Establishment and characterization of a novel ‘double-hit’ follicular lymphoma cell line, FL-SJC

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Abstract
About 5 per cent of follicular lymphoma (FL) cases are double-hit (DH) lymphomas. Double-hit follicular lymphoma (DHFL) cell lines can improve our understanding and drug development on FL. But there are only few DHFL cell lines. Here, we established a new MYC/BCL2 DHFL cell line, FL-SJC. The cells were obtained from the hydrothorax of a patient with MYC/BCL2 DHFL and cultured for 140 passages in vitro. FL-SJC cells demonstrated CD19++, CD20+, CD22++, HLA-DR+, CD10+, CD38+, Lambda+CD23−, CD5− and Kappa−. The chromosome karyotypic analysis confirmed the co-existence of t(8;22)(q24;q11) and t(14;18)(q32;q21), as well as additional abnormalities involving chromosomes 2 and 3. Fluorescence in situ hybridization analysis (FISH) showed IGH/BCL2 fusion gene and the MYC rearrangement. In addition, the FL-SJC cells displayed KMT2D/MLL2 and CREBBP gene mutations. After subcutaneous inoculation of FL-SJC cells, the SCID mice developed solid tumour masses within 6-8 weeks. FL-SJC cells were proven to be free of Epstein-Barr (EB) virus infection and be multidrug-resistant. In conclusion, the FL-SJC cell line has been identified as a novel MYC/BCL2 double-hit follicular lymphoma that can be used as a potentially available tool for the clinical and basic research, together with the drug development for MYC/BCL2 DHFL.

KEYWORDS
BCL2, Double-hit lymphoma, FL-SJC cell line, Follicular lymphoma, MYC
1 | INTRODUCTION

Follicular lymphoma (FL) which accounts for 20%-25% of all non-Hodgkin’s lymphoma (NHL) cases is the second most common form of NHL.1-3 Cytogenetically, 85%-90% of patients have a typical translocation t(14;18)(q32;q21) that leads to overexpression of BCL2 protein.4,5 Although FL is considered as indolent, most patients are still incurable and 15% to 28% of cases will turn into an invasive phenotype, usually diffuse large B-cell lymphoma (DLBCL) within 10 years.2

Recently, the concept of double-hit (DH) lymphoma has attracted considerable attention. DH lymphoma is defined as a chromosomal translocation between the MYC gene which locates in 8q24.2 and another recurrent oncogene, such as BCL2, BCL6 or rarely other genes.6-8 MYC is a transcription factor that regulates the expression of several target genes related to the cell cycle, DNA damage repair, metabolism, protein synthesis and stress response.9 BCL2 usually plays an anti-apoptotic role.6 MYC/BCL2 DH lymphomas represent approximately 60%-85% of all cases of DH lymphoma.7,8 DH lymphoma (all types) is considered to be ‘high-grade’ B-cell lymphoma,6,10 has an aggressive clinical course, has poor prognosis, and often involves the bone marrow and the central nervous system with a median overall survival no more than 1-2 years.10,11

The studies on the pathogenesis of DH lymphoma depend on well-validated DH lymphoma cell lines.12 The major advantages of cell lines include the probability of unlimited supply, the global availability, the certainty of background and the infinite viable storability in liquid nitrogen. Until 2016, nearly 30 cell lines meet the diagnosis of DH lymphoma, bearing both MYC and BCL2 rearrangement.13,17 Among them, most of them were derived from patients with DLBCL, or Burkitt lymphoma (or B-ALL), while only 4 cell lines were from patients with FL,13 including FLK-1,3 FL-18,18,19 SC-120,21 and TAT-1.22 FLK-1 holding t(2;8)(p12;q24) and t(14;18)(q32;q21), established in 2001, was found to depend on a follicular dendritic cells. When follicular dendritic cells were removed, FLK-1 cells stopped growing and eventually died.3 So FLK-1 is unstable and inconvenient as a cell line.3 FL-18 was established in 1985, in which the translocation [t(8;22)(q24;q13) and t(14;18)(q32;q21)] was not verified by fluorescence in situ hybridization (FISH), Southern blot, polymerase chain reaction (PCR) or other method.13,18,19 probably due to the previous technical restriction. Only SC1 with t(8;14)(q24;q32;q21) 20,21 and TAT-122 with t(8;14;18)(q24;q32;q21) had fully documented genetic background.

Here, we established and characterized a novel lymphoma cell line, FL-SJC, that held chromosomal abnormalities of t(8;22)(q24;q11), t(14;18)(q32;q21), del2q and del3, as well as gene mutations of KMT2D/MLL2 and CREBBP. This cell line could provide a useful tool for studies on the malignant nature of FL.

