Expression of ERG in Lymphoblastic Lymphoma Patients: A Potential Diagnostic Pitfall

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Short report

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Expression of ERG in Lymphoblastic Lymphoma Patients: a Potential Diagnostic Pitfall

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Abstract

Background: The Ets-related gene (ERG) is the member of ETS family of transcription factors, which commonly expressed in Ewing's sarcoma. Recently, we found that ERG can also express in lymphoblastic lymphoma. The aim of this article is to analyze the ERG expression in lymphoblastic lymphoma.

Methods: The patients of lymphoblastic lymphomas who had undergone fine needle aspiration or surgical operation from 2017 to 2021 in the second affiliated hospital of Nan-Chang university were collected and examined. Immunohistochemistry (IHC) was performed to evaluate the expression of ERG.

Results: In this study, 20 T-lymphoblastic lymphomas and 4 B-lymphoblastic lymphomas were investigated for the expression of ERG. Our findings showed that ERG was expressed in 8 of the 20 (40%) T-lymphoblastic lymphomas, and 3 of the 4 (75.0%) B-lymphoblastic lymphomas.

Conclusions: This report shows that ERG can express in lymphoblastic lymphomas, and highlights a potential diagnostic pitfall in the diagnosis of Ewing sarcoma, which urges pathologists to exercise caution in cases where ERG-positivity and illustrates the need for further immunohistochemical examination to avoid misdiagnosis.

Keywords: ERG, Ewing's sarcoma, Lymphoblastic lymphomas, Diagnostic Pitfall

Background

Lymphoblastic lymphomas are aggressive hematological malignancies consisting of small to medium-sized blast cells which are similar to other small round cell tumours. Most often, these malignancies manifest with extensive marrow and blood affectation,[1] and sometimes as a mass lesion in the thymus or extranodal soft tissue. Actually, lymphoblastic lymphomas involving in the soft tissue may be confused with Ewing sarcoma, particularly when a limited panel of antibodies such as ERG, FLi-1 and CD99 are positive in both of them, which may lead to an erroneous diagnosis.

ERG is an ETS-family TF, which is a potent oncogene associated with both solid organ and hematologic malignancies.[2] Chromosomal translocations that result in the expression of oncogenic ERG fusion proteins have been identified in leukemia, in Ewing sarcoma, and in 40%-80% of prostate carcinomas.[3-5] Except the positive expression of ERG in Ewing sarcoma, recent research also showed that the Ewing sarcoma gene (EWS)–ERG fusion protein can not only transform mesenchymal progenitors into sarcomas, but also transform committed lymphocytes into T-cell leukemias in vivo. Besides, ERG is also temporally regulated during B lymphopoiesis, suggesting it may regulate B lymphoid development.[6-8,9] Hererin, we hypothesis that ERG may play a key role in the development of lymphoblastic lymphoma.

To determine the expression of ERG in lymphoblastic lymphoma, we investigated 20 cases of T-lymphoblastic lymphoma and 4 cases of B-lymphoblastic lymphoma for the expression of ERG by immunohistochemistry staining, and evaluated the expressional pattern of ERG in lymphoblastic lymphoma, especially for the small needle biopsy specimens, to avoid a possible misdiagnosis between lymphoblastic lymphoma and Ewing sarcoma.

Methods

Tissue samples

This study included 20 T-lymphoblastic lymphoma and 4 B-lymphoblastic lymphoma patients, the pathological specimens come from the tissue archives of the department

of pathology, the second hospital of Nanchang university. The lymphoblastic lymphomas specimens were derived from patients who had undergone fine needle aspiration or surgical excision between 2017 and 2021. The histological sections were evaluated by four pathologists. Pathological diagnosis was determined by the use of histological features and immunohistochemical marker panels according to the newest World Health Organisation classification.

Immunohistochemical staining

Immunohistochemistry was performed on formalin fixed, paraffin-embedded tissue sections using the Dako Envision (Dako, Carpinteria, CA) automated system for the following antigens: ERG (ZA-0405, 1:100; ZSGB-Bio, Beijing, China), FLI-1 (ZA-0105, 1:50; ZSGB-Bio, Beijing, China), TdT (EP212, 1:100; ZSGB-Bio, Beijing, China), CD3 (EP41, 1:100; ZSGB-Bio, Beijing, China), Pax-5(EP156, 1:100; ZSGB-Bio, Beijing, China) and CD99 (PCB1, 1:100; ZSGB-Bio, Beijing, China). All stainings were performed with appropriate positive and negative controls.

