Published in final edited form as:
J Natl Cancer Inst Monogr. 2008 ; (39): 25–31. doi:10.1093/jncimonographs/lgn011.

Characterization of MYC Translocations in Multiple Myeloma Cell Lines

Amel Dib, **Ana Gabrea**, **Oleg K. Glebov**, **P. Leif Bergsagel**, and **W. Michael Kuehl**
National Cancer Institute, Bethesda MD (AD, AG, OG, WMK); Mayo Clinic, Scottsdale, AZ (PLB).

Abstract

Translocations involving an MYC gene (c ≫ N ≫L) are very late tumor progression events and provide a paradigm for secondary translocations in multiple myeloma. Using a combination of fluorescent *in situ* hybridization and comparative genomic hybridization arrays (aCGH), we have identified rearrangements of an MYC gene in 40 of 43 independent myeloma cell lines. A majority of MYC translocations involve an Ig locus (IgH > Ig λ ≫Ig κ), but the breakpoints only infrequently occur near or within switch regions or V(D)J sequences. Surprisingly, about 40% of MYC translocations do not involve an Ig locus. The MYC translocations mostly are nonreciprocal translocations or insertions, often with the involvement of three chromosomes and sometimes with associated duplication, amplification, inversion, and other associated chromosomal abnormalities. High-density aCGH analyses should facilitate the cloning of MYC breakpoints, enabling the determination of their structures and perhaps elucidating how rearrangements not involving an Ig gene cause dysregulation of an MYC gene.

Translocations involving an IgH locus are present in nearly 50% of premalignant monoclonal gammopathy of undetermined significance (MGUS) tumors, approximately 60% of fully malignant multiple myeloma (MM) tumors and nearly 90% of human myeloma cell lines (HMCL) (1–4). There are five recurrent IgH translocation chromosomal partners in MGUS and MM. Together, the combined prevalence of these translocations is approximately 40% of MM tumors, with approximately 15% 4p16 (FGFR3 and MMSET), 3% 6p21 (CYCLIN D3), 15% 11q13 (CYCLIN D1), 5% 16q23 (c-MAF), and 2% 20q12 (MAFB). The mostly simple reciprocal translocations involving the five recurrent loci appear to be primary translocations that usually are mediated by errors in IgH switch recombination, and perhaps less often errors in somatic hypermutation, during the maturation of B cells in germinal centers (5,6).

About 3% of MM tumors have an IgH translocation that targets c-MYC at 8q24.(7) By contrast, in Burkitt's lymphoma and murine plasmacytoma, chromosomal translocations that juxtapose c-MYC with one of the Ig loci (IgH > Igλ ~ Igκ) represent a virtually invariant, early oncogenic event (8,9). The physiological consequence of these translocations is dysregulated, increased expression of c-MYC as a result of its juxtaposition to intronic and/or 3′ IgH or IgL enhancers. The nontranslocated c-MYC allele is expressed at very low levels, presumably reflecting little or no expression of c-MYC in resting B cells, germinal center B cells, memory B cells, and terminally differentiated plasma cells (10,11).

Several years ago, we showed that there is selective expression of L-MYC or one c-MYC allele in nine informative HMCL (12). We also showed that 21 of 22 HMCL have complex rearrangements that involve c-MYC or L-MYC. Seventeen of these MYC rearrangements

Correspondence to: W. Michael Kuehl, Genetics Branch, Naval Hospital, Bldg 8, Rm 5101, Bethesda, MD 20889-5105 (e-mail: wmk@helix.nih.gov).

involved juxtaposition to an Ig locus (IgH, 15 and Igλ, 2), but four (19%) had no detectable involvement of an Ig locus. We found similar kinds of complex rearrangements involving c-MYC in advanced MM tumors. We have extended our analysis of MYC rearrangements to a panel of 43 independent HMCL but have now included fluorescent *in situ* hybridization (FISH) mapping and aCGH studies to characterize the MYC rearrangements.

Methods

General Procedures

Procedures for cell culture, isolation of RNA and DNA, and sequence analysis of polymerase chain reaction (PCR) products generated from c-MYC genomic DNA and RNA has been described previously (12) The V00568 c-MYC RNA reference sequence is used to indicate the positions of sequence changes in c-MYC.

Gene Expression Profiling

The expression of c-, L-, and N-MYC was determined using HG-U133-2.0-plus Affymetrix chips for 46 HMCL (www.broad.mit.edu/mmgp). For each HMCL, the expression of c-MYC was normalized (MYCR in Table 1) to the median expression of c-MYC in 22 samples of bone marrow plasma cells purified from healthy individuals (GEO accession GSE5900) (11).

FISH Analyses

Probes and FISH analyses on metaphase chromosomes were as described previously, except that Vλ and Vκ cosmid clones containing sequences from the centromeric end of the respective IgL loci were used in some experiments (12). The following BAC/PAC clones (size of insert, distance of the middle of the clone from the c-MYC gene) were used in FISH mapping experiments: RP1-316L14 (130 kb, −5000 kb), RP11-622O11 (195 kb, −1700 kb), RP11-645E10 (187 kb, −800 kb), Sts-N5487 (130 kb, −650 kb), R11-751J13 (150 kb, −500 kb), R11-570C7 (200 kb, −450 kb), GS-252H19 (200 kb, −400 kb), GS-93F05 (150 kb with *c-myc* locus), GS-123D17 (400 kb, +200 kb), R11-709E21 (186 kb, +400 kb), GS-02D9 (57 kb, +550 kb), R11-18K20 (191 kb, +900 kb), GS-25K15 (100 kb, +1050 kb), and R11-259L23 (171 kb, +1900 kb).

