Chapter 8
Immunoglobulin and *MYC* Rearrangements in Multiple Myeloma Pathogenesis

P. Leif Bergsagel and W. Michael Kuehl

Contents

Abstract Multiple myeloma is a post-germinal centre plasma cell tumour that is usually preceded by a pre-malignant condition, monoclonal gammopathy of undetermined significance. Both are characterized by hyperdiploidy and recurrent immunoglobulin gene translocations that all result in the direct or indirect dysregulation of the CCND/RB1 pathway. Analysis of the translocation breakpoints suggests that they most frequently occur as a result of an error during class switch recombination, but also VDJ recombination, and sometimes somatic hypermutation. A rearrangement of the *MYC* locus is identified in nearly one half of untreated patients with MM, most frequently (>60 %) those with hyperdiploidy, and less frequently (<25 %) those with t(11;14). The rearrangements juxtapose *MYC* to super-enhancers from elsewhere in the genome, resulting in dysregulated expression of *MYC*. One-third of

P.L. Bergsagel (✉)
Comprehensive Cancer Center, Mayo Clinic Arizona, Scottsdale, AZ, USA
e-mail: bergsagel.leif@mayo.edu

W.M. Kuehl
Genetics Branch, Center for Cancer Research, National Cancer Institute, Bethesda, MA, USA

J.D. Rowley et al. (eds.), *Chromosomal Translocations and Genome Rearrangements in Cancer*, DOI 10.1007/978-3-319-19983-2_8

the rearrangements involve an immunoglobulin gene enhancer (*IGH>IGL>>IGK*), and two-thirds one of a variety of non-immunoglobulin gene enhancers that are frequently associated with plasma cell gene expression (e.g., *PRDM1, IGJ, FAM46C, TXNDC5, FOXO3*). It is likely that early rearrangements of the *MYC* locus cause the progression of monoclonal gammopathy to multiple myeloma in many patients, and that late rearrangements of the *MYC* locus, frequently involving an immunoglobulin gene enhancer, contribute to further, often extramedullary, tumour growth.

Keywords Multiple myeloma • Chromosome translocation • Plasma cell neoplasm • Monoclonal gammopathy of undetermined significance • MYC oncogene

8.1 Multiple Myeloma Is a Post-germinal Centre Plasma Cell Tumour

Multiple myeloma (MM) is a malignant tumour of post-germinal centre B cells, with a phenotype that is similar to long-lived bone marrow plasma cells (BMPCs) [1, 2]. MM mostly – perhaps always – is preceded by a pre-malignant MGUS (monoclonal gammopathy of undetermined significance) tumour, which is present in 4 % of individuals over the age of 50 [3, 4]. Presently there are no molecular or phenotypic markers that unequivocally distinguish MGUS and MM tumour cells, but MGUS can sporadically progress to symptomatic MM expressing the same monoclonal immunoglobulin at an average rate of ~1 % per year. Asymptomatic/smouldering MM (SMM) has a larger tumour mass than MGUS, but sporadically progresses to symptomatic MM at an average rage of ~10 % per year for the first 5 years, 3 % for the next 5 years, and 1 % for the next 10 years [5]. Unlike MGUS, therefore, the rate of progression of SMM is not fixed, but decreases over time. This indicates that SMM likely represents a mix of patients with MGUS, and others with MM who have not yet developed end-organ damage. Extramedullary MM, often manifested as primary or secondary plasma cell leukaemia (PCL), is a more aggressive stage of disease [2, 6]. MM cell lines (MMCLs) usually are generated only from extramedullary MM tumours [7].

