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Secondary Genomic Rearrangements Involving Immunoglobulin or MYC Loci Show Similar Prevalences in Hyperdiploid and Nonhyperdiploid Myeloma Tumors

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Abstract

The pathogenesis of multiple myeloma (MM) is thought to involve at least two pathways, which generate hyperdiploid (HRD) or nonhyperdiploid (NHRD) tumors, respectively. Apart from chromosome content, the two pathways are distinguished by five primary immunoglobulin heavy chain (*IGH*) rearrangements (4p16, *FGFR3*, and *MMSET*; 6p21, *CCND3*; 11q13, *CCND1*; 16q23, *MAF*; 20q12, *MAFB*) that are present mainly in NHRD tumors. To determine the prevalence and structures of *IGH*, immunoglobulin (*IG*) light chain, and *MYC* genomic rearrangements in MM, we have done comprehensive metaphase fluorescent in situ hybridization analyses on 48 advanced MM tumors and 47 MM cell lines. As expected, the prevalence of the five primary *IGH* rearrangements was nearly 70% in NHRD tumors, but only 12% in HRD tumors. However, *IGH* rearrangements not involving one of the five primary partners, and *IG* light chain rearrangements, have a similar prevalence in HRD and NHRD tumors. In addition, *MYC* rearrangements, which are thought to be late progression events that sometimes do not involve an *IG* heavy or light chain locus, also have a similar prevalence in HRD and NHRD tumors. In contrast to the primary *IGH* rearrangements, which usually are simple balanced translocations, these other *IG* rearrangements usually have complex structures, as previously described for *MYC* rearrangements in MM. We conclude that *IG* light chain and *MYC* rearrangements, as well as secondary *IGH* rearrangements, make similar contributions to the progression of both HRD and NHRD MM tumors.[†]

INTRODUCTION

It has been hypothesized that the pathogenesis of non-IgM/plasma cell monoclonal gammopathy of undetermined significance (MGUS) and multiple myeloma (MM) involve two distinct pathways that result in a similar prevalence of hyperdiploid (HRD) and nonhyperdiploid (NHRD) tumors (Smadja et al., 1998, 2003; Fonseca et al., 2003b, 2004). HRD tumors have 48–75 chromosomes, typically with extra copies of four to eight odd-numbered chromosomes (3, 5, 7, 9, 11, 15, 19, and 21), or derivatives of these chromosomes. NHRD tumors have <48 and/or >75 chromosomes, but only infrequently

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have extra copies of these same chromosomes. The two pathways are also distinguished by five recurrent immunoglobulin heavy chain (*IGH*) rearrangements (4p16, *FGFR3*, and *MMSET*; 6p21, *CCND3*; 11q13, *CCND1*; 16q23, *MAF*; 20q12, *MAFB*) that are present in nearly 70% of NHRD tumors but only in about 10% of HRD tumors (Bergsagel and Kuehl, 2005). The five recurrent *IGH* rearrangements are thought to be mediated by errors in switch recombination or somatic hypermutation in germinal center B cells (Bergsagel and Kuehl, 2001; Gabrea et al., 2006). These primary translocations mostly appear to be one of the earliest events involved in the pathogenesis of MM.

Dysregulation of a *MYC* gene, involving complex genomic rearrangements that sometimes but not always include immunoglobulin (IG) sequences, is a very late progression event in MM (Shou et al., 2000; Avet-Loiseau et al., 2001b; Gabrea et al., 2006; Dib et al., in press). Not surprisingly, these rearrangements rarely involve *IGH* switch or *JH* regions, since *IGH* switch recombination and somatic hypermutation are thought to be inactive in normal plasma cells and plasma cell tumors. *MYC* rearrangements have been proposed to provide a paradigm for secondary genomic rearrangements in MM. Genomic rearrangements involving the *IGH* locus and other chromosomal partners, or an *IG* light chain locus, also have been identified in plasma cell MGUS and MM (Fonseca et al., 2002; Kuehl and Bergsagel, 2002). The goal of this study was to do comprehensive metaphase fluorescent in situ hybridization (FISH) analyses to determine the prevalence and characteristics of secondary genomic rearrangements, involving *IG* or *MYC* (with or without juxtaposition of *IG* and *MYC* sequences), that occur in advanced HRD and NHRD intramedullary MM tumors, and in human myeloma cell lines (HMCL) that reflect an even later stage of tumor progression.

MATERIALS AND METHODS

Tumors and Cell Lines

Bone marrow aspirates were processed for routine chromosome studies as previously described (Shaughnessy et al., 2001). Metaphase spreads were generated from 48 independent MM tumor samples that were obtained from patients at the University of Arkansas for Medical Sciences. All the samples had abundant chromosomal material and an abnormal karyotype as determined by conventional cytogenetic analyses. Most samples that had a t(11;14)(q13;q32) by conventional cytogenetics were not available for this study. Similar to advanced relapsed tumors, 70% of tumors (14 of 20 with gene expression profiling data) in this study had an expression proliferation index >2.0 (Bergsagel et al., 2005; Kuehl and Shaughnessy, unpublished). Metaphase chromosomes from all 48 tumors (Table 1) and 47 HMCL (Table 2) were studied for *IG* and *MYC* rearrangements using a variety of FISH probes (later).

FISH and SKY Probes

The following probes were described elsewhere: CH BAC, C λ BAC, C κ cos, all three including strong 3' enhancers, VH telomeric cos, *LMYC* genomic fragment, *MYC* BAC (GS-93F05), *MYC* plasmid, *CCND1* (6.22 cosmid), *CCND3* (ccnd3cy13 cosmid), *FGFR3* (L184d6 cosmid), and *MMSET* (L190b4 cosmid) (Chesi et al., 1998b; Gabrea et al., 1999; Shou et al., 2000; Shaughnessy et al., 2001; Dib et al., in press). *NMYC* (*MYCN*) (AC010145), *MAFB* (AL035665), *NIK* (*MAP3K14*) (AC003963) are identified by their GenBank accession numbers. *MAF* and *IRF4/MUM1* were obtained by screening Genome Systems BAC libraries. V λ and V κ cosmids, each containing the most centromeric V sequences, were prepared by subcloning V λ and V κ BAC fragments (Genome Systems) into Supercos. SKY probes were obtained from Applied Spectral Imaging (Vista, CA). FISH probes were labeled by nick translation with either biotin-16-dUTP (Roche, Indianapolis,

IN) followed by avidin-FITC (Sigma, St. Louis, MO) detection or digoxigenin-11-dUTP (Roche, Indianapolis, IN) followed by TRITC antibody (Sigma, St. Louis, MO) detection. Whole chromosome painting (wcp) probes were generated in our laboratory by direct PCR labeling with Cy5-5-dUTP of chromosome-specific template DNA, generously provided by Dr. Thomas Ried (Center for Cancer Research, National Cancer Institute, Bethesda, Maryland).

FISH and SKY Hybridization

FISH hybridizations of metaphase chromosomes have been described in detail elsewhere (Shou et al., 2000). SKY analyses were carried out according to the manufacturer's protocol ASI. For sequential hybridizations, slides were analyzed and the position on the slide of 5–10 metaphases was recorded. The slides were then prepared for rehybridization by shaking twice in 4×SSC/Tween (15 min each time), rinsing briefly with water, dehydrating in ethanol, and stripping of the old probes in 70% formamide denaturing solution (72°C for 2 min).

Strategy for Detection of Structural Chromosome Abnormalities

An initial screen was conducted on all MM tumor samples and HMCL for each *IG* locus and the *MYC* locus. For the *IG* loci, three-color FISH hybridizations were performed using constant (C) region probes that include strong enhancer sequences, variable (V) region probes, and whole chromosome painting probes. For the *MYC* locus, two-color FISH hybridizations were performed using the *MYC* and wcp8 probes. For translocation partners easily identified by their morphology, a second round of FISH hybridization was performed using a wcp probe and a specific target gene probe. For complex rearrangements, sequential SKY and FISH hybridizations were performed on the same metaphase chromosomes.

RESULTS

Hyperdiploid and Nonhyperdiploid Tumors

The 48 advanced tumors studied were subdivided into two groups based on the metaphase chromosome number (Table 1). The first 22 tumors, which have <48 and/or >75 chromosomes, were classified as NHRD. The remaining 26 tumors, which have 48–75 chromosomes, were classified as HRD. Consistent with published studies, extra copies or partial copies of eight odd-numbered chromosomes (3, 5, 7, 9, 11, 15, 19, and 21) occur frequently in HRD tumors, but only infrequently in NHRD tumors. Extra copies of at least four of these chromosomes are present in 24 of 26 HRD tumors, with the two exceptions having the minimum (#48, 48 chromosomes) or close to the maximum (#23, 70 chromosomes) number of chromosomes that define this group. By contrast, only two (#1, 13) of 22 NHRD tumors had extra copies, or partial copies, of at least four of these chromosomes. Curiously, one NHRD tumor (#1) has only 32 chromosomes, with two copies of each of the eight odd chromosomes and only one copy of most other chromosomes (see Discussion).