High-Resolution Array Comparative Genomic Hybridization

This was performed on the Human Genome 44B microarray (Agilent Technologies) following the protocol suggested by the manufacturer. Briefly, 800 ng of test or normal female reference (Promega) genomic DNA was directly labeled with either Cy5 or Cy3. The hybridization sample containing equal amounts of test and reference DNA was hybridized to the microarray at 65°C for 40 hours. The slides were washed and scanned using the Agilent G2505B DNA microarray scanner. The generated microarray images were analyzed using Feature Extraction software V8.1 (Agilent). The Log2-transformed ratio data were analyzed with GeneSpring V7.1 and CGH Analytics V3.4.27 (Agilent Software). The aCGH data in Table 1 are shown as the signal in the test sample normalized to the normal reference sample. The aCGH data is available at www.broad.mit.edu/mmgp.

Results

Most MM Cell Lines Express c-MYC But Two LinesExpress L- or N-MYC

We analyzed a panel of 46 HMCL, including three pairs (KMS-12BM/-12PE, KMS-28BM/-28PE, OPM-1/-2) derived independently from three patients (Table 1) (13). As results were virtually identical for each of these three pairs of HMCL, we will refer to results only for 43 HMCL derived from different patients. Expression arrays show that 40 of the 43

HMCL express c-MYC RNA at levels that are higher than normal plasma cells (Table 1). A notable exception is the IL-6-dependent PE-1 HMCL that expresses quite low levels of c-MYC RNA. The remaining two HMCL express little or no c-MYC RNA but instead express high levels of N-MYC (PE-2) or L-MYC (U266) (not shown). Consistent with the low prevalence of N-MYC expression in the HMCL, eight of 559 (1.4%) primary MM tumors express N-MYC, whereas none of these tumors express L-MYC (GEO accession GSE2658) (14).

Only One c-MYC Allele Is Expressed in All 11 Informative MM Cell Lines

Genomic DNAs from 31 HMCL that express c-MYC were screened for exon mutations that would distinguish the two parental c-MYC alleles. Eleven HMCL had genetically distinguishable alleles. For untranslated exon 1, we found G56A in JJN-3 and XG-7, and C170T in FLAM-76, KMS-11, and KHM-11. For exon 2, we found A590G in OCI-MY1, G807C in MM.1, and G1251A in FR-4, KHM-11, KMS-18, MM-S1, and OPM-1/-2. The only missense mutation is A590G, which converts codon 11 from ASN to SER. Excepting XG-7, we determined the sequence of cDNA in 10 of the HMCL with c-MYC mutations and found selective expression of the wild-type allele (FR-4, KMS-18, MM.1), the variant allele (FLAM-76, JJN-3, KMS-11, MM-S1, OCI-MY1, OPM-2), or one of two variant alleles (G1251A in KHM-11). Therefore, all 11 informative HMCL (including H929 which expresses a truncated allele) selectively express only one of the two genetically distinguishable c-MYC alleles, whereas U266 and PE-2 express, respectively, L- and N-MYC but no c-MYC. We conclude that there is tumor-specific dysregulation of L-MYC, N-MYC, or only one c-MYC allele in all 13 informative HMCL.

FISH Analyses of Metaphase Chromosomes Detect MYC Rearrangements in 40 of 46 HMCL

We analyzed all 46 HMCL for rearrangements of a c-MYC gene by performing three-color FISH analyses on metaphase chromosomes, using combinations of probes flanking all three Ig loci, a MYC probe, and chromosome painting probes. We previously had reported the results of these analyses for 22 of these HMCL (12) but now briefly summarize the results here for the 43 unrelated HMCL (Table 1). Using these molecular cytogenetic assays, only six HMCL have no detectable rearrangement involving an MYC gene.

Twenty-two HMCL have rearrangements that juxtapose an MYC gene with 3′ enhancer sequences from one of the three Ig loci: 16 with 3′ IgH enhancer sequences, although only three (FR-4, JK6L, KMS-28BM) are simple, balanced t(8;14) translocations; five with 3′ Igλ enhancer sequences, none of which are simple, balanced translocations; and one (PE-2) has an intrachromosomal inversion that repositions 3′ Igκ enhancer sequences (2p12) near N-MYC at 2p25 (Figure 1).

Fifteen HMCL have rearrangements of c-MYC or L-MYC (U266) that do not involve detectable Ig sequences. With the exception of L363, which has a balanced t(5;8) translocation but two copies of der8 and one copy of der5, all these rearrangements appear to be unbalanced translocations or insertions. The complex rearrangements of an MYC gene, sometimes with and other times without close juxtaposition to 3′ Ig enhancer sequences, frequently involve three chromosomes near the site of the MYC breakpoint (eg, FLAM-76). In addition, these complex rearrangements sometimes are associated with duplication (eg, MM-S1), duplication and inversion (eg, KMS-11), or amplification (eg, KAS-6) of MYC or MYC/3′ Ig enhancer sequences that are detected by FISH analyses of metaphase chromosomes. Several examples of rearrangements involving an MYC gene are illustrated in Figure 1 .

Mapping MYC Rearrangements in 14 HMCL Using FISH Probes Flanking the c-MYC Gene

To map the approximate location of breakpoints relative to the c-MYC gene, we assembled a contig of 13 BAC or PAC clones that cover a region 5 Mb centromeric and 1.9 Mb telomeric

to c-MYC. We then used combinations of these clones, a c-MYC BAC, and appropriate painting probes to characterize relevant chromosomes in 14 HMCL that did not have rearrangements that juxtaposed an MYC gene with 3′ Ig enhancer sequences (Table 2). Although five of these HMCL had no detectable rearrangement involving a c-MYC gene in the FISH analyses described above, this more complete analysis showed an internal deletion that included sequences located approximately 200–550 kb telomeric to c-MYC in the UTMC-2 cell line. For the nine HMCL that had rearrangements of c-MYC in our initial analyses, we obtained the following results. Three HMCL (FLAM-76, SKMM-2, XG-7) had chromosomes with insertions of c-MYC associated with loss of both centromeric and telomeric sequences. Two HMCL (H929 and OCI-MY7) had chromosomes with loss of sequences telomeric to c-MYC. The results for H929 were somewhat surprising because a translocation breakpoint located within the 3′ untranslated region had been cloned (15), which was consistent with the der8t(8;20)(q24;q11) detected by FISH. Yet by the FISH mapping studies there was an internal deletion of at least 1700 kb of centromeric sequences and retention of some sequences telomeric to c-MYC on the der8t(8;20)(q24;q11) chromosome. Two HMCL (KMS-18, XG-2) had chromosomes with loss of sequences centromeric to c-MYC. Finally, two HMCL (KHM-11, L363) had loss of sequences centromeric to c-MYC on one or more chromosomes, as well as loss of sequences telomeric to c-MYC on one or more other chromosomes. In both cases, the mapping results were consistent with balanced translocations, but for KHM-11, the chromosome structure was more complex than would be expected for a balanced reciprocal translocation.