MGUS and MM tumour cells secrete monoclonal immunoglobulin (Ig) that has a high prevalence of somatic mutations in the variable regions of the heavy and light chains, consistent with repeated rounds of somatic hypermutation (SHM) and antigen selection in precursor germinal centre B cells [8, 9]. Similar to long-lived BMPCs, most MGUS and MM tumours have undergone productive *IGH* class switch recombination (CSR), resulting mostly in expression of IgG or IgA and, rarely, IgE or IgD. However, about 1 % of tumours express IgM. In addition, about 15 % of MGUS and newly diagnosed MM tumours express an Ig light chain but no Ig heavy chain, most likely a consequence of post-germinal centre inactivation of *IGH* expression by a variety of mechanisms, including *IGH* translocations [4, 10].

8.2 Three Specific DNA Modifications Mediate Translocations in B Cell Tumours

Many kinds of B cell tumours have chromosomal translocations that involve the *IGH* locus (14q32.3), or less often one of the light chain loci: kappa (*IGK*, 2p12) or lambda (*IGL*, 22q11.2) [11]. Most of these translocations appear to be a consequence of errors in three B cell-specific DNA modification processes: (1) V(D)J recombination (VDJR), which is recombination activation gene complex (RAG1/RAG2) dependent, mostly occurs at very early stages of B cell development but sometimes during receptor editing or receptor revision at later stages of B cell development; (2) somatic hypermutation (SHM), which is activation-induced cytidine deaminase (AID) dependent, occurs almost exclusively in germinal centre B cells; and (3) IgH class switch recombination (CSR) which is also AID dependent and occurs mainly but not exclusively in germinal centre B cells (Table 8.1).

CSR involves recombination between long repetitive switch regions (Sμ, Sγ, Sα, Sε) located upstream of all *IGH* constant regions except for *IGH* delta [12]. The μ>δ CSR, involves Sμ and/or 422 bp duplicated sequences (σμ and σδ) positioned, respectively, upstream of Sμ and δ sequences [13, 14].

Each of these three mechanisms, which appear to be operative only at specific stages of B cell development, can generate double-strand breaks at or near specific sites in an *IG* locus, but sometimes can cause mutations or double-strand breaks in non-*IG* loci [11, 12, 15, 16]. The consequence of IG translocations typically is dysregulation and increased expression of an oncogene that is positioned near one or more of the strong *IGH* enhancers (Eμ, Eα1, Eα2), the *IGK* enhancers (E.IK, E.3′K), or the *IGL* enhancer (E.3′L) (Fig. 8.1).

Translocations mediated by the first two mechanisms typically generate a translocation breakpoint upstream of all enhancers, so that all enhancers are relocated to one of the two derivative chromosomes, der(14) for *IGH*, der(non-2) for *IGK*, and der(non-22) for *IGL*. By contrast, the CSR mechanism generates a breakpoint within or near a switch region, so that Eα1 and/or Eα2 are relocated to der(14) whereas Eμ (and sometimes Eα1) are relocated to der(non-14). As a result an oncogene can be dysregulated by an *IGH* enhancer on both chromosomes, as first demonstrated for *FGFR3* on der(14) and *WHSC1/MMSET* on der(4) in MM [17].

Table 8.1 B-cell specific DNA modifications that contribute to chromosome translocations in multiple myeloma

DNA modification	Mechanism	Timing
VDJ recombination	RAG1/RAG2	Mostly early B-cell, but sometimes during receptor editing or receptor revision in mature B cells
Somatic hypermutation	AID	Germinal centre B cells
Class switch recombination	AID	Mainly, but not exclusively, germinal centre B cells