Four Types of IGH Rearrangements in MM Tumors

Nine of the 48 tumors (#6, 7, 10, 11, 15, 31, 34, 46, and 48) had *IGH* rearrangements characterized previously by conventional cytogenetic and SKY analyses, and four other tumors were shown to have an add(14)(q32) (#12, 17, 20, and 22) (Supplemental Table 1) (Sawyer et al., 1998, 2001). For a comprehensive analysis, we subjected all 48 tumor samples to specific *IGH*FISH analyses. An initial screen, using a CH probe, which hybridizes with *Ea1* and *Ea2* enhancer sequences, a distal VH probe located within 100 kb of the 14q telomere, and a wcp14 probe revealed 36 *IGH* rearrangements in 26 tumors

(Table 1). There were four kinds of rearrangements: (1) classical balanced *IGH* translocations; (2) variant *IGH* translocations; (3) unbalanced (but nonvariant) *IGH* translocations; and (4) *IGH* insertions.

Classical balanced (B) *IGH* translocations are distinguished by a FISH hybridization pattern of dissociated CH and VH signals, with CH remaining on der(14) and VH relocating to the telomere of the other derivative (Fig. 1B and D). Tumors #2, 4, 5, 8, 9, 10, 14, 18, 23, 37, and 47 have the karyotypically cryptic t(4;14)(p16;q32) with the *FGFR3* gene colocalizing with CH on der(14), while VH is on der(4) (Fig. 1D). Other balanced classical translocations, which were confirmed by FISH analyses, were found in tumors #6 and 12 (*CCND1*), #7 and 15 (*MAF*), #10 (*NMYC*), #22 (*MYC*) and #11, 17, 46, and 48 (unidentified target genes).

Variant (V) *IGH* translocations are distinguished by a FISH hybridization pattern in which the VH and CH probes, without wcp14, colocalize at the telomere of another chromosome, and der(14) has no CH signal. One tumor (#34) had a balanced t(3;14)(q?27;q32) variant *IGH* translocation, with der(3) having CH and VH signals colocalizing at the telomere, and der(14) lacking CH or VH signals. Three other tumors (#17, 21, and 46) had unbalanced variant *IGH* translocations, with no der(14), but VH and CH probes colocalizing at the telomeric end of another chromosome (adjacent to *MYC* at 8q24.1 in tumors #17 and 46) (Fig. 1C). None of the variant *IGH* translocations would be detected by interphase FISH analyses designed to identify dissociation of the VH and CH probes. In addition, it should be noted that it is only the balanced variant *IGH* translocation that might be suspected by conventional cytogenetic or SKY analyses.

Excluding variant translocations, unbalanced (UB) *IGH* translocations are distinguished by a FISH hybridization pattern in which there is either a der(14) having a CH signal or another chromosome having a telomeric VH signal. This kind of unbalanced translocation was found in four tumors. Tumor #31 had a CH signal on der(14)t(1;14)(q11~12;q32), but no der(1) with VH. Tumors #17 and #44 had CH signals at the telomere of der(14) but no detectable chromosome sequences telomeric to CH, and no derivative with VH, which limits our ability to identify the partner chromosomes or target genes. Tumor #5 had no der(14) with CH, but two copies of VH colocalize with the *MMSET* gene, one copy on der(4) and the other on a more complex derivative chromosome.

The final group of *IGH* rearrangements involves simple insertions (I). The CH signals, which are dissociated from telomeric VH signals, are found internally on recipient chromosomes, usually without a detectable wcp14 signal. In half of the tumors that have a simple CH insertion (#11, 34, 42, and 46), the inserted CH probe colocalized with the *MYC* gene at 8q24. In tumor #8 the recipient chromosome is 17, but a target gene has not been identified. In tumors #12 and 21 our FISH and SKY analyses failed to classify the recipient chromosome because of poor metaphase quality, although we were able to eliminate *MYC* as the target gene. In tumor #47, which also has a balanced t(4;14) (see above), inserted CH sequences colocalized with *CCND1* at 11q13 (Fig. 1E). In this tumor we therefore have identified two independent, karyotypically silent *IGH* rearrangements, neither of which was detected by conventional cytogenetic or SKY analyses.

Remodeling of Chromosomes Carrying *IGH* Rearrangements

In each of the four kinds of *IGH* rearrangements described above, we have found tumors with complex derivative chromosomes that result from further rearrangements of translocated chromosomes. New breakpoints may occur far from the *IGH* rearrangement, and thus should not affect the juxtaposed target gene and CH sequences (e.g., tumor #17). Alternatively, new breakpoints may occur in very close proximity to the *IGH* rearrangement,

thereby masking the target gene juxtaposed to CH sequences (tumors #5, 12, 20, and 46) (Fig. 2C). Tumor #20 has a complex, balanced translocation, which the conventional cytogenetic analysis detected as an add(14)(q32). Our initial FISH screen revealed one CH signal on der(14)t(4;14) (q22;q32), and one VH signal on an unknown small chromosome (not shown). A subsequent hybridization confirmed a der(14)t(4;14) but no colocalizing CH and *FGFR3* signals (Fig. 2A). Sequential SKY analysis was carried out on the same metaphase chromosomes, which revealed both that the VH sequences are on der(20) and that there is a thin strip of chromosome 20 sequences at the t(4;14) breakpoint (Fig. 2B). A final hybridization confirmed colocalization on der(14)t(14;20)(q32;q11) t(4;20)(q22;q?) of CH and *MAFB* (not shown). It appears that an initial t(14;20)(q32;q11) juxtaposed CH and *MAFB* sequences, resulting in the presumptive dysregulation of *MAFB*, and that der(14) subsequently underwent a second translocation with chromosome 4.

***IGH* Rearrangements in HMCL**

FISH analyses showed that 43 of 47 HMCL had 68 different *IGH* rearrangements, whereas the other four HMCL had *IG* light chain rearrangements (Table 2 and below). The same four kinds of structural abnormalities identified in MM tumors also were identified for the HMCL.

Classical balanced translocations (B) juxtapose CH sequences mostly to recurrent target genes, *FGFR3/MMSET* (13 cases), *CCND1* (four cases), *MAF* (five cases), *MAFB* (three cases), and *MYC* (seven cases); but also to unidentified genes in XG-2 and in OCI-MY7, which also had an unbalanced translocation involving *CCND1*. XG-7 has a complex, apparently balanced classical t(4;14), with der(4) having colocalizing telomeric *MMSET* and VH sequences, but with der(14) having colocalizing CH, *IRF4* and 4p telomeric sequences, but no *FGFR3* sequences. It seems likely that the original t(4;14) in XG-7 generated a der(14) that was modified by an insertion of *IRF4* (6p25) and deletion of *FGFR3*.

Five variant *IGH* translocations (V) were found in HMCL, four of which were unbalanced (*IRF4* in FR4; *MYC* in LP1 and XG-1; unknown target gene in LP1), and one of which was balanced (t(14;17) in Kp6). The balanced translocation in XG-2 is similar to a balanced variant translocation since it has both CH and VH signals on der(12). However, it does not meet our definition of a variant *IGH* translocation since it has a CH signal on der(14). In any case, 3' *IGH* enhancer sequences potentially could dysregulate a gene on der(14), der(12), or both in XG-2.

Excluding variant *IGH* translocations, approximately one fourth of *IGH* translocations are unbalanced (U). Target genes include *MMSET* (one case), *CCND1* (four cases), *CCND3* (one case), *MAF* (one case), *IRF4* (two cases), and *MYC* (two cases). In addition, both 8226 and Karpas-620 have CH signals on der(14)t(1;14)(q12;q32), but a target gene has not been identified. Of the unbalanced translocations, about half are highly complex, resulting in rearranged derivative chromosomes, in which the CH sequences can be juxtaposed to more than one target gene. For example, Karpas-620 has a der(14)t(8;14) with CH and *MYC* sequences, and a der(8)t(8;11) with CH, *MYC*, and *CCND1* sequences (Fig. 4A). More complete FISH mapping analyses (not shown) suggest that der(14)t(11;14) underwent a subsequent translocation involving the *MYC* locus that generated the two chromosomes described above. See Figure 4A for other examples of complex unbalanced *IGH* translocations.

