Frequent Expression of p53 Protein without Mutation in the Atypical Epithelium of Human Bronchus

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Human carcinomas of the lung are thought to develop from a series of consecutive independent molecular events. These involve activation of oncogenes such as K-ras, myc, or her2/neu, or loss of repressive oncogenes such as p53 and probably one or more tumor-suppressor genes on chromosome 3p (1). The most commonly identified genetic change in human cancers is mutation of the p53 gene, located at band 13 on the short arm of chromosome 17 (2). This gene is a tumor-suppressor gene and encodes a 53-kD nuclear phosphoprotein capable of binding to DNA and acting as a transcriptional factor (3, 4). The wild-type p53 protein inhibits cell proliferation, and loss of this activity is important in neoplastic transformation (3). The inactivation of tumor-suppressor genes is thought to be important in the development of many human malignancies (5, 6). Inactivation of these genes, through deletion or mutation, presumably allows a cell to escape normal growth controls.

Recent studies have elucidated a model of colorectal tumorigenesis in which the steps required for the development of cancer involve the mutational activation of an oncogene coupled with the loss of several genes that normally suppress tumorigenesis (7). The sequence of morphologic change is consistent with a multistage model of carcinogenesis, and it is thought that the genetic changes found in advanced lung cancers also occur in a stepwise fashion accompanied by morphologic changes (8-11). In studies of human atypical bronchial epithelium (ABE), adenocarcinoma in resection specimens, concomitant mutations in p53 and other genetic abnormalities have been noted in areas of ABE (12, 13). However, sample numbers in these reports have been small, and no associations between immunohistochemical findings and p53 mutations have been reported in ABE found in benign diseases or in tissues separated from the lung carcinomas.

In this study we have investigated the correlation between p53 protein expression and gene mutations in ABE biopsy specimens derived from patients with or without lung cancer, using immunohistochemical detection and poly-
The chromatin has a ground-glass appearance (Figure 1, center panel). In hyperplasia, the thickest stratum of nuclei was observed and the nuclei were rounded or oval in shape. The chromatin of the nuclei is visible and the nuclei are larger than those in normal bronchial epithelium.

Microscopic findings and the presence of p53 protein

We used a modification of a previously described preceq microdissection technique (16) to collect atypical epithelial cells under direct microscopic observation from human lung tissues and cores were embedded in paraffin. The tissue sections were deparaffinized in xylene and dehydrated in ethanol series. Subsequently, the slides were autoclaved in 121°C for 20 min and then incubated for 3 min at 60°C. The slides were dehydrated and cleared in xylene.

Immunohistochemistry

The method of staining was used for immunostaining to enhance immunoreactivity in formalin-fixed paraffin-embedded tissue samples. The primary anti-p53 monoclonal antibody was DO-1 (Abcam, Cambridge, UK) which recognizes both wild-type and mutant forms of p53. The slides were incubated overnight at 4°C with DO-1 at a dilution of 1:200 (0.15 μg/ml). Secondary antibodies conjugated with biotin (Nichirei, Japan) and avidin-biotin complex method were used for immunostaining.

The primary antibody was blocked by 10% rabbit serum placed on the slides for 20 min. The avidin-biotin complex method was used for immunostaining. The slides were then dehydrated in xylene and dried in air. The slides were mounted in DPX and examined under a light microscope.

Microdissection of specimens

The tissue sections were digitized using a digital microscope (Axiolab, Carl Zeiss, Germany) and then transferred to a computer. The epithelial cells were microdissected under the microscope and then purified using a microcapillary tube that was pulled to a fine tip by a micropipette (World Precision Instruments, Sarasota, FL). The microdissected cells were then purified by Microcon centrifugal filters (Amicon, Beverly, MA) and quantified using a hemacytometer. The microdissected cells were then stored at -20°C for future use.

Screening of p53 gene

The screening of the p53 gene was performed as described previously (17). In a total of 100 patients treated at the National University Hospital, Singapore, and 50 patients with lung cancer and 20 patients with nonmalignant diseases, including seven with pulmonary tuberculosis, four with chronic bronchitis, four with interstitial pneumonitis, three with pulmonary fibrosis, and one with bronchial asthma, ABE was graded independently by two pathologists (Abe and Kataoka) (18). Briefly, each preclinical lesion was diagnosed as follows: hyperplasia, the deepest stratum of the epithelium comprises several layers thick; occasional slightly enlarged polyhedral or oval cell with slight irregularity of the nuclear membrane and a recognition of p53 expression in more than 10% of the cells was considered positive (Figure 1, right panel). The cells were considered to be positive if those with 5 to 10% of positivity were considered as associated with the presence of p53 protein.

