Am J Blood Res 2022;12(4):136-143
www.AJBlood.us /ISSN:2160-1992/AJBR0143498

Original Article

Canonical and non-canonical Wnt signal pathway in classic Hodgkin lymphoma and the prognostic significance of LEF1, β-catenin, FZD6 and Wnt5a/b

Linlin Gao[1], Wei Cui[1], Sarah Kelting[1], Janet Woodroof[1], Hua Li[2], Linheng Li[1,2], Da Zhang[1]

[1]Department of Pathology and Laboratory Medicine, University of Kansas Medical Center, 3901 Rainbow Boulevard, Kansas City, KS 66160, USA; [2]Stowers Institute for Medical Research, 1000 E 50 St, Kansas City, MO 64110, USA

Received April 12, 2022; Accepted June 27, 2022; Epub August 15, 2022; Published August 30, 2022

Abstract: Aberrant Wnt signaling has been found in many solid organ cancers, as well as hematological malignancies. However, its role in classic Hodgkin lymphoma (CHL) remains unclear. We evaluated the expression of Wnt signaling components LEF1, β-catenin, FZD6 and Wnt5a/b and their correlation with the prognosis in 50 CHL patients by immunohistochemical stains. The neoplastic Hodgkin/Reed-Sternberg (HRS) cells showed expression of LEF1, FZD6, and Wnt5a/b but absent nuclear β-catenin. Wnt5a/b expression was seen in a significantly higher percentage of stage IV patients (67%) compared to other stages (p=0.03). However, there was no correlation between the expression of LEF1, FZD6 and Wnt5a/b and patients' stage or survival. In summary, our results confirmed decreased β-catenin expression in HRS. Non-canonical Wnt pathway may play a role in the microenvironment that facilitates HRS migration, however, it is not sufficient to consider LEF1, β-catenin, FZD6 and Wnt5a/b as prognostic factors for CHL.

Keywords: Classic Hodgkin lymphoma, Wnt signal pathway, prognosis

Introduction

Wnt signaling is a critical regulator in many physiological processes, including tissue development, differentiation, and cell homeostasis [1, 2]. Classically, Wnt signaling is divided into canonical and non-canonical pathways. The key mediator of canonical pathway is β-catenin. In the absence of Wnt ligands, a destruction complex is formed, which includes APC (adenomatous polyposis coli), Axin (axis inhibitor), and GSK-3β (glycogen synthase kinase-3 beta). The destruction complex leads to phosphorylation and ubiquitination-mediated degradation of β-catenin. When Wnt ligand binds to the Frizzled (FZD) receptor, some components of the destruction complex are recruited to the cell membrane, which leads to dissociation of the destruction complex and accumulation of β-catenin. Accumulated β-catenin translocates to the nucleus, interacts with transcription factors TCF (T-cell factor)/LEF (lymphocyte-enhancer-binding factor), and promotes the transcription of Wnt target genes including *VEGF, CCND1, and cMYC* [3].

Non-canonical Wnt signaling pathways are further divided into Wnt/planar cell polarity (PCP) signaling and Wnt-cGMP/calcium signaling, which are involved in the process of polarity and cell motility. Wnt proteins are grouped as canonical Wnts such as Wnt1, Wnt2, Wnt3 and Wnt8a, and non-canonical Wnts, including Wnt4, Wnt5a, Wnt5b, Wnt6, Wnt11, etc. [4].

Dysregulated Wnt signaling has been associated with the pathogenesis of many solid organ cancers such as colorectal cancer, breast cancer and hepatocellular carcinoma, as well as hematological malignancies including classic Hodgkin lymphoma (CHL) [5]. CHL is a B cell lymphoma characterized by mononuclear Hodgkin cells and multinucleated Reed-Sternberg cells. Hodgkin/Reed-Sternberg (HRS) cells usually comprise a minority of the total cellularity and are embedded in a background of non-neo-

Table 1. Clinicopathologic characteristics of CHL patients

No. of patients	50
Age (range)	38 (18-84)
Gender	F: 25; M: 25
Histology, *n* (%)	
Nodular sclerosis	40 (80%)
Mixed cellularity	5 (10%)
Lymphocyte-rich	5 (10%)
Ann Arbor stage, *n* (%)	
I	6 (12%)
II	17 (34%)
III	12 (24%)
IV	15 (30%)
Bulky disease	13 (26%)
Nodal sites > 2, *n* (%)	36 (72%)
Extranodal disease, *n* (%)	17 (34%)
Bone marrow involvement, *n* (%)	8 (16%)
ESR > 50 mm/h, *n* (%)	19 (38%)

