

IGH rearrangement in myeloid neoplasms

Though immunoglobulin genes are typically expressed in B lymphocytes, recent studies found ectopic immunoglobulin expression in non-B-cell tumor cells including acute myeloid leukemia (AML). The immunoglobulin genes, including immunoglobulin heavy chain genes (*IGH*), light kappa (κ) chain genes (*IGK*) and light lambda (λ) chain genes (*IGL*), are frequently rearranged in B-cell tumors. These rearrangements result in a juxtaposition of IG enhancers to the vicinity of onco-

genes such as *MYC* and *BCL2*, leading to their overexpression and activation. Here, we found two *IGH* rearrangements in myeloid tumors, including an *IGH-MECOM* in a myelodysplastic syndrome (MDS) and an *IGH-CCNG1* in an AML. Our studies provide the first evidence that a once believed B-cell tumor-specific oncogenic mechanism is also present in myeloid tumors.

Case #1: A 60-year-old female with a history of breast cancer presented with fatigue, bilateral flank pain, and hematuria. Complete blood count showed $24.82 \times 10^9/L$ of white blood cells, 11.2 g/dL of hemoglobin, 33.9% of hematocrit, and $68 \times 10^9/L$ of platelets. The bone marrow

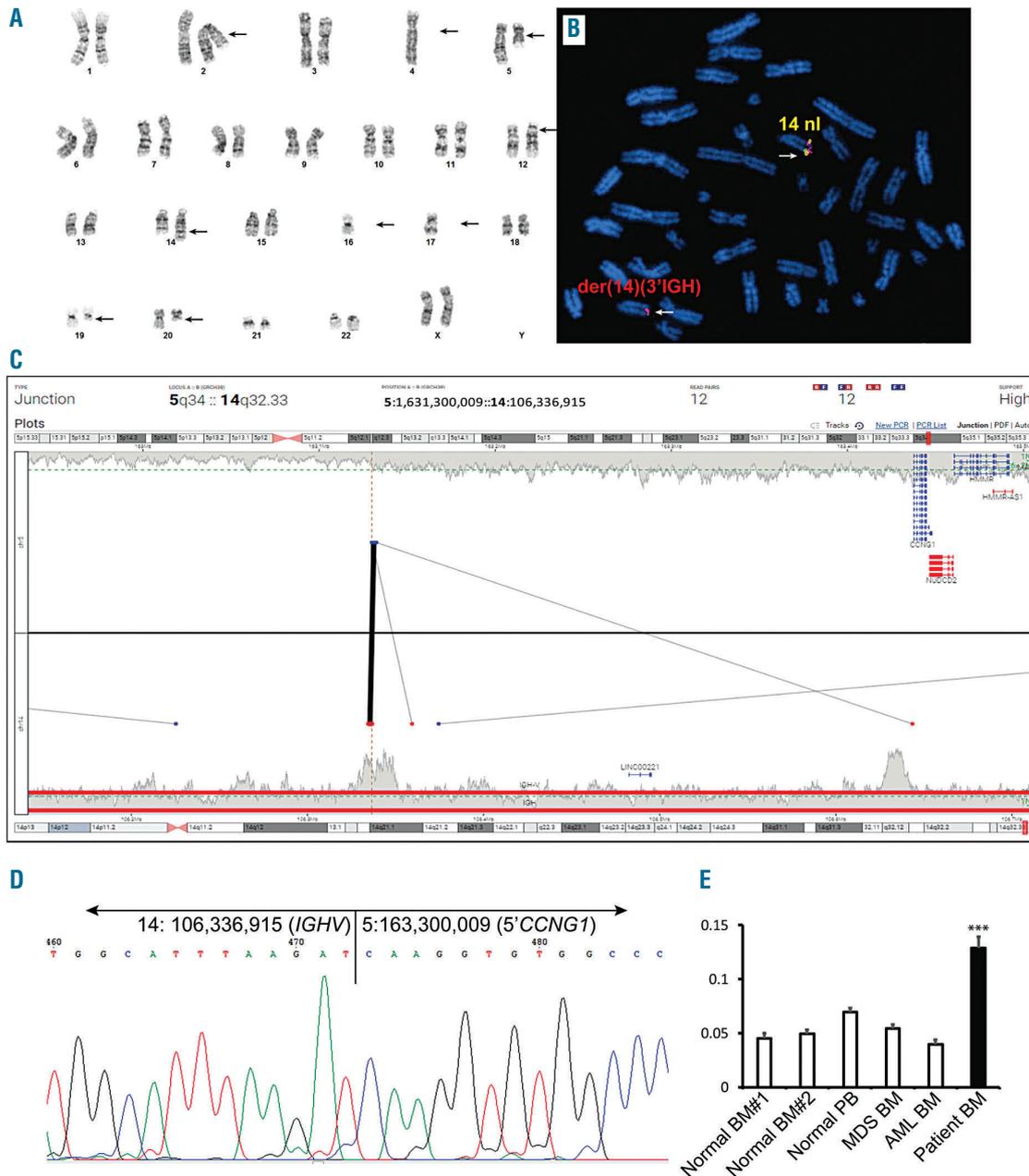


Figure 1. Acute myeloid leukemia with an *IGH-CCNG1* rearrangement. (A) Chromosome analysis of the bone marrow aspirate showed a complex karyotype, including an unbalanced translocation involving chromosomes 5, 14 and 19. Arrows indicate clonal