There are two kinds of *IGH* insertions (I) in the HMCL. First, CH sequences without associated wcp14 are inserted at a single site in an apparently normal chromosome region, e.g., FR4, JJJ-3, KMS-12PE, MM-S1, and U266. Second, CH sequences with or without

associated wcp14 are inserted at the breakpoint of two chromosomes, e.g., EJM, MM.1, OPM-1, and KMS-11 (Fig. 4A). For JJN-3, the telomeric end of chromosome 8 moves onto the p arm of chromosome 14, with CH and *MYC* juxtaposed at the breakpoint (Fig. 4A). For KMM-1, FISH analyses and cloning results show that chromosome 21 (without wcp21) and *MYC* (without wcp8) sequences are inserted into the *IGH* locus at 14q32 (Shou et al., 2000).

***IGL* Rearrangements in MM Tumors**

Conventional cytogenetic and SKY analyses detected *IGL* rearrangements in 5 of 48 tumors (#7, 13, 14, 32, and 40) (Supplemental Table 1). FISH analyses of metaphase chromosomes from all 48 tumor samples revealed nine *IGL* rearrangements in eight tumors (Table 1), so that three tumors had karyotypically silent rearrangements.

Similar to *IGH* rearrangements, there are four kinds of *IGL* rearrangements (Fig. 3). Classical balanced *IGL* translocations (B) are distinguished by a FISH hybridization pattern that identifies dissociation of V λ and C λ signals, with V λ remaining on der(22) and C λ relocating to the breakpoint of the other derivative chromosome. Only one tumor sample (#13) had a balanced (B) translocation, and this involved *MYC*. A variant *IGL* translocation (V) is distinguished by a FISH hybridization pattern in which der(22) retains both V λ and C λ sequences. Only one tumor (#40) had a variant *IGL* translocation, and this was an unbalanced variant translocation that involved *MYC*. Excluding the tumor with the variant translocation, five tumors have unbalanced translocations (U) in which there was no der(22), but only another chromosome derivative with a C λ signal. The target genes in these tumors included *MYC* (tumors #14 and 44), *CCND3* (tumor #32), and unknown genes (tumors #7 and 40). Finally, two tumors (#18 and 36) had insertions (I) of C λ sequences without a detectable wcp22 signal within chromosome 8, and juxtaposed to *MYC* at 8q24.1.

***IGL* Rearrangements in HMCL**

Using the same hybridization techniques and *IGL* probes described earlier, we found that 8 of 47 HMCL analyzed had 12 distinct *IGL* rearrangements (Table 2, Fig. 4B). Four HMCL have both light and heavy chain rearrangements: 8226, KMS-26, OCI-MY1, and XG-2, but four HMCL have only light chain rearrangements: Delta-47, KHM-1B, L363, and XG-6.

Delta-47, KMS-26, and L363 have balanced translocations with breakpoints at 11p14, 8q24 (*MYC*), and 17q21 (*NIK*), respectively. The rearrangement in L363 has a cloned breakpoint 6.8 kb upstream of C λ 1, ~48 kb from the 3' C λ enhancer (Annunziata et al., 2007). OCI-MY1, 8226, and XG-6 had unbalanced translocations, with the der(22) and associated V λ signal being absent in all three lines. In OCI-MY1, C λ and *MYC* were juxtaposed at 8q24 on der(8). In 8226, C λ and *MAF* were juxtaposed at 16q23 on der(16), and there was associated over-expression of *MAF* (Chesi et al., 1998a). In XG-6, C λ is juxtaposed to BAC sequences that include *MAF* at 16q23 on der(16). We did not, however, find over-expression of *MAF* in XG-6, suggesting that a different unidentified gene may be the target for dysregulation (Kuehl, unpublished).

Delta-47, KHM-1B, and XG-2 have simple insertions that are detected by C λ probe signals, without associated wcp22 probe signals. In Delta-47 and KHM-1B, C λ signals were juxtaposed near *MYC* at 8q24, with an additional C λ insertion at 9p24 also detected in Delta-47. Finally, in XG-2, a C λ signal was inserted near *MAFB* at 20q11.

In addition to insertions of C λ sequences into other chromosomes, two HMCL have insertions of other genes near a C λ sequences. In 8226, *MYC* sequences without an associated wcp8 signal were found at the breakpoint of the der(16)t(16;22) unbalanced translocation (Fig. 4B). This suggests that the t(16;22) that dysregulates *MAF* occurred initially, but was followed by insertion of a *MYC* gene so that both *MAF* and *MYC* are

dysregulated by Cλ 3' enhancer sequences on der(16)t(16;22). In XG-2, the over-expression of *CD40* was reported to be associated with insertion of the *CD40* gene near Cλ and Vλ sequences on chromosome 22 (Keats et al., 2007). Importantly, the detection of both of these insertions required an assay that used target gene specific probes, and could not have been detected by assays that used only *IGL* probes (similar to *MYC* insertion into *IGH* locus in KMM-1 or *IRF4* insertion replacing *FGFR3* on der(14) in XG-7).

MYC Rearrangements in MM Tumors

We confirmed *MYC* abnormalities in 7 of 48 tumors (#4, 7, 13, 14, 26, 43, and 47) and clarified or corrected cytogenetic and SKY findings in three other tumors (#22, 37, and 44) (Supplemental Table 1). We also identified new *MYC* rearrangements in 11 other tumors (#5, 11, 17, 18, 29, 34, 36, 38, 40, 42, and 46). For tumor #10, we confirmed a classical balanced t(2;14)(p23;q32) that results in the juxtaposition of CH and *NMYC* sequences.

The *MYC* rearrangements, which are found in 22 of 47 MM tumors, include involvement of the following: (1) an *IGH* locus (#10, 11, 17, 22, 34, 42, and 46); (2) an *IGL* locus (#13, 14, 18, 36, 40, and 44); (3) an *IGK* locus (#7); or (4) no detectable *IG* sequences (#4, 5, 26, 29, 37, 38, 43, and 47) (Table 1). The eight tumors without apparent involvement of an *IG* locus deserve further comment. First, tumors #4, 5, 43, and 47 have simple unbalanced translocations, three of which involve chromosome 1. Second, tumors #29 and 38 have *MYC* sequences without a wcp8 signal inserted into un-identified chromosomes. Third, tumor #37 had three highly complex rearrangements that involve *MYC*, suggesting that sequential breaks and reunions involving the *MYC* locus occurred, giving rise to these complex derivative chromosomes. Finally, tumor #26 has three copies of an i(8)(q10) and thus a *MYC* amplification without any evidence for a rearrangement near the *MYC* gene.

MYC Rearrangements in HMCL

Previously, we have characterized in detail the *MYC* rearrangements found in 30 HMCL (Table 2 *MYC*) (Shou et al., 2000; Dib et al., in press). We extended our FISH analyses to encompass the remaining 17 HMCL available to us, and identified partner chromosomes that were previously reported as unknown. For example, we found here that Delta47 has a Cλ insertion into der(19)t(8;19). Overall, FISH hybridizations reveal *MYC* rearrangements in 39 of 47 HMCL, plus one *LMYC* rearrangement (U266) and one *NMYC* rearrangement (PE-2). Six HMCL (ANBL6, EJM, H1112, KP-6, PE-1, and XG-6) have no karyotypic abnormality of a *MYC* gene, but array CGH analyses detected rearrangements near the *MYC* gene in two HMCL (EJM and XG-6) (Dib et al., in press).

Rearrangements that involve the *IGH* locus (19 HMCL), the *IGL* locus (5 HMCL), and the *IGK* locus (inversion to *NMYC* in PE-2) have been described earlier or in Table 2 (see also Fig. 4). For 14 HMCL that have *MYC* rearrangements not involving an *IG* locus, we find balanced and unbalanced translocations as well as simple and complex insertions (Table 2 and Fig. 4B), with some of these results having been presented in detail elsewhere (Shou et al., 2000; Dib et al., in press). One HMCL (UTMC-2) has two different chromosomes: i(8)(q10) and der(5)t(5;8)(?:q11), both of which have an ~500 kb deletion immediately telomeric to *MYC*. Finally, in the U266 HMCL, which expresses *LMYC* but not *MYC*, *LMYC* was found to have been relocated from 1p34 close to the centromere at 1p13 as the result of a translocation, with an associated inversion.

DISCUSSION

Identification of *IG* and *MYC* Genomic Rearrangements in MM Tumors and Cell Lines

A comprehensive analysis of *IG* and *MYC* rearrangements was performed on 48 advanced MM tumors and 47 independent HMCL. Selection of tumors was dependent on the availability of an abnormal karyotype and enough additional metaphase chromosome material to enable multiple metaphase FISH analyses. Unfortunately, most tumors with a t(11;14) were not included in the samples available for analysis. The initial strategy was to do separate three-color metaphase FISH analyses for the three *IG* loci and the *MYC* locus. Additional FISH and SKY analyses—sometimes done sequentially on marked metaphase spreads—were used to characterize more fully rearrangements detected in the initial analyses, as well as to identify potential target genes for dysregulation by strong enhancer elements found in *IG* loci. In all HMCL and the few tumors where expression microarray data were available, we confirmed over-expression of target genes identified by FISH (Kuehl and Shaughnessy, unpublished). Apart from insertion of unknown sequences, inversions, and small (less than 5 Mb) deletions, or amplifications, most nonphysiological *IG* and *MYC* rearrangements should be detected by this strategy.

