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Original Paper

Analysis of aberrant somatic hypermutation (SHM) in non-Hodgkin's lymphomas of patients with chronic HCV infection

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Abstract

Hepatitis C virus (HCV) and aberrant somatic hypermutation (SHM) have each been suggested to contribute to the development of B-cell non-Hodgkin's lymphoma (NHL). The incidence of *PIM-1, PAX-5, RhoH/TTF*, and c-*MYC* mutations in tumour biopsy specimens from 32 HCV-infected B-cell NHL patients was analysed to determine whether the extent of aberrant SHM among these patients differed from that previously reported for HCV-negative B-cell NHL patients. Mutation of *PIM-1, PAX-5, RhoH/TTF*, and c-*MYC* was detected in 4 (13%), 5 (16%), 4 (13%), and 4 (13%) of 32 samples, respectively. In HCV-positive B-cell NHL patients, the frequency of aberrant SHM was lower than that already found in HCV-negative B-cell NHL patients. This indicates that, unlike B-cell lymphomas from HCV-negative patients, aberrant SHM may not contribute significantly to malignant transformation in HCV-associated B-cell lymphomas.

Keywords: HCV; B-NHL; aberrant SHM

Introduction

Hepatitis C virus (HCV) infection is associated with chronic hepatitis, cirrhosis, and hepatocellular carcinoma [1]. Recent evidence has also suggested that HCV infection contributes to the development of autoimmune diseases and non-Hodgkin's lymphoma (NHL) [2–4]. A systematic review of 66 studies revealed that HCV infection was common among B-cell NHL patients [5]. The link between HCV infection and the development of B-cell NHL is also supported by recent case–control studies [6,7].

The mechanisms by which HCV infection promotes B-cell NHL development remain unclear. While HCV infection has been detected in stromal cells associated with B-cell lymphoma tissue, it has not been detected in neoplastic B-lymphocytes. VDJ rearrangement analysis supports the possibility that chronic antigenic stimulation of B-cells arising from HCV infection contributes to B-cell expansion and its evolution to lymphoma [8].

Somatic hypermutation (SHM) is a process that enhances antibody affinity for a particular antigen by introducing nucleotide substitutions within the immunoglobulin variable (IgV) genes of germinal centre (GC) B-cells [9]. Specific features of SHM are the predominance of single base substitutions, preference for transitions over transversions, and specific targeting of AG/G/CT/AT (RGYW) motifs. The *BCL-6* proto-oncogene is another target of SHM in GC B-cells. BCL-6 is a transcriptional repressor that regulates B-cell maturation [10].

Aberrant SHM has been suggested to contribute to the development of diffuse large B-cell lymphomas (DLBCLs) [11]. The majority of DLBCLs exhibit evidence of aberrant SHM of the coding sequence or 5′ untranslated region (UTR) of proto-oncogenes that have been implicated in the pathogenesis of lymphoid malignancies, including *PIM-1, PAX-5, RhoH/TTF*, and c-*MYC* [11]. Frequent aberrant SHM of *PIM-1, PAX-5, RhoH/TTF*, and c-*MYC* has also been observed in NHLs associated with AIDS [12] as well as primary central nervous system lymphomas (PCNSLs) [13].

The potential involvement of aberrant SHM in the development of B-cell NHL among HCV-infected individuals has not previously been investigated. In

the present study, tumour biopsy specimens from 32 HCV-infected B-cell NHL patients were analysed for evidence of aberrant SHM.

Materials and methods

Tumour panel

This study was based on 32 samples of B-cell NHL from patients with chronic HCV infection. All patients were observed at the Centro di Riferimento Oncologico of Aviano in Italy from 1994 to 2003 and were sero-negative for human immunodeficiency virus (HIV). Informed consent was obtained from all patients and tissue collection was approved by the Institutional Review Board. The cases were selected on the basis of the availability of frozen tumour tissue samples. Sera obtained at disease diagnosis were all positive for anti-HCV antibodies [by the enzyme-linked immunosorbent assay (HCV 3.0; Ortho Diagnostic Systems, Raritan, NJ, USA) and the recombinant-based immunoblot assay (Chiron RIBA; Ortho Diagnostic Systems)] and for HCV-RNA [14].

