

CXCR4 upregulation is an indicator of sensitivity to B-cell receptor/PI3K blockade and a potential resistance mechanism in B-cell receptor-dependent diffuse large B-cell lymphomas

Linfeng Chen,^{1,2} Jing Ouyang,^{1*} Kirsty Wienand,^{1*} Kamil Bojarczuk,^{1,3*} Yansheng Hao,^{1,4} Bjoern Chapuy,^{1,5} Donna Neuberg,⁶ Przemyslaw Juszczynski,^{1,3} Lee N. Lawton,¹ Scott J. Rodig,⁷ Stefano Monti⁸ and Margaret A. Shipp¹

¹Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA, USA; ²Current address: H3 Biomedicine, Cambridge, MA, USA; ³Current address: Department of Experimental Hematology, Institute of Hematology and Transfusion Medicine, Warsaw, Poland; ⁴Current Address: Department of Pathology, Mount Sinai Hospital, New York, NY, USA; ⁵Current Address: Department of Hematology and Oncology, University Medical Center Göttingen, Göttingen, Germany; ⁶Department of Biostatistics and Computational Biology, Dana-Farber Cancer Institute, Boston, USA; ⁷Department of Pathology, Brigham and Women's Hospital, Boston, MA, USA and ⁸Section of Computational Biomedicine, Boston University School of Medicine, Boston, MA, USA

*JO, KW and KB contributed equally to this work.

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Correspondence: MARGARET A. SHIPP - margaret_shipp@dfci.harvard.edu

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Supplementary Methods

Cell surface Ig flow cytometry, analyses of cellular proliferation and immunoblotting were performed as previously described².

Quantitative RT-PCR (qRT-PCR)

Total RNA was prepared with TRIzol (Invitrogen) and reverse-transcribed with the Superscript III first-strand cDNA synthesis kit (Invitrogen) and random hexamer primers. Expression of specific genes was evaluated relative to peptidylprolyl isomerase A (cyclophilin A, PPIA) by qRT-PCR with appropriate oligonucleotide primers (Table 1, below) and Power SYBR Green PCR Master Mix (Applied Biosystems). PCR was performed using an ABI 7500 thermal cycler (Applied Biosystems) and threshold Cycle (CT) values were generated using the Sequence Detection Software, version 1.2 (Applied Biosystems). Target gene transcript abundance was calculated relative to the housekeeping control using the $2^{-(\Delta CT_{\text{target gene}} - \Delta CT_{\text{PPIA}})}$ method. Standard deviations were calculated from triplicate ΔCT values. Primer Sequences are listed in Table 1.

Table 1. Sequences of primers used in qRT-PCR experiments

Primer	5'- 3' Sequence
CXCR4, Forward	
CXCR4, Reverse	
PPIA, Forward	AGGGTTTATGTGTCAGGGTGTT
PPIA, Reverse	GGACCCGTATGCTTTAGGATGA
BCL2A1, Forward	
BCL2A1, Reverse	
SYK, Forward	
SYK, Reverse	

Lentiviral-mediated shRNA transduction

Cells were infected with lentiviral particles containing either the negative control vector (pLKO.1-emptyT, TRCN0000208001, Broad Institute, Cambridge, MA) or specific SYK or FOXO1 shRNAs, and subsequently selected for 48 h with puromycin and analyzed thereafter for knockdown efficacy and cellular proliferation.

Accession number

The gene expression profiling of five DLBCL cell lines treated with SYK inhibitor (R406) was reported previously under Gene Expression Omnibus accession number (GSE43510)³.

Supplementary Figures

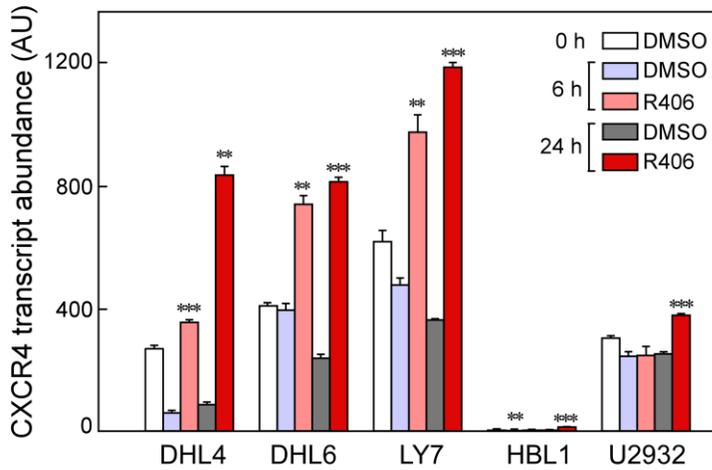


Figure S1. CXCR4 transcript abundance in vehicle- and R406-treated BCR-dependent DLBCL cell lines. CXCR4 transcript abundance after R406 treatment for 6 or 24 h was assessed by gene expression profiling as described (Cancer Cell 2013 23:826), data available via GSE43510. The differences in CXCR4 expression between control (DMSO) and R406 treated groups were determined with a one-sided Welch t-test. *** $p < 0.0001$; ** $p < 0.001$; * $p < 0.01$; # $p < 0.05$.

