



Clinical Significance of TIPE2 Protein Upregulation in Non-Hodgkin's Lymphoma

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Summary

Non-Hodgkin's lymphoma (NHL), which includes diffuse large B-cell lymphoma (DLBCL) and peripheral T-cell lymphoma, is a refractory malignant tumor originated from the lymphatic system. TNFAIP8L2 (TIPE2 or tumor necrosis-alpha-induced protein-8 like 2) is a negative regulator for inflammation and an inhibitor for carcinogenesis. However, whether TIPE2 plays a role in lymphomagenesis is unknown. In this study, we determined TIPE2 expression in NHL by immunohistochemistry and investigated its clinicopathological significance in DLBCL. We found that TIPE2 expression was upregulated in both DLBCL and peripheral T-cell lymphoma. But the expression of TIPE2 in T lymphomas was weaker than that in DLBCL. Interestingly, among DLBCL, TIPE2 expression was significantly stronger in the germinal center B-cell (GCB) type than in the non-GCB type. These results suggest that the expression of TIPE2 protein could be a predictor of better prognosis for DLBCL. (J Histochem Cytochem 64:556–564, 2016)

Keywords

diffuse large B-cell lymphoma, germinal center, non-Hodgkin's lymphoma, TNFAIP8L2

Introduction

Non-Hodgkin's lymphoma (NHL) including diffuse large B-cell lymphoma (DLBCL) and peripheral T-cell lymphoma is considered as a refractory malignant tumor originated in the lymphatic system.¹ DLBCL, which traditionally has been defined as a diffuse proliferation of large B lymphoid cells, is the most common type of NHL comprising of greater than 30% of adult NHLs in the West and an even higher percent in developing countries,^{2,3} whereas peripheral T-cell lymphoma appears to be more common in China.⁴ However, the exact mechanism of NHL remains unclear. More and more evidence has showed that inflammatory response gene may play an important role in lymphomagenesis.^{5–7}

The malignant B cells of DLBCL show extensive morphological and phenotypic similarities. Traditionally, DLBCL has been classified by the morphology and

immunophenotype of the malignant B cells. However, more and more recent studies report molecular classification for DLBCL. Cell-of-origin classification based on gene expression profiling (GEP) has demonstrated that a germinal center (GC) phenotype is associated with a better survival than an activated B-cell-like phenotype.^{8,9} However, GEP is not readily applicable in routine clinical practice. According to Hans' criteria, the combined immunostaining of CD10, BCL-6, and MUM-1/IRF4 (multiple myeloma oncogene 1/interferon regulatory factor 4) has been developed for use

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on paraffin-embedded tissues, with division into GC DLBCL and non-GC DLBCL subtypes.^{10,11} However, Hans' criteria are unsatisfactory for subclassification into better and poorer prognosis groups in the rituximab era.^{12,13} Therefore, it is important to investigate novel clinicopathological factors associated with clinical prognosis of DLBCL. Ichikawa et al.¹⁴ reported that the expression level of BACH2 is a promising predictor of prognosis for DLBCL. Here, we report that TIPE2 may be a novel predictor associated with clinical prognosis of DLBCL.

TNFAIP8L2, tumor necrosis-alpha-induced protein-8 like 2 (also known as TIPE2), is a new member of TNFAIP8 (also known as TIPE) family. TIPE2 was originally identified as a negative regulator for immune homeostasis, which is mainly expressed in lymphoid tissues and inflammatory tissues.¹⁵ Subsequent work has shown that murine TIPE2 protein is also expressed in endocrine cells and germ cells of the reproductive organs and possesses a unique structure shared by all members of the TIPE family,^{16,17} whereas human TIPE2 was detected in a variety of non-immune cell types, including hepatocytes, neurons, and epithelial cells.¹⁸ Its germline deletion resulted in fatal inflammation and hypersensitivity to Toll-like receptor and T-cell receptor signaling.^{15,19} In human, TIPE2 is abnormal in chronic inflammatory disease such as hepatitis,^{20,21} atherosclerosis,^{22,23} and colitis.²⁴ Furthermore, TIPE2 could promote cell death and significantly inhibited tumorigenesis in mice.^{25–27} Thus, TIPE2 is not only a negative regulator for inflammation but also an inhibitor for carcinogenesis.^{15,25,26,28} However, it remains unclear whether TIPE2 plays roles in lymphomagenesis. Therefore, in this article, we determine TIPE2 expression in NHL by immunohistochemistry and investigate its clinicopathological significance in DLBCL. To our knowledge, this is the first report about the association of TIPE2 and NHL. Therefore, findings of this study were performed to contribute to our understanding of the significance of TIPE2 in the genesis and progression of DLBCL.

