A Comparative Study on Aberrations of Chromosome 17 and Proliferating Cell Fraction in Lung Cancer

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To better understand the relationship between specific chromosome changes found in human lung tumors and their phenotypic consequences a the tissue level, an in situ hybridization (ISH) procedure of chromosome 17 and immunohistochemistry of proliferating cell nuclear antigen (PCNA) were done. The deparaffinized sections were stained with pericentromeric probes for chromosome 17 and an immunohistochemical study of a monoclonal antibody against PCNA were performed. The numbers of chromosome signals were than compared with the positivity of PCNA expression. The mean numbers of chromosome were 1.62 in normal lymphocytes and 2.48 in lung cancer cells. Tumors showed a high mean positivity of PCNA of 43.4%. Mean PCNA expression was higher in squamous carcinomas than in adenocarcinomas ($p < 0.05$). A linear correlation between numbers of ISH signals and PCNA expression was not demonstrated, but there was a tendency of increasing PCNA positivity according to increasing numbers of ISH signals in adenocarcinomas of the lung and the tumor tissues which were over 50% positive PCNA expression. There was no linear correlation between numbers of ISH signals, PCNA positivity and tumor stages, and keratinization of squamous cell lung cancer. These results suggest that ISH will prove to be an important tool for determining the underlying genetic basis for tissue phenotypic heterogeneity by allowing genetic determinations to be made on paraffin-embedded tissue sections where histologic architecture is preserved, and immunohistochemical nuclear staining with anti-PCNA on routinely processed tissue is a simple technique for the assessment of proliferation in non-small cell lung carcinoma.

Key Words: In Situ Hybridization, Proliferating Cell Nuclear Antigen, Lung Cancer

INTRODUCTION

Tumor cells often show numerical or structural chromosomal abnormalities. These aberrations may, in some cases, provide clues to the initial changes which caused the tumor to arise. Such aberrations may also correlate with the behaviour of the tumor and have prognostic value. For solid tumors, however, several

disadvantages are inherent in the cytogenetic analysis (Teyssier et al., 1989). The classic technique of karyotyping Giemsa banded metaphase chromosomes is still mostly used to generate detailed information on the chromosomal content of neoplastic cells. But tumor tissue often has to be cultured to obtain sufficient metaphases, and the success rate is only 60% in obtaining good metaphases. When successful, the number of metaphase spreads is often small, and because of culture effects it is questionable whether these are representative of the original tumor cell population. As a consequence, knowledge of cytogenetic aberrations in solid tumors is poor when compared with that in haematological malignancies (Limon et al.,

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1986). An alternative method for the study of chromosomal abnormalities is the measurement of abnormal DNA content of cell nuclei using flow cytometry. This technique is extremely rapid and results are representative of the cells in the sample. In 1983 Hedley described the use of nuclei isolated from formalin-fixed, paraffin was-embedded tissue in DNA flow cytometry (Hedley et al., 1983). To evaluate the prognostic and therapeutic significance, tumor DNA content was determined by flow cytometry in paraffin-embedded tissue samples obtained surgically from patients with non-small cell lung cancer (Aysegul et al., 1990; Hiroshi et al., 1990; Camille et al., 1990; Andrew et al., 1992). A major drawback, however, is the "limited cytogenetic sensitivity"; a "small" change in nuclear DNA content like the loss of one complete chromosome often remains undetected. Furthermore, when abnormal DNA content is found, the question remains as to what cytogenetic changes have occurred (Cornelisse et al., 1991). A third technique for the study of cytogenetic changes in solid tumors is ISH using chromosome specific DNA probes. DNA sequences can be detected in interphase nuclei as well as in metaphase spreads, thereby obviating the need to culture tumor material (Cremer et al., 1988). Application of interphase cytogenetics has been described for solid tumors of the bladder (Hopman et al., 1988; Hopman et al., 1989), breast (Devilee et al., 1988), stomach (Dekken et al., 1990), and brain (Arnoldus et al., 1991). But, most of the previous studies were performed on single cell suspensions where the original tumor architecture was lost. Recently, ISH method applications on paraffin-embedded tissue sections was introduced (Emmerich et al., 1989). Therefore, it became possible to detect genetic abnormality on paraffin-embedded tissue sections using chromosome ISH. Furthermore, it also became possible to investigate the genetic abnormalities with the consequent phenotypic changes on paraffin-embedded tumor tissue, which might give us an avenue to understand the pathobiology of carcinogenesis. Up to now, variable numerical and/or structural chromosomal aberrations have been reported in lung cancer. But the pathobiological meanings of those changes were not completely understood because of several limitations of conventional cytogenetic analysis on solid tumors.