plastic reactive immune cells. Morrison et al. reported expression of nuclear and cytoplasmic β-catenin in 15% of Epstein-Bar virus (EBV)-positive and 7% of EBV-negative Hodgkin lymphomas [6]. Sohlbach et al. investigated the expression of the components of Wnt signaling in a CHL cell line, L1236 cells, and found overexpression of Wnt5a, Wnt5b, Wnt8a, β-catenin, FZD6, LEF1, and cMYC. However, β-catenin expression was not identified in the HRS cells of the 28 cases of CHL, but instead was found in the dendritic processes of stromal cells to varying degrees [7]. As one of the major components of the destruction complex, an activated form of GSK-3β, pY216 GSK-3β, was found to be present in 100 of 100 (100%) CHL cases, while β-catenin was detected in the cytoplasm of only 12% of the cases. The accumulation of cytoplasmic β-catenin was associated with the co-existence of an inhibitory form of GSK-3β, pS9 GSK-3β [8]. Linke et al. reported overexpression of a non-canonical Wnt protein, Wnt5a, stimulated migration of CHL cell lines and promoted lymphoma engraftment and vascularization in chick chorioallantoic membrane (CAM) assay [9]. The same research group also studied the role of canonical Wnt signaling in the interactions between CHL cells and vascular endothelial cells (EC). LEF1 and β-catenin were found to be required for CHL cell migration toward ECs and promoted angiogenesis in vitro. Low levels of nuclear β-catenin were detected in CHL cell lines, such as L428 cells and KM-H2 cells, indicating a basal canonical Wnt signaling activity. Nuclear LEF1-positive HRS cells were found in 3 of 9 (33%) mixed-cellularity CHL cases and 1 of 18 (6%) nodular sclerosis CHL cases [10].

Although the canonical Wnt signaling pathway was reported to be activated at basal levels in CHL cell lines [10], nuclear β-catenin accumulation in CHL cases has been reported in variable ratios, ranging from 0% to 15% in different patient groups [6-8]. A non-canonical Wnt protein, Wnt5a, and canonical Wnt signaling components, LEF1 and β-catenin, were demonstrated to promote cell migration in vitro [9, 10], but their expression levels in relation to prognosis in CHL patients has not been evaluated to date. In our study, we evaluated the expression of multiple Wnt signaling components including β-catenin, LEF1, FZD6 and Wnt5a/b in 50 CHL patients and correlated their expression with the stage and survival of patients.

Materials and methods

Case selection

We selected a total of 50 patients diagnosed with CHL in the University of Kansas Health System and available paraffine tissue from 2008 to 2016. The cases that did not have paraffin embedded tissue blocks in our archive were excluded. All cases were diagnosed according to the current World Health Organization Classification of Tumors of Hematopoietic and Lymphoid Tissue (2017). Clinical, morphologic and immunophenotypic data were reviewed. This retrospective study was approved by the institutional review board of University of Kansas Medical Center.

Immunohistochemical methods

Immunohistochemical analyses were performed using formalin-fixed, paraffin-embedded tissue sections. The antibodies used were as follows: anti-phospho-β-catenin Ser 552 rabbit polyclonal antibody (Stowers Institute for Medical Research, Kansas City, MO, USA) [11]; Wnt5a/b rabbit polyclonal antibody (1:200; Thermo Fisher Scientific, Waltham, MA, USA); LEF1 rabbit monoclonal antibody (1:50; Cell

