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Genetic analysis of de novo CD5$^+$ diffuse large B-cell lymphomas suggests an origin from a somatically mutated CD5 $^+$ progenitor B cell

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Brief report

Genetic analysis of de novo CD5+ diffuse large B-cell lymphomas suggests an origin from a somatically mutated CD5+ progenitor B cell

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CD5+ diffuse large B-cell lymphomas (DLBLs) have recently been described as a particular subgroup of DLBLs. Classical banding and interphase cytogenetic analyses targeting *ATM, TP53,* and *P16INK4a* genes and the *D13S25* locus from 13 CD5+ DLBLs were compared with 55 CD5− DLBLs. Additionally, analysis of somatic mutations of the immunoglobulin heavy chain variable region (IgVH) genes were performed in CD5+ DLBLs. CD5+ DLBLs were somatically mutated (7 of 8 cases) and were negative for t(11;14)(q13;q32) and t(14;18)(q32;q21), whereas t(3;14)(q27;q32) was found in only one tumor. Trisomy 3 and gains on chromosomes 16/16p and 18/18q were significantly overrepresented in CD5+ DLBLs. No *ATM* deletions were detected. The prevalence of deletions at the *D13S25* locus was significantly higher in CD5+ DLBLs (4 of 12 [33%]) compared with CD5− DLBLs (4 of 42 [10%]), as were p*16INK4a* deletions (33% versus 8%). On the basis of these findings, CD5+ DLBLs are likely to arise from the same progenitor cell as the mutated variant of CD5+ lymphocytic lymphoma/B-cell chronic lymphocytic leukemia (B-CLL). (Blood. 2003;101:699-702)

Introduction

Diffuse large B-cell lymphomas (DLBLs) are the most common type of non-Hodgkin lymphoma (NHL).[1] They represent a heterogeneous category with respect to morphology, immunophenotype, cytogenetics, and gene expression profiles.[2-4] Studies identified de novo DLBL with CD5 expression (CD5+ DLBL) as a particular subgroup, suggesting that these tumors may be different from both CD5+ DLBL developing in the setting of small lymphocytic lymphoma/chronic lymphocytic leukemia (Richter syndrome) and CD5− DLBL.[5-9] Their clinical characteristics are elderly onset, female predominance, frequent involvement of extranodal sites, and a poor clinical course.[9] No *BCL1* rearrangements or CyclinD1 expression were demonstrated in those tumors, excluding a possible association with the blastoid variant of mantle-cell lymphoma (MCL).[5,7,10] Moreover, most CD5+ DLBLs have been shown to carry somatic mutations in their immunoglobulin heavy chain variable region (IgVH) genes, further separating them from MCL.[11] Interestingly, in one study, the extent of somatic mutations in de novo CD5+ DLBL was determined to be similar to that of CD5+ (mutated) B-cell chronic lymphocytic leukemia (B-CLL), thus allowing for speculations on the derivation of CD5+ DLBL and CD5+ B-CLL from the same cell of origin, the B-1 lymphocyte.[6]

Until now, only rare genetic data,[5] and especially no cytogenetic data on CD5+ DLBL, have been reported. In this work, we describe classical cytogenetic results in 13 CD5+ DLBLs. Because deletions at the *D13S25* locus and of the *ATM* gene are frequent findings in CD5+ B-CLL,[12] we analyzed these chromosomal regions by interphase cytogenetics (fluorescence in situ hybridization [FISH]) and also performed FISH for *TP53* and *P16INK4a* genes, the inactivation of which has been strongly associated with lymphoma progression.[13] These data were compared with respective findings in 55 nodal CD5− DLBLs.

Study design

Thirteen CD5+ DLBLs were compared with 55 CD5− DLBLs according to their morphologic features, classical and interphase cytogenetic data, and clinical characteristics. In addition, the molecular configuration of the IgVH genes in CD5+ DLBL was determined. Classification of the tumors was performed on high-quality Giemsa-stained slides according to the World Health Organization (WHO) classification.[2]

Cytogenetic investigations were done following established protocols,[14] and metaphases were evaluated according to the International System for Cytogenetic Nomenclature (ISCN) guidelines.[15]

Bicolor interphase FISH for *TP53*, *D13S25*, and *BCL1* (cases 9 and 10) was carried out according to the manufacturer's advice (VYSIS, Stuttgart, Germany). The *P16INK4a* deletion status was analyzed by a cosmid contig of approximately 200 kb that had previously been shown to reliably detect genomic deletions of the INK4a cluster region.[16] For deletions affecting *ATM*, biotin-dUTP (deoxyuracil triphosphate)–labeled P1-derived artificial chromosome (PAC) probes specific for the *ATM* gene locus[17] were applied as previously described.[18,19] Signal visualization was accomplished by using a Zeiss Axioskop2 fluorescence microscope (ZEISS, Jena, Germany), and illustrations were made by using the ISIS imaging system (MetaSystems, Altlussheim, Germany).

