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Brief Report

LYMPHOID NEOPLASIA

The double-hit signature identifies double-hit diffuse large B-cell lymphoma with genetic events cryptic to FISH

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KEY POINTS

- **Gene expression identifies DHITsig$^+$ DLBCL tumors with *BCL2* and *MYC* translocations detectable by whole-genome sequencing, but not by breakapart FISH.**
- **Additional genetic mechanisms of MYC dysregulation include focal *MYC* and *MIR17HG* copy-number gains and *PVT1* promoter deletions.**

High-grade B-cell lymphomas with *MYC* and *BCL2* and/or *BCL6* rearrangements (HGBL-DH/THs) include a group of diffuse large B-cell lymphomas (DLBCLs) with inferior outcomes after standard chemoimmunotherapy. We recently described a gene expression signature that identifies 27% of germinal center B-cell DLBCLs (GCB-DLBCLs) as having a double-hit–like expression pattern (DHITsig) and inferior outcomes; however, only half of these cases have both *MYC* and *BCL2* translocations identifiable using standard breakapart fluorescence in situ hybridization (FISH). Here, 20 DHITsig$^+$ GCB-DLBCLs apparently lacking *MYC* and/or *BCL2* rearrangements underwent whole-genome sequencing. This revealed 6 tumors with *MYC* or *BCL2* rearrangements that were cryptic to breakapart FISH. Copy-number analysis identified 3 tumors with *MYC* and 6 tumors with *MIR17HG* gains or amplifications, both of which may contribute to dysregulation of *MYC* and its downstream pathways. Focal deletions of the *PVT1* promoter were observed exclusively among DHITsig$^+$ tumors lacking *MYC* translocations; this may also contribute to *MYC* overexpression. These results highlight that FISH fails to identify all HGBL-DH/THs, while revealing a range of other genetic mechanisms potentially underlying *MYC* dysregulation in DHITsig$^+$ DLBCL, suggesting that gene expression profiling is more sensitive for identifying the biology underlying poor outcomes in GCB-DLBCL. (*Blood*. 2019;134(18):1528-1532)

Introduction

Diffuse large B-cell lymphomas (DLBCLs) are a genetically heterogeneous group of neoplasms with variable outcomes after standard chemoimmunotherapy (eg, R-CHOP [rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone]). There is an ongoing interest in delineating subgroups within DLBCL that share targetable biology, with the potential for precision medicine to overcome biology currently associated with treatment failure. Among the subgroups with the worst outcomes, high-grade B-cell lymphomas with *MYC* and *BCL2* (and sometimes *BCL6*) rearrangements (HGBL-DH/TH-*BCL2*s) mostly belong to the germinal center B-cell (GCB) molecular subgroup (the subgroup generally considered to have favorable prognosis).[1,2] We recently defined a gene expression signature that identifies nearly one-third of GCB-DLBCL tumors as having a double-hit–like gene expression pattern (DHITsig), with only half harboring rearrangements of both *MYC* and *BCL2* as determined by breakapart fluorescence in situ hybridization (FISH).[3] In that cohort, the outcomes for the 20 non–HGBL-DH/TH-*BCL2* patients were comparable to those of patients with HGBL-DH/TH-*BCL2*.

In contrast to Burkitt lymphoma (BL), where the partner of *MYC* is universally an immunoglobulin gene, in approximately half of HGBL-DH/THs with DLBCL morphology, the partner is nonimmunoglobulin.[4] For this reason, a breakapart FISH strategy is favored for detecting *MYC* rearrangements in DLBCL rather than *MYC*/*IGH* dual fusion. Breakapart FISH purportedly fails to detect 2% to 4% of *MYC* rearrangements when the false-negative cases are identified using an *MYC*/*IGH* dual-fusion approach.[5-7] Recently, a BL tumor was described where the coding exons of *MYC* (8.6 kb) were inserted into the *IGH* locus, an event cryptic to both FISH approaches.[8] This raises the possibility that a proportion of DLBCLs harbor *MYC* and/or *BCL2* rearrangements that are cryptic to FISH.

