Gaziantep Tıp Dergisi 2001; 1: 16-21

Significance of p53, bcl-2 and Fas oncogenes in the laryngeal squamous cell carcinoma

Yıldırım A. Bayazıt[1], Kemal Bakır[2], Ramazan Uçak[2], Enver Özer[1], Semih Mumbuç[1], Muzaffer Kanlıkama[1]

[1]Department of Otolaryngology Head and Neck Surgery, [2]Department of Pathology, Faculty of Medicine, Gaziantep University, Gaziantep, Turkey

SUMMARY

Objective: To assess whether the expression of oncoproteins, p53, bcl-2 and fas, correlated with the clinical and pathological parameters in squamous cell laryngeal carcinoma (SCLC).

Study design: Medical records of 30 patients who were operated for SCLC, were reviewed, and histopathological and immunhistochemical assessments of the corresponding tissue samples were performed.

Methods: The monoclonal antibodies to p53 bcl-2 and fas proteins were used for immunhistochemical staining. Streptavidin-Biotin, horseradish method was used. The data obtained was compared with the clinical and histopathological results.

Results: A positive staining for p53 was obtained in 19 (63.3%) of 30 tumors. Only one (3.3%) of 30 tumors positively stained for bcl-2. A positive staining for fas was obtained in 27 (90%) of the tumors. The results of p53 and bcl-2 did not correlate with mitotic count (MC), grade, T and N, and smoking. There was a correlation between fas and MC ($p=0.007$). But, the results of fas did not correlate with grade, T, N and smoking.

A correlation was not found between; p53 vs.bcl-2 ($p<0.05$); p53 vs. fas ($p<0.05$); and bcl-2 vs. fas ($p<0.05$).

Conclusion: There was no association between the oncoproteins (p53, bcl-2 and fas) and clinical or pathological parameters studied. The expression of the oncoproteins between smokers and non smokers did not show significant difference as well.

Key words: P53, fas, bcl-2, oncoprotein, squamous cell laryngeal carcinoma

ÖZET

p53, bcl-2 VE fas onkogenlerinin yassı hücreli larinks karsinomlarındaki anlamı

Amaç: p53, bcl-2 ve fas onkoprotein ekspresyonlarının yasıı hücreli larinks karsinomlarındaki (YHLK) klinik ve patolojik korelasyonlarını incelemek.

Çalışma Dizaynı: YHLK tanısıyla tedavi edilen 30 hastanın tıbbi kayıtları inclendi ve bu hastalara ait tümör dokularının histopatolojik ve immunhistokimyasal incelemeleri yapıldı.

Metod: Anti p53, bcl-2 ve fas monoclonal antikorları kullanılarak immunhistokimyasal boyama yapıldı. Streptavidin-Biotin, horseradish metodu kullanıldı. Elde edilen sonuçlar klinik ve sitopatolojik sonuçlarla karşılaştırıldı.

Bulgular: Positif p53 boyanması toplam 30 tümörün 19'unda (63.3%) gözlendi. Sadece 1 (3.3%) tümörde bcl-2 pozitifliği vardı. Yirmi yedi (90%) tumorde fas boyanması pozitifti. p53, fas ve bcl-2 sonuçlarıyşa mitoz sayımı, grade, T, N ve sigara içimi arasında ilişki bulunamadı. Fas ve mitoz sayımı arasında ilişki vardı ($p=0.007$). Fakat, fas sonuçları ile grade, T, N ve sigara içimi arasında ilişki yoktu. p53 ile bcl-2 ($p<0.05$), p53 ile fas ($p<0.05$) ve bcl-2 ile fas ($p<0.05$) arasında ilişki yoktu:

Sonuç: Çalışılan onkoproteinler (p53, bcl-2 ve fas) ile klinikopatolojik parametreler arasında ilişki yoktur. Aynı zamanda, bu onkogen ekpresyonları ile sigara kullanımı arasında ilişki bulunamamıştır.

Anahtar kelimeler: p53, fas, bcl-2, onkoprotein, yassı hücreli larinks karsinomu

INTRODUCTION

Although squamous cell carcinoma is the most common cancer of the larynx, genetic alterations with their clinical relevance requires being elucidated precisely. One of the genetic alterations in cancer disease occurs in the apoptotic mechanisms.