Primary tumour biopsy specimens were collected under sterile conditions during routine procedures according to a standard protocol developed by the Division of Pathology of the Institution. In all instances, the specimen was collected at diagnosis before specific therapy. A portion of unfixed tissue was snap-frozen in liquid nitrogen immediately after collection and stored at $-80\,^{\circ}C$. According to morphological and immunophenotypic analysis, the fraction of malignant cells in the pathological specimen was 90% or more in all cases. All B-cell NHL cases were reviewed (by AC) for the purposes of this study and were classified according to the 2001 *World Health Organization (WHO) Classification of Tumours of Haematopoietic and Lymphoid Tissues* [15]. They included 20 DLBCLs, three mucosa-associated lymphoid tissue (MALT) lymphomas, four chronic lymphocytic leukaemia/small lymphocytic lymphomas (CLL/SLLs), three follicular lymphomas (FLs) (one FL classified as G3 and two FLs classified as G2), one mantle cell lymphoma (MCL), and one extra-osseous plasmacytoma.

Immunophenotyping

Dewaxed and cryostat sections were used for immunophenotyping and lineage assignment of lymphoma cases as previously described [12].

DNA extraction

Genomic DNA was isolated from tumour biopsy specimens by precipitation in ethanol as previously described [16].

Oligonucleotides

Sequences of the oligonucleotide primers used for amplification of *PIM-1, PAX-5, RhoH/TTF*, c-*MYC* exon 1, c-*MYC* exon 2, and *BCL-6* by the polymerase chain reaction (PCR) were PIM1-F, 5′-CCC TCA GTT GTC CTC CGA CTC-3′; PIM-1-R, 5′-GAT GTT TTC GTC CTT GAT GTC; PAX-5-F, 5′-TGT CCA CAC GCA GCG GGT CC -3′; PAX-5-R, 5′-AAG AGC TGA AAT GTC GCC GCC G -3′; RhoH/TTF-F, 5′-CTG CCC CAC ACA CAC TAA C-3′; RhoH/TTF-R, 5′-AAC TCT TCA AGC CTG TGC TG-3′; MYC-1-F, 5′-CAC CGG CCC TTT ATA ATG CG-3′; MYC-1R, 5′-CGA TTC CAG GAG AAT CGG AC-3′; MYC2-F, 5′-CCG CTG GTT CAC TAA GTG CG-3′; MYC-2R, 5′-GGA TGG GAG GAA ACG CTA AAG-3′; BCL6-10F, 5′-CTC TTG CCA AAT GCT TTG-3′; and BCL6-12R, 5′-CAC GAT ACT TCA TCT CAT C-3′.

Analysis of *PIM-1, PAX-5, RhoH/TTF*, c-*MYC*, and *BCL-6* mutations

Analysis of *PIM-1, PAX-5, RhoH/TTF*, c-*MYC*, and *BCL-6* mutations was performed on regions of these genes previously shown to contain more than 90% of mutations introduced by aberrant SHM in B-cell lymphomas [11,12,17]. Purified PCR products were sequenced using an ABI 3100 Genetic Analyser (Perkin Elmer, Foster City, CA, USA) with the dye-terminator protocol. Both strands of each PCR product were sequenced. The sizes of the *PIM-1, PAX-5, RhoH/TTF*, c-*MYC* exon 1, c-*MYC* exon 2, and *BCL-6* PCR products were 1009, 861, 1109, 1301, 1167, and 739 bp, respectively. The sequence of each PCR product was compared with the corresponding wild-type sequence. GenBank accession numbers of wild-type *PIM-1, PAX-5, RhoH/TTF*, c-*MYC*, and *BCL-6* sequences are AF386792, AF386791, AF386789, X00 364, and AY189 709, respectively.

Statistical analysis

Mutation data were managed with Excel software (Microsoft, Redmond, WA, USA). SPSS software (version 6.0 for Windows) was utilized for statistical analysis. Differences in the mutation frequency were defined to be statistically significant for p values less than 0.05. Mutation frequencies were normalized based on the base composition of the sequences analysed. The normalized mutation frequencies of each individual nucleotide were compared with the expected mutation frequency by the goodness-of-fit χ^2 test [12]. The mutation frequency for nucleotides occurring in the context of an RGYW/WRCY motif was compared with the expected mutation frequency by the goodness-of-fit χ^2 test [12].

