Growing clinical evidence for the interaction of the p53 genotype and response to induction chemotherapy in advanced non–small cell lung cancer

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Objective: The objective of this study is to establish clinical evidence that the p53 genotype can serve as a predictive marker for response to cisplatin-based induction therapy.

Methods: Patients with advanced non–small cell lung cancer who had received neoadjuvant chemotherapy in the context of a prospective phase II trial were analyzed for the p53 genotype of their tumors. Response to induction therapy was then correlated to the p53 genotype as assessed by complete direct DNA sequencing. Patients had received 3 cycles of cisplatin and etoposide, and 1 cycle of simultaneous radiochemotherapy. All 3 treatment components mediate their cytotoxic effect through induction of apoptosis, which is suggested to require an intact p53 gene. In addition, the results from a previously published hypothesis-finding study are updated to demonstrate the consistency of clinical results and summarize currently available clinical evidence.

Results: In the phase II trial, 35 patients underwent resection after induction chemotherapy, allowing a pathohistologic response assessment. The presence of a mutant p53 genotype was highly indicative of resistance to induction chemotherapy ($P < .002$). The sensitivity of a mutant p53 genotype to identify nonresponders was 94% (71.3–99.9 confidence interval). A normal p53 gene was significantly associated with radical resection ($P < .004$) and survival advantage ($P = .02$).

Conclusion: This is the second clinical evaluation demonstrating a significant relation between p53 genotype and response to induction therapy in non–small cell lung cancer. We conclude that the p53 genotype should be evaluated as a predictive marker for response to induction therapy in prospective randomized protocols.

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Presented in part at the Forty-first Annual Meeting of the American Society of Clinical Oncology, Orlando, Florida, May 13–15, 2005.

Supported by national grant: Medizinisch wissenschaftlicher Fonds des Bürgermeisters der Bundeshauptstadt Wien, number 2495.

Received for publication May 2, 2007; revisions received Sept 26, 2007; accepted for publication Oct 22, 2007.

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J Thorac Cardiovasc Surg 2008;135:1036–41

0022-5222/\$34.00

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doi:10.1016/j.jtcvs.2007.10.072

Induction chemotherapy as treatment strategy for advanced stage non–small cell lung cancer (NSCLC) remains controversial. Despite the advantages of neoadjuvant chemotherapy (reduction of tumor burden, activity against micrometastases, patient compliance, response assessment), it is not yet widely accepted as standard treatment in advanced NSCLC.¹ Multiple randomized phase III trials have shown that the use of inductive cisplatin-based chemotherapy or chemoradiation before surgery can improve survivals up to 30% at 3 years.^{2–7} In an update of a systematic review, a 12% relative survival benefit equivalent to an absolute improvement in survival of 5% at 5 years was suggested.⁸ However, the beneficial effect of neoadjuvant chemotherapy seems to be restricted to responders only. In a recently published large randomized trial (MRC LI22/NVALT"/EORTC 08012), neoadjuvant chemotherapy resulted in a good response rate in 49% of patients.⁸ However the beneficial effect of neoadjuvant chemotherapy appears to be restricted to responders only. This implies that approximately 50% of patients with NSCLC currently do not benefit from standard neoadjuvant therapy. The efficacy of chemotherapy needs to be improved, and the identification of predictive markers is a promising strategy. Tumor-specific

Abbreviations and Acronyms

IHC = immunohistochemistry
 NSCLC = non-small cell lung cancer

genetic alterations affecting possible pathways for chemotherapy resistance may serve as candidates to individualize and improve treatment.

The p53 gene is a well-known candidate gene for predicting response to chemotherapy. It is generally accepted that the cytotoxicity of drugs interacting with DNA is not mediated through DNA damage itself but through induction of apoptosis. DNA damage is the strongest trigger for the activation of the p53 gene, which crucially controls the apoptotic pathway.⁹⁻¹¹ Drugs such as cisplatin and ifosfamide, etoposide, doxorubicin, cyclophosphamide, and gemcitabine, as well as radiation treatment, damage the DNA and consequently activate p53. Inactivation of the p53 gene by mutation may affect response to certain chemotherapies.^{12,13} Because the p53 gene seems to be the most frequent mutated gene associated with cancer, this hypothesis was addressed in various studies.¹⁴⁻¹⁶ In an attempt to avoid DNA sequencing, p53 was analyzed by immunohistochemistry (IHC) or screening techniques in the majority of these studies. The sensitivity of these methods to detect p53 mutations varies; consequently, inconsistent findings have been published. In the meantime, DNA sequencing is accepted as the gold standard for p53 testing.¹⁷ In vitro studies consistently show that the p53 genotype plays an important role for response to treatment.^{14,18} However, clinical results are still rare.