2 | MATERIALS AND METHODS

2.1 | Patient

A 52-year-old male patient with cough, chest tightness, asthma, night sweat, weight loss and enlarged lymph nodes was admitted to our hospital in July 2016 (no fever or chill) (Figure 1). B-ultrasonic examination showed enlargement of bilateral cervical, supraclavicular, axillary, inguinal and retroperitoneal lymph nodes. Positron emission tomography/computed tomography (PET/CT) showed multiple extensively enlarged lymph nodes with increased maximum of standard uptake value (SUVmax) as well as spleen and bone infiltration (Figure 2A). Bilateral pleural effusion was also detected (Figure 2A). A right cervical lymph node with high SUVmax in PET/CT was excised for biopsy. The biopsy showed BCL2+, CD10+, CD38+ (few), CD20+, CD43-, CD5-, CD21+, CyclinD1-, SOX-11- and ZAP-70 (few), which fulfilled the diagnostic criteria of follicular lymphoma with grade II. Bone marrow smear revealed 21% of lymphoma cells, and peripheral blood smear revealed 2% of lymphoma cells (Figure 2B). These cells had large nuclei with obvious nucleoli (Figure 2B). Thoracentesis was performed, and pleural effusion was collected. The cells from pleural effusion expressed typical immunophenotype for follicular lymphoma (Figure 2C, see below). Conventional chromosome karyotype analysis of bone marrow cells confirmed the existence of t(14;18)(q32;q21) and t(8;22)
(q24;q11) (see below). FISH of mononuclear cells from patient’s pleural effusion confirmed IGH(14q32)/BCL2(18q21) fusion gene (Figure 2D, see below). The laboratory examination showed β₂-microglobulin (β₂-MG) 5.12 μg/mL (0.9 ~ 2.7 mg/mL), lactate dehydrogenase (LDH) 188U/L (135 ~ 266 U/L), ferritin >2000 μg/L (21.81 ~ 274.6 μg/L), haemoglobin 95 g/L (100 ~ 300×10⁶/L), platelet count 108 × 10⁹/L (100 ~ 300×10⁹/L) and EB virus DNA 1.94 × 10⁵ copies/mL (<5 × 10² copies/mL). According to the biopsy, clinical symptom and PET/CT, the patient was diagnosed as follicular lymphoma with grade II, stage IV and B group. According to Follicular Lymphoma International Prognostic Index-2 (FLIPI-2), the patient was in high risk.

From August 2016, the patient was treated with R-CHOP therapy (rituximab, cyclophosphamide, epirubicin, vincristine and prednisone) for four cycles. But the disease did not achieve remission with progressive pleural effusion and emerging pericardial infiltration. The second-line chemotherapy regimen of R-DHAP (rituximab, cisplatin, high-dose cytarabine and dexamethasone) was performed in December 2016. Subsequently, the patient complained with headache and a large number of lymphoma cells were found in the cerebrospinal fluid. An invasion of central nervous system was confirmed. High-dose methotrexate and cytarabine combined with bendamustine were given since January 2017. However, he suffered from an epileptic seizure frequently and the lymphoma gradually invaded into the reproductive system. A puncture on the mass located between testis and pubic symphysis for biopsy was performed in June 2017. The biopsy showed FL transforming into germinal centre B cell-like (GCB) DLBCL with AE1/AE3⁺, Vimentin⁺, CD45⁺, BCL2⁺, CD10⁺, CD20⁺, CD79a⁺, CD38⁺, Ki67⁺(75%), c-Myc⁺, PAX-5⁺, BCL6⁺, CD3⁺, CD21⁺, CD5⁺, CyclinD1⁺, MUM-1⁺, CD138⁺, Kappa⁺ and Lambda⁺ (Figure 2E). The laboratory examination showed β₂-MG 5.4 mg/L, LDH 1262 U/L, white blood cell 2.1 × 10⁹/L, haemoglobin 62 g/L and platelet count 37 × 10⁹/L. Although intensive chemotherapy regimens were chosen, the patient died in June 2017 (Figure 1).

2.2 | Cell culture

The pleural effusion was collected through thoracentesis in August 2016. The cells were harvested and cultured in RPMI 1640 medium with 10% foetal bovine serum and 1% penicillin/streptomycin without external growth factors or stimulatory cytokines. The cells passaged for at least 140 times were named as the ‘FL-SJC’.

2.3 | Cell growth and viability assay

Cell viability of FL-SJC cell line was assessed using cell counting kit-8 (Beyotime Biotechnology) according to the directions of the kit. Another two FL cell lines, Dohh2 and SC1 (as a gift generously provided by Prof. Chuanxin Huang, Shanghai Jiao Tong University College of Basic Medical Science), were served as controls. The doubling time of these cells was calculated.