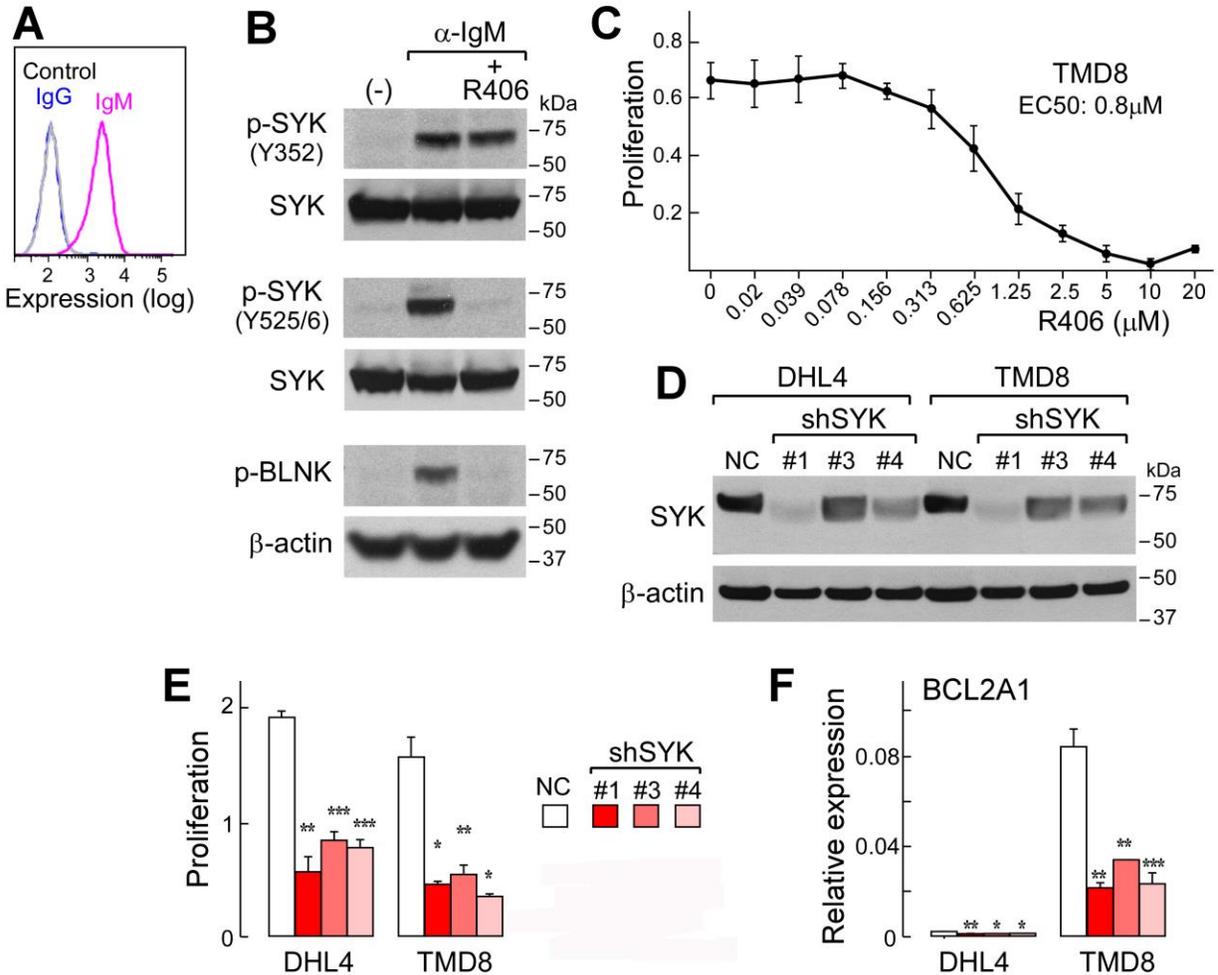


Figure S2. BCR signaling in the TMD8 DLBCL cell line. (A) TMD8 cells were stained with PE-conjugated anti-IgG (blue) or -IgM (purple) and analyzed by flow cytometry. Unstained control cells in gray. (B) TMD8 cells were treated with vehicle or 1 μ M R406 for 1 h and subsequently stimulated with anti-IgM or not, then analyzed for p-SYK352, p-SYK525/526, p-BLNK84 and total SYK by immunoblotting. β -actin, loading control. (C) Cellular proliferation of R406-treated (72 h) TMD8 cells was measured by MTS assay. (D) The SYK protein level in cell lysates prepared from the indicated cell lines transduced with a negative control (NC) shRNA or the indicated SYK shRNAs was assessed by immunoblotting. β -actin, loading control. (E) Cellular proliferation of SYK-depleted DHL4 and TMD8 cells was assessed by MTS assay. (F) BCL2A1 expression in SYK-depleted DLBCL cell lines was assessed by qRT-PCR. In (E) and (F), the p-values for NC versus SYK shRNA