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Genomic DNA of 8 CD5$^+$ DLBLs was extracted from cryopreserved tissue blocks. VH-DH-JH gene rearrangements were amplified by polymerase chain reaction (PCR) by using family-specific VH forward primers and consensus JH reverse primers according to the modified protocol of Küppers et al[20] and Campbell et al.[21] PCR products were ligated into pPCR-Script Amp SK (+) cloning vector and transformed into Epicurian Coli XL-Gold Kan ultracompetent cells (Stratagene, La Jolla, CA). Usually, 9 clones per case were sequenced bidirectionally and compared with the nucleotide sequence database Entrez Blast (www.ncbi.nlm.nih.gov/blast/). VH-DH-JH rearrangements were analyzed by using DNAPLOT (www.dnaplot.de/).

Results and discussion

On morphology, the series presented here displayed a remarkable heterogeneity. The CD5$^+$ DLBLs consisted of 8 centroblastic and 3 immunoblastic DLBLs. Two tumors (cases 9 and 10) displayed particular features: their neoplastic cells were medium- to large-sized with scant cytoplasm and slightly irregular nuclei, giving the morphologic impression of blastoid MCL (Figure 1). All cases were CD5$^+$ and negative for CD23 and CyclinD1. IgD expression was found in 5 of 13 (39%) CD5$^+$ tumors. In the CD5$^-$ DLBLs, 8 of 39 cases tested were IgD$^+$ (difference not statistically significant). Cases 6 and 12, in addition, stained weakly for CD10. Nuclear expression of BCL6 was demonstrated in more than 10% of cells in 12 of 12 CD5$^+$ DLBLs and in 46 of 54 (85%) CD5$^-$ DLBLs. There was also no statistically significant difference between the groups, if only high BCL6 expression ($>$ 60% of cells) was taken into account (4 of 12 [33%] CD5$^+$ DLBL versus 29 of 54 [54%] CD5$^-$ DLBL).

All 68 patients enrolled in this study were treated by a conventional anthracycline-containing regimen (cyclophosphamide, hydroxydaunomycin, vincristine, and prednisone [CHOP]) with or without radiotherapy. In contrast to recently published clinical data,[9] there was no statistically significant difference in survival between the CD5$^+$ and CD5$^-$ DLBLs.

Analysis of somatic hypermutations of IgVH genes revealed a germ line pattern in one case and somatically mutated or hypermutated IgVH genes in 3 (37%) and 4 (50%) of 8 cases, respectively (Table 1). VH4 gene family usage was demonstrated in 7 of 8 (88%) tumors, and VH4-34 gene usage in 4 of 7 cases. Only case 6 showed ongoing somatic mutations. These findings are in accordance with published data, suggesting that the cell of origin of CD5$^+$ DLBL predominantly is of postgerminal origin.[6,8,11]

On banding analysis, the majority (11 of 13 [85%]) of CD5$^+$ DLBLs exhibited complex karyotypic alterations with 12 structural or numerical aberrations on average. All cases were negative for the t(11;14)(q13;q32) and t(14;18)(q32;q21) chromosome translocations, and t(3;14)(q27;q32) targeting the *BCL6* gene was found in only 1 tumor (case 2). In contrast, CD5$^-$ DLBLs harbored *BCL2* and *BCL6* rearrangements in 9 of 55 (16%) and 4 of 55 cases (7%), respectively. The sole recurrent structural aberration was deletion del(10)(q22q24) in cases 5 and 8. Trisomies for chromosomes 3 (7 of 13 [54%] versus 7 of 55 [13%]), 16/16p (5 of 13 [38%] versus 8 of 55 [15%]), and 18/18q (6 of 13 [46%] versus 7 of 55 [13%]) were significantly more frequent in CD5$^+$ DLBLs than in CD5$^-$ DLBLs ($P < .05$).

FISH analysis failed to reveal *ATM* deletions, being strongly associated with B-CLL[12] and MCL,[17] in both CD5$^+$ and CD5$^-$ DLBLs. In contrast, the prevalence of deletions at the *D13S25* locus was significantly higher in CD5$^+$ DLBLs (4 of 12 [33%]) compared with CD5$^-$ DLBLs (4 of 42 [10%], $P < .05$). Deletions of *TP53* were equally distributed in both groups, whereas p*16^{INK4a}* hemizygous deletions were more frequent in CD5$^+$ tumors (4 of 12 [33%]) than in the CD5$^-$ group (3 of 38 [8%], $P < .05$).