Apoptosis or programmed cell death is one of the critical mechanisms involved in the tissue homeostasis. Dysregulation of apoptotic mechanisms are involved in the malignancies through prevention of the normal programmed cell turnover, blockage of apoptosis induced by activated oncogenes, prevention of cell death triggered by DNA damage, and interference with apoptosis induced by loss of cell attachment to the extracellular matrix (1). The oncogenes, p53,

✉ *Dr. Yıldırım A. Bayazıt, Gaziantep Üniversitesi, Tıp Fakültesi, Kulak, Burun, Boğaz Anabilim Dalı, 27070, Gaziantep, Turkey*

bcl-2 and fas (APO-1 or CD95), are involved in the apoptosis.

p53 is a tumor suppressor gene that initiates DNA repair mechanisms, and functions as a transcriptional activator of genes that block progression from G1 to S phase (2). p53 allows some time for cell to repair its damaged DNA, and otherwise it initiates apoptotic mechanisms. Loss of p53 function is associated with neoplasia (3).

Bcl-2 family includes death promoting and death inhibiting members. Among the death inhibiting members is bcl-2 that counteracts apoptosis initiated by various signals including p53 (4).

The fas which belongs to tumor necrosis factor and nerve growth factor superfamily, is a membrane protein of 43 kd molecular weight that triggers apoptosis in the cells (5). Interaction of a functional fas receptor and its ligand initiates apoptosis in cells. The fas is a key regulator of apoptosis both in normal and pathological states and expressed in various organs and cells including lymphocytes, heart, lung, kidney and ovary (6).

Oncoproteins which are representatives oncogenes and tumor suppressor genes, can be detected immunohistochemically. In this study, immunohistochemical methods were used to detect the oncoproteins, p53, bcl-2 and fas, and the purpose was to find out the changes at the oncoprotein level and to assess whether expression of these oncoproteins correlated with the clinical and pathological parameters of the patients who were treated for squamous cell laryngeal carcinoma (SCLC).

MATERIALS AND METHOD

Thirty patients who were operated for SCLC between 1992 and 1997, were included in the study. Medical records of the patients were reviewed, and archival paraffin embedded tumor tissues were obtained for histopathological and immunhistochemical examinations.

Conventional histopathology:

Five micron thick sections were cut from the paraffin embedded tumor specimens, routinely processed and stained with hematoxylin and eosin. Several sections from each case were reviewed to select the best preserved and most representative portion of the tumor tissue. The corresponding blocks were also used for immunohistochemistry.

A histopathological grading ranging from I (well differentiated) to III (poorly differentiated) was performed for each tumor. Grading was made according to tissue differentiation and nuclear pleomorphism. The perineural, vascular and cartilage invasions, positivity of the surgical margins were assessed. T stage was determined according to clinical assessment (indirect and direct laryngoscopy, and videostroboscopy or computed tomography) and intraoperative examination of the material when laryngectomy was performed. N stage was determined according to the histopathology results of the patients who underwent neck dissection, and was determined according to clinical and radiological (CT) assessments for those who did not require a neck dissection.

Mitotic counting (MC) of the tumors also was performed according to number of mitosis at 10 consecutive high power field. The areas selected for cell counting were from the most mitotically active sites, and usually were at the tumor periphery.

Immunohistochemistry:

After deparaffinization of the tumor blocks, five micron tumor sections were placed on the adhesive lams (poly-L-lysin, Sigma no; P8920) and microwaved with antigen retrieval solution for ten minutes. Monoclonal antibodies were used for p53 (Biogenex, AM 195-5M), bcl-2 (Dako N1587) and fas (Dako M3554). Streptavidin-Biotin, Horseradish method was used for immunhistochemical staining (Dako LSAB (2) KIT, KO675, 10 ml). The sections were washed with PBS (phosphate buffer solution) to prevent drying. Dense staining areas were selected under light microscope and 1000 cells were counted. Number of the cells staining positive for p53, bcl-2 and fas were calculated, and more than 5% staining of the tumor cells was considered positive. Immunhistochemical assessment of each sample was made by two different pathologist. Tonsil tissue was used as the control of fas and bcl-2 immunhistochemistry, and breast cancer tissue was used as the control of p53. The intensity of positivity was also graded as (+), (++) and (+++) when the percentages of the positively staining cells were 5 to 10, 11 to 15 and more than 15, respectively.

