

Case Report

Translocation (5; 11) in a conjunctival MALT lymphoma

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Abstract: Lymphoma is the most frequent malignant tumor of the ocular adnexa with the most common histologic type being extranodal marginal zone B-cell lymphoma (EMZL) of mucosa-associated lymphoid tissue (MALT lymphoma). Here we report a case of a 28 year-old male who presented with a left conjunctival mass of one year duration. A diagnosis of primary MALT lymphoma of the conjunctiva was made based on morphologic and immunophenotypic studies. Chromosome analysis revealed a male karyotype with a translocation t (5;11) (q33;p11.2) as the primary chromosomal abnormality, which, to the best of our knowledge, is the first reported translocation in MALT lymphomas and ocular MALT lymphomas as well.

Keywords: Translocation, MALT lymphoma, ocular, conjunctiva

Introduction

Lymphoma is the most frequent malignant tumor of the ocular adnexa [1, 2]. The majority of ocular adnexal lymphomas are primary extranodal neoplasms, with 10-32% presenting as secondary tumors in patients with disseminated lymphoma [3]. The most common histologic type of primary ocular adnexal lymphoma is reported to be the extranodal marginal zone B-cell lymphoma (EMZL) of mucosa-associated lymphoid tissue (MALT lymphoma), accounting for 35-80% of cases. The most frequent site of origin of ocular adnexal MALT lymphoma is the orbit (~40%), followed by conjunctiva (35-40%), lacrimal gland (10-15%), and eyelid (~10%) [3, 4].

Ocular adnexal lymphomas are usually low-grade lymphomas of MALT origin, that present with localized disease in the fifth to seventh decade of life (median age 65 years) with female preponderance [3]. In general, nodal and EMZL are very rare in children and young adults. A review of the literature revealed that virtually all patients with a MALT lymphoma in the conjunctiva and lacrimal gland reported were adults, with only one case of a conjunctival MALT lymphoma reported in a 10 year-old kid [5].

Several genetic abnormalities have been described in MALT lymphomas. Trisomy 3, 18 or less commonly of other chromosomal abnormalities is a non-specific but not infrequent finding in MALT lymphomas [6]. Chromosomal translocations associated with MALT lymphomas include t (11;18) (q21;q21), juxtaposing the *API2* (apoptosis inhibitor 2) gene on chromosome 11 and the *MALT1* gene on chromosome 18, creating the *API2-MALT1* fusion protein [7, 8]; the t (14;18) (q32;q21) which juxtaposes the *MALT1* gene and the *IgH* promoter on chromosome 14, inducing constitutive expression of the *MALT1* gene [8]; the t (1;14) (p22;q32) translocation, induces juxtaposition of the *BCL-10* gene on chromosome 1 and the *IgH* promoter, leading to constitutive expression of the *BCL-10* gene [9]. These three translocations appear to be linked through the roles of *BCL10* and *MALT1* in activating the necrosis factor (NF)-[kappa] B pathway [3, 11]. More recently, the t (3;14) (p14.1;q32) translocation was described, juxtaposing the *FOXP1* gene on chromosome 3 and the *IgH* promoter [12]. Cytogenetic analysis has proved useful by demonstrating similar alterations in MALT lymphomas from different anatomic sites. Here we report on a case of primary MALT lymphoma of the conjunctiva with a novel translocation t (5;11) (q33;p11.2) as the primary chromosomal