Results

Nuclear staining of cells with an anti-p53 antibody was assessed by immunohistochemical analysis, as shown in Figure 2. Although we have classified the degree of immunostaining according to the percentage of p53-immunopositive nuclei, as described in Materials and Methods, all the ABE specimens that were considered to be positive expressed p53 protein in more than 90% of atypical cells, and negative samples expressed p53 in fewer than 1% of cells. By this classification, 44 of the 78 ABE cases expressed p53 in more than 90% of atypical cells, and negative samples expressed p53 in fewer than 1% of cells. By this classification, 44 of the 78 ABE cases expressed p53 in more than 90% of atypical cells, and negative samples expressed p53 in fewer than 1% of cells. By this classification, 44 of the 78 ABE cases expressed p53 in more than 90% of atypical cells, and negative samples expressed p53 in fewer than 1% of cells. By this classification, 44 of the 78 ABE cases expressed p53 in more than 90% of atypical cells, and negative samples expressed p53 in fewer than 1% of cells. By this classification, 44 of the 78 ABE cases expressed p53 in more than 90% of atypical cells, and negative samples expressed p53 in fewer than 1% of cells. By this classification, 44 of the 78 ABE cases expressed p53 in more than 90% of atypical cells, and negative samples expressed p53 in fewer than 1% of cells. By this classification, 44 of the 78 ABE cases expressed p53 in more than 90% of atypical cells, and negative samples expressed p53 in fewer than 1% of cells. By this classification, 44 of the 78 ABE cases expressed p53 in more than 90% of atypical cells, and negative samples expressed p53 in fewer than 1% of cells. By this classification, 44 of the 78 ABE cases expressed p53 in more than 90% of atypical cells, and negative samples expressed p53 in fewer than 1% of cells. By this classification, 44 of the 78 ABE cases expressed p53 in more than 90% of atypical cells, and negative samples expressed p53 in fewer than 1% of cells. By this classification, 44 of the 78 ABE cases expressed p53 in more than 90% of atypical cells, and negative samples expressed p53 in fewer than 1% of cells. By this classification, 44 of the 78 ABE cases expressed p53 in more than 90% of atypical cells, and negative samples expressed p53 in fewer than 1% of cells. By this classification, 44 of the 78 ABE cases expressed p53 in more than 90% of atypical cells, and negative samples expressed p53 in fewer than 1% of cells. By this classification, 44 of the 78 ABE cases expressed p53 in more than 90% of atypical cells, and negative samples expressed p53 in fewer than 1% of cells. By this classification, 44 of the 78 ABE cases expressed p53 in more than 90% of atypical cells, and negative samples expressed p53 in fewer than 1% of cells. By this classification, 44 of the 78 ABE cases expressed p53 in more than 90% of atypical cells, and negative samples expressed p53 in fewer than 1% of cells. By this classification, 44 of the 78 ABE cases expressed p53 in more than 90% of atypical cells, and negative samples expressed p53 in fewer than 1% of cells. By this classification, 44 of the 78 ABE cases expressed p53 in more than 90% of atypical cells, and negative samples expressed p53 in fewer than 1% of cells. By this classification, 44 of the 78 ABE cases expressed p53 in more than 90% of atypical cells, and negative samples expressed p53 in fewer than 1% of cells. By this classification, 44 of the 78 ABE cases expressed p53 in more than 90% of atypical cells, and negative samples expressed p53 in fewer than 1% of cells.
Figure 2: H&E staining (upper panel) and immunostaining (lower panel) of bronchial epithelium with dysplasia. Original magnification ×250.

and at least 50 cells were used for each PCR. As described previously, more than 90% of the cells microdissected were positively stained for p53.

We screened for the presence of mutations in exons 5, 6, 7, and 8 in the microdissected materials. Because more than 90% of p53 gene mutations occur in these exons (19, 20), a single specific amplification product was detected in provided suitable template were positively stained for p53.

all cases, including that all microdissected materials preserved suitable template were positively stained for p53. In the four specimen with mobility shifts, the highest incidence found in squamous-cell carcinomas (100%) and the lowest incidence in adenocarcinomas (30%) (19, 24, 25). It has been reported that the expression of p53 protein in cancer cells is generally associated with mutations in the p53 gene (20, 26). However, it is not clear whether this tendency exists in premalignant lesions as well.