Mapping c-MYC Rearrangements in 46 HMCL by aCGH

Unlike FISH experiments, aCGH, which measures a cellular population average of specific sequences on all relevant chromosomes, cannot detect balanced rearrangements or balanced changes in ploidy and is limited in measuring gain or losses of a single copy sequence if there are multiple (three or four) copies of that sequence in a cell. However, it has the potential advantage of detecting several fold amplification of sequences, whereas FISH is less quantitative. Examples of this are illustrated by XG-6 and EJM, for which aCGH shows, respectively, a threefold increase and a 35% increase in MYC compared to flanking sequences (Table 1 and Results), whereas FISH analyses did not detect these abnormalities (Table 2). From both FISH and aCGH analyses (Table 1 and Table 2), it is possible to map the sites of rearrangements near c-MYC. However, high-density CGH arrays have the advantage of more precisely identifying the location of breakpoints so that they can be cloned by conventional methods or more direct methods such as inverse PCR.

Because most c-MYC rearrangements appear to be unbalanced based on the studies described above, it seemed likely that aCGH would detect most of these abnormalities. In fact, data generated from analyses using Agilent 44K arrays confirm this expectation, as summarized in Table 1. Compared to normal cells, the content of c-MYC DNA was increased at least twofold in 16 of 43 HMCL. Regardless of c-MYC content, for 15 HMCL, there was loss of both centromeric and telomeric sequences that flank the c-MYC gene, a result that is consistent with insertion or amplification of c-MYC and variable extents of immediately flanking sequences. In most cases, the c-MYC karyotypic abnormality enables one to distinguish insertion from amplification. There are three instances (gray shading in Table 1) in which the loss of flanking sequences relative to c-MYC are substantially less than twofold. However, the median value for 21 probes centromeric to c-MYC (not shown) was significantly decreased relative to c-MYC (average of four probes) for all three HMCL: 0.79 ($P = .014$) for EJM, 0.77 ($P = .004$) for Karpas-620, and 0.70 ($P < .001$) for KMS-11. Similarly, the median value for 24 probes telomeric to c-MYC (not shown) was significantly decreased relative to c-MYC: 0.77 ($P = .005$) for EJM, 0.69 ($P < .001$) for Karpas-620, and 0.55 ($P < .00001$) for KMS-11. Even though

the loss of flanking sequences appears to be significant for these three HMCL, it is not possible to determine the precise breakpoints from this data set.

For 12 and 4 HMCL, there is a significant loss, respectively, of only telomeric or of only centromeric sequences, again consistent with c-MYC karyotypic abnormalities. For XG-2, the loss of centromeric sequences begins >2.5 Mb from c-MYC, consistent with the FISH mapping results (above and Table 2). The aCGH data also confirmed the internal deletion in UTMC-2 that was detected by the FISH mapping studies (above and Table 2). Twelve HMCL, which includes three with no apparent karyotypic abnormality and two with rearrangements of L- or N-MYC showed no apparent loss of either centromeric or telomeric flanking sequences. The result for MM.S1 was somewhat unexpected, but perhaps explained by the presence of a der3t (3;8) and a der13t(8;13), which would be consistent with no loss of chromosome 8 sequences in the c-MYC locus.

Significantly, the aCGH analyses detected MYC rearrangements in three (EJM, UTMC-2, XG-6) of the six HMCL for which we had found no karyotypic abnormalities in our initial FISH analyses. The EJM and XG-6 HMCL show loss of both centromeric and telomeric sequences immediately flanking c-MYC by the aCGH analysis (above). The remaining three HMCL (ANBL6, PE-1, H1112) had no detectable rearrangements of MYC. However, they could have cryptic rearrangements, such as insertions or internal inversions, or may have found some other mechanism to enable MYC expression. Unfortunately, these three HMCL did not have genetically distinguishable c-MYC alleles, so that we do not know if MYC is expressed selectively from one or both parental alleles.

The positions of the breakpoints relative to c-MYC deserve comment. Although 16 HMCL have both centromeric and telomeric breakpoints, 13 HMCL have breakpoints telomeric of c-MYC, whereas only four HMCL have breakpoints centromeric of c-MYC. The distances of both telomeric and centromeric breakpoints from the c-MYC gene are mostly >200 kb, although there seem to be some examples of breakpoints that can occur >1 Mb from the MYC gene, including XG-2 for which the breakpoint appears to be >2.5 Mb centromeric to c-MYC (Table 1 and Table 2). This issue is considered further in the Discussion.

