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

Detecção do Rearranjo da Proteína BCL2/JH em Carcinomas Epidermoides de Boca e Faringe

Jair Montovani*, Magaly M. Sales**, Maria Inês M. C. Pardini.***

* Full Professor in Otorhinolaryngology. Full Professor, Adjunct.
** Doctoral and Postdoctoral Fellow in Genetics at UNESP.
*** PhD. Professor, Department of Medicine, Blood Bank Division.
Institution: Faculdade de Medicina de Botucatu - UNESP Department of Ophthalmology and Otolaryngology and Head and Neck. Botucatu / SP - Brazil.
Mail Address: Jair Cortez Montovani - Faculty of Medicine of Botucatu - UNESP Department of Ophthalmology and Otolaryngology and Head and Neck District Rubiao Junior s / n - Botucatu / SP - Brazil - Zip code: 18603-970 - Telephone / Fax: (+55 14) 3811-6256 / 3811-6081 - E-mail: montovan@fmb.unesp.br
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SUMMARY

Introduction: The BCL2 protein found in the internal mothocondrial membrana 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 contradictious.

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

Method: Sixteen DNA samplers were examinated being 13 of squamous cells carcinoma (SCC) and 3 of epidermoid (CE), y 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.

RESUMO

Introdução: A proteína BCL2 encontrada na membrana mitocondrial interna, regula a apoptose inibindo a morte celular programada. A translocação (14;18), detectada em 70 a 85% dos linfomas foliculares, leva a superexpressão da proteína BCL2, pela justaposição do gene BCL2 ao segmento JH do gene da cadeia pesada da imunoglobulina. Porém, os achados da expressão da BCL2 em carcinoma de cabeça e pescoço são contraditórios.

Objetivo: Investigar a presença da translocação (14;18) do gene BCL2 em carcinomas de cabeça e pescoço.

Método: Foram examinadas 16 amostras de DNA, sendo 13 de carcinomas de células escamosas (CCE) e 3 de epidermoide (CE), por meio da reação em cadeia da polimerase (PCR).

Resultados: O rearranjo BCL2/JH foi encontrado em 2 (15%) dos 13 casos de CCE e em nenhum dos 3 casos de CE. A média de frequência de moléculas com rearranjo foi de 46,44 x 107. Não foi observada associação entre a presença de rearranjo e a exposição ao tabaco e álcool (p=0,6545).

Conclusão: Diferente dos resultados encontrados em linfomas foliculares a presença da translocação (14;18) em carcinomas de cabeça e pescoço não é comum e, quando ocorre, pode ser uma mutação ocasional não associada a exposição ao tabaco e álcool.

Palavras-chave: translocação genética, biologia molecular, neoplasias bucais, neoplasias faríngeas.

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

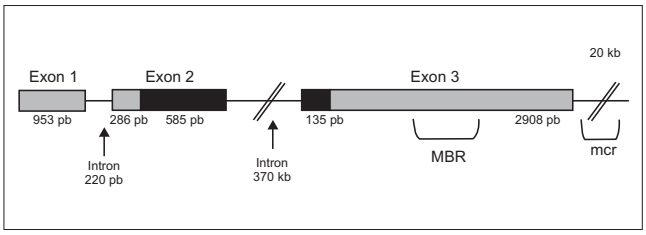


Figure 1. BCL2 gene structure. The BCL2 contains three exons and two introns. The untranslated regions of the gene are illustrated with solid bars of gray, while black bars represent the translated regions.

(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^{0}C for 1 minute, 55^{0}C for 1 minute and 72^{0}C for 1 minutes. 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^{0}C for 1 minute, 58^{0}C for 1 minute and 72^{0}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	Exposure 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 BCL2/JH	Number of cases	No Exposure Tobacco e Alcohol	Exposure Tobacco e Alcohol	Exposure Tobacco
Positive	2	0	2	0
Negative	14	5	7	2
Total	16	5	9	2

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 (1µg of DNA contains 5×10^{-19} moles of each single copy sequence = ~ 300 000 molecules), the estimate λ was given by the usual Poisson estimator: $\lambda = 1 / M \cdot \ln (1 - p)$, where p is the fraction of trials with at least one rearrangement.

