Detection of Protein BCL2/JH Rearrangement in Epidermoid Carcinomas of Mouth and Pharynx

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SUMMARY

Introduction: The BCL2 protein found in the internal mitochondrial membrane regulates the apoptosis preventing the programmed cell death. The translocation (14:18), detected in 70 to 85% of the follicular lymphoma, lead the super expression of BCL2 protein, by juxtaposition of BCL2 gene to the JH segment of the immunoglobulins' heavy chain gene. However, the found of the BCL2 expression in head and neck carcinoma are contradictory.

Objective: To investigate the presence of the translocation (14:18) of the BCL2 gene in head and neck carcinoma.

Method: Sixteen DNA samplers were examined being 13 of squamous cells carcinoma (SCC) and 3 of epidermoid (CE), by means of chain reaction of the polymerase (PCR).

Results: The BCL2/JH rearrangement in 2 (15%) of the CCE 13 cases and in none of the 3 cases of CE. The average of the frequency of molecules with rearrangement was 46,44x107. Was not observed association between the rearrangement presence and the exhibition to tobacco and alcohol (p=0, 6545).

Conclusion: Different from the results found in follicular lymphoma, the presence of the translocation (14; 18) in head and neck carcinomas is not common and, when it occurs, it can be an occasional mutation not associated to exhibition to the tobacco and alcohol.

Keywords: genetics translocation, molecular biology, buccal neoplasia, pharyngal neoplasia.
INTRODUCTION

The human BCL2 gene is located on chromosome 18q21 in a telomere-centromere orientation and consists of three exons (Figure 1). The BCL2 protein found in the inner mitochondrial membrane regulates apoptosis by inhibiting the cells from programmed cell death (1). Translocation (14; 18) (q32, q21), detected in 70-85% of follicular lymphomas, leads to overexpression of BCL2 protein by the juxtaposition of the BCL2 gene to JH gene segments of immunoglobulin heavy chain (IGH). Most of the points of breaks of t (14; 18) occur in non-coding region of 18q21.3 BCL2. These points are the regions of breaks MBR (Major Breakpoint Region), found in approximately 60% of lymphomas with t (14; 18) and mcr (Minor Cluster Region), and located 20 kb away from the MBR region (2, 3, 4, 5).

Data from recent studies are contradictory on the correlation between the presence of rearrangements and changes in gene expression as predictive values of BCL2/JH prognosis of follicular lymphomas (RADOJKOVIE et al., 2008) (6). However, for DEGHIED et al. (2007) (7) the expression of this protein may be used, including retrospectively for the diagnosis of doubtful cases, such as reactive hyperplasia. In patients with minimal expressions BCL2/JH in samples of peripheral blood or bone marrow would be the correct retest the rearrangement BCL2/JH to exclude false positives (8, 9).

Unlike the findings in lymphomas, the over expression of BCL2 protein has been detected in 30% of carcinomas of the head and neck (10, 11, 12) and is not associated with presence of the t (14; 18) (HARN et al., 1996). Few epidemiological studies have indicated that the use of tobacco and high consumption of alcohol are important etiologic factors in the induction of genetic alterations such as chromosomal translocation t (13, 14, 21). However, the rearrangement BCL2/JH has been found in lymphocytes from healthy individuals who smoke (14, 15, 16, 17, 18) and in bone marrow cells from patients with diseases other than lymphoid (8, 17).

The aim of this study was to investigate the presence of BCL2 rearrangement (MBR) / JH in tissue samples from patients with carcinomas of the mouth and pharynx and the possible correlation with the same exposure to tobacco and alcohol.

METHOD

Genomic DNA was extracted from 16 fresh biopsies of cancers of the mouth (n = 13) and pharynx (n = 3) obtained from patients with histological confirmed epidermoid carcinoma, maid to any treatment by digestion of tumor tissue by proteinase-K (20 mg / ml) and DNA extraction without phenol-chloroform. The tumor biopsies were performed in the service of Otolaryngology School of Medicine, UNESP, Botucatu, SP, Brazil, after the patient and or guardian becomes aware of the research objectives and signed a consent form and approved by the Ethics in Research in humans of our institution.