Results

The characteristics of patients with lymphoblastic lymphoma are presented in Table 1. All T-lymphoblastic lymphomas and B-lymphoblastic lymphomas had already been diagnosed using immunohistochemical staining. The age ranged from 8 to 55 years, and the median age is 31 years old. Cases 1 to 20 were T-lymphoblastic lymphomas, and Case 21, 22, 23 and 24 were B-lymphoblastic lymphomas.

The expression profiles of ERG in T-lymphoblastic lymphoma and B-lymphoblastic lymphoma are summarised in Table 1. Representative images of ERG immunohistochemical results in lymphoblastic lymphoma are shown in Fig. 1.

ERG positive expression was observed in 8 (40%) of the 20 T-lymphoblastic lymphomas, and 3 (75.0%) of the all 4 B-lymphoblastic lymphomas. We confirmed positive ERG expression in the vascular endothelial cell of the tumor as a positive control.

Besides, the expression of FLI-1 was also observed in this study, and positive expression was found in 8 (66.6%) of the 12 T-lymphoblastic lymphomas, all 4

(100.0%) of the B-lymphoblastic lymphomas showed strong positive expression (Fig. 2).

Discussion

Given to the overlapping histologic and immunohistochemical features, the differentiation of Ewing's sarcoma from lymphoblastic lymphoma are challenging, particularly in the small needle biopsy specimens. Immunohistochemical detection of ERG, FLI-1 and CD99 may be valuable in confirming the diagnosis of Ewing's sarcoma, while several reports have showed that ERG and FLI-1 are associated with the development of lymphoblastic lymphomas, and positive expression of lymphoblastic lymphoma had reported in literatures.[10,11] In this study, we further evaluated the immunohistochemical staining with ERG in lymphoblastic lymphoma, and review literatures to avoid misdiagnosis.

Ewing sarcoma family tumors are characterized by the presence of non-random chromosomal translocations producing fusion genes that encode aberrant transcription factors. The t(11;22)(q24;q12) translocation is associated with 85% of tumors and leads to EWS-FLI-1 formation, whereas t(21;12)(22;12) and other less common translocations induced EWS-ERG fusion comprises the remaining 10% to 15% of cases.[12] Hence, the expression of ERG and FLI-1 are widely used to the diagnosis of Ewing sarcoma and conformed to be the relatively sensitive markers of Ewing sarcoma.

However, ERG can also express in many other small round cell tumours except for the Ewing sarcoma. According to the recent reports, ERG is capable of promoting the development of leukemia and is crucial for its maintenance,[6,9] which illustrates the possibility of the positive expression of ERG in lymphoblastic lymphoma. In this study, we found that ERG expressed in parts of lymphoblastic lymphoma. This reminds us that ERG is not a very specific marker for Ewing sarcoma, and we should exclude the possibility of lymphoblastic lymphoma when the small round cells positive express ERG. Interesting, we found that ERG shows a more frequency of negative expression in T-lymphoblastic lymphomas of thymus than other sites in this

study. The reason for this phenomenon still unclear, one of the reasons we hypothesis is that the lymphoblastic lymphoma located in thymus is associated with *DUX4* rearrangement which induces deregulation of ERG.[13]

As ERG and FLI-1 are usually combining used to the diagnosis of Ewing sarcoma, here we also investigated the expression of FLI-1 in lymphoblastic lymphoma. In normal tissues, FLI-1 was found to be restricted to haematopoietic cells and endothelial cells. FLI-1 was mainly expressed in EWS with a specificity of over 90%, and later on it was added to CD99 as a useful marker in the histological diagnosis of EWS.[14,15] However, further studies showed that FLI-1 was frequently seen in various tumour types, including vascular tumours, Merkel cell carcinoma (MCC) and desmoplastic small round cell tumour (DSRCT).[16,17]In this study, we further investigated the expressional pattern of FLI-1 in lymphoblastic lymphoma and found that FLI-1 was positive expression in 8 (66.6%) of the 12 T-lymphoblastic lymphomas and all 4 (100.0%) of the B-lymphoblastic lymphomas. All these findings above indicates that ERG and FLI-1 are not enough in the diagnosis of Ewing sarcoma especially for the small needle specimens, and more extensive antibodies may be necessary to differentiate lymphoblastic lymphomas from Ewing sarcoma.