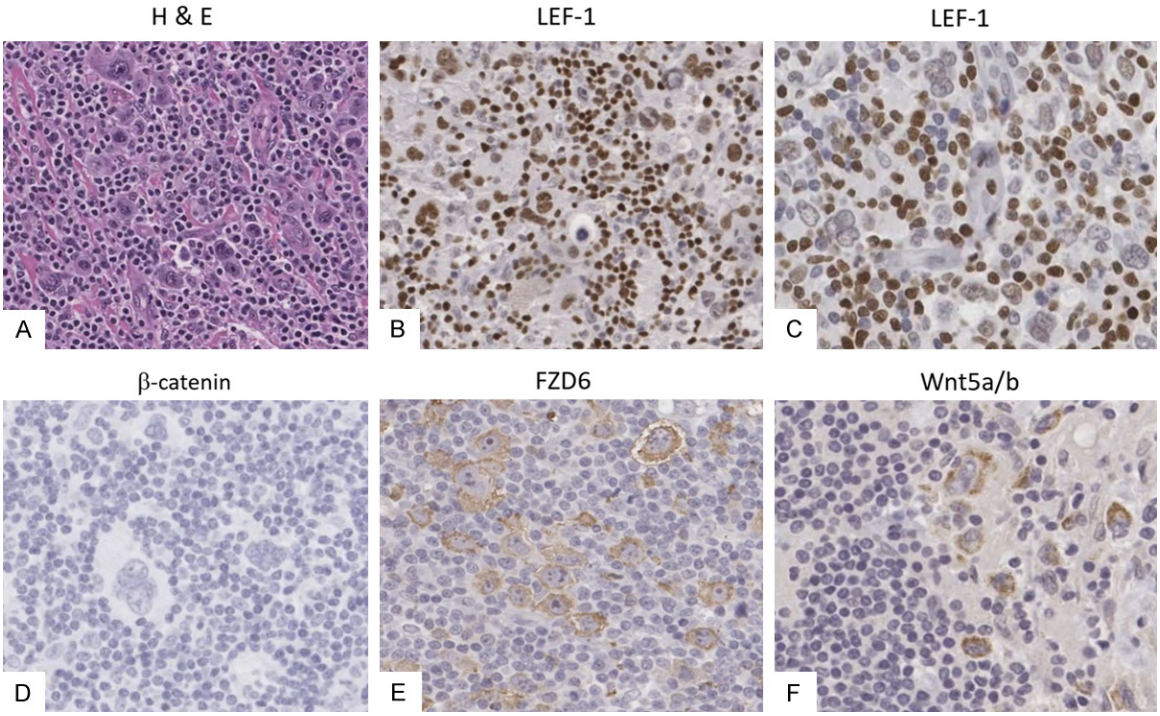

Figure 1. Expression of LEF-1, β-catenin, FZD6 and Wnt5a/b in CHL patients. A. Prominent Hodgkin/Reed-Sternberg (HRS) cell in classic Hodgkin lymphoma (CHL), H&E, 200×; B. Positive nuclear staining of LEF1 in HRS cells, 200×; C. Negative LEF1 staining in HRS cells, 200×; D. Negative β-catenin staining in HRS cells, 200×; E. Positive membranous staining of FZD6 in HRS cells, 200×; F. Positive cytoplasmic staining of Wnt5a/b in HRS cells, 200×.

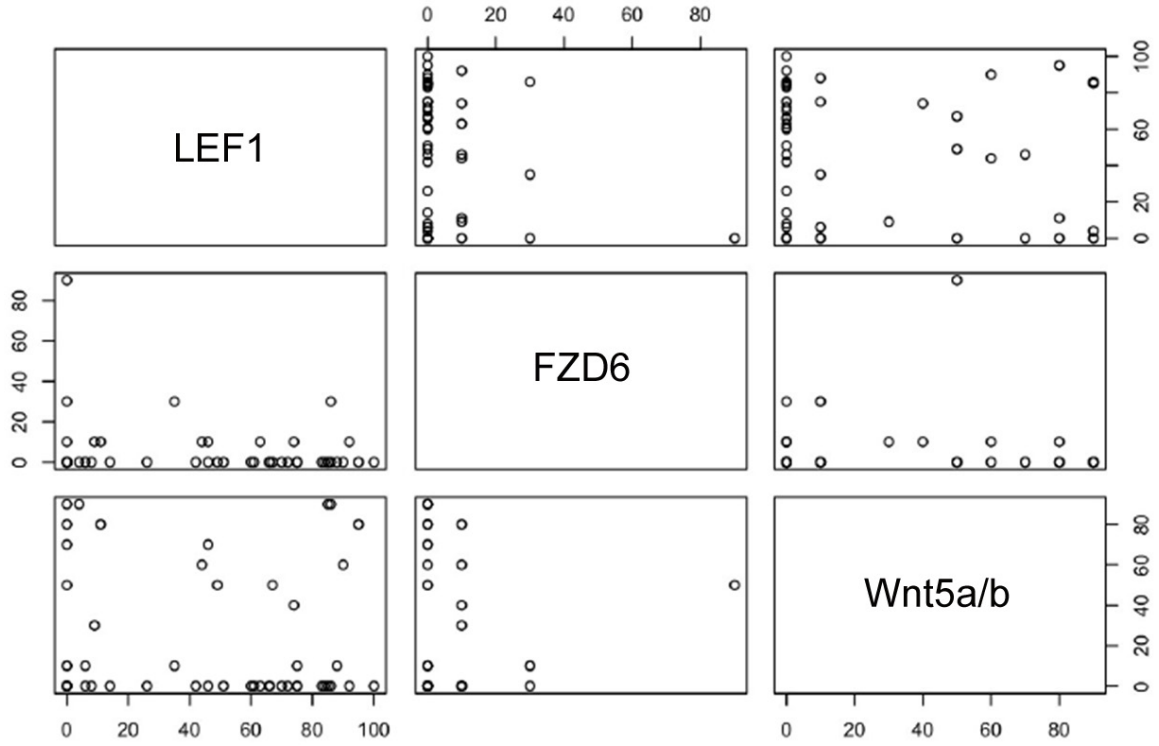

Figure 2. Scatterplots of expression for LEF-1, FZD6 and Wnt5a/b.