DISCUSSION

In addition to providing further support for our hypothesis (below) regarding the role of MYC translocations in MM, the data in this paper illustrate the potential utility of aCGH to characterize complex MYC rearrangements. As summarized in the Results, the MYC rearrangements tend to be complex, but a few specific examples serve to further highlight the level of complexity. A first example is H929, which has multiple and apparently discontinuous abnormalities [a der8t(8;20)(q24;q11), a cloned breakpoint within the 3′ untranslated region of c-MYC, internal deletion of centromeric sequences, and retention of at least some telomeric sequences] (15) A second example is KMM-1, for which the c-MYC gene and a small piece of DNA from chromosome 21 are inserted together just upstream of the delta gene in the IgH locus (12). A third example is XG-2, in which there is an apparent breakpoint approximately 2.5 Mb centromeric to MYC by aCGH analyses. It seems prudent to remain skeptical that MYC can be dysregulated when it is positioned several megabases away from a breakpoint (16), even though we previously showed that the t(14;16) translocation in HMCL dysregulates c-MAF when it located up to 1.3 Mb from the 3′ IgH enhancer (6,17) Given the extreme complexities of MYC rearrangements, one must consider the possibility that the breakpoint in XG-2 might actually be much closer if there were, for instance, an associated internal inversion that positioned MYC closer to the centromeric break-point. A final example is the variant IgH t (8;14) translocation (Figure 1) that is seen in myeloma but not Burkitt’s lymphoma. As summarized elsewhere (6), we have proposed an hypothesis to explain why the B cell–specific

mechanisms mediating the simple reciprocal, classical t(8;14) translocation in Burkitt's lymphoma are unlikely to generate the variant IgH t(8;14) translocation that must be mediated by non–B cell–specific recombination mechanisms in myeloma.

Based on previous work, we proposed that secondary translocations involving a MYC gene, occur as a late progression events as MM tumors become less stromal cell dependent and more proliferative (12). Together with results published by others and the results published here, we are now able to more fully summarize the evidence that supports this hypothesis (5,7,16). First, most MYC rearrangements involve c-MYC. By contrast, N-MYC and L-MYC rearrangements, respectively, have an approximately 10-fold and perhaps 100-fold lower prevalence, consistent with little or no expression of these genes in plasma cells. Second, MYC rearrangements are rare or absent in MGUS or smoldering MM tumors but are present in about 15% of MM tumors, approximately 45% of advanced MM tumors, and 90% or more of HMCL (1,7,12). Third, MYC rearrangements often are present in only a subset of primary tumor cells, providing definitive evidence for a progression event in these cases (1,7,12). Fourth, rearrangements of an MYC gene in HMCL involve a 3′ Ig enhancer about 60% of the time (IgH, 47%; Igλ , 13%; Igκ, 2%) and no apparent Ig enhancer nearly 40% of the time. For the rearrangements not involving an Ig locus, there does not appear to be involvement of other recurrent chromosomal loci. We have similar results for advanced MM tumors (18). Surprisingly, Avet-Loiseau et al. found that c-MYC rearrangements in primary MM tumors involve an Ig locus 26% of the time (IgH, 17% and Igλ, 9%), whereas they found no involvement of an Ig enhancer in 74% of tumors with c-MYC rearrangements (7). Fifth, c-MYC rearrangements rarely have breakpoints near or within IgH switch or JH regions, consistent with the absence of B cell–specific recombination mechanisms in plasma cells or plasma cell tumors (5). By contrast, the five recurrent and presumptive primary IgH translocations in MM mostly have breakpoints near or within IgH switch, or less often JH, regions. Sixth, for the HMCL reported here, >80% of MYC rearrangements are unbalanced (74% of IgH, 100% of Igλ, >87% with no involvement of an Ig locus), whereas only ~ 20% of five recurrent IgH translocations are unbalanced. As described above, MYC rearrangements typically are complex translocations and insertions, with frequent involvement of three chromosomes, and sometimes with duplication, amplification, inversion, and additional insertion or deletion events. Seventh, it is notable that the levels of c-MYC RNA expression are fully comparable in HMCL that have MYC rearrangements either with or without involvement of an Ig locus (Table 1). It is tempting to speculate that a non–Ig enhancer is juxtaposed near MYC in these cases, but currently we have no evidence to support this. Finally, all 13 informative HMCL express L-MYC, N-MYC, or one of the two parental c-MYC alleles, as summarized above. All these HMCL have MYC rearrangements, including five without involvement of 3′ Ig enhancer sequences.

It seems apparent that five recurrent IgH translocation partners (11q13, CYCLIN D1; 4p16, FGFR3 and MMSET; 16q23, c-MAF; 20q12, MAFB; 6p21, CYCLIN D3) in MM occur as early, primary events that are mediated mostly by errors in IgH switch recombination or somatic hypermutation as activated B cell pass through germinal centers (1). In contrast to MYC rearrangements, which mostly—if not always—represent late progression events, the five recurrent translocations can be present in premalignant MGUS tumors and usually are present in all tumor cells, as expected for a primary event. In addition to the approximately 40% of MM tumors that have one of the five recurrent translocations, another 15%–20% of tumors have IgH translocations that do not involve one of the five recurrent partners or an MYC gene. In our characterization of translocations in the panel of 46 HMCL and 47 MM tumors, we have found that these IgH translocations have the same kinds of structural complexity as MYC translocations, and thus seem likely to represent secondary, progression events (6,18).