In conclusion, we performed ERG and FLI-1 in lymphoblastic lymphomas by immunophenotype analysis and showed that ERG and FLI-1 are positive in majority of lymphoblastic lymphomas, which urges pathologists to exercise caution in the diagnosis of Ewing sarcoma when ERG and FLI-1 are positivity, and illustrates the need for further immunohistochemical examination to avoid misdiagnosis.

Abbreviations

ERG: Ets-related gene; EWS: Ewing sarcoma gene; IHC: Immunohistochemistry; MCC: Merkel cell carcinoma; DSRCT: desmoplastic small round cell tumour

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None.

Author contributions

Wenyong Huang and Wen Zhang performed the immunohistochemical expression

experiments, and prepared the figures and the manuscript. Lei Zeng, Yueer Zheng, Fangfang Hu, Fanrong Liu, Weisong Li and Guangxiu Guo contributed to the collection of the samples and corrected the data. Lixiang Li contributed with pathological diagnosis of the cases. Wenyong Huang and Lixiang Li designed the research. All authors have read and approved the final manuscript.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The study protocol was approved by the ethics committee at the second affiliated hospital of Nanchang university, China.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Table 1. Clinical and immunohistochemical findings in patients with lymphoblastic lymphoma

Case NO.	Age(y)	Sex	Tissue	Diagnosis	ERG staining
1	29	F	LN	T-LBL	Positive
2	15	M	Mediastinum	T-LBL	Negative
3	52	M	LN	T-LBL	Positive
4	28	M	Mediastinum	T-LBL	Negative
5	20	F	LN	T-LBL	Negative
6	8	M	LN	T-LBL	Positive
7	12	M	Mediastinum	T-LBL	Negative
8	21	F	Tonsil	T-LBL	Positive
9	32	M	Mediastinum	T-LBL	Negative
10	42	M	Mediastinum	T-LBL	Positive
11	46	M	LN	T-LBL	Positive
12	28	F	LN	T-LBL	Negative
13	22	M	Mediastinum	T-LBL	Negative
14	25	M	LN	T-LBL	Positive
15	14	M	Mediastinum	T-LBL	Negative
16	55	F	LN	T-LBL	Positive
17	54	M	LN	T-LBL	Negative
18	12	M	LN	T-LBL	Positive
19	43	F	LN	T-LBL	Positive
20	39	M	LN	T-LBL	Negative
21	44	F	Lumbar vertebrae	B-LBL	Positive
22	16	F	Pelvic	B-LBL	Positive
23	36	F	LN	B-LBL	Positive
24	50	F	Retroperitoneal	B-LBL	Negative

