomal dominant disorder, Rubinstein-Taybi syndrome, in general populations are quite low (~1 in 30,000; ref. 25), and the regions of homozygous deletions in these three cases were different from those detected thus far in patients with Rubinstein-Taybi syndrome. Therefore, it was indicated that homozygous deletions detected in the three lung cancer cell lines were somatic and not constitutional.

Mutations of the CBP Gene. Direct sequencing of all coding exons led us to detect 42 different types of nucleotide substitutions among the 59 cell lines. Among them, seven were considered as being somatic mutations as described below, and the remaining 35 were concluded as being genetic polymorphisms from the analysis of a large number of noncancerous cell samples and from the information of genetic polymorphism databases on the web. A base substitution detected in H2122 was confirmed as being a somatic mutation because this substitution was not detected in the corresponding lymphoblast cell line (Fig. 1B). Another six different types of substitutions detected in five cell lines, H322, H520, H1703, H1184, and Lu65, were likely to be somatic mutations and not genetic polymorphisms. This was because these substitutions were not detected in noncancerous cells of 95 different individuals, and each of them was detected in only one of the 59 lung cancer cell lines and in none of the 95 primary tumors (Table 1). One of these seven mutations was nonsense, and the remaining six were missense. Two different missense mutations were detected in H1703 (Table 1). PCR amplification of a CBP cDNA fragment encompassing the two mutation sites and subsequent cloning and sequencing of 12 independent clones revealed that the two mutations were present on different alleles; therefore, both CBP alleles were mutated in H1703 cells. Five other mutations detected in five cell lines were heterozygous; that is, the wild-type allele was retained in these cases. In total, mutations of the CBP gene were detected in six of the 59 (10.2%) lung cancer cell lines, and expression of these mutant alleles were confirmed by the reverse transcription-PCR (RT-PCR) products sequencing of these cell lines (Fig. 1B).

Five (5.3%) of the 95 surgical specimens were concluded as having somatic mutations because these sequence variants were detected only in cancer cells but not in the corresponding noncancerous cells. One was 1-bp deletion, three were missense mutations, and the remaining one was a silent mutation (Table 1). One missense mutation, which was detected in S31T (Fig. 1B), was homozygous, and the other four mutations were heterozygous. Expression of mutant alleles in Na98T (Fig. 1B) and Na79T was confirmed by RT-PCR product sequencing of these samples.

All nine missense mutations (Fig. 2A) caused substitutions of amino acids conserved between human and mouse CBP, and moreover seven of them, except for mutations in codons 893 and 2111, changed amino acids conserved between human CBP and p300. In particular, mutations in codons 1411, 1446, and 1472 caused substitutions of amino acids in the HAT domain (26). A nonsense mutation at codon 1835, which leads to the production of a truncated protein without the COOH terminus, was detected in the H1184 cell line. A one-base deletion in Na98T was a frame-shift mutation leading also to production of a truncated protein without the C terminus (Fig. 2B). Thus, it is highly possible that these mutations have some effects on the physiologic function of the CBP gene.

Expression of CBP mRNA and Protein. Expression of the CBP gene was examined in 59 lung cancer cell lines and two primary tumors (Na98T and Na79T) by RT-PCR analysis. CBP cDNA fragments for exons 30 to 32 were not amplified in two cell lines, H209 and H1963, with homozygous deletions, whereas they were amplified in the remaining 57 cell lines and the primary tumors, including those with missense, nonsense, and frame-shift mutations. The levels of CBP transcripts were not drastically different among the 57 cell lines (Fig. 3A). A normal sized cDNA fragments for exons 1 to 3 transcripts were amplified in H209, suggesting that a small CBP protein lacking a large C-terminal region was suggested to be produced in H209 cells (Fig. 2B). Exons 1 to 3 cDNA fragments were not amplified in H1963; thus, CBP expression was suggested to be lacking in H1963 cells due to loss of the promoter region sequence upstream of exon 1. CBP mRNA transcripts for
exons 30 to 32 were detected in LK-2, in which exon 3 was homozygously deleted. However, RT-PCR for exons 2 to 4 as well as exons 2 to 3 revealed the absence of the exon 3 sequence in the transcripts (Fig. 3A and B), indicating that the exon 3 deletion caused production of a small and truncated CBP protein without a large portion for the COOH terminus (Figs. 2B and 3B). It was also possible that a truncated CBP protein without the NH2 terminus was produced by using a methionine codon in exon 2 or 4 as a translation start site (Figs. 2B and 3B).