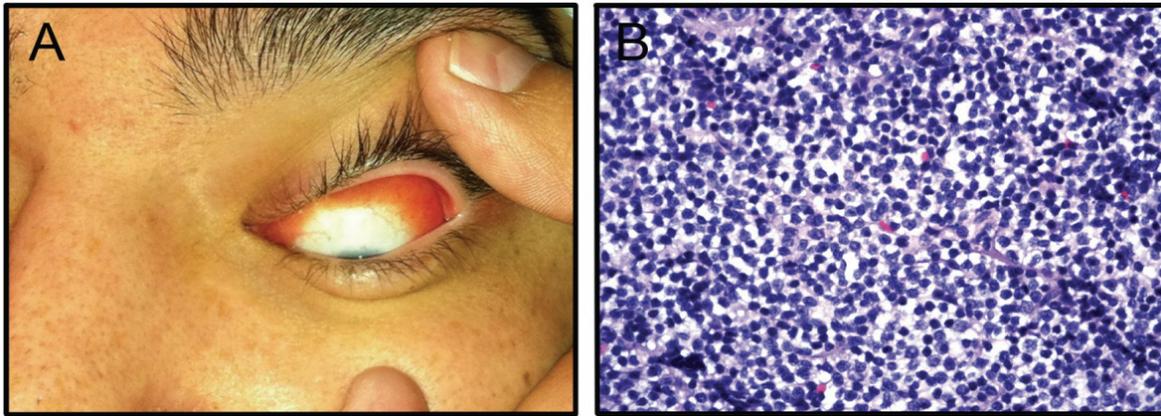


Figure 1 (A) Conjunctival MALT lymphoma with involvement of the superior bulbar conjunctiva nasally and temporally. (B) Diffuse lymphoid infiltrate composed of mostly medium sized lymphoid cells demonstrating monocytoid appearance (400x).

abnormality.

Material and methods

Case history

A 28 year-old hispanic man was admitted to the hospital for a left conjunctival mass of one year duration. His past medical history was unremarkable. There was no family history of cancer. Upon physical examination, there was a salmon-colored, fleshy mass measuring 1.0 x 0.8 cm, involving the left superior bulbar conjunctiva nasally and temporally and spreading superiorly into the palpebral conjunctiva of the upper eyelid. (**Figure 1A**). The right eye was unremarkable. A computed tomography (CT) scan performed with contrast revealed a soft tissue thickening anterior to the left globe, with preservation of the orbit and optic nerve. An excisional biopsy was performed under local anesthesia, which rendered the diagnosis of extranodal marginal zone lymphoma. Clinical staging was performed, which included whole body positron emission tomography-computed tomography (PET-CT) scan, and bone marrow aspirate and biopsy. These studies confirmed the localized conjunctival MALT lymphoma. Currently the patient is asymptomatic, and has been scheduled for radiation therapy.

Surgical pathology

The excisional biopsy specimen was fixed in 10% neutral-buffered formalin, embedded in paraffin, cut at 5 μ m, and stained with hema-

toxylin-eosin. The following immunoperoxidase stains were performed using an automatic immunostainer (Dako Corporation, Carpinteria, Calif) and a streptavidin-biotin-peroxidase complex technique (LSAB2 system, Dako): CD20 (clone L26; Dako, Carpinteria, CA, USA), BCL-2 (clone 124, Dako), CD5 (clone SP19, Dako), CD10 (clone 56C6; BioCare medical, Concord, CA), BCL-1 (clone SP4; Neomarkers, Fremont, CA, USA), CD3 (polyclonal rabbit antihuman CD3; Dako), and CD35 (clone Ber-MAC-DRC; Dako).

Cytogenetic and molecular cytogenetic studies

A fresh sample of the conjunctival mass was collected in RPMI tissue culture medium and was sent directly to Dynagene/LabCorp laboratory (Houston, TX). The tumor tissue was carefully minced to separate cells. These cells were cultured in Minimal Essential Medium (MEM) for 13 to 14 days on a coverslip (Falcon) and in the original dish (Falcon) used for mincing the tissue. After adding 50 μ L of Colcemid (10 μ g/mL, Roche diagnostics, Indianapolis, IN) to each dish for 30 minutes, the cells were harvested. The slides were prepared using both standard *in situ* technique as well as a conventional slide dropping method. Individual chromosomes were identified by the use of a GTG banding procedure. The chromosome aberrations were designated according to the International System for Human Cytogenetic Nomenclature (2009). Fluorescence *in situ* hybridization (FISH) analysis was performed with dual color probe sets for *PDGFRB*, *IGH/BCL2* and *MALT1* (Abbott Molecu-

