Purpose: To investigate the expression and significance of the expression of nucleostemin (NS) and proliferating cell nuclear antigen (PCNA) protein in non-small cell lung cancer (NSCLC).

Methods: Immunohistochemistry (streptavidin-peroxidase method) was used to detect NS and PCNA expression in 53 NSCLC samples and 15 normal lung samples.

Results: NS protein expression was detected in 54.7% (29/53) of the NSCLC samples and 0% (0/15) of the normal lung samples (p<0.01). Furthermore, the positive expression rate of PCNA was 6.67% (1/15) in normal lung samples and 71.7% (38/53) in NSCLC samples (p<0.05). Also, the NS protein expression rate was 65.2% (15/23) in adenocarcinoma tissue samples, significantly higher than that in squamous tissues, where the NS expression rate was 46.7% (14/30) (p<0.05). In addition, the NS expression rate of 42.9% (15/35) in well or moderately differentiated tumor tissues was lower than the rate of 77.8% (14/18) in poorly differentiated tumor tissues (p<0.05). The grade of differentiation had no correlation with tumor-node-metastasis (TNM) stage and lymph node metastasis (p>0.05). Also, the positive expression rate of PCNA was significantly higher in NSCLC samples than in normal lung samples (p<0.05). In addition, a positive correlation was found between NS and PCNA expression in NSCLC (p<0.05).

Conclusion: The highly valuable tumor molecular markers, NS and PCNA, had higher expression levels in NSCLC samples. Combined detection of NS and PCNA may be important for the early diagnosis of lung cancer and individualized therapy, having also an important role in predicting tumor prognosis.

Key words: non-small cell lung cancer, NS gene, nucleostemin, proliferating cell nuclear antigen

Introduction

Lung cancer has the highest incidence and mortality rate worldwide [1]. NSCLC accounts for approximately 85% of lung cancers [2]. In China, investigation and analysis of the data on incidence and mortality from 10 registries covering the past 18 years showed that the incidence of lung cancer is increasing every year, with an average annual increase rate of 1.63%, while mortality has increased by 46.5% in the past 30 years, thus seriously greatly affecting the citizens’ health. Despite major efforts to improve the therapeutic results the prognosis is still poor, the cure rate is also dissapointing, and the overall 5-year survival rate is less than 15% [3,4]. One of the most important reasons for this unwanted situation is that early diagnosis for lung cancer is very low, and more than half of the patients have advanced or terminal cancer at diagnosis [5,6]. Therefore, effective and accurate diagnosis and timely treatment of lung cancer are still important issues and...
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NS is a protein located in the nucleolus, and is found in embryonic stem cells, central nervous system stem cells, and cancer cells. Under certain conditions, it is released into the cytoplasm, and affects cell differentiation and tissue cell maturation, while it helps maintain continued proliferation of tumor cells and stem cells [5,6]. NS is a type of p53-binding protein under physiological conditions its function is related to p53 expression in the nucleolus. Research has confirmed that NS plays an important role in cell cycle regulation of stem cells and tumor cells, and that it has increased expression in a variety of tumors [7-11]. NS also plays an important role in the occurrence of lung cancer. In a study, NS expression was not only higher, but also played an important role in promoting proliferation of A549 cells [12]. Therefore, NS expression may be an important indicator for early diagnosis of lung cancer.

PCNA is a DNA polymerase-associated protein. Clinical studies have confirmed that PCNA is an essential accessory protein in DNA replication, which can directly reflect proliferative activity, and plays an important regulatory role in DNA replication; there are also some links between content changes and cell proliferation [13-16]. PCNA expression in lung cancer is controversial [17-19], and there are few studies about the joint use of NS and PCNA expression for the prediction and diagnosis of NSCLC. Therefore, this study aimed to detect and analyze the NS expression combined with the PCNA protein expression in NSCLC and adjacent tissues by using the immunohistochemical streptavidin-peroxidase method, and to provide a theoretical basis for further diagnosis and treatment of lung cancer.