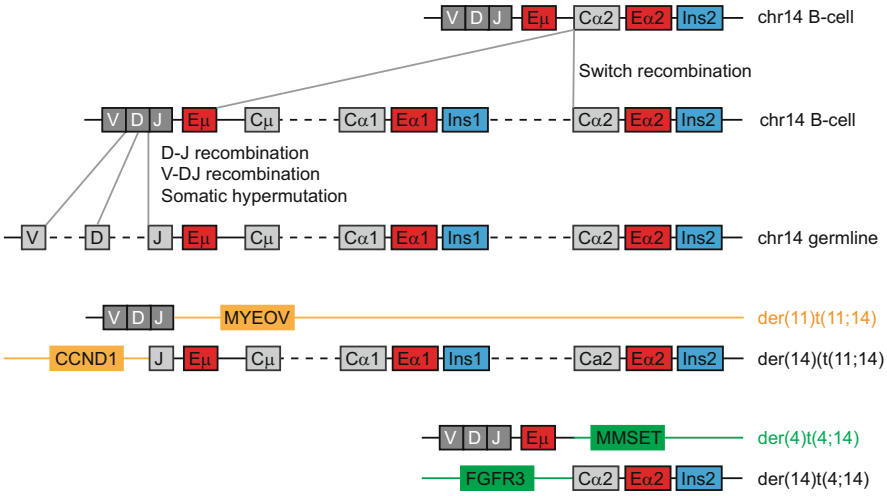

Fig. 8.1 Schema of *IGH* translocations mediated by B-cell specific DNA modifications in multiple myeloma. VDJ recombination, somatic hypermutation and class switch recombination remodel the germline *IGH* locus during B cell development (*top*). Errors in VDJ recombination and somatic hypermutation contribute to translocations that juxtapose all three *IGH* enhancers (*red*) to the translocated oncogene (e.g., CCND1) on der(14)t(11;14)(q13.3;q32.3) (*middle*). In contrast, errors in class switch recombination dissociate the *IGH* enhancers, so that the intronic enhancer (Eμ) dysregulates MMSET on der(4)t(4;14)(p16.3;q32.3), and the 3′ *IGH* enhancer (3′α2) dysregulates *FGFR3* on der(14)t(4;14)(p16.3;q32.3) (*bottom*). Insulators (*blue*) downstream of the 3′ enhancers prevent downstream gene dysregulation

8.3 *IGH* Rearrangements Are Present in About 50 % of MM Tumours

Conventional cytogenetics of MM greatly underestimated the prevalence of *IGH* translocations, and rarely identified the partner chromosomal loci, except for t(11;14). This was a consequence of: a low mitotic index, the telomeric location of the *IGH* locus together with telomeric or subtelomeric location of many partner loci, and the marked complexity of karyotypes [13, 18]. Interphase FISH assays largely solved the problem of detecting *IGH* translocations. Quantitative interphase FISH procedures are performed using a three colour cytoplasmic immunoglobulin method (cIg FISH) [19], or on purified MGUS/MM cells that are selected on anti-CD138 magnetic beads [20]. It is worth noting that the widely used Vysis *IGH* probes efficiently detect translocations but do not efficiently identify insertions of *IGH* sequences; fortunately other *IGH* probes that detect Ea1 and Ea2 sequences – including a Cytocel commercial probe – can efficiently detect both *IGH* translocations and *IGH* insertions [21]. Results from many studies indicate that the prevalence of *IGH* translocations is ~45 % for MGUS tumours, ~55 % for intramedullary MM tumours, ~80 % in primary PCL, and ~80 % in MMCL [6, 20, 22–27]. There is very

little information about *IGK* or *IGL* rearrangements. However, *IGL* rearrangements were identified in about 15–20 % of MMCL or advanced MM tumours, whereas IGK rearrangements are much less frequent, i.e., ~2 % of MMCL or advanced MM tumours [27].

8.4 Primary *IGH* Translocations: An Early Oncogenic Event in ~40 % of MM Tumours

Primary *IGH* translocations are defined as recurrent translocations that are present in both MGUS and MM tumours, although it is unknown if they represent the initial oncogenic event [28]. The seven recurrent *IGH* translocations comprise three translocation groups, with the chromosomal site, target oncogene(s), and approximate prevalence in MM as indicated [1, 20, 25, 29–31]:

Cyclin D group:	
11q13.3 (*CCND1*)	15 %
12p13.3 (*CCND2*)	~1 %
6p21.1 (*CCND3*)	2 %
MAF group:	
16q23 (*MAF*)	5 %
20q12 (*MAFB*)	2 %
8q24.3 (*MAFA*)	<1 %
WHSC1/MMSET and FGFR3 group:	
4p16.3 (*WHSC1/MMSET* and *FGFR3*)	14 %