Table 2. Spearman correlation coefficient

	LEF-1	FZD6	Wnt5a/b
LEF-1	1.0000	-0.0947	-0.0597
FZD6	-0.0947	1.0000	0.0924
Wnt5a/b	-0.0597	0.0924	1.0000

Marque, MilliporeSigma, Burlington, MA, USA), and FZD6 rabbit polyclonal antibody (1:100; Thermo Fisher Scientific, Waltham, MA, USA). Immunostaining procedures were performed using the Biocare IntelliPath autostainer. Epitope retrieval was performed using the Biocare Decloaking Chamber. Slides were counterstained with hematoxylin and permanently mounted.

LEF1 positivity was defined as at least 10% of the HRS cells having nuclear staining [12]. FZD6 positivity was defined as at least 10% of the HRS cells having membranous staining and Wnt5a/b was defined as at least 10% of the HRS cells having cytoplasmic staining. β-catenin positivity was defined as positive nuclear stained cells per 40x high power filed [11]. Expression of β-catenin, LEF1, FZD6 and Wnt5a/b was evaluated manually by 3 pathologists, the percentage of positive cells per 100 HRS cells was counted, and a score of the average count was assigned to each case.

Statistical analysis

Overall survival (OS) was defined as date of diagnosis to date of death from any cause. Patients who were lost to follow-up were censored on their last follow-up date. Fisher's exact test was used to evaluate the relationship between the expression of LEF1, FZD6, Wnt5 and stage and OS. Regression analysis was performed using the statistical software R-4.0, and ggplot2 to correlate the expression of proteins with the patients' overall survival (OS). Wilcoxon rank sum test was used to assess their expression in alive versus dead patients. Two-tailed Fisher exact test was performed using the statistical software R-4.0. P value < 0.05 was considered statistically significant.

Results

Clinicopathologic characteristics of CHL patients

The study included 25 female and 25 male CHL patients with a mean age of 38 years (range, 18-84 years). The clinicopathologic characteristics of CHL patients are summarized in **Table 1**, which includes age, gender, histologic subtypes, Ann arbor stage, bulky disease, number of involved lymph nodes, extranodal disease, bone marrow involvement and erythrocyte sedimentation rate (ESR). The main histologic subtype was nodular sclerosis (80%), and the other subtypes were mixed cellularity (10%) and lymphocyte-rich (10%). According to the Ann Arbor staging system, 23 patients (46%) had stage I/II disease, while 27 patients (54%) had stage III/IV disease. A total of 36 patients (72%) had more than 2 lymph nodes involved by lymphoma and 13 (26%) had bulky mediastinal mass. Extranodal disease was detected in 17 patients (34%) and bone marrow involvement was found in 8 cases (16%). A total of 19 patients (38%) had an ESR that is over 50 mm/h.

Expression of LEF1, β-catenin, FZD6 and Wnt5a/b in CHL patients

Figure 1A demonstrates the typical features of mononucleated Hodgkin cells and multinucleated Reed-Sternberg cells with large nuclei and prominent eosinophilic nucleoli. Thirty eight out of 50 (76%) CHL patients had positive nuclear LEF1 staining in HRS cells as well as in some of the surrounding nonneoplastic cells (**Figure 1B**). **Figure 1C** shows that the HRS cells were negative for LEF1, while the surrounding lymphocytes were LEF1-positive. None of the 50 CHL patients (0%) had β-catenin expression in HRS cells or nonneoplastic cells (**Figure 1D**). A total of 12 patients (24%) showed membranous staining of FZD6 in HRS cells only (**Figure 1E**) and 22 patients (44%) showed cytoplasmic staining of Wnt5a/b in HRS cells only (**Figure 1F**). To evaluate the correlation between LEF1, FZD6, and Wnt5a/b, Spearman's rank correlation was used. The scatterplots and near-zero coefficients indicated that there was no correlation between LEF1, FZD6, and Wnt5a/b (**Figure 2** and **Table 2**).

