Expression of mutant p53 in oral squamous cell carcinoma is correlated with the effectiveness of intra-arterial chemotherapy

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Abstract. The aim of the present study was to evaluate the correlation between the positive expression rate of mutant p53 and the clinical characteristics of patients with oral squamous cell carcinoma (OSCC), as well as the effectiveness of intra-arterial chemotherapy. Expression of mutant p53 in tumor tissues was determined by immunohistochemical analysis of 51 OSCC patients, prior to and following intra-arterial chemotherapy. Prior to intra-arterial chemotherapy, mutant p53 positive rates in patients with higher pathological grades were significantly higher than those of the patients with lower pathological grades. The mutant p53 positive rate in patients with lymph node metastasis was 73% (19/26), which was significantly higher than that of the patients without lymph node metastasis (20%, 5/25). Mutant p53 was expressed in 17% (3/18) of clinical phase II patients, while 64% (21/33) of clinical phase III and IV patients exhibited positive expression of mutant p53 ($P<0.05$). The mutant p53 positive rate in chemotherapy non-responsive patients was 69% (11/16), which was significantly higher than that in the chemotherapy-responsive patients (37%, 13/35). Mutant p53 positive rates were not significantly correlated with age, gender or the location of the tumor. The mutant p53 positive rate prior to chemotherapy was 47% (24/51), and decreased to 18% (9/51) following chemotherapy. Expression of mutant p53 was decreased in all 13 (100%) chemotherapy-responsive patients, while only 5 (45%) chemotherapy non-responsive patients exhibited reduced expression levels of mutant p53 ($P<0.05$). In conclusion, mutant p53 has a significant role in the differentiation, development and treatment guidance of OSCC. Intra-arterial chemotherapy with 5-fluorourcil and carboplatin potentially exerts a therapeutic effect by reducing the expression of mutant p53.

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Introduction

Oral squamous cell carcinoma (OSCC) is the most common tumor of the head and neck in India (1) and is the most prevalent oral malignancy worldwide, with ~274,300 cases diagnosed annually (2). Increasing attention has been paid to OSCC due to its high risk of malignancy and invasion, rapid metastasis and increasing occurrence. Although a variety of novel approaches have been proposed for the treatment of OSCC, surgery remains the most effective strategy (3), while radiotherapy and chemotherapy are recommended for malignancies. Chemotherapy has been widely applied as a significant component of comprehensive treatment for OSCC. Surgery complemented with neoadjuvant chemotherapy is the most common comprehensive treatment program for OSCC. Neoadjuvant chemotherapy may achieve short-term clinical remission of tumors and reduce the size of the primary tumor (4), thereby enhancing tumor resection rate, increasing the negative rate of the resection edge, inhibiting tumor metastasis (5), reducing postoperative tissue defects and improving the quality of life of patients, particularly those requiring oral and maxillofacial surgery.

Regional arterial infusion may achieve a high concentration of anticancer drugs surrounding the tumor, and maximize the apoptotic effect on tumor cells without apparent toxicity. Therefore, this treatment strategy provides a precise, simple, long-term, low-toxicity and highly effective method of drug administration (6). Eckardt *et al* (7), achieved marked clinical remission for patients with advanced head and neck squamous cell carcinoma by utilizing regional arterial infusion chemotherapy. A combination of 5-fluorourcil (5-Fu) and carboplatin has been demonstrated to be an effective treatment regimen for OSCC (8,9). Regional arterial infusion chemotherapy with 5-Fu and carboplatin for the treatment of OSCC has previously been utilized by our group, and achieved marked clinical efficacy (10,11).

Various studies have demonstrated that mutation of the p53 gene has a significant role in tumor development (12-17). When DNA is damaged by physical and/or chemical factors, p53 gene transcription is increased and wild-type p53 protein is concentrated, which results in arrest of the cell cycle at G1/S phase and apoptosis of cells with cancerous characteristics (18). When the p53 gene is mutated, mutant p53 protein loses its cancer inhibition function and promotes the trans-

formation of normal cells to malignant cells. Mutant p53 is present in almost all types of human tumor (12,19-21), and is closely correlated with the development of OSCC. However, to the best of our knowledge, the effect of neoadjuvant chemotherapy on the expression of mutant p53 has not previously been investigated. Therefore, the present study aimed to determine the expression of mutant p53 in patients with OSCC prior to and following intra-arterial chemotherapy, in order to provide novel approaches for the treatment of OSCC.