Together the combined prevalence of primary *IGH* translocations is approximately 40 % in MM but somewhat lower in MGUS. Compared to MM, MGUS has a significant decrease in the prevalence of t(4;14) (~4 %) and possibly t(14;16), and an apparent increase in t(11;14). It is possible but unproven that the lower prevalence of t(4;14) and t(14;16) in MGUS results from a more rapid progression from MGUS to MM, and vice versa for the increased prevalence of t(11;14) in MGUS. Surprisingly, the prevalence of t(11;14) is markedly increased (~40 %) for MGUS tumours that are associated with primary amyloidosis, but presently there is not a good explanation for this observation [32, 33]. Most of the primary *IGH* translocations appear to be generated as simple reciprocal translocations, usually with both derivative chromosomes present at all disease stages. However, for the t(4;14), about 20 % of MM tumours and MMCL have lost the der(14) so that *FGFR3* no longer is dysregulated [34–36]. There are no examples of MM tumours or MMCL that have lost the der(4). This suggests that dysregulation of *MMSET* on the der(4) is essential both for early pathogenesis but also for maintenance of the tumour throughout the course of the disease. Some MM tumours and MMCLs with the t(11;14) do not have the der (11), and others have two copies of the der(14) and one copy of the der(11) [27, 37, 38]. There are very few examples of insertions that juxtapose *IGH* enhancer sequences

with one of the seven recurrent *IGH* partners [27]. Rare monoclonal tumours have two independent *IGH* translocations or insertions that involve two different *IGH* translocation groups, e.g., 4p16.3 and 11q13.3; 4p16.3 and 16q23; 6p21.1 and 16q23; and possibly 11q13.3 plus 20q12 [24, 27]. Curiously, *IGL* and IGK translocations rarely involve one of the seven recurrent *IGH* partners, but *IGL* translocations involving 6p21.1 [39] or 16q23 [40], and insertions involving 20q12 and *IGK* [41] or *IGL* [27] have been identified.

8.5 Primary *IGH* Breakpoints Generated by All 3 B Cell Specific DNA Modifications in MM

Metaphase and interphase FISH analyses, together with conventional cytogenetics and spectral karyotypic (SKY) analyses, can efficiently identify most IG rearrangements, including the apparent partner chromosomal loci. However, identification of the rearrangement breakpoints and target genes has been a more involved process. Initially this was done by a combination of Southern blotting to map breakpoints, plus conventional cloning or various PCR procedures to isolate more than 50 primary *IGH* breakpoints [reviewed in [28]]. Unfortunately, this was a somewhat biased approach, which focused mainly on *IGH* switch regions, and to a somewhat lesser extent on sequences between JH and Sμ. More recently, mate pair sequencing (PLB&WMK, unpublished), whole genome sequencing [42], and targeted sequencing of captured genomic regions containing *IG* sequences [43] have resulted in more than 60 additional primary *IGH* breakpoints that are less biased for specific regions within the *IGH* locus. A summary from the analysis of more than 100 primary *IGH* breakpoints includes the following [18, 28, 43]. First, the locations of breakpoints in the *IGH* locus suggest that all three B cell specific DNA modification processes (CSR>VDJR>SHM) can be implicated (Table 8.1). Second, the positioning of breakpoints near or within V, D, or J sequences suggests errors in D>J joining and less often V>DJ joining or V region replacement, all of which are mediated by the VDJR mechanism. Third, two t(14;16) breakpoints are located near one of two nearly identical non-adjacent sequences in the *IGH* locus but with no homology to the partner chromosome; the authors suggested a homologous recombination mechanism, but the molecular basis for this event is unclear [43]. Fourth, in all cases, the t(4;14) involves *IGH* switch regions. Fifth, about half of the t(11;14) breakpoints occur near or within switch regions, about one third at locations suggesting errors in VDJR, and most of the others at locations consistent with errors in SHM [18, 28, 43]. Sixth, there is less data for the t(6;14), t(14;16), and t(14;20) breakpoints, but it appears that about 50 % are located near or within switch regions.