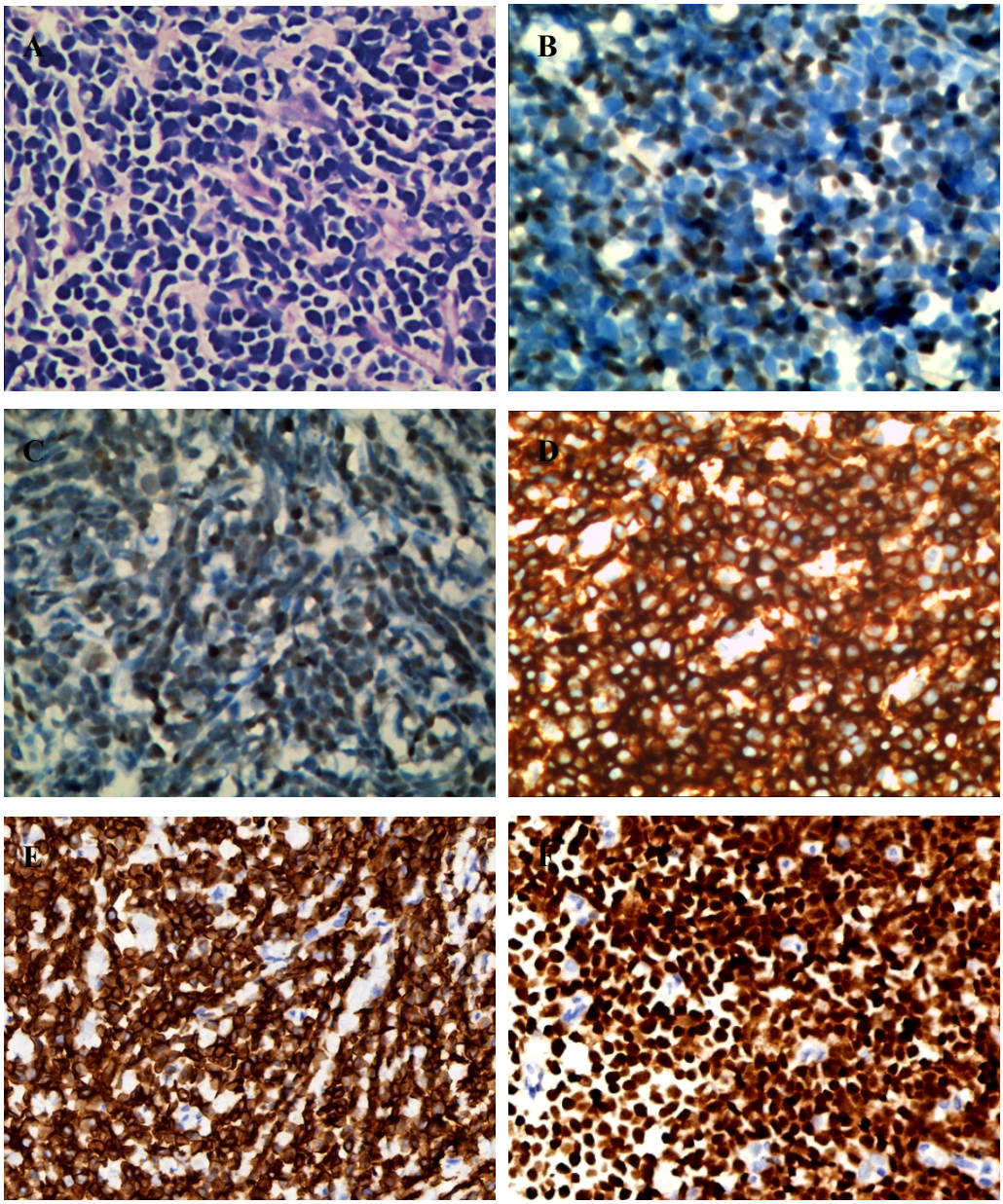

Fig. 1 Morphological and immunohistochemical features in T-LBL. A) H&E stain of T-LBL**.** B) ERG, C) FLI-1, (D) CD99, (E) CD3 and (F) TdT positive expression in T-lymphoblastic lymphoma.

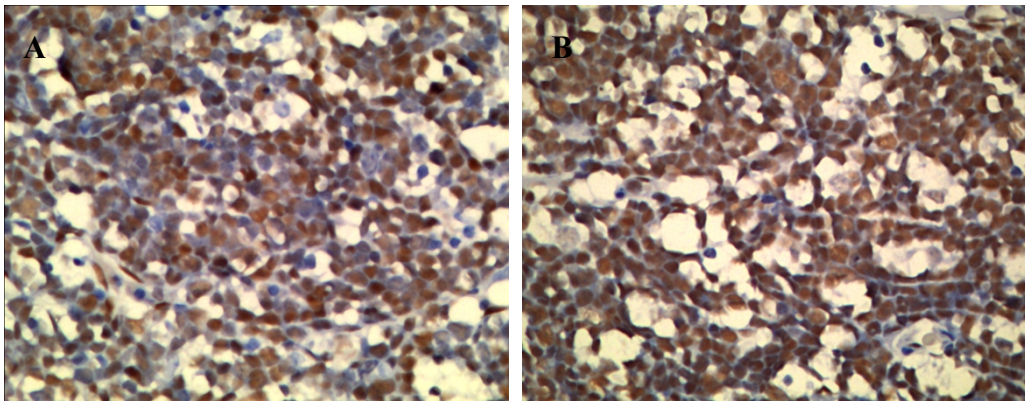

Fig. 2 A) ERG and B) FLI-1 positive expression in B-lymphoblastic lymphoma of lumbar vertebrae.