References

1. Bergsagel PL, Kuehl WM. Molecular pathogenesis and a consequent classification of multiple myeloma. J Clin Oncol 2005;23(26):6333–6338. [PubMed: 16155016]
2. Bergsagel PL, Kuehl WM, Zhan F, Sawyer J, Barlogie B, Shaughnessy J Jr. Cyclin D dysregulation: an early and unifying pathogenic event in multiple myeloma. Blood 2005;106(1):296–303. [PubMed: 15755896]
3. Fonseca R, Bailey RJ, Ahmann GJ, et al. Genomic abnormalities in monoclonal gammopathy of undetermined significance. Blood 2002;100(4):1417–1424. [PubMed: 12149226]
4. Fonseca R, Barlogie B, Bataille R, et al. Genetics and cytogenetics of multiple myeloma: a workshop report. Cancer Res 2004;64(4):1546–1558. [PubMed: 14989251]
5. Bergsagel PL, Kuehl WM. Chromosomal translocations in multiple myeloma. Oncogene 2001;20(40): 5611–5622. [PubMed: 11607813]
6. Gabrea A, Leif Bergsagel P, Michael Kuehl W. Distinguishing primary and secondary translocations in multiple myeloma. DNA Repair (Amst) 2006;5(9–10):1225–1233. [PubMed: 16829212]
7. Avet-Loiseau H, Gerson F, Margrangeas F, Minvielle S, Harousseau J-L, Bataille R. Rearrangements of the c-myc oncogene are present in 15% of primary human multiple myeloma tumors. Blood 2001;98 (10):3082–3086. [PubMed: 11698294]
8. Dalla-Favera, R.; Gaidano, G. Molecular biology of lymphoma. In: DeVita, VT.; Hellman, S.; Rosenberg, SA., editors. Cancer: Principles and Practice of Oncology. Vol. 6th ed. Philadelphia, PA: Lippincott Williams and Wilkins; 2001. p. 2215-2235.
9. Potter M. Neoplastic development in plasma cells. Immunol Rev 2003;194(1):177–195. [PubMed: 12846815]
10. Klein U, Tu Y, Stolovitzky GA, et al. Transcriptional analysis of the B cell germinal center reaction. Proc Natl Acad Sci USA 2003;100(5):2639–2644. [PubMed: 12604779]
11. Zhan F, Barlogie B, Arzoumanian V, et al. Gene-expression signature of benign monoclonal gammopathy evident in multiple myeloma is linked to good prognosis. Blood 2007;109(4):1692–1700. [PubMed: 17023574]
12. Shou Y, Martelli ML, Gabrea A, et al. Diverse karyotypic abnormalities of the c-myc locus associated with c-myc dysregulation and tumor progression in multiple myeloma. Proc Natl Acad Sci USA 2000;97(1):228–233. [PubMed: 10618400]
13. Drexler HG, Matsuo Y. Malignant hematopoietic cell lines: in vitro models for the study of multiple myeloma and plasma cell leukemia. Leuk Res 2000;24(8):681–703. [PubMed: 10936422]
14. Zhan F, Huang Y, Colla S, et al. The molecular classification of multiple myeloma. Blood 2006;108 (6):2020–2028. [PubMed: 16728703]
15. Hollis GF, Gazdar AF, Bertness V, Kirsch IR. Complex translocation disrupts c-myc regulation in a human plasma cell myeloma. Mol Cell Biol 1988;8(1):124–129. [PubMed: 3275865]
16. Fabris S, Storlazzi CT, Baldini L, et al. Heterogeneous pattern of chromosomal breakpoints involving the MYC locus in multiple myeloma. Genes Chromosomes Cancer 2003;37(3):261–269. [PubMed: 12759924]
17. Chesi M, Bergsagel PL, Shonukan OO, et al. Frequent dysregulation of the c-maf proto-oncogene at 16q23 by translocation to an Ig locus in multiple myeloma. Blood 1998;91(12):4457–4463. [PubMed: 9616139]
18. Gabrea A, Martelli ML, Qi Y, et al. Secondary genomic rearrangements involving immunoglobulin or MYC loci show similar prevalences in hyperdiploid and nonhyperdiploid myeloma tumors. Genes Chrom Cancer 2008;47(7):573–590. [PubMed: 18381641]

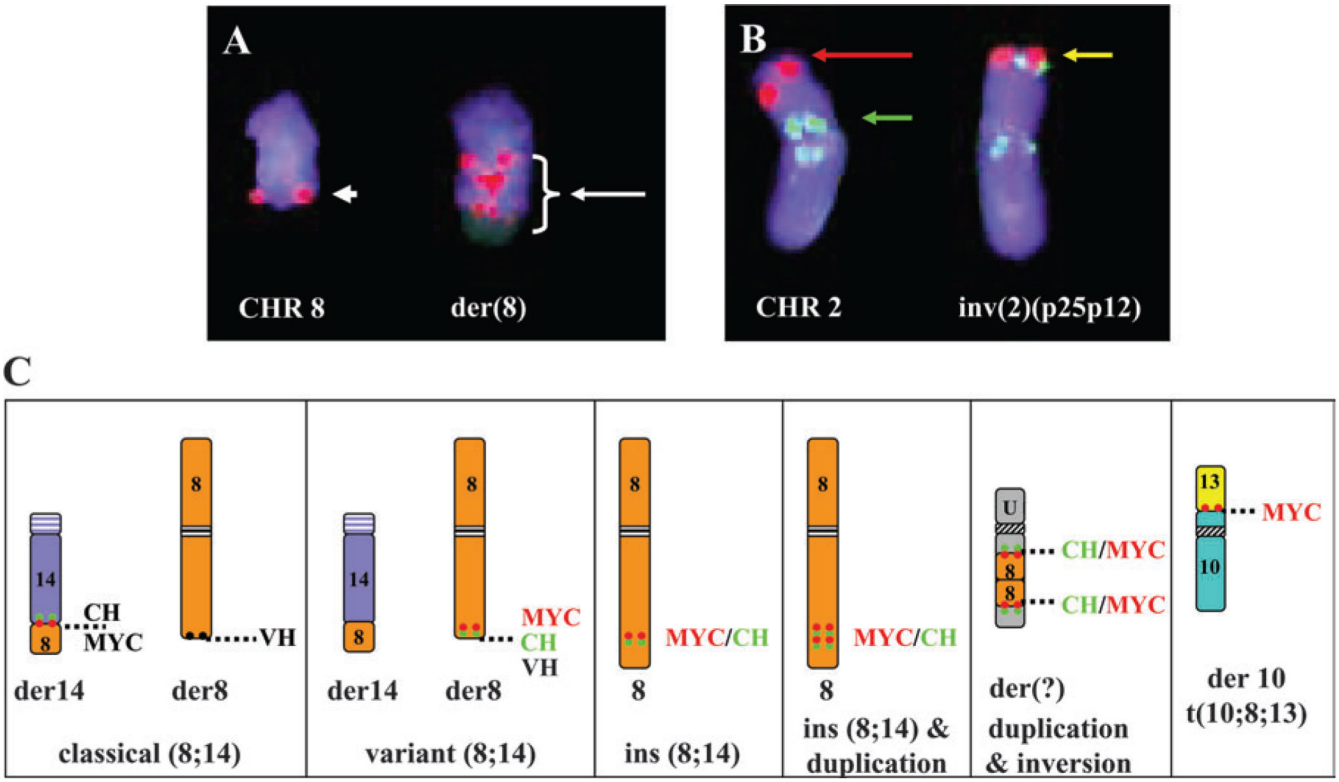

Figure 1.