aberrations. (B) Fluorescence *in situ* hybridization (FISH) on abnormal metaphase showed the 3'*IGH* (red) remaining on the derivative chromosome 14 and the 5'*IGH* lost, consistent with an unbalanced *IGH* rearrangement. (C) A mate-pair next-generation sequencing (NGS) found an *IGH-CCNG1* rearrangement (3'*IGH-V3*::5'*CCNG1*). (D) *IGH-CCNG1* breakpoints were confirmed by PCR followed by Sanger sequencing. (E) The *CCNG1* expression level was evaluated by a real-time quantitative RT-PCR with the patient's bone marrow, a normal blood sample, two normal bone marrow samples, a myelodysplastic syndrome (MDS) with a normal karyotype and an acute myeloid leukemia (AML) with a complex karyotype without 14q (*IGH*) rearrangement. *CCNG1* expression was normalized to *ACTB*. ***: $P < 0.001$.

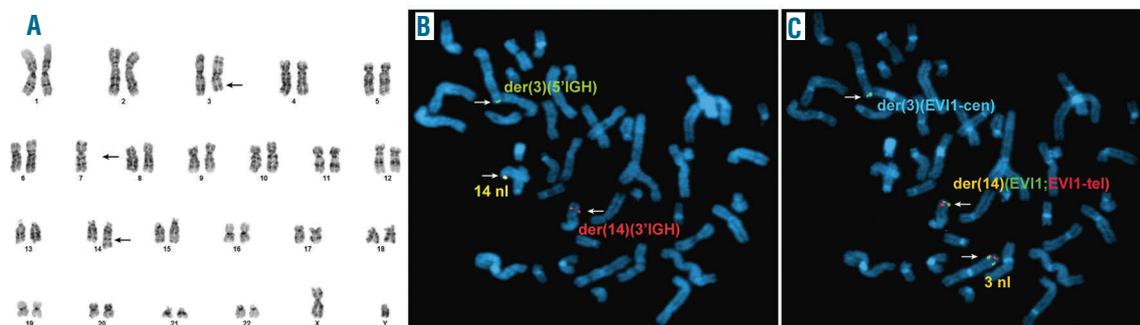


Figure 2. Myelodysplastic syndrome with an *IGH-MECOM* rearrangement. (A) Chromosome analysis of the bone marrow aspirate showed monosomy 7 and a balanced translocation t(3;14). Arrows indicate clonal aberrations. (B) Fluorescence *in situ* hybridization (FISH) on an abnormal metaphase with a *MECOM* (*EVI1*) probe showed the centromeric *MECOM* (aqua) remaining on chromosome 3 and both the *MECOM* (green) and the telomeric *MECOM* (red) translocated to chromosome 14. (C) FISH on the same abnormal metaphase (after probe stripping) with a *IGH* probe showed the 3'*IGH* (red) remaining on chromosome 14 and the 5'*IGH* translocated to chromosome 3.