Kim et al. (1993) reported the hyperploidy of chromosome 7, 17 in lung cancer tissues, and Lee et al. (1990) reported that proliferating cell nuclear antigen was a useful marker for accessing proliferation cell fraction *ex vivo*. To better understand the relationship between specific chromosome changes found in human lung

tumors and their phenotypic consequences at the tissue level, an *in situ* hybridization procedure of chromosome 17 and immunohistochemical detection of PCNA was done on the same formalin-fixed paraffin-embedded tissue sections of human lung tumors.

MATERIAL AND METHODS

(1) *In Situ* Hybridization

1) Tissue Section Preparation

Tissue blocks were obtained from fourteen primary non-small cell lung cancer tissues (4 adenocarcinomas, 10 squamous cell carcinomas) that had been fixed in buffered formaldehyde and embedded in paraffin. Six- μ m-thick sections were cut with a microtome and placed on clean microscope slides treated with poly-L-lysine. Prior to further processing, the slides were frozen at -70°C and thawed at room temperature five times at 30-minutes intervals to enhance probe accessibility, and then incubated at 65°C in an oven overnight to enhance adhesion of the tissue section to the slide. The slides were then deparaffinized with xylene followed by 100% ethanol, three times each for 10 minutes, and then treated with 3% H_2O_2 in methanol for 5 minutes to block endogenous peroxidase activity.

To facilitate DNA probe accessibility to the cellular DNA, air-dried slides were digested with pepsin/hydrochloric acid (0.4% pepsin in 0.2 M HCl 37°C for 45 minutes after soaking at 4°C for 15 minutes). After washing in deionized water (3 times for 3 minutes each), the slides were dehydrated through a graded ethanol series and acetone (2 minutes each), and finally air dried.

2) DNA Probe

The repetitive satellite DNA probes for detecting sequences in the pericentromeric regions of chromosome 17 [p17H8 (D17Z1)] were purchased in the biotin-labelled from Oncor, Inc. (Gaithersburg, MD).

3) *In Situ* Hybridization

The slides were treated with 0.15-1.0mg/ml RNase A at 37°C for 1 hour, washed in 2X standard sodium citrate (SSC) times for 3 minutes each, and then dehydrated through a graded ethanol series. The hybridization mixture contained 60% formamide, 2X SSC, 5% dextran sulfate, 0.1 μ g/ μ l salmon sperm DNA, and 0.8ng/ μ l probe DNA: 30 μ l of probe mixture was added to each slide. The slide were then covered with HCl-precleaned coverslip and sealed with rubber cement. Next the slides and probe were denatured

together at 95°C for 3 minutes in a wet chamber, and then allowed to hybridize overnight at 37°C. Following hybridization, the rubber cement and coverslip were removed, and the slides were washed in 50% formamide/1X SSC (pH 7.0) at room temperature twice for 15 minutes. The slides were then washed in 0.1X SSC for 30 minutes at 37°C.

4) Detection on Hybridized DNA Probes

Biotinylated DNA probe that had hybridized to the tissue section was visualized by an indirect peroxidase reaction. For the peroxidase reaction, the slides were first incubated with 3% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) for 10 minutes at room temperature, then incubated with avidin (Vector) for 30 minutes at 37°C, washed three times in PBS for 5 minutes each, incubated with antiavidin (Vector) for 30 minutes at 37°C, and then washed in PBS again. The slides were then incubated with preformed avidin-biotin-peroxidase complex (Vector) for 30 minutes at 37°C, washed in PBS, and then incubated with a solution containing 50mg diaminobenzidine (DAB, Sigma) and 10 μ l of 30% H₂O₂ in 50ml PBS for 2-5 minutes to allow the color reaction to form. For color intensification of the DAB reaction product, optionally 35mg of NiCl₂ · 6H₂O was added to DAB/H₂O₂ solution. The slides were then counterstained with Mayer's hematoxylin.