Materials and Methods

Human Subjects and Specimens

Tissue specimens from 82 DLBCL and 30 peripheral T-cell lymphomas of patients who were admitted to Shandong Qilu Hospital between 2009 and 2013 were investigated in this study. No patients had received prior treatment. The diagnosis of lymphoma was made in accordance with the "WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues." The specimens were fixed in 10% buffered formalin and paraffin

embedded, and serial sections about 4 mm were made for each case. Histological grade was graded according to hematoxylin and eosin staining. Histopathological diagnosis was carried out according to the criteria of the World Health Organization.²⁹ Among DLBCL, 34 cases were assigned to the germinal center B-cell (GCB) group and 31 cases to the non-GCB group according to the phenotype determined by Hans' algorithm.¹¹ A total of 16 normal lymph nodes were used as controls. All procedures were pre-approved by the Institutional Review Board of the Shandong University.

Immunohistochemistry and Pathological Examination

In most cases, immunohistochemistry was performed using the following primary antibodies: antibodies against the expression of pan-B-lineage markers such as CD19, CD20, and CD79a, and of other routine phenotype markers such as CD10, CD23, CD30, BCL-2, BCL-6, and MUM-1 (ZSGB-BIO, Beijing, China). Among DLBCL cases, GCB and non-GCB types were determined according to criteria presented by Hans et al.¹¹ Utilizing the remaining paraffin-embedded sections described above, investigational immunohistochemical analyses for TIPE2 were performed.

Immunostaining was performed as described with minor modifications.¹⁶ Paraffin-embedded sections were deparaffinized by rehydration with decreasing concentrations of alcohol. After inhibition of endogenous peroxidase with 3% hydrogen peroxide (Sigma, St. Louis, MO) and blocking of nonspecific staining by incubation in goat serum (Invitrogen, Carlsbad, CA), the sections were incubated with primary antibodies (rabbit anti-TIPE2, 1:200, or rabbit IgG as isotype-matched control antibodies, 1:200; Invitrogen) for 3 hr at room temperature. Slides were washed in PBS solution, and antirabbit horseradish peroxidase-conjugated antibody (HRP-streptavidin system; Invitrogen) was applied for 30 min. After washing, immunoperoxidase staining was performed using a diaminobenzidine chromogen kit (ZSGB-BIO). Nuclei were stained with hematoxylin (ZSGB-BIO). The sections were examined under an optical microscopy (Olympus BX51, Tokyo, Japan).

The distribution of TIPE2 protein in tumor cells was determined and scored independently by two hematopathologists (Y.H.C. and Z.G.Y.). For each stained slide, the percentage of tumor cells showing positive staining for TIPE2 was recorded and the intensity of staining was scored as follows: (–) = no staining cell detected; (1+) and (2+) = weak staining and moderate

Table 1. Analysis of TIPE2 Expression in Biopsies by Immunostaining.

Patients	TIPE2 Expression			
	Intense (+++)	Moderate (++)	Weak (+)	Negative (-)
T lymphoma (n=30)	0	10	15	5
DLBCL (n=82)	21	35	19	7
GCB types (n=38)*	17	15	6	0
Non-GCB types (n=44)*	4	20	13	7

Abbreviations: TIPE2, tumor necrosis-alpha-induced protein-8 like 2; DLBCL, diffuse large B-cell lymphoma; GCB, germinal center B cell; Non-GCB, non-germinal center B cell.

* $p < 0.0001$.

staining, respectively; and (3+) = strong staining. A case was scored as positive if at least 50% of tumor cells stained positive for TIPE2 with an intensity of 1+, 2+, or 3+. Positive staining cells showed reactivity for TIPE2 in both the cytoplasm and nucleus.