Patients and methods

Patients and samples. A total of 51 patients with OSCC diagnosed at the First Affiliated Hospital of Chongqing Medical University (Chonqing, China) between 2001 and 2011 were included in the present study. The following criteria were required for the patients to be included in the present study: i) All patients had a complete clinical history; ii) all patients were recently diagnosed with OSCC by biopsy; iii) biopsy specimens were maintained and available; and iv) chemotherapy or other treatments had not been applied prior to diagnosis. Following diagnosis, an arterial chemotherapy pump was implanted by superficial temporal artery retrograde intubation. Carboplatin (300 mg/m^2; Qilu Pharmaceutical Co., Ltd., Jinan, China) on the first day, and 5-Fu (500 mg/m^2; Shanghai Xudong Haipu Pharmaceutical Industry Co., Ltd., Shanghai, China) from the first day to the fifth day, were delivered by arterial infusion. Measurable lesions were determined by computed tomography (CT) and magnetic resonance imaging (MRI). Chemotherapeutic efficacy was divided into: Complete response (CR), partial response (PR), stable disease (SD) and progressive disease (PD), according to World Health Organization (WHO) criteria (22). All patients received two cycles of treatment prior to the evaluation of therapeutic efficacy. CR and PR were considered to be responsive, while SD and PD were considered to be non-responsive. Following two cycles of chemotherapy, primary cancer resection and radical neck dissection surgery were performed, primary cancer and neck metastases were determined by pathological examination. Tissue specimens were fixed with 10% formalin (Biyuntian Biotech Co., Ltd., Shanghai, China), embedded in paraffin (Biyuntian Biotech Co., Ltd.) and sectioned into 4-μm slices prior to hematoxylin-eosin (Biyuntian Biotech Co., Ltd.) and immunohistochemical staining.

Clinical data. Clinical data, including age, gender, symptoms, disease duration, surgical records (resection range and involvement of the surrounding tissues) and imaging data, were obtained from the patients' original medical records. The average age of the 51 patients with OSCC (male, 37; female, 14) was 55.5±10.6 years. Complete resection of the tumors was performed for all cases. Based on the location of the tumor, there were 15 cases of buccal cancer, 14 cases of mouth floor cancer, 12 cases of tongue cancer, 5 cases of palate cancer and 5 cases of gum cancer. According to the WHO histological classification of OSCC (23), there were 19 well-differentiated, 21 moderately-differentiated and 11 poorly-differentiated cases of squamous cell carcinoma. Post-operative pathological examination confirmed that 26 cases possessed cervical lymph node metastasis, while the remaining 25 cases did not. Union for International Cancer Control (UICC) clinical phase classification (24) revealed that there were 18 cases of phase II, 27 cases of phase III and 6 cases of phase IV cancer. A total of 35 cases were responsive to chemotherapy, while 16 cases were non-responsive. The present study was approved by the Medical Ethics Committee at the First Affiliated Hospital of Chongqing Medical University. All patients provided written informed consent prior to undergoing chemotherapy.

Immunohistochemistry. Tissue paraffin blocks of each specimen were divided into 4-μm sections. The sections were then dewaxed by placing in pure xylene (Biyuntian Biotech Co., Ltd.) at 60˚C for 10 min, followed by xylene at room temperature for 3 min. Subsequently, the sections were dehydrated in 100, 95, 80 and 75% alcohol (Biyuntian Biotech Co., Ltd.) (3 min at each concentration). Sections were then rinsed with distilled water for 3 min until they were clean and transparent. The endogenous peroxidase activity was blocked by placing the sections in 3% hydrogen peroxide (Biyuntian Biotech Co., Ltd.) for 10 min, followed by one rinse with distilled water and three rinses with phosphate-buffered saline (PBS; Biyuntian Biotech Co., Ltd.). Immunohistochemical staining of mutant p53 was performed by incubating the sections with mouse anti-human mutant p53 monoclonal antibody (cat. no. 0010-2; 1:200; Fuzhou Maixin Biotech., Co., Ltd., Fuzhou, China) overnight at 4˚C, biotinylated rabbit anti-mouse immunoglobulin G (cat. no. 9710; 1:10; Fuzhou Maixin Biotech., Co., Ltd.) for 30 min at room temperature and streptavidin-horseradish peroxidase and diaminobenzidine solution (Zymed, San Francisco, CA, USA) for 30 min at room temperature. The sections were stained with hematoxylin and mounted with neutral gum (Biyuntian Biotech Co., Ltd.). Cells with brown particles in the nucleus were considered to be mutant p53 positive. Based on the number of mutant p53-positive cells, the specimens were classified into the following categories: Mutant p53-negative, no brown particles observed; weakly positive (+), <30% cells were mutant p53-positive; moderately positive (++), 30-70% cells were mutant p53-positive; and strongly positive (+++), >70% cells were mutant p53-positive.

