Gene \textit{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 \textit{p73} gene encoding a product structurally and functionally similar to the protein \textit{p53} and the pathogenesis of non-small-cell lung carcinoma (NSCLC; 83 patients). Loss of one of the \textit{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 \textit{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 \textit{p73}, contrary to the six NSCLC samples which preserved both of the \textit{p73} alleles and showed biallelic expression. Enhanced expression of \textit{p73} mRNA in tumor tissue compared with normal bronchogenic epithelium was found in 28 of 34 (82.4\%) NSCLC patients. Expression of \textit{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 \textit{p73} is not a classical tumor suppressor gene in NSCLC. However, alterations of \textit{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 \textit{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 \textit{p53}, usually caused by deletions or point mutations [3].

The first homolog of the gene \textit{p53}, gene \textit{p73} has recently been identified [4, 5]. Protein \textit{p73} shows considerable similarity with \textit{p53} in three domains: transactivation domain, DNA-binding domain, and oligomerization domain [4, 6, 7]. As shown in \textit{in vitro} experiments, protein \textit{p73} similarly to \textit{p53} is able to activate transcription of the negative cell-cycle regulator \textit{p21^{waf}} 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 \textit{p53}- and \textit{p73}-dependent mechanisms [8–11].

Mutations of the \textit{p73} gene in human tumor cells are extremely rare [12–16]. Extensive search revealed no \textit{p73} mutations in lung tumors [17, 18]. At the same time, \textit{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 \textit{p73} in normal bronchogenic epithelium [18], though the existing data are contradictory. Therefore, deletion of the untranscribed allele of the gene \textit{p73} probably affects the expression of this gene and contributes to development of NSCLC.

In this work we studied monoallelic deletion of the gene \textit{p73} and expression of \textit{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
the TNM system according to the International Anti-
cancer Organization rules (UICC, version of 1989).

The tissues were analyzed histopathologically and the malignancies were identified at the Institute of Clinical Oncology, Cancer Research Center. Serial tissue sections were prepared, first and last slides were stained with hematoxylin–eosine. Stained sections were compared with parallel unstained ones.

The cells from the region of interest on a section were removed from the glass with scalpel and used for isolation of nucleic acids. The Trireagent solution (Molecular Genetic Center Inc., USA) was used to isolate RNA as recommended by the manufacturer. DNA was isolated as described in [20].

**Study of p73 allelic deletion.** The deletions were studied using a polymorphic C/T site in the second exon of the p73 gene [4]. The polymorphic region was amplified by PCR with primers P1 (5'-caggaggacagag-
cacgag-3') and P2 (5'-cgaaggtggctgaggctag-3') (Fig. 1). The reaction mixture (50 μl) contained 100–200 ng DNA, 150 ng each primer, 100 μM each nucleotide, 1.5 μCi [α-33P]dCTP, buffer ×1 for Taq polymerase, 1.5 μM MgCl2, and 2.5 units Taq polymerase (BioMaster). The reaction was run as follows: first 94°C, 5 min; then 30 cycles of 94°C, 30 s; 61°C, 30 s; 72°C, 30 s; last 72°C, 10 min. The PCR product (3 μl) was treated with restriction endonuclease StyI which cleaves specifically only the T but not the C allele of p73. The restriction fragments were separated by 6% PAGE and exposed onto X-ray film. The deletions were detected by 50% or lower intensity of the signal (compared with the signal from normal tissue DNA of the same patient) indicating the presence in the tumor sample of only one of the two DNA alleles.

**Study of allele-specific expression of p73.** Reverse transcription was run with 2 μg total RNA and reverse transcriptase of the murine breast tumor virus (Gibco BRL) according to manufacturer's instructions. One fifth of the reverse transcription reaction product (5μl) was used for PCR. The polymorphic site of the second p73 exon was amplified by PCR with primers P3 (5'-gacggctccagaggtc-3') and P4 (5'-gagagctccagaggtc-3') (Fig. 1) as follows: 25 cycles, 94°C, 30 s; 61°C, 30 s; 72°C, 30 s; then 2 μl of the PCR product was reamplified under the same conditions in the presence of 1.5 μCi [α-33P]dCTP. The resulting PCR product (3 μl) was digested with endonuclease StyI. The restriction fragments were separated by 6% PAGE, the gel was dried and exposed with X-ray film.

**Semiquantitative PCR** was run with one fifth part of the reverse transcription product (5 μl) Primers P5 (5'-aagctgcccacaccggag-3') and P6 (5'-ggcgttcgctg-
ccctaca-3') were used to amplify the p73 product (Fig. 1); primers P7 (5'-acgtgcctgctgctgtaa-3') and P8 (5'-tccaccacggtgctgta-3') were used to amplify a control fragment of the gene encoding glyceralde-
hyde-3'-phosphate dehydrogenase. The reaction was run as follows: 25 cycles, 94°C, 30 s; 61°C (55°C for the control), 30 s; 72°C, 30 s in the presence of 1.5 μCi [α-33P]P]dCTP. The PCR products were separated by 6% PAGE, the gel was dried and exposed with the X-ray film. Autoradiographs were scanned and analyzed using the Image Quant software for Windows, version 3.3, and Excel for Windows, version 7.0.

The two-sided exact Fisher test was used for statistical analysis of the results.

**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.