Oral follicular lymphomas. A short report of 8 cases with assessment of the IGH/BCL2 gene fusion with fluorescence in situ hybridization

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Objective. To present the clinicopathologic features and confirm the presence of the IGH/BCL2 gene fusion in an oral follicular lymphoma (OFL) series.

Study design. Cases of OFLs were retrieved from a data base of non-Hodgkin lymphomas (NHL). Fluorescence in situ hybridization (FISH) was performed to confirm the IGH/BCL2 fusion.

Results. Eight (8.7%) of 92 NHL were OFLs. Six (75%) patients were male and two female (mean age: 73.4 ± 14.8). The most frequent site was the palate. Five of the 8 patients are alive and without disease. Five (three grade 1 and two grade 2) of six successfully hybridized cases revealed the IGH/BCL2 gene fusion. The sixth case, a grade 3 follicular lymphoma (FL), demonstrated multiple BCL2 signals without IGH/BCL2 fusion.

Conclusions. OFLs exhibit an indolent clinical behavior. In the present study, 5/6 cases in which FISH was successful had an IGH/BCL2 fusion as would result from the t(14;18)(q32;q21) translocation commonly seen in FL of extraoral sites. (Oral Surg Oral Med Oral Pathol Oral Radiol 2013;116:343-347)

Follicular lymphomas (FL) are neoplastic proliferations of germinal center B-cells exhibiting at least a partially follicular architectural pattern.1 According to recent epidemiological data, FLs account for 15%-20% of all lymphomas, affecting predominantly adults (median age > 50 years) with a slight female preponderance (male:female, 1:1.7).1-3 FL is the most common subtype of indolent (low grade) lymphoma; however, transformation to a high-grade, aggressive histologic subtype, usually diffuse large B-cell lymphoma (DLBCL), occurs in 20%-30% of cases.4

The molecular mechanism underlying the pathogenesis of FL has been well characterized.5,6 A t(14;18)(q32; q21) translocation is detected in up to 90% of stage I and II FLs.5,7 The t(14;18) translocation results in IGH/BCL2 gene fusion, leading to constitutive overexpression of the anti-apoptotic intracellular protein Bcl-2. This overexpression allows neoplastic B-cells to abrogate the programmed apoptotic process, thus resulting in prolonged survival.5

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Statement of Clinical Relevance
This paper presents the intraoral manifestation of a series of follicular lymphomas. Immunohistochemical and molecular techniques are useful in rendering an accurate final diagnosis.
probe set (Abbott Molecular, Des Plaines, IL, USA) to the IGH (14q32) and BCL2 (18q21) loci. Unstained 3- to 5-μm-thick paraffin sections were cut and placed on positively charged slides. The slides were baked at 90 °C for several hours, deparaffinized in SafeClear II (Fisher Scientific, Pittsburgh, PA, USA), and dehydrated in a series of ethanol washes. After tissue digestion in a pepsin/hydrochloric acid solution, the slides were subjected to denaturation and hybridization with 8 μL probe/buffer mixture. After overnight hybridization, slides were washed, dehydrated in an ethanol series, and counterstained with 4',6-diamidino-2-phenylindole dihydrochloride (DAPI).

RESULTS

Ninety-two cases of NHL of the oral cavity were identified between 1992 and 2006, eight (8.7%) of which were classified as OFL, including five grade 1 (62.5%), two grade 2 (25%) and one grade 3 (12.5%), the latter with areas of diffuse large B cell lymphoma. Six patients (75%) were male and two female (25%), ranging in age from 49 to 92 years (mean: 73.4 ± 14.8). The most frequent site of involvement was the palate (6/8, 75%) with one case each occurring in the maxillary gingival and the buccal mucosa. Five patients (5/8, 62.5%) were alive, three with no evidence of disease and two in remission, including the patient with grade 3 OFL (mean follow-up period: 3.7 ± 1.3 years). The two oldest patients died of unrelated causes and one patient was lost to follow-up. The clinical and epidemiological information regarding the 8 cases of OFL, as well as the follow-up period and the outcome, are presented in Table I. Data concerning therapy for each case were not available.