The location of breakpoints within the *IGH* locus has been used to infer not only the occurrence of an error in a particular B cell specific DNA modification process but also the time of occurrence during B cell development. The latter makes two assumptions: (1) that we know at what stage(s) of normal B cell development a

particular modification occurs; and (2) that a particular modification occurs only during the stage(s) implicated for normal B cell development, but not at another stage in a pathological situation. For translocations involving switch regions there are several lines of evidence indicating that most – if not all – of these rearrangements occur during normal *IGH* switch recombination. First, cloning and sequencing of *IGH* switch breakpoints in 40 t(4;14) MM tumours or MMCLs show that the 5′ switch breakpoint on the der(4) always is μ-, μγ-, or μα- and the 3′ switch breakpoint on the der(14) involves -μ, -γ, or -α, and less often –μγ or -μα; one caveat is that t(4;14) rearrangements may be uniquely constrained to have breakpoints close to Eμ so that *MMSET* can be dysregulated on the der(4) [28]. Second, in the U266 MMCL, a productive μ>ε switch was associated with a second switch event that excised a portion of the intervening switch sequences (including Eα1), and inserted them near the *CCND1* gene at 11q13.3 [44]. Third, when 3′ switch breakpoints involve distal switch regions (-γ2, -γ4, -α2), Eα1 almost always is deleted as would be predicted if a normal switch event involved Sμ and a distal switch region [28].

The timing of translocations that appear to be mediated by errors in VDJR or SHM presents a more complex situation. It was suggested that rearrangements involving V segments associated with JH mutations could represent either receptor revision or SHM in germinal centre B cells, whereas the lack of JH mutations was more consistent with revision in B cells that have not yet undergone SHM [43]. Most interestingly, the authors described seven examples [six with the t(11;14)] of translocation breakpoints suggesting an apparent error in D>J joining. As a result they suggested that some translocations can occur in pro-B cells that are undergoing D>J rearrangements. This provocative result is consistent with the fact that Eμ. CCND1 transgenic mice show only minimal abnormalities of normal B cell development [45, 46]. However, an alternative explanation that D>J joining can occur in mature human B cells – perhaps only as a rare pathological event – cannot be excluded.

8.6 Locations of Breakpoints on Primary *IGH* Translocation Partner Chromosomes

The primary *IGH* translocation partner chromosome breakpoints have a number of interesting features [28, 43]:

1. The breakpoints can be located more than 1000 kb centromeric to the oncogene that is dysregulated on the der(14), e.g., 20q12 breakpoints are 300–1200 kb downstream of *MAFB* and 16q23 breakpoints are 480–1280 kb downstream of *MAF*.
2. The 16q23 breakpoints are located mostly within the ultimate intron of *WWOX*, which includes the FRA16D fragile site, and has been proposed to be a tumour suppressor gene in some tumours.