biopsy was markedly hypercellular with less than 5% fat. Approximately 80-90% of the cellularity was composed of intermediate-sized blasts. Flow cytometric analysis of a peripheral blood specimen demonstrated 23% of immature cells that was positive for CD45(dim), CD34, HLA-DR, the myeloid markers CD13(subset), CD56 (subset), CD33, CD15 (subset), CD11b, CD117 (subset), and CD123, but was negative for other monocytic, B- and T-lymphoid markers, consistent with myeloblasts. A targeted next-generation sequencing (NGS) gene panel for leukemia showed *DNMT3A* c.1322delG p.V441fs* (VAF, 55.5%), *TET2* c.5618T>C p.I1873T (85.4%), and *TP53* c.814G>A p.V272M (85.1%), all common mutations in AML. Bone marrow cytogenetic analysis showed a complex karyotype with an unbalanced three way translocation involving chromosomes 5, 14 and 19, among other changes (43,XX,add(2)(p23),-4,der(5)t(5;14;19)(q34;q32;q13.2)del(5)(q14q34),i(11)(q10),der(12)del(12)(p11.2)dup(12)(q14q23),der(14)t(5;14;19),-16,-17, der(19)t(5;14;19),del(20)(q11.2)[cp20] (Figure 1A). Because the translocation t(5;14;19) involved 14q32, the *IGH* locus, fluorescence *in situ* hybridization (FISH) was performed with an *IGH* split-apart probe. While the 3'*IGH* remained on the derivative chromosome 14, the 5'*IGH* was lost, consistent with an unbalanced *IGH* rearrangement (Figure 1B). A mate-pair NGS¹ showed that the chromosome 14 breakpoint was within *IGH* at the *IGHV3-30*, which fused to a chromosome 5 fragment approximately 308 Kb upstream of the 5' *CCNG1* gene (NM_004060). The fusion led to a juxtaposition of the 3'*IGH*, including the intact intragenic enhancer (E_{μ}) and the 3'-regulatory region (3'-RR), to *CCNG1* on the der(14) (Figure 1C). The *IGH-CCNG1* rearrangement was confirmed by PCR amplification and Sanger sequencing, using primers specific to *IGH* and *CCNG1* (Figure 1D and Online Supplementary Table S1). A real-time quantitative RT-PCR with *CCNG1*-specific primers showed *CCNG1* overexpression in the patient's bone marrow compared to normal blood and bone marrow specimens and myeloid tumors without 14q (*IGH*) rearrangement (Figure 1E and Online Supplementary Table S2). The patient was diagnosed with therapy-related AML. She received a standard 7+3 regimen with no clinical response followed by another 10-day course of Decitabine, which also failed. The patient succumbed to the disease two months later.

Case #2: A 46-year-old male with a history of acute promyelocytic leukemia presented with thrombocytopenia and anemia. The bone marrow biopsy showed a fragmented, moderately hypocellular marrow with 80% fat.

Erythroid elements were moderately proportionally decreased and exhibited maturation. Myeloid elements were moderately proportionally increased, exhibiting left-shifted maturation and including increased numbers of early and eosinophilic forms. Blasts comprised up to 10% of the marrow cellularity, as confirmed by immunoperoxidase study for CD34. A differential count of the bone marrow aspirate found 10% of blasts and dysplastic myeloid elements. Flow cytometry of the bone marrow showed that the immature cells were positive for CD45(dim), CD34, HLA-DR, myeloid markers CD13, CD33, CD117, and 11B(subset), but that the bone marrow was negative for other monocytic, B- and T-lymphoid markers, consistent with myeloblasts. Bone marrow cytogenetics showed monosomy 7 and a balanced translocation between chromosomes 3 and 14 (45,XY,-7,t(3;14)(q26.2;q32)[19]/46,XY[1]) (Figure 2A). FISH assays on abnormal metaphases with a *MECOM* triple-colored probe confirmed a *MECOM* rearrangement, with part of the probe, including the intact *MECOM*, translocated to chromosome 14 (Figure 2B). The cells were then hybridized to an *IGH* split-apart probe, which showed an *IGH* rearrangement, with 3'*IGH* remaining on the chromosome 14 and the 5'*IGH* translocated to chromosome 3 (Figure 2C). Together these FISH results are consistent with an *IGH-MECOM* fusion. Unfortunately, no materials were available for mate-pair NGS or RT-PCR for further characterization of the rearrangement. The patient was diagnosed with therapy-related MDS, best regarded as refractory anemia with excess blasts-2. After four cycles of decitabine, his blast count was <5% and he underwent a stem cell transplant (SCT) with his brother as the donor. He is now six years after SCT and healthy.