Prognostic impact of LEF1, FZD6 and Wnt5a/b expression in CHL patients

The number of patients with and without expression of LEF1, FZD6 and Wnt5a/b at different Ann Arbor stages were showed in **Figure 3**. Fisher's exact test was used to determine if there was any association between the clinical stage and the expression of these 3 proteins. Wnt5a/b expression was seen in a higher per-

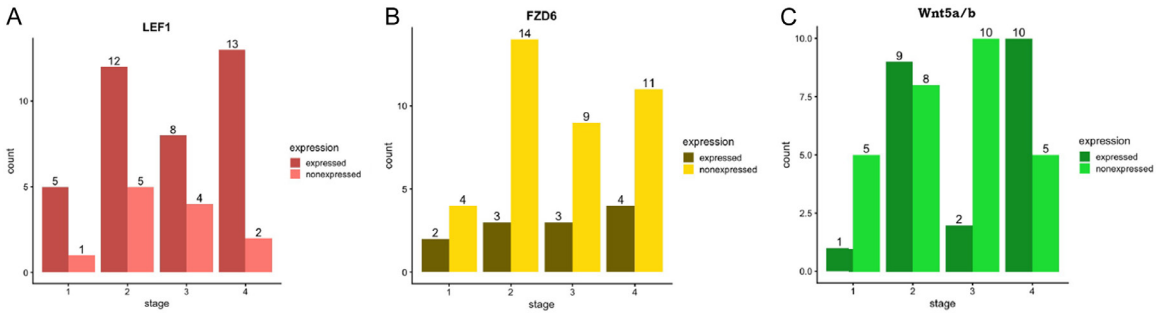

Figure 3. Expression of LEF-1, FZD6 and Wnt5a/b in patients at different Ann Arbor stages. The number of patients at different stages with and without expression of LEF1 (A), FZD6 (B) and Wnt5a/b (C).

Table 3. Comparison of LEF-1, FZD6 and Wnt5a/b expression in alive and dead patients

	Alive	Dead	*p* value
LEF-1-positive	31	7	0.66
LEF-1-negative	11	1	
FZD6-positive	10	2	1.00
FZD6-negative	32	6	
Wnt5a/b-positive	20	2	0.44
Wnt5a/b-negative	22	6	

centage of stage IV patients (10/15 cases, 67%) compared to other stages (p=0.03). However, there was no difference in LEF1 and FZD6 expression in patients at different stages.

We also compared the expression of LEF1, FZD6 and Wnt5a/b in alive patients versus dead patients using Fisher exact test and Wilcoxon rank sum test (data not shown), and no significant difference was identified between the two groups in terms of LEF1, FZD6 and Wnt5a/b expression (**Table 3**). We further evaluated whether the expression of these 3 proteins correlated with the patients' overall survival (OS). The coefficients of determination (R^2) derived from regression analysis were close to zero, which indicated there was no correlation between OS and expression of LEF1, FZD6 and Wnt5 (**Figure 4**).

Discussion

The roles of Wnt signaling in many cancers are well established. However, in the case of CHL, the impact of aberrant Wnt signaling is less clear. As the indicator of canonical Wnt signaling activation, the expression of β-catenin in CHL cases has been reported inconsistently. β-catenin expression in HRS cells were variable [6-8]. In this study, we investigated 50 CHL cases and did not identify β-catenin expression in the HRS cells, indicating β-catenin-dependent canonical Wnt signaling was unlikely to play an important role in CHL tumorigenesis or progression. Consistent with the low or absent expression of β-catenin in mature lymphocytes [13], β-catenin was not detected in the lymphocytes surrounding the HRS cells either.

LEF1 was found to be consistently overexpressed in chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) and was usually absent in other small B-cell lymphomas, which made LEF1 a highly specific marker for CLL/SLL [12]. LEF1 expression was also identified in a subset of cases of Burkitt lymphoma, follicular lymphoma and diffuse large B-cell lymphoma [14, 15]. In the case of CHL, LEF1 expression was reported with variable rates in several studies. One study observed positive LEF1 expression in 6% (1/18) of nodular sclerosis and 33% (3/9) of mixed-cellularity CHL cases [9]. Another study found that LEF1 was expressed in 28% (12/43) of de novo CHL cases and 60% (12/20) of CHL associated with Richter transformation [16]. Rea et al. reported LEF1 positivity in 15 of 19 (70%) cases of CHL and all 4 cases of CHL with Richter transformation [17]. Our investigation of LEF1 revealed that 76% (38/50) of CHL cases had positive nuclear LEF1 staining in HRS cells. High LEF1 expression was reported to be a favorable prognostic factor in patients with cytogenetically normal acute myeloid leukemia [18]. However, the expression of LEF1 in the CHL cases included in our study did not correlate with the patients' stages or survival.