In 1999 we published a small hypothesis-finding study showing for the first time a strong relationship between the presence of a normal p53 gene and response to cisplatin/ifosfamide-based induction chemotherapy in 25 patients with NSCLC.¹⁶ For that study, we retrospectively recruited the patients at the University of Vienna; therefore, the study generated level-of-evidence 3b data (retrospective cohort study).*

The aim of the current study was to advance the clinical evidence level and provide level-of-evidence 2b data by analyzing a study cohort that had been recruited prospectively. Therefore, patients with advanced NSCLC who had received neoadjuvant chemotherapy in the context of a prospective phase II trial at the University of Essen, Germany, were analyzed.¹⁹ The induction treatment consisted of 3 cycles of cisplatin and etoposide and 1 cycle of simultaneous radiochemotherapy. Because the cytotoxic effect of all 3 treatment components is based on their induction of DNA damage, this cohort seemed suitable for testing the hypothesis.

This study adds clinical evidence to the suggested interaction between the p53 genotype and the chemotherapy response. In addition, our previously published hypothesis-finding study is updated to demonstrate the consistency of clinical results and summarize currently available clinical data because the number of patients is still small.

Materials and Methods

In 1999, Stamatis and coworkers¹⁹ reported the results of a prospective phase II trial that included 56 patients with stage IIIA and IIIB NSCLC. In this trial, patients received 3 cycles of cisplatin (60 mg/m² days 1 + 7) and etoposide (150 mg/m² days 3–5), followed by 1 cycle of simultaneous radiochemotherapy (cisplatin 50 mg/m² days 2 + 9, etoposide 100 mg/m² days 4–6, thoracic irradiation 45 Gy). After induction therapy, 35 of 56 patients were surgically resectable. For the present analysis, tumor material and response data of the 35 surgically resected patients were provided for p53 analysis.

Patient data and genetic material were handled at the p53 research laboratory at the Surgical Research Laboratories of the Medical University of Vienna under the oversight of the university's ethics committee. The laboratory is officially certified by the national ministry of health in performing molecular genetic analyses of the p53 gene and handling patient data.

Archived paraffin-embedded tumor material from the diagnostic biopsies of patients who were included in the prospective phase II trial was sent to our laboratory for p53 sequencing. The sequencing results were then correlated to the response data provided. As a control, the provided response data were compared with the data from the pathohistologic reports.

Response Category

The 4 response evaluation criteria in solid tumors (RECIST categories)²⁰—complete remission, partial remission, stable disease, and progressive disease—were grouped in responders (including complete remission and partial remission) and nonresponders (including stable disease and progressive disease) for statistical evaluation.

Statistical Methods

The 2 (grouped) categories of response to induction treatment (responders, nonresponders) were correlated with the p53 genotype (mutated or normal).

The data in the various categories were described with frequencies and percentages, and corresponding associations among the data were tested with chi-square tests. In cases in which only few data were available, a Fisher exact test was calculated for 2 × 2 tables and an exact Pearson test was calculated for 2 × 3 tables. The diagnostic value of the p53 gene with regard to response to induction treatment was assessed with sensitivity, specificity, and corresponding 2-sided 95% confidence intervals. In cases of 100% sensitivity or specificity, corresponding 1-sided 95% confidence intervals were provided. Survival data are graphically described with Kaplan-Meier curves and tested with log-rank test and Wilcoxon tests.^{21,22} All *P* values are 2-sided.

p53 Gene Sequencing

Complete DNA sequencing was carried out to assess the p53 genotype and identify mutations in the p53 gene using complete direct DNA sequencing. Tumor DNA was prepared from sections from

*Oxford Centre of Evidence-based Medicine levels of Evidence, May 2001: www.cebm.net

TABLE 1. Characteristics of investigated patient cohorts

	Hypothesis-finding study	Actual study	Combined evaluation
	Cisplatin/ifosfamide	Cisplatin/etoposide/radiation	
No. of patients	24	35	59
Mean age (y)	59.3 ± 10.7	54.9 ± 7.9	56.9 ± 9.3
Median follow-up (mo)	43.6	64.7	53.2
No. of p53 mutations	8 (33%)	11 (31%)	19 (32%)

The actual study describes the new patient cohort. The hypothesis-finding study contains updated information on our previously published data.

