*The Journal of Laryngology & Otology*
August 2002, Vol. **116,** pp. 610–612

# Lack of *Bcl10* gene mutations in laryngeal squamous cell carcinoma

Domenica Ronchetti, Ph.D., Daniela Intini, Ph.D.*, Giancarlo Pruneri, M.D.†, Antonino Neri, M.D.*, Lorenzo Pignataro, M.D.

**Abstract**

The *Bcl10* gene encodes a protein probably involved in some apoptotic regulatory pathways. *Bcl10* mutations lead to the translation of truncated proteins that show gain-of-function transforming activity; it has been suggested that *Bcl10* may represent a major target gene for inactivation in many human cancers. To define the frequency of *Bcl10* mutations in laryngeal squamous cell carcinoma and their possible association with tumour progression, we investigated a large panel of tumours representative of all grades and stages of malignancy.

To detect pathogenic mutations in exons 1, 2 and 3 of the *Bcl10* gene, we performed a silver-staining polymerase chain reaction – single-strand conformation polymorphism (PCR-SSCP) analysis followed by direct DNA sequencing. We revealed the presence of SSCP variants in 18 out of 91 laryngeal tumours. Direct DNA sequencing showed previously described polymorphisms but no pathogenic mutations. We have strong evidence that the *Bcl10* gene is not involved in laryngeal carcinogenesis.

**Key words: Larynx; Carcinoma, Squamous Cell; Genes; Apoptosis**

## Introduction

The *Bcl10* gene has been identified recently as a result of its direct involvement in the t(1;14)(p22;q32) chromosomal translocation currently associated with extranodal (MALT-type) marginal zone lymphomas.[1,2] It encodes a protein that is probably involved in some apoptotic regulatory pathways insofar as functional studies have indicated that it activates NF-κB, induces apoptosis in MFC-7 and 293 cells, and is capable of suppressing *in vitro* transformation.[1–5]

In lymphoid tumours such as MALT and follicular lymphomas, and in cell lines derived from solid tumours such as germ cell tumours and mesotheliomas, *Bcl10* cDNAs present a wide range of mutations, most of which lead to the translation of truncated proteins that maintain their ability to activate NF-κB but do not induce apoptosis and have gain-of-function transforming activity.[1,2] On the basis of this evidence, it has been suggested that *Bcl10* may represent a major target gene for inactivation in many human cancers.[1]

Recent data indicate that the frequency of *Bcl10* gene mutations is low in lymphoid neoplasms,[6] although they occur in some lymphoma subtypes such as MALT lymphoma, in which they are associated with tumour progression.[7] The *Bcl10* gene is rarely mutated in breast, epithelial ovarian, endometrial, cervical, colorectal, or renal carcinomas,[8–13] and two different studies[10,13] failed to detect any *Bcl10* gene mutation in a total of 40 cases of head and neck carcinomas whose site was not specified. Similar results have been reported in the case of 32 laryngeal tumours of unspecified grade and stage.[14]

In order to define the frequency of *Bcl10* somatic mutations in laryngeal squamous cell carcinoma and their possible association with tumour progression, we investigated a large panel of 91 tumours representative of all grades or stages of malignancy. Single-strand conformation polymorphism (SSCP) and direct DNA sequencing failed to detect any pathogenic mutations in exons 1, 2 and 3.

## Materials and methods

### *Patients*

A total of 91 laryngeal squamous cell carcinomas were selected from the 1983–1995 files of Milan School of Medicine's Otolaryngology Clinic I. The

From [1]Clinica Otorhinolaringoiatrica I, Università degli Studi di Milano, [2]Laboratorio di Ematologia Sperimentale e Genetica Molecolare, Servizio di Ematologia, Dipartimento di Scienze Mediche, Ospedale Maggiore IRCCS, [3]Istituto Europeo di Oncologia (IRCCS) divisione di Anatomia Patologica e Medicina di Laboratorio, Università di Milano, 20122 Milano, Italy.
This work was supported by grants from the Associazione Italiana Ricerca sul Cancro (AIRC) (to A.N.), and the Ministero Italiano della Sanità (to the Ospedale Maggiore IRCCS, Milan, Italy).
Accepted for publication: 6 February 2002.