We presented here two cases of therapy-related myeloid tumors with *IGH* rearrangements, which have never been reported before. *IGH* rearrangement is one of the most common findings in mature B-cell tumors, which leads to a juxtaposition of the potent *IGH* enhancers nearby otherwise silent oncogenes, leading to their expression and activation. More than 40 genes are fused with *IGH*, with the common ones being *MYC*, *BCL2* and *BCL6*, *CCND1*, *FGFR3*, as seen in Burkitt lymphoma, follicular lymphoma, mantle cell lymphoma, and multiple myeloma, respectively. These rearrangements are clinically important for the diagnosis and classification of these B-cell tumors. In B lymphocytes, *IGH* enhancers interact with *IGH* promoters to regulate a tissue-specific and temporally regulated expression.² While the intragenic E_{μ} enhancer controls the V(D)J recombination, the 3'RR controls class switch

recombination (CSR) and somatic hypermutation (SHM). Early studies showed an unexpected V(D)J rearrangement in AML (not to be confused with the *IGH* rearrangement leading to gene fusion seen in our cases),³ suggesting that these non-B-cell tumors somehow activate the complex *IGH* recombination and maturation process. *IGH* expression has subsequently been detected in several types of non-B-cell tumors, including breast cancer, colon cancer, lung cancer, hepatoma, cervical cancer and AML.⁴ In AML, the V(D)J recombination and the SHM of V regions were found in myeloblasts, but not in monocytes or neutrophils from healthy controls or patients with non-hematopoietic neoplasms.⁵ How these non-B-tumor cells activate an otherwise cell-specific *IGH* program is unknown. Several attributes of tumors may contribute to ectopic *IGH* expression. (1) Global changes in the epigenetic landscape are a hallmark of cancer, which may lead to local chromatin accessibility of the *IGH* locus. (2) Deregulation of the *IGH* enhancer-binding transcription factors is frequent in tumors, which may lead to ectopic activation of *IGH* enhancers. Ets proteins and Ikros, both major regulators of B-cell development, are deregulated in myeloid tumors.⁶ OCT-2 is a co-activator of *IGH* enhancer E μ and is expressed in AML with the t(8;21),⁷ and (3) The Epstein-Barr virus (EBV) infection can be seen in various tumors including AML.⁸ The EBV protein LMP1 increased the enhancer activity of IG κ light chain by activating the NF- κ B and AP-1 pathways in nasopharyngeal carcinoma cells.⁹ Similar activation mechanisms may also be present for the *IGH* locus.

IGH is fused with two well-known oncogenes including *MECOM* and *CCNG1* in myeloid tumors. *MECOM* is often rearranged in MDS and AML. The classic inv(3q)/t(3;3) bring the GATA2 distal hematopoietic enhancer (*G2DHE*) to the vicinity of *MECOM*, leading to its overexpression.¹⁰ *MECOM* is a transcription factor that, when overexpressed, induces upregulation of cell cycle and blocks granulocytic differentiation of hematopoietic stem cells.¹¹ *CCNG1* is a non-canonical cyclin involved in the regulation of both Tp53 and cMYC. *CCNG1* binds to Ser/Thr protein phosphatase 2A (PP2A), which dephosphorylates and activates mouse double minute 2 (Mdm2). Activated Mdm2 serves as an E3 ubiquitin ligase of Tp53, leading to its proteasome degradation (cyclin G1/Mdm2/Tp53 axis).¹² *CCNG1* also binds to CDK2 or CDK5, which phosphorylates and activates cMYC (cyclin G1/CDk2/c-Myc axis).¹³ Because our case with *IGH-CCNG1* rearrangement had monosomy 17 and a *TP53* mutation, it is likely that the cyclin G1/CDk2/c-Myc axis, instead of cyclin G1/Mdm2/Tp53 axis, plays a role in its tumorigenesis. *CCNG1* overexpression or amplification is found in many tumors.¹⁴ Clinical trials with a dominant-negative *CCNG1* retroviral expression (DeltaRex-G) showed impressive results, including several metastatic tumors being cancer-free ten years after DeltaRex-G monotherapy.¹⁵ *TP53* loss or mutation is relatively rare in myeloid tumors, suggesting an intact cyclin G1/Mdm2/p53 axis. *MYC* is located on chromosome 8. Trisomy 8 is one of the most common findings in myeloid tumors, which leads to *MYC* copy number gain. In addition, double minutes or homogeneously staining regions containing *MYC* amplification are often seen in AML. These studies are consistent with an active *MYC* signaling. It will be interesting to evaluate how frequent the *CCNG1* is overexpressed in myeloid tumors, and whether an anti-*CCNG1* strategy such as DeltaRex-G is effective in treating

myeloid tumors, a group of diseases typically with very poor prognosis.

In summary, we find that the *IGH* rearrangement can happen in myeloid tumors. Similar to those in B-cell tumors, *IGH* rearrangements lead to the juxtaposition of the *IGH* enhancers to the vicinity of oncogenes, leading to their overexpression.

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doi:10.3324/haematol.2020.246744

Information on authorship, contributions, and financial & other disclosures was provided by the authors and is available with the online version of this article at www.haematologica.org.

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