5) Evaluation of ISH Signals

More than 400 nuclei per slide were evaluated for each tumor area analyzed and more than 200 nuclei per slide for normal areas of each tissue section. The following criteria were applied to the evaluation of the ISH signal: (a) nuclei should be free of cytoplasmic material; (b) nuclei from different cells should not overlap; (c) the signal intensity should be more or less homogenous in the nucleus; (d) minor binding sites resulting from cross-hybridization to other centromeric regions should be excluded; (e) labelled regions are included only when the signals are completely separated from one another; and (f) paired or split spots are counted as one (Hopman et al., 1989).

To estimate ploidy of chromosome 17 in a tumor area, the average number of labelled spots per nucleus in the tumor cells was divided by the average number of labelled spots found in normal cell nuclei such as normal lymphocytes. The ratio was termed the chromosome index. Since portions of nuclei were cut in the preparation to 6 μ m tissue sections, the average number of labelled spots per nucleus in normal cells was less than the expected

2, which would have been observed on whole diploid nuclei.

(2) Immunohistochemical Staining of Proliferating Cell Nuclear Antigen

1) Immunohistochemistry of PCNA

We stained the PCNA by 'One hour immunohistochemistry' using the MicroProbe system. Six- μ m-thick tissue sections were placed on pretreated slides. The slides were deparaffinized with xylene and histoclear at 60°C. The slides were then treated with endoblocker (Biomed M69) at 40°C to block endogenous peroxidase activity. After treating with tissue conditioner, the slides were incubated with primary antibody (DAKO) at 40°C for 9 minutes. After washing, the slides were incubated with secondary antibody (Biomed kit) at 40°C for 4 minutes and washed. The slides were then incubated with avidin-peroxidase reagents and washed with peroxidase enhancer (Biomed M68). Then the slides were incubated with chromogen (3-amino-9-ethylcarbazole, AEC, Biomed) at 40°C for 10 minutes twice. The slides were counterstained with hematoxylin.

2) Detection of Positivity of PCNA

Each slide was evaluated for PCNA positivity. Cells were considered positive for PCNA only when definite reddish-brown staining of the nucleus was identified. The extent of PCNA positivity in each tissue was evaluated by determining the percentage of positive nuclei present in at least 400 malignant cells.

(3) Data Analysis

An analysis of the results was carried out by correlation analysis and Wilcoxon rank sum test with the PC statistic programs of SAS.

RESULTS

(1) In Situ Hybridization of Chromosome 17

The mean signal number of normal lymphocytes as a normal control was 1.62 (range 1-2), and that of cancer cells had more signals than normal lymphocytes ($p < 0.05$) (Fig. 1). In normal lymphocytes, 38% showed one signal and 62% showed 2 signals. In lung cancer cells, 11.3% showed one signal, 44.8% showed 2 signals, 32.1% showed 3 signals, 8.2% showed 4 signals, 2.7% showed 5 signals, and 0.9% showed 6 signals. In squamous cell lung cancer, it was 10.1%, 45.4%, 32.8%, 8.4%, 2.9%, and 0.4%, respectively

and in adenocarcinoma, it was 14.2%, 43.2%, 30.2%, 7.8%, 2.4%, and 2.2%, respectively (Table 1). These results revealed that tumor cells had more ISH signals than normal lymphocytes, all normal lymphocytes had two or less signals, and only 56.1% of tumor cells had two or less signal numbers. A mean chromosome index for each tumor derived by dividing the mean number of chromosome signals of the tumor cells by the mean number of chromosome signals of normal lymphocytes was 1.5 (range 1.3-1.8).