tumor tissues that were obtained in the course of diagnostic biopsies or surgery. The methods of DNA extraction, polymerase chain reaction amplification of the p53 gene, and purification of the polymerase chain reaction product have been published.¹⁶

Sequencing with the ABI Prism 310

Direct sequencing was done using the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems, Foster City, Calif). Amplification was performed in a total volume of 10 μ L for 25 cycles using 2 μ L of purified polymerase chain reaction product, 2 pmol of primer (sense or antisense), and 2 μ L of Terminator Ready Reaction Mix. For T1 Thermocycler (Biometra, Göttingen, Germany), the following thermal profiles were used: 10-second denaturation at 96°C, 5-second annealing at 50°C, and 4-minute extension at 60°C. Sequence reaction was then purified by Centri Sep spin columns (Princeton Separations P/N CS-901, Adelphia, NJ). Sequence analysis was performed with the ABI Prism 310 Genetic Analyzer (PE Applied Biosystems).

Results

Patient Characteristics

Patient characteristics are shown in Table 1. Patients of the actual prospective study differed from patients of the hypothesis-finding study in that the proportion of squamous cell carcinoma was higher (74% vs 50%). No significant association between histologic type and p53 mutation could be found (Table 2). The proportion of patients with more advanced disease (stage IIIB) was higher in the actual study cohort compared with the hypothesis-finding study cohort. The

summarized analysis of the 59 patients reveals that mutations in the p53 gene are more frequently present in the advanced stage (IIIB) (Table 2), which is consistent with the literature (International Agency for Research on Cancer database).

Predicting the Response to Induction Treatment

We identified a strong correlation between patients with a normal p53 gene and an effective response to cisplatin-based induction treatment in both studies (Table 3). A mutant p53 genotype was highly indicative of resistance to the induction treatment regimens used in the 2 studies (cisplatin/ifosfamide in the hypothesis-finding study and cisplatin/etoposide/radiation in the actual study).

The sensitivity of predicting response to treatment by p53 status was as high as 94% in the actual study compared with 100% in the hypothesis-finding study and 97% when both studies were considered together. A normal p53 gene was significantly associated with radical resection in both studies (Table 3).

Overall Patient Survival

Patients with a normal p53 gene had a significant survival advantage over patients with mutated p53 (Figure 1).

Characterization of p53 Mutation

Table 4 characterizes the p53 mutations found in the actual study: Three mutations (numbers 864, 874, and 875) were found to result in the formation of a STOP codon (representing

TABLE 2. Association of clinicopathologic variables and p53 genotype

	Hypothesis-finding study			Actual study			Combined evaluation		
	No	Yes	P	No	Yes	P	No	Yes	P
Histologic type									
Adeno	7	4		7	1		14	5	
Squamous	8	4		16	10		24	14	
Large cell	1	0	NS	1	0	NS	2	0	NS
Stage									
IIIA	11	5		18	4		31	10	
IIIB	5	3	NS	6	7	.057	9	9	.053

NS, Not significant.

TABLE 3. P53 genotype in relation to response to neoadjuvant therapy and radical resection

	Hypothesis-finding study			Actual study			Combined evaluation		
	p53 normal	p53 mutant	P value	p53 normal	p53 mutant	P value	p53 normal	p53 mutant	P value
Pathologic response									
Responder	16 (100%)	0		16 (94%)	1 (6%)		32 (97%)	1 (3%)	
Nonresponder	0	8 (100%)	<.001	8 (44%)	10 (56%)	.002	8 (31%)	18 (69%)	<.001
	16	8		24	11		40	19	
Sensitivity	100% (16/16)	CI: 82.9–100		94% (16/17)	CI: 71.3 – 99.9		97% (32/33)	CI: 84.3–99.9	
Specificity	100% (8/8)	CI: 68.7–100		56% (10/18)	CI: 30.7 – 78.5		69% (18/26)	CI: 48.2–85.7	
Radical resection									
Yes	16 (100%)	4 (50%)		17 (90%)	2 (10%)		33 (85%)	6 (15%)	
No	0	4 (50%)	.007	7 (44%)	9 (56%)	.004	7 (35%)	13 (65%)	<.001
	16	8		24	11		40	19	

CI, Confidence interval.

2 frameshifts and 1 nonsense mutation), which is deleterious for the biologic activity of the p53 gene.

One mutant was found to be a silent mutation (number 842), which does not alter the amino acid sequence of the encoded protein. Therefore, the affected patient was considered as “p53 normal” for our analyses.