Statistical Analysis

Data were presented as means \pm standard deviations. The Wilcoxon two-sample test was used to determine the significance of the differences in TIPE2-positive cells. A value of $p < 0.05$ was considered statistically significant. All analyses were performed using the SAS for Windows 9.13.

Results

TIPE2 Expression Was Upregulated in Non-Hodgkin's Lymphoma

Immunohistochemical analysis for TIPE2 revealed moderate to strong staining of malignant B cells in DLBCL. TIPE2 expression in these cells localized to the cytoplasm and, to a variable extent, the nuclei. DLBCL is the most common large cell lymphoma and commonly identified by pan-B-cell antigen expressed by malignant B cells. We stained 82 cases of DLBCL for TIPE2 protein and detected positive expression in the tumor cells for 75 of 82 cases (Table 1). As shown in Fig. 1A and B and Fig. 2, TIPE2 protein was detected mainly in the cytoplasm of tumor cells. Strong and moderate staining of TIPE2 were detected in 21 and 35 cases, respectively. Low levels or weak staining of TIPE2 protein was detected in 19 cases and was not detectable in seven cases. The results suggest that TIPE2 is commonly expressed by malignant B cells of DLBCL.

The expression of TIPE2 protein in T lymphomas was weaker than that in DLBCL cells (Fig. 1C and D, Fig. 3). Only moderate or low-level staining of TIPE2 protein was detected in 25 cases of total of 30 cases (Table 1).

Furthermore, we found that TIPE2 protein was detectable in both T-cell zone and GC of the normal lymph node by immunohistochemistry (Fig. 1E and F). The staining was specific for TIPE2 because isotype-matched antibody controls showed no staining (Fig 1G, H, and I).

Clinicopathological Characteristics of DLBCL

Patients

The clinical features of the DLBCL patients are summarized in Table 2. There were 39 females and 43 males with a median age of 59 years (range, 23–91 years). Thirty-seven patients had nodal DLBCL, and 45 samples were obtained from extranodal sites, such as the stomach, small intestine, colon, skin, ovary, lung, brain, adrenal gland, liver, and breast. All patients received no treatment. Among DLBCL, according to immunohistochemistry algorithm described by Hans et al.,¹¹ 38 patients with MUM-1 (-), CD10 (-), and BCL-6 (+), or MUM-1 (-), CD10 (+), and BCL-6 (+/-), were diagnosed with the GCB subtype, whereas 44 patients with MUM-1 (+), CD10 (-), and BCL-6 (+/-) were non-GCB phenotype (data not shown).

The Staining of TIPE2 Protein Was Stronger in GCB Phenotype Than That in Non-GCB Type

Among DLBCL, according to the phenotype determined by Hans' algorithm, cases with immunostaining of CD10 positive, BCL-6 positive, and MUM-1 negative were considered as the GCB phenotype, whereas CD10 negative, BCL-6 negative, and MUM-1 positive cases were considered as the non-GCB type (data not shown). As a result, 38 cases were assigned to the GCB phenotype and 44 cases to the non-GCB phenotype. We found that TIPE2 staining was much stronger in GCB groups than that in non-GCB groups (Figs. 4 and 5). In patients with the GCB phenotype, strong and moderate staining of TIPE2 protein were detected