Examples of molecular cytogenetic analyses of MYC rearrangements in multiple myeloma. **A, B**) Primary data and **C**) schematic representations of FISH analyses of metaphase chromosomes found in different cell lines. MYC probe is red and Ig C (3′ enhancer) probe is green. **A**) Normal chromosome 8 (CHR 8) and amplification of MYC gene and flanking sequences on der(8) in KAS-6 HMCL; **B**) normal chromosome 2 (CHR 2) and juxtaposition of Ck probe from 2p12 near N-MYC at 2p25 on inv(2)(p25p12) in PE-2 HMCL. The C_{κ} probe cross-hybridizes with unknown sequences at 2q11 on both CHR 2 and on inv(2)(p25p12). **C**) Diagrams of some of the structural rearrangements involving c-MYC that have been identified by FISH analyses in different HMCL and primary MM tumor samples.

Table 1

Mapping c-MYC rearrangements in HMCL by aCGH

	Centromeric								Distance (kb) of probe from 5′ end of c-MYC gene (0)				Telomeric								
HMCL	**2711**	**2646**	**2584**	**1359**	**1176**	**574**	**268**	**40**	**0**	**1**	**2**	**4**	**138**	**400**	**758**	**1155**	**1342**	**Ig**	**c-MYC karyotypic abnormality**	**#CH**	**MYCG**	**MYCR**
JK6L	0.6	1.0	0.9	1.1	0.9	1.3	0.8	0.8	1.5	1.8	1.7	1.4	0.9	1.1	1.2	1.1	1.3	H	t(8:14):MYC duplication	50	1.6	16.2
KMM-1	0.7	0.9	1.0	1.3	1.0	1.2	0.7	1.3	1.6	1.8	1.7	1.5	0.7	0.8	0.9	0.9	0.8	H	MYC ins into IgH	80	1.6	12.9
KMS-12BM	1.9	1.9	2.2	2.3	1.9	1.8	3.0	3.2	4.0	3.9	4.7	3.8	3.2	3.9	1.8	1.9	1.6	H	IgH ins near MYC	47	4.1	22.5
MM-S1	1.8	1.8	1.8	1.8	1.5	3.4	1.4	3.4	3.1	3.9	3.9	3.3	3.0	3.6	3.5	1.8	1.5	H	IgH ins near MYC&duplication	47	3.6	18.8
KARPAS-620	1.1	1.5	1.4	1.4	1.2	1.9	1.8	1.6	1.8	2.1	2.1	1.7	1.6	2.1	1.5	1.4	1.7	H	t(8;14):duplication	68	2.0	6.2
KMS-11	1.3	1.3	1.2	1.6	1.5	1.2	1.0	1.7	1.8	1.9	2.2	1.6	1.5	1.9	1.3	1.1	0.8	H	CH/MYC ins; duplic&inversion	70	1.9	3.9
8226	1.0	1.1	0.8	1.2	1.1	0.9	0.8	0.9	2.3	1.8	2.3	1.9	1.1	1.2	0.8	1.0	1.2	L	der16t(16;8;22)	60	2.1	10.3
DELTA47	0.7	0.9	0.9	1.5	1.1	1.1	0.9	0.9	1.8	1.9	1.8	1.5	0.2	0.9	1.2	0.9	1.0	L	ins CL on der(19)t(8;19)	45	1.8	19.5
FLAM-76	0.4	0.6	0.5	0.8	0.9	0.7	0.6	1.0	1.1	1.4	1.2	0.9	0.6	0.5	0.6	0.6	0.7	O	ins MYC on der(10)t(10;13)	42	1.2	4.8
INA-6	0.9	0.9	1.0	1.3	1.1	3.7	0.8	2.3	2.4	2.9	3.2	2.3	0.9	1.0	1.1	1.0	0.9	O	ins MYC on?	82	2.7	8.8
SKMM-2	1.5	1.7	1.4	1.5	1.2	2.4	2.4	3.0	3.3	3.2	3.0	3.1	0.6	1.7	1.8	1.4	1.6	O	MYC ins on?	37	3.2	15.9
XG-7	1.1	1.0	1.3	1.0	1.0	1.4	0.7	1.1	2.0	1.9	2.1	2.6	1.0	1.1	0.9	1.1	1.0	O	MYC ins on 17 and 19	43	2.2	6.5
EJM	1.1	1.2	1.1	1.6	1.3	0.7	1.0	0.9	1.3	1.5	1.6	1.4	0.9	1.3	1.1	1.4	0.8	O	NONE	58	1.5	10.1
XG-6	1.0	1.3	1.2	1.5	1.2	1.3	1.0	1.3	3.3	3.4	4.0	3.5	1.0	1.5	1.1	1.3	1.2	O	NONE	77	3.6	7.5
H929	1.2	1.5	1.3	0.6	0.6	0.8	0.4	0.4	1.4	1.6	1.5	1.4	1.3	1.7	1.4	0.6	0.5	O	der(8)t(8;20)	45	1.5	3.1
LP-1	1.5	1.8	2.5	1.7	1.6	2.1	1.5	2.3	2.2	2.0	2.1	2.1	1.8	1.0	0.8	0.9	1.5	H	der(8)t(8;14); VARIANT IgH	80	2.1	9.9
OPM-1	1.2	1.3	1.3	1.4	1.1	1.8	1.0	1.1	1.2	1.5	1.2	1.1	0.9	1.2	1.5	0.6	0.6	H	der(8)t(1;8) with ins CH	74	1.3	2.4
XG-1	1.2	1.2	1.1	1.5	1.2	0.6	1.0	0.8	1.2	1.5	1.4	1.4	0.9	1.3	0.6	0.6	0.5	H	der(8)t(8;14); VARIANT IgH	44	1.4	2.6
KMS-26	1.8	1.7	2.0	2.6	2.2	2.0	1.1	1.9	1.8	1.9	2.5	1.7	1.0	1.9	1.7	2.4	0.4	L	der(8)t(8;22)	75	2.0	6.3
OCI-MY1	0.9	1.2	1.1	1.2	0.8	2.2	0.9	1.3	1.1	1.2	1.4	1.5	1.4	1.6	1.0	0.6	0.7	L	der(8)t(8;22)	49	1.3	3.3
ARP-1	1.1	1.1	1.3	1.6	1.3	1.0	1.0	1.1	1.1	1.3	1.6	1.4	0.9	1.3	0.6	0.8	0.7	O	der(8)t(8;?)	69	1.3	11.2
JIM-3	1.4	1.1	1.3	1.7	1.4	1.9	0.8	1.1	1.4	1.8	2.3	1.4	0.6	0.6	0.8	0.8	0.4	O	der(der8)t(8;?)	61	1.7	5.9
KAS-6	1.2	1.4	1.5	2.5	1.9	3.3	1.9	2.2	2.1	2.5	2.4	2.1	1.7	0.7	0.8	0.7	0.8	O	der 8/amplification)	70	2.3	7.1
KHM-11	1.8	2.0	2.3	1.9	1.6	1.8	1.5	1.8	2.1	2.2	2.3	2.1	0.9	0.9	0.8	0.9	0.8	O	der(8)t(3;8)	75	2.2	7.1
KMS-34	1.3	1.4	1.6	1.5	1.3	1.4	1.2	1.4	1.6	1.7	1.8	2.4	0.9	1.4	1.1	1.4	1.0	O	der8t(8;?)	71	1.9	19.7
L363	1.3	1.5	1.4	1.6	1.4	1.3	1.3	1.4	1.5	1.5	1.8	1.6	1.4	1.9	0.9	1.1	0.9	O	(t(5;8)); der8×2+der5	46	1.6	6.6
OCI-MY7	0.9	1.2	1.0	1.5	1.1	1.8	0.9	1.1	1.1	1.4	1.3	1.0	1.1	1.2	0.6	0.6	0.6	O	der8(t3;8))	78	1.2	15.9
OCI-MY5	0.9	1.1	0.9	1.3	1.2	1.4	1.8	1.7	2.0	2.7	2.1	1.8	1.8	2.1	2.3	2.1	2.0	H	der(der14)t(8;14);dup&inversion	46	2.1	2.2

