"Rearrangement of NOTCH1 or BCL3 can independently trigger progression of CLL"

De Keersmaecker, Kim ; Michaux, Lucienne ; Bosly, André ; Graux, Carlos ; Ferreiro, Julio Finalet ; Vandenberghe, Peter ; Cools, Jan ; Wlodarska, Iwona

Document type: Article de périodique (Journal article)

Référence bibliographique

De Keersmaecker, Kim ; Michaux, Lucienne ; Bosly, André ; Graux, Carlos ; Ferreiro, Julio Finalet ; et. al. Rearrangement of NOTCH1 or BCL3 can independently trigger progression of CLL. In: Blood, Vol. 119, no. 16, p. 3864-3866 (2012)

DOI : 10.1182/blood-2011-10-388124
Rearrangement of NOTCH1 or BCL3 can independently trigger progression of CLL

Kim De Keersmaecker, Lucienne Michaux, André Bosly, Carlos Graux, Julio Finalet Ferreiro, Peter Vandenberghe, Jan Cools and Iwona Wlodarska
To the editor:

Rearrangement of NOTCH1 or BCL3 can independently trigger progression of CLL

Recent data indicate that NOTCH1 mutations significantly increase the risk of CLL progression toward Richter syndrome (RS) and chemoresistance,1,2 and that activation of NOTCH1 at time of CLL diagnosis is an independent prognostic factor of poor survival.1,3 We report here a case of CLL with a novel rearrangement of NOTCH1 identified at the time of RS. The patient, a 58-year-old male, was diagnosed with CLL (unmutated VH) in RS in June 2003. Cytogenetic analysis and FISH on peripheral blood (PBL), bone marrow (BM), and lymph node (LN) cells showed 2 related clones: one with an isolated +12 and a second with +12 and dic(9;14)(q34;q32) (supplemental Table 1, available on the Blood Web site; see the Supplemental Materials link at the top of the online article). FISH analysis of dic(9;14)(q34;q32) indicated that this aberration resulted in juxtaposition of 3'IGH and 5'NOTCH1, as evidenced by the loss of sequences telomeric to the breakpoints (Figure 1A-D). These imbalances were confirmed by array CGH (data not shown). The targeting of NOTCH1 by dic(9;14) was evidenced by qRT-PCR analysis, which showed a 10-fold up-regulation of NOTCH1 mRNA (Figure 1E) and a low expression of the neighboring genes (GPM11, CARD9, DNL2). Immunoblotting of a cell lysate from LN with a NOTCH1 antibody recognizing active, cleaved NOTCH1 (Val1744) identified a band corresponding to activated intracellular NOTCH1 (Figure 1F), suggesting an additional truncating mutation. Indeed, sequence analysis identified a 2 bp frame deletion, ΔCT7544–7545/P2515fs. Despite treatment, 2 clones harboring BCR (B-BCR) or BCR + CpG (B-BCR/CpG) for 3 days and expression of surface markers (% positive cells) was analyzed (n = 3 experiments). The statistical significance as determined by 2-tailed paired Student ttest is indicated (*P < .05 vs Ctrl; **P < .05 vs DC + LPS, and ***P < .05 vs B-BCR).
samples (supplemental Table 1). Four months later, the patient developed peripheral T-cell lymphoma, a rare recurrent event in CLL.\(^7\) Altogether, the present case allows us to deduce the sequence of multiple genetic defects driving development and progression of CLL. An initial clone with \(+/H11001\) likely present at a presymptomatic CLL phase, later acquired an activating mutation of \(\text{NOTCH1}\). Subsequent acquisition of \(\text{dic}(9;14)(q34;q32)\) with \(\text{NOTCH1}\)-mutated clone underwent another hit, \(\text{t}(14;19)/(\text{IGH}-BCL3)\), which initiated the second progression that was followed by a fatal CLL-unrelated T-cell lymphoma. Our findings confirm the risk of activating mutations of \(\text{NOTCH1}\) in Richter transformation of CLL,\(^1,2\) particularly CLL with \(+/H11001\) and highlight that a residual chemotherapy-