Fig. 1. ISH signals using chromosome 17 centromeric probe in lung cancer cells

(2) Correlation between Signal Numbers of ISH and Tumor Stages, Differentiation

There was no significant correlation between signal numbers of ISH and tumor stages (5 patients with stage I, 2 patients with stage II, 7 patients with stage III). Also there was no significant correlation between signal numbers of ISH and keratinization in squamous cell lung cancer (Table 2).

(3) PCNA Expression in Lung Cancer Cells

Tumor cells showed 43.4% (range 3-90%) of mean positivity of PCNA expression, of which adenocarcinoma showed 7% and squamous cell lung cancers showed 58% positivity. There were significant differences between adenocarcinoma and squamous cell carcinoma in PCNA expression ($p < 0.005$) (Table 3) (Fig. 2).

(4) Correlation between PCNA Expression and Tumor Stages, Differentiation

Tumor stages had no significant correlation with PCNA expression, also keratinization of squamous cell lung cancer had no significant correlation with PCNA expression (Table 4).

Table 1. Signal Number of In Situ Hybridization in Lung Cancer

Tissue	Percentages of number of spots per nucleus								Mean numbers of spots (T/N)
	Tumor cells						Normal lymphocytes		
	1	2	3	4	5	6	1	2	
AD	7.6	34.3	35.2	12.4	4.8	5.7	36.4	63.6	2.9/1.6
AD	7.8	47.6	34.0	7.8	1.9	1.0	36.6	63.4	2.5/1.6
AD	16.8	46.5	29.7	5.0	0	2.0	36.4	63.6	2.3/1.6
AD	24.7	44.5	21.9	6.2	2.7	0	36.5	63.5	2.2/1.6
SQ	5.9	51.0	34.3	5.9	2.9	0	40.6	59.4	2.5/1.6
SQ	8.8	46.1	35.3	6.9	2.0	1.0	39.8	60.2	2.5/1.6
SQ	12.2	49.5	29.3	7.1	2.0	0	43.6	56.4	2.4/1.6
SQ	6.7	53.3	33.3	4.8	1.9	0	34.7	65.3	2.4/1.7
SQ	7.8	38.2	43.1	8.8	2.0	0	50.5	49.5	2.6/1.5
SQ	10.8	33.3	34.3	14.1	5.9	1.0	40.6	59.4	2.7/1.6
SQ	14.4	42.3	31.7	7.7	3.8	0	39.6	60.4	2.4/1.6
SQ	15.1	43.0	29.0	8.6	4.3	0	29.3	70.7	2.4/1.7
SQ	9.8	49.0	25.5	12.7	2.0	1.0	32.3	67.7	2.5/1.7
SQ	9.8	48.0	32.4	6.9	2.0	1.0	32.4	67.6	2.5/1.7
Mean									
AD	14.2	43.2	30.2	7.8	2.4	2.2	36.1	63.5	2.5/1.6
SQ	10.1	45.4	32.8	8.4	2.9	0.4	38.3	61.7	2.5/1.6
toal	11.3	44.8	32.1	8.2	2.7	0.9	37.8	62.2	2.5/1.6

AD: Adenocarcinoma, SQ: Squamous cell lung cancer, T/N: Tumor cells/Normal lymphocytes

(5) Correlation between Numbers of Signals of ISH and PCNA Expression

Tumor showed higher mean positivity of PCNA than normal lymphocytes and almost all of the tumor cells showed positive PCNA expression, but there was no linear correlation. There was a tendency of increasing PCNA positivity according to increasing numbers of ISH signals in adenocarcinoma of the lung (Pearson correlation coefficient 0.85, p value 0.15), and a similar tendency was demonstrated in the tissues

which were over 50% of positive PCNA expression (Pearson correlation coefficient 0.75, p value 0.09) (Table 5).

(6) Correlations between Chromosome Index and PCNA Expressions

There was no significant correlation between chromosome index of ISH and PCNA expression of immunohistochemistry (Table 6).