CBP protein expression was examined in several lung cancer cell lines including those carrying CBP deletions or mutations by Western blot analysis with antibodies for NH2-terminal and COOH-terminal proteins (Fig. 4). Normal sized CBP protein was not detected in the H209 and LK-2 cell lines; however, small sized proteins were detected in H209 and LK-2 only with NH2-terminal and COOH-terminal CBP antibodies, respectively. Whereas the size of CBP protein in H209 cells was smaller than normal, it was larger than predicted suggesting the possibility that a fusion protein of a part of the CBP sequence and an unknown sequence was produced in this cell line (Fig. 2B). The size of CBP protein detected in LK-2 corresponded to the protein lacking the NH2 terminus described above (Fig. 2B). Although expression of truncated protein was expected in H1184, no abnormal sized CBP protein was detected by these antibodies. No CBP protein was detected in H1963 with any of the two antibodies as expected from the mRNA analysis. Apparently, normal-sized CBP proteins were detected in other cell lines, including those with CBP mutations as well as those without mutations, and the amounts of CBP protein were not drastically different among the cell lines as in the case of mRNA expression.
Loss of Heterozygosity and Allelic Imbalance at the CBP Locus. LOH and AI analyses were done for 10 lung cancer cell lines and 95 surgical specimens, respectively, in which the corresponding noncancerous cells were available for the analysis. Two of the 9 (22.2%) informative lung cancer cell lines, H2141 and H2347, had LOH, and 20 of 90 (22.2%) informative primary tumors had AI for the CBP locus, respectively. None of the cell lines with LOH and one (S31T) of the primary tumors with AI had CBP mutation.

Transcription Activation Ability of CBP Mutants. Previously, human and mouse CBP/Cbp proteins fused to the DNA binding domain of the yeast transcription factor Gal4 (Gal4DBD) were shown to stimulate HAT-dependent transcription of reporter genes through binding to Gal4 binding sites in their promoter regions (19, 22, 27). To examine whether CBP mutations detected in lung cancer cells have functional defects, wild-type CBP protein, and three HAT mutant CBP proteins, 1411Glu, 1446Cys, and 1472Cys, fused to Gal4DBD were transduced in 293T cells by constructing plasmid vectors to express those fusion proteins (Fig. 5). Consistent with previous results, the Gal4DBD-wild-type CBP fusion showed 20-fold more transcriptional activity than Gal4DBD alone in this study.