Discussion

The present publication clinically demonstrates a significant relation between the p53 genotype and the response to induction therapy. In this study, the “p53 genotype” is analyzed by complete DNA sequencing and shows consistent predictive power compared with our previously published hypothesis-finding study. Both studies suggest that the p53 genotype could serve as a predictive marker for a patient’s response to cisplatin-based induction therapy in advanced NSCLC.

P53 Analysis Method

The impact of the p53 gene on the response to chemotherapy has been addressed in vitro and in some clinical trials. However, because of conflicting results obtained in previous studies, p53 has not been considered a useful predictive marker in NSCLC.²³

Several factors account for the conflicting results. First, a wide range of techniques have been used for p53 analysis to avoid complete direct sequencing of the whole gene. Different electrophoresis-based screening techniques have been applied in selecting patients to be sequenced. Additional sequencing, if applied, was often restricted to parts of the gene (exons 5–8). Therefore, the mutation frequency is likely to be underreported in such studies.

The use of p53 IHC is based on the observation that missense mutations alter the protein structure, inhibiting rapid degradation of the protein. Therefore, accumulation of p53 protein was suggested to indicate the presence of p53 gene mutations. Different antibodies and cutoffs were used to determine a tumor as p53-accumulation positive. Apart from this lack of standardization, the sensitivity and specificity

of IHC are affected by false-positive and false-negative IHC results: Accumulation of the p53 protein can occur as a result of physiologic stabilization or overexpression resulting from genotoxic stress. Because most antibodies are not able to discriminate between wild-type and mutant p53 protein, this will result in false-positive IHC. Complete absence of p53 protein can be caused by deleterious gene mutations. The latter prevent the transcription of the gene and result in false-negative IHC results.²⁴

Because of the inherent bias concerning the analytic techniques that have been used, the results of existing studies assessing the predictive value of p53 are of limited value. In our recent review of the literature on the predictive value of p53 in the treatment of breast cancer, response to treatment seems to be consistent and linked to the p53 sequencing results but not to IHC.¹⁷

Complete direct sequencing of the entire p53 gene (exons 2–11) is currently the most reliable, standardized, and sensitive method to evaluate p53 gene mutations.

Chemotherapy Resistance in NSCLC

d’Amato and colleagues²⁵ recently published preclinical data on the resistance of NSCLC tumor cell cultures to several chemotherapy agents. They found that tumor cells were frequently resistant to at least 1 agent in chemotherapy doublet regimens and expressed a possible association between drug resistance and p53 overexpression. They observed that many tumors exhibit simultaneous resistance to platinum agents and gemcitabine. Because both drugs act via induction of DNA damage, these findings support the hypothesis that functional p53 is required to induce apoptosis as a result of DNA damage. The reported resistance rates of 60% to 80% can be explained by the high p53 mutation rates frequently observed in cell cultures.²⁶

Conclusions

We provide clinical evidence that a normal p53 genotype can identify patients with NSCLC who will benefit from standard

A

B

C

Figure 1. Survival benefit after neoadjuvant therapy is related to a normal p53 genotype in both clinical evaluations, A, Four-year update of the retrospective, hypothesis finding study; B, Prospective, actual study; C, Combined calculation of 59 patients.

TABLE 4. Characterization of p53 mutations in the actual study cohort

Patient No.	TP53 mutant	Mutant codon
835	c.578A>T (p.His193Leu)	CTT
836	c.743G>T (p.Arg248Leu)	CTG
842	c.489C>T (p.Tyr163Tyr)	TAT silent*
850	c.746G>T (p.Arg249Met)	ATG
857	c.524G>A (p.Arg175His)	CAC
859	c.747G>T (p.Arg249Ser)	AGT
860	c.461G>T (p.Gly154Val)	GTC
864	c.521 537del (p.Arg174_Arg 179fsX6)	Frameshift
872	c.746G>T (p.Arg249Met)	ATG
874	c.731del G (p.Gly244 AlafsX3)	Frameshift
875	c.511G>T (p.Glu171X)	TAG
876	c.747G>T (p.Arg249Ser)	AGT

*This mutation does not alter the amino acid residue and therefore was calculated as p53 normal. However, this alteration can affect mRNA splicing, stability, and so forth, and clinically the patient did not respond to chemotherapy.

cisplatin-based induction treatment. We demonstrate that the presence of a normal p53 gene results in response to induction therapy. In the group of responders, we observed a high rate of radical resections and prolonged overall survival.

On the basis of the consistent results of our clinical analyses, we conclude that the p53 genotype should be evaluated as a predictive marker in randomized, neoadjuvant protocols for NSCLC. Upcoming protocols should consider the technical aspects of p53 analysis and allow correct response assessment.

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