Results

Frequency of aberrant hypermutation in HCV-associated B-cell NHL

Tumour biopsy specimen DNA from 32 HCV-infected B-cell NHL patients was analysed for mutation of *PIM-1, PAX-5, RhoH/TTF*, and c-*MYC* (Table 1). Mutation of at least one of these genes was detected in 10 of 32 (31%) NHLs, including 8 of 20 (40%) DLBCLs, 1 of 3 MALT lymphomas, and 1 of 1 mantle cell lymphoma (MCL). Mutation of more than one of these genes was observed in 5 of 32 (16%) NHLs, including 4 of 20 (20%) DLBCLs and 1 of 1 MCL. Each gene was mutated in a significant fraction of tumour biopsy specimens. Mutation of *PIM-1, PAX-5, RhoH/TTF*, and c-*MYC* was detected in 4 (13%), 5 (16%), 4 (13%), and 4 (13%) of 32 patients, respectively.

Distribution of mutations in *PIM-1, PAX-5, RhoH/TTF*, and c-*MYC* in B-cell NHL tissue

The detailed characterization of *PIM-1, PAX-5, RhoH/TTF*, and c-*MYC* mutations in HCV-associated B-cell NHL is reported in Table 2, and their general features are summarized in Table 3.

Table 1. Distribution of *PIM-1, PAX-5, RhoH/TTF*, c-*MYC*, and *BCL-6* mutations throughout the clinicopathological spectrum of HCV-associated NHL

Histology	Mutated/tested (%)				
	PIM-1	***PAX-5***	***RhoH/TTF***	**c-*MYC* exons 1 and 2**	***BCL-6***
HCV-associated NHL (all)	4/32 (13%)	5/32 (16%)	4/32 (13%)	4/32 (13%)	14/32 (44%)
Diffuse large B-cell lymphoma	4/20 (20%)	4/20 (20%)	4/20 (20%)	2/20 (10%)	10/20 (50%)
MALT lymphoma	0/3	0/3	0/3	1/3	1/3
Mantle cell lymphoma	0/1	1/1	0/1	1/1	0/1
Chronic lymphocytic leukaemia/small lymphocytic lymphomas; follicular lymphomas; extra-osseus plasmacytoma	0/8	0/8	0/8	0/8	3/8 (38%)

Table 2. Analysis of *PIM-1, PAX-5, RhoH/TTF*, c-*MYC*, and *BCL-6* mutations in HCV-associated NHL

Patient	Histology*	***PIM-1***	***PAX-5***	***RhoH/TTF***	**c-*MYC* exons 1 and 2**	***BCL-6***
4079	DLBCL	—	—	—	—	T570C, C780G, C786G, T838A
4082	DLBCL	1097ΔG, C1345T, G1495A, G1497A	G948A, G1017C, C1185T, G1206A, G1266A, G1287A, G1333A, G1347A, C1350T, G1381A	A295C, T300C, A373T, A421G, T488C, C515T, G538T, A582C, A748G, G884A, C885A, C890A, A894G, G900A, ΔTCACCGGTTT (868–878)	—	G734T, G809C
4085	DLBCL	—	—	G700A, C885T	—	A465C, A4787G
4090	DLBCL	—	—	—	G2397A	—
4093	DLBCL	C1119T, G1127C, G1242A, C1875T, C1720G	—	T956G	—	G479C, A508G, G1067C
4100	DLBCL	C1158G, T1177C, C1191A, A1746G	C848T, C1052A	—	—	T510C, G541A, T633G
4159	DLBCL	—	G1134A	—	—	T488A, G954A
4160	DLBCL	—	—	—	—	C958T
4161	DLBCL	C1325T	G1045C	A852G, C885G	—	A495G, T557C, C786G, G779A,
4163	DLBCL	—	—	—	—	G859A, G954A,
4164	DLBCL	—	—	—	G2709A	—
4166	DLBCL	—	—	—	—	T892G, C905T, G953A, C936A
4076	MALTL	—	—	—	G3031A	—
4165	MALTL	—	—	—	—	G805A,
4083	CLL/SLL	—	—	—	—	A1065G
4089	CLL/SLL	—	—	—	—	A583G, C789G, G1003A
4081	FL G2	—	—	—	—	G722C, T736C
4087	MCL	—	G847T	—	G3004A	—