Table 2. Percentages of Nuclei with a Certain Spot Number according to Stages and Keratinization

	Number of spots per nucleus (%)						Mean numbers of spots
	1	2	3	4	5	6	
Stage							
I (n=5)	11.3	44.7	32.1	8.2	2.7	0.9	2.5
II (n=2)	12.0	43.1	31.7	9.0	3.0	1.2	2.5
III (n=7)	13.2	41.2	31.2	9.8	3.1	1.4	2.5
Type of squamous cell carcinoma							
K (n=4)	11.0	44.6	32.1	8.6	3.4	0.2	2.5
NK (N=6)	9.5	45.9	33.3	8.3	2.5	0.5	2.5

AD: Adenocarcinoma, SQ: Squamous cell lung cancer K: Keratinizing type, NK: Nonkeratinizing type

Table 3. PCNA Expression in Lung Cancer

Cell type	Percentages of PCNA positive nuclei					Mean positivity of PCNA (%)
	0-4	5-25	26-50	51-75	76-100	
AD (n=4)	1	3				7
SQ (n=10)	0	1	3	2	4	58
Total	1	4	3	2	4	43

AD: Adenocarcinoma, SQ: Squamous cell lung cancer

Table 4. PCNA Expression according to Stages and Keratinization.

	Percentages of PCNA positive nuclei					Mean positivity of PCNA (%)
	0-4	5-25	26-50	51-75	76-100	
Stages						
I (n=5)	0	1	1	1	2	43.4
II (n=2)	0	0	1	1	0	38.0
III (n=7)	1	3	1	0	2	34.0
Type of squamous cell carcinoma						
K (n=4)	0	0	2	1	1	55.0
NK (N=6)	0	1	1	1	3	59.8

AD: Adenocarcinoma, SQ: Squamous cell lung cancer

K: Keratinizing type, NK: Nonkeratinizing type

Fig. 2. Expression of monoclonal PCNA antibody applied to a paraffin section (a) Squamous cell carcinoma, (b) Adenocarcinoma

DISCUSSION

Irrefutable evidence exists that cancer is a genetic disease. However, there are myriad changes (mutations) in the genome that appear to contribute to cancer etiology. In the broadest context, any alteration in the genome resulting in abnormal gene regulation or expression (e.g., point mutations, translocations, deletions, amplification) could be a viable candidate for aiding the initiation (or progression) of human cancer. Two fundamental concepts are used to describe the process of carcinogenesis in the aerodigestive tract. First the concept of "field cancerization" which was first introduced by Slaughter et al. (1953), hypothesizes that the whole field of tissue is exposed to the carcinogenic insult (e.g., cigarette smoke) and is at increased risk for developing cancer. In the case of aerodigestive tract cancers, this notion is supported clinically by the frequent association of tumors with premalignant lesions in the field at risk [e.g., oral leukoplakia (Silverman et al., 1984), or bronchial metaplasia (Auerbach et al., 1961), and/or dysplasia (Lippman et al., 1989)] and by the synchronous or metachronous development of multiple primary tumors. A second concept describing tumorigenesis in general is that of the multistep carcinogenic process. The driving forces behind the process is thought to be genetic damage caused by continuous exposure to carcinogens, as evidenced by an increased frequency of micronuclei in the high risk tissue and in premalignant lesions (Farber et al., 1984), and its ac-

Table 5. Correlation between Number of ISH Spots and PCNA Expression

	Percentages of PCNA positive nuclei					
	0-4	5-25	26-50	51-75	76-100	
PCNA (n = 14, %)	7	29	21	14	29	
	Numbers of ISH spots					
	1	2	3	4	5	6
ISH (n = 14, %)	11.3	44.8	32.1	8.2	2.7	0.9

p-value > 0.5

Table 6. Correlation between Chromosome Index and PCNA Expression

Cases	AD	AD	AD	AD	SQ	SQ	SQ	SQ	SQ	SQ	SQ	SQ	SQ	SQ
CI	1.8	1.5	1.4	1.3	1.6	1.6	1.5	1.5	1.7	1.7	1.5	1.4	1.5	1.5
PCNA (%)	10	10	5	3	30	80	30	65	90	45	80	5	80	75

p-value < 0.5, AD: adenocarcinoma, SQ: squamous cell carcinoma CI: chromosome index