The presence of rearrangement BCL2/JH (Figure 1), breaking MBR region was investigated by nested PCR. 1.0μg of DNA from each sample was amplified with 200 nmol of MBR and JH primers (GRIBBEN et al., 1994) in 50 μl of buffer (Tris-HCl pH 7.5 and 5 mM, 25 mM KCl MgCl2 and 1.5 mM), 0.1 mM dNTPs and 1.25 U Taq DNA polymerase. This was followed by 25 amplification cycles at 94°C for 1 minute, 58°C for 1 minute and 72°C for 1 minute. In the second reaction (nested) 10μl of the product’s first reaction was amplificated again with primers MBR-JH-N and N (200 nmol) in 50μl of buffer (Tris-HCl pH 7.5 to 8 mm, the KCl 40 mM MgCl2 and 1.5 mM), 0.1 mM dNTPs and 1.25 U Taq DNA polymerase. This was followed by 25 amplification cycles at 94°C for 1 minute, 55°C for 1 minute and 72°C for 1 minute. In the second reaction (nested) 10μl of the product’s first reaction was amplificated again with primers MBR-JH-N and N (200 nmol) in 50μl of buffer (Tris-HCl pH 7.5 to 8 mm, the KCl 40 mM MgCl2 and 1.5 mM), 0.1 mM dNTPs and 1.25 U Taq DNA polymerase. This was followed by 30 cycles of amplification at 94°C for 1 minute, 58°C for 1 minute and 72°C for 1 minute. The products of nested PCR were subjected to electrophoresis on polyacrylamide gel and stained with 5% solution of ethidium bromide (50 mg / ml).

DNA extracted from cell line RL with the MBR rearrangement was used as positive control of PCR reaction. Human DNA negative at (14, 18) and a tube of PCR without DNA were used as negative controls of the reaction.

The Fisher exact test was used to assess the correlation between the presence of BCL2 rearrangement (MBR) / JH and exposure to tobacco and alcohol.
Table 1. Incidence of cases of epidermoid carcinoma of the mouth and pharynx.

| Patient | Age/Gender | Exposure | Tobacco | Alcohol | Site            | Histology | Degree | BCL2/JH |
|---------|------------|----------|---------|---------|-----------------|-----------|--------|---------|
| 1       | 43/M       | •        | •       | •       | Floor of the mouth | EC        | B      | -       |
| 2       | 44/M       | •        | •       | •       | Floor of the mouth | EC        | B      | +       |
| 3       | 49/M       | •        | •       | •       | Floor of the mouth | EC        | B      | -       |
| 4       | 52/M       | •        | •       | •       | Oropharynx        | EC        | M      | -       |
| 5       | 53/M       | •        | °       | °       | Palate           | EC        | M      | -       |
| 6       | 56/M       | •        | •       | •       | Palate           | EC        | B      | +       |
| 7       | 60/M       | •        | •       | •       | Palate           | EC        | M      | -       |
| 8       | 63/M       | •        | •       | •       | Retromolar Mucosa | EC        | M      | -       |
| 9       | 64/M       | °        | °       | °       | Oral mucosa      | EC        | B      | -       |
| 10      | 65/M       | •        | •       | •       | Palate           | EC        | M      | -       |
| 11      | 67/M       | °        | °       | °       | Palate           | EC        | M      | -       |
| 12      | 69/M       | •        | °       | °       | Oral mucosa      | EC        | B      | -       |
| 13      | 72/F       | °        | °       | °       | Oral mucosa      | EC        | B      | -       |
| 14      | 72/M       | •        | •       | •       | Tongue           | EC        | B      | -       |
| 15      | 80/M       | °        | °       | °       | Tongue           | EC        | B      | -       |
| 16      | 83/F       | °        | °       | °       | Tongue           | EC        | B      | -       |

Legend: EC: epidermoid carcinoma, M: male, F: female; B: well differentiated; MD: moderately differentiated, (-): negative expression, (+): positive expression.

Table 2. Association between the presence of MBR rearrangement and exposure to tobacco and alcohol in patients with head and neck tumors.

| Rearrangement presence | Number of cases | No Exposure | Exposure |
|------------------------|-----------------|-------------|----------|
| BCL2/JH Positive       | 2               | 0           | 2        |
| BCL2/JH Negative       | 14              | 5           | 7        |
| Total                  | 16              | 5           | 9        |

Legend: P = 0.6545 (Fisher’s exact test).

RESULTS

The incidence of BCL2 rearrangement (MBR) / JH was 12.5% (2 / 16) for cases of epidermoid carcinoma of the mouth and pharynx (Table 1).

The analysis of the frequency of BCL2 rearrangement (MBR) / JH was made by molecule (λ) in the two positive patients (Figures 2, 3 and 4) and used a Poisson model of statistical analysis for the number of rearrangements per sample (21). Because all trials had the same number of molecules (1 μg of DNA contains 5 x 10^-19 moles of each single copy sequence = ~ 300 000 molecules), the estimate λ was given by the usual Poisson estimator: λ = 1 / M ln (1 - p), where p is the fraction of trials with at least one rearrangement.

No association was found between the presence of BCL2 rearrangement (MBR) / JH and exposure to tobacco and alcohol (Table 2) (P = 0.6545).

