Histological Type-selective, Tumor-predominant Expression of a Novel CHK1 Isoform and Infrequent in Vivo Somatic CHK2 Mutation in Small Cell Lung Cancer

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Abstract

Inactivation of p53, which represents the most prevalent genetic alteration in lung cancer, has been shown to play a crucial role in the acquisition of genomic instability. We examined 44 lung cancer specimens to search for mutations in the CHK1 and CHK2 genes, which have been suggested to play roles in regulating p53 after DNA damage. We found that the CHK2 gene was somatically mutated in lung cancer in vivo, although at a low frequency, and that a previously undescribed shorter isoform of CHK1 was expressed preferentially in small cell lung cancer in a tumor-predominant manner. Additional studies are warranted to investigate the functional significance of these changes as well as the potential involvement of other components in this important pathway to maintain genomic stability.

Introduction

Lung cancer currently claims more than 160,000 lives annually as the number one cause of cancer deaths in the United States, and it has also become the leading cause of cancer deaths in Japan, claiming more than 47,000 lives annually (1). Recent molecular biological studies have clearly indicated that lung cancer is a disease caused by the accumulation of multiple genetic defects in both tumor suppressor genes and dominant oncogenes (2, 3). In addition, cytogenetic studies have shown that lung cancer frequently contains chromosomal abnormalities, as also seen in most other cancers (4). A number of surveillance mechanisms exist in cells to ensure the maintenance of genomic stability against various types of damage to the genome. The G1 checkpoint prevents replication of damaged DNA, whereas genomic integrity before mitosis is monitored by the G2 checkpoint, which promotes G2 arrest on detection of DNA damage. Failure of such checkpoint functions results in genomic instability, a mutagenic condition that predisposes cells to neoplastic transformation and tumor progression (5).

Among the genetic lesions identified in lung cancer, the p53 gene is the most frequently altered, suggesting an important role of this gene in the pathogenesis of lung cancer (6). The p53 gene plays an essential role in the G1 checkpoint, whereas cells lacking p53 function are completely defective in the G1 checkpoint in response to DNA damage such as ionizing radiation (7). Whereas the ATM gene activates p53 in response to DNA damage and leads to G1 arrest, previous studies in yeast have also shown that Chk1 in Schizosaccharomyces pombe as well as Cds1 in S. pombe and Rad53 in Saccharomyces cerevisiae play roles as downstream mediators of ATM involved in G2 arrest in response to DNA damage (8–10). When the presence of damaged DNA is sensed, both Chk1 and Cds1/Rad53 phosphorylate Cdc25C at serine-216, which leads to the binding of Cdc25C to a 14-3-3 protein, resulting in its export from the nucleus. The Cdc2/cyclin B complex is thereby prevented from becoming activated and initiating mitosis, and the cells are arrested in G2. Although the G2 checkpoint genes are potential targets for genetic alterations in human cancers, virtually no information is available about such defects in human lung cancer.

In the present study, we examined 44 lung cancer specimens to investigate the potential involvement of CHK1 (11) and CHK2 (12–15), human homologues of Chk1 and Cds1/Rad53, respectively, in the pathogenesis of lung cancers.

Materials and Methods

Lung Cancer Specimens. Tumor samples, along with uninvolved lung tissue when available, were collected from 44 patients diagnosed histologically as having lung cancer (9 SCLCs, 15 adenocarcinomas, 11 squamous cell carcinomas, and 9 large cell carcinomas). All tissues were quickly frozen in liquid nitrogen and stored at −80°C until analysis.

Southern Blot Analysis. Southern blot analysis was carried out using PCR-generated cDNA probes, which covered the entire open reading frame of the CHK1 and CHK2 genes. The following oligonucleotide primer pairs were used for probe generation: (a) CHK1, S1 (sense; 5'-ACAGTCGGCGAGGTGCCT) and AS9 (antisense; 5'-TCACACGAGATTCCCAAGA); and (b) CHK2, F1 (sense; 5'-GGGTGTGGATGTTCTCCGC) and R9 (antisense; 5'-TTGCTGCTGTTCAACACCGG). PCR amplification consisted of 35 cycles of 94°C for 1 min, 53°C for 45 s, and 72°C for 2 min after an initial denaturation step at 94°C for 5 min.