2.4 | Flow cytometry

The original FL cells from pleural effusion and FL-SJC cells were centrifuged, respectively, and tested for immunophenotypic analysis by flow cytometry. The panel of monoclonal antibodies, including those specific for CD45, CD19, CD20, HLA-DR, CD19, CD5, CD22, CD23, CD10, CD38, and Kappa and Lambda light chains, was bought from BD Biosciences Co.

2.5 | Cytogenetic analysis

Chromosome analysis was performed using conventional R-banding. FISH was performed using a dual-colour break apart MYC probe, and a dual-colour break apart IGH probe, as well as IGH(14q32)/BCL2(18q21), CCND1(11q23)/IGH(14q32), and TP53(17p13)/CEP17(17p11-q11) dual-colour dual-fusion translocation probes (Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College). The cut-offs to define positive results for rearrangement of MYC, rearrangement of BCL2, IGH/BCL2, CCND1/ IGH and TP53/ CEP17 probes are 2.53%, 4.18%, 2.4%, 2.67%, 2.85% and 2.89%, respectively.

2.6 | Gene mutation of FL-SJC cells

Exon sequencing of lymphoma-associated 38 genes was performed (Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College). According to the literature, these genes included ARID1A, ATM, BCL2, BCL6, BIRC3, CCND1, CCND3, MYC, CREBBP, DNMT3A, ECT2L, EP300, EPHA7, EZH2, FBXW7, IDH2, IKZF1, JAK1, JAK2, JAK3, KIT, MLL2, MUM1, MYD88, NOTCH1, NOTCH2, PAX5, PDGFRA, PHF6, PTEN, RELN, TEL, TET2, TNFAIP3, TP53, WHSC1, WT1 and XPO1.

2.7 | Quantitative RT-PCR

Total RNA was extracted from FL-SJC cells, Dohh2, SC1 and K562, using the DNA/RNA/protein co-extraction kit (TianGen Biotech). Peripheral blood mononuclear cells from healthy volunteers were served as a control. Reverse transcription was performed using PrimeScript™ RT Master Mix (TaKaRa RR336A, Otsu, Japan) according to the manufacturer’s instructions. The qPCR was performed using the SYBR Premix Ex Taq (TaKaRa RR420A) according to the manufacturer’s protocol. GAPDH was used as an internal reference for normalization, and the relative expression of MLL2 mRNA was evaluated by the 2−ΔΔCT method. The following primers according to the literature synthesized by Sangon Biotech (Shanghai, China) were used: GAPDH (forward) 5’-GGTGAAGGTCGGAGTCAACG-3’ and (reverse) 5’-GGGTGCCTCGTCAAGACGATGTG-3’; MLL2 (forward)5’-GGGAGGCTTCAATGTATCTTAT-3’ and (reverse) 5’-CGCGGGATCTGGTGCAAGCTATCTTTG-3’.
The FL-SJC cells were lysed using cell lysates. Peripheral blood mononuclear cells from healthy volunteers were served as a control. The boiled proteins were electrophoresed with 10% SDS-PAGE and then transferred onto PVDF membrane. Proteins were detected using the rabbit anti-MLL2 polyclonal antibody (sc-292359) purchased from Santa Cruz Biotechnology, Inc, and mouse anti-β-actin monoclonal antibody (mAbcam8226) purchased from Abcam, followed by HRP-conjugated secondary antibodies. Immunoreactive bands were detected by ECL. The signal intensity of the respective bands was measured by the NIH Image J software.

2.9 Xenograft transplantation in vivo

FL-SJC cells (10 × 10⁶) were inoculated into the left armpits of 4-week-old female SCID mice (Gempharmatech Co., Ltd) by subcutaneous injection (n = 3). Animals were marked and checked every two weeks for lump formation. After 6 to 8 weeks, solid tumour mass under the armpits from all of the 3 mice inoculated with FL-SJC cells was detected. The mice were sacrificed under euthanasia, and tumour masses were resected for biopsy. All animal experiments have been reviewed and approved by the Institutional Animal Care and Use Committee of Jiangsu University.

2.10 Epstein-Barr (EB) virus detection

Genomic DNAs from FL-SJC cells were extracted to detect whether the cells were infected by the EBV or not by PCR. Raji cells (received as a gift from Nanjing Medical University, Nanjing, China) were used as a positive control and Jeko-1 cells (as a gift from Prof. Xiaodong Xi of Ruijin Hospital, Shanghai Jiao Tong University School of Medicine) were used as a negative control. The primers for the detection of EBV were as follows: EBNA1-F: GGTAGAAGGCGATTCTTCCAC; EBNA1-R: CTCCATCGCTCAAGCTGCAC; EBNA2-F: CAGGTACATGCCAACACCTT; EBNA2-R: CCAACAAAAATTGTAGTTGGAAT. For the internal control, a PCR of β-actin was performed. The primers for the detection of β-actin were as follows: β-actin-F: CTCCATCCCTGCGCTGCCTGT; β-actin-R: GCTGTCACCTTACCGTTCC.