were determined with a one-sided Welch t-test. ***, $p < 0.0001$; **, $p < 0.001$; *, $p < 0.01$. In (C), (E) and (F), the error bars represent the SD of 3 independent assays from a representative experiment.

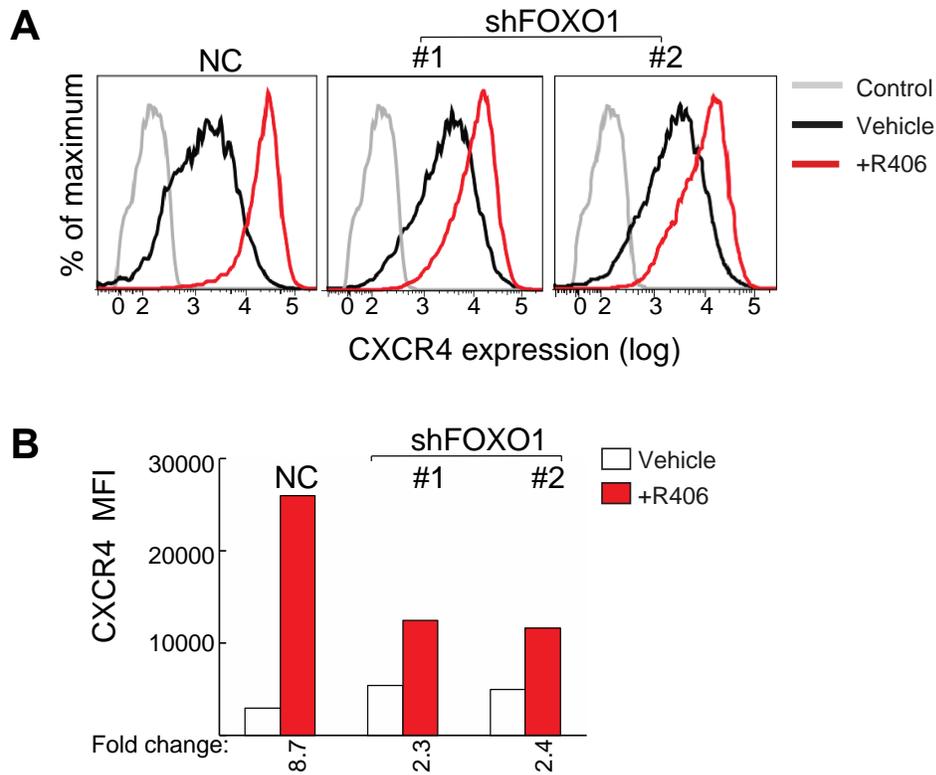


Figure S3. CXCR4 upregulation in R406-treated DHL4 cells is reduced by FOXO1 depletion. DHL4 cells were transduced with either negative control (NC) shRNA, FOXO1 shRNA (shFOXO1#1 or shFOXO1#2). After 2 days of puromycin selection, cells were treated with vehicle (empty bar) or R406 (red) for 24 h and analyzed thereafter for cell surface CXCR4 expression by flow cytometry. A) CXCR4 expression by flow cytometry. B) Fold change in CXCR4 expression (y-axis, mean fluorescence intensity [MFI], from A) in DHL4 cells (normal control [NC] or shFOXO1 depletion [hairpin #1 or #2]). Fold change for R406/vehicle indicated below. Results are from one of two independent experiments with comparable fold changes in R406/vehicle-treated cells.