The role of Bak expression in apoptosis of the oral squamous cell carcinoma (OSCC) and metastases to lymph nodes (LNMs)

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Abstract

The immunohistochemical method was applied to show Bak expression in oral squamous cell carcinoma and its metastases to lymph nodes (LNMs). Bak expression was evaluated by immunohistochemical methods in specimens with oral squamous cell carcinomas and their lymph node metastases. Immunohistochemical studies were performed, using goat polyclonal Bak antibodies (Santa Cruz Biotechnology, USA) at 1:200 dilution. Our studies revealed over expression (64%) of Bak in the cytoplasm of epithelial cells in primary tumours (PTs) and in (75%) LNMs. No statistically significant correlations were observed between Bak immunoreactivity and age, pT and G of the carcinoma in PTs and LNMs. We conclude that expression of Bak may be useful for better characterising and predicting the prognosis of OSCC but cooperative studies are needed to assess its applications in the clinical practice.

Key words: apoptosis, Bak, Squamous Cell Carcinoma, lymph nodes, metastases.

Introduction

At least 95% of cancers of the oral cavity (including tongue) are squamous cell carcinomas (SCC). Although most of them are readily accessible to discovery and biopsy, many are detected late and 50% of these lesions prove fatal. On histological examination, SCC range from well-differentiated keratinizing neoplasms to anaplastic, sometimes, sarcomatoid tumours. They infiltrate local tissues and subsequently metastasize to lymph node, the mediastinum, the lungs, the liver and bones [1]. The tumour growth is characterised by an imbalance between cell proliferation and programmed cell death (PCD)-apoptosis. The regulation of PCD is very important for neoplastic transformation. Bak is a pro-apoptotic member of the Bcl-2 genes family that are involved in the regulation of apoptosis [2]. In the present study, we examined the expression of Bak in SCC of the oral cavity and its metastases to lymph nodes.

Material and methods

Bak expression was evaluated in formalin-fixed, paraffin-embedded specimens from 47 males and 11 females, ranging in age from 30-76 years, with a median of 56,8 years with T1-T4 oral SCC and, out of whom, 25 presented with lymph node metastases. In our examination, we used TNM system and the 3rd degree grading system of histological malignancy. Immunohistochemical studies were performed, using goat polyclonal Bak antibodies (Santa Cruz Biotechnology, USA) at 1:200 dilution. The reaction was performed by the Labelled Streptavidin Biotin (LSAB) technique (DAKO). The slides were diagnosed, using a light microscope at 100-fold magnification. The immunostaining was evaluated by counting at least 500 cells in the tumour areas of each section. In negative controls, the primary antibodies were omitted. Bak expression was estimated semi-quantitatively as the percentage of positive cells as follows: 15-30% stained cancer cells - slight reaction (+), 31-50% stained cancer cells - moderate reaction (++), >50% stained cancer cells - strong reaction (+++) and <15% stained tumour cells were considered negative. Correlation of Bak and various clinicopathological features was done, using the Chi - square test. The significance level was set to p=0.05.

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Results

The expression of Bak in oral cancer and lymph node metastases is summarised in Tables 1 and 2. Our studies revealed over expression of bak in the cytoplasm of epithelial cells in 37/58 (64%) primary tumours (PTs) and 18/24 (75%) lymph node metastases (LNMs). Bak was detectable in higher percentage in pT4 groups (78%-of PTs and 80% of LNMs). According to the grading system of histological malignancy, we noticed a higher expression of Bak in G2 groups (68% in PT and 78.9 in LMNC). In G3 group, we found a difference between the primary tumours (33.3%- positive cells) and lymph node metastases (66.7% positive cells). In twelve cases, we observed a positive immunohistochemical reaction for Bak in PTs and LNMs between the primary tumours and their corresponding lymph node metastases. In five cases, it was a positive reaction for Bak in PTs and negative immunohistochemical staining in LNMs, then, in five cases, we noticed positive staining in LNMs and negative in PTs. There was no significantly statistical correlation in either age, pT or the grading system between primary tumour and lymph nodes metastases.

Table 1. The expression of Bak, according to staging (pT)

STAGING	PRIMARY TUMOURS		METASTASES	
	A	B	C	D
pT1	25	60%	9	67%
pT2	18	67%	8	75%
pT3	6	50%	3	67%
pT4	9	78%	5	80%

A- the number of primary tumours with expression of Bak in different pT groups
B- the percentage of primary tumours with expression of Bak in different pT groups
C- the number of tumours with LNMs and expression of Bak in different pT groups
D- the percentage of tumours with LNMs and expression of Bak in different pT groups

Table 2. The expression of Bak, according to grading (G).

GRADING	PRIMARY TUMOURS		METASTASES	
	A	B	C	D
GI	4	57%	2	50%
GII	31	69%	19	79%
GIII	2	33%	3	66%

A- the number of primary tumours with expression of Bak in different G groups
B- the percentage of primary tumours with expression of Bak in different G groups
C- the number of tumours with LNMs and expression of Bak in different G groups
D- the percentage of tumours with LNMs and expression of Bak in different G groups

Discussion

Lymph node metastases are crucial in the prognosis of cancer process. We have not found any scientific reports about Bak proteins in SCCs of the oral cavity and their lymph node metastases. The aim of our study was to assess the role of Bak, as a pro-apoptotic protein in primary SCCs of the oral cavity and lymph nodes metastases. Tumour progression is characterised by an imbalance between cell proliferation and apoptosis [3]. The process of apoptosis plays a very important role in elimination of abnormal cells from the tissues. The inhibition of this process can aid progress of cancer [4]. Proteins of the Bcl-2 family are central regulators of apoptosis and are thought to act primarily on the mitochondria [5]. Members of the Bcl-2 family are cellular homologous which are divided into three subfamilies: Bcl-2, Bax and BH3, which can perform either anti-apoptotic or pro-apoptotic functions. Proteins from the Bcl-2 family are known as inhibitors of apoptosis but Bax and BH3 as promotors of apoptosis. Bak belongs to the Bax subfamily [6]. Members of this family interact and form hetero- and homodimers, which control apoptosis. Some authors have noticed that Bak can form heterogeneous dimers with Bcl-2 or Bcl-xl to inhibit their anti-apoptotic functions [7]. In our experiment, we noticed that the expression of Bak is correlated with staging of the cancer (TNM system). The highest percent of Bak expression was observed in pT4 staging groups. Our observations are similar to those of Rosen at al., who indicated that Bak deficiency is correlated with the occurrence and development of tumours [8] and Kondo et al. who found that positive ratios of Bak expression negatively correlated with pathological and clinical stages of gastric cancer [9]. Although there are still many unanswered questions, regarding the exact mechanism by which Bak modulates apoptosis, we assume that Bak plays an important role in the process of apoptosis in SCCs of the oral cavity and their metastases to lymph nodes.

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