2.11 Drug-resistance assay

The cell counting kit-8 was utilized to assess chemosensitivity of FL-SJC cell lines. The anticancer drugs, including cytarabine (Ara-c), methotrexate (MTX) and cyclophosphamide (CTX), were added at various concentrations. The absorbance, defined as optical density (OD), was detected with a microplate reader at a wavelength of 450 nm. Inhibition rate was defined as. Dohh2 and SC1 cells were served as controls.

2.12 Statistical analysis

Statistical analyses were performed using the GraphPad Prism 5.0. The measurement data were expressed as mean ± standard error. The t test and the analysis of variance were used as appropriate. P < .05 indicates that the difference is statistically significant.

3 RESULTS

3.1 Establishment of the FL-SJC cell line

Original cells were obtained from the patient’s hydrothorax and cultured without any external stimuli. The cells proliferated stably with a doubling time of 24 hours. The cells demonstrated non-adherent growth without cellular clump formation.

FL-SJC cells were small-to-medium sized and blast-like lymphocytes, and the cytoplasm was moderately rich and basophilic (Figure 3A). The large nuclei were round to ovoid with rough chromatin and obvious nucleoli (Figure 3A). The morphologic features of FL-SJC cells were similar to that of original FL cells (Figure 2B).

The viability of FL-SJC cells was high, while a proliferative rate was not as high as that of Dohh2 and SC1 cells (Figure 3B).

3.2 Immunophenotypic characterization

Original FL cells from the patient’s hydrothorax expressed CD19, CD20, CD22, CD10, CD38, Lambda and HLA-DR, partially expressed CD5 and CD23, but did not express Kappa (Figure 2C, Figure 3G). FL-SJC cells were positive for CD19, CD20, CD22, CD10, CD38, Lambda and HLA-DR, and were negative for CD5, CD23 and Kappa (Figure 3G).
3.3 Chromosome karyotyping and FISH analysis

Conventional chromosome karyotype analysis of FL cells from patient’s bone marrow revealed 44X, -Y, -22, t(8;22)(q24;q11), t(14;18) (q32;q21) [3]/46XY [7], while FL-SJC cells demonstrated a complex karyotype: 45,XY, del(2)(q34;q27),-3,del(7)(q11),t(8;22)(q24;q11),t(14;18) (q32;q21)[5]/ 45,XY, del(2)(q34;q27),t(8;22)(q24;q11),t(14;18)(q32;q21)[10]/[20] (Figure 3C). The karyotype of FL-SJC cells was similar to but more complex than the original FL cells.

FISH of original FL cells from patient’s pleural effusion showed 65% cells had \(IGH(14q32)/BCL2(18q21)\) fusion gene, 96% had BCL2(18q21) gene rearrangement (the cut-off value was 4.18%). 96% cells had \(IGH(14q32)\) gene rearrangement. The proliferation of \(IGH\) was also detected (Figure 3D). The detection rate of FISH was higher in FL-SJC cells than that in FL cells, probably due to the purification of long culture.

3.4 Gene mutation of FL-SJC cells

FL-SJC cells had MLL2 (Exon14, c.4135_4136del, p.M1379Vfs*52) and CREBBP (Exon30, c.5039_5041del, p.S1680delS) gene mutations. The other gene mutations were also detected, including CREBBP (Exon25, c.4174C > T, p.R1392X), CCND3 (Exon5, c.869_872dup, p.L292Tfs*56), BCL2 (Exon2, c.256C > T, p.S86L), MLL2 (Exon14, c.4135_4136del, p.M1379Vfs*52), CREBBP (Exon30, c.5039_5041del, p.S1680delS), and other mutations were also detected.
p.L86F), RELN (Exon43, c.6575G > A, p.R2192Q), MYC (Exon2, c.221C > T, p.P74L) and NOTCH1 (Exon11, c.1837C > T, p.R613C) (Table 1).

3.5 | mRNA level and protein expression of MLL2

The quantitative RT-PCR results showed that mRNA level of MLL2 was significantly up-regulated in K562 compared with that in the normal control cells (P < .05), consistent with the previous report. However, mRNA expression of MLL2 in FL-SJC, as well as other two follicular lymphoma cell lines (Dohh2 and SC1), had no statistically significant difference (P > .05) compared with the normal control (Figure 3E). The protein expression of MLL2 in FL-SJC cells was decreased compared with that in normal control cells (Figure 3F).