We hypothesized that genetic dysregulations of *MYC* and *BCL2* and their downstream signaling pathways are among the fundamental genetic events underlying the DHITsig in most, if not all, DHITsig$^+$ DLBCL tumors. Using whole-genome sequencing (WGS) of 20 tumors lacking *MYC* and/or *BCL2* rearrangements by FISH, we identified 6 cases with rearrangements cryptic to

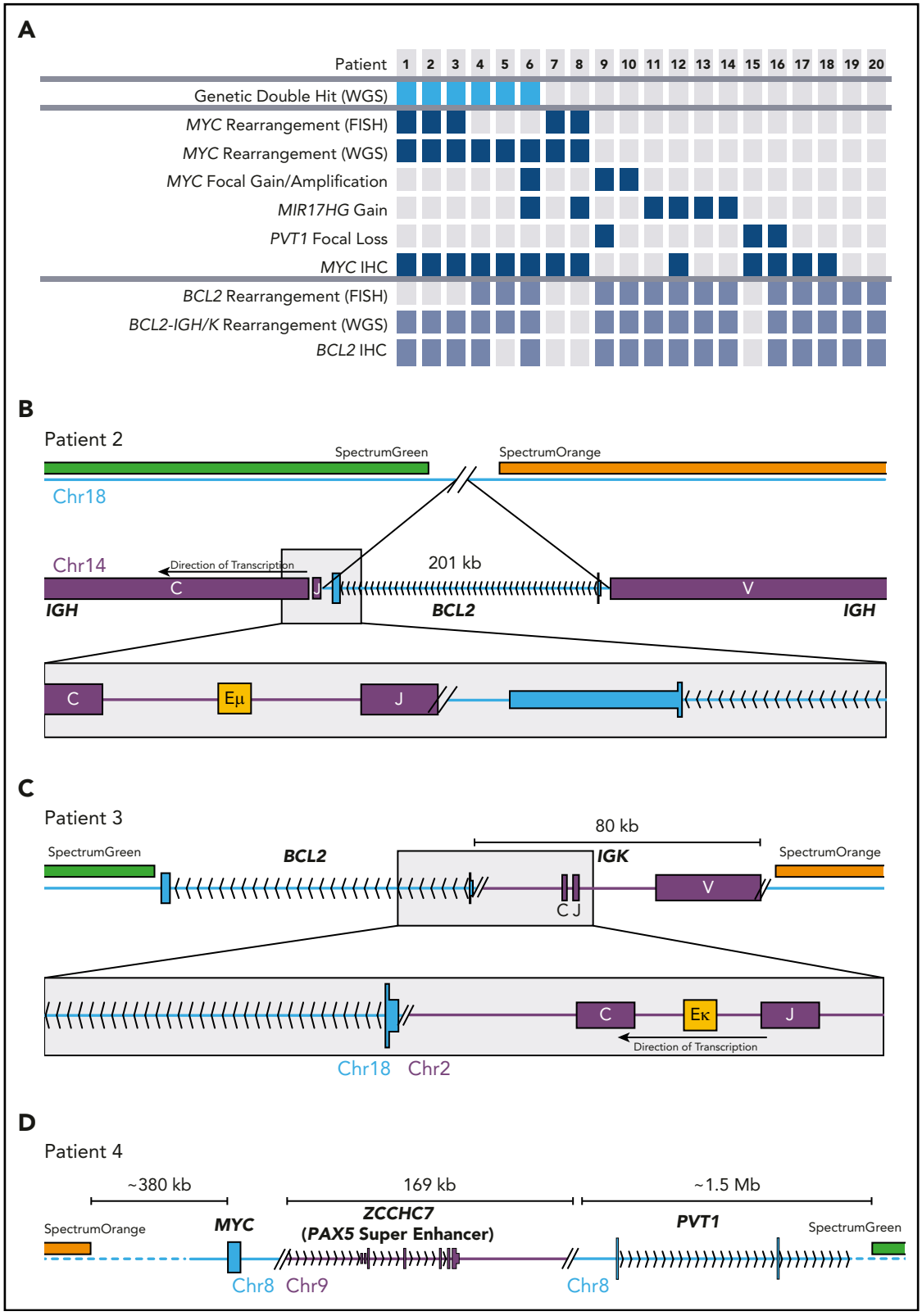

Figure 1. FISH-cryptic *MYC* and *BCL2* rearrangements identified by WGS in DHITsig⁺ DLBCL tumors. (A) Diagram summarizing the occurrence of each genetic event identified in the 20 non–HGBL-DH/TH-*BCL2* DHITsig⁺ genomes. The thresholds for MYC and BCL2 immunohistochemistry (IHC) positivity were 40% and 50%, respectively. (B-C) Cryptic *BCL2* translocations identified in tumors that were negative for *BCL2* rearrangement by breakapart FISH. (D) A cryptic *MYC* rearrangement identified in a tumor that was negative for *MYC* rearrangement by breakapart FISH.