* DLBCL = diffuse large B-cell lymphoma; MALTL = mucosa-associated lymphoid tissue lymphoma; CLL/SLL = chronic lymphocytic leukaemia/small lymphocytic lymphomas; FL = follicular lymphoma; MCL = mantle cell lymphoma.

Table 3. Features of *PIM-1, PAX-5, RhoH/TTF*, c-*MYC*, and *BCL-6* mutations in HCV-associated NHL

Locus	Mutations/ 100 bp	Deletions or insertions	Single bp substitutions	Transitions/ transversions	G + C	A + T	G + C mutations/ 100 bp†	A + T Mutations/ 100 bp*	RGYW†
PIM-1	0.19 (0.05–0.28)	1	13	9/4 (2.25)	11	2	0.20	0.079	4 ($p = 0.88$)
PAX-5	0.17 (0.06–0.58)	0	15	11/4 (2.75)	15	0	0.26	0	6 ($p = 0.36$)
RhoH/TTF	0.22 (0.06–0.89)	1	19	11/8 (1.37)	9	10	0.18	0.26	8 ($p = 0.18$)
c-*MYC* exon 1	0.038	0	4	4/0	4	0	0.067	0	2 ($p = 0.52$)
c-*MYC* exon 2	0	0	0	0/0	0	0	0	0	0
Total	NA	2	51	35/16 (2.18)	39	12	0.19	0.084	20 ($p = 0.08$)
BCL-6	0.16 (0.07–0.27)	0	34	19/15 (1.26)	19	15	0.18	0.15	12 ($p = 0.01$)

* Calculated from the number of G + C and A + T nucleotides in the wild-type gene sequences.
† The frequency of mutations within RGYW motifs was compared with the frequency of mutations outside RGYW motifs by the χ^2 test.

The average frequency of mutation, calculated taking into account only mutated cases, ranged from 0.38×10^{-3} per bp in the case of c-*MYC* exon 1 to 6.76×10^{-3} per bp in the case of *RhoH/TTF*. The majority of the mutations included single base-pair substitutions ($n = 51$), whereas deletions/insertions of a short DNA stretch were observed in only one case (Tables 2 and 3). Of the 51 single base-pair substitutions observed, 35 were transitions and 16 were transversions, with a transition-to-transversion ratio of 2.18 (expected 0.5, $p < 0.001$, χ^2 test; Table 3). Analysis of the nucleotide mutational pattern, when considering the cumulative effects of nucleotide substitutions observed in PIM-1, PAX-5, RhoH/TTF, and c-MYC, revealed that G + C base pairs were targeted at a frequency 3.5-fold higher than for A + T.

Although mutations seemed to occur mostly within RGYW/WRCY motifs, the prevalence was not statistically significant (PIM-1, 6.7% versus 5.41%; PAX-5, 10% versus 5.8%; RhoH/TTF, 13% versus 7%; c-MYC exon 1, 2% versus 0.8%) (Table 3).

Six PIM-1 mutations in two B-cell NHL cases from HCV-infected patients were located within the coding region. In particular, two mutations caused amino acid changes (Pro79Ala in case 4093 and Asn7Lys in case 4100), probably altering the biochemical and structural properties of the protein. The remaining four mutations were silent.

BCL-6 mutations in HCV-associated B-cell NHL

For comparison, the mutational analysis of the *BCL-6* gene was also performed in all 32 B-cell NHLs from HCV-infected patients. *BCL-6* mutations were detected in 14 of 32 (44%) cases, including 10 of 20 (50%) DLBCLs, 1 of 3 MALT lymphomas, 2 of 4 CLL/SLLs and 1 of 3 FLs (Tables 1 and 2). Of the 34 single base-pair substitutions observed, 19 were transitions and 15 transversions, with a transition-to-transversion ratio of 1.26 (expected 0.5, $p = 0.06$, χ^2 test; Table 3). Nucleotide substitutions showed a predominance of G + C over A + T with a ratio of 1.26. Moreover, as expected, mutations occurred predominantly within RGYW/WRCY motifs (10% versus 4%, $p = 0.01$) (Table 3).