3.6 | Xenotransplantation of FL-SJC cells into SCID mice

FL-SJC cells (10 × 10⁶) were seeded into the left armpits of SCID mice by subcutaneous injection. Within 6-8 weeks, all the mice (3 mice) gradually developed into solid tumour masses in the armpits (Figure 4A). Further biopsy analysis showed that the tumour mass removed (Figure 4B) consisted of lymphocytes from human B cell, morphologically consistent with FL-SJC cells. Immunohistology results showed BCL2++, CD10++, CD19++, CD138++, CD43, Lambda+, Ki67++, MYC++, CD20±, BCL6, CD3, CD5, CD23, Cyclin D1, SOX11- and Kappa- (Figure 4C-T).

3.7 | EBV status in FL-SJC cells

PCR analysis showed EBV negative in FL-SJC cells. As expected, the genomes of EBV1 (type 1) and EBV2 (type 2) were detected in the Raji cell line (as positive control), but not in the Jeko-1 cell line (as negative control) (Figure 5A).

3.8 | Drug resistance

The chemosensitivity of FL-SJC, Dohh2 and SC1 to Ara-c, MTX and CTX is shown in Figure 5B, 5C and 5D. The inhibition rate of Ara-c in FL-SJC was lower than that in Dohh2 and SC1, and the inhibition rate of MTX in FL-SJC was lower than that in Dohh2, suggesting that FL-SJC demonstrated a high drug resistance.

4 | DISCUSSION

Although therapeutic outcomes of FL have markedly improved in the last few decades, 15% to 28% of cases will transform into an aggressive phenotype, typically DLBCL. The therapeutic approaches for FL are most often based on stage, FLIPI and burden of disease. In the beginning, the disease was diagnosed as FL with grade II, stage IV and B group, and with high risk FLIPI. The patient showed an unsatisfactory outcome associated with normal chemotherapy including R-CHOP, R-DHAX and high-dose MTX. Autologous hematopoietic stem cell transplantation should have been performed; unfortunately, the patient died during the induction treatment.

The aggressive characteristics of disease may result from the gene rearrangements. In this patient, conventional chromosome karyotype analysis and FISH examination confirmed the co-existent of t(14;18)(q32;q21) and t(8;22)(q24;q11) involving gene rearrangement of MYC/BCL2, which recently is named as ‘double hit (DH)’. The ‘DH’ lymphomas are defined as a chromosomal breakpoint affecting the MYC/8q24 and another recurrent genetic abnormality, mainly involving BCL2, BCL6, BCL3 or other genes. Among them, most are MYC/BCL2. MYC/BCL2 double-hit lymphomas tend to demonstrate themselves with advanced stage and poor prognostic parameters including extranodal infiltration and elevated LDH, and a high FLIPI score. This patient had high LDH, infiltration in the bone marrow, reproductive system and the central nervous system. All these features were fulfilled in this patient. Most common transformation of DHFL is transformation into DLBCL; the biopsy from infiltrated mass near the pubic symphysis in this patient confirmed the DLBCL transformation at the late stage of disease.

**TABLE 1** Gene mutation of FL-SJC cells

| Gene       | Transcript | Site     | DNA          | Amino acid       | Mutation ratio (%) |
|------------|------------|----------|--------------|------------------|--------------------|
| KMT2D/MLL2 | NM_003482  | Exon14   | c.4135_4136del | p.M1379Vfs*52    | 45.8               |
| CREBBP     | NM_004380  | Exon30   | c.5039_5041del | p.S1680delS      | 47.5               |
| CREBB      | NM_004380  | Exon25   | c.4174C > T   | p.R1392X         | 51.1               |
| CCND3      | NM_001760  | Exon5    | c.869_872dup  | p.L292Tfs*56     | 38                 |
| BCL2       | NM_000633  | Exon2    | c.256C > T    | p.L86F           | 39.9               |
| RELN       | NM_005045  | Exon43   | c.6575G > A   | p.R2192Q         | 48.5               |
| MYC/MYC    | NM_002467  | Exon2    | c.221C > T    | p.P74L           | 61.2               |
| NOTCH1     | NM_017617  | Exon11   | c.1837C > T   | p.R613C          | 49.7               |
One of the main limitations of the successful treatment of MYC/BCL2 DH lymphoma is lack of comprehensive understanding of disease pathogenesis and mechanisms of chemotherapeutic resistance, as well as knowledge of potential therapeutic targets. Cell lines are critical for mechanism exploration and drug discovery.