Statistical analysis. The χ^2 test was performed for statistical analysis using SPSS 10.0 (SPSS, Inc., Chicago, IL, USA). $P<0.05$ was considered to indicate a statistically significant difference.

Results

Correlations between mutant p53 expression and clinicopathological features of OSCC. Mutant p53 was expressed mainly in the nucleus of tumor cells in OSCC specimens, and thus the nuclei were stained brown (Figs. 1-3). Mutant p53 was not expressed in well-differentiated OSCC. Expression of mutant p53 occurred in 55% of moderately-differentiated OSCC cases and 100% of poorly-differentiated OSCC specimens ($P<0.05$). The mutant p53-positive rate in patients with lymph node metastasis was 73% (19/26), which was significantly higher than that in the patients without lymph node metastasis (20%, 5/25; $P<0.05$). Mutant p53 was expressed in

Table I. Expression of mutant p53 and its association with the clinicopathological features of patients with oral squamous cell carcinoma.

		p53 expression		
Parameter	Cases, n	Negative	Positive	P-value
Age, years				>0.05
≥60	19	9	10	
<60	32	18	14	
Gender				>0.05
Female	14	8	6	
Male	37	19	18	
Tumor differentiation				<0.05
Well	19	19	0	
Moderate	21	8	13	
Poor	11	0	11	
Lymph node metastasis				<0.05
No	25	20	5	
Yes	26	7	19	
Clinical stage				<0.05
II	18	15	3	
III or IV	33	12	21	
Tumor site				>0.05
Cheek	15	7	8	
Floor of mouth	14	8	6	
Tongue	12	7	5	
Palate	5	3	2	
Gum	5	2	3	
Therapeutic effectiveness				<0.05
Responsive	35	22	13	
Non-responsive	16	5	11	

χ^2 test was performed for statistical analysis. P<0.05 was considered statistically significant.

Table II. Changes in mutant p53 expression in OSCC tissues following intra-arterial chemotherapy.

		Mutant p53 expression				
Intra-arterial chemotherapy	Cases, n	-	+	++	+++	P-value
Prior to	51	27	7	8	9	<0.05
Following	51	42	3	0	6	

χ^2 test was performed for statistical analysis. P<0.05 was considered statistically significant.

17% (3/18) of clinical phase II patients, while 64% (21/33) of clinical phase III and IV patients had positive expression of mutant p53 (P<0.05). The mutant p53 positive rate in chemotherapy non-responsive patients was 69% (11/16), which was significantly higher than that in the chemotherapy-responsive patients (37%, 13/35; P<0.05). The mutant p53 positive rate was not significantly correlated with age, gender or the location of the tumor (P>0.05; Table I).

Correlations between mutant p53 expression and the efficacy of intra-arterial chemotherapy. The mutant p53 positive rate prior to chemotherapy was 47% (24/51), which decreased to 18% (9/51) following chemotherapy (P<0.05; Table II). Expression of mutant p53 was decreased in all 13 (100%) chemotherapy-responsive patients, while only 5 (45%) chemotherapy non-responsive patients exhibited a reduced level of mutant p53 expression (P<0.05; Table III).

Table III. Correlation between mutant p53 expression and the efficacy of intra-arterial chemotherapy.

Efficacy	p53 positive cases, n	Expression of mutant p53		P-value
		No reduction	Reduction	
Responsive	13	0	13	<0.005
Non-responsive	11	6	5	

χ^2 test was performed for statistical analysis. P<0.05 was considered statistically significant. Comparisons were made between the mutant p53 positive rate in chemotherapy responsive patients and that of the chemotherapy non-responsive patients.

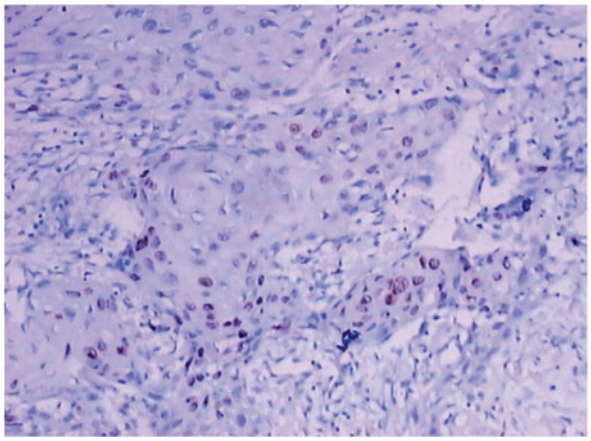

Figure 1. Weak expression of mutant p53 in oral squamous cell carcinoma (+) detected by immunohistochemical analysis (magnification, x100).