RT-PCR-SSCP Analysis. PCR amplification using random-primed first-strand cDNAs was performed with the aid of the following oligonucleotide primers in the presence of [32P]dCTP, followed by electrophoretic separation on 6% nondenaturing polyacrylamide gels in both the presence of 5% glycerol at room temperature and the absence of glycerol at 4°C. The primer pairs used for amplification of CHK1 were as follows: (a) S1 (sense; see the description above) and AS1 (antisense; 5'-GGACAGTCTACGGACGCC); (b) S2 (sense; 5'-GGAAGCTGGAGTGAGAGAG) and AS2 (antisense; 5'-CGAGCTCTAGCTGC); (c) S3 (sense; 5'-TGATGAGGAAGGATGCT) and AS3 (antisense; 5'-GGTAGCAACACCTGAG); and (d) S4 (sense; 5'-CTTGGGTGGTGCAACAGT) and AS4 (antisense; 5'-CCAAGTCAAATGCTGCG) and S5 (sense; 5'-ACCAAAAAAGGCTGACGAG) and AS5 (antisense; 5'-CTGTCGCGGAGGACTCTG); (f) S6 (sense; 5'-GGAGCTTCTCGGATGCTGGACGAGG) and AS6 (antisense; 5'-GAAGCGAGTGGGTTGGGGG); (g) S7 (sense; 5'-TGAGAGGAGGAGGAGGAGG) and AS7 (antisense; 5'-GAGAGGAGGAGGAGGAGGAGG) and AS8 (antisense; 5'-GAGAGGAGGAGGAGGAGGAGG) and AS8
CHK1 AND CHK2 IN HUMAN LUNG CANCER

Results

Preferential Expression of a Previously Undescribed Isoform of CHK1 in SCLC. We investigated the potential involvement of CHK1 and CHK2 in the pathogenesis of lung cancers by examining 44 lung cancer specimens obtained directly from patients. We first conducted Southern blot analysis, but we failed to identify any gross alterations in either of the G2 checkpoint genes (data not shown). RT-PCR-SSCP analysis was then performed to search for mutations in the CHK1 gene, yielding one example with a unique electrophoretic mobility shift (case 33, PCR primers S2 and AS2; Fig. 1A). The same mobility shift was also detected in the corresponding normal lung tissue. Subsequent sequence analysis revealed that this mobility shift was due to a nucleotide substitution at codon 46 (GTA to ATA) resulting in the substitution of isoleucine for valine within the kinase domain. In an additional screening of 125 normal lung RNAs, the distinct band detected in case 33 was found to be unique, suggesting that it may represent a very rare polymorphism or possibly a germ-line mutation. Unfortunately, detailed past and family histories of case 33 were not available.

During the course of RT-PCR-SSCP analysis of CHK1, we often detected the presence of the faster-migrating band preferentially in SCLC cases with the S5 and AS5 primers (data not shown). Agarose gel electrophoresis of the RT-PCR products showed readily detectable expression of a shorter isoform of CHK1 mRNA in 4 of 9 cases of SCLC in contrast to almost negligible expression of this isoform in only 2 of 35 NSCLC cases and in normal lung tissues (Fig. 1B). Sequence analysis revealed that this shorter CHK1 isoform lacks 32 amino acids between codons 240 and 271 corresponding to the conserved subdomain XI within the catalytic domain of this kinase (11, 16).

Identification of an in Vivo Somatic Mutation of CHK2 in SCLC. Mutations in the CHK2 gene were also searched for by RT-PCR-SSCP analysis in the same cohort, resulting in the identification of two cases with distinct mobility shifts (Fig. 2). The distinct mobility shift in SCLC case 6 detected with the F6 and R6 PCR primers was present only in the tumor specimen and not in the corresponding normal lung, whereas the mobility shift in case 9 was seen in both normal and tumor specimens.
mutation (GAC to GTC) at codon 311, which resulted in a nonconservative amino acid substitution of valine for aspartic acid (Fig. 3). Loss of the wild-type CHK2 allele was clearly detected by both RT-PCR-SSCP and direct sequencing analyses. The substituted aspartic acid at codon 311 of human CHK2 is conserved in murine and rat Chk2 as well as in its yeast homologues, Cds1 in S. pombe and Rad53 in S. cerevisiae, suggesting potential functional importance (12). The other distinct mobility shift detected in case 9 was found to be present in both normal and tumor specimens (Fig. 2), and subsequent sequence analysis showed that it represents a silent polymorphism (GAA to GAG) at codon 84.