However, as far as we know, there have been only a few published manuscripts describing the establishment and characterization of defined DHFL cell lines. Some of them are not in full detail in genetic messages. In this report, we established a novel ‘double-hit’ follicular lymphoma cell line, FL-SJC, which we wish as a useful tool for the improved understanding of MYC/BCL2 ‘double-hit’ FL.

The success rate for the establishment is low, and it is impossible to predict whether it is successful for specimens from source. Those lymphoma cell lines which were established successfully were high-grade lymphomas and from the ascitic fluid, or pleural fluid. FL-SJC cell line was from the pleural effusion and passed for at least 140 times. It took us 2 years to establish it. We then identified the cell line according to the published guidelines. The clinical characteristics and cell culture data, as well as immunological and cytogenetic features were described. Established FL-SJC cells demonstrated small-medium size, blast-like morphology.

The biopsy from initial lymph node fulfilled the immunophenotype of follicular lymphoma. The biopsy from late infiltrated mass near the pubic symphysis confirmed FL transforming into DLBCL. The positive rate of Ki67 gradually increased suggesting aggressive conversion. The FL-SJC cells expressed CD19, CD10, CD22, HLA-DR, CD38, Lambda and CD20, but did not express CD23, CD5 and Kappa, which was similar to that of the original FL cell from pleural effusion. The FL-SJC cells were fully negative for CD23 and CD5 which were 8% and 5% positive, respectively, in primary FL cell, suggesting that the FL-SJC cells have a higher purity than original tumour cells.

Xenotransplantation of FL-SJC cells into SCID mice can cause the solid tumour mass, suggesting the high proliferative viability. The immunohistology of tumour showed the loss of CD20. Pham LV et al ever reported that the RC cell line showed gradually reduced CD20 expression with the culture. Denyssevych T et al also observed that the expression of the CD20 antigen on the surface of Tat-1 cells grown in mice gradually decreased. It is possibly due to selection of more immature lymphoma cells for its growth advantage during the culture and inoculation, because CD20 is mainly expressed on mature B lymphocytes.

FISH and karyotypic analysis confirmed the existence of t(8;22)(q24;q11) and t(14;18)(q32;q21) double fusion genes. Chromosomal translocations join MYC in 8q24 to the light chain IG gene 22q11 (Lambda light chain) and BCL2 in 18q21 to the heavy chain IG gene 14q32. Unfortunately, the test of IGL/MYC gene fusion was not confirmed due to unavailability of dual-fusion translocation probes. The FL-SJC hold more chromosome change than FL did, having deletion of chromosome 2(q34;q27) and 3.

Additionally, recently some genes had been reported to be associated with the prognosis of lymphoma. We tested the prognosis-related genes. EZH2 mutation which was associated with good
prognosis, according to the newest risk prognostication developed in FL, the M7-FLIPI, was not found in FL-SJC, while CREBBP (a histone modification enzyme) mutation, which was associated with poor prognosis, was found in FL-SJC. MLL2, also known as KMT2D or ALR, is another histone modification enzyme, gene mutations of which are mainly found in lymphomas (especially DLBCL and FL from indolent to aggressive) and myeloma. MLL2 acts as a central tumour suppressor in FL. The frameshift mutation of MLL2 gene, which has 54 exons, also was found in Exon14 in FL-SJC cells. By RT-PCR, we found mRNA level of MLL2 in FL-SJC cells was similar to that in normal control cells. While MLL2 protein expression was obviously decreased in FL-SJC cells by Western blot. The anti-MLL2 antibody (sc-292359) we used is raised against amino acid 4021-4320 mapping which is at the downstream of mutation site. It is possible that the frameshift mutation of MLL2 in FL-SJC cells causes a stop of translation from mRNA (20635bp) to protein. In this study, we only tested 38 genes, in the future we will perform RNA-sequencing to show the global changes of gene expression and find some useful clues.

The expression of Epstein-Barr virus associated antigen (EBNA-I) is often detected in established lymphoma lines. EBV can immortalize cell lines and truly malignant cell lines must be distinguished from EBV-immortalized normal cells. This FL-SJC cell line was negative for EBV according to PCR result suggesting that the immortality of FL-SJC results from intrinsic gene changes.

We had made a preliminary estimation of FL-SJC sensitivity to chemotherapeutic drugs, Ara-c, MTX and CTX. FL-SJC demonstrated a higher resistance to Ara-c than Dohh2 and SC1 did, and to MTX than Dohh2 did. This may reflect subtle changes in cell growth regulation.

5 | CONCLUSION

We described the establishment and characterization of a new MYC/BCL2 DHFL cell line, FL-SJC, which would be useful in studies on human DHFL, and therapeutic drug development.

