Typical Waldenstrom macroglobulinemia is derived from a B-cell arrested after cessation of somatic mutation but prior to isotype switch events

Surinder S. Sahota, Francesco Forconi, Christian H. Ottensmeier, Drew Provan, David G. Oscier, Terry J. Hamblin, and Freda K. Stevenson

There exists a wide spectrum of IgM-secreting B-cell tumors with different clinical behavior. Knowledge of the V_H gene status can reveal their origin and clonal history. For Waldenstrom macroglobulinemia (WM), a distinct subtype of lymphoplasmacytic lymphoma, early data on limited sequences showed evidence for somatic mutation. A recent report of one case demonstrated intraclonal mutational activity occurring after transformation, a characteristic of germinal center lymphomas. To extend the investigation, we have analyzed 7 cases of WM. V_H genes were somatically mutated with no evidence of intraclonal variation in all cases. In contrast to IgM-secreting multiple myeloma, there was no evidence for isotype switch transcripts in any of the cases. These data support the concept that typical WM is derived from a B cell that has undergone somatic mutation prior to transformation, at a point where isotype switch events have not been initiated. (Blood. 2002;100:1505-1507) © 2002 by The American Society of Hematology

Introduction

According to recent World Health Organization (WHO) proposals, typical Waldenstrom macroglobulinemia (WM) is a specific clinical syndrome commonly associated with a low-grade lymphoplasmacytic lymphoma (LPL). It has a characteristic monoclonal serum IgM of more than 5 g/L. WM involves primarily the bone marrow and possibly other lymphoid tissues. Tumor morphology shows small lymphocytes with a variable degree of plasma cell maturation, an immunophenotype of CD19+/CD20+CD5−CD10− cIg/cIgM+×, and distinct histopathology. These considerations distinguish it from other B-cell neoplasias with a serum monoclonal IgM, including B-chronic lymphocytic leukemia (B-CLL), splenic marginal zone lymphoma (SMZL), mucosa-associated lymphoid tissue (MALT) lymphoma, follicular lymphoma (FL), and mantle cell lymphoma.

Variable region (V) gene analysis is now providing a robust means of identifying tumor origins in B-cell tumors, because neoplastic transformation imprints the clonal history of the B cell in these genes. It has become feasible to classify B-cell tumors in relation to the germinal center (GC), a site of somatic mutation. V gene somatic mutation status defines tumors as those that never enter the GC, those that remain in the GC, and those that transit the GC and exit. Tumors located in the GC display intraclonal heterogeneity of V gene sequence, reflecting continual targeting by the mutation mechanism following neoplastic arrest.

Analysis of isotype switch events via V gene probes provides another tier of maturation status in tumor cells. The presence of tumor-derived isotype-switched transcripts can reveal whether tumor cells have activated switch mechanisms, at least at the RNA level. It is an assay of at least a log-scale greater sensitivity than Southern blotting to assess isotype switch.

In the case of WM, previous V gene analysis had suggested origins from a somatically mutated B cell. These data consisted of V_H analysis from complementarity-determining region 3 to framework region 4 (CDR3-FR4), and a few V_H sequences from Epstein-Barr virus–transformed lines from WM patients secreting IgM with cold agglutinin activity. V_H gene use had also been analyzed in some cases. Intraclonal variation in WM remained unresolved, because analysis of the CDR3-FR4 regions excluded other domains in each V_H gene. Recently, Ciric et al reported a single WM case where V_H gene sequence analysis revealed intraclonal heterogeneity in tumor cells, suggesting a continual influence on tumor cells of the mutator mechanism in the GC environment.

To resolve the stage of arrest in WM, we have analyzed the V_H gene status of 7 patients with typical WM. In all cases, tumor V_H genes revealed somatic mutation and a complete lack of intraclonal variation, and no evidence for any isotype-switched transcripts in 6 of 6 cases. These results suggest tumor origins in WM from an IgM-secreting B cell that transforms before the onset of isotype switch, with somatic mutation silenced.

Study design

Patients

Unselected patients from the hematology clinic were studied. Diagnosis of typical WM (cases WM1-7) was made using the WHO/Revised European and American Classification of Lymphoid Neoplasm (REAL) criteria. Monoclonal serum IgM was raised, more than 30 g/L in 6 of 7 patients and 18 g/L in the other patient, with no evidence of other monoclonal

From the Molecular Immunology Group, T enovus Laboratory, Southampton University Hospitals, Southampton, United Kingdom; Department of Haematology, St Bartholomew’s and The Royal London School of Medicine and Dentistry, London, United Kingdom; Department of Haematology, Royal Bournemouth Hospital, Bournemouth, United Kingdom.

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Reprints: Surinder S. Sahota, Molecular Immunology Group, T enovus Laboratory, Southampton University Hospitals, Southampton, United Kingdom; e-mail: s.s.sahota@soton.ac.uk.