TABLE I
CLINICAL AND PATHOLOGIC CHARACTERISTICS OF THE 91 STUDY PATIENTS

| Characteristic | No. | % |
|---|---|---|
| *Age, years* | | |
| ⩽60 | 34 | 37.3 |
| >60 | 57 | 62.7 |
| *Site* | | |
| SG | 49 | 53.8 |
| GL | 42 | 46.2 |
| *Histological grade* | | |
| G1 | 16 | 17.6 |
| G2 | 48 | 52.7 |
| G3 | 27 | 29.7 |
| *Tumour extension* | | |
| T1–2 | 43 | 47.2 |
| T3–4 | 48 | 52.8 |
| *Lymph nodes* | | |
| NO | 56 | 61.5 |
| N+ | 35 | 38.5 |
| *Clinical stage* | | |
| I–II | 40 | 43.9 |
| III–IV | 51 | 56.1 |

patients had to have primary squamous cell carcinoma of the larynx only, with no history of previous malignancies, nor previous radiotherapy or chemotherapy, and the tumour had to have been surgically excised by means of partial or total laryngectomy. All of the enrolled patients gave their written informed consent.

Their main clinical and pathologic characteristics are shown in Table I. Clinical staging and the anatomical identification of the tumour site were based on the International Union Against Cancer (UICC) TNM classification of malignant tumours.[15] Forty-nine tumours were supraglottic (SG; 53.8 per cent) and 42 glottic (GL; 46.2 per cent); 40 patients were in stage I–II (43.9 per cent), and 51 in stage III–IV (56.1 per cent). We diagnosed and assessed the grade of differentiation of the tumours according to the method of Shanmugaratnam *et al.*,[16] and thus selected 16 well-differentiated (G1; 17.6 per cent), 48 moderately-differentiated (G2; 52.7 per cent), and 27 poorly differentiated squamous cell carcinomas (G3; 29.7 per cent) (Table I).