Defining Hyperdiploid and Nonhyperdiploid Tumors

The classification of tumors strictly by chromosome number instead of chromosome content is problematic (Table 1, Results), particularly since we do not understand the molecular basis for this classification (Chng et al., 2006, 2007). Perhaps this is illustrated best by tumor #1, which is markedly hypodiploid (32 chromosomes), and thus classified as a NHRD tumor. However, this tumor has single copies of most chromosomes, but extra copies of all eight odd-numbered chromosomes, with the chromosomal imbalance suggesting that this tumor might have a “HRD” phenotype. This extreme hypodiploid phenotype is associated with a similar kind of chromosome imbalance in other tumors: one tumor included cells with 31 or 61–63 chromosomes, both associated with extra copies of chromosomes 3, 7, 9, 11, 15, and 19 (Sawyer, unpublished); a second tumor (#129) included cells with 30 or 57–58 chromosomes, both associated with extra copies of at least five of the eight odd-number chromosomes (Smadja et al., 2001); and a third tumor (#21) had 30 chromosomes, again with extra copies of at least five of the eight odd-numbered chromosomes (Avet-Loiseau et al., 2001a). As summarized elsewhere, HMCL appear to be selectively generated from NHRD tumors, and it is possible that some “HRD” HMCL are not generated from HRD tumors (Bergsagel et al., 2005).

Prevalence of *IGH* Rearrangements in MM Tumors and Cell Lines

Similar to results published by others, *IGH* rearrangements were identified in 26 (54%) of 48 tumors (Table 1) (Avet-Loiseau et al., 2002; Fonseca et al., 2003a, 2004). As expected, there was a marked difference in the prevalence of *IGH* rearrangements in NHRD versus HRD tumors (Fonseca et al., 2003b; Smadja et al., 2003). *IGH* rearrangements were present in 17 (77%) of 22 NHRD tumors, but only nine (35%) of 26 HRD tumors. More strikingly, four (4p16, *FGFR3/MMSET*; 11q13, *CCND1*; 16q23, *MAF*; 20q12, *MAFB*) of the five recurrent *IGH* rearrangements were present in 13 (59%) of the 22 NHRD tumors [18(67%) of 27 assuming sample selection excluded the analysis of five tumors with a t(11;14)], but only three (12%) of 25 HRD tumors. In contrast, *IGH* rearrangements were identified in 43 (91%) of 47 HMCL, and the five recurrent rearrangements were identified in 34 (72%) of 47 HMCL (Table 2), consistent with the hypothesis that most HMCL are generated from NHRD tumors (Bergsagel et al., 2005).

Excluding the five recurrent *IGH* rearrangements, there are 19 additional *IGH* rearrangements in 13 (27%) of the 48 tumors. Seven (37%) of these 19 rearrangements

involve a *MYC* gene. However, unlike the recurrent *IGH* rearrangements, these *IGH* rearrangements have a similar prevalence in NHRD and HRD tumors, with 11 in seven (32%) of 22 tumors (0.50/tumor) and 8 in six (23%) of 26 tumors (0.32/tumor), respectively. Significantly, reexamination of SKY analyses on 150 MM tumors identified apparent *IGH* rearrangements not involving the five recurrent partners with the same prevalence (0.28 per tumor) in HRD and NHRD tumors (Sawyer et al., 1998, 2001). In addition to the 35 *IGH* rearrangements that affect the five recurrent partners, there are 33 additional *IGH* rearrangements in 27 of the 47 HMCL (0.72/HMCL). Nineteen (56%) of these 33 rearrangements involve a *MYC* gene. The higher prevalence of *IGH* rearrangements that do not involve one of the five recurrent partners in HMCL compared to primary tumors is consistent with the hypothesis that they represent secondary *IGH* rearrangements that occur during tumor progression.

Prevalence of *IG* Light Chain Rearrangements in MM Tumors and Cell Lines

We detected 10 *IG* light chain (nine immunoglobulin lambda *IGL*) rearrangements in eight (17%) of the 48 MM tumors (0.21/tumor), with five each in NHRD and HRD tumors. Seven rearrangements involved the *MYC* gene, and one involved *CCND3*, a recurrent *IGH* translocation partner. In addition, we detected 13 *IG* light chain (12 *IGL*) rearrangements in nine (19%) of the 47 HMCL (0.28/HMCL). Five *IG* light chain rearrangements involved *MYC*, one involved *NMYC*, and two (*MAF* and *MAFB*) are recurrent *IGH* translocation partners. The *IG* light chain rearrangements are three- to fivefold less prevalent than *IGH* rearrangements. Also, immunoglobulin kappa (*IGK*) rearrangements appear to be ~10-fold less frequent than *IGL* rearrangements. Although our estimate of *IGK* rearrangements might be somewhat low since we only did FISH analyses for *IGK* rearrangements in 20 tumors and 20 HMCL, SKY analyses on 150 MM tumors identified apparent rearrangements of *IGL* in 24 tumors (15 involving *MYC*) and *IGK* in two tumors (both involving *MYC*) (Sawyer et al., 1998, 2001).

Prevalence of *MYC* Rearrangements in MM

MYC rearrangements were identified in 22 (46%) of 48 MM tumors, and were equally distributed between the NHRD (43%) and HRD (48%) groups, a result that is consistent with reported SKY analyses on 150 MM tumors (Sawyer et al., 1998, 2001). Among the 22 *MYC* rearrangements (21 *MYC* and one *NMYC*), seven involved an *IGH* locus (32%), six involved an *IGL* locus (27%), one involved an *IGK* locus (2%), and eight did not appear to involve an *IG* locus (32%). The recurrent involvement of 1p11~13 (tumors #4, 5, and 43; plus six published tumors), and to a lesser extent 6q21 (tumors #37 and 47; plus five published tumors) (Lewis and MacKenzie, 1984; Sawyer et al., 1998, 2001; Smadja et al., 1998), are the only apparent recurrent non-*IG* chromosomal loci involved in *MYC* rearrangements. *MYC* rearrangements were identified in 41 (87%) of 47 HMCL, although two additional HMCL (EJM, XG-6) had *MYC* rearrangements detected by array CGH (Dib et al., in press). Among the 41 *MYC* rearrangements (39 *MYC*, one *LMYC*, and one *NMYC*), 19 rearrangements involved the *IGH* locus (46%), five involved the *IGL* locus (12%), one involved the *IGK* locus (2%), and 16 had no apparent involvement of an *IG* locus (39%). These *MYC* rearrangements have additional complexities, often with involvement of three chromosomes and sometimes with associated amplification, duplication, or inversion (Shou et al., 2000) (also Tables 1 and 2). More than a third of *MYC* abnormalities detected in the MM tumors and HMCL would not have been detected by either conventional cytogenetic or SKY analyses, and only half of the rearrangements not involving an *IG* locus would have been detected by interphase FISH analyses using probes that flank the *MYC* locus.

Anatomy of IG Rearrangements

As described earlier, we have classified nonphysiological rearrangements of the *IG* loci into four groups: classical balanced rearrangements (B); variant rearrangements (V); unbalanced but nonvariant rearrangements (U); and insertions (I). Balanced rearrangements can be either reciprocal translocations or inversions (e.g., *IGK/NMYC* inversion in PE-2, Table 2). We use the term “variant” *IGH* rearrangement when there is no dissociation of V and C probes on either derivative chromosome, as depicted in Figure 1. This variant configuration has not been described for primary *IG* translocations in other kinds of B cell tumors, and has not been found among the 52 *IGH* rearrangements that involve the five recurrent partners in MM tumors and HMCL (Tables 3 and 4). However, for rearrangements not involving the five recurrent partners, we find that eight of 53 *IGH* rearrangements have the variant configuration.

There is a striking difference in the distribution of the four kinds of rearrangements for *IGH* translocations involving the five recurrent partners compared to all other *IGH* and *IG* light chain rearrangements (Tables 3 and 4). The former have a much higher proportion of balanced translocations, and a much lower proportion of rearrangements having the variant or insertion configuration.

Multiple Independent Aberrant IG Rearrangements in a Tumor Cell

Two or more independent *IGH* rearrangements have been thought to be rare in MM tumors (Avet-Loiseau et al., 2002). However, we found nine of 48 advanced MM tumors and 19 of 47 HMCL with two or more *IGH* rearrangements, and five HMCL with three or more different *IGH* rearrangements. Coincident *IGH* and *IG* light chain rearrangements in the same tumor cell also occur, with one tumor (#7) having rearrangements that involve each of the three *IG* loci.