Statistics:

The data was processed by an IBM PC computer and statistical analyses were performed by using SPSS statistical package

(SPSS for windows 6.0). Pearson correlation coefficient was used for the following assessments; to compare the results of p53, bcl-2 and fas for any correlation in between; to compare the results of oncoprotein immunohistochemistry with MC, grade, tumor stage (T) and nodal stage of the neck (N). T and N stages of the patients were also compared with the immunohistochemistry results by using chi square tests.

All patients had the history of smoking. The number of daily cigarette consumption and how long the patients smoked were learnt by the patient history. Daily cigarette consumption was multiplied by the length of smoking and the smoking index was calculated. Smoking indexes of the immunohistochemistry positive and immunohistochemistry negative patients were compared by Mann Whitney U test.

Survival and distant metastases were not compared with the immunhistochemistry results, because there was not adequate number of patients for such a statistical analysis.

RESULTS

Clinical parameters

There were 2 female and 28 male patients with the ages ranging from 45 to73 (mean 59 years), and with a mean follow up period of 51 months (from 21 to 85 months).

Eight patient had low stage (5 stage I, 3 stage II) and 22 patients had high stage (8 stage III, 14 stage IV) tumors. There were supraglottic, glottic, transglottic and subglottic tumors in 14, 10, 5 and 1 of the patients, respectively.

Conventional histopathology:

There were 11 well differentiated (grade I) and 19 moderately or poorly differentiated (grade II in 14 and grade III in 5) tumors. The necks were N0 and N+ in 18 (60%) and 12 (40%) patients, respectively. The MC values ranged from 3 through 78 (mean, 17.1). A perineural or vascular invasion and positive tumor margins were not encountered on the histopathologic examination.

The clinical and pathological results of the patients are shown in the Table 1.

Immunohistochemistry:

P53: A positive staining for p53 was obtained in 19 (63.3%) of 30 tumors, of which 7 (23.3%), 8 (26.7%) and 4 (13.3%) were (+), (++) and (+++), respectively. The results of p53 did not correlate with MC (r= .323, p= .082), grade (r= -.045, p= .815), T stage(r= -.119, p= .532 and x^2=3.615, p=.306), N stage (r= .260, p=.165 and x^2= .0957, p=.992) and smoking (z=.175, p=.356).

Bcl-2: Only one (3.3%) of 30 tumors positively stained for bcl-2 that was (+), and 29 tumors were negative for bcl-2. The results of bcl-2 did not correlate with grade (r= 0.317, p= 0.088), T stage (r= 0.051, p=0.787 and x^2=0, p=0.931), N stage(r=0.268, p=0.152 and x^2=1.551, p=0.670) and smoking (z= 0.264, p= 0.189).

*Fas:*A positive staining for fas was obtained in 27 (90%) tumor samples, of which 5 (16.7%), 14 (46.7%) and 8 (26.6%) were (+), (++) and (+++), respectively. There was a correlation between fas and MC (r=0.482, p=0.007). But, the results of fas did not correlate with grade (r=0.282, p=0.130), T stage (r=0.0457, p=0.810 and x^2=0.017, p=0.999), N stage (r=0.126, p=0.505 and x^2 =0.062, p=0.995) and smoking (z=0.209, p=0.268).

*Relationship between p53, bcl-2 and fas:*A correlation was not found between; p53 vs.bcl-2 (r= -0.203, p=0.282); p53 vs. fas (r=0.258, p=0.168); and bcl-2 vs. fas (r=0.225, p= 0.232).