cumulation in the target tissue. Hypothetically, the greater the degree of genetic damage in the premalignant lesions, the greater the risk for cancer development. Moreover, these genetic alterations then give rise to phenotypic alterations in the tissues, such as dysregulation of the proliferation and differentiation pathway. So the generation of one-to-one correlations between specific chromosome changes previously detected by karyotypic evaluation and specific phenotypic changes in tissue is very important. A major advance in the field of cytogenetics was the introduction of nonradioactive *in situ* hybridization with cloned DNA probes capable of detecting specific repetitive target sequences. With this method, it has become possible to localize individual chromosomes in interphase nuclei (Arnoldus et al., 1991). ISH with these probes permits fast identification of (marker) chromosomes in metaphase spreads, the determination of the number of copies of selected chromosomes in interphase nuclei, and interphase topographical studies. However, with the increasing availability of chromosome-specific repetitive probes and libraries, the simultaneous application of probes detecting different target sequences make it possible to detect structural chromosome aberrations in interphase nuclei. This technique obviates the need to culture tumor material. Other major advantages of this technique are the absence of radiation hazards and the speed of analysis of the hybrid target sequences (Arnoldus et al., 1991). If interphase cytogenetics could be used routinely on paraffin was embedded material, this would imply that archival tumor material could be used for possible chromosomal abnormalities. As the histopathological grading of this material and the clinical outcome is usually known, potential correlations with the chromosomal aberrations might be studied relatively easily. Unlike the flow cytometric methods, it can detect a small change in nuclear DNA content and intratumoral heterogeneity of DNA content in lung cancer (Marion et al., 1991). Devilee et al. (1988) detected numerical aberrations of the target sites of chromosome 1 and 18 with ISH in breast tumors. Cremer et al. (1988) reported that the number or spots detected in interphase nuclei of neuroectodermal tumor cells correlated well with metaphase counts of chromosomes specifically labeled by ISH. Hopman et al. (1988) reported bi-color detection of two target DNAs by non-radioactive ISH in bladder tumors. Nederlof et al. (1990) developed a method for multiple fluorescence ISH allowing the simultaneous detection of more than three target sequences with only three fluorescent dyes (FITC, TRITC, AMCA) respectively emitting in the green, red, and blue, which would be impor-

tant for the prenatal diagnosis and detection of numerical and/or structural chromosomal aberrations in tumor diagnosis. Kim et al. (1993) reported polysomic changes of chromosome 7 and/or 17 in human lung cancer using ISH.

Biological, histological, and clinical studies have suggested that human tumors are heterogenous in nature having evolved through a multistep process. For example, histological analyses of human lung tumors often demonstrate regions with distinctly different histological phenotypes ranging from mixtures of adenomatous, squamous, undifferentiated, and even sarcomatous components (Olcott, 1955). Francis et al. (1990) reported that over 90% of lung cancer tissues had varying degrees of intratumoral heterogeneity of DNA content in flow cytometric study. In this paper, we performed ISH of chromosome 17 with repetitive alpha-satellite DNA probes on formalin-fixed, paraffin-embedded tissue sections of human lung tumors to evaluate cytogenetic aberrations. The mean numbers of chromosome 17 were 1.62 in normal lymphocytes and 2.48 in lung cancer cells. 43.9% of tumor cells had more than 3 numbers of signals of chromosome 17. A chromosome index for each tumor was derived by dividing the mean number of chromosome signals found on the tumor cells by the mean number of chromosome signals on normal cells in the same section. The overall chromosome index was 1.5, which was hyperploidy. There was no statistically significant correlations between numbers of ISH signals and keratinization of squamous cell lung cancer and stages of cancers.