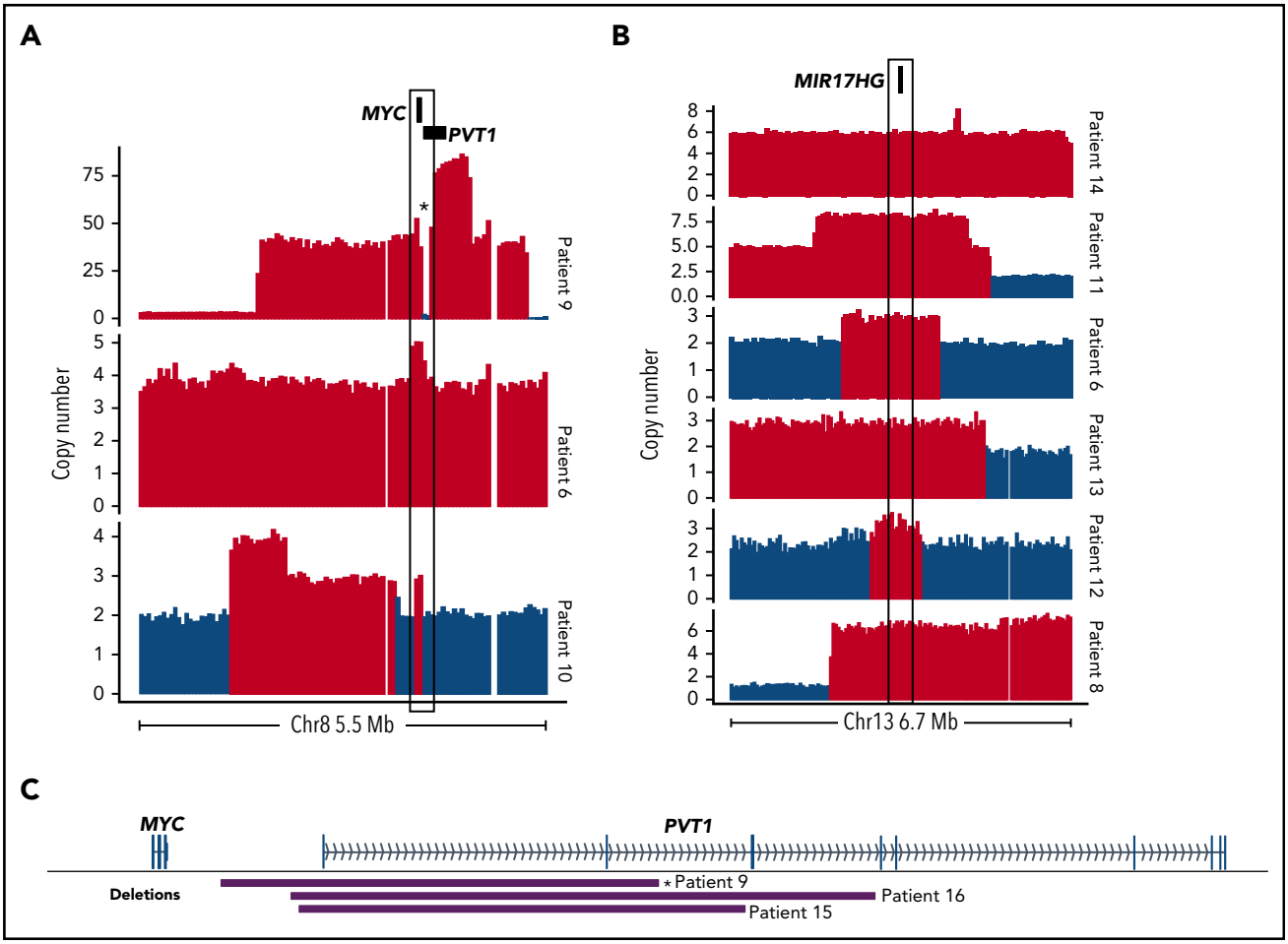

Figure 2. Somatic CNVs affecting *MYC*, *MIR17HG*, and *PVT1* identified by WGS among DHITsig$^+$ DLBCL tumors. Focal copy-number gains of *MYC* (A) or *MIR17HG* (B) identified among DHITsig$^+$ DLBCL tumors. Red bars indicate regions where Control-FREEC identified a significant increase in copy number ($P < .05$). Compared with 162 GCB tumors without a *MIR17HG* amplification as determined by single-nucleotide polymorphism (SNP) arrays, expression of *MIR17HG* was significantly elevated among these 6 cases with *MIR17HG* copy gains (log2 fold change, 0.85; Wilcoxon $P = .032$). (C) The boundaries of focal *PVT1* transcription start site (TSS) deletions identified in DHITsig$^+$ tumors. *Indicates the *PVT1* deletion identified in the double-minute chromosome.

breakapart FISH, along with other genetic mechanisms of dysregulation of *MYC* and downstream pathways.

Methods

DNA was extracted from the 20 fresh-frozen GCB DHITsig$^+$ tumors lacking *MYC* and/or *BCL2* translocations (breakapart FISH) identified by Ennishi et al.[3] Polymerase chain reaction–free libraries were constructed from sheared DNA that was end repaired and A-tailed using the NEBNext Ultra II DNA library prep kit for Illumina (New England Biolabs) before the ligation of dual-indexed TruSeq adapters and cleanup using 0.8X AMPure XP beads (Beckman-Coulter). Libraries were sequenced to an average depth of ×60 on an Illumina HiSeqX system. Reads were aligned using BWA-MEM (version 0.7.6a).[9] Structural variants were identified using Manta.