	Centromeric								Distance (kb) of probe from 5′ end of c-MYC gene (0)				Telomeric								
HMCL	**2711**	**2646**	**2584**	**1359**	**1176**	**574**	**268**	**40**	**0**	**1**	**2**	**4**	**138**	**400**	**758**	**1155**	**1342**	**Ig**	**c-MYC karyotypic abnormality**	**#CH**	**MYCG**	**MYCR**
SACHI	0.6	0.6	0.7	0.8	0.7	0.6	1.0	1.3	1.1	1.4	1.4	1.3	1.1	1.4	1.2	1.5	0.9	H	der(14)t(8;14)	69	1.3	17.2
KMS-18	2.2	2.1	1.7	2.7	1.9	2.4	2.1	2.3	4.0	4.1	4.6	3.5	4.2	4.0	2.7	3.2	3.3	O	der(6&21)t(6&21;8)	74	4.1	5.4
XG-2	0.9	1.0	1.3	1.3	1.3	1.3	1.3	1.4	1.3	1.6	2.1	1.6	1.3	2.3	1.5	1.8	1.5	O	der(2)t(2;8)	49	1.7	5.1
UTMC-2	2.2	2.4	2.6	2.1	1.7	2.8	2.2	2.8	2.5	2.6	2.5	2.7	0.8	0.8	2.3	2.2	2.8	O	NONE	77	2.6	6.3
FR-4	0.9	0.9	1.0	1.1	1.0	0.8	1.1	1.1	1.2	1.3	1.4	1.3	0.9	1.3	1.1	1.3	0.9	H	t(8;14):balanced	100	1.3	2.2
JJN-3	1.9	2.3	1.9	1.9	1.5	2.9	1.9	2.2	2.2	2.5	2.6	2.5	1.9	2.6	2.6	2.5	1.9	H	IgH ins on 8 & complex t(8;14)	60	2.5	16.7
KMS-28BM	1.4	1.6	1.5	1.6	1.1	2.9	1.2	1.5	1.6	1.8	1.8	1.6	1.6	2.0	2.2	1.6	1.7	H	t(8;14):balanced	42	1.7	6.6
MM.1S	0.9	1.1	1.1	1.1	1.0	1.2	0.9	1.1	1.1	1.1	1.2	1.1	0.9	1.4	1.0	1.0	1.3	H	IgH ins at bkpt of der3t(3;8)	44	1.1	3.4
SKMM-1	1.0	1.0	1.0	1.2	1.1	1.8	0.9	0.9	1.1	1.3	1.2	1.0	1.0	1.1	1.6	1.2	1.1	H	t(8;14);complex	79	1.1	13.3
KHM-1B	1.1	1.3	1.3	1.3	1.1	1.5	1.2	1.6	1.2	1.4	1.4	1.3	0.9	1.5	1.1	1.2	1.4	L	IgL ins near MYC	59	1.3	7.5
ANBL6	1.0	0.9	0.9	1.1	1.0	1.2	0.7	0.7	0.9	1.1	0.9	0.8	0.9	0.8	1.0	0.9	0.9	O	NONE	46	0.9	2.6
H1112	0.8	1.2	1.0	1.2	0.9	1.5	0.8	0.8	1.0	1.1	1.1	1.0	0.8	1.0	1.1	0.9	0.9	O	NONE	46	1.0	1.1
PE-1	1.4	1.2	1.4	1.4	1.2	2.2	1.1	1.2	1.4	1.6	1.6	1.3	1.2	1.3	1.4	1.3	1.2	O	NONE	79	1.5	0.3
PE-2	1.7	1.7	2.0	2.0	1.8	1.4	1.5	1.8	1.7	1.8	2.3	1.9	1.4	2.0	1.6	2.1	1.8	K	N-MYC/IgK inversion	72	1.9	0.1
U266	1.1	0.9	1.0	1.2	1.1	1.7	0.7	0.9	1.0	1.3	1.4	0.9	0.9	1.0	1.1	1.0	0.7	O	L-MYC/translocation and inversion	39	1.1	0.1