DISCUSSION

p53 mutation is the most frequent genetic alteration in the solid tumors. It was postulated that p53 overexpression correlated with low grade tumors of the head and neck (7), and that overexpression of p53 had been determinant of decreased survival in laryngeal carcinoma (8). On the other hand, it was proposed that p53 did not correlate with clinicopathological parameters or survival, but correlated with heavy smoking and drinking (9). Some other studies also could not find a correlation between p53 expression and tumor differentiation and lymph node metastasis (10, 11). Further, it was suggested that p53 had been a useless prognostic marker (12). In this study, almost two-third (63.3%) of the tumors were positive for p53 that is comparable with the previous reports (13, 14). The results showed that p53 values did not correlate with the histopahological (MC and grade) and clinical (T and N stage) parameters.

It was reported that bcl-2 correlated with survival, and could have a prognostic value in some cancer types (15). In patients with small cell lung cancer who had bcl-2-positive tumors, survival time tended to be shorter than in those with bcl-2-negative tumors. Absence of significant correlation between bcl-2 expression and clinical factors (smoking and staging) was

also reported (16). Although bcl-2 was shown to be a significant prognostic indicator in the squamous cell head and neck carcinoma (17), it

detection techniques, heterogeneity of the examined tumor samples, expression levels of death promoting or death inhibitor members of

Table 1. Clinical and pathological results of the patients.

Patient	P53	FAS	BCL-2	GRADE	No. mitosis	Tumor location	T	N
1. YT	-	+	-	1	3	G	3	0
2. HA	-	++	-	1	5	TG	3	1
3. AT	-	-	-	2	5	SG	3	0
4. MT	++	+++	-	1	3	SG	3	0
5. ÖA	+++	++	-	1	16	TG	3	2b
6. BÇ	+	++	-	3	15	SG	2	0
7. AU	-	+++	-	3	35	G	3	1
8. CC	-	-	-	2	5	SG	1	0
9. SÖ	+	+++	-	3	17	SG	4	0
10. HS	++	++	-	2	8	TG	3	0
11. BA	++	++	-	2	7	SG	4	2c
12. Hİ	-	++	-	2	10	G	3	0
13. VK	++	++	-	2	39	SG	1	0
14. MY	+	-	-	2	15	SG	4	2c
15. AS	-	+++	-	3	14	G	3	0
16. EÖ	+	++	-	1	18	G	2	0
17. ST	++	+++	-	2	6	G	1b	0
18. AY	-	++	-	1	10	SG	4	1
19. HE	+++	+++	-	2	50	SG	3	2c
20. AY	+	++	-	1	17	SG	2	2b
21. MD	-	+	-	1	4	SubG	3	0
22. AD	-	+++	+	3	54	G	3	2b
23. ND	++	++	-	1	24	G	1b	0
24. MD	-	+	-	1	7	TG	3	0
25. SU	++	+	-	2	11	SG	3	2b
26. AD	+++	+++	-	2	78	TG	3	2b
27. AA	++	++	-	2	16	G	4	0
28. KK	+	++	-	2	2	SG	4	2b
29. BO	+	++	-	1	13	G	1b	0
30. IM	+++	+	-	2	7	SG	2	0

G; glottic, TG; transglottic, SG; supraglottic, SubG; subglottic.

was also suggested that bcl-2 had not been a prognostic discriminator in laryngeal carcinoma (8). In a previous study., bcl-2 positivity was found in 26% of the SCLC, and p53 and bcl-2 immunoreactivity was significantly associated with poor histological differentiation and lymph-node metastases (18). In this study, very few number (3.3%) of SCLC tissues were bcl-2 positive, and the values of bcl-2 did not correlate with grade, and T and N stage. Similar results were also reported previously in which bcl-2 expression was found consistently low and did not correlate with prognosis or metastasis (8). Briefly, prognostic significance of the bcl-2 is controversial in laryngeal carcinoma. Factors like genetic or racial differences, cancer promotion by different environmental or chemical carcinogens, utilization of different bcl-2 family, immunohistochemical level of discrimination, or some unknown factors might have a role in the differences in the expression of bcl-2.

In leukemia, it was shown that presence of fas reflected favorable prognosis whereas its absence showed unfavorable prognosis (19). The significance of fas in SCLC has not been elucidated yet. There is only one report in the literature referring to significance of fas in the benign epithelial hyperplastic lesions of the larynx (20). In this study, fas positivity was obtained in 90% of the tumors, and correlated with MC whereas it did not correlate to T or N stage as was reported previously (21). The relationship between fas and may indicate that both active cell proliferation and cell death were present concomitantly in LSCC, and that fas

receptor protein was functional and increased fas balanced the active cell proliferation.