[10] Copy-number variants (CNVs) were identified with Control-FREEC.[11] Copy-number data were obtained for the discovery cohort using Affymetrix SNP6.0 arrays as previously described.[1,12] All statistical tests were performed in R software (version 3.5.1). This study was reviewed and approved by the University of British Columbia–BC Cancer Research Ethics Board in accordance with the Declaration of Helsinki.

Results and discussion

FISH-cryptic rearrangements of BCL2 and MYC

All FISH-detected *BCL2* and *MYC* rearrangements were detected by WGS structural variant calling, confirming the sensitivity of WGS to these events (Figure 1A; supplemental Table 1, available on the *Blood* Web site). Additional *BCL2* and *MYC* rearrangements were identified in the genomes of 3 cases each. Figure 1B describes 1 of 2 tumors in which the *BCL2* gene was inserted into the *IGH* locus, placing *BCL2* in close proximity to the Eμ enhancer. The breakpoints in *IGH* and near the 3′ untranslated region of *BCL2* were consistent with typical *BCL2-IGH* translocations.[13] Importantly, the removal of *BCL2* from chromosome 18 left the regions targeted by breakapart FISH probes adjacent (supplemental Figure 1). In another case, 80 kb of the *IGK* locus, including the Eκ enhancer, was inserted telomeric to *BCL2* (Figure 1C). Two tumors harbored cryptic enhancer insertions near *MYC* (eg, 170 kb of the *ZCCHC7* locus including the *PAX5* super-enhancer inserted telomeric to *MYC*; Figure 1D). None of these enhancer insertions were large enough to separate the breakapart probes so as to be detectable by FISH (supplemental Figure 1).

