Expression of P53 and Bcl-2 in laryngeal squamous cell carcinoma

Shanting Liu[1,2,*], Weihua Lou[1], Jianzhong Sang[1], Ming Zhao[2], Zhaozhong Meng[2]

[1]*Department of Laryngology, The First Affiliated Hospital, Zhengzhou University, Zhengzhou, Henan* 450052, *China*; [2]*Department of Head and Neck, Henan Tumor Hospital, Zhengzhou, Henan* 450008, *China*

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Abstract

Objective. To investigate the expression of P53 and Bcl-2, their relationship, and their roles in the pathogenesis and development of laryngeal squamous cell carcinoma. *Methods*. Immunohistochemistry was used to study 110 cases of laryngeal carcinoma and 20 cases of normal laryngeal tissues to detect the expression of P53 and Bcl-2. *Results*. Among 110 cases of laryngeal squamous cell carcinoma, the rate of expression of P53 and Bcl-2 were 62.7% and 47.3% respectively; the expression of P53 was closely correlated with lymph node metastasis and Bcl-2 correlated with pathological grade, lymph node metastasis. P53 expression was related to Bcl-2 expression. *Conclusion*. P53 is correlated to Bcl-2. They play important roles in tumor matestasis and progression and will be the target of gene therapy in laryngeal carcinoma. [Life Science Journal. 2007;4(1):29-31] (ISSN: 1097-8135).

Keywords: laryngeal squamous cell carcinoma; p53; bcl-2; immunohistochemistry

1 Introduction

It is generally accepted that malignant tumor is caused by hyperproliferation. p53 is a type of tumor suppressor gene, whose mutation is the most common genetic aberration which has been identified as closely related to cancer so far. The apoptosis inhibition molecular of Bcl-2 family is an important component of the miscellaneous apoptosis inhibition moleculars[1]. This research is to detect the expression of P53 and Bcl-2 in laryngeal squamous carcinoma, and try to illuminate the underlying pathogenesis of laryngeal carcinoma.

2 Materials and Methods

2.1 Clinical data

Samples of 110 cases of laryngeal squamous cell carcinoma in the First Affliated Hospital of Zhengzhou University and Henan Tumor Hospital from June 1998 to June 2001 were collected, among which 102 cases were male and 8 cases were female. The age ranges from 42 to 70, with the average age of 58.8. According to the Union International Contre Le Cancer(UICC) standard of 1997, 47 cases of them were glotticcarcinoma, 58 cases were supraglottic carcinoma, and 5 cases were subglottic carcinoma. In terms of clinical stage, there were 5 cases of stage 0, 29 cases of stage Ⅰ, 33 cases of stage Ⅱ, 25 cases of stage Ⅲ, and 18 cases were stage Ⅳ. As to the differentiation, there were 43 cases of well differentiated squamous cell carcinoma, 51 cases of moderate differentiated squamous cell carcinoma, and 16 cases of poorly differentiated squamous cell carcinoma. In regard to the lymphatic metastasis, 48 cases were cervical lymph node metastasis and 63 cases without cervical lymph node metastasis. In adddition, 20 cases of normal mucosa were collected in the operation as control.

2.2 Immunohistochemistry

All reagents were purchased from the Wuhan Boster Biology Engineering Corportion (Wuhan, China). Paraffin sections were dewaxed and dehydrated. Followed the first antibody and the second antibody core-streptavidin-peroxidases was added. After dripping by the newly confected diaminobenzidine(DAB) solution, it was observed under microscope. It was stained with hematoxylin. Grade alcohol was used for dehydration and dryness. Dimethylbenzene was used for clarity, and neutral balata was used for seal.

2.3 Results judgement

The positive signals of Bcl-2 showed as brown yellow granule or diffusing shape distribution. P53 was mainly expressed in cell nucleus, and positive cells were brown yellow. In the samples, less than 10% of positive cells were regarded as negative, and the cases of more than 10% positive cells were regarded as positive.

2.4 Statistical method

χ^2 test was operated to show the correlation between P53 and Bcl-2 expression and clinical data. Spearman correlation test conducted for the correlation between P53 and Bcl-2. The statistic software sas 9.0 was used and the significance level was set at 0.05.