Although our finding that nearly 20% of advanced MM tumors have two *IGH* rearrangements is unexpected, we note that in one study, two of 59 MGUS/SMM tumors were found to have two different *IGH* rearrangements (Fonseca et al., 2002). For most tumors with two different *IGH* rearrangements, there is involvement of one of the five recurrent *IGH* partners plus a *MYC* gene (Tables 1 and 2). Rarely, however, two of the five different recurrent *IGH* partners are involved in *IGH* rearrangements in the same tumor cell, i.e., t(4;14) plus insertion of 3' E α near *CCND1* in tumor #47 (Table 1); and t(4;14) plus t(14;16) in KMS-11 HMCL (Table 2). Other published examples include two MGUS/SMM tumors with t(4;14) plus t(14;16) or t(4;14) plus a fraction of cells with t(11;14) (Fonseca et al., 2002); and t(14;16) plus t(6;14) in one of 150 MM tumors analyzed by SKY (Sawyer et al., 2001). A sixth MM tumor most likely has t(11;14) plus t(14;20) based on the marked over-expression of *CCND1* and *MAFB* by microarray analysis (Shaughnessy, unpublished). Thus, all combinations of the three types of recurrent rearrangements (4p + a *MAF* gene or a *CCND* gene, and a *CCND* gene plus a *MAF* gene) have been seen, suggesting that there can be at least partial complementation between these different translocation partners.

Timing of IG and MYC Rearrangements in MM

IGH rearrangements that involve the five recurrent partners in MM are thought to represent primary translocations that are mediated by errors in *IGH* switching or somatic hypermutation as B cells pass through germinal centers (Bergsagel and Kuehl, 2001; Gabrea et al., 2006). We have summarized detailed evidence supporting the hypothesis that *MYC* (*MYC* > *NMYC* > *LMYC*) rearrangements in MM, either with or without juxtaposition to an *IG* locus, are very late progression events that occur at a time when tumors are becoming more proliferative and less stromal cell dependent (Dib et al., in press). The findings reported here support this hypothesis, since *MYC* rearrangements were identified in nearly

90% of HMCL and about 45% of advanced, intramedullary MM tumors, most of which have a high proliferation index as determined from expression arrays. By contrast, it has been reported that *MYC* rearrangements are rare in MGUS or smoldering MM (SMM), but are present in about 15% of MM tumors at the time of diagnosis (Avet-Loiseau et al., 2001b). Therefore, *MYC* rearrangements provide a paradigm for secondary genomic rearrangements not involving the mechanisms responsible for B-cell-specific DNA modifications.

We have shown here that the five recurrent *IGH* rearrangements are mostly simple balanced translocations, whereas all other *IGH*, *IG* light chain, and *MYC* rearrangements are mostly insertions, and variant or unbalanced translocations. Moreover, in contrast to the five recurrent *IGH* rearrangements that occur predominantly in NHRD tumors, all other *IGH*, *IG* light chain, and *MYC* rearrangements occur with a similar frequency in NHRD and HRD tumors. Unlike *MYC* rearrangements, *IG* light chain rearrangements and *IGH* rearrangements not involving one of the five recurrent partners occur with a significant frequency in MGUS (Fonseca et al., 2002). Therefore, we suggest that *IG* light chain rearrangements, *IGH* rearrangements not involving one of the five recurrent partners, and perhaps some *IGH* rearrangements involving the five recurrent partners (especially tumors with two recurrent *IGH* translocations, see above) represent secondary rearrangements that can occur at all stages of pathogenesis, including MGUS tumors (Fig. 5).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Structures of *IGH* translocations in MM tumors. (A) The 1 Mb *IGH* locus is depicted with boxed coding regions, enhancer elements, and direction of transcription (arrow). Eα1 and Eα2 enhancers control gene expression bidirectionally (flanking arrowheads), but insulator sequences (thick vertical lines) may block centromeric effects. Eμ is the bidirectional intronic enhancer. The VH cosmid (red line) located 100 kb from the telomere is essentially a 14q tel probe. The CH BAC (green line) detects Eα1 and Eα2 sequences. (B) FISH analysis detected a classical balanced t(8;14) in the ARK HMCL. CH (green) and MYC (red) colocalize on der(14) (purple). The diagram depicts a breakpoint centromeric to MYC (8q24) and telomeric to the Eα1 enhancer (shaded box) that presumably dysregulates MYC. (C) FISH analysis detected a variant unbalanced t(8;14) in tumor #17. CH (green) and VH (red) colocalize on mod der(8) (C1). MYC (red) is juxtaposed to CH (green) on mod der(8) (purple) (C2). The diagram depicts a breakpoint telomeric to MYC and centromeric to Eα2; MYC may be juxtaposed and thus dysregulated either by an inverted Eα2 enhancer or an Eα2 enhancer with deleted insulator sequences (alternative shaded boxes). (D, E) FISH detection of classical t(4;14) and CH insertion at 11q13 in tumor #47. (D) One copy of CH (green) colocalizes with VH (green) on normal chromosome 14, a second copy of CH colocalizes with *FGFR3* (red) on der(14), one copy of VH is on der(4) (purple), and one copy of *FGFR3* is on chromosome 4 (purple) (E). A third copy of CH (green) is shown to

colocalize with *CCND1* (red) on der(11) (purple), while a second copy of *CCND1* is on normal chromosome 11 (purple).

Figure 2. Complex chromosome rearrangements in MM tumors. Sequential FISH (A) and SKY (B) analysis of the complex *IGH* translocation t(14;20)t(4;20) in tumor #20. Metaphase chromosomes were hybridized with CH (green), VH (green), *FGFR3* (red), and wcp4 (purple). The arrowhead shows a copy of CH at the t(4;14) breakpoint, while the arrow indicates a copy of VH on a small chromosome. The same metaphase chromosomes were stripped and rehybridized with SKY probes to show that the VH is on der(20) (arrow) and that chromosome 20 sequences are inserted at the t(4;14) breakpoint (arrowhead). Examples of chromosomes with rearrangements of the *IGH* (C), *IGL* (D), and *MYC* (E) loci are shown for selected MM tumors. Relative positions of CH, VH, CL, and target gene sequences (*MAFB*, *CCND1*, *MMSET*, *MYC*) are indicated.

Figure 3.

Structures of *Igλ* translocations in MM tumors. (A) The 1 Mb *IGL* locus is depicted with boxed coding regions and direction of transcription (arrow). The 3' enhancer (E) is depicted as controlling gene expression bidirectionally, except for putative telomeric insulator sequences (thick vertical line). The Vλ cosmid (red line) detects centromeric sequences, the Cλ-BAC detects JC and 3'E sequences (green line) and Cλ-cos detects only the 3'E. (B) FISH analysis detected a classical balanced t(8;22) in tumor #13. Cλ (green) and telomeric 22 sequences (purple) translocate to der(8) (B1). Cλ (green) colocalizes with MYC (red) on der(8) (purple) (B2). (C) FISH analysis detected a variant unbalanced and complex t(8;22) in tumor #40. Cλ (green) and Vλ (red) colocalize on der(22) (purple) (C1). MYC (red) is juxtaposed to Cλ (green) on der(22) (C2).

Figure 4.

Complex chromosome rearrangements in HMCL. Examples of chromosomes with complex rearrangements of the *IGH* (A), *IGL* (B), and *IGK* (C) loci are shown for selected HMCL. Relative positions of CH, VH, C λ , C κ , and target gene sequences (*CCND1*, *MAF*, *MYC*, and *NMYC*) are indicated. Karyotypic abnormalities for the *MYC* locus that do not involve an *IG* locus are shown (D).

Figure 5. Primary and secondary genomic rearrangements during MM pathogenesis. There are two proposed pathways of pathogenesis for MM: hyperdiploid (HRD) and nonhyperdiploid (NHRD). Primary translocations that are mediated mainly by errors in *IGH* switch recombination or somatic hypermutation in germinal center B cells occur mostly in NHRD tumors. Secondary *IG* genomic rearrangements (TLC) that can occur at all stages of pathogenesis and *MYC* genomic rearrangements that occur at late stages of pathogenesis have a have a similar prevalence in HRD and NHRD tumors.