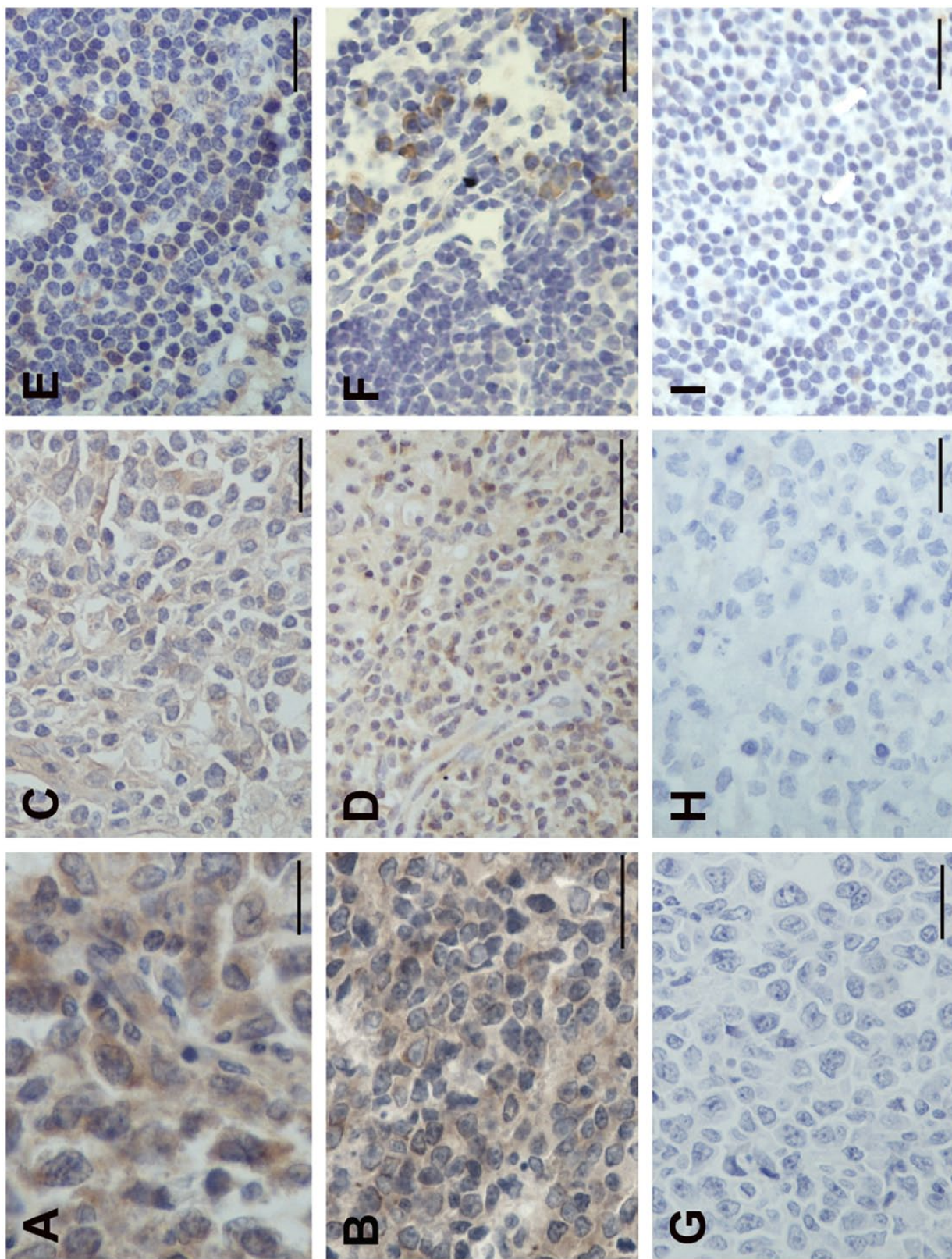


Figure 1. Tumor necrosis-alpha-induced protein-8 like 2 (TIPE2) expression in non-Hodgkin's lymphoma. A and B show the detection of TIPE2 protein in diffuse large B-cell lymphoma (DLBCL) by immunohistochemistry (A, x400; B, x200). C and D show that moderate levels of TIPE2 protein were detected in T lymphomas (C, x400; D, x200). E and F show that the staining of TIPE2 protein was detected in the human normal lymph node (x400). G, H, and I: No staining was detectable with the rabbit isotype antibody for DLBCL, T lymphomas, and normal lymph nodes, respectively (x400).