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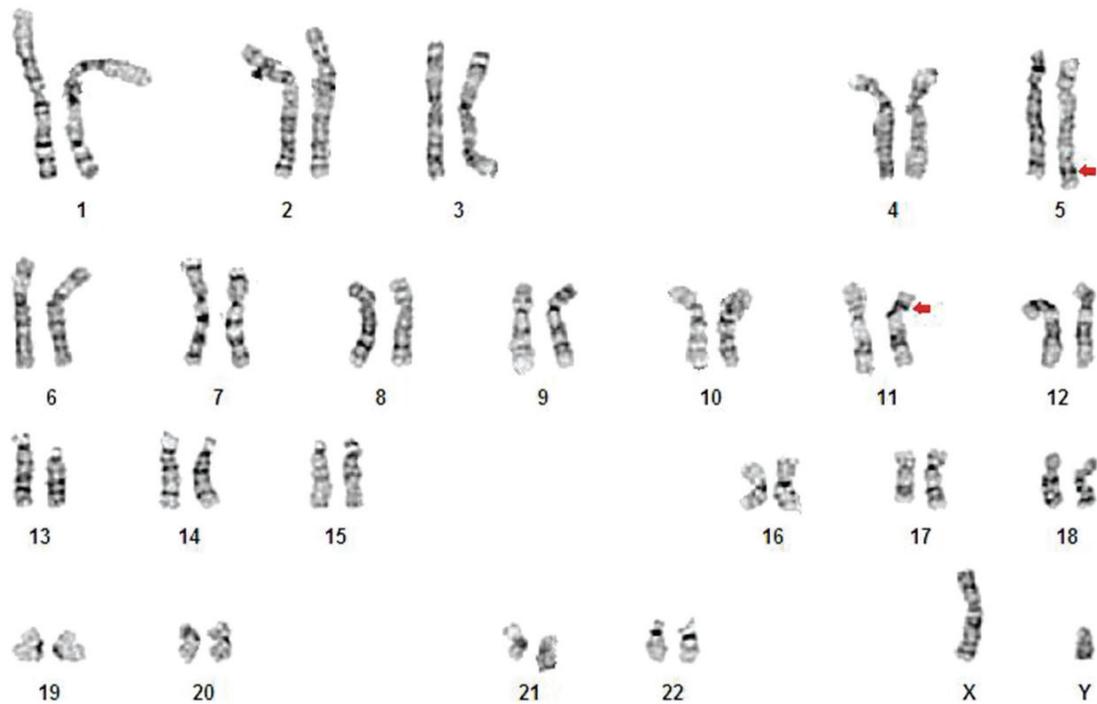


Figure 2 GTG banded analysis showing the karyotype: 46, XY, t (5;11) (q33;p11.2) [cp4].

lar, Des Plaines, IL).

Results

Microscopically, the specimen (0.9 x 0.6 x 0.3 cm) revealed conjunctival epithelium overlying sheets of monotonous lymphoid infiltrate, consisting of mostly medium sized cells with round to slightly irregular nuclear contour, and inconspicuous nucleoli. Some lymphoid cells demonstrated monocytoid appearance with pale cytoplasm. Large atypical lymphoid cells were sparse. No appreciable lymphoepithelial lesion noted (**Figure 1B**). By immunohistochemical studies, the abnormal lymphoid cells were immunoreactive to CD20 and BCL-2, but negative for CD5, CD10 and BCL-1, with few interspersed CD3 positive T lymphocytes. CD35 highlighted the residual germinal center that was negative for BCL-2. Flow cytometry performed at ARUP laboratories (Salt Lake City, UT) detected a population of monoclonal B cells expressing CD19, CD20 and CD22 but negative for CD5 and CD10.

The cytogenetic analysis revealed a male karyotype with a normal cell line and two abnormal cell lines of different clonal origin. The primary

chromosomal abnormality was a reciprocal translocation between the long arm (q) of chromosome 5 and the short arm (p) of chromosome 11, and the minor clone had a reciprocal translocation between 14q and 16q: 46, XY, t (5;11) (q33;p11.2) [cp4]/46, XY, t(14;16) (q24;q22) [3]/46, XY [13] (**Figure 2**). Normal hybridization patterns were observed for all the probe sets indicating no rearrangements involving these genes located on the long arms of chromosomes 5, 14 and 18 respectively.

Discussion

Lymphoproliferative lesions of the ocular adnexa include a wide spectrum of disorders ranging from benign lymphoid hyperplasia to malignant lymphoma. The distinction of diffuse hyperplasia from MALT lymphoma may be difficult using morphology alone, without immunophenotyping and molecular techniques [3, 14]. Morphologic features of ocular adnexal MALT lymphomas are similar to EMZL in other sites, such as infiltration of small B-cells, centrocyte-like, plasmacytoid and monocytoid cells, with scattered larger centroblast-like cells, although lymphoepithelial lesions are very infrequently seen [3, 15, 16].