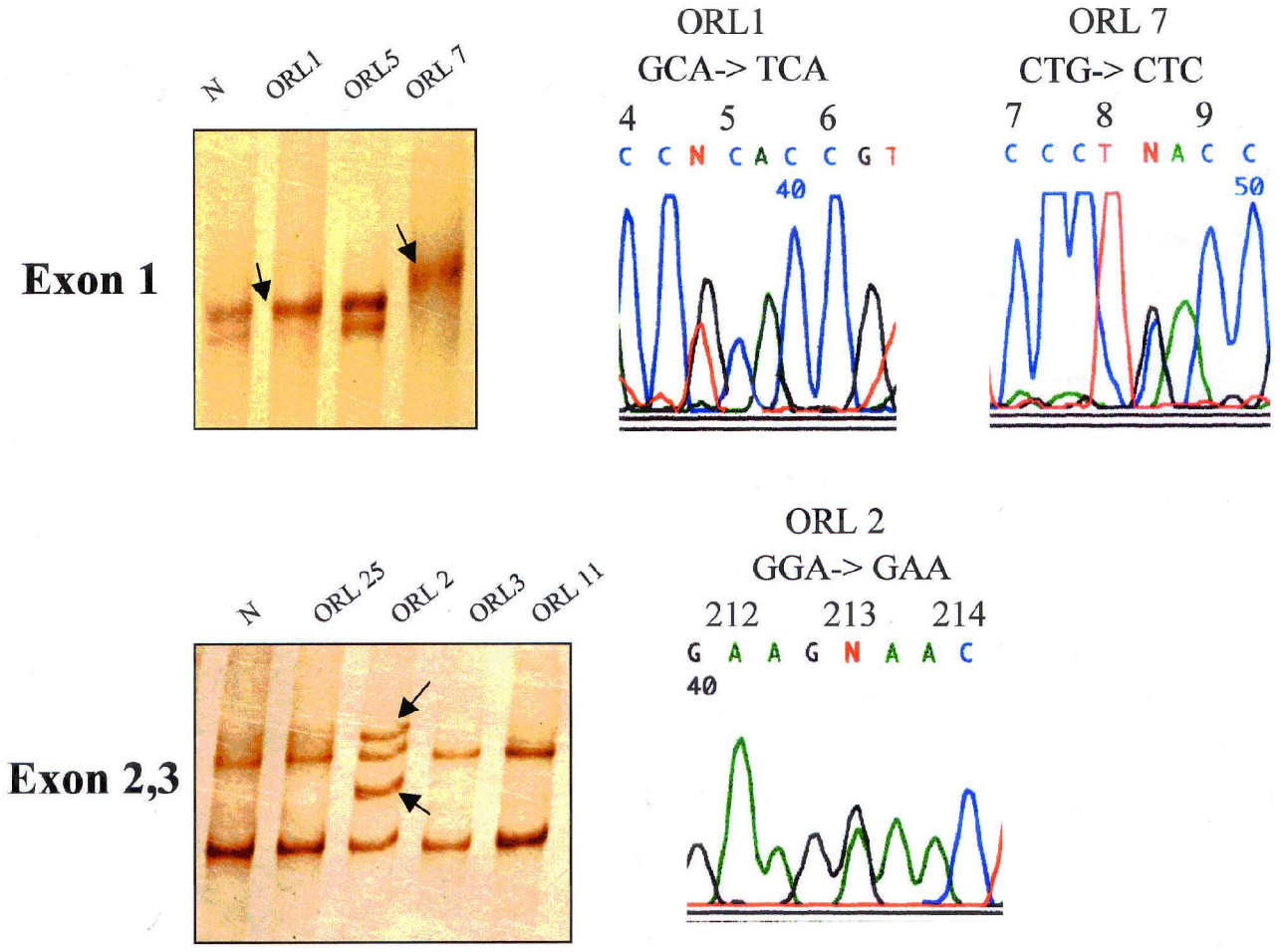


FIG. 1

*Bcl10* gene mutation analysis. Representative examples of SSCP analysis of exon 1 and exons 2, 3 of the *Bcl10* gene. Migrating fragments not observed in normal control (N) are indicated by arrows. Direct sequencing of PCR-amplified fragments from ORL cases with abnormal migration patterns: electropherogram of ORL 1 DNA shows the G/T polymorphic change at codon 5; electropherogram of ORL 7 DNA shows the G/C polymorphic change at codon 8; electropherogram of ORL 2 DNA shows the G/A polymorphic change at codon 213.

https://doi.org/10.1258/00222150260171605 Published online by Cambridge University Press

*DNA preparation*

The pathologic samples were collected during surgery and immediately frozen in liquid nitrogen. They were minced on dry ice, and the DNA was purified by means of proteinase K digestion, phenol-chloroform extraction, and ethanol precipitation.

*PCR-SSCP and direct DNA sequencing*

Exons 1, 2 and 3 of the *Bcl10* gene were amplified from 100 ng of genomic DNA by means of the polymerase chain reaction (PCR) using the five (1, 2.1, 2.2, 3.1 and 3.2) primer sets described by Willis *et al.*[1] The PCR and SSCP conditions were previously optimized for each set of primers.[6] The colonic carcinoma-derived Lovo cell line mutated at codon 46 of the *Bcl10* gene[1] was used as a positive control for SSCP sensitivity. Direct DNA sequencing was performed on the PCR-amplified fragments using the 5′ and 3′ primers used for PCR-SSCP analysis. The DNA fragments were purified by means of agarose gel extraction, and sequenced in both directions using the Big Dye Terminator Cycle Sequencing Kit in an ABI Prism 310 automated sequencer (Perkin Elmer, Norwalk, CT).

## Results

Exons 1, 2 and 3 of *Bcl10* gene were analysed by the silver-staining PCR-SSCP method and direct DNA sequencing.

Analysis of exon 1 revealed the presence of SSCP variant in 11 cases (data not shown): direct DNA sequencing of these samples showed two previously described genetic polymorphisms, including G/T in the first base of codon 5 (three cases) and G/C in the third base of codon 8 (eight cases) (Figure 1).[8–13]

SSCP variants in exons 2 and 3 were identified in seven samples and direct DNA sequencing of all of them revealed the presence of the known G/A polymorphism at the second base of codon 213 in exon 3 (Figure 1).[8,9,13]

## Discussion

The aim of this study was to verify the frequency of *Bcl10* gene mutations in a large panel of 91 laryngeal tumours representing all grades and stages of malignancy (see Table I), as well as in 18 normal mucosa specimens taken from some of these patients. The types of *Bcl10* transcript could not be investigated because no RNA was available.

In order to screen for *Bcl10* gene mutations, we used the same PCR-SSCP based method as that used by Willis *et al.*[1] and optimized by Luminari *et al.*[6] The colonic carcinoma-derived Lovo cell line mutated at codon 46 of the *Bcl10* gene[1] was used as a positive control for SSCP sensivity: our SSCP conditions also make it possible to detect a monoallelic *Bcl10* mutation in about 10 per cent of a given cell population (data not shown), as described in previous reports.[17]

Our results revealed that no pathogenic mutations were present in 91 tumours, strongly suggesting that *Bcl10* is not involved in laryngeal carcinogenesis.

