Chronic Lymphocytic Leukemia

Karyotype complexity and V_H gene status in B-cell chronic lymphocytic leukemia

B-cell chronic lymphocytic leukemia-related genomic changes were analyzed by karyotyping, fluorescence *in situ* hybridization, and V_H gene sequencing in a prospective clinical evaluation. The V_H mutational status correlated with high-risk cytogenetic aberrations while no such relation could be demonstrated for specific V_H gene usage (V_{3-21} and V_{1-69}). Complex karyotypes were highly indicative of disease progression.

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Genomic testing recently gained a significant role in the characterization of chronic lymphocytic leukeima (CLL). Lack of somatic V_H hypermutation was found indicative of instable disease with relatively short survival[1,2] and specific V_H gene usage (V_{3-21} and V_{1-69}) could be related with disease aggressiveness.[3-5] At the chromosomal level, deletions of p53 and ATM locus are the strongest predictors of poor outcome, while normal karyotype or isolated deletion of 13q14 locus is an indicator of long, symptom-free survival.[6,7] The role of additional aberrations and complex karyotypes in this system of novel high-risk prognostic markers has not been evaluated in detail, although their total frequency seems to be significant. Following the genomic analysis of 103 peripheral blood samples from CLL patients we questioned how V_H gene variables are related with individual or complex chromosomal changes.

In order to determine this, we evaluated results from karyotyping, fluorescence *in situ* hybridization (FISH) and V_H gene data. Metaphase G-banding was performed after culturing blood cells with the B-cell mitogen tetradecanoyl-phorbol-myristate-acetate (TPA, Sigma-Aldrich, St. Louis, USA) for 72 hours. FISH analysis was done on the same material using direct fluorochrome-conjugated DNA probes (13q14; ATM, cen12 and p53, Vysis, Inc., Des Plaines, IL, USA). IgV_H gene sequencing data were obtained following the extraction of the genomic DNA and multiplex polymerase chain reaction using family-specific FR1 and consensus J_H primers, as described elsewhere.[8]

Karyotyping was successful in 76.7% of the cases (79/103) and FISH could be interpreted in 91.3% (94/103). Recurrent chromosome changes were observed at similar frequencies as reported previously (50-75%) and the correlation between high-risk aberrations and unmutated V_H status was reproduced in our series (Table 1). Among the 31 different V_H genes that we identified, the V_{1-69} gene (13/64, 20.31%) was the most frequent. All were of unmutated nature showing 100% sequence homology with one exception. Five out of the 13 cases presented with cytogenetic aberrations, two with del(17)(p13), two with +12 and one case with three deletions including the loci 13q14, 6q22-24 and 11q22-23.

The second most frequent gene, V_{3-21}, was identified in 7.85% (5/64). Hypermutation was demonstrated in two out of five cases. One of them showed a normal cytogenetic profile while the other displayed an isolated deletion of the 13q14 locus. Of the three unmutated cases using V_{3-21}, one appeared to be normal by cytogenetics, while the remaining two presented with del(13)(q14) and additional aberrations.

Table 1. Correlation between IgV_H hypermutational status V_H gene usage and the occurrence of cytogenetic aberrations in 64 samples sequenced for the relevant variable region.

		Chromosome abnormality						
IgV_H	*n*	*del (17p)*	*del (11q)*	*+12**	*del (6q)**	*isolated (del13q)*	*del(13q) combined*	*normal*
Mutation status								
Mutated	31	2	1	0	1	12	1	16
Unmutated	33	4	8	4	3	2	8	8
Gene usage								
V_{1-69}	13	2	1	2	1	–	1	8
V_{3-21}	5	1	–	–	–	1	2	2
non-V_{1-69} or V_{3-21}	46	3	8	2	3	13	6	14
Total	64	6	9	4	4	14	9	24

*V segment hypermutation was determined using a cut-off of 98% sequence homology (*determined in part only by karyotyping).*

Table 2. V_H gene status and disease outcome in CLL with aberrations other than del17p, del11q, del13q and +12.

Case	*V_H usage*	*V_H hyper mutation*	*Karyotype*	*Outcome*
1	V3-20	U	46, XY, t(4;11)(p15;q21), +3, +8 [8]	DOD
2	V1-8	M	44, X, -Y, t(1;14)(q31;q32), add(6)(qter), -14 [22]	DOD
3	V1-69	U	46, XY, t(1;10)(q12 ;q21), del(6)(q22q26), del(11)(q21q23), del(13)(q13q14) [4]	P
4	nd	nd	46, XX, der(12)t(?;12)(?;?) [3]	P
5	V4-39	U	46, XY, inv(4)(p?q?), del(6)(q22q26), del(11)(q21q23), del(13)(q14q22) [3]	P
6	V3-21	U	46, XX, del(13)(q14q22), add(16)(pter) [12]	D

DOD: died of disease within 30 months of observation, P: clinical progression; D: died of other cause, S: stable disease, nd: no data; U: unmutated; M: mutated.

The overall frequencies of V_{1-69} and V_{3-21} gene usage were in the ranges reported previously (15.7-22.6% and 2.9-11.2%, respectively).[4,5] Neither V_{1-69} nor V_{3-21} usage was associated with specific chromosome aberrations.

The most frequent chromosomal aberrations were deletions of 13q, 11q, 17p, 6q as well as trisomy 12; however, a few other abnormalities (numeric and translocations) were also demonstrated in the present series, in part obviously accompanying the mentioned standard aberrations.

Two or more chromosomal aberrations were found in 16/103 cases (15.5%) which were in good association with unmutated V_H status but not with any specific V_H gene usage. Early transition to Rai stage III-IV was observed, in conjunction with complex chromosome aberrations, supporting the role of accumulating genetic failures during disease progression. In six cases such clonal structural changes were identified which were not

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challenging our four probe FISH approach (Table 2). Moreover, in three cases (no. 1, 2 and 4, Table 2) none of the clonal karyotype changes was covered by FISH. According to our clinical records two of these cases (cases no. 1 and 2.) proved to be highly aggressive and terminated in early death within 30 months of follow-up despite intensified treatment. Lack of V_H mutation was demonstrated in only one of them and these cases were not characterized by specific high-risk V_H usage.

We conclude that complex karyotypes in CLL may consist of combinations of standard recurrent chromosomal changes but also of infrequent translocations and of aneusomies. These represent progressive disease arising on the basis of sequential genomic events. Karyotype evolution not following the classical route in CLL seems to be rare but highly indicative of aggressiveness; moreover, such cases may escape proper risk assessment if evaluated by targeted molecular methods. An extended study should clarify whether such cytogenetic profiles characterize a group with highly unfavorable disease and how these changes are related to established high-risk prognostic factors.

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