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Short communication

Rarity of IgH translocations in Waldenström macroglobulinemia

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Abstract Comparatively little is known of the cytogenetics of Waldenström macroglobulinemia (WM). This is primarily due to the low proliferation of the clonal B cells, which precludes conventional karyotyping in many cases. Translocations involving the immunoglobulin heavy chain (*IGH*) gene at 14q32 are characteristic of many B-cell lymphomas and myelomas. Initial reports suggested that the t(9;14) was characteristic of lymphoplasmacytic lymphoma (the underlying pathological diagnosis in WM), but subsequent studies have failed to confirm the uniqueness of the translocation. To clarify this, we examined 69 cases of WM with interphase fluorescence in situ hybridization and failed to demonstrate an IgH translocation in 67 (97%). We conclude that *IGH* translocations are not a feature of WM, and the implications of this finding are discussed.

1. Introduction

Waldenström macroglobulinemia (WM) is a B-cell lymphoproliferative disorder characterized by IgM monoclonal gammopathy and bone marrow infiltration by small lymphocytes, plasmacytoid cells, and plasma cells [1–5]. The underlying pathologic diagnosis is considered to be lymphoplasmacytic lymphoma (LPL), as defined by World Health Organization criteria [6]. Clinical features may be due to tissue infiltration, such as cytopenias, lymphadenopathy, and splenomegaly, or may be secondary to the IgM paraprotein (e.g., hyperviscosity syndrome and autoantibody phenomena) [1–5].

Comparatively little is known of the cytogenetics of WM, which is primarily due to the low proliferation of the clonal B cells, precluding conventional karyotyping in many cases [7]. However, several small studies describing a variety of nonspecific numerical and structural abnormalities have been published [8–15]. Deletion of 6q, however, appears to be the most common abnormality, occurring in up to 50% of patients [7,9,16].

Translocations involving the immunoglobulin heavy chain (*IGH*) locus at 14q32 are characteristic of many B-cell lymphoproliferative disorders and myeloma. Initial reports suggested that the t(9;14)(p13;q32), which deregulates the *PAX5* gene as a consequence of its juxtaposition to the *IGH* locus, was characteristic of LPL [17–21]. This, however, has not been confirmed in other studies [7,8,12,14,22–24]. In particular, Schop et al. [7] failed to demonstrate the translocation in 42 cases of WM examined by interphase FISH. Their analysis failed to demonstrate evidence of any translocation involving the *IGH* locus. To clarify this apparent contradiction, we used interphase fluorescence in situ hybridization (FISH) to assess 69 patients with WM for the presence or absence of *IGH* translocations.

2. Methods

Sixty-nine cases of WM were included in this analysis (38 male, 31 female, median age 73 years). All cases were characterized by IgM paraprotein, bone marrow infiltration by small lymphocytes, plasmacytoid cells, and plasma cells, and fulfilled the diagnostic criteria for WM, as established by the consensus panel recommendations from the Second International Workshop on WM [5]. All cases had adequate bone marrow aspirate samples for analysis, and the following techniques were performed on these samples.

Interphase FISH was performed using a *BCL2*/*IGH* dual-color dual-fusion probe set (LSI IgH/Bcl2, 5J7101; Abbott Molecular Diagnostics, Maidenhead, UK). This probe set is used routinely in our laboratory to investigate all CD5− lymphoproliferative disorders. This was considered an adequate screening method for all *IGH* translocations because the *IGH* probes span the entire locus. Bone marrow aspirate

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smears containing morphologically identifiable disease were fixed in fresh cold methanol/acetic acid (3:1) and codenatured with probe at 73°C for 5 minutes and then hybridized for 16 hours at 37°C. This was followed by a stringency wash in a 0.4% standard saline citrate (SSC)/ 0.3% Nonidet P-40 mixture carried out twice for 2 minutes each, followed by a single wash in 2% SSC at room temperature and counterstained in 4',6-diamidino-2-phenylindole (DAPI; Vysis Downer's Grove, IL). Hybridization signals were visualized using a Zeiss AxioPlan2 imaging fluorescence microscope (Carl Zeiss Jena GmbH, Jena, Germany) with narrow bandpass filters for DAPI, SpectrumGreen, and SpectrumRed dyes. Images were captured and processed using the ISIS3 image capture system (MetaSystems, Altlussheim, Germany). Cases in which additional *IGH* signals were identified were further investigated using an *IGH* dual-color break-apart strategy, while cases with additional *BCL2* signals were further investigated using an α-18 probe [LSI *IGH* dual-color break-apart (5J7301) and CEP #18 SpectrumAqua (5J0918); Abbott Molecular Diagnostics, Maidenhead, UK]. Codenaturation was performed at 73°C for 5 and 3 minutes, respectively, while hybridization in both instances was for 16 hours at 37°C.