Methods

Source of samples

The study included 53 patients who had undergone surgery for pathologically confirmed NSCLC between January 2010 and January 2012 in our hospital. This study was conducted in accordance with the declaration of Helsinki and after approval from the Ethics Committee of Henan Provincial Chest Hospital. Written informed consent was obtained from all patients. There were 31 men and 22 women aged between 37 and 76 years (mean 75±12.3). Among them, 25 (45.4%) patients had adenocarcinoma and 30 (56.6%) had squamous cell carcinoma. None of the patients had received radiotherapy and chemotherapy before surgery. The histological grades were as follows: 18 (34.0%) cases of poorly differentiated, 25 (47.2%) cases of moderately differentiated, and 10 (18.9%) cases of well-differentiated tumors. According to the TNM classification of the International Union Against Cancer [20], the stage distribution was as follows: 15 (28.5%) cases with stage III, 54 (64.1%) cases with stage II, and 4 (7.5%) cases with stage I. For the control group, we selected 15 specimens of adjacent (>4cm) normal lung tissue, verified on pathological examination.

Immunohistochemical staining

The sections were subjected to conventional deparaffinization followed by incubation with 3% H2O2 deionized water to eliminate the activities of endogenous peroxidase, antigen retrieval and completion with normal goat serum-containing working fluid (Shanghai qbio Science & Technologies Co., Ltd). Mouse anti-human NS monoclonal antibody (Shanghai FengShou Biological Technology Co., Ltd) and mouse anti-human PCNA monoclonal antibody (Shanghai FengShou Biological Technology Co., Ltd) were used for overnight incubation at 4°C. Then, biotin-labeled goat anti-rabbit IgG was added and the incubation continued at room temperature for 15 min, followed by incubation with horseradish peroxidase-labeled streptavidin working solution for 15 min at room temperature. Subsequently, diaminobenzidine staining was performed, followed by thorough rinsing with distilled water, hematoxylin staining, conventional dehydration, and mounting. For the negative control, phosphate-buffered saline was used to replace the primary antibody, while the other steps remained unchanged. Known positive samples were used as the positive control.

Analysis

At x200 magnification, 5 fields were randomly selected to observe positive NS protein expression in the cytoplasm and positive PCNA protein staining in the nucleus. Furthermore, 200 cells per field were selected for calculations from a total of 1000 cells.

Statistics

All data were analyzed using SPSS 17.0 software. Categorical data are presented as percent frequency of occurrence, and study groups were compared using x² test or Fisher’s exact test. Pearson’s correlation analysis was used to analyze the relation between NS and PCNA in NSCLC. A p value <0.05 denoted a statistically significant difference.

Results

NS protein expression in NSCLC and adjacent normal tissues

Immunohistochemistry results demonstrated that NS protein was present in the cytoplasm (Figure 1A). The rate of positivity for NS protein...
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Positive PCNA staining was observed in the nucleus, in the form of brown particles (Figure 1B, C). The rate of PCNA positivity was 6.67% (1/15) in adjacent normal tissues and 71.7% (38/53) in NSCLC tissues, which was higher than that observed in normal tissues (p<0.01; Table 2).

NS protein expression rate in squamous cell carcinoma and adenocarcinoma

The NS protein expression rate in squamous cell carcinoma was 46.7% (14/30), while in adenocarcinoma it was 65.2% (15/23), resulting in a significant difference (p<0.05; Table 3).

NS protein expression in highly, moderately, and poorly differentiated tissues

The NS protein expression rate in highly and moderately differentiated tissues was 42.9% (15/35), while in poorly differentiated tissues, it was 77.8% (14/18), resulting in a significant difference (p<0.05). Meanwhile, lymph node metastasis and TNM stage of patients had no significant impact on NS protein expression (p>0.05; Table 4).
Correlation between NS protein and PCNA expression

On analysis, a positive correlation was found between NS and PCNA expression in NSCLC (r = 0.79, p<0.05).