TABLE I
Numeric Chromosomal Abnormalities and Structural Abnormalities of *IG* or *MYC* Loci in 48 MM Tumors

Trisomies ^a													<i>IG</i> and <i>MYC</i> Rearrangements		
Sort#	Chr#	3	5	7	9	11	15	19	21	Locus ^b	Target gene ^c	Breakpoints (BP) ^d	Derivatives ^e		
1	32	D	d	d	D	D	D	D	D						
2	36					t				<i>IGH</i>	<i>FGFR3, MMSET</i>	4p16 :: 14q32	B: der(4), der(14)		
3	39					t									
4	41									<i>IGH</i>	<i>FGFR3, MMSET</i>	4p16 :: 14q32 1p11~13 :: 8q24	B: der(4), der(14) U: der(1)		
5	42	t			T				T	<i>IGH</i>	<i>MMSET</i>	4p16 :: 14q32 1? :: 8q24	U*: der(4), mod der(14) see Fig.2C U*: mod der(1) see Fig.2E		
6	42									<i>IGH</i>	<i>CCND1</i>	11q13 :: 14q32	B: der(11), der(14)		
7	43								T	<i>IGH</i>	<i>MAF</i>	14q32 :: 16q23 Xp? :: 22q11	B: der(14), der(16) U: der(X)		
										<i>IGK</i>	<i>MYC</i>	2p11 :: 8q24	U: der(8)		
8	43									<i>IGH1</i>	<i>FGFR3, MMSET</i>	4p16 :: 14q32	B: der(4), der(14)		
										<i>IGH2</i>	?	ins CH @ 17q?21 on 17	I: 17 (wcp17+, wcp14-, CH+)		
9	43									<i>IGH</i>	<i>FGFR3, MMSET</i>	4p16 :: 14q32	B: der(4), der(14)		
10	44									<i>IGH1</i>	<i>FGFR3, MMSET</i>	4p16 :: 14q32	B: der(4), der(14)		
										<i>IGH2</i>	<i>NMYC</i>	2p23 :: 14q32	B: der(2), der(14)		
11	45								T	<i>IGH1</i>	?	14q32 :: 22q13	B: der(14), der(22)		
										<i>IGH2</i>	<i>MYC</i>	ins CH @ 8q24 on 8	I: 8 (wcp8+,MYC+, wcp14-, CH+)		
12	45									<i>IGH1</i>	<i>CCND1</i>	11q13 :: 14q32	B*: der(11), der(14), der(?) see Fig.2C		
										<i>IGH2</i>	?	ins CH @ ?	I: ? (wcp14-, CH+)		

Trisomies ^a													IG and MYC Rearrangements		
Sort#	Chr#	3	5	7	9	11	15	19	21	Locus ^b	Target gene ^c	Breakpoints (BP) ^d	Derivatives ^e		
13	45		T	T			t		T	IGH	MYC	8q24 :: 22q11	B: der(8), der(22)		
14	45						T			IGH	FGFR3, MMSET	4p16 :: 14q32	B: der(4), der(14)		
										IGH	MYC	ins CA @ 8q24 on der(8)t(8;?11)(q24;? p13)	I*: der(8) (wcp8+,MYC+,wcp22-,CA+) ×2		
15	45									IGH	MAF	14q32 :: 16q23	B: der(14), der(16)		
16	45														
17	46			t	T			T		IGH1	?	7q32 :: 14q32	B: der(7), der(14)		
										IGH2	MYC	8q24 :: 14q32	V (U*): mod der(8) (wcp8+, MYC+, wcp14 -, CH +, VH+)		
										IGH3	?	? :: 14q32	U: der(14)		
18	47					T	T			IGH	FGFR3, MMSET	4p16 :: 14q32	B: der(4), der(14)		
										IGH	MYC	ins CA @ 8q24 on 8	I: 8 (wcp8+,MYC+, wcp22-, CA+)		
19	76			t											
20	76			t	tt	t				IGH	MAFB	14q32 :: 20q11	B*: der(14), der(20) see Fig.2C		
21	77			t		TT		T		IGH1	?	? :: 14q32	V (U): der(?) (CH+,VH+)		
										IGH2	?	ins CH @ ?	I: ? (wcp14 -, CH+)		
22	78									IGH	MYC	8q24 :: 14q32	B: der(8), der(14)		
23	70			T				T	t	IGH	FGFR3, MMSET	4p16 :: 14q32	B: der(4), der(14)		
24	70	T	T	T			Tt	T							
25	60	T	T	T	T	TTt	TTT	T	TT						
26	56	T	t	T	t		tt		T	?	MYC	8q10 :: 8q10	Amplification MYC: i(8)(q10) ×3		
27	55	t	t	T		T	TT	T							

		Trisomies ^a										IG and MYC Rearrangements		
Sort#	Chr#	3	5	7	9	11	15	19	21	Locus ^b	Target gene ^c	Breakpoints (BP) ^d	Derivatives ^e	
28	55	t	T		t	t	TT	T	T					
29	55	T	T	T	TT	T	TT	T	TT	?	MYC	ins MYC @ ?	I: ? (wcp8-,MYC+)	
30	54	T	t	T	Tt	t	T	t						
31	54		T	t	T	t	T	t	T	IGH	?	1q11~12 :: 14q32	U: der(14)	
32	54	T	T	T	T	t	T	T	T	IGL	CCND3	6p21 :: 22q11	U: der(6)	
33	53	T	t	T	T	T	T	T	T					
34	53	t		Tt	T	T	T	t		IGH1	?	3q?27 :: 14q32	V (B): der(14) (wcp14+,CH-, der(3) (wcp3+,CH+,VH+,wcp14-)	
										IGH2	MYC	ins CH @ 8q24 on 8	I: 8 (wcp8+,MYC+, wcp14 -, CH+)	
35	52	T	T	T	T		T	T	T					
36	52			T	T	T	T	T	T	IGL	MYC	ins Cλ @ 8q24 on 8	I: 8 (wcp8+,MYC+,wcp22-, Cλ+)	
37	51	t	T	TT			TT			IGH	FGFR3, MMSET	4p16 :: 14q32	B: der(4), der(14)	
										?	MYC	6? :: 8q24	U*: mod der(6)	
										?	MYC	8q24 :: 15?	U: der(15) (×2)	
										?	MYC	8q24 :: 16?	U: der(16)	
38	51	t	t	t	T	T	T	t	T	?	MYC	ins MYC @ ?	I: ? (wcp8-,MYC+)	
39	51		T	T	T		T	T						
40	51		T	T	t	T	T	t		IGL1	?	? :: 22q11	U*: der(?) see Fig.2D	
										IGL2	MYC	8q24 :: 22q11	V (U*): der(22) (Vλ+,Cλ+,wcp22+,MYC+,wcp8-)	
41	51	T		T	T	T	T	t	T					
42	51	T		T	t	T	T	T		IGH	MYC	ins CH @ 8q24 on 8	I: 8 (wcp8+,MYC+, wcp14 -, CH+)	
43	51	T		T	T	T	T	T	T	?	MYC	1p13 :: 8q24	B: der(1) ×2, der(8) ×2 (MYC+)	

IG and MYC Rearrangements											
Trisomies ^a											
Sort#	Chr#	3	5	7	9	11	15	19	21	Locus ^b	Target gene ^c
Breakpoints (BP) ^d											
44	51	T		T	T		TT	T		IGH IGL	? MYC
Derivatives ^e											
										? :: 14q32 ins CA @ 8q24 on der(8)t(8;11) (q24;q23)	U: der(14) I*: der(8) (wcp8+,MYC+,wcp22-,CA+,wcp11+) see Fig.2D
45	50	Tt		T	T		TT	T			
46	50	T		T	T	Tt	T		T	IGH1 IGH2	? MYC
Derivatives ^e											
										I?42 :: 14q32 8q24 :: 14q32 & ins CH @ 8q24 on mod der(8)	B: der(1), der(14) V (U*), I: mod der(8) (wep8+,MYC+,wcp14 -,CH+) & (wcp14 -,CH+,VH+,wcp8-,MYC+) see Fig.2C
47	49	T		T	T				T	IGH1 IGH2 ?	FGFR3, MMSET CCND1 MYC
Derivatives ^e											
										4p16 :: 14q32 ins CH @ 11q13 on 11 6q21 :: 8q24	B: der(4), der(14) I: 11 (wcp11+,CCND1+,wcp14-,CH+) B: der(6) (MYC+), der(8) (MYC+)
48	48				T			Tt		IGH	?
Derivatives ^e											
										3q23 :: 14q32	B: der(3), der(14)

^aExtra copies of the odd number chromosomes are shown, where **T** or **D** represents a full copy and **t** or **d** represents a rearranged copy of that chromosome.

^bLoci containing *IG* or unknown (?) regulatory elements that dysregulate the target genes.

^cTarget genes found by FISH to colocalize with *IG* or unknown (?) loci sequences; for t(4;14), *MMSET* is presumed on der(4) for all tumors and HMCL with the exception of tumor #5 and XG-7 where *MMSET* was confirmed by FISH. In contrast to cell lines (Table 2), *IRF4* was not a target in any of the 48 advanced tumors.

^dBreakpoint information is restricted to the particular area on the chromosome that affects an *IG* or *MYC* locus; full karyotype is provided in the Supplemental Tables.