The functions of Wnt5a in solid organ cancers remain controversial. Some studies dem-

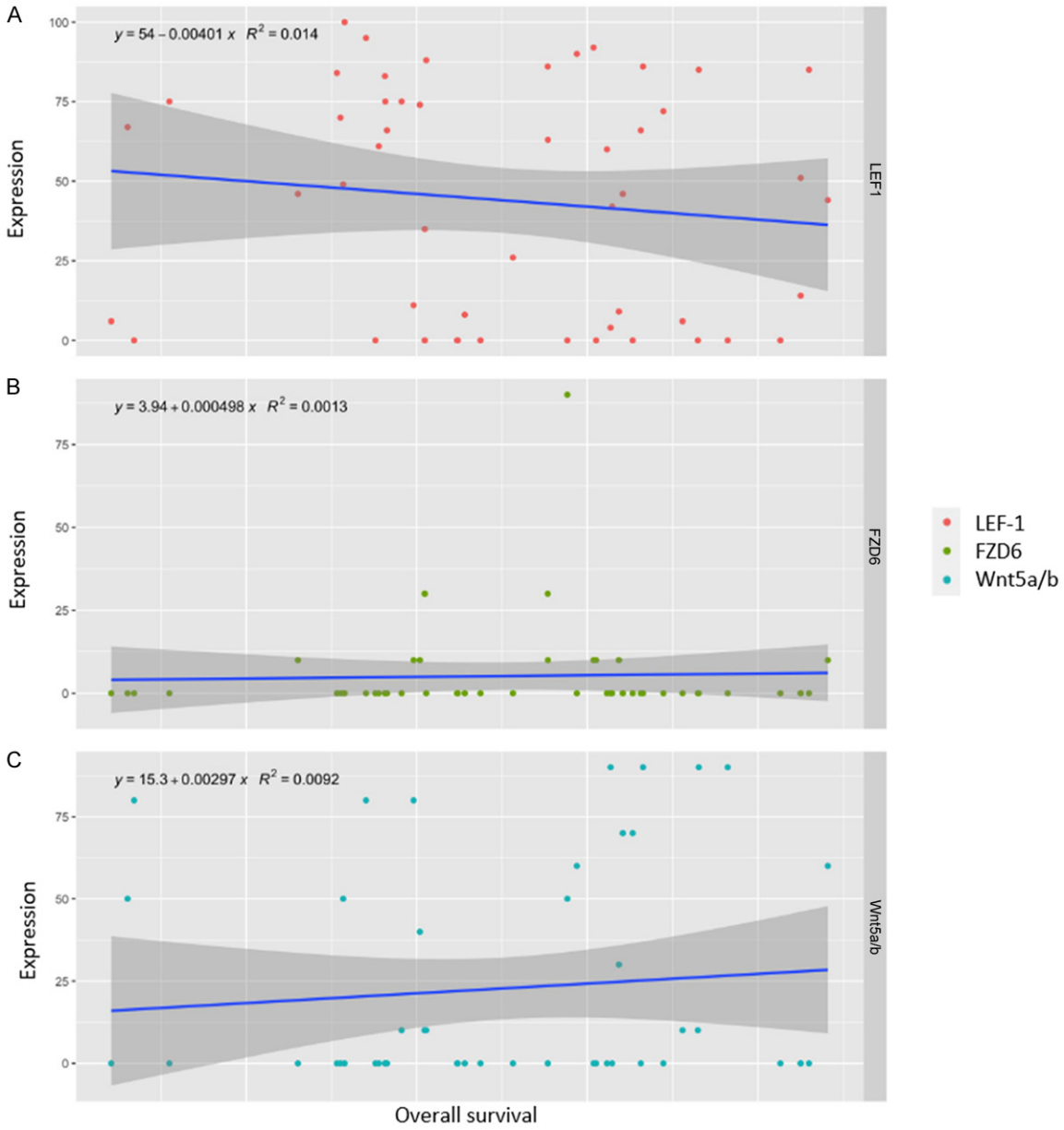

Figure 4. Correlation of LEF-1 (A), FZD6 (B) and Wnt5a/b (C) expression with overall survival.