## References


1 Willis TG, Jadayel DM, Du MQ, Peng H, Perry AR, Abdul-Rauf M, *et al. Bcl10* is involved in t(1;14)(p22;q32) of MALT B cell lymphoma and mutated in multiple tumor types. *Cell* 1999;**96**:35–45

2 Zhang Q, Siebert R, Yan M, Hinzmann B, Cui X, Xue L, *et al.* Inactivating mutations and overexpression of *Bcl10*, a caspase recruitment domain-containing gene, in MALT lymphoma with t(1;14)(p22;q32). *Nature Genet* 1999;**22**:63–8

3 Koseki T, Inohara N, Chen S, Carrio R, Merino J, Hottiger MO, *et al.* CIPER, a novel NF κB-activating protein containing a caspase recruitment domain with homology to herpesvirus-2 protein E10. *J Biol Chem* 1999;**274**:9955–61

4 Yan M, Lee J, Schilbach S, Goddard A, Dixit V. mE10, a novel caspase recruitment domain-containing proapoptotic molecule. *J Biol Chem* 1999;**274**:10287–92

5 Thome M, Martinon F, Hofmann K, Rubio V, Steiner V, Schneider P, *et al.* Equine herpesvirus-2 E10 gene product, but not its cellular homologue, activates NFκB transcription factor and c-Jun N-terminal kinase. *J Biol Chem* 1999;**274**:9962–8

6 Luminari S, Intini D, Baldini L, Berti E, Bertoni F, Zucca E, *et al. Bcl10* gene mutations rarely occur in lymphoid malignancies. *Leukemia* 2000;**14**:905–8

7 Du MO, Peng H, Liu H, Hamoudi RA, Diss TC, Willis TC, *et al. Bcl10* gene mutation in lymphoma. *Blood* 2000;**15**:3885–90

8 Fakruddin JM, Chaganti RSK, Murty VS. Lack of *Bcl10* mutations in germ cell tumors and B-cell lymphomas. *Cell* 1999;**97**:683–4

9 Apostolou S, De Rienzo A, Murthy SS, Jhanwar SC, Testa J. Absence of *Bcl10* mutations in human malignant mesothelioma. *Cell* 1999;**97**:684–6

10 Lambers AR, Gumbs C, Ali S, Marks JR, Iglehart JD, Berchuck A, *et al. Bcl10* is not a target for frequent mutation in human carcinomas. *Br J Cancer* 1999;**80**:1575–6

11 van Schothorst EM, Mohkamsing S, van Gurp RJ, Oosterhuis JW, van der Saag PT, Looijenga LH. Lack of *Bcl10* mutations in testicular germ cell tumours and derived cell lines. *Br J Cancer* 1999;**80**:1571–4

12 Stone JG, Rowan AJ, Tomlinson IP, Houlston RS. Mutations in *Bcl10* are very rare in colorectal cancer. *Br J Cancer* 1999;**80**:1569–70

13 Gill S, Broni J, Jefferies S, Osin P, Kovas G, Maitland NJ, *et al. Bcl10* is rarely mutated in human prostate carcinoma, small-cell lung cancer, head and neck tumours, renal carcinoma and sarcomas. *Br J Cancer* 1999;**80**:1565–8

14 Lee SH, Shin MS, Kim HS, Park WS, Kim SY, Lee HK, *et al.* Point mutations and deletions of the *Bcl10* gene in solid tumours and malignant lymphomas. *Cancer Res* 1999;**15**:5674–7

15 Hermanek P, Sobin LH. UICC: *TNM Classification of Malignant Tumors*. Berlin: Springer Verlag, 1992;25–8

16 Shanmugaratnam K, Sobin LH. *Histological Typing of Tumors of the Upper Respiratory Tract and Ears*. Berlin: Springer Verlag, 1987

17 Neri A, Baldini L, Trecca D, Cro L, Polli E, Maiolo AT. p53 gene mutations in multiple myeloma are associated with advanced forms of malignancies. *Blood* 1993;**81**:128–35



Address for correspondence:
Lorenzo Pignataro, M.D.,
Clinica ORL,
Dipartimento di Scienze Otorinolaringologiche,
Università di Milano,
Ospedale Maggiore di Milano, IRCCS,
Via Francesco Sforza 35,
20122 Milano, Italy.

Fax: +39-02-55033929
E-mail: lorenzo.pignataro@unimi.it



L. Pignataro M.D., takes responsibility for the integrity of the content of the paper.
Competing interests: None declared