Discussion

Recent molecular biological studies have clearly indicated that lung cancer is a disease caused by an accumulation of multiple genetic defects (2, 3). We and others have shown that inactivation of the p53 gene, which disrupts the G1 checkpoint, plays a major role in the pathogenesis of this fatal disease (5). Because aberrations in the CHK1 and CHK2 genes are expected to result in the impairment of another important checkpoint, i.e., the G2 checkpoint, it was conceivable that these genes may also be altered with high frequency and contribute to the acquisition of genetic instability in lung cancer cells. However, there have been few reports concerning aberrations in the G2 checkpoint genes in human cancers (17, 18). Bell et al. (17) recently reported the identification of germ-line CHK2 mutations in one of four classical and two of 18 variant LFS families that had wild-type p53. Interestingly, their additional search for mutations in 49 cancer cell lines in vitro, including three cell lines of lung origin, resulted in the identification of only a single colon cancer cell line with a potential somatic mutation. The present study extends their observation and clearly shows that the CHK2 gene is somatically mutated in lung cancer in vivo with clear loss of the wild-type allele, although CHK2 alterations were infrequent. This is the first description of a CHK2 point mutation within the kinase domain, which shows a clear difference from germ-line CHK2 mutations in LFS (i.e., two of the three LFS mutations reported thus far were frameshifts, and the other was a missense mutation within the forked head-associated domain). As for the low prevalence of CHK2 alterations in lung cancers, it is possible that frequent p53 mutations in lung cancer (reportedly ~90% of SCLCs and ~50% of NSCLCs have p53 inactivation) might preclude the necessity of the acquisition of additional CHK2 mutations. In this regard, a direct functional link of CHK2 with p53 in a checkpoint control program was reported recently, and CHK2 was found to play a role as an upstream regulator of the p53 protein (19, 20). However, it is noteworthy that case 6 with a CHK2 mutation also had a missense mutation (TAC to TGc, Tyr to Cys) at codon 234 in the p53 gene, which suggests the possibility that other cases without a CHK2 mutation might carry mutations in p53-independent G2 checkpoint component(s) downstream of CHK2.

Alterations in a polyadenosine stretch within the CHK1 coding region have been reported in colon cancers with microsatellite instability (18). In the present study, we did not observe any somatic CHK1 mutations in lung cancer. However, we identified histological type-selective, tumor-predominant expression of a previously undescribed alternative isoform of CHK1 in SCLC, which lacks the conserved subdomain XI of the catalytic domain of this kinase (11, 16). It should be mentioned in this context that Chen et al. (21) recently reported on the crystal structure of the CHK1 kinase domain and suggested that Glu-248 and Arg-253, two amino acids that are removed by the alternative splicing described here, may be important for substrate selectivity through support of the activation loop structure. This finding is also interesting from a clinicopathological point of view because SCLC and NSCLC represent two major categories of lung cancer and exhibit very distinct characteristics such as prominent neuroendocrine differentiation and highly aggressive clinical course in SCLC (2, 3). It will be interesting to investigate the functional significance of the alternative CHK1 isoform in relation not only to lung carcinogenesis but also to the development and differentiation of the lung because our preliminary results indicated readily detectable expression of the alternative CHK1 isoform in the fetal lung.

The present findings mark CHK2 as a potential target for genetic alterations in lung cancer, although the frequency of such mutations is low. It thus remains to be determined whether there are as yet unidentified DNA damage checkpoint genes with mutation frequencies comparable to that of p53 or whether there might be a number of other affected genes that each play a role in a small proportion of cases. In this connection, accumulating information from studies in yeast on DNA damage checkpoints will be valuable to better understand the molecular pathogenesis of lung cancer.

References


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