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\textsuperscript{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 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
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'–caggaggcagag-
cagac-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 x1 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
claves specifically only the T but not the C allele of
p73. The restriction fragments were separated by 6% PAGE in nondenaturing conditions, the gel was dried
and exposed onto X-ray film. The deletions were
detected by 50% or lower intensity of the signal (com-
pared 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 poly-
morphic site of the second p73 exon was amplified by
PCR with primers P3 (5'–gagagctcagaggtgctc-3')
and P4 (5'–gagagctcagaggtgctc-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'–aactgctccaaccacccagag-3') and P6 (5'–gacctgcatgc-
cctcatac-3') were used to amplify the p73 product
(Fig. 1); primers P7 (5'–aacctgccctgcttagaa-3') and
P8 (5'–tccacccggtgcgtgta-3') were used to amplify a
control fragment of the gene encoding glyceralde-
yde-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]dCTP. The PCR products were sepa-
rated 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, ver-
sion 7.0.

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

RESULTS AND DISCUSSION

Our study of the gene p73 monoallelic deletion fre-
cuency 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 fre-
quent than allele C deletion, six and nine cases,
respectively. The results obtained are in good agree-
ment 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 parame-
ters: age and sex of the patients, histological type, dif-
ferentation 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.