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Livin, a novel marker in lymphoma type distinction ☆

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ABSTRACT

Despite advances in immunohistochemical and molecular diagnostics, there are persistent difficulties in differentiating between several subtypes of non-Hodgkin lymphoma (NHL) and classic Hodgkin lymphoma (CHL). Considering high level of livin expression in hematologic malignancies, we aimed to examine the utility of livin expression ratio, as an ancillary biomarker, in distinguishing CHL from NHL in ambiguous cases. We evaluated livin expression in 38 CHL, 23 NHL, and 39 nonneoplastic lymph nodes in paraffin-embedded blocks. Tissue microarray–based semiquantitative immunoflourecent staining was applied for protein expression. Criterion standard of diagnosis was based on selection of only definite cases and not the cases suspected by hemathopathologists. A significant difference was found in the livin/GAPDH mean ratio (M.R) of expression between NHL and CHL cases. A receiver operating characteristic curve analysis confirmed 0.6370 to be the best diagnostic cut-off value for the livin/GAPDH expression M.R in diffuse large B-cell lymphoma (DLBCL) (area under the curve = 0.944); it yielded 92% sensitivity, 94% specificity, likelihood ratios positive 17.5, and likelihood ratios negative 0.07 for diagnosing DLBCL from CHL. Mean ratio of livin/Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) expression seems to be a valuable index in differentiating DLBCL from CHL. We suggested an optimal cut-off point for livin/GAPDH expression M.R with a high sensitivity and specificity. Thus, in diagnostically difficult cases of DLBCL and CHL, focus on livin as marker may provide useful corroborative information.

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1. Introduction

In recent years, overlap in biological and morphologic characteristics has been recognized between classic Hodgkin lymphoma (CHL) and various subtypes of B-cell non-Hodgkin lymphoma (NHL) such as diffuse large B-cell lymphoma (DLBCL) and anaplastic large cell lymphoma (ALCL) [1].

On the other hand, this diagnostic gray zone is not just of theoretical importance; it presents a practical problem, as the therapeutic approaches for CHL differ from those for aggressive B-cell lymphomas [2,3]. Genomics and proteomics approaches have led to identification of novel biological and clinical subgroups of lymphoma. However, the application of such studies in clinical practice on a routine basis was premature because many of the appropriate techniques are not available in the clinical laboratories up to now [1]. For example, in spite of advances in immunohistochemical analysis and molecular diagnostics, there are persistent difficulties in differentiating between certain subtypes of NHL and CHL in many cases [1,3]. A number of immunohistochemical markers such as Pax-5 [4], octamer-binding transcription factor 2, B-cell octamer–binding protein 1 [5,6], and fascin have been introduced to help in classifying a subset of such cases [7,8], but each of them had some practical problems or controversies in discriminative diagnosis especially for DLBCL [1,4-8].

Livin is a member of the inhibitors of apoptosis protein gene family, which has important roles in apoptosis, cell proliferation, and cell cycle control. Overexpression of livin has been reported in a variety of human neoplasms including hematologic malignancies during the process of cancer formation and/or progression [9,10]. It is worth noting that increased livin expression is variable in different tumors and might have a potential role in categorizing subsets of malignancies [11]. Furthermore, a recent study has shown that the livin messenger RNA (mRNA) and protein are highly expressed in NHL lymph node samples. The same study showed a positive correlation between livin expression and clinical stages of NHL [12]. Thus,

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regarding overly aggressive behavior of NHL in comparison with CHL [13-15], we hypothesized that livin expression is higher in DLBCL than CHL lymph node samples. We conducted this study to examine the utility of livin expression ratio in differentiating CHL cases from morphologically close forms of NHL (with Reed-Sternberg [RS]–like cells) such as DLBCL and ALCL.

2. Materials and methods

2.1. Patients

Livin expression was examined in 100 paraffin-embedded lymph node blocks including 38 CHL blocks, 22 NHL tissue blocks, 30 normal lymph nodes, and 9 reactive hyperplasia lymph nodes. The lymph nodes were excised in the department of surgery from 2006 to 2011, in Seyed-Alshohada and Alzahra hospitals, which are affiliated by Isfahan University of Medical Sciences.

Primary diagnosis of CHL was made based on histologic examination through hematoxylin and eosin staining and confirmed morphology diagnosis of RS cells by expert panel, and also, based on immunohistochemical examination, we accepted RS cells (to consider as CHL cases), which are expressed CD15 (85% of cases) and CD30 (100% of cases) and lack of global expression of pan-B (CD19, CD20, and CD79a) and pan-T (CD3 and CD7) antigens.