Thus, 6 apparently non–HGBL-DH/TH tumors were confirmed to be HGBL-DH/TH through the use of WGS. All of the cases with cryptic *BCL2* or *MYC* rearrangements were positive for BCL2 or MYC protein by immunohistochemistry, respectively (Figure 1A). Cryptic *MYC* translocations exclusively involved a nonimmunoglobulin partner, whereas *BCL2* rearrangements all involved immunoglobulin partner loci. In 2 tumors, the *MYC* locus was translocated adjacent to the *BCL6* superenhancer (supplemental Table 1), an event cryptic to both *MYC* and *BCL6* FISH. No other cryptic *BCL6* rearrangements were detected in this patient group. Considering these findings, breakapart FISH failed to detect a clinically significant proportion of HGBL-DH/THs, with cryptic HGBL-DH/TH-*BCL2* tumors representing 19% (6 of 31) of all HGBL-DH/TH-*BCL2* tumors in the original DHITsig discovery cohort.

CNVs enriched in DHITsig$^+$ tumors

Although focal *MYC* gains were not exclusive to DHITsig$^+$ tumors, several of the genomes had notable CNVs affecting *MYC* and/or pathways downstream of *MYC*, including 2 focal *MYC* gains and 1 case of double minute (Figure 2A). *MIR17HG* encodes the miR-17∼92 microRNA cluster, which is a direct target of MYC.[14,15] Copy gains affecting *MIR17HG* were identified in 6 of 20 DHITsig$^+$ cases (Figure 2B) and may represent an avenue for dysregulation of *MYC* and its downstream targets in these cases.

A comparison of SNP array copy-number profiles from 179 GCB-DLBCLs, including the 20 cases described here, revealed that amplifications (copy number >3) of *MIR17HG* and *FCGR2B* and deletions of *CDKN2A* and 22q11.22 (*IGL*) were significantly enriched among DHITsig$^+$ tumors (supplemental Figure 2). *FCGR2B* amplification is associated with rituximab resistance and may therefore contribute to treatment resistance among DHITsig$^+$ tumors.[12,16] Deletions of *CDKN2A* lead to decreased TP53 protein[17] and represent an additional mechanism of TP53 inactivation in DHITsig$^+$ tumors, along with the frequent *TP53* mutations described previously.[3] The 22q11.22 deletion breakpoints observed by WGS were consistent with *IGL* VJ recombination.

A single genome harbored the 11q aberration pattern, which has been described among *MYC*$^-$ BLs.[18] The SNP array copy-number data identified 6 cases with the 11q aberration: 1 each in GCB DHITsig$^+$ cases with or without genetic double hit, and 4 in GCB DHITsig$^-$ tumors lacking *MYC* or *BCL2* rearrangements. Four 11q aberrations were confirmed by FISH (supplemental Figure 3). These results suggest that the 11q aberration is not associated with the DHITsig in DLBCL.

Among the genomes, there were 3 instances of focal promoter deletions of *PVT1*, including 1 in the double-minute chromosome (Figure 2C). The SNP array copy-number data did not identify any additional cases with this deletion. The *PVT1* long noncoding RNA gene contains intragenic enhancers that drive expression of *PVT1*, but in the absence of an active *PVT1* promoter they instead drive expression of *MYC*.[19] Although the number of occurrences limits our power to determine whether *PVT1* deletions are associated with DHITsig positivity, these results suggest that this is a rare, albeit recurrent, event that could promote *MYC* expression in DHITsig$^+$ tumors.

In summary, genetic events affecting both *MYC* and *BCL2* were identified in 13 of 20 DHITsig$^+$ tumors that were deemed non–HGBL-DH/THs by breakapart FISH. The identification of FISH-cryptic *MYC* and *BCL2* rearrangements in 6 cases highlights the limitation of FISH in identifying HGBL-DH/TH. The DHITsig phenotype can thus be attributed to a range of genetic events affecting *MYC*, *BCL2*, and downstream pathways, some of which are not revealed even by WGS, demonstrating that gene expression profiling is a more appropriate method to identify this biological group that constitutes high-risk GCB-DLBCL.

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Authorship

Contribution: L.K.H., B.M.G., J.T., M.A., C.K.R., A.J., and R.D.M. analyzed and interpreted the data; L.K.H. generated all figures; M.B., B.M., and S.B.-N. processed the tissues and performed FISH assays; and L.K.H., D.W.S., and R.D.M. conceived the study and wrote the manuscript.

Conflict-of-interest disclosure: A.J., D.W.S. and R.D.M. are named inventors on a patent application pertaining to the DHITsig. The remaining authors declare no competing financial interests.

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Footnotes

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