3. Results

Sixty-nine cases of WM were investigated using a *BCL2*/*IGH* dual-color dual-fusion probe set. In 67 of the 69 cases (97%), a normal hybridization pattern was demonstrated, and these cases were not investigated further. In two cases, additional *IGH* signals were encountered and this was considered to be suspicious of an underlying *IGH* translocation. This was confirmed with the dual-color *IGH* break-apart strategy in one case, but there was insufficient material to investigate the remaining case. There are currently no commercially available *PAX5* probes, and the t(9;14) therefore was not specifically sought in those cases with additional *IGH* signals. In five cases, additional *BCL2* signals were demonstrated, and subsequent analysis with the α-18 probe set confirmed trisomy 18 in all cases.

4. Discussion

The cytogenetics underlying WM remains poorly characterized, and no disease defining abnormalities have been identified. Conventional G-banding studies frequently demonstrate a normal karyotype, which is primarily due to the low proliferative capacity of the clonal B cells [7]. This is a common problem in other indolent lymphoproliferative disorders and myelomas, in which conventional cytogenetic studies have similarly been shown to substantially underestimate the incidence of clonal karyotypic abnormalities.

In B-cell lymphomas and myeloma, translocations involving the *IGH* gene locus are frequently seen and may be disease-defining. For example, follicular lymphoma, Burkitt lymphoma, and mantle cell lymphoma are defined by the t(14;18), t(8;14), and t(11;14), respectively. Similarly, *IGH* translocations are common in myeloma occurring in most cell lines and in up to 60% of patient samples. These translocations occur through errors of isotype switch recombination and involve a number of partner chromosomes, including 11q13, 4p16, 16q23, and 6p25 [25].

There are conflicting data in the published literature regarding the incidence of *IGH* translocations in WM. Isolated case reports have described patients with the t(14;18) and t(8:14) [10,26,27]. The clinical features of these patients, however, were not typical of WM, and these translocations would be more characteristic of other entities. Offit et al. [20] demonstrated the t(9;14) in 8 of 400 karyotypically abnormal lymphomas. In four of these eight patients, the underlying pathologic diagnosis was LPL, and in an additional two patients, there was a preceding history of LPL before the transformation to diffuse large B-cell lymphoma [20]. On the basis of these data, the t(9;14) has been considered to be the key genetic event in LPL [6]. This data have not, however, been corroborated by other studies. Louviaux et al. [8] found no evidence of 14q32 abnormalities in 45 cases of WM assessed by conventional karyotyping, although deletion of 6q appeared to be common. Two other smaller studies also used conventional karyotyping in 24 and 17 patients with WM, respectively, and found no evidence of 14q32 abnormalities [12,14]. In a more recent analysis of a cohort of 74 patients with WM, there was no evidence of the t(9;14) in any case by Southern blot assay ($n = 12$), conventional karyotyping ($n = 37$), or interphase FISH ($n = 42$) [7]. Indeed, there was no evidence of any translocation involving the *IGH* locus in their cases. In this report, we have also demonstrated a very low incidence of *IGH* translocations in WM, although we were unable to look specifically for the t(9;14). However, a slightly higher incidence has been reported by Chang et al. [24], who demonstrated *IGH* translocations in 3 of 22 (14%) cases assessed by *IGH* break-apart FISH.

This apparent contradiction is difficult to explain. It is interesting to note that none of the patients in the series from Offit and colleagues [20] had IgM monoclonal gammopathy and, therefore, did not fulfil the recognized diagnostic criteria for WM. Indeed, it has been suggested that the t(9;14) is not compatible with the secretion of monoclonal immunoglobulin [17,19,28], although *IGH* translocations do not appear to alter monoclonal protein secretion in multiple myeloma because they involve the nonproductive allele.

The apparent rarity of *IGH* translocations in WM is intriguing and is consistent with the hypothesis that WM is derived from IgM^+ post-germinal center memory B-cells. Along with other researchers, we have previously shown that WM is consistently characterized by the following immunophenotype: CD5−, CD10−, CD23−, CD19+, CD20+, CD75−, CD79+, CD27+, CD138−, Pax5+,