All cases of OFL exhibited a predominantly follicular pattern with closely-packed follicles of neoplastic B-cells (Figure 1A and B). Occasional neoplastic follicles were poorly defined, large and irregularly shaped. Neoplastic populations were intensely and diffusely positive for CD20 and Bcl-2 (Figure 1C and D). Of the six cases (6/8, 75%) that hybridized successfully with the IGH/BCL2 FISH probe set, five (three grade 1 and two grade 2) had a dual-fusion signal pattern consistent with IGH/BCL2 gene fusion (Figure 2). The sixth, a grade 3 FL, had multiple (>5) BCL2 signals with no apparent IGH/BCL2 fusion (Figure 3) (Table I).

DISCUSSION

FL accounts for approximately 30% of all cases of NHL. FL primarily involves lymph nodes, although spleen and bone marrow are also well-documented sites of involvement. Reportedly, less than 10% of all FLs demonstrate primary extranodal manifestation that encompasses the gastrointestinal (GI) tract, skin, head and neck region, breast and testis. Interestingly, despite the collective categorization under the nomenclature “FL,” certain extranodal subtypes including primary cutaneous and primary intestinal FL exhibit distinct clinico-pathological features and perhaps a more favorable outcome than the nodal counterpart.

OFL accounts for approximately 15% (6/40 cases) of NHL. However, information regarding the clinical, epidemiological or pathologic characteristics of OFL is limited. In general, extranodal lymphomas of the oral cavity comprise <5% of all intraoral malignancies with the vast majority affecting predominantly the hard or soft palate and tongue. In the present study, of 92 NHLs localized to the oral cavity, 8 (8.7%) were diagnosed as FL.

Microscopically, FL is a proliferation of neoplastic follicle center B-cells composed of both centrocytes (small cleaved cells) and centroblasts (large, non-cleaved cells) and characterized by closely apposed, often poorly circumscribed follicles with attenuated or absent mantle zones. Few if any tingible body macrophages may be appreciated. By immunohistochemistry, FL is typically positive for CD19, CD20, CD22, CD79a, as well as BCL2, BCL6, and CD10. Specifically, interfollicular positivity against the latter immunohistochemical marker is particularly useful in establishing the diagnosis of FL. Interestingly, a small number of FLs, usually of grade 3 morphology, may lack CD10 expression entirely. Approximately 85% of diagnosed cases feature BCL2 expression by the neoplastic cells. The interfollicular spaces are filled by neoplastic centrocytes, which may present cytologic and immunohistochemical differences from germinal center cells.

Recent studies have attempted to elucidate the molecular events underlying the pathogenesis of FL. The t(14; 18)(q32; q21) translocation associated with FL results in juxtaposition of the promoter of the immunoglobulin heavy-chain (IGH) gene at 14q32 with BCL2 at 18q21, resulting in a chimeric IGH/BCL2 fusion gene. The IGH/BCL2 gene fusion results in constitutive overexpression of the anti-apoptotic intracellular protein Bcl-2 which permits neoplastic B-cells to abrogate the programmed apoptotic process; consequently, these cells have prolonged survival. Furthermore, most tumors are characterized by recurrent secondary epigenetic alterations including genomic gains, losses, and mutations. Alterations targeting certain genes such as MLL2, EPHA7, TNFRSF14, and EZH2 are usually encountered and may provide a growth advantage in the neoplastic population participating in the pathogenesis of FL.

The t(14; 18)(q32; q21) translocation is identified in up to 85%-90% of FLs. FISH on paraffin-embedded tissue sections is a sensitive and specific method to detect IGH/BCL2 gene fusion in FL. This BCL2 rearrangement is more commonly encountered in grade 1-2 FL than in grade 3 cases, with reported
incidences of 90% and 50% respectively.\textsuperscript{1,9,21} Additionally, in high-grade FL lacking a \(t(14; 18)\) translocation, amplification of \(\text{BCL2}\) has been appreciated,\textsuperscript{2} as was observed in the grade 3 tumor of the present study. The incidence of \(\text{IGH}/\text{BCL2}\) gene fusion varies between nodal and extranodal FLs and among the different extranodal locations. Specifically, Fernández de Larrea et al. reported identification of the aforementioned gene fusion in 65% of nodal FLs and 54% of non-cutaneous extranodal FLs. On the contrary, primary cutaneous FLs demonstrated a \(t(14; 18)\) translocation in a significantly lower percentage, approaching 14%.\textsuperscript{22} Furthermore, Goodlad et al. investigated the presence of \(t(14; 18)\) translocation in non-cutaneous extranodal sites, including certain cases of OFL, and detected the \(\text{IGH}/\text{BCL2}\) chimeric gene in approximately 14.3% (2/14) of extranodal FLs compared with 56.3% (9/16) stage 1 nodal FLs.\textsuperscript{12}

