Gene p73: Deletions and Expression in Non-Small-Cell Lung Carcinoma Cells

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Abstract—We studied the relation between genetic anomalies in the p73 gene encoding a product structurally and functionally similar to the protein p53 and the pathogenesis of non-small-cell lung carcinoma (NSCLC; 83 patients). Loss of one of the p73 alleles was revealed in 44% (15/34) informative cases. Presence of deletions correlated with the features common for tumor development: metastatic affection of regional lymph nodes (p = 0.045), large tumor size (p = 0.037), advanced stage of the disease (p = 0.017). Allele expression of the gene p73 was studied basing on analysis of the polymorphic C/T site in the second exon. All ten studied samples of normal bronchogenic epithelium showed monoallelic expression of p73, contrary to the six NSCLC samples which preserved both of the p73 alleles and showed biallelic expression. Enhanced expression of p73 mRNA in tumor tissue compared with normal bronchogenic epithelium was found in 28 of 34 (82.4%) NSCLC patients. Expression of p73 in NSCLC cells showed correlation neither with deletions of one of the alleles, nor with any parameter reflecting clinical pathology. The results suggest that p73 is not a classical tumor suppressor gene in NSCLC. However, alterations of p73 expression are common for NSCLC. This may be important for our understanding of the NSCLC origin and development.

Key words: non-small-cell lung carcinoma, gene p73, deletions, expression, carcinogenesis

INTRODUCTION

The lung cancer is at present the most widespread form of malignancy and becomes more and more frequent [1]. This disease is usually of aggressive type and has bad prognosis, therefore a search for the genes involved in tumor origin and progression appears one of the important tasks in molecular oncobiology. The development of various malignancies and carcinomas of the lung is known to be related to impairment of oncogenes and tumor suppressor genes [2]. Lung carcinoma often (70% patients) shows inactivation of the gene p53, usually caused by deletions or point mutations [3].

The first homolog of the gene p53, gene p73 has recently been identified [4, 5]. Protein p73 shows considerable similarity with p53 in three domains: transactivation domain, DNA-binding domain, and oligomerization domain [4, 6, 7]. As shown in in vitro experiments, protein p73 similarly to p53 is able to activate transcription of the negative cell-cycle regulator p21<sup>wt</sup> and to induce apoptosis [5]. Activation of protein kinase c-Abl induced in the cell by DNA-damaging agents resulted in induction of apoptosis involving both p53- and p73-dependent mechanisms [8–11].

Mutations of the p73 gene in human tumor cells are extremely rare [12–16]. Extensive search revealed no p73 mutations in lung tumors [17, 18]. At the same time, p73 is located at the chromosomal locus 1p36.33 [4], which forms a part of the minimal deletion overlap region (1p36.2–1p36.3) recently identified by us for non-small-cell lung carcinoma (NSCLC) [19]. Some data show monoallelic expression of p73 in normal bronchogenic epithelium [18], though the existing data are contradictory. Therefore, deletion of the untranscribed allele of the gene p73 probably affects the expression of this gene and contributes to development of NSCLC.

In this work we studied monoallelic deletion of the gene p73 and expression of p73 mRNA in the cells of NSCLC and of normal bronchogenic epithelium.

EXPERIMENTAL

Tumor samples and isolation of nucleic acids. The samples of lung tumors and normal lung tissue were obtained from 83 NSCLC patients (50 cases of flat-cell carcinoma and 33 cases of adenocarcinoma) operated at the Cancer Research Center, Russian Academy of Medical Sciences, in 1993–1998; none of the patients received chemotherapy or X-ray treatment before operation. Immediately after lung resection, tissue samples were frozen and stored in liquid nitrogen. All cases of malignancies were classified by
DNA was isolated as described in [20]. Isolation of nucleic acids. The Tri-reagent solution (Molecular Genetic Center Inc., USA) was used to isolate RNA as recommended by the manufacturer.

For p73 expression study, we found significant correlation of a deletion in the second exon of p73 gene [4]. Monoallelic deletions were detected in 44% informative cases (15/34); allele T deletion was somewhat more frequent than allele C deletion, six and nine cases, respectively. The results obtained are in good agreement with the data on the deletion frequency in the region where p73 is located [19]. We analyzed the relation between deletion of one of the p73 alleles with conventional clinical and pathological parameters: age and sex of the patients, histological type, differentiation and size of carcinoma, regional lymph nodes metastasis, and stage of the disease.

No correlation was revealed for deletion of an allele in p73 and either patient age, patient sex, tumor histological type, or tumor differentiation.

RESULTS AND DISCUSSION

Our study of the gene p73 monoallelic deletion frequency and allele-specific expression was based on the presence of a polymorphic C/T site in the second exon of this gene [4]. Monoallelic deletions were studied using 83 NSCLC samples (Fig. 2a). Deletions of a p73 allele were detected in 44% informative cases (15/34); allele T deletion was somewhat more frequent than allele C deletion, six and nine cases, respectively. The results obtained are in good agreement with the data on the deletion frequency in the region where p73 is located [19]. We analyzed the relation between deletion of one of the p73 alleles with conventional clinical and pathological parameters: age and sex of the patients, histological type, differentiation and size of carcinoma, regional lymph nodes metastasis, and stage of the disease.

No correlation was revealed for deletion of an allele in p73 and either patient age, patient sex, tumor histological type, or tumor differentiation. However, we found significant correlation of a deletion in p73 with the features characterizing tumor progression: affection of the regional lymph nodes (N1–N3, \( P = 0.045 \)), large tumor size (T3–T4, \( P = 0.037 \)), and advanced stage of the disease (stage III–IV, \( P = 0.017 \)) (table). From these results we suppose that one of the p73 alleles is lost mainly at the later stages of tumor progression.

**Fig. 1.** Positioning of primers to amplify various fragments of the gene p73.

![Diagram of primer positioning](image-url)