PCR products of patients positive for the rearrangement BCL2/JH can be observed in Figure 2. Fragments of different sizes were observed in two patients with squamous cell carcinoma.

Figures 2, 3 and 4 show the amplifications carried out on multiple screens, rearrangement of BCL2 (MBR) / JH of the two patients who were positive.

The second patient, a smoker since childhood, and chronic alcoholics (~two liters of alcohol per day), remained positive in 3 of 8 more screens performed (Figure 2A). We observed fragments of the same size, suggesting that it is the same clone. The frequency of the MBR rearrangement was 19.32 x 10^-7 molecules positive.

Patient 6, chronic smoker and drinker, he remained positive in seven of eight more trials performed (Figure 2B). Fragments were observed even sizes (~200 bp). The
The frequency of the MBR rearrangement was $73.57 \times 10^{-7}$ molecules positive.

**Discussion**

A high expression of BCL2 has been observed in squamous cell carcinomas (LAVIELLE et al. 1998; DRENNING et al., 1998), however, there are reports of the presence of BCL2/JH rearrangement in tumors of head and neck. In this study, the BCL2 rearrangement (MBR)/JH was investigated in fresh biopsies of 13 squamous cell carcinomas and 3 carcinomas, from patients with head and neck tumors by nested PCR.

HARN et al. (1996) (12) reported the absence of the MBR rearrangement by analyzing 32 cases of nasopharyngeal carcinoma by means of PCR. However, the study by HARN et al. (1996) (12) were used for fixed tissue samples. Thus, this discrepancy in the incidence of BCL2/JH rearrangement may be due to technical differences or sampling.

SALO et al. (1997) (20) studied the status of the BCL2 gene in nine human cell lines of squamous cell carcinoma. The BCL2 cDNA was amplified in five cell lines, showing that the mRNA was expressed in these cells. The five cDNAs were sequenced; however, point mutations in the BCL2 gene were not detected, indicating that translocation is probably a typical change in these neoplastic cells. For VERCIES et al. (2004) (18) these results suggest that one mechanism of BCL2 overexpression in squamous cell carcinomas, may be due to chromosomal translocation t (14;18).

**Figure 2.** Amplifications of BCL2 rearrangement (MBR) / JH in patients with squamous cell carcinoma (EC). Polyacrylamide gel electrophoresis in 5%.

**Figure 2A.** Amplifications multiple BCL2 rearrangement (MBR) / JH in patients with squamous cell carcinoma (EC). Polyacrylamide gel electrophoresis in 5%.

**Figure 2B.** Amplifications multiple BCL2 rearrangement (MBR) / JH in patients with squamous cell carcinoma (EC). Polyacrylamide gel electrophoresis in 5%.

**Figure 2A.** Amplifications multiple BCL2 rearrangement (MBR) / JH in patients with squamous cell carcinoma (EC). Polyacrylamide gel electrophoresis in 5%.

**Figure 2B.** Amplifications multiple BCL2 rearrangement (MBR) / JH in patients with squamous cell carcinoma (EC). Polyacrylamide gel electrophoresis in 5%.

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**Figure 2B.** Amplifications multiple BCL2 rearrangement (MBR) / JH in patients with squamous cell carcinoma (EC). Polyacrylamide gel electrophoresis in 5%.

**Figure 2B.** Amplifications multiple BCL2 rearrangement (MBR) / JH in patients with squamous cell carcinoma (EC). Polyacrylamide gel electrophoresis in 5%.
Still, in this study, one of the smokers showed a positive high frequency (73.57 x 10^{-7}) of BCL2 rearrangement (MBR) JH and this increase may be due to the expansion of a single dominant clone and sequencing of the product PCR can confirm that this individual observation. This data could have predictive value for diagnosis as shown by Bue et al. (1995), when one of the smokers (three packs of cigarettes a day), which showed a high frequency of rearrangement in peripheral blood BCL2/JH developed melanoma (21). This individual was positive four times in four trials conducted and remained positive on April 2 more screens retested eight months later. Thus, measuring the rate of translocation t (14, 18) could identify individuals with a greater risk of developing lymphoma or other cancers or that responded poorly to therapy (6,7,21).

Finally, one can say that the BCL2 rearrangement (MBR) JH is not restricted to lymphoproliferative diseases and can be detected in neoplastic cells of primary carcinomas of the mouth and pharynx. The translocation t (14; 18) may therefore be a secondary mutation found in squamous cell carcinoma of the mouth and pharynx but in our case, due to the small number of patients tested, cannot assess the prognostic value of the presence of BCL2 rearrangement / JH in these tumors.

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