3. For two translocations the oncogene apparently can be dysregulated even when other genes are closer to the breakpoint. For t(4;14) tumours, *FGFR3* is dysregulated, whereas *LETM1*, which is located between *FGFR3* and the breakpoint, is not dysregulated. For t(11;14) tumours, the breakpoints are mostly scattered throughout the 400 kb region upstream of *CCND1* and downstream of *MYEOV*. However, breakpoints in five tumours were located in a 90 kb region upstream of *MYEOV*, and the breakpoint in one tumour was located in the *PPP6R3* gene, which is located centromeric to both *TPCN2* and *MYEOV* [43].
4. The mechanism(s) responsible for creating double-stranded breaks on the partner chromosomes are not well understood; However, the frequent involvement of AID was suggested based on the presence of CpG dinucleotide sequences or WGCW sequences, known AID recognition sites, near the breakpoint in up to 61 % of samples [43].
5. The breakpoints in t(4;14) tumours are invariably located upstream of *MMSET* or clustered in 5′ introns in *MMSET* [17, 35, 43]. Therefore *FGFR3* is positioned 30–80 kb telomeric to the 3′*IGH* enhancers on the der(14). Full length or variously truncated portions of *MMSET* are positioned near the Eμ enhancer on the der(4), with consequent expression of hybrid transcripts that contain JH or Iμ exons. Importantly, the hybrid transcripts, unique for this translocation, can be detected by a very specific and sensitive PCR assay. This invariant scenario is consistent with the need to simultaneously dysregulate both *FGFR3* and *MMSET* early in tumourigenesis, despite the fact that the der(14) is not present in ~20 % of MM tumours, presumably because subsequent oncogenic events (e.g. *RAS* gene mutations) obviate the need for dysregulation of *FGFR3*.

8.7 Two Major Pathways of MM Pathogenesis: Primary *IGH* Translocations and Hyperdiploidy

Approximately half of MM tumours are hyperdiploid (HRD), typically containing 48–60 chromosomes and, typically with a characteristic pattern of multiple trisomies involving eight chromosomes (3, 5, 7, 9, 11, 15, 19, 21) [47, 48]. Non-hyperdiploid (NHRD) MM tumours usually are hypodiploid, pseudodiploid, or subtetraploid, often with near diploid and subtetraploid cells in the same tumour. Interphase FISH studies indicate that HRD tumours constitute a somewhat lower fraction in MGUS compared to MM [20, 49]. Primary *IGH* translocations are present in ~70 % of NHRD MM tumours but only ~15 % of HRD MM tumours. Similar to MM, primary *IGH* translocations are found mostly in NHRD MGUS tumours [20]. Despite some overlap, it generally is thought that the two major pathways of MGUS/MM pathogenesis are associated with primary *IGH* translocations or hyperdiploidy, respectively, as early if not initiating events. The causes and molecular consequences of hyperdiploidy have not been elucidated at this time. However, it is notable that the fraction of MM tumours with hyperdiploidy increases with age and the fraction of tumours with *IGH* translocations decreases with age [50].

8.8 *MYC* Rearrangements: A Paradigm for Secondary Rearrangements in MM

Prior to the development of FISH and spectral karyotyping technology, conventional cytogenetic analysis rarely identified *MYC* rearrangements in MM tumours or MMCLs [18]. The finding that only one *MYC* allele was expressed in all nine informative MMCLs examined prompted metaphase FISH studies, which showed that 19 of 20 MMCLs and 7 of 14 advanced MM tumours had complex rearrangements of *MYC* or *MYCL*, many of which did not involve one of the three *IG* loci [40]. Collectively, comprehensive metaphase FISH studies, which utilized *IGH*, *IGL, IGK, MYC*, *MYCL*, *MYCN*, and whole chromosome painting probes, identified rearrangements of *MYC*, *MYCN*, or *MYCL* in 42/53 (79 %) MMCLs and 28/62 (45 %) advanced MM tumours [27, 40]. There was heterogeneity of *MYC* rearrangements in some MM tumours. Less comprehensive spectral karyotyping studies detected a somewhat lower prevalence of *MYC* rearrangements in advanced MM tumours [37, 38]. The *MYC* rearrangements were mostly unbalanced translocations or insertions, often with involvement of three chromosomes, and sometimes with associated amplification, duplication, deletion, or inversion (Fig. 8.2).