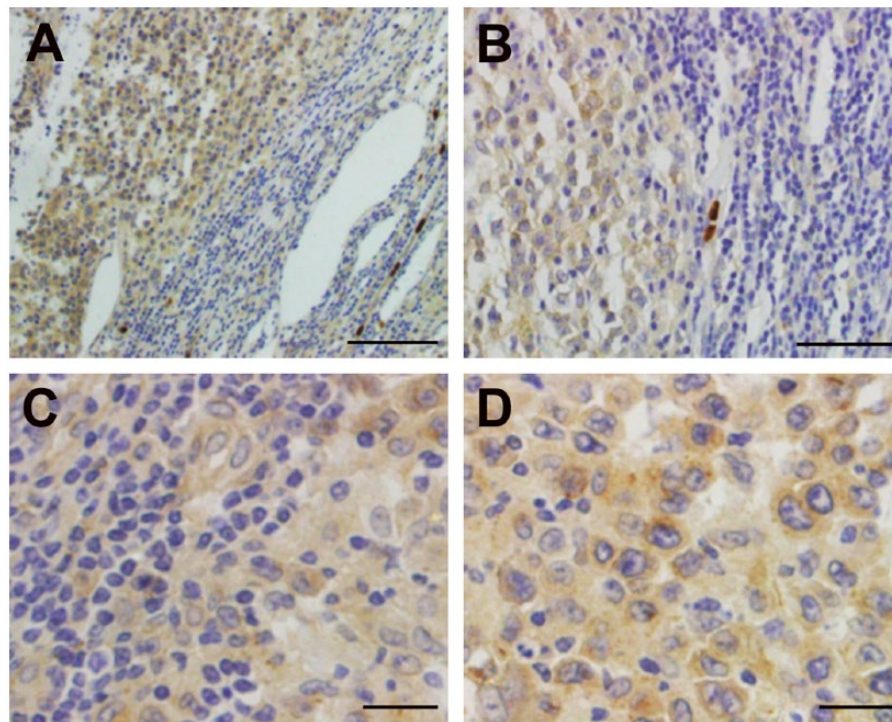


Figure 2. Tumor necrosis-alpha-induced protein-8 like 2 (TIPE2) expression in diffuse large B-cell lymphoma by immunohistochemistry. A and B show that the staining of TIPE2 was detected in malignant cells (A, $\times 100$; B, $\times 200$). C and D show that TIPE2 protein was expressed mainly in the cytoplasm of tumor cells ($\times 400$).

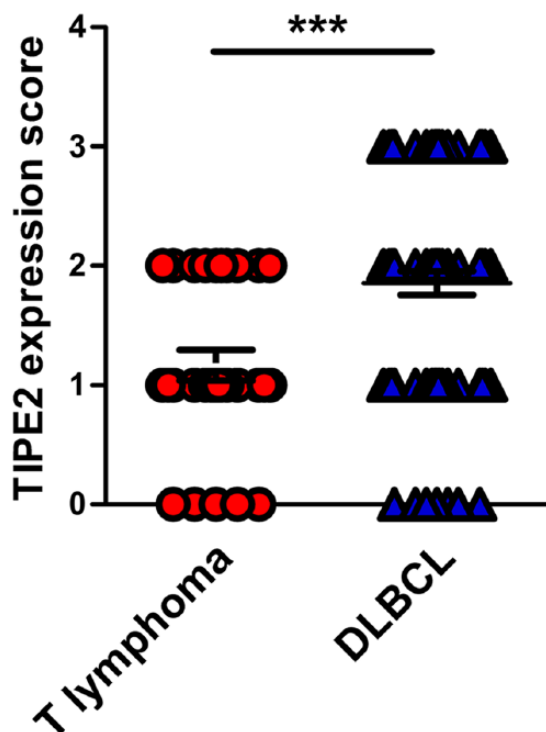


Figure 3. Statistical analysis on the levels of tumor necrosis-alpha-induced protein-8 like 2 (TIPE2) expression in malignant cells between diffuse large B-cell lymphoma (DLBCL) and T lymphoma patients. $***p < 0.001$.

Table 2. Clinicopathological Characteristics of Diffuse Large B-Cell Lymphoma.

Characteristics	Patients (n)
Gender	
Female	39
Male	43
Age in years, median (range)	59 (23–91)
Phenotype	
Germinal center	38
Non-germinal center	44
Ki67 $\geq 80\%$	39
Presentation	
Nodal	37
Extranodal	45

in 17 cases (45%) and 15 cases (39%), respectively. Low-level or weak staining of TIPE2 protein was detected in six cases (16%), and no negative case was found, whereas in patients with the non-GCB phenotype, only four cases (9%) with strong staining were detected. Moderate and low levels of TIPE2 protein were detected in 20 cases (45%) and 13 cases (30%), respectively. There was a significant difference about TIPE2 levels between GCB and non-GCB phenotypes ($p < 0.0001$; Table 1).