Immunophenotypically, MALT lymphomas have a characteristic profiles allowing their distinction from benign lymphoproliferative disorders and other small B-cell lymphomas [3]. The demonstration of immunoglobulin light chain restriction is important in the differential diagnosis with benign lymphoid infiltrates. The tumor cells of MALT lymphoma are characterized by CD20, CD79a positive, CD10, CD5, and CD23 negative, and CD43 and CD11c variably positive B-cell lymphocytic infiltrates, with scattered CD3 positive T-cell lymphocytes. Staining for CD21 and CD35 also typically reveals expanded meshworks of follicular dendritic cells corresponding to colonized follicles [6].

Cytogenetic analysis has demonstrated similar alterations in MALT lymphomas from different anatomic sites [12]. However, the frequencies at which the translocations or trisomies occur vary markedly with the primary anatomic site of disease [11, 16, 17]. The t (11;18) (q21;q21) is mainly detected in pulmonary and gastric tumors, t (14;18) (q32;q21) in ocular adnexa and salivary gland lesions and t (3;14) (p14.1;q32) in MALT lymphomas arising in the thyroid, ocular adnexa and skin [11, 17]. Only a small number of studies have fully examined the cytogenetic findings in MALT lymphomas involving the ocular adnexa. Most of these studies have focused only on detection of t (11;18) (q21;q21) by RT-PCR or interphase FISH, reporting this translocation in 0% to 16% of cases [16]. A report of recurrent abnormalities in ocular adnexa MALT lymphomas by Streubel et al [17] in 37 European cases described +3/+18 in 35%, *IGH/MALT1* in 24%, and *API2/MALT1* in 3%. Ruiz et al studied cases of ocular MALT lymphomas from two North American institutions and found +3/+18q in 79% of 31 patients. However, all cases were negative for *MALT1* translocations. *IGH* translocations were identified in 3 cases (10%), although the translocation partner gene could not be identified [16]. Thus, it appears that there is a variation in the frequency of these recurring cytogenetic abnormalities in MALT lymphomas not only by primary site of disease but there may also be a geographic variability [11, 16].

During the last two decades, the ability to identify chromosomal abnormalities has been markedly improved by the development of molecular cytogenetic technologies such as FISH and comparative genomic hybridization (CGH). However, molecular cytogenetic techniques are only able

to detect defined chromosomal aberrations searched for by a specific assay. Thus, a number of other genetic alterations may be missed that can be disclosed by conventional cytogenetic analyses [18]. However, conventional cytogenetic analyses of lymphomas can also be limited by a low metaphase yield. Translocations in total have been identified in only about 25% of primary MALT lymphomas, thus it is possible that other recurrent translocations remain to be discovered, as suggested by Remstein et al [11].

In the present study we have identified a novel chromosomal aberration in MALT lymphoma involving a reciprocal translocation between chromosomes 5 and 11-t (5;11) (q33;p11.2) as the primary chromosomal abnormality; and a minor clone with a reciprocal translocation between 14q and 16q. An initial FISH analysis was carried out to investigate whether *PDGFR-β* was rearranged on the derivative chromosome 5. The dual color probe set for *PDGFR-β* was negative for a rearrangement of the *PDGFR-β*. Similarly, FISH analysis was also performed with dual color probe sets for *IGH/BCL2* and *MALT1*. Normal hybridization patterns were observed for all the probe sets indicating no rearrangements involving these genes located on the long arms of chromosomes 14 and 18 respectively. Further cytogenetic studies remain to be done to elucidate the rearranged genes involved in this case.

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References

- [1] Margo CE, Mulla ZD. Malignant tumors of the orbit: analysis of the Florida Cancer Registry. *Ophthalmology* 1998; 105: 185-190.
- [2] Shields JA, Shields CL, Scartozzi R. Survey of 1264 patients with orbital tumors and simulating lesions: The 2002 Montgomery Lecture, part 1. *Ophthalmology* 2004; 111: 997-1008.
- [3] Stefanovic A, Lossos IZ. Extranodal marginal zone lymphoma of the ocular adnexa. *Blood* 2009; 114: 501-510.
- [4] Moslehi R, Devessa SS, Schairer C, Fraumeni JF Jr. Rapidly increasing incidence of ocular non-Hodgkin lymphoma. *J Natl Cancer Inst* 2006; 98: 936-939.
- [5] Tiemann M, Häring S, Heidemann M, Reichelt J, Claviez A. Mucosa-associated lymphoid tis-