There was not a correlation between p53 and bcl-2 in a previous study (22). However, the correlation between fas, p53 and bcl-2 has not been searched as yet. According to our results, which to our knowledge is the first, there was not correlation between p53, bcl-2 and fas results.

It is well established that glottic cancers respond better to irradiation and have better prognosis than the supraglottic cancers. Tumors without functional p53 genes may demonstrate a poor response to radiation and chemotherapeutic agents. In this study, p53 overexpression was more frequent in the supraglottic cancers (79%) than the glottic cancers (50%). Although we did not search for a relationship between radiation response and p53 expression, less frequent loss of the functional p53 in glottic cancers than the supraglottic cancers in this study may be indicating the radiation sensitivity of the glottic cancers. This issue necessitates further assessments.

Although nicotine has been implicated as a potential factor in the pathogenesis of cancers, its mechanism of action in the development of this cancer remains largely unknown. Nicotine activates the mitogen-activated protein kinase signaling pathway in lung cancer cells, specifically extracellular signal-regulated kinase (ERK2), resulting in increased expression of the bcl-2 protein and inhibition of apoptosis in these cells; and blocks the inhibition of protein kinase C and ERK2 activity in lung cancer cells by anti-cancer agents, and thus can adversely affect cancer therapy. In lung cancers, no association was between smoking and; tumor grade, stage, or patient performance status; p53 or c-erbB2 immunohistochemical staining, or p53 mutations (23). In this study, there was no association between smoking and p53, bcl-2 and fas expressions as well. Another study also showed that fas, bcl-2, and p53 expressions were not significantly different between normal individuals with chronic cigarette smoking and those without smoking (24). It is not only the nicotine, but also might some other environmental and genetic factors be involved in p53 overexpression in head and neck cancers (25). Therefore, due to lack of an association between smoking and oncoproteins in this study, it is plausible to postulate that smoking may be exerting its carcinogenic action through some other genetic mechanisms.

Because of high levels of p53 and fas, which are apoptotic, and low levels of bcl-2, which is anti-apoptotic, the tumors in this study were considered to have high apoptotic thresholds.

In conclusion, there was no association between the oncoproteins (p53, bcl-2 and fas) and clinical or pathological parameters studied. The expression of the oncoproteins between smokers and non smokers did not show significant difference.

REFERENCES

1. Kitada S, Krajewska M, Zhang X, et al. Expression and location of proapoptotic bcl-2 family protein BAD in normal human tissues and tumor cell lines. Am J Pathol 1998; 152:51-61.
2. Aupperle KR, Boyle DL, Hendrix M, et al. Regulation of synoviocyte proliferation, apoptosis and invasion by p53 tumor suppressor gene. Am J Pathol 1998; 152:1091-1098.
3. Hollstein M, Sidransky D, Vogelstein B, Harris CC. P53 mutations in human cancers. Science 1991; 23:49-53.
4. Soini Y, Paakko P, Lehto VP. Histopathological evaluation of apoptosis in cancer. Am J Pathol 1998; 153:1041-1053.
5. Moller P, Koretz K, Leithauser F, et al. Expression of APO-1 (CD95), a member of the NGF/TNF receptor superfamily, in normal and neoplastic colon epithelium. Int J Cancer 1994; 57:371-377.
6. Pan G, Vickers SM, Pickens A, et al. Apoptosis and tumorigenesis in human cholangiocarcinoma cells. Involvement of fas/APO-1 (CD 95) and calmodulin. Am J Pathol 1999; 155:193-203.
7. Gandour-Edwards R, Trock BJ, Gumerlock P, Donald PJ. Heat shock protein and p53 expression in head and neck squamous cell carcinoma. Otolaryngol Head Neck Surg 1998; 118:610-615.
8. Spafford MF, Koeppe J, Pan Z, Archer PG, Meyers AD, Franklin WA. Correlation of tumor markers p53, bcl-2, CD34, CD44H, CD44v6, and Ki-67 with survival and metastasis in laryngeal squamous cell carcinoma. Arch Otolaryngol Head Neck Surg 1996; 122:627-632.
9. Field JK, Pavelic ZP, Spandidos DA, Stambrook PJ, Jones AS, Gluckman JL. The role of the p53 tumor suppressor gene in squamous cell carcinoma of the head and neck. Arch Otolaryngol Head Neck Surg 1993; 119:1118-1122.