The findings of our study, despite the small number of samples, support the presence of \(\text{IGH}/\text{BCL2}\) gene fusion in 75% of cases and suggest a major participation of the \(t(14; 18)(q32; q21)\) translocation in the pathogenetic pathway of OFL. The difference between our results and those deriving from other studies may be attributed to the different molecular techniques applied for the detection of the \(\text{IGH}/\text{BCL2}\) gene fusion. Polymerase chain reaction (PCR) studies, including the one performed by Goodlad et al.,\textsuperscript{12} may reveal limited detection rates associated predominantly with difficulties in designing highly sensitive primers able to recognize a broad spectrum of variations in translocation breakpoints. Thus, FISH is generally preferable to PCR in routine practice.\textsuperscript{20,23} However, the presence of the aforementioned chromosomal abnormality is not considered sufficient to cause B-cell lymphomagenesis\textsuperscript{19} as supported by the observation that \(t(14; 18)\)
positive clonal B cells can be identified in the blood and lymphoid tissues of up to two-third of healthy individuals.24,25 Interestingly, this translocation is not a pathognomonic feature of FL as it is detectable in other types of lymphoproliferative disorders as well, including DLBCL and chronic lymphocytic leukemia.26,27

Due to its innocuous clinical behavior, the majority of patients with FL present with widespread disease at the time of the initial diagnosis.28 Reportedly, less than 10% of patients with FL are diagnosed with stage I/II disease.7 Radiation therapy is considered the treatment of choice for limited stage FL resulting in a 10-year overall survival rate of 60%-80%.7 Nevertheless, in 10% to 60% of patients with FL, the disease evolves abruptly toward an aggressive NHL-like diffuse large B cell lymphoma, lymphoblastic lymphoma or Burkitt lymphoma.4,29

Estimated median overall survival for patients with FL is 8-10 years.29 Interestingly, as stated previously, various studies have identified important differences concerning the biologic behavior and clinical outcome between nodal and extranodal FL. Goodlad et al. concluded that despite the appreciated high relapse rate, patients with extranodal FL are more likely to achieve complete remission and demonstrate a more favorable long-term prognosis than those with equivalent nodal disease.12 Additionally, certain subtypes of extranodal FL exhibit better prognosis than others. According to Fernández de Larrea et al.,22 5-year overall survival was 100% for cutaneous and 83% for non-cutaneous FL, respectively, while Yamamoto et al. reported that the majority of diagnosed cases of primary GI FL are grade 1-2 with only 4.3% being grade 3, compared with approximately 20% for nodal FL.30 In the present study, in spite of the relatively short period of follow-up (3.7 ± 1.3 years), the majority of patients with OFL (62.5%) were alive at follow-up. The previous finding indicates a better prognosis for OFL, resembling other categories of extranodal FL such as the cutaneous counterpart.

In summary, we reviewed the clinicopathologic characteristics of examples of OFL and confirmed, by FISH, the association with IGH/BCL2 gene fusion in five grade 1 and 2 lesions. We also showed BCL2 amplification in the absence of IGH/BCL2 gene fusion in one grade 3 tumor. Although we were not able to perform FISH in two cases, this method can be routinely utilized to evaluate histologically diagnosed cases of FL for the presence of IGH/BCL2 gene fusion or BCL2 amplification. Furthermore, our study supports the indolent biologic behavior of FL of the oral cavity. Additional, more thorough and extensive investigation is required in order to clarify the clinical features and prognosis of OFL.

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REFERENCES
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