Table 1 CBP Mutations in human lung cancer

<table>
<thead>
<tr>
<th>Sample</th>
<th>Histology</th>
<th>Exon (change)</th>
<th>Predicted in gene product change</th>
<th>Expression mRNA</th>
<th>Expression Protein</th>
<th>p53 mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell line</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H209</td>
<td>SCC</td>
<td>4-32 (homozygous deletion)</td>
<td>Fusion</td>
<td>+</td>
<td>+</td>
<td>* IVS6-2 A/G</td>
</tr>
<tr>
<td>H1963</td>
<td>SCC</td>
<td>1-3 (homozygous deletion)</td>
<td>Null</td>
<td>–</td>
<td>–</td>
<td>Val147Asp</td>
</tr>
<tr>
<td>NK-2</td>
<td>SqC</td>
<td>3 (homozygous deletion)</td>
<td>Truncation</td>
<td>+</td>
<td>+</td>
<td>Val272Met</td>
</tr>
<tr>
<td>H2122</td>
<td>AdC 3</td>
<td>(A248C: heterozygous)</td>
<td>Asn83Thr</td>
<td>+</td>
<td>+</td>
<td>Gln16Leu</td>
</tr>
<tr>
<td>H322</td>
<td>AdC</td>
<td>15 (C2678T: heterozygous)</td>
<td>Ser893Leu</td>
<td>+</td>
<td>+</td>
<td>Arg248Ile</td>
</tr>
<tr>
<td>H520</td>
<td>SqC</td>
<td>27 (C4336T: heterozygous)</td>
<td>Arg1446Cys</td>
<td>+</td>
<td>+</td>
<td>Trp146stop</td>
</tr>
<tr>
<td>H1703</td>
<td>SqC</td>
<td>28 (G4416T: heterozygous)</td>
<td>Trp1472Cys</td>
<td>+</td>
<td>+</td>
<td>IVS8+1G/T</td>
</tr>
<tr>
<td>H1184</td>
<td>SCC</td>
<td>32 (G5503T: heterozygous)</td>
<td>Glu1835Stop</td>
<td>+</td>
<td>+</td>
<td>Asp259Val</td>
</tr>
<tr>
<td>Lu65</td>
<td>LCC</td>
<td>32 (A6332G: heterozygous)</td>
<td>Asn2111Ser</td>
<td>+</td>
<td>+</td>
<td>Gln11Gln</td>
</tr>
<tr>
<td>Surgical specimen</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na98T</td>
<td>AdC</td>
<td>3 (DG91 or 92: heterozygous)</td>
<td>Truncation</td>
<td>+</td>
<td>NT</td>
<td>Gly271Lys</td>
</tr>
<tr>
<td>N501T</td>
<td>AdC</td>
<td>8 (C1651A: heterozygous)</td>
<td>Leu551Ile</td>
<td>NT</td>
<td>NT</td>
<td>His193Tyr</td>
</tr>
<tr>
<td>S31T</td>
<td>SCC</td>
<td>26 (G4232A: homozygous)</td>
<td>Gly1411Glu</td>
<td>NT</td>
<td>NT</td>
<td>ND</td>
</tr>
<tr>
<td>Na79T</td>
<td>SqC</td>
<td>31 (C4926G: heterozygous)</td>
<td>silent</td>
<td>+</td>
<td>NT</td>
<td>ND</td>
</tr>
<tr>
<td>T10-28T</td>
<td>SqC</td>
<td>32 (C6131G: heterozygous)</td>
<td>Ala2044Gly</td>
<td>NT</td>
<td>NT</td>
<td>Tyr220Cys</td>
</tr>
</tbody>
</table>

Abbreviations: SCC, small cell carcinoma; AdC, adenocarcinoma; SqC, squamous cell carcinoma; LCC, large cell carcinoma; NT, not tested; ND, not detected.

*Aberrant size.
†Corresponding noncancerous tissue DNA was not available.

Loss of Heterozygosity and Allelic Imbalance at the CBP Locus. LOH and AI analyses were done for 10 lung cancer cell lines and 95 surgical specimens, respectively, in which the corresponding noncancerous cells were available for the analysis. Two of the 9 (22.2%) informative lung cancer cell lines, H2141 and H2347, had LOH, and 20 of 90 (22.2%) informative primary tumors had AI for the CBP locus, respectively. None of the cell lines with LOH and one (S31T) of the primary tumors with AI had CBP mutation.

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Fig. 2 A, schematic of CBP protein illustrating functional domains with the location of missense mutations. The CBP protein spans 2,442 amino acids. Closed arrowhead, homozygous mutation; open arrowheads, heterozygous mutations. B, CBP proteins deduced to be produced in lung cancer cells with homozygous deletions (H209 and LK-2), nonsense mutation (H1184), and frame-shift mutation (Na98T). RID, receptor-interacting domain; TAZ1, transcriptional adaptor zinc finger 1; CREB, CREB-binding domain; BD, bromodomain; PBD, plant homeodomain; CoA, putative CoA-binding domain; ZZ, ZZ zinc finger motif; TAZ2, transcriptional adaptor zinc finger 2;QP, glutamine- and proline-rich domain; HAT, histone acetyltransferase domain.
However, transcriptional activities of all three mutant CBP fusions were significantly lower than that of the wild-type CBP fusion (Fig. 5). Similar results were obtained in H1299 lung cancer cells (data not shown), indicating that the phenotype of the mutants is not cell-type specific. Thus, it was indicated that at least three mutants examined here are accompanied by reduced transcription activation abilities.