In the present study we have shown that expression of p53 protein was found in 56% of the specimens with ABE and that increased p53 expression correlated with the severity of cellular atypia, consistent with data reported by other investigators using both surgically resected specimens (8, 9) and transbronchial biopsy specimens (10, 11, 27). Nakamura and coworkers (8) found a significant correlation between p53 expression in bronchial dysplasia and their related squamous-cell carcinoma, suggesting that the p53 expression could be an early event in the development of a squamous-cell carcinoma of the lung. Bennett and colleagues (9) reported an increased frequency of p53 protein accumulation in dysplasia, and that suggested p53 alterations (but not mutations) occur before invasion. Investigators Boers and associates (11) and Walker and coworkers (10) have shown that the proportion of p53-positive cells was correlated with the existence or development of lung cancer (11). However, Walker and colleagues (10) also found that expressions of p53 protein in the normal epithelium of resection margins in five of 10 were cancerous. The proportion of p53-positive cells was correlated with the existence or development of lung cancer (11). However, Walker and coworkers (10) also found that expressed p53 protein in the normal epithelium of resection margins in five of 10 were cancerous.

Discussion

Of the known tumor-suppressor genes, p53 is the gene most frequently mutated in human malignancies (21). This gene is mutated in many common tumors, including breast, ovarian, and colon cancers. In lung cancer, approximately 90% of small-cell lung cancers (22) and 50% of non-small-cell lung cancers have mutated p53 (23), with the highest incidence found in squamous-cell carcinomas (100%) and the lowest incidence in adenocarcinomas (30%) (19, 24, 25). It has been reported that the expression of p53 protein in cancer cells is generally associated with mutations in the p53 gene (20, 26). However, it is not clear whether this tendency exists in premalignant lesions as well.

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The pathologic diagnosis of bronchial dysplasia, and all of them were detected in the microdissected materials. The p53 gene mutation was identified by direct sequencing (Figure 4, upper panel). The DNA sequencing reaction of the PCR-amplified exon 7 showed the same point mutation at codon 248 (CGG-to-CTG transversion) in all four specimens. The same point mutation in both sample (data not shown).

Figure 3. Data show p53 immunoreactivity in each grade of ABE. The rating shown at the top of each bar is the number of positive samples/total number of lesions of a specific histologic grade.

Table 3. Relationship between p53 expression and patients' clinical backgrounds.

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Table 4. Summary of SSCP analysis of p53 exon 7 from normal (N), dysplasia (D), and carcinoma (C) tissues from four cases (upper panel). Mobility shifts were observed in dysplasia and carcinoma samples. DNA sequencing of PCR amplified exon 7 fragments from normal and dysplastic tissue in case 1 is shown (lower panel). The codon at which the mutation occurs is indicated.
mutations. There are only a few studies reporting that p53 gene mutation in the dysplastic tissue adjacent to the carcinoma tissue and that the gene alterations were concordant with those found in the carcinoma. Soon and associates [12] reported in autopsy cases of CGC that p53 gene mutation was found in both the tissue of lung cancer and the tissue of severe dysplasia taken at the resection margin. In that report, investigators analyzed a detailed p53 gene status using a PCR-SSCP method followed by a direct sequencing method. Hata and coworkers [13] reported that all cases in lung cancer tissues and normal lungs treated with aminopterin showed more than 90% of the ABE samples expressed wild-type p53 protein. This result suggests that mutation of the p53 gene in human ABE is a common event and that p53 may act to inhibit the carcinogenesis of precancerous ABE lesions.

In summary, the p53 protein was expressed in more than 90% of the samples with ABE. In addition, in p53-positive samples with ABE, gene analysis of p53 by PCR-SSCP. We investigated gene status in a single individual with widespread dysplastic changes of the lung. With the p53 protein half-life [33], altered expression of the p53 gene was identified in ABE tissues derived from an individual with widespread dysplastic changes of the lung. This result suggests that mutation of the p53 gene in human ABE may be a frequent target for protective mechanisms in lung cancer. By 1990, it was useful to note that mutations in the p53 gene were found in many human cancers. In particular, mutations in the p53 gene were found in human breast cancer. In this review, the p53 gene was found to be associated with the development of lung cancer. In addition, the p53 gene was found to be associated with the development of lung cancer in high-risk patients. In the future, the p53 gene will be a useful target for therapeutic strategies.

References