In all cases of suspected NHL, the initial panel of immunohistochemical stains that had been performed included LCA, CD20, CD3, Pax-5 (pan-B cell antigen), and proliferation marker Mib1 (Ki-67). Additional markers used in some cases included CD10, BCL2, CD5, CD21, etc. In cases of suspected ALCL, the initial panel of markers included LCA, CD20, CD3, CD30, and ALK protein. Most cases of CHL in our study were negative for LCA, B- and T-cell markers and positive for CD30 and CD15. In some cases, the tumor cells were positive for CD20. Most cases of DLBCL in our study were positive for LCA, CD20, and Pax-5 and were negative for CD30. Most cases of ALCL were negative for LCA, CD20, CD3, and CD15 and were positive for CD30.

For minimizing the selection bias, all cases were run over by 2 independent blinded pathologists. The sampling method was a base on randomized selection but try to omit ambiguous cases by use of pathologic expert panel point of view. The so-called criterion standard of our diagnosis was the cases marked as definite, by our experienced hematopathologists. Finally, eligible lymph node blocks were confirmed again by the hematopathologists with supplementary immunohistochemical staining. We enrolled 38 CHL lymph node blocks including 18 nodular sclerosis CHL, 14 mixed cellularity CHL, 4 lymphocyte-rich CHL, and 2 lymphocyte depleted CHL. The NHL group included 14 DLBCL, 3 ALCL, and 6 follicular lymphoma (FL) blocks.

Clinical features of all patients including sex, age, B symptom (fever, night sweats, and weight loss), lymph node involvement site (cervical, auxiliary, mediastinum, paraaortic, perihilar, and inguinal), and extralymphatic organ involvement (lung, liver, spleen, and bone marrow) were recorded, and clinical staging was performed by an oncologist based on Ann Arbor criteria.

2.2. Immunostaining

The slide preparation and semiquantitative immunostaining method used in this study are discussed in our previous studies [13-15]. In summary, we used 2 antibodies against livin and GAPDH. Because the expression of GAPDH is constant in normal and neoplastic lymphoid tissue, any differences in livin/GAPDH ratio indicate changes in livin protein expression. Livin and GAPDH protein expressions were measured using semiquantitative immunofluorescent assay. For livin detection, we used rabbit anti-Human IgG polyclonal antibody (Lot ID:LS-B456/10844; LifeSpan, Seattle, WA BioSiences) that recognizes amino acids 264 to 280 of the short form and 281 to 298 of the long form of human livin. This sequence is identical between α and β isoforms of the livin proteins, so this antibody detects both α and β isoforms of intake livin protein, not truncated livin protein. GAPDH primary antibody was mouse anti-Human IgG monoclonal antibody (Lot ID: LS-B520; LifeSpan BioSiences). Fluorochrome-conjugated secondary antibodies were fluorescein (FITC) AffiniPure Goat anti-rabbit whole IgG Heavy + Light chains (H + L) and Texas Red AffiniPure Goat anti-mouse IgG (H + L).

2.3. Image analysis

Image analysis was performed as described in our previous study [13]; briefly slides were examined using an Leica fluorescence microscope (BZ00, Wetzlar, Germany) with filter sets suitable for FITC and Texas Red dyes. Two images were taken from each microscope field, one with blue filter (350-450 nm) and another with green filter (550-650 nm). Furthermore, for each sample, between 20 to 30 images were taken from randomly selected fields of the tissue section. Then, we analyzed the images using image processing algorithms in MATLAB (Natick, Massachusetts, USA) 7 software (http://www.mathworks.com). In each image, the whole boundary of desired cells (morphologically typical lymphocytes) in both green and red planes was selected, and then, the ratio for intensity between the mean of pixels in the green and red plane of selected cells was computed by following the ratio formula:

$$\text{Ratio} = \frac{\begin{array}{c}\text{Mean of pixel intensity for a cell in the green plane}\\ \text{(indicates livin expression)}\end{array}}{\begin{array}{c}\text{Mean of pixel intensity for the same cell in the red planet}\\ \text{(indicates GAPDH expression)}\end{array}}$$

2.4. Statistical analysis

Independent *t* test and one-way analysis of variance were run to compare the mean level of livin expression ratio in both cases (NHL and CHL), control groups, and their subdivides.

Table 1
Clinical features of patient with CHL

Sex (no. of cases)	Male			Female		
	21			17		
Age (y)	33.6 ± 15.1					
B symptom (no. of cases)	Positive			Negative		
	14			17		
Fever	9/14					
Night sweat	7/14					
Weight loss	8/14					
Lymph node Involvement (no. of cases)	Cervical	Axillary	Mediastinal	Paraaortic	Perihilar	Inguinal
	26	5	9	3	3	4
Extralymphatic organ involvement (no. of cases)	Lung		Liver	Spleen	Bone marrow	Others
	3		2	3	0	4

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