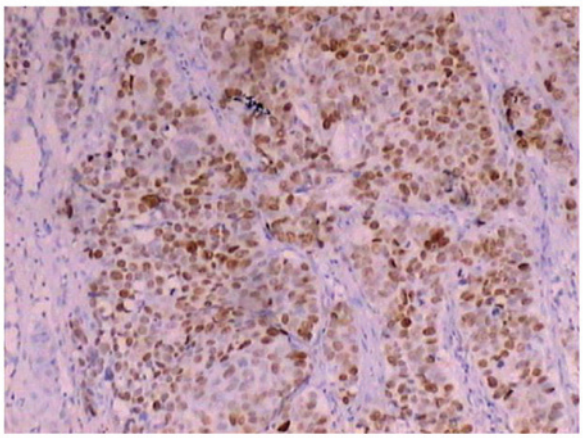

Figure 3. Strong positive expression of mutant p53 in oral squamous cell carcinoma (+++) detected by immunohistochemical analysis (magnification, x100).

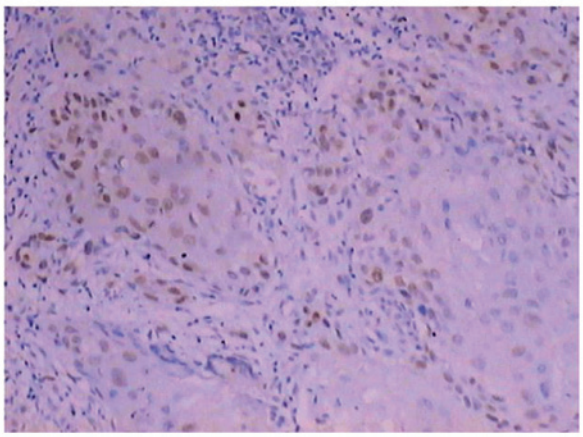

Figure 2. Moderately positive expression of mutant p53 in oral squamous cell carcinoma (++) detected by immunohistochemical analysis (magnification, x100).

Discussion

In the present study, the expression of mutant p53 was observed in OSCC tissues, suggesting that p53 mutation was present in OSCC. Mutated p53 loses its ability to suppress the function of oncogenes. Furthermore, mutant p53 may function as an oncogene to stimulate cell division and promote the growth of tumor cells (25). The results of the present study indicated that with the increase of OSCC pathological grading, mutant p53 positive rate was also increased, which was consistent with the results of previous studies (26-32), suggesting that the mutation of p53 may be significant in the pathological differentiation of OSCC. The negative correlation detected between the mutant p53 expression and the therapeutic efficacy indicated that mutant p53 may be used to predict the sensitivity of OSCC to 5-Fu and carboplatin chemotherapy. For example, positive expression of mutant p53 in OSCC tissues would indicate poor efficacy of chemotherapy with 5-Fu and carboplatin, and therefore an alternative chemotherapy scheme should be used. The results also indicated that the mutant p53 positive rate was higher in OSCC patients with lymph node metastasis than those without metastasis (33), suggesting that neck dissection should be performed for OSCC patients with expression of mutant p53. Mutant p53 positive rate in later clinical phases is higher than that in the early clinical phase (34), suggesting that mutant p53 promotes the development of OSCC. Overall, expression of mutant p53 is associated with the occurrence and development of OSCC, and contributes to the effective design of OSCC treatment programs.

Regional arterial infusion of carboplatin and 5-Fu was used to treat OSCC. 5-Fu is a pyrimidine antagonist, and may be transformed into floxuridine to inhibit thymidine synthase and interfere with the DNA synthesis (35). Carboplatin is a cytotoxic drug. Binding of platinum with guanine residues results in inter- and intra-DNA chain crosslinking, which prevents DNA synthesis and induces cell toxicity (36). The results demonstrated that intra-arterial chemotherapy with carboplatin and 5-Fu inhibited the expression of mutant p53 in OSCC. Gao *et al* (37) demonstrated that 5-Fu upregulates the expression

of p53 through the p38-MAPK pathway in HepG2 cells, leading to apoptosis. The antitumor effects of 5-Fu may be achieved by reducing mutant p53 expression in OSCC. The results of the present study indicate that all chemotherapy-responsive patients exhibited reduced levels of mutant p53 expression, indicating that carboplatin and 5-Fu achieve their therapeutic effect on OSCC through reduction of mutant p53 expression. These results also suggest that the reduction of mutant p53 expression may represent an effective treatment strategy for OSCC. Therefore, the use of intra-arterial chemotherapy with 5-Fu and carboplatin was suggested for the treatment of OSCC.

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