Discussion

Our findings indicate that detection of NS combined with PCNA expression could be important for the early diagnosis and treatment of patients with lung cancer, because there is a trend of significantly high NS expression and PCNA protein expression in NSCLC and adjacent tissues. In recent years, several studies have confirmed that both proteins are major factors in regulating cell proliferation [21,22].

This observation showed that NS protein expression in adjacent normal tissues was significantly lower than that observed in NSCLC tissues (Figure 1A). The possible cause for higher NS expression could be an early event caused by NSCLC. Many studies suggested that NS may have effects on cell cycle regulation through the p53 pathway, and is highly expressed in a variety of primary tumors and cancer cells, while it is not expressed in terminally differentiated cells [5]. High NS protein expression in NSCLC increases the capacity of normal cells and tissues to become malignant. On the other hand, NSCLC differentiation has no direct relationship with the NS protein. NS protein expression in highly and moderately differentiated tissues was significantly lower than that observed in poorly differentiated tissues; as the NS protein expression increased, the grade of differentiation decreased. As tumor cells exited the high NS protein expression stage, a transition in the G2/M and G1/S stages of the cell cycle was observed. During the cell cycle, tumor cells will gradually increase and block differentiation, and increase the speed of mitosis. Therefore high NS expression could be used as an indicator to evaluate the malignancy of tumor cells during malignant cell proliferation. By combining immunohistochemistry techniques with fiberoptic bronchoscopy, NS protein expression can be determined, and this would help early detection of NSCLC, thus improving the early diagnosis rate. This is consistent with recent studies suggesting that NS is not only highly expressed, but it also plays an important role in the promotion of cell proliferation [12].

On the other hand, tumor cells have strong proliferative activity, and PCNA is an indicator of cell proliferation. Hence, PCNA has been studied in many tumors in China and worldwide; many studies have evaluated the correlations between PCNA and tumor development, grade, stage, radiosensitivity, prognosis, recurrence, metastasis, cause of death, tumor markers, and other aspects [25,26]. PCNA is a cell cycle protein, which is directly involved in

![Expressions of NS protein and PCNA protein in non-small-cell lung carcinoma. A: NS high expression in adenocarcinoma (SP x200); B: PCNA high expression in adenocarcinoma (SP x400); C: PCNA high expression in squamous cell carcinoma streptavidin/peroxidase (SP x400). SP: streptavidin/peroxidase.](image-url)
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DNA replication during cell proliferation as a DNA polymerase accessory protein; its synthesis and expression are associated with the cell proliferation cycle [27,28]. PCNA expression increases in the early G1 and S phase of the cell cycle, reaches its peak in the S phase, but declines in G2; it is lowest in the mitotic phase and thus it can represent the cell proliferation status. Altered PCNA expression is one of the reasons for abnormal cell proliferation and carcinogenesis, and can cause loss of cell cycle control. Correspondingly, this result indicated that the rate of PCNA positivity in NSCLC tissues was significantly higher than that observed in the adjacent normal tissues (p<0.01) (Figure 1B,C). Therefore, PCNA expression may directly reflect the level of DNA replication activity in tumor cells, and it has an important role in determining the malignancy of tumors. In patients with NSCLC, differentiation and lymph node metastasis were closely related to the level of PCNA-positive expression [29], and, therefore, PCNA could be used to determine treatment and prognosis in patients with lung cancer.

In summary, patients with NSCLC had a trend of significantly high expression of NS protein. The PCNA-positive rate was significantly higher, and it was an important indicator of malignant proliferation of tumor cells. Both these proteins are tumor molecular markers with high clinical value. Therefore, detection of NS combined with PCNA expression could play an important role in the early diagnosis and individualized treatment of patients with lung cancer, and could also be important for determining the prognosis. Further investigation into these two proteins’ function will enable a better understanding of how to affect cancer cell development in NSCLC.

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