**DISCUSSION**

We showed here that the CBP gene is genetically altered in human lung cancers: 15.3% in cancer cell lines and 5.3% in primary tumors. Thus, genetic alterations of the CBP gene were indicated to be involved in the pathogenesis of a subset of lung cancers. Recently, frame-shift mutations in mononucleotide repeats and internal tandem duplications of the coding region in the CBP gene have been reported in colon cancer and esophageal squamous cell carcinoma, respectively (10, 11). However, there was no lung cancer case that showed mutations at mononucleotide repeats in the coding exons of the CBP gene. We also searched for internal tandem duplications in 59 lung cancer cell lines by RT-PCR covering all coding exons; however, such alterations were not observed among them (data not shown). Instead, genetic alterations detected in lung cancer were point mutations and homozygous deletions. Therefore, molecular mechanisms for the occurrence of CBP gene alterations in lung cancer are likely to be different from those in colon and esophageal cancers.

In this study, identification of homozygous deletions in three lung cancer cell lines supported the role of the CBP gene as a tumor suppressor. The finding that three mutations with amino acid substitutions in the HAT domain caused significant reduction in the transcription activation ability of CBP protein in vivo also supported the idea. However, wild-type CBP allele was retained in 9 of 11 cases with CBP mutations, and both the wild-type and mutant alleles were expressed in all the six cases with heterozygous CBP mutations examined. In two of the three cases with homozygous CBP deletions, altered-sized CBP proteins were expressed. Thus, at present, it is unclear whether the CBP gene is functionally inactivated in lung cancer cells.
with genetic CBP alterations. It is possible that some of the mutated CBP proteins have dominant negative effects to the wild-type CBP protein. Alternatively, reduction of CBP activities due to the mutations might have some effects on the genesis and/or progression of lung cancer. The fact that CBP mutations were not clustered in the HAT domain but dispersed in the whole gene region may indicate that biological effects of these CBP mutants are diverse.

CBP is involved in multiple pathways controlling cell growth, differentiation, and apoptosis as a transcriptional coactivator (4, 5). Recently, it was found that function of p53 tumor suppressor protein is regulated by acetylation by CBP and other histone acetyltransferases (4, 5, 28). Our mutational analysis (29) revealed that all the six lung cancer cell lines and three of the five primary tumors with CBP gene alterations had p53 mutations (Table 1). Coexistence of CBP and p53 alterations in lung cancer cells, therefore, indicated that CBP alterations contribute to lung carcinogenesis by disturbing pathways other than those involving p53. In preliminary studies, we transfected a wild-type CBP expression vector into a lung cancer cell line with homozygous CBP deletions. However, neither apoptosis nor growth arrest was induced in these cells under standard culture conditions (data not shown). Therefore, at present, the biological and pathogenetic significance of CBP alterations in lung cancer remains unclear. To elucidate the issue, analyses of changes in a variety of cellular properties, such as tumorigenic and metastatic potentials, due to exogenous expression of wild-type and/or mutant CBP are in progress for lung cancer cell lines carrying different genetic alterations. Phenotypic changes of cultured lung epithelial cells by knocking down CBP are also being investigated.

Importantly, as for the EP300 gene, which also encodes a histone acetyltransferase and structurally and functionally relates to CBP (4), somatic mutations have been identified in several types of human cancers, including colorectal and breast cancers (10, 30, 31), although their prevalence in human lung cancer is unclear. The present findings on CBP further strengthened the idea that aberrations of histone acetyltransferases are involved in the development and/or progression of human cancers, including lung cancers. Thus, further genetic and functional studies of CBP and EP300 would help us understand how aberrations of histone acetyltransferases are involved in human carcinogenesis.

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