**Figure 1. Genetic and molecular analysis of \(\text{NOTCH1}\) aberrations.** (A) Graphic representation of \(\text{dic}(9;14)(q34;q32)\) with indicated probes applied for FISH mapping of both breakpoints and their hybridization pattern. (B-D) Examples of FISH analysis performed on a diagnostic LN sample with (B) LSI IGH dual color break apart probe and CEP12 (green), (C) RP11-70703 (green) and RT11-769N4 (red), (D) WI2-569D3 (G248P90019F4, green) and WI2-1851N4 (G248P9957B2, red). BAC and fosmid clones were selected using the UCSC Genome Browser on Human May 2004 (NCBI35/hg17) Assembly. Note in panel B the presence of the \(3'\)/IGH/reed signal on \(\text{dic}(9;14)\) and loss of the distal /IGH/ green signal in cell with trisomy 12, in panel C hybridization of RP11-70703 (green) with \(\text{dic}(9;14)\) and loss of the distal RT11-769N4 (red) sequences, and in panel D hybridization of both fosmids covering \(\text{NOTCH1}\) with \(\text{dic}(9;14)\). (E) qRT-PCR analysis of \(\text{NOTCH1}\) mRNA expression levels. The patient was analyzed at 2 different timepoints, at diagnosis (05/2003; PBL; 57% of cells with \(\text{dic}(9;14)\)) and during disease evolution (06/2011; PBL; \(\text{dic}(9;14)\)-negative), and relative \(\text{NOTCH1}\) expression levels were compared with 2 control CLL samples (CLL1 and CLL2) with unmutated VH and trisomy12. (F) Western blot analysis of protein extract of diagnostic LN cells of the index case (15% of cells with \(\text{dic}(9;14)\)) and 2 control CLLs (unmutated VH, trisomy12); CLL3 with unmutated \(\text{NOTCH1}\) and CLL4 positive for \(\Delta\text{CIT}7544-7545/P2515fs\). The top panel shows detection of active, cleaved \(\text{NOTCH1}\) (Val 1744 antibody; Cell Signaling Technology) in CLL4 and the index case, but not in CLL3 cells. The bottom panel shows expression of \(\text{NOTCH1}\) with a general anti-\(\text{NOTCH1}\) antibody (c-20 Santa Cruz Biotechnology). Because of a low content of cells with \(\text{dic}(9;14)\) in the only available LN sample, overexpression of an activated form of \(\text{NOTCH1}\) could not be evidenced. (G) Sanger chromatogram illustrating a heterozygous \(\Delta\text{CIT}7544-7545/P2515fs\) of the \(\text{NOTCH1}\) cDNA in the diagnostic BM sample.
resistant NOTCH1-mutated clone is at risk of acquiring further progression-associated hits. Besides the known t(14;19)(q32; q13)/IGH-BCL3,8-10 we also identified dic(9;14)(q34;q32)/IGH- NOTCH1, which so far has not been reported in B-cell leukemia/lymphoma, as a novel genomic aberration capable of triggering RS.

Kim De Keersmaecker
Center for Human Genetics, KU Leuven,
Center for the Biology of Disease, Vlaams Instituut voor Biotechnologie,
Leuven, Belgium

Lucienne Michaux
Center for Human Genetics, KU Leuven,
Leuven, Belgium

André Bosly
Mont-Godinne University Hospital,
Yvoir, Belgium

Carlos Graux
Mont-Godinne University Hospital,
Yvoir, Belgium

Julio Finalet Ferreiro
Center for Human Genetics, KU Leuven,
Leuven, Belgium

Peter Vandenberghe
Center for Human Genetics, KU Leuven,
Leuven, Belgium

Jan Cools
Center for Human Genetics, KU Leuven,
Center for the Biology of Disease, Vlaams Instituut voor Biotechnologie,
Leuven, Belgium

Iwona Wlodarska
Center for Human Genetics, KU Leuven,
Leuven, Belgium

The online version of this article contains a data supplement.

Acknowledgments: This work was supported by the European Research Council (ERC-starting grant to J.C.), KU Leuven (concerted action grant to J.C., P.V., I.W.), and the Stichting Tegen Kanker (grant to P.V.). K.D.K. is a postdoctoral researcher of the Research Foundation-Flanders (FWO) and P.V. is a senior clinical investigator of FWO. The authors thank Ursula Pluys for technical assistance and Rita Logist for editorial help.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Correspondence: Iwona Wlodarska, Center for Human Genetics, KU Leuven, Gasthuisberg, Herestraat 49, Box 602, B-3000 Leuven, Belgium; e-mail: iwona.wlodarska@uzleuven.be.