Discussion

The aim of the present study was to investigate the role of aberrant SHM in the process of lymphomagenesis in the context of HCV infection. We report that, similar to DLBCL from both immuno-competent and immuno-deficient hosts, *PIM-1, PAX-5, RhoH/TTF*, and c-*MYC* are also targeted by mutations in B-cell NHL from HCV-infected patients. In HCV-infected patients, the overall incidence of cases mutated in at least one locus (31%) was lower than that reported in non-HCV-infected lymphomas (75%). However, when the mutated loci are considered, the mutation frequency per 100 bp was comparable to that reported in non-HCV carriers [11]. Moreover, the mutational profile of *PIM-1, PAX-5, RhoH/TTF*, and c-*MYC* in HCV-associated NHL was mainly represented by single nucleotide substitutions, with a prevalence of G + C over A + T substitutions and transitions over transversions, similarly to that reported previously [11,12].

In the HCV-associated immunocytomas, the presence of SHM and intraclonal variation in V-gene sequences has been observed, suggesting a role for antigen stimulation in the growth and clonal evolution of these tumours [18]. Since the *BCL-6* gene is a physiological target of the SHM process, we analysed the *BCL-6* sequence as a read-out of the penetrance of SHM in our series. The number of HCV-associated NHLs mutated in the *BCL-6* gene reflected the frequencies reported previously, as well as the prevalence of mutations within RGWY motifs of the gene. These results confirmed that a physiological SHM process was normally active in HCV-associated NHL and hit physiological targets.

In the present study, all cases with mutations in at least one of the three proto-oncogenes *PIM-1, PAX-5* or *RhoH/TTF* were also mutated in the *BCL-6* gene. Conversely, the cases with c-*MYC* (exon 1) mutations were invariably wild type for the *BCL-6* gene and rarely showed mutations in the other three loci studied. This is different from what was previously observed in both immuno-competent and immuno-compromised hosts [11,12].

In HCV-associated NHL, the mutational spectrum of the proto-oncogenes analysed failed to reveal a

clear-cut clustering of mutation within the RGYW motifs. In fact, although a trend could be observed, the mutations detected in *PIM-1, PAX-5, RhoH/TTF*, and c-*MYC* loci were not significantly associated with aberrant SHM activity. Conversely, in B-cell lymphomas from immuno-competent hosts, specific RGYW targeting was significantly recognizable in each of the four hypermutated loci [11]. Our findings are in agreement with most recent data reported by Machida *et al* [19]. The authors showed that the mutational spectrum of the β-catenin and p53 genes failed to show an RGYW/WRCY preference in HCV-associated B-cell lymphomas. The pattern was instead compatible with a peculiar mutator phenotype determined by error-prone DNA polymerases activated by HCV [19]. The authors conclude that HCV-associated lymphomas are likely to experience mutational hits that are specifically HCV-induced. However, although occasional HCV-infected lymphoid cells have been detected in HCV carriers, the HCV genome has never been detected within neoplastic lymphoid tissue, but rather in the tumour-associated stromal cells [20,21].

Overall, our data indicate that in HCV-associated B-cell lymphomas the phenomenon of aberrant SHM is not a major mechanism contributing to malignant transformation.