*Corresponding author. Email: liushanting@163.com

3 Results

There was almost no expression of P53 and Bcl-2 in normal laryngeal mucosa. Weak positive expression in basal layer cells was in few samples. The expression rates of P53 and Bcl-2 in laryngeal carcinoma were 62.7% and 47.3% respectively. P53 was mainly expressed in cell nucleus, and positive cells were brown yellow(Figure 1). Bcl-2 signal was brown yellow granules and was mainly expressed in cytoplasm and nuclear membrane (Figure 2).

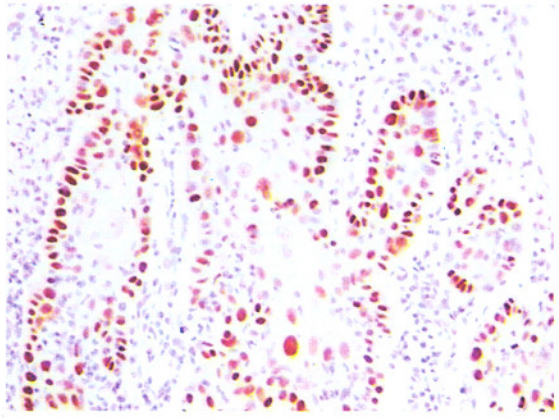

Figure 1. Expression of P53 in laryngeal squamous carcinoma by IHC. (HE×200)

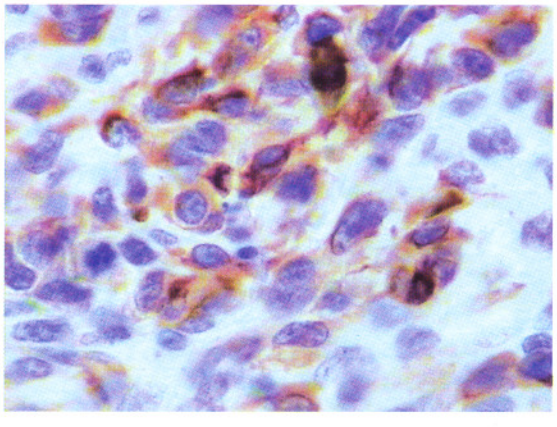

Figure 2. Expression of Bcl-2 in laryngeal squamous carcinoma by IHC. (HE×400)

Correlation analysis revealed: P53 was related to lymphatic metastasis but not to clinical stage, age, gender, type and differentiantion (Table 1); Bcl-2 was related to differentiation and lymphatic metastasis, but not to age, gender, clinical stage and type (Table 2); P53 expression and Bcl-2 expression were positively correlated (Table 3).

4 Discussion

It is generally accepted that malignant tumor is caused by gene mutation under outside stimulation. The accumulation of this mutation will activate oncogenes and inactivate tumor suppressor genes.

p53 is an important tumor suppressor gene, located at 17P13.1, with the length of 16 – 20kb, comprising 11 exons, with the transcription of 2.8 kb of mRNA recorded. The encoded protein is P53. So far, p53 has been identified as a gene that is most closely related to human tumor. Its major function is to regulate cells' response to DNA damage and inhibit the growth of cells by bringing about cell cycle retardance and/or apoptosis. It plays an important role in maintaining the stability of nucleus or promoting the cell apoptosis[2]. Mutant p53 has some new characteristics, such as prolonged half life and altered conformation, and loses the function of maintaining the stability of nucleus. The half life of wild type p53 in normal tissues is short and is easily hydrolyzed. It can not be tested in cells. In this study, no positive expression of p53 was detected in the 20 cases of normal mucosa. In the 110 cases of laryngeal carcinoma, 69 cases were positively detected. The positive rate was 62.7%, which is close to most reports. Its expression is related to cervical lymph node metastasis, but not to age, gender, clinical stage, type and differentiation, which is in accordance with the reports abroad[3]. It can be established that p53 is closely related to the occurrence and development of laryngeal carcinoma and lymphatic metastasis. Mutant p53 not only directly inhibits apoptosis and stimulates cell proliferation, but also accumulates themselves in cells. The highly positive rate of 62.7% in this study confirmed this. In addition, the high expression rate can lead to the instability of hereditary substance subsequently and the imbalance of caner-related genes, which will cause oncogenesis and metastasis.