^eTypes of rearrangements for loci of interest are given (B, UB, V, D) followed by the derivatives detected (hybridization signals not shown for most balanced and unbalanced translocations); for complex (*) rearrangements, the hybridization signals detected are specified for each derivative; detailed descriptions of complex chromosomes are given in Supplemental Table 2.

Chr#, average number of metaphase chromosomes.

Rearrangements: **B**: simple balanced translocations or inversions with respect to the breakpoint of interest: *IGH* with der(14)(wcp14+, CH+, VH-, target gene+), der(14)(wcp14 -,CH-, VH+); *IGL* with der(22)(wcp22+, Vλ+, CA-), der(22)(wcp22-, Vλ-, CA+), target gene+; **U**: unbalanced translocations with respect to the breakpoint of interest, with only one of the derivatives present. **V**: variant translocations: *IGH* with der(14)(wcp14+, CH-, VH-), der(14)(wcp14 -,CH-, VH+), target gene+; *IGL* with der(22)(wcp22+, Vλ+, CA-, target gene+), der(22)(wcp22-, Vλ-, CA-), target gene+; **I**: insertion of *IG* sequences into another chromosome juxtaposed to target genes (with or without associated wcp). **I(tg)**: insertion of target gene sequences into an *IG* locus.

Note: bold **der** indicates that additional rearrangements occurred in addition to the translocation of interest, without affecting the centromere; **mod der** indicates that additional rearrangements occurred in addition to the translocation of interest, with the modification also replacing the centromere.

TABLE 2

Structural Abnormalities of *IG* or *MYC* Loci in 47 HMCL

IG and MYC Rearrangements					
HMCL	Chr#	Locus	Target Gene	Breakpoints (BP)	Derivatives
8226	60	IGH	?	1q12 :: 14q32	U: der(14) ×2
		IGL	MAF, MYC(§)	ins MYC @ BP on der(16)t(16;22)(q23;q11)	I(tg) & U*: der(16) (wcp16+,MAF+,wcp8-,MYC+,wcp22+,Cλ+) see Fig.4B
ANBL6	82	IGH	MAF	14q32 :: 16q23	B*: mod der(14) ×3, der(16) ×2
ARK	46	IGH	MYC (§)	8q24 :: 14q32	B: der(8), der(14)
ARP-1/ARP-1C ^a	69/43	IGH	MAF	14q32 :: 16q23	B: der(14) ×2, der(16) ×2
		?	MYC	? :: 8q24	U: der(8)
Delta47	45	IGL1	?	11?p14 :: 22q11	B: der(11) (Vλ-, Cλ+), der(22) (Vλ+, Cλ+)
		IGL2	?	ins Cλ @ 9p24 on 9	I: 9 (wcp9+, wcp22-, Cλ+)
		IGL3	MYC (§)	ins Cλ @ 8q24 on der(19)t(8;19)(q13;q13)	I*: der(19) (wcp19+, wcp22-, Cλ+, wcp8+, MYC+)
DP6-DJ	47	IGH	MYC	ins CH @ 8q24 on 8	I: 8 (wcp8+, MYC+, wcp14 -, CH+)
EJM	58	IGH1	MAFB	14q32 :: 20q11	B*: mod der(14), der(20)
		IGH2	?	ins CH @ BP on der(18)t(7;18)(?;q23)	I: der(18) (wcp18+, wcp14 -, CH+, wcp7+)
Flam76	42	IGH	CCND1	11q13 :: 14q32	B*: der(11), der(14), der(14) see Fig.4A
		?	MYC (§,§)	ins MYC @ BP on der(10)t(10;13)	I: der(10) (wcp10+, wcp8-, MYC+, wcp13+) see Fig.4D
FR4	100	IGH1	IRTA	ins CH @ 1q21 on der(1)(1;?)(p?;?)	I*: der(1) (wcp1+, wcp14 -, CH+)
		IGH2	IRF4	6p25 :: 14q32	V (U): der(6) (wcp14 -, CH+, VH+, wcp6+, IRF4+) ×4
		IGH3	MYC (§)	8q24 :: 14q32	B: der(8) ×2, der(14) ×2
H1112	46	IGH	CCND1	11q13 :: 14q32	B: der(11), der(14)
H929	45	IGH	FGFR3, MMSET	4p16 :: 14q32	B: der(4), der(14)
		?	MYC (§,§)	8q24 :: 20q11	U: der(8) ×2

IG and MYC Rearrangements					
HMCL	Chr#	Locus	Target Gene	Breakpoints (BP)	Derivatives
INA-6	82	IGH ?	CCND1 MYC	11q13 :: 14q32 ins MYC @ ?	U: der(14) ×2 I: ? (wcp8-, MYC+) ×3
JIM-3	60	IGH ? ?	FGFR3, MMSET MYC MYC	4p16 :: 14q32 6q?21 :: 8q24 ? :: 8q24	B: der(4), der(14) U*: mod der(8) ×2 U*: mod der(8)
JIN3	60	IGH1 IGH2	MAF MYC (S)	14q32 :: 16q23 ins CH @ 8q24 on 8 & der(14)	B*: der(14), der(14), der(16) ×2 see Fig.4A I*: 8 (wcp8+, MYC+, wcp14-, CH+) ×2 & der(14) (wcp8+, MYC+, wcp14+, CH+) see Fig.4A
JK-6L	50	IGH	MYC	8q24 :: 14q32	B: der(14) (complex der(14) in 40% of metaphases) (wcp14+, CH+, VH-), der(8) (wcp14-, CH+, VH-)
Karpas 620	68	IGH1 IGH2 IGH3	CCND1, MYC (S) MFC (S) ?	ins CH @ 8q24 on der(8)(8;11)(q24;q13) 8q24 :: 14q32 1q12 :: 14q32	I*: der(8) (wcp8+, MYC+, wcp14-, CH+, wcp11+, CCND1+) see Fig.4A U: der(14) U: der(14)
Kas-6	65	IGH ?	FGFR3, MMSET MYC	4p16 :: 14q32 1? :: 8q24	B*: der(4), der(4) ×2, mod der(14) ×2 U: der(8)dup(8)(q22q24) (MYC+) see Fig.4D
KHM-1B	59	IGL	MYC	ins CA @ 8q24 on 8	I: 8 (wcp8+, MYC+, wcp22-, CA+)
KHM-11	75	IGH ?	FGFR3, MMSET MYC (S)	4p16 :: 14q32 6?p24 :: 8q24	B: der(4), der(14) B: der(6), mod der(8) ×2 (MYC+)
KMM1	80	IGH1 IGH2	CCND3 MYC (S)	6p21 :: 14q32 ins MYC @ 14q32 on der(12) & der(4)	U*: der(14) (wcp14+, CH+, wcp6+, CCND3+) ×2 see Fig.4A I(tg)*: der(12) (wcp14+, CH+, VH+, wcp8-, MYC+) & der(4) (wcp14+, CH+, VH+, wcp8-, MYC+)
KMS-11	70	IGH1 IGH2 IGH3	FGFR3, MMSET MAF MYC (S)	4p16 :: 14q32 14q32 :: 16q23 ins CH @ 8q24 on der(?) & dup(8) (q24.3q24)	B: der(4), der(14) B: der(14), der(16) I*: der(?) (wcp14-, CH+, wcp8+, MYC+; wcp8+, MYC+, wcp14-, CH+) see Fig.4A