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CONFLICT OF INTEREST
The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTION
Min Chen: Formal analysis (lead); Methodology (lead); Writing-original draft (equal). Guoxiong Jiang: Formal analysis (equal); Methodology (equal); Writing-original draft (equal). Yichen Liu: Formal analysis (equal); Methodology (equal); Writing-original draft (equal). Dongya Li: Methodology (equal); Supervision (equal); Writing-original draft (equal). Jie Peng: Methodology (equal); Qian Jiang: Formal analysis (equal); Methodology (supporting); Software (equal). Haiyan You: Formal analysis (equal); Methodology (supporting); Software (equal). Rong Ba: Formal analysis (equal); Methodology (supporting); Software (equal). Jinlan Pan: Formal analysis (equal); Methodology (supporting). Mei Li: Formal analysis (equal); Methodology (supporting); Software (equal). Weiguo Long: Formal analysis (equal); Methodology (supporting); Writing-review & editing (equal). Yan Zhu: Investigation (equal); Resources (equal). Yun Wang: Methodology (supporting); Software (equal). Xiaodong Xi: Formal analysis (equal); Methodology (equal); Writing-review & editing (equal). Jianhua Mao: Conceptualization (lead); Data curation (equal); Methodology (equal); Supervision (equal); Writing-original draft (equal). Xiaofeng Shi: Conceptualization (lead); Data curation (equal); Funding acquisition (lead); Investigation (lead); Methodology (equal); Resources (lead); Supervision (lead); Writing-original draft (lead); Writing-review & editing (lead).