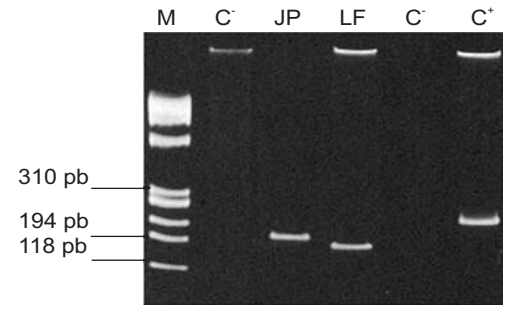
No association was found between the presence of BCL2 rearrangement (MBR) / JH and exposure to tobacco and alcohol (Table 2) (P = 0.6545).

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.

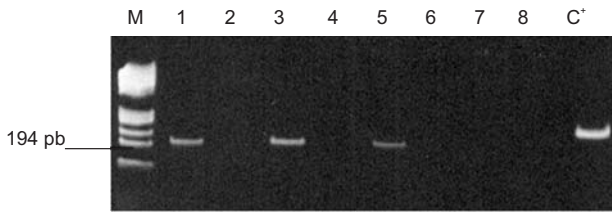
The second patient, a smoker since childhood, and chronic alcoholics (~ two liters of alcohol per day), remained positive in 3 of 8 more screens performed (Figure 2A). Were observed fragments of the same size, suggesting that it is the same clone. The frequency of the MBR rearrangement was 19.32×10^{-7}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



M = molecular weight marker: Ø 174 Hae III digest.
C- = negative control: normal DNA.
JP = patient BCL2/JH+ with CCS.
LF = patient BCL2/JH+ with CCS.
C- = negative control: no DNA.
C+ = positive control: MBR.

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



M = molecular weight marker: Ø 174 Hae III digest.
1 a 8) Screening of Patient 2 that was positive for MBR rearrangement: wells 1, 3, 5 in the gel.
C+ = Positive control: MBR.

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

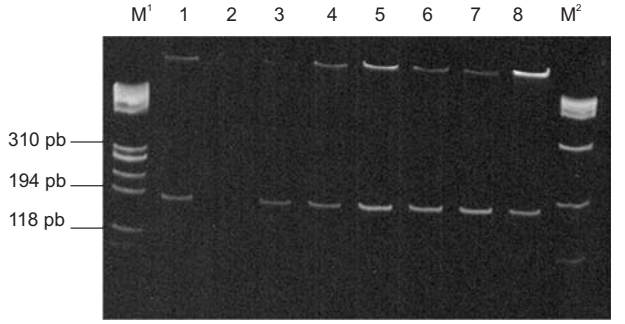
frequency of the MBR rearrangement was 73.57 x 10^{-7} molecules positive.

Discussion

A high expression of BCL2 has been observed in squamous cell carcinomas (Lavieille 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 Vergies 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).



M1 = molecular weight marker: Ø 174 Hae III digest.
1-8) Screening of patient 6 was positive for MBR rearrangement: wells 1, 3, 4, 5, 6, 7 and 8 in the gel.
M2 = marker Low DNA mass ladder: 2000, 1200, 800, 400, 200 and 100 pb.

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

Bell et al. (1995) (13) and Shuler et al. (2003) (17) reported a significant association between smoking and the frequency of cells with at (14, 18) in peripheral blood of healthy volunteers.Other authors have found these same translocations in individuals with cancer are associated with tobacco (8,9,15,16). In this study, there was an association between positive MBR/BCL2 rearrangement and exposure to tobacco and alcohol in patients with head and neck tumors. Because BCL2 mutation rate was similar for both smokers and nonsmokers (8.9 15), the relationship of tobacco antigens stimulating the emergence of clones of rare rearrangements BCL2/JH pre-existing was not observed in our patients. However, no such association was impaired by the low number of subjects studied.

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 Bell 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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