onstrated that Wnt5a promoted metastasis in lung cancer and thyroid cancer, while other studies indicated its function as a tumor suppressor in colon cancer and thyroid cancer [4]. Knowledge of the roles of Wnt5a in CHL is limited. One study proved Wnt5a to be a motility-promoting factor as overexpression of Wnt5a stimulated migration of Hodgkin lymphoma cells in culture and promoted lymphoma engraftment and vascularization [10]. In our study, a total of 22 patients (44%) were positive for Wnt5a/b in HRS cells and Wnt5a/b expression was seen in a significantly higher percentage of stage IV patients (67%) compared to other stages, which was consistent with the above proposed function of Wnt5a. CHL is unique for its scattered tumor cells and complex microenvironment, which consists of non-malignant T and B lymphocytes, plasma cells, histiocytes, eosinophils, neutrophils, mesenchymal stromal cells and endothelial cells that surround the HRS cells. Tumor microenvironment can promote cancer progression through its influences on cell migration and angiogene-

sis. The Vascular endothelial growth factor (*VEGF*) family is one of the downstream targe genes of Wnt signaling. Linke et al. reported that LEF1 and β-catenin promoted angiogenesis in vitro by regulating the expression of *VEGF-A*. Analyses of *VEGF-A* expression in CHL patients demonstrated that low *VEGF-A* expression was associated with a better overall survival of CHL patients [9]. In a large cohort study of 194 CHL cases, Dimtsas et al. performed immunohistochemical evaluation of VEGF-A, VEGFR1 and VEGFR2 and found the HRS cells expressed VEGF-A, VEGFR1 and VEGFR2 in over 90% of cases. Although no correlation with patients' outcome was observed, all 3 molecules were positively correlated with microvessel branching and VEGFR-2 was positively correlated with serum albumin levels ≥ 4 g/dL [19]. Touati et al. reported CD68+ tumor-associated macrophages were associated with the progression of CHL and affected response to treatment and OS [20]. However, in our study, the low CD68 expression group (< 25%) and the high CD68 expression group (≥ 25%) did not show statistical significance related to the stage or OS (data not shown). The correlation between LEF1, Wnt5a, CD68 and the VEGF family may be of interest for future studies.

In conclusion, multiple factors may be involved with the dissemination or migration of HRS cells, and our study further confirms the high levels of expression of LEF1 in the HRS cells in CHL. All the CHL cases included in this study lack β-catenin expression, indicating the β-catenin-dependent canonical Wnt signaling may not play a critical role in the tumorigenesis migration of HRS cells or progression of CHL. Wnt5a/b expression was correlated with a higher stage but not overall survival of the patients. Our case study is small; however, these findings indicate the non-canonical Wnt pathway may play a role as part of microenvironment that contributes to migration or dissemination of HRS cells. Based on our results, it is not sufficient to consider LEF1, β-catenin, FZD6 and Wnt5a/b or CD68 alone as prognostic factors for CHL.

Acknowledgements

Financial support from University of Kansas Department of Pathology & Laboratory Medicine.

Approval from the Institutional Review Board of The University of Kansas Medical Center and patient informed consent were obtained for this study.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Da Zhang, Department of Pathology, University of Kansas Medical Center, KUMC, Mail Stop 3045, 3901 Rainbow Boulevard, Kansas City, KS 66160, USA. Tel: 913-588-0388; Fax: 913-588-8780; E-mail: dzhang@kumc.edu

References

[1] Logan CY and Nusse R. The Wnt signaling pathway in development and disease. Annu Rev Cell Dev Biol 2004; 20: 781-810.

[2] Xiao Q, Chen Z, Jin X, Mao R and Chen Z. The many postures of noncanonical Wnt signaling in development and diseases. Biomed Pharmacother 2017; 93: 359-369.

[3] Duchartre Y, Kim YM and Kahn M. The Wnt signaling pathway in cancer. Crit Rev Oncol Hematol 2016; 99: 141-149.

[4] Chen Y, Chen Z, Tang Y and Xiao Q. The involvement of noncanonical Wnt signaling in cancers. Biomed Pharmacother 2021; 133: 110946.

[5] Grainger S, Traver D and Willert K. Wnt signaling in hematological malignancies. Prog Mol Biol Transl Sci 2018; 153: 321-341.

[6] Morrison JA, Gulley ML, Pathmanathan R and Raab-Traub N. Differential signaling pathways are activated in the Epstein-Barr virus-associated malignancies nasopharyngeal carcinoma and Hodgkin lymphoma. Cancer Res 2004; 64: 5251-5260.

[7] Sohlbach K, Moll R, Gossmann J, Nowak O, Barth P, Neubauer A and Huynh MQ. beta-catenin signaling: no relevance in Hodgkin lymphoma? Leuk Lymphoma 2012; 53: 996-998.

[8] Agostinelli C, Carloni S, Limarzi F, Righi S, Laginestra MA, Musuraca G, Fiorentino M, Napolitano R, Cuneo A, Vergara D, Zinzani PL, Sabattini E, Pileri SA and De Matteis S. The emerging role of GSK-3beta in the pathobiology of classical Hodgkin lymphoma. Histopathology 2017; 71: 72-80.