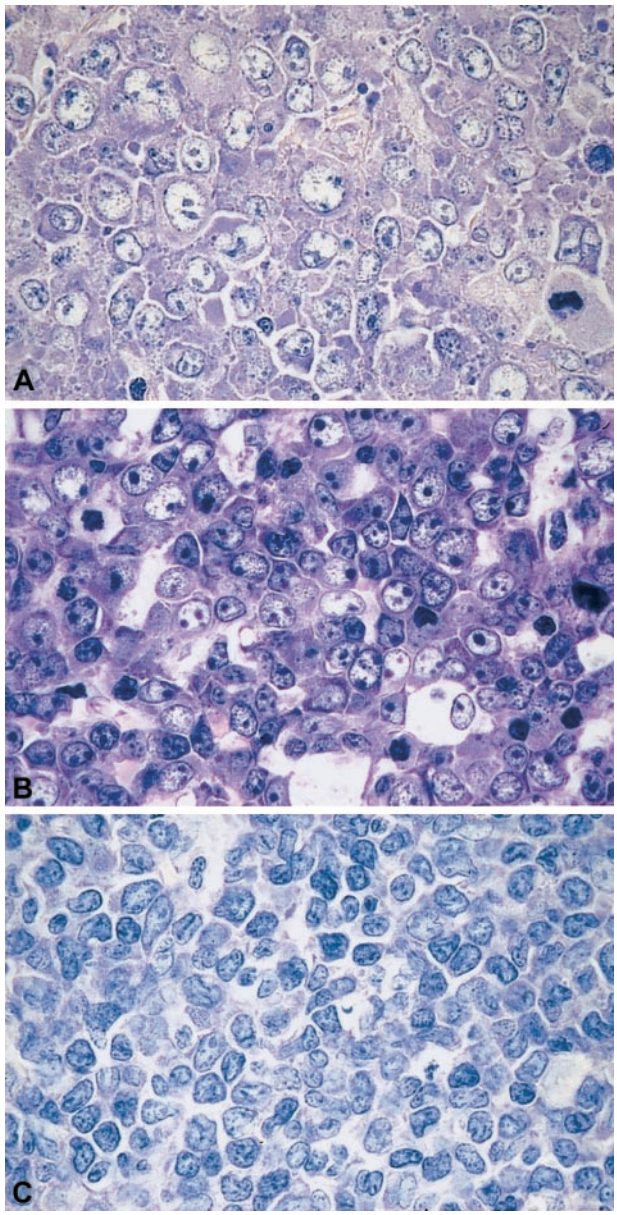

Figure 1. The morphologic spectrum of CD5$^+$ DLBLs. (A) DLBL centroblastic. (B) DLBL immunoblastic. (C) DLBL unclassified. Giemsa stain, original magnifications × 400.

Deletion of *D13S25* is a frequent finding in B-CLL and MCL, but it is rarely found in other malignant lymphomas of B-cell type.[12,22,23] The strong association of *D13S25* deletions with CD5$^+$ DLBL may, therefore, indicate that this aberration hits a CD5$^+$ progenitor B cell at the same differentiation level as in B-CLL. Interestingly, deletions of *ATM* commonly found in B-CLL[12] were not detectable in CD5$^+$ DLBLs. It recently turned out, however, that *ATM* deletions in B-CLL occur almost exclusively in the unmutated variant of the disease,[24] whereas *D13S25* deletions cluster in mutated B-CLL.[25] Because most of our CD5$^+$ cases displayed mutated IgVH genes, their cell of origin is likely to be different from the pregerminal progenitor cell of the unmutated B-CLL cases that are frequently characterized by ATM inactivations. CD5$^+$ DLBLs may, therefore, be viewed as an early transformed aggressive DLBL variant related to CD5$^+$ lymphocytic lymphoma with additional transforming events having occurred before the clonal expansion of the low-grade neoplasm. According to the results of this first study comparing cytogenetic alterations in CD5$^+$ and CD5$^-$ DLBLs, one likely candidate gene for this early transforming event is the loss of one p*16^{INK4a}* allele. The frequent association of