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES
1. Maddocks K, Barr PM, Cheson BD, et al. Recommendations for clinical trial development in follicular lymphoma. J Natl Cancer Inst. 2017;109(3):djw255.
2. Castellino A, Santambrogio E, Nicolosi M, et al. Follicular lymphoma: the management of elderly patient. Mediterr J Hematol Infect Dis. 2017;9:e2017009.
3. Kagem Y, Jung J, Choi YS, et al. Establishment of a follicular lymphoma cell line (FLK-1) dependent on follicular dendritic cell-like cell line HK. Leukemia. 2001;15:148-156.
4. Taira T, Nagasaki A, Tomoyose T, et al. Establishment of a human herpes virus-8-negative malignant effusion lymphoma cell line (STR-428) carrying concurrent translocations of BCL2 and c-MYC genes. Leuk Res. 2007;31:1285-1292.
5. Korfi K, Ali S, Heward JA, et al. Follicular lymphoma, a B cell malignancy addicted to epigenetic mutations. Epigenetics. 2017;12:370-377.
6. Rosenthal A, Younes A. High grade B-cell lymphoma with rearrangements of MYC and BCL2 and/or BCL6: Double hit and triple hit lymphomas and double expressing lymphoma. Blood Rev. 2017;31:37-42.
7. Li S, Saksena A, Desai P, et al. Prognostic impact of history of follicular lymphoma, induction regimen and stem cell transplant in patients with MYC/BCL2 double hit lymphoma. Oncotarget. 2016;7:38122-38132.
8. Kikuchi H, Higuchi T, Hashida Y, et al. Generation and characteristics of a novel "double-hit" high grade B-cell lymphoma cell line DH-My6 with MYC/IGH and BCL6/IGH gene rearrangements and potential molecular targeted therapies. Oncotarget. 2018;9:33482-33499.
9. Dang CV, O’Donnell KA, Zeller KL, et al. The c-Myc target gene network. Semin Cancer Biol. 2006;16:253-264.
10. Landsburg DJ, Petrich AM, Abramson JS, et al. Impact of oncogene rearrangement patterns on outcomes in patients with double-hit non-Hodgkin lymphoma. Cancer. 2016;122:559-564.
11. Oki Y, Noorani M, Lin P, et al. Double hit lymphoma: the MD anderson cancer center clinical experience. Br J Haematol. 2014;166:891-901.
12. Drexler HG, Matsuo AY, MacLeod RA. Continuous hematopoietic cell lines as model systems for leukemia-lymphoma research. Leuk Res. 2000;24:881-911.
13. Drexler HG, Eberth S, Nagel S, et al. Malignant hematopoietic cell lines: in vitro models for double-hit B-cell lymphomas. Leuk Lymphoma. 2016;57:1015-1020.
14. Pham LV, Lu G, Yamato AT, et al. Establishment and characterization of a novel MYC/BCL2 "double-hit" diffuse large B cell lymphoma cell line. RC. J Hematol Oncol. 2015;8:121.
15. Goy A, Ramdas L, Remache YK, et al. Establishment and characterization by gene expression profiling of a new diffuse large B-cell lymphoma cell line, EJ-1, carrying t(14;18) and t(8;14) translocations. Lab Invest. 2003;83:913-916.
16. Johnson-Farley N, Veliz J, Bhagavathi S, et al. ABT-199, a BH3 mimetic that specifically targets Bcl-2, enhances the antitumor activity of chemotherapy, bortezomib and JOQ in "double hit" lymphoma cells. Leuk Lymphoma. 2015;56:2146-2152.
17. Rahmani M, Aust MM, Benson EC, et al. PI3K/mTOR inhibition markedly potentiates HDAC inhibitor activity in NHL cells through BIM- and MCL1-dependent mechanisms in vitro and in vivo. Clin Cancer Res. 2014;20:4849-4860.
18. Ohno H, Doi S, Fukushima S, et al. A newly established human lymphoma cell line, FL-18, carrying a 14;18 translocation. Jpn J Cancer Res. 1985;76:563-6.
19. Doi S, Ohno H, Tatsumi E, et al. Lymphoma cell line (FL-18) and Epstein-Barr virus-carrying cell line (FL-18-EB) obtained from a patient with follicular lymphoma: monoclonal derivation and different properties. Blood. 1987;70:1619-23.
20. Th'ng KH, Garewal G, Kearney L, et al. Establishment and characterization of three new malignant lymphoid cell lines. Int J Cancer. 1987;39:89-93.
21. DSMZ L-l. Catalogue human and animal cell lines. Braunschweig. 2015; [accessed 2015 Oct 28]: Available from: http://www.dsmz.de/catalogues/catalogue-human-and-animal-cell-lines.html
22. Denysyvevych T, Lestou VS, Knesevich S, et al. Establishment and comprehensive analysis of a new human transformed follicular lymphoma B cell line, Tat-1. Leukemia. 2002;16:276-283.
23. Pastore A, Jurinovic V, Krdiel R, et al. Integration of gene mutations in risk prognostication for patients receiving first-line immunotherapy for follicular lymphoma: a retrospective analysis of a prospective clinical trial and validation in a population-based registry. Lancet Oncol. 2015;16:1111-1122.
24. Qu J, Ero R, Feng C, et al. Kindlin-3 interacts with the ribosome and regulates c-Myc expression required for proliferation of chronic myeloid leukemia cells. Sci Rep. 2015;5:18491.
25. Sun P, Wu T, Sun X, et al. KMT2D inhibits the growth and metastasis of bladder Cancer cells by maintaining the tumor suppressor genes. Biomed Pharmacother. 2019;115:108924.
26. Issaeva I, Zonis Y, Rozovskaia T, et al. Knockdown of ALR (MLL2) reveals ALR target genes and leads to alterations in cell adhesion and growth. Mol Cell Biol. 2007;27:1889-1903.
27. Aukema SM, Siebert R, Schuuring E, et al. Double-hit B-cell lymphomas. *Blood*. 2011;117:2319-2331.
28. Mirabelli P, Coppola L, Salvatore M. Cancer cell lines are useful model systems for medical research. *Cancers*. 2019;11(8):1098.
29. Close DA, Wang AX, Kochanek SJ, et al. Implementation of the NCI-60 Human Tumor Cell Line Panel to Screen 2260 Cancer Drug Combinations to Generate >3 Million Data Points Used to Populate a Large Matrix of Anti-Neoplastic Agent Combinations (ALMANAC) Database. *SLAS Discov*. 2019;24:242-263.
30. Ford RJ, Goodacre A, Ramirez I, et al. Establishment and characterization of human B-cell lymphoma cell lines using B-cell growth factor. *Blood*. 1990;75:1311-1318.
31. Drexler HG, Matsuo Y. Guidelines for the characterization and publication of human malignant hematopoietic cell lines. *Leukemia*. 1999;13:835-842.
32. Geraghty RJ, Capes-Davis A, Davis JM, et al. Guidelines for the use of cell lines in biomedical research. *Br J Cancer*. 2014;111:1021-1046.
33. Barry TS, Jaffe ES, Kingma DW, et al. CD5+ follicular lymphoma: a clinicopathologic study of three cases. *Am J Clin Pathol*. 2002;118:589-598.
34. Andor N, Simonds EF, Czerwinski DK, et al. Single-cell RNA-Seq of follicular lymphoma reveals malignant B-cell types and coexpression of T-cell immune checkpoints. *Blood*. 2019;133:1119-1129.
35. Morin RD, Mendez-Lago M, Mungall AJ, et al. Frequent mutation of histone-modifying genes in non-Hodgkin lymphoma. *Nature*. 2011;476:298-303.
36. Drexler HG, Minowada J. History and classification of human leukemia-lymphoma cell lines. *Leuk Lymphoma*. 1998;31:305-316.

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