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Table 1. Analysis of WM tumor–derived V_H genes

<table>
<thead>
<tr>
<th>Patient</th>
<th>V_H family</th>
<th>Germline gene</th>
<th>Homology, %</th>
<th>R/S mutation</th>
<th>J_H</th>
<th>Tumor-derived clones/clones screened</th>
<th>Tumor clones sequenced</th>
</tr>
</thead>
<tbody>
<tr>
<td>WM1</td>
<td>V_H 1</td>
<td>V1-02</td>
<td>92.8</td>
<td>6/9 (0.7)</td>
<td>J_H 4b</td>
<td>7/10</td>
<td>7</td>
</tr>
<tr>
<td>WM2</td>
<td>V_H 3</td>
<td>V3-30</td>
<td>91.0</td>
<td>9/3 (3.0)</td>
<td>J_H 4b</td>
<td>8/6</td>
<td>6</td>
</tr>
<tr>
<td>WM3</td>
<td>V_H 3</td>
<td>V3-30</td>
<td>95.5</td>
<td>5/3 (1.7)</td>
<td>J_H 6c</td>
<td>8/10</td>
<td>6</td>
</tr>
<tr>
<td>WM4</td>
<td>V_H 3</td>
<td>V3-30</td>
<td>93.9</td>
<td>5/1 (5.0)</td>
<td>J_H 6b</td>
<td>9/9</td>
<td>9</td>
</tr>
<tr>
<td>WM5</td>
<td>V_H 3</td>
<td>V3-23</td>
<td>89.4</td>
<td>10/8 (1.3)</td>
<td>J_H 3b</td>
<td>17/18</td>
<td>16</td>
</tr>
<tr>
<td>WM6</td>
<td>V_H 3</td>
<td>V3-72</td>
<td>88.4</td>
<td>10/8 (1.3)</td>
<td>J_H 3b</td>
<td>11/11</td>
<td>11</td>
</tr>
<tr>
<td>WM7</td>
<td>V_H 1</td>
<td>V1-02</td>
<td>93.2</td>
<td>12/4 (3.0)</td>
<td>J_H 4b</td>
<td>8/24</td>
<td>8</td>
</tr>
</tbody>
</table>

Figure 1. Deduced amino acid sequences of tumor-derived V_H genes from WM cases, indicating extent of somatic mutation and primer design for alternative transcripts. Comparisons for WM (WM1-7) are made with the closest germline V_H gene: uppercase, replacement (R) mutation (underlined in J_H); lower case, silent (S) mutation (EMBL accession nos. AJ488538-44). Each mutation was defined by nucleotide exchanges in a single codon, with successive mutations leading in some cases to 2 or 3 distinct R or S events. These are shown as vertically aligned amino acid changes at specific sites. Nucleotide mutations in the last codons of tumor V_H genes were assessed only if followed by at least a 2-base pair homology to the germline gene. Tumor V_H sequence specific 5' primers to assess switched transcripts are double-underlined in Figure 1.

Results and discussion

Potentially functional, in-frame tumor-derived V_H sequences (5 V_H 3, 2 V_H 1) were readily identified in each case by a signature CDR3 sequence in multiple clones from separate PCRs (Table 1 and Figure 1).

Each WM tumor V_H gene revealed extensive somatic mutations, indicating exposure of the cell of origin to somatic hypermutation. This confirms previous observations of V gene status in WM, suggesting a common feature. Analysis of the pattern of somatic mutations for antigen imprint by clustering algorithms is now considered flawed, first because analysis of CDRs is compromised by the existence of hot-spots in nonfunctional V(D)J rearrangements. Second, an overall low R/S ratio of less than 1.7 in FRs, although predictive of structural conservation, is clearly insufficient; 4 of 7 WM patients (Table 1) did not meet this requirement, despite the fact that the immunoglobulin is being expressed.

Significantly, there was no evidence for any intraclonal variation in V_H sequence in any of the WM cases, within 6 to 16 (mean, 9) clones sequenced in each case (Table 1). Our findings establish a postfollicular tumor origin for typical WM, now confirmed very recently in 3 WM cases lacking intraclonal variation. This is a characteristic feature of LPL" and IgM-secreting and typical multiple myeloma. In contrast, FL, MALT lymphoma, and some SMZL and monoclonal gammopathies of undetermined significance cases display intraclonal variation in tumor V_H sequences, analyzed in comparable numbers of clones, suggesting an earlier stage of arrest.

To further delineate the stage of arrest, tumor-associated isotype switch events were examined. No tumor-derived isotype switch variants could be identified in 6 of 6 WM cases, using a strategy that has
successfully identified such transcripts in a number of other B-cell tumors in small cohort studies.\textsuperscript{5,12,15} This suggests an origin for WM from an IgM\textsuperscript{+} mature B cell that transforms before the isotype switch stage and indicates that tumor cells do not activate switch mechanisms during tumor maintenance. Our findings are supported by emerging preliminary evidence that WM cells do not undergo switch recombination when assessed by Southern blotting.\textsuperscript{23}

Malignant B cells arise at distinct stages of maturation and the patterns of V gene sequences can reveal events likely to be occurring during normal B-cell maturation. Tumors also occupy multiple sites and may be able to respond to environmental signals in those sites. The fact that IgM-secreting tumors commonly evidently occurs prior to isotype switch, with ongoing somatic mutation silenced. Absence of somatic mutation in the bone marrow is also suggested by IgM-secreting myeloma.\textsuperscript{12} In contrast to WM, these myeloma cases harbor isotype variant transcripts within the tumor clone,\textsuperscript{22} indicating that they have activated the switch mechanism. In myeloma, illegitimate switching has been implicated as a causative mechanism in transformation.\textsuperscript{7} Preliminary evidence from interphase fluorescent in situ hybridization analysis in WM suggests that the 14q32 locus is free of aberrant switch events.\textsuperscript{23} abrogating such potentially neoplastic mechanisms in WM. Our cases of WM have consistent features, but exceptions clearly can occur, and serve to remind us of the heterogeneity within our current tumor categories.

Acknowledgments

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References

8. Aoki H, Takishita M, Kosaka M, Saito S. Frequent somatic mutations in D and/or J\textsubscript{5} segments of Ig gene in Waldenstrom’s macroglobulinemia and chronic lymphocytic leukemia (CLL) with Richter’s syndrome but not in common CLL. Blood. 1995;85:1913-1919.
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