The microscopic grading of non-small cell lung carcinomas (squamous cell carcinoma, adenocarcinoma and large cell undifferentiated carcinoma) is subjective and does not have a prognostic value (Greenberg et al., 1987). Useful information about tumor biology has been gained by studying tumor cell kinetics in lung carcinoma in addition to histopathologic classification of tumors. Methods for assessing the state of cellular proliferation such as thymidine-or bromodeoxyuridine-labelling and flow cytometry are not easily practicable for routine diagnosis. On the other hand, immunohistochemical cell-cycle markers such as antibodies against Ki-67 and PCNA can easily be applied in routine surgical pathology laboratories. The advantage of anti-PCNA is that it can be used with routinely processed formalin-fixed and paraffin-embedded tissue, in contrast to the Ki-67 antibody which requires frozen tissue sections (Theunissen et al., 1992). PCNA, also known as cyclin, is a 36kDa DNA polymerase- δ auxiliary protein, and plays a critical role in cell proliferation due to its functions as a

cofactor for DNA synthesis (Robbins et al., 1987). Its expression fluctuates dramatically during the cell cycle, and it appears in trace amounts in the G1 phase, increases to its maximum in the S phase, and slowly declines thereafter. Recently, data on cell kinetics obtained by immunostaining for PCNA in paraffin-embedded tissue samples have been reported (Garcia et al., 1989). In vitro studies with very careful fixation conditions have identified two types of nuclear staining with anti-PCNA: a distinct granular pattern which is S-phase-specific and a more diffuse form which is not S-phase-specific (Bravo et al., 1987). In the present study, both types were found. PCNA positivity correlated with mitotic activity and tumor grade in breast and colon cancer (Dierendonck et al., 1991). But Hall et al. (1990) reported that in normal tissue and lymphoid neoplasms, PCNA immunolocalization could be used as an index of cell proliferation. However, in some forms of neoplasia, including breast and gastric cancer and in vitro cell lines, the simple relation between PCNA expression and cell proliferation was lost. In some breast and pancreatic tumors there was apparent deregulation of PCNA with increased expression in tissues adjacent to the tumors. The overexpression in some tumors and in adjacent morphologically normal tissue might represent autocrine or paracrine growth factor influence on PCNA gene expression. Carey et al. (1992) studied the correlations between immunohistochemical PCNA expression and flow cytometric DNA ploidy in lung cancer, and reported that mean PCNA expression was higher in squamous carcinomas than in adenocarcinomas but there was intra-tumor variation in the PCNA index. Lee et al. (1992) reported that since an important component of carcinogenesis is unregulated growth, they employed antibodies against the proliferation markers topoisomerase II, DNA polymerase- α , Ki-67, and PCNA and found PCNA to be quite useful as a marker for proliferating cells, even in the routinely processed, formalin-fixed, paraffin-embedded tissue samples. And they reported that there was an inverse relationship between histological grade and the proportion of PCNA-positive cells. They examined PCNA expressions in chronic smokers (over 15 pack-year smoking) whose bronchial biopsy had revealed squamous metaplasia, they suggested a significant correlation between increase in proliferative activity and histological progression from normal to hyperplasia to squamous metaplasia/dysplasia, and suggested that PCNA might have a role as biomarkers of intermediate end points of chemoprevention.

In this study, an immunohistochemical study of a monoclonal antibody against PCNA was performed

with the deparaffinized sections that showed numerical aberrations of chromosome 17, and the overall positivity of PCNA was 3-90% in lung cancer tissues, of which adenocarcinomas showed 7% positivity and squamous cell carcinomas showed 58% positivity. There was a statistically significant difference and this result was similar with that of Lee et al. (1992). A linear correlation between numbers of ISH signals and PCNA expression was not demonstrated, but there was a tendency of increasing PCNA positivity according to increasing numbers of ISH signals in adenocarcinomas of the lung and tumor tissues which were over 50% positive PCNA expression. There was no linear correlation between numbers of ISH signals, PCNA positivity and tumor stages and keratinization of squamous cell lung cancer. A few cases of metastatic nodes showed similar PCNA positivity with primary lung cancer tissues but had no statistic significance. To better understand the relationship between specific chromosome changes found in human lung tumors and their phenotypic consequences at the tissue level, more extensive studies are needed. Also further expansion of these studies and exploration of panels of biomarkers, including PCNA, ABH blood group antigen, genes at the chromosome 3p deletion site, growth regulatory genes, and dominant and recessive oncogenes (e.g., those coding for transforming growth factor- β and EGFR, c-myc, ras, and p53) are expected to generate exciting results in the fields of carcinogenesis, cancer treatment by the use of biomarkers, and chemoprevention in at high risk patients of lung cancer development.

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