10. Schipper JH, Kelker W. Expression of tumor suppressor gene p53 and Rb in patients with epidermoid carcinoma of the head and neck. HNO 1994; 42:270-274.
11. Franchi A, Gallo O, Boddi V, Santucci M. Prediction of occult neck metastases in laryngeal carcinoma: role of proliferating cell nuclear antigen, MIB-1, and E-cadherin immunohistochemical determination. Clin Cancer Res 1996; 2:1801-1808.
12. Nakashima T, Wang XF, Masuda M, et al.Overexpression of p53 nuclear protein in premalignant and malignant laryngeal lesions. Eur Arch Otorhinolaryngol 1996; 256 (Suppl):56-59.
13. Hotz M, Bosq J, Zbaeren P, et al. Spontaneous apoptosis and expression of p53 and bcl-2 family proteins in locally advanced head and neck cancer. Arch Otolaryngol Head Neck Surg 1999; 125:417-422.
14. Greenblatt MS, Bennett WP, Hollstein M, Horris CC. Mutations in the p53 tumor suppressor gene: clues to cancer etiology and molecular pathogenesis. Cancer Res 1994; 54:4855-4878.
15. Earola AK, Ruokolainen H, Soini Y, Raunio H, Paakko P. Accelerated Apoptosis and Low Bcl-2 Expression Associated with Neuroendocrine Differentiation Predict Shortened Survival in Operated Large Cell Carcinoma of the Lung. Pathol Oncol Res 1999; 20:179-186.
16. Takayama K, Ogata K, Nakanishi Y, et al. Bcl-2 Expression as a Predictor of Chemosensitivities and Survival in Small Cell Lung Cancer. Cancer J Sci Am 1996; 2:212.
17. Friedman M, Grey P, Venkatesan TK, Bloch I, Chawla P, Caldarelli DD. Prognostic significance of bcl-2 expression in localize squamous cell carcinoma of the head and neck. Ann Otol Rhinol Lryngol 1997; 106:445-450.
18. Pruneri G, Pignataro L, Carboni N, et al. Clinical relevance of p53 and bcl-2 protein over-expression in laryngeal squamous-cell carcinoma. Int J Cancer 1998; 79:263-8.
19. Baryshnikov AYu, Polosukhina ER, Tupitsin NN, Gavrikova NV, Andreeva L Yu, Zabotina TN. CD95 (FAS/APO-1) antigen is a new prognostic marker of blast cells of acute lymphoblastic leukaemia patients. Adv Exp Med Biol 1999; 457:251-258.
20. Hellquist HB. Apoptosis in epithelial hyperplastic laryngeal lesions. Acta Otolaryngol Suppl (Stockh) 1997; 527:25-29.
21. Bayazit Y, Mumbuc S, Ucak R, Kanlıkama M, Bakır K. Significance of fas protein in the squamous cell carcinoma of the larynx. Acta Otolaryngol 2000; 120:557-561.
22. Lavieille JP, Gazzeri S, Riva C, Reyt E, Brambilla C, Brambilla E. p53 mutations and p53, Waf-1, Bax and Bcl-2 expression in field cancerization of the head and neck. Anticancer Res 1998; 18:4741-4749.
23. Fleming MV, Guinee DG Jr, Chu WS, et al. Bcl-2 immunohistochemistry in a surgical series of non-small cell lung cancer patients. Hum Pathol 1998; 29:60-64.
24. Suzuki N, Wakisaka S, Takeba Y, Mihara S, Sakane T. Effects of cigarette smoking on Fas/Fas ligand expression of human lymphocytes. Cell Immunol 1999; 192:48-53.
25. van Oijen MG, van de Craats JG, Slootweg PJ. p53 overexpression in oral mucosa in relation to smoking. J Pathol 1999; 187:469-474.