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ORIGINAL ARTICLE

High expression of LMO2 in Hodgkin, Burkitt and germinal center diffuse large B cell lymphomas

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Abstract *Background and aim:* The LMO2 gene encodes a transcription factor that regulates key events in erythropoiesis, angiogenesis, and embryogenesis and is highly expressed at the most immature stages of lymphopoiesis. Its implication in Hodgkin lymphoma (HL), Burkitt lymphoma (BL) and diffuse large B cell lymphoma (DLBCL) is limited in the literature.
Material and methods: An immunohistochemical study was performed on 68 lymphoma specimens showing different types including Hodgkin lymphoma (23), Burkitt lymphoma (10) and diffuse large B cell lymphoma (35). Also, seven specimens of the reactive nodal tissue were included as control. A monoclonal anti-human antibody has been used to detect LMO2.
Results: LMO2 was detected in all cases of HL (100%), in nine cases of BL (90%) and in all cases of DLBCL of germinal center (GC) subtype 20/35 (57.1%) but is completely negative in non-germinal center (NGC) DLBCL. In normal control of reactive nodes, LMO2 was expressed in germinal center area but not expressed in other areas including mantle, marginal, or T cell zones. In DLBCL; there was no statistically significant relation between LMO2 positive cases and the studied clinicopathological parameters including patient's age, sex and tumor site, stage and histological subtype. On the other hand, it was statistically significant regarding immunophenotyping of GC versus NGC.
Conclusions: LMO2 expression is a special feature of GC DLBCL which can be used as a diagnostic marker and therapeutic target. Further studies regarding its prognostic role in patients are recommended.
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Introduction

In Egypt, lymphoma is considered as the fourth ranking adult tumor, it represents (11.6%), 76.6% non-Hodgkin lymphoma (NHL) and 23.4% Hodgkin lymphoma (HL). The clinically aggressive subtypes such as diffuse large B-cell lymphoma (DLBCL) and Burkitt lymphoma (BL) are the most frequent diagnostic subtypes of non-Hodgkin lymphoma, representing 40–50% of all adult non-Hodgkin lymphomas [1]. In the United States the number of estimated new cases for lymphoma is 74,030 with an estimated cancer death of 21,530 [2].

Recently, gene expression profiling has been used to identify prognostic subsets of DLBCL, with the germinal center B-cell (GCB) DLBCL subgroup, demonstrating a superior 5-year survival compared with the activated B-cell (ABC) DLBCL subgroup [3,4]. Because gene expression profiling is impractical for routine clinical practice, a few studies have used the immunohistochemical expression of CD10, bcl-6, or MUM1 to classify cases of DLBCL into GCB (CD10+ or CD10−, BCL6+, and MUM1−) and non-GCB (CD10− and MUM1+) subgroups [5–9]. However, the resulting data are conflicting, with 2 studies showing a significantly better survival for the GCB group [6,9] whereas two others have found no difference in survival between the GCB and non-GCB groups [7,8]. Accordingly, the search for novel diagnostically relevant markers and therapeutic targets continues.

LMO2 is the Lim Domain. Only two genes encode a cystein-rich LIM domain containing transcription factor that is required for the complete hematopoiesis in mice [10] (it is also termed RBTN2 and TTG2). While it was expressed in different tissues during fetal life its expression in hematopoietic cells is tightly regulated and varies at different stages of maturation. In normal B-cell differentiation and maturation, LMO2 is expressed mainly at two stages: at early lymphopoiesis within the bone marrow and in germinal centers (GC) of secondary lymphoid organs [11].

Expression of LMO2 has not been identified in a number of different epithelial malignancies [11], to the best of our knowledge, there is no published full length literature directly evaluating its expression in DLBCL, BL and HL. Therefore the aim of our study was to evaluate the expression of LMO2 in a series of DLBCL, BL and HL specifically to determine its utility in discriminating B cell lymphoma of GC origin from those of the non-GC origin and then correlate its expression with different clinicopathological parameters.

Material and methods

Tissue samples

The cases were consecutively retrieved from the surgical pathology files of the Suez Canal University hospital during the period from January 2003 to December 2009. The study included 68 lymphoma specimens divided into 35 DLBCL, 23 HL and 10 BL. Additional seven normal lymph node tissue specimens were included. The specimens selected for this study were based on the availability of complete clinical data and the possibility of retrieving paraffin blocks from the diagnostic biopsy samples. Cases of DLBCL were classified according to the WHO classification [12] into centroblastic when more than 90% of the cells were centroblasts, centroblastic polymorph when the proportion of immunoblasts ranged from 10% to 90%, and immunoblastic when more than 90% of tumor cells were immunoblasts.

Formalin-fixed, paraffin-embedded samples for LMO2 immunohistochemical staining

Hematoxylin and eosin-stained sections from each paraffin-embedded, formalin-fixed block were used to define diagnostic areas and then representative sections of 5 μm were cut from each paraffin block and stained with LMO2 monoclonal antibody. Then the cases of DLBCL were stained with other antibodies such as CD20, CD10, bcl-6, MUM1, bcl-2, and MIB1 for their classification. The antibodies, their sources, clones, antigen retrieval buffer and dilutions are illustrated in (Table 1).

All of the slides were deparaffinized using xylene and then rehydrated in decreasing concentrations of ethanol. Antigen retrieval (AR) using microwave heating (three times of 10 min) was performed after the inhibition of endogenous peroxidase activity (0.3 hydrogen peroxidase for 15 min). The slides were incubated with the primary antibody at room temperature, and then washed using phosphate buffered saline (PBS) and then incubated with secondary antibody for 15 min followed by PBS wash. Finally the detection of bound antibody was accomplished using the ABC reagent (ultra-vision detection system, anti-polyvalent, ready to use, LAB VISION, USA) for 20 min followed by PBS washes, 0.1% solution of diaminobenzidine (DAB) was used for 5 min as a chromogen and slides were counterstained with Mayer's hematoxylin for 5–10 min. Negative controls were obtained by omitting the primary antibody and reactive nodes were used as a positive control for all antibodies.

A given lymphoma was judged positive when 20% positive cells were immunostained by each antibody except MIB-1, the percentage of positive cells for MIB-1 was estimated. The number of MIB-1 positive cells and all tumor cells were determined by an actual count in areas (2.5 mm^2) counted at high power field (400×), background of small lymphocytes and starry sky macrophages were excluded, a mean of three areas in each case was taken as the MIB-1 index and then the cases classified into two groups with a cutoff point of 50% [13]. For LMO2 expression, The mean percentage of positive tumor cells was determined in at least 10 fields at 400-fold magnification and assigned one of the following four categories: 0, $<30\%$; 1, 30–$<50\%$; 2, 50–$<75\%$; 3, $>75\%$. Cases were considered positive if 30% or more of the tumor cells were nuclear stained with an antibody then positive staining intensity was scored into three categories, weak (1+), moderate (2+) and strong (3+) [11]. Immunoperoxidase results for CD10, bcl-6, and MUM1 were used to subclassify the cases of DLBCL (Diagram 1). Cases were subdivided into GC like B cells and NGC (ABC or type 3), based on the positivity for CD10, bcl-6 and Mum-1. A case was considered of GC like B cell origin if Mum-1 was negative and CD10 alone or both CD10 and bcl-6 were positive [14–16]. ABC was considered when cells were only positive for Mum-1 and type 3 when a case was negative for CD10 but double positive or negative for both bcl-6 and Mum-1.

A CD20 stain was performed to evaluate each section for involvement of tumor, and each slide was evaluated independently by two pathologists for the percentage of tumor cells

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