Interphase FISH studies using *IGH*, *IGL*, and *MYC* probes, together with a pair of probes flanking the *MYC* locus, were done on 22 MMCL, 529 newly diagnosed MM tumours, 53 relapsed MM tumours, and 63 MGUS tumours [51]. *MYC* locus rearrangements, which often were present in only a fraction of primary tumour cells, were detected in 55 % of MMCL and 16 % of MM tumours, but only 3 % of MGUS tumours. Another study reported a smouldering MM tumour with no *MYC* rearrangement that progressed to symptomatic MM with a *MYC* rearrangement [52]. Finally, in contrast to primary *IGH* translocations (above), the *IGH* breakpoints in *MYC*:*IGH* translocations are rarely within or near switch regions or VDJ sequences [28, 29]. Therefore, they are unlikely to be caused by errors in the B cell specific DNA modification mechanisms that are thought to be inactive in plasma cells and plasma cell tumours. Together, these results indicate that *MYC* rearrangements are a paradigm for secondary rearrangements that are uncommon in MGUS but have an increasing prevalence during progression of MM.

The combination of metaphase FISH (see above) and Agilent 244 K CGH arrays detected *MYCN* (one MMCL), *MYCL* (one MMCL) or *MYC* locus (chr8: 126000000–130000000[hg19]) rearrangements in 47/53 (89 %) MMCL [29]. Rearrangements in five MMCLs were detected only by FISH and in five other tumours only by CGH. For the 47 MMCL with *MYC* rearrangements, 28 (60 %) repositioned a *MYC* family member near one of the *IG* 3′ enhancer sequences that are included in the FISH probes (21 with *IGH*, 5 with *IGL*, and 2 with *IGK*).

A combination of 244 K CGH, interphase FISH to detect *MYC*:*IGH* and *MYC*:*IGL* fusions, and gene expression profiling identified *MYC* locus rearrangements or *MYCN* expression (two tumours) in 108/218 (49 %) MM tumours [29]. The prevalence of rearrangements was not significantly different for treated (52 %) vs newly diagnosed (46 %) vs smouldering (55 %) MM tumours. Of these, 33/218 (15 %)