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- sue lymphoma in the conjunctiva of a child. *Virchows Arch* 2004; 444: 198-201.
- [6] Isaacson PG, Chott A, Nakamura S, Müller-Hermelink HK, Harris NL, Swerdlow SH. Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma). WHO classification of tumours of haematopoietic and lymphoid tissues. Edited by Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J, Vardiman JW. Lyon, International Agency for Research on Cancer (IARC) 2008, pp: 214-217.
- [7] Auer IA, Gascoyne RD, Connors JM, Cotter FE, Greiner TC, Sanger WG, Horsman DE. t (11;18) (q21;q21) is the most common translocation in MALT lymphomas. *Ann Oncol* 1997; 8: 979-985.
- [8] Ye H, Liu H, Attygalle A, Wotherspoon AC, Nicholson AG, Charlotte F, Leblond V, Speight P, Goodlad J, Lavergne-Slove A, Martin-Subero JI, Siebert R, Dogan A, Isaacson PG, Du MQ. Variable frequencies of t (11;18) (q21;q21) in MALT lymphomas of different sites: significant association with CagA strains of *H pylori* in gastric MALT lymphoma. *Blood* 2003; 102: 1012-1018.
- [9] Streubel B, Lamprecht A, Dierlamm J, Cerroni L, Stolte M, Ott G, Raderer M, Chott A.T (14;18) (q32;q21) involving *IGH* and *MALT1* is a frequent chromosomal aberration in MALT lymphoma. *Blood* 2003; 101: 2335-2339.
- [10] Willis TG, Jadayel DM, Du MQ, Peng H, Perry AR, Abdul-Rauf M, Price H, Karran L, Maje-kodunmi O, Wlodarska I, Pan L, Crook T, Hamoudi R, Isaacson PG, Dyer MJ. Bcl-10 is involved in t (1;14) (p22;q32) of MALT B cell lymphoma and mutated in multiple tumor types. *Cell* 1999; 96: 35-45.
- [11] Remstein ED, Dogan A, Einerson RR, Pater-noster SF, Fink SR, Law M, Dewald GW, Kurtin PJ. The incidence and anatomic site specificity of chromosomal translocations in primary extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) in North America. *Am J Surg Pathol* 2006; 30: 1546-53.
- [12] Streubel B, Vinatzer U, Lamprecht A, Raderer M, Chott A. t (3;14) (p14.1;q32) involving *IGH* and *FOXP1* is a novel recurrent chromosomal aberration in MALT lymphoma. *Leukemia* 2005; 19: 652-658.
- [13] Reiser M, Josting A, Diehl V, Engert A. Primary lymphoma of the conjunctiva-a rare manifestation of indolent non-Hodgkin's lymphoma. *Ann Hematol* 2001; 80: 311-313.
- [14] McKelvie PA, McNab A, Francis IC, Fox R, O'Day J. Ocular adnexal lymphoproliferative disease: a series of 73 cases. *Clin Exp Ophthalmol* 2001; 29: 387-393.
- [15] Isaacson PG. Extranodal marginal zone lymphoma: MALT lymphoma. *Hematopathology: expert consult*. Edited by Jaffe ES, Vardiman JW, Campo E, Arber D, Harris NL. Philadelphia: Elsevier Saunders 2011; pp: 291-305.
- [16] Ruiz A, Reischl U, Swerdlow SH, Hartke M, Streubel B, Procop G, Tubbs RR, Cook JR. Extranodal marginal zone B-cell lymphomas of the ocular anexa: multiparameter analysis of 34 cases including interphase molecular cytogenetics and PCR for *Chlamydia psittaci*. *Am J Surg Pathol* 2007; 31: 792-802.
- [17] St Streubel B, Simonitsch-Klupp I, Müllauer L, Lamprecht A, Huber D, Siebert R, Stolte M, Trautinger F, Lukas J, Püspök A, Formanek M, Assanasen T, Müller-Hermelink HK, Cerroni L, Raderer M, Chott A.. Variable frequencies of MALT lymphoma-associated genetic aberrations in MALT lymphomas of different sites. *Leukemia* 2004; 18: 1722-1726.
- [18] Siebert R, Weber-Matthiesen K. Fluorescence in situ hybridization as a diagnostic tool in malignant lymphomas. *Histochem Cell Biol* 1997; 108: 391-402.