References

Persistently high quality of life conferred by coexisting congenital deficiency of terminal complement C9 in a paroxysmal nocturnal hemoglobinuria patient

Paroxysmal nocturnal hemoglobinuria (PNH) clone bears a PIGA mutation and fails to express glycosylphosphatidylinositol-linked membrane proteins such as complement-regulatory CD55 and CD59, leading to complement-mediated intravascular hemolysis and thrombosis. The advent of eculizumab, an inhibitor of terminal complement CD59, leading to complement-mediated intravascular hemolysis reactions by generation of C5a and C5b-8. Currently, virtually all PNH cases have normal C9 and C5b-8 levels, which so far has not been reported in B-cell leukemia/lymphoma, as a novel genomic aberration capable of triggering RS.

To the editor:

Persistently high quality of life conferred by coexisting congenital deficiency of terminal complement C9 in a paroxysmal nocturnal hemoglobinuria patient

Paroxysmal nocturnal hemoglobinuria (PNH) clone bears a PIGA mutation and fails to express glycosylphosphatidylinositol-linked membrane proteins such as complement-regulatory CD55 and CD59, leading to complement-mediated intravascular hemolysis and thrombosis. The advent of eculizumab, an inhibitor of terminal complement CD59, leading to complement-mediated intravascular hemolysis reactions by generation of C5a and C5b-8. Currently, virtually all PNH cases have normal C9 and C5b-8 levels, which so far has not been reported in B-cell leukemia/lymphoma, as a novel genomic aberration capable of triggering RS.

To the editor:

Persistently high quality of life conferred by coexisting congenital deficiency of terminal complement C9 in a paroxysmal nocturnal hemoglobinuria patient

Paroxysmal nocturnal hemoglobinuria (PNH) clone bears a PIGA mutation and fails to express glycosylphosphatidylinositol-linked membrane proteins such as complement-regulatory CD55 and CD59, leading to complement-mediated intravascular hemolysis and thrombosis. The advent of eculizumab, an inhibitor of terminal complement CD59, leading to complement-mediated intravascular hemolysis reactions by generation of C5a and C5b-8. Currently, virtually all PNH cases have normal C9 and C5b-8 levels, which so far has not been reported in B-cell leukemia/lymphoma, as a novel genomic aberration capable of triggering RS.

To the editor:

Persistently high quality of life conferred by coexisting congenital deficiency of terminal complement C9 in a paroxysmal nocturnal hemoglobinuria patient

Paroxysmal nocturnal hemoglobinuria (PNH) clone bears a PIGA mutation and fails to express glycosylphosphatidylinositol-linked membrane proteins such as complement-regulatory CD55 and CD59, leading to complement-mediated intravascular hemolysis and thrombosis. The advent of eculizumab, an inhibitor of terminal complement CD59, leading to complement-mediated intravascular hemolysis reactions by generation of C5a and C5b-8. Currently, virtually all PNH cases have normal C9 and C5b-8 levels, which so far has not been reported in B-cell leukemia/lymphoma, as a novel genomic aberration capable of triggering RS.

To the editor:

Persistently high quality of life conferred by coexisting congenital deficiency of terminal complement C9 in a paroxysmal nocturnal hemoglobinuria patient

Paroxysmal nocturnal hemoglobinuria (PNH) clone bears a PIGA mutation and fails to express glycosylphosphatidylinositol-linked membrane proteins such as complement-regulatory CD55 and CD59, leading to complement-mediated intravascular hemolysis and thrombosis. The advent of eculizumab, an inhibitor of terminal complement CD59, leading to complement-mediated intravascular hemolysis reactions by generation of C5a and C5b-8. Currently, virtually all PNH cases have normal C9 and C5b-8 levels, which so far has not been reported in B-cell leukemia/lymphoma, as a novel genomic aberration capable of triggering RS.

To the editor:

Persistently high quality of life conferred by coexisting congenital deficiency of terminal complement C9 in a paroxysmal nocturnal hemoglobinuria patient

Paroxysmal nocturnal hemoglobinuria (PNH) clone bears a PIGA mutation and fails to express glycosylphosphatidylinositol-linked membrane proteins such as complement-regulatory CD55 and CD59, leading to complement-mediated intravascular hemolysis and thrombosis. The advent of eculizumab, an inhibitor of terminal complement CD59, leading to complement-mediated intravascular hemolysis reactions by generation of C5a and C5b-8. Currently, virtually all PNH cases have normal C9 and C5b-8 levels, which so far has not been reported in B-cell leukemia/lymphoma, as a novel genomic aberration capable of triggering RS.