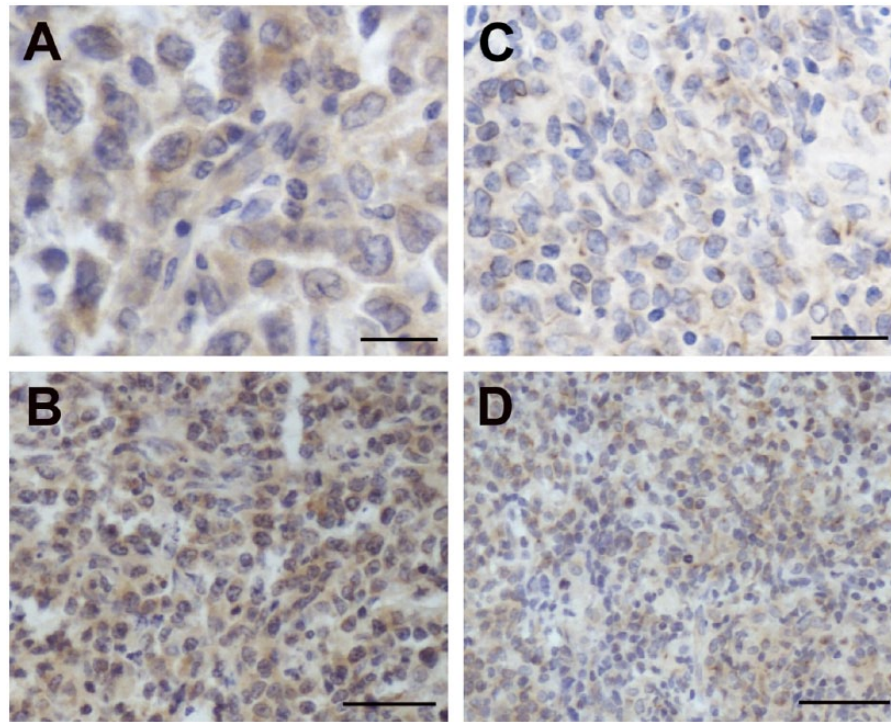


Figure 4. Detection of tumor necrosis-alpha-induced protein-8 like 2 (TIPE2) protein in germinal center B-cell (GCB) phenotype and non-GCB type of diffuse large B-cell lymphoma (DLBCL) patients. A and B show the expression of TIPE2 protein in non-GCB phenotype of DLBCL by immunohistochemistry (A, $\times 400$; B, $\times 200$). C and D show TIPE2 expression in GCB phenotype (C, $\times 400$; D, $\times 200$).

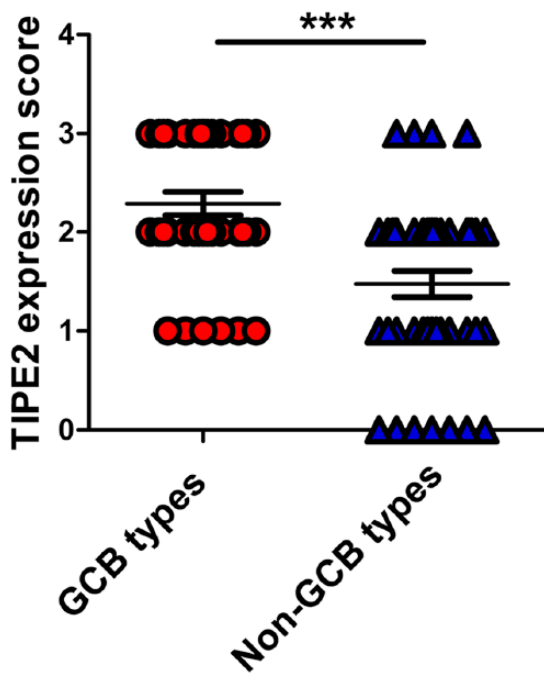


Figure 5. Statistical analysis on the levels of tumor necrosis-alpha-induced protein-8 like 2 (TIPE2) expression between germinal center B-cell (GCB) type and non-GCB type. *** $p < 0.001$.