IG and MYC Rearrangements					
HMCL	Chr#	Locus	Target Gene	Breakpoints (BP)	Derivatives
KMS-12 PE/BM ^b	4/7/5	IGH1	CCND1	11q13 :: 14q32	B*: der(11), der(14), mod der(14)
		IGH2	MYC (S)	ins CH @ 8q24 on 8	I: 8 (wcp8+, MYC+, wcp14 -, CH+) ×2
KMS-18	74	IGH	FGFR3, MMSET	4p16 :: 14q32	B*: der(4), mod der(14) ×2
		?	MYC (S)	6? :: 8q24	U: der(6) ×2
		?	MYC (S)	8q24 :: 21?	U: der(21) ×2
KMS-20	41	IGH	MYC	8q24 :: 14q32	B*: der(8), mod der(14)
KMS-26	71	IGH	FGFR3, MMSET	4p16 :: 14q32	B*: mod der(4), mod der(14)
		IGL	MYC	8q24 :: 22q11	U*: mod der(8) (wcp8+, MYC+, wcp22+, CA+) ×3 see Fig.4B
KMS-28 PE/BM ^b	42/42	IGH1	FGFR3, MMSET	4p16 :: 14q32	B*: der(4), mod der(14)
		IGH2	MYC	8q24 :: 14q32	B: der(8), der(14)
KMS-34	76	IGH	FGFR3, MMSET	4p16 :: 14q32	B*: der(4), der(14), mod der(14)
		?	MYC	? :: 8q24	U: der(8)
KP6-DJ	47-89	IGH	?	14q32 :: 17?p	V, B: der(14) (wcp14+, CH-, VH-) ×3, der(17) (wcp14 -, CH+, VH+, wcp17+) ×3
L363	46	IGL	NIK	17q21 :: 22q11	B: der(17) ×3, der(22) ×2
		?	MYC (S, S)	5? :: 8q24	B: der(5), der(8) ×2 (MYC+)
LP1	80	IGH1	FGFR3, MMSET	4p16 :: 14q32	B: der(4), der(14)
		IGH2	?	ins CH @ ?	I: ?(wcp14 -, CH+)
		IGH3	?	? :: 14q32	V (U)*: mod der(8) (wcp14 -, CH+, VH+) ×2
		IGH4	MYC (S)	8q24 :: 14q32	V (U)*: der(8) (wcp8+, MYC+, wcp14 -, CH+, VH+) ×3 & mod der(8) (wcp8+, MYC+, wcp14 -, CH+, VH+) ×2
MM-1	44	IGH1	MAF	14q32 :: 16q23	B: der(14), der(16)
		IGH2	MYC (S)	ins CH @ BP on der(3)(3:8)(?;q24)	I*: der(3) (wcp3+, wcp14 -, CH+, wcp8+, MYC+)
MM-S1	47	IGH1	CCND1	11q13 :: 14q32	B: der(11), der(14)
		IGH2	MYC (S)	ins CH @ 8q24 on 8 & i(8q)	I*: 8 & i(8q) (wcp8+, MYC+, wcp14 -, CH+)

IG and MYC Rearrangements					
HMCL	Chr#	Locus	Target Gene	Breakpoints (BP)	Derivatives
OCI-MY1	49	<i>IGH1</i>	?	ins CH @ ?	I: ? (wcp14-, CH+)
		<i>IGH2</i>	??	ins CH @ ??	I: ?? (wcp14-, CH+)
		<i>IGL</i>	<i>MYC</i>	8q24 :: 22q11	U: der(8)
OCI-MY5	46	<i>IGH1</i>	<i>MAF</i>	14q32 :: 16q23	U: der(14)
		<i>IGH2</i>	<i>MYC</i> (§)	8q24 :: 14q32 & dup(14)(q32;q?22)	U*: der(?) (wcp8+, MYC+, wcp14+, CH+, wcp8+, MYC+) see Fig.4A
OCI-MY7	78	<i>IGH1</i>	<i>CCND1</i>	11q13 :: 14q32	U: der(14) ×2
		<i>IGH2</i>	?	?X?q :: 14q32	B: der(?)X, der(14)
		?	<i>MYC</i> (§)	3? :: 8q24	U: der(8) ×2
OPM-1/2 b	75/75	<i>IGH1</i>	<i>FGFR3, MMSET</i>	4p16 :: 14q32	B: der(4), der(14)
		<i>IGH2</i>	<i>MYC</i> (§)	ins CH & wcp14 @ 8q24 on der(8)(8; 14)(q24;q32)t(1;14)(?q12;q?)	I*: der(8) (wcp8+, MYC+, wcp14+, CH+)
PE-1	83	<i>IGH</i>	<i>CCND1</i>	11q13 :: 14q32	U*: der(14) ×2, mod der(14)
PE-2	72	<i>IGH</i>	<i>FGFR3, MMSET</i>	4p16 :: 14q32	B: der(4), der(14)
		<i>IGK</i>	<i>NMYC</i>	2p12 :: 2p25	B: der(2)inv(2)(p25p12)(wcp2+, Cx+, NMYC+) see Fig.4C
Sachi	69	<i>IGH1</i>	<i>MAFB</i>	14q32 :: 20q11	B*: der(14) ×2, der(20) ×2
		<i>IGH2</i>	<i>MYC</i>	8q24 :: 14q32	B: der(8), der(14)
SKMM1	79	<i>IGH1</i>	<i>MAFB</i>	14q32 :: 20q11	B: der(14), der(20)
		<i>IGH2</i>	<i>IRF4</i>	6p25 :: 14q32	U*: der(8) (wcp14+, CH+, wcp6-, IRF4+)
		<i>IGH3</i>	<i>MYC</i> (§)	8q24 :: 14q32	U*: der(8) (wcp8+, MYC+, wcp14+, CH+)
SKMM2	37	<i>IGH</i>	<i>CCND1</i>	11q13 :: 14q32	U: der(14)
		?	<i>MYC</i> (§)	ins <i>MYC</i> @ ?9	I: ?9 (wcp8-, MYC+)
U266	39	<i>IGH</i>	<i>CCND1</i>	ins CH @ 11q13 on der(11)(11;?)q14;q7)	I: der(11) (wcp11+, CCND1+, wcp14-, CH+)
		?	<i>LMYC</i> (§)	1p13 :: 1p34	U: der(1)inv(p34p13) (LMYC @ p13)

IG and MYC Rearrangements					
HMCL	Chr#	Locus	Target Gene	Breakpoints (BP)	Derivatives
UTMC-2	77	<i>IGH</i> ?	<i>FGFR3</i> , <i>MMSET</i> <i>MYC</i> ([‡])	4p16 :: 14q32 del(8q24)	B: der(4) ×2, der(14) ×2 U: del (.5-1 Mb tel of MYC)
XG-1	44	<i>IGH1</i> <i>IGH2</i>	<i>CCND1</i> <i>MYC</i>	11q13 :: 14q32 8q24 :: 14q32	U: der(14) ×2 V (U)*: der(8) (wcp8+, MYC+, wcp14 -, CH+, VH+)
XG-2	49	<i>IGH</i> <i>IGL1</i> <i>IGL2</i> ?	? <i>MAFB</i> <i>CD40</i> <i>MYC</i> ([‡])	12q24.31 :: 14q32 ins Cλ @ 20q11 on 20 ins <i>CD40</i> @ 22q11 on 22 2q? :: 8q24	B: der(12) (wcp14 -, CH+, VH+), der(14) (wcp14+, CH+, VH-) I: 20 (wcp20+, MAFB+, wcp22-, Cλ+) I(tg): 22 (wcp22+, Vλ+, Cλ+, wcp20-, CD40+) ×2 U: der(2)
XG-6	77	<i>IGL</i>	7	16q23 :: 22q11	B: der(16) (wcp16+, MAF+, wcp22-, Cλ+) ×2, der(22)
XG-7	43	<i>IGH1</i> <i>IGH2</i> ? ?	<i>MMSET</i> <i>IRF4</i> <i>MYC</i> ([‡]) <i>MYC</i> ([‡])	4p16 :: 14q32 ins <i>IRF4</i> @ 14q32 on der(14)(4;14) (p16;q32) ins <i>MYC</i> @ ? on 17 ins <i>MYC</i> @ ? on 19	U: der(4) (VH+, MMSET+, FGFR3-) I(tg)*: der(14) (wcp14+, CH+, FGFR3-, wcp6-, IRF4+, 4p16+) I: 17 (wcp17+, wcp8-, MYC+) I: 19 (wcp19+, wcp8-, MYC+)

See Table I legend.

^aTwo sublines; ARP-IC subline was incorrectly identified as CAG in Annunziata et al., 2007.

^bTwo independent lines from the same patient.

(^s) *MYC* rearrangements previously described (Shou et al., 2000).

([‡]) *MYC* rearrangements recently described (Dib et al., 2008).

TABLE 3

Anatomy of *IG* and *MYC* Rearrangements in 48 MM Tumors

Rearrangements	#	B	U	V	I	%B	%V+I
<i>IGH</i> 5 recurrent	17	15	1	0	1	88	6
<i>IGH</i> . <i>MYC</i>	7	2	0	1	4	29	71
<i>IGH</i> . Other	12	4	3	2	3	33	42
<i>IGL</i> / <i>IGK</i>	10	1	4	1	4	10	50
<i>IGL</i> / <i>IGK</i> . <i>MYC</i>	7	1	1	1	4	14	71

#Number of rearrangements. As described in the Results, the different kinds of rearrangements are: B, balanced; U, unbalanced; V, variant; and I, insertion.

TABLE 4

Anatomy of *IG* and *MYC* Rearrangements in 47 HMCL

Rearrangements	#	B	U	V	I	%B	%V+I
<i>IGH</i> recurrent	35	26	7	0	2	74	6
<i>IGH.MYC</i>	19	6	3	2	8	32	53
<i>IGH</i> . Other	15	2	4	3	6	13	60
<i>IGL/IGK</i>	13	4	3	0	6	31	46
<i>IGL/IGK.MYC</i>	6	1	2	0	3	17	50

#Number of rearrangements. As described in the Results, the different kinds of rearrangements are: B, balanced; U, unbalanced; V, variant; and I, insertion.