Comparative genomic hybridization arrays (aCGH) data in c-MYC locus, with distances of probes from 5′ end of c-MYC gene. Ig indicates juxtaposition of a MYC gene with IgH (H), Ig λ (L), Ig κ(K), or no Ig (O) sequences. #CH = modal number of chromosomes per cell. MYCG = DNA content normalized to normal reference DNA; MYCR = RNA content relative to normal plama cells. Dark shading = approximately twofold or more loss of sequences compared to c-MYC; light shading = less than twofold loss of equences. See Methods and other text for additional information.

Table 2

Mapping of c-MYC rearrangements in HMCL by FISH

	Centromeric	Distance (kb) from c-MYC gene (0)												Telomeric	
HMCL	**5000**	**1700**	**800**	**650**	**500**	**450**	**400**	**0**	**200**	**400**	**550**	**900**	**1050**	**1900**	**No. of copies of chromosome 8 and derivatives per cell**
ANBL6	+		+	+				+	+		+	+	+		2N8
	+		+	+				+	+		+	+	+		2der14t(8;14)(q11;q32)
EJM	+		+	+		+	+	+			+		+		1N8
	+		+	+		+	+	+			+		+		1der8t(8;19)(p21;?)
	+		+	+		+	+	+			+		+		der?8t(7;16;8)(?;?;q11)
FLAM-76	+			+	+		+	+	+		+				1N8
	–			–	–		–	+	–		–				der10t(10;13)w ins. of c-myc
H1112	+		+	+				+	+		+	+	+		2N8
H929	+	+	+	+			+	+			+	+	+		1N8
	+	–	–	–			–	+			+	+	–		2der8t(8;20)(q24;q11)
KHM-11	+		+			+	+	+	+	+	+				2N8
	+		+			+	+	+	–	–	–				2der2t(2;8)(?;q21)
	+		+			+	+	+	–	–	–				2der1t(1;8)(?;q21)
	–		–			–	–	+	+	+	+				1der1t(1;8)+1der6t(6;8)(?;q24)*
	+		+			+	+	+	–	–	–				1der8 t(3;8)(?;q24)
KMS-18	+		+	+	+	+		+	+		+	+	+		2N8
	+		+	+	+	+		+	+		+	+	+		2der14t(8;14)+2der12t(8;12)(q11;?)
	–		–	–	–	+		+	+		+	+	+		2der21t(8;21)(q24;?)
	–		–	–	–	+		+	+		+	+	+		2der6t(6;8)(?;q24)
	+		+	+	+	+		+	+		+	+	+		2der2t(2;8)(q24;?)
L363	+			+				+		+	+	–	–		2der8t(5;8)(?;q24)
	–			–				–		–	–	+	+		1der5t(5;8)(?;q24)
	+			+				+		+	+	+	+		1der8 del q24-qter
OCI-MY7							+	+	+		+	+	+		2N8
							+	+	+		+	–	–		2der8 t(3;8)(?;q24)
SKMM-2			+	+		+	+	+	+	+	+	+			1N8
			+	+		+	+	+	+	+	+	+			1iso8
			–	–		–	–	–	–	–	–	–			1der16t(8;16)

J Natl Cancer Inst Monogr. Author manuscript; available in PMC 2009 September 3.

	Centromeric		Distance (kb) from c-MYC gene (0)										Telomeric	No. of copies of chromosome 8 and derivatives per cell
HMCL	5000	1700	800	650	500	450	400	0	200	400	550	900	1050	1900	
			–	–		–	+	+	–	–	–	–			1der9 t(8;9;ins8,9,13)
			–	–		–	+	+	–	–	–	–			der?(14;?1;?9,ins8,9)
UTMC-2	+		+	+				+	+	+	+	+	+	+	2N8
	+		+	+				+	–	–	–	+	+	+	2iso8q
	+		+	+				+	–	–	–	+	+	+	2der5t(5;8)(?;q11)
XG-2	+		+	+				+	+		+	+			2N8
	+		+	+				+	+		+	+			1der10t(8;10)(q11;q22)
	–	–	+	+				+	+		+	+			1der2t(8;?)(q24;q?)
	–	–	–	–				–	–		–	–			1der8delq24-q?
XG-6				+				+	+		+	+			2N8
				+				+	+		+	+			1derY?t(8;?)(q11;q?)
XG-7				+	+	+		+	+		+			+	1N8
				+	+	+		+	+		+			+	1der8t(8;13)(p 12;?)
				–	–	–		+	–		–			–	Ins of c-myc on chr.17 and chr.19

Centromeric to telomeric FISH data for c-MYC locus, with distances in kilobases of probes from c-MYC gene (dark shading). Chromosome 8 (N8) and derivative chromosomes that contain portions of chromosome 8 are shown, including copy numbers of each chromosome per cell. FISH signals are indicated as present (+) or absent (−), with negative regions (and regions in between negative regions) highlighted by light shading.

* indicate positive signal with c-MYC BAC clone but negative with c-MYC plasmid probe. See Methods and other text for additional information.