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References

1. Lauer GM, Walker BD. Hepatitis C virus infection. *N Engl J Med* 2001; **345**: 41–52.
2. Silvestri F, Pipan C, Barillari G, *et al*. Prevalence of hepatitis C virus infection in patients with lymphoproliferative disorders. *Blood* 1996; **87**: 4296–4301.
3. Dammacco F, Sansonno D, Piccoli C, Racanelli V, D'Amore FP, Lauletta G. The lymphoid system in hepatitis C virus infection: autoimmunity, mixed cryoglobulinemia, and overt B-cell malignancy. *Semin Liver Dis* 2000; **20**: 143–157.
4. Quinn ER, Chan CH, Hadlock KG, Foung SK, Flint M, Levy S. The B-cell receptor of a hepatitis C virus (HCV)-associated non-Hodgkin lymphoma binds the viral E2 envelope protein, implicating HCV in lymphomagenesis. *Blood* 2001; **98**: 3745–3749.
5. Negri E, Little D, Boiocchi M, La Vecchia C, Franceschi S. B-cell non-Hodgkin's lymphoma and hepatitis C virus infection: a systematic review. *Int J Cancer* 2004; **111**: 1–8.
6. Mele A, Pulsoni A, Bianco E, *et al*. Hepatitis C virus and B-cell non-Hodgkin lymphomas: an Italian multicenter case–control study. *Blood* 2003; **102**: 996–999.
7. Talamini R, Montella M, Crovatto M, *et al*. Non-Hodgkin's lymphoma and hepatitis C virus: a case–control study from northern and southern Italy. *Int J Cancer* 2004; **110**: 380–385.
8. De Re V, De Vita S, Marzotto A, *et al*. Pre-malignant and malignant lymphoproliferations in an HCV-infected type II mixed cryoglobulinemic patient are sequential phases of an antigen-driven pathological process. *Int J Cancer* 2000; **87**: 211–216.
9. Kuppers R, Klein U, Hansmann ML, Rajewsky K. Cellular origin of human B-cell lymphomas. *N Engl J Med* 1999; **341**: 1520–1529.
10. Shen HM, Peters A, Baron B, Zhu X, Storb U. Mutation of BCL-6 gene in normal B cells by the process of somatic hypermutation of Ig genes. *Science* 1998; **280**: 1750–1752.
11. Pasqualucci L, Neumeister P, Goossens T, *et al*. Hypermutation of multiple proto-oncogenes in B-cell diffuse large-cell lymphomas. *Nature* 2001; **412**: 341–346.
12. Gaidano G, Pasqualucci L, Capello D, *et al*. Aberrant somatic hypermutation in multiple subtypes of AIDS-associated non-Hodgkin lymphoma. *Blood* 2003; **102**: 1833–1841.
13. Montesinos-Rongen M, Van Roost D, Schaller C, Wiestler OD, Deckert M. Primary diffuse large B-cell lymphomas of the central nervous system are targeted by aberrant somatic hypermutation. *Blood* 2004; **103**: 1869–1875.
14. De Vita S, Sacco C, Sansonno D, *et al*. Characterization of overt B-cell lymphomas in patients with hepatitis C virus infection. *Blood* 1997; **90**: 776–782.
15. Jaffe ES, Harris NL, Stein H, Vardiman JW. *World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues*. IARC Press: Lyon, 2001.
16. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988; **16**: 1215.
17. Capello D, Vitolo U, Pasqualucci L, *et al*. Distribution and pattern of BCL-6 mutations throughout the spectrum of B-cell neoplasia. *Blood* 2000; **95**: 651–659.
18. Ivanovski M, Silvestri F, Pozzato G, *et al*. Somatic hypermutation, clonal diversity, and preferential expression of the VH 51p1/VL kv325 immunoglobulin gene combination in hepatitis C virus-associated immunocytomas. *Blood* 1998; **91**: 2433–2442.
19. Machida K, Cheng KT, Sung VM, *et al*. Hepatitis C virus induces a mutator phenotype: enhanced mutations of immunoglobulin and protooncogenes. *Proc Natl Acad Sci U S A* 2004; **101**: 4262–4267.
20. De Vita S, De Re V, Sansonno D, *et al*. Gastric mucosa as an additional extrahepatic localization of hepatitis C virus: viral detection in gastric low-grade lymphoma associated with autoimmune disease and in chronic gastritis. *Hepatology* 2000; **31**: 182–189.
21. De Vita S, Sansonno D, Dolcetti R, *et al*. Hepatitis C virus within a malignant lymphoma lesion in the course of type II mixed cryoglobulinemia. *Blood* 1995; **86**: 1887–1892.