Discussion

NHLs, solid tumors of lymphocyte origin, are the most common hematopoietic cancers in both men and women in the developed world.^{29,30} More and more evidence suggests that the occurrence of NHL is associated with abnormal homeostasis, such as severe immunodeficiency including both hereditary immunodeficiency disorders and acquired conditions, and autoimmune conditions, which is a strong risk factor for NHL.^{31,32} In this report, we used a specific antibody and a standard immunohistochemical staining technique to determine the expression of TIPE2 protein in malignant cells of NHL, including DLBCL and peripheral T-cell lymphoma. We show that TIPE2 protein is mainly expressed in the malignant B cells and T cells of NHL.

First, we found that TIPE2 expression was upregulated in malignant B and T cells. TIPE2 was originally identified as a negative regulator for immune homeostasis, which is mainly expressed in lymphoid tissues and inflammatory tissues.¹⁵ Within the lymphoid compartment, T cells appear to express high level of TIPE2 protein, whereas B cells and B-cell zones of lymphoid organs were devoid of TIPE2.¹⁶ This expression pattern can explain why TIPE2 deficiency preferentially affected

cellular but not humoral immunity in mice.¹⁵ In this article, we reported that the levels of TIPE2 protein were markedly upregulated in malignant B cells and T cells, especially malignant B cells. Immunohistochemical analysis revealed that moderate to strong staining of TIPE2 protein was detected in malignant B cells of most DLBCL patients, whereas in T lymphoma cells, TIPE2 staining was weaker than that in DLBCL cells. These data suggest that TIPE2 is commonly expressed by malignant B cells and T cells. But much work needs to be done about how TIPE2 protein was upregulated during lymphomagenesis in the future.

Furthermore, we demonstrated that TIPE2 expression may be a predictor of better prognosis in cases of DLBCL. Studies showed that a GC phenotype might be associated with a better survival.^{8,9} According to the phenotype determined by Hans' algorithm, cases with immunostaining of CD10 positive, BCL-6 positive, and MUM-1 negative were considered as the GCB phenotype. Bellas et al.³³ also suggested that BCL-6 protein expression conferred a trend toward better outcomes. In this study, we found that the staining of TIPE2 protein was stronger in the GCB phenotype compared with the non-GCB type ($p < 0.0001$) of DLBCL patients. Furthermore, TIPE2 staining in T lymphoma cells was weaker than that in DLBCL cells. This expression pattern suggests that TIPE2 expression may be a predictor of better prognosis in lymphomas. Much work is required to confirm this notion.

TIPE2 plays important roles in controlling inflammation and tumorigenesis by suppressing RAS signaling.^{15,26} Studies showed that TIPE2 plays atheroprotective roles by regulating the functions of macrophages and vascular smooth muscle cells (VSMCs).^{22,23} In the physical circumstances, TIPE2 is highly expressed in macrophages but very low in VSMCs.¹⁶ Interestingly, TIPE2 was downregulated in macrophages, whereas it was upregulated in VSMCs during atherogenesis.^{22,23} TIPE2 is also an inhibitor for carcinogenesis by promoting cell death and was found to be downregulated in hepatoma cells.^{25,26} Similar events have been observed in this article. TIPE2 is highly expressed in T cells, but downregulated in T lymphoma cells, the same as the downregulation in Hepatitis B virus-specific T cells,²¹ while upregulated in DLBCL, especially was stronger in GC-subtype than that in non-GC type. These data suggest that TIPE2 may be a new inhibitor for carcinogenesis with a different expression pattern from other tumor inhibitory genes. It is very interesting to study the mechanism of TIPE2 expression, especially under physiological and pathological conditions.

Collectively, our data demonstrate that TIPE2 expression was upregulated in malignant cells of

DLBCL and peripheral T-cell lymphoma. Among DLBCL patients, TIPE2 expression was significantly stronger in the GCB phenotype compared with that in the non-GCB type. These results suggest that TIPE2 protein may be a predictor of better prognosis in cases of DLBCL.

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Competing Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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