[9] Linke F, Harenberg M, Nietert MM, Zaunig S, von Bonin F, Arlt A, Szczepanowski M, Weich HA, Lutz S, Dullin C, Janovska P, Krafcikova M, Trantirek L, Ovesna P, Klapper W, Beissbarth T, Alves F, Bryja V, Trumper L, Wilting J and Kube D. Microenvironmental interactions between

endothelial and lymphoma cells: a role for the canonical WNT pathway in Hodgkin lymphoma. Leukemia 2017; 31: 361-372.
[10] Linke F, Zaunig S, Nietert MM, von Bonin F, Lutz S, Dullin C, Janovska P, Beissbarth T, Alves F, Klapper W, Bryja V, Pukrop T, Trumper L, Wilting J and Kube D. WNT5A: a motility-promoting factor in Hodgkin lymphoma. Oncogene 2017; 36: 13-23.
[11] Zhang D, O'neil MF, Cunningham MT, Fan F, Olyaee M and Li L. Abnormal Wnt signaling and stem cell activation in reactive lymphoid tissue and low-grade marginal zone lymphoma. Leuk Lymphoma010; 51: 906-910.
[12] Menter T, Dirnhofer S and Tzankov A. LEF1: a highly specific marker for the diagnosis of chronic lymphocytic B cell leukaemia/small lymphocytic B cell lymphoma. J Clin Pathol 2015; 68: 473-478.
[13] Chung EJ, Hwang SG, Nguyen P, Lee S, Kim JS, Kim JW, Henkart PA, Bottaro DP, Soon L, Bonvini P, Lee SJ, Karp JE, Oh HJ, Rubin JS and Trepel JB. Regulation of leukemic cell adhesion, proliferation, and survival by beta-catenin. Blood 2002; 100: 982-990.
[14] Walther N, Ulrich A, Vockerodt M, von Bonin F, Klapper W, Meyer K, Eberth S, Pukrop T, Spang R, Trumper L and Kube D. Aberrant lymphocyte enhancer-binding factor 1 expression is characteristic for sporadic Burkitt's lymphoma. Am J Pathol 2013; 182: 1092-1098.
[15] Tandon B, Peterson L, Gao J, Nelson B, Ma S, Rosen S and Chen YH. Nuclear overexpression of lymphoid-enhancer-binding factor 1 identifies chronic lymphocytic leukemia/small lymphocytic lymphoma in small B-cell lymphomas. Mod Pathol 2011; 24: 1433-1443.
[16] Lyapichev KA, Sakhdari A, Khoury JD, O'Malley DP, El Hussein S, Yin CC, Patel KP, Thakral B, Young KH, Medeiros LJ and Konoplev S. Lymphoid enhancer binding factor 1 (LEF1) expression is significantly higher in Hodgkin lymphoma associated with richter syndrome relative to de novo classic Hodgkin lymphoma. Ann Diagn Pathol 2020; 49: 151636.
[17] Rea B and Bailey NG. Additional characterization of LEF1 expression in nodular lymphocyte predominant Hodgkin lymphoma and classic Hodgkin lymphoma. Ann Diagn Pathol 2021; 52: 151730.
[18] Metzeler KH, Heilmeier B, Edmaier KE, Rawat VP, Dufour A, Dohner K, Feuring-Buske M, Braess J, Spiekermann K, Buchner T, Sauerland MC, Dohner H, Hiddemann W, Bohlander SK, Schlenk RF, Bullinger L and Buske C. High expression of lymphoid enhancer-binding factor-1 (LEF1) is a novel favorable prognostic factor in cytogenetically normal acute myeloid leukemia. Blood 2012; 120: 2118-2126.
[19] Dimtsas GS, Georgiadi EC, Karakitsos P, Vassilakopoulos TP, Thymara I, Korkolopoulou P, Patsouris E, Kittas C and Doussis-Anagnostopoulou IA. Prognostic significance of immunohistochemical expression of the angiogenic molecules vascular endothelial growth factor-A, vascular endothelial growth factor receptor-1 and vascular endothelial growth factor receptor-2 in patients with classical Hodgkin lymphoma. Leuk Lymphoma 2014; 55: 558-564.
[20] Touati M, Delage-Corre M, Monteil J, Abraham J, Moreau S, Remenieras L, Gourin MP, Dmytruk N, Olivrie A, Turlure P, Girault S, Labrousse F, Preux PM, Jaccard A and Bordessoule D. CD68-positive tumor-associated macrophages predict unfavorable treatment outcomes in classical Hodgkin lymphoma in correlation with interim fluorodeoxyglucose-positron emission tomography assessment. Leuk Lymphoma 2015; 56: 332-341.