Table 1. Correlation between P53 expression and clinical pathological features of the patients

Clinical pathological feature		*n*	P53 expression		χ^2	*P* value
			Negative	Positive		
Age	≤60 years	48	18	30	0.0019	0.9654
	>60 years	62	23	39		
Gender	Male	102	38	64	0.0002	0.9890
	Female	8	3	5		
Clinical stage	0 + Ⅰ + Ⅱ	67	25	42	0.0001	0.9912
	Ⅲ + Ⅳ	43	16	27		
Type	Glottic	47	17	30		
	Supraglottic	58	22	36	0.0511	0.9748
	Infraglottic	5	2	3		
Differentiation	Well	43	18	25		
	Moderate	51	19	32	1.4179	0.4922
	Poor	16	4	12		
Lymph node metastasis	Negative	62	27	33	4.6232	0.0315*
	Positive	48	12	36		

*$P < 0.05$, expression of P53 was closely correlated with lymph node metastasis.

Table 2. Correlation between Bcl-2 expression and clinic-pathological features of the patients

Clinical pathological feature		*n*	Bcl-2 expression		χ^2	*P* value
			Negative	Positive		
Age	≤60 years	48	25	23	0.9035	0.0142
	>60 years	62	33	29		
Gender	Male	102	55	47	0.8026	0.3703
	Female	8	3	5		
Clinical stage	0 + Ⅰ + Ⅱ	67	36	31	0.0693	0.7923
	Ⅲ + Ⅳ	43	22	21		
Type	Glottic	47	25	22		
	Supraglottic	58	30	28	0.1336	0.9354
	Infraglottic	5	3	2		
Differentiation	Well	43	29	14		
	Moderate	51	27	24	8.5708	0.0138*
	Poor	16	4	12		
Lymph node metastasis	Negative	62	38	24	4.1798	0.0409*
	Positive	48	20	28		

*$P<0.05$, expression of Bcl-2 was closely correlated with differentiation and lymph node metastasis.

Table 3. Correlation of Bcl-2 and P53

P53 expression	*n*	Bcl-2		*r*	*P* value
		Positive	Negative		
P53 (+)	69	40	29	0.2780	0.0036
P53 (−)	41	12	29		

From the aspect of cell apoptosis, the mechanism of tumor can be explained as the result of inhibiting apoptosis and hindering the decrease of tumor cells[4]. Bcl-2 family plays an important role in controlling cell apoptosis[1,5]. bcl-2 is a type of proto-oncogenes found in B cell lymphoma. The B cell lymphoma is often of characteristic t translocation (14:18). In most cases, bcl-2 gene on 18q21 is translocated to 14q32 which is adjacent to Ig heavy chain genes. Ig heavy chain gene has powerful promoter and enhancer, which can lead to unusually high bcl-2 expression and thus inhibits cell apoptosis and cause cancer. The data in this research suggest that there was almost no Bcl-2 expression in normal laryngeal mucosa. Bcl-2 expression rate in laryngeal carcinoma was 47.3%. A research about 176 cases of laryngeal carcinoma has found that Bcl-2 expression is related to differentiation and lymph node metastasis[6]. The present research obtained the similar result. The data of this research also showed that Bcl-2 expression was not correlated to age, gender, clinical stage and type. The high expression of Bcl-2 in the tissue of laryngeal carcinoma plays a vital role inhibiting cell apoptosis and oncogenesis. It is proposed that different Bcl-2 expression level may inhibit cell apoptosis differently and thus lead to different accumulation degree of damaged cells. As a result, different malignant phenotypes, such as different pathology grades, are observed.

The encoded protein of wile type p53 can be combined with the segments of 5′ non-coded zone of bcl-2 genes, and inhibit the expression of bcl-2. The mutant p53 will lose the function of inhibiting bcl-2, which may lead to the increase of Bcl-2 expression. This research results showed p53 and bcl-2 were correlated, which further confirmed the hypothesis that oncogenesis is the cooperation of various factors.

To damaged normal cells, apoptosis is the ultimate event, whereas to mutant cells, apoptosis inhibition is the early event and runs through the whole process of oncogenesis. Based on this, some scholars proposed that p53 and bcl-2 be used as therapeutic targets for the treatment of malignant tumor[7]. It is also suggested by the authors of this paper that the unique expression of p53 and bcl-2 in cancer tissues be hopefully used as an index for early treatment.

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