Table 1. Cytogenetic and interphase cytogenetic data of CD5+ DLBL

Case no.	Diagnosis	Karyotype	IgVH status	*D13S25*	*ATM*	*TP53*	*P16INK4a*
1	CB	33-50,X,?del(X)(q13)[5]	ND	n	n	n	n
2	CB	43-50,XX,t(3;14)(q27;q32),del(6)(q15q21),+11,+18,+19[11]	ND	ND	ND	ND	ND
3	CB	86-91P⟨4n⟩,XXXX,−1[4],del(1)(q11)[4],+3[3],t(3;14)(p21;q32)[3],der(6)t(1;6)(q21;q21)[4], +der(6)t(1;6)(q21;q21)[3],t(8;11)(p23;p12)x2[4],der(16)t(1;16)(q21;q21)[4], +der(16)t(1;16)(q21;q21)[3],−17[4],−17[3][cp4]	Mutated	tet	tet	del	del
4	IB	44/48,XY,−6,+21[2]	Hypermutated	del	n	n	n
5	IB	43-45,XY,del(2)(q21q31)[15],−6[15],del(9)(p13)[15],del(10)(q22q24)[15],+mar[2][cp15]	ND	del	n	n	del
6	CB	46-52,XY,+2[2],+3[8],+5[9],+7[6],+17[5],+18[4],+19[2],+20[3],+22[3][cp13]	Mutated*	n	n	n	n
7	CB	48-50,XX,+3[7],del(4)(q21q25)[7],dup(11)(q21q24)[7],+16[7],add(17)(p10)?t(13;17)(q22;p10)[6],+18x2[7][cp7] 50,XX,+3[11],del(4)(q21q25)[11],trp(11)(q21q24)[11], +16[11],iso(17)(p10)[10],+18x2[11][cp11]	Hypermutated	n	tri/tet	del	n
8	IB	48-50,XX,t(2;8)(p13or14;q24.1)[14],add(2)(p11)[14],+3[13],del(6)(?q15q23)[14], ?der(9)t(?X;9)(?p11;p11)[14],del(10)(q22q24)[14],t(13;15)(q14;q24)[14],+16[14], iso(18)(q10)[14],+iso(18)(q10)x2[14][cp14]	Hypermutated	del	n	del	del
9	Unclass	47-49,X,−Y[15],del(1)(p31or32)[15],+3[15],idic(6)(q16)[15],+idic(6)(q16)[15], del(12)(q11q22)[15],+del(12)(q11q22)[15],+der(12)t(5;12)(q13;p13)[15], −15[15],add(17)(q25)[15],+mar[14][cp15]	Mutated	tri	n	n	n
10	Unclass	84-87⟨4n⟩,XXYY,add(2)(q37)[5],add(2)(q37)[2],add(6)(q11)[3],add(6)(q11)[2],del(9)(q12)x2[5], der(12)t(12;15)(p11;q15)[4],der(12)t(12;15)(p11;q15)[2],add(14)(q32)[4][cp6],inc	Unmutated	del	tet	tet	tet
11	CB	39-48,XX,−X[5],add(3)(q21)?t(3;12)(q21;q15)[20],+5[3],−6[5],−8[5],−9[4],−10[5],+12[5], +14[2],add(14)(q32)[20],+15[11],−18[4],+20[7],+20[2],−21[4],−22[5],+mar[7][cp20]	ND	tri	n	n	n
12	CB	48-51,X,del(X)(q22orq24)[3],+3[3],+16[3],+der(16)t(X;16)(q22orq24;q13)[2],+18[2], der(19)t(5;19)(q14q31;q13)[3][cp3]/47–49,X,del(X)(q22orq24)[10],+3[10],−8[12], der(9)t(8;9)(q13;p21orp22)[12],+16[12],+der(16)t(X;16)(q22orq24;q13)[5],+18[11], der(19)t(5;19)(q14q31;q13)[12][cp12]	Hypermutated	n	n	n	n
13	CB	91-97⟨4n⟩,XXXX,−X[3],+X[2],−1[6],+1[2],add(1)(p22)[7],−2[3],del(2)(p23)[6],add(2)(q31)[15],+3[2],−4[3],+4[3],−6[5],del(6)(q21)[15],del(6)(q21)[9],del(6)(p22)[15],del(6)(p22)[12],+7[2],der(7)t(7;12)(q21;q13)[6],+8[6],+9[2],−10[6],ins(10;?)(q22;?)[2], +11[4],del(11)(q23or24)[2],add(11)(p15)[6],+13[3],−14[8],−14[3],−15[4],+16[2], −17[4],?add(17)(p13)[3],?add(17)(p13)[2],−18[6],+18[2],−19[3],+19[3],+20[5], +21[5],+mar[7],+mar2[3],+mar3[5],+mar4[5],+mar6[2],+mar7[3],+mar8[2][cp15],inc	ND	tet	tet	del	del

CB indicates centroblastic; ND, not done; n, normal; Mutated, VH homology in 95% to 98%; tet, tetrasomy; del, deleted; IB, immunoblastic; Hypermutated, VH homology in < 95%; tri, trisomy; Unclass, unclassified; and Unmutated, VH homology in > 98%.

*Ongoing mutations.

p*16INK4a* inactivations with transformed low-grade lymphomas[13] would be in excellent agreement with this hypothesis.

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