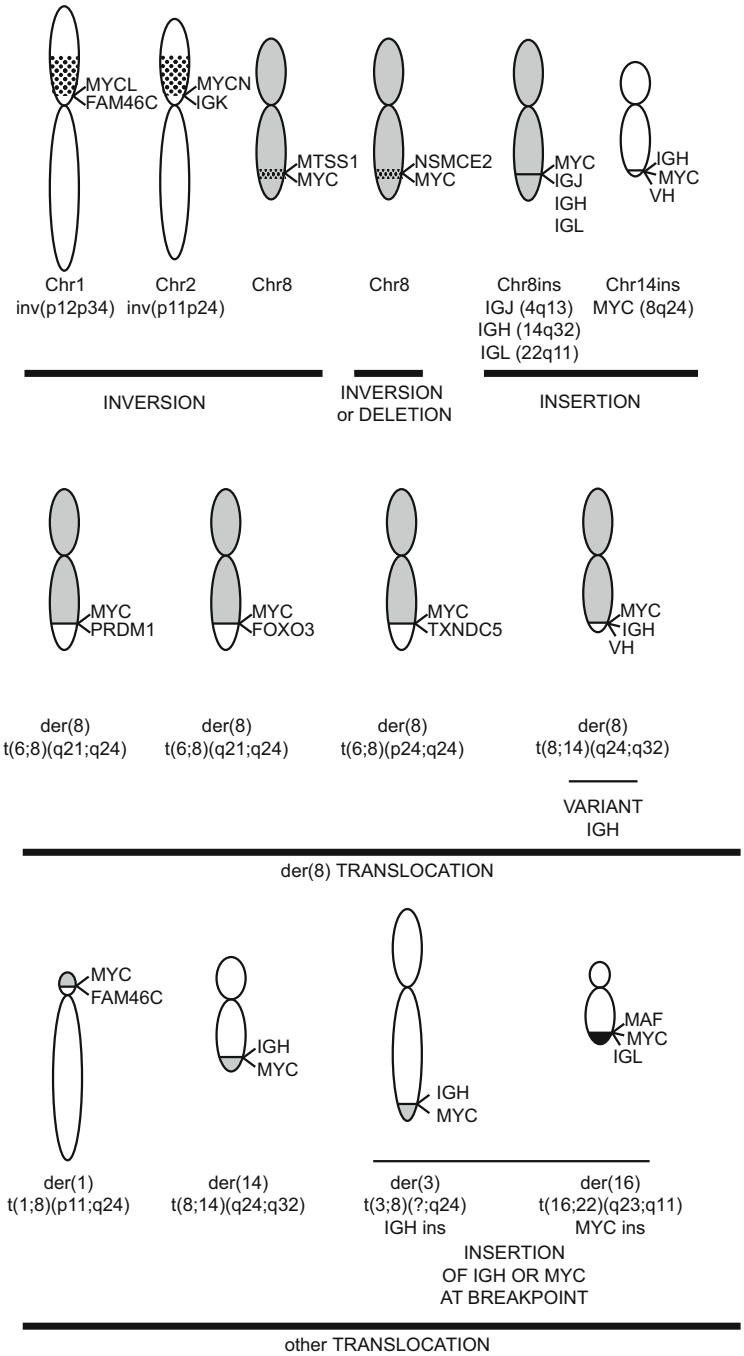

Fig. 8.2 Rearrangements of *MYC*, *MYCN* and *MYCL* in multiple myeloma. Ideograms of chromosomal rearrangements including inversions (*stippled*), deletions (*stippled*), insertions (*black line*), and translocations (chr8 is shaded in *grey*). A variant *IGH* translocation has a small inversion near the breakpoint so that the insulator is not positioned between *MYC* and the 3′ *IGH* enhancer

tumours had *MYC*:*IGH* (11 %) or *MYC*:*IGL* (4 %) fusions, with 22 of these tumours also having a rearrangement detected by CGH. There was significant heterogeneity of the *MYC*:*IG* fusion signal in 6 of the 33 tumours. Using *MYC* as a reference location, the *MYC* locus copy number abnormalities detected by CGH, which were similar for MMCL and MM tumours, comprised four groups: centromeric changes (mostly segmental loss), segmental *MYC* gain, telomeric segmental gain that was located mostly in a region 350–500 kb downstream of *MYC*, and other telomeric CNA. *MYC*:*IGH* or *MYC*:*IGL* fusions were present in only 4 % of the tumours with centromeric changes, but in about 30 % of the tumours in each of the other three groups.

MYC expression in MMCL and MM tumours was mostly monoallelic with rearrangements, but mostly biallelic without rearrangements. The mean level of *MYC* RNA expression was about threefold higher in tumours or MMCL with vs. those without *MYC* rearrangements. Surprisingly, for both MMCL and MM tumours, the mean level of *MYC* expression was similar for rearrangements that did or did not involve *IG* loci.

Given that the CGH and FISH assays would miss some *MYC* rearrangements (e.g., balanced translocations; some inversions and insertions), it was estimated that *MYC* rearrangements are likely to have been present in ~60 % of the MMRC MM tumour samples [29]. Therefore, it seems that *MYC* is the most frequent target of genomic rearrangements in MM. It is clear that many *MYC* rearrangements occur during progression of MM. However, even though the increased expression of *MYC* in MM compared to MGUS often is mediated by a biallelic mechanism, it is possible that in some tumours, increased monoallelic expression of *MYC* mediated by *MYC* rearrangements is a driver of the MGUS to MM transition.

8.9 Complex *MYC* Rearrangements Mostly Hijack Nonrandom Super Enhancers

Enhancer DNA regulatory elements, which can directly regulate the transcriptional activity of genes, typically are located near or within the relevant gene(s), but sometimes can be located more than 1 Mb away from the gene [53, 54]. Enhancers can be identified by distinct patterns of histone marks (e.g., H3K27Ac), and the presence of specific transcription factors (e.g., *MED1* or *BRD4*) [55–57] (Table 8.2). Super enhancers (SE) typically are associated with genes that generally are expressed at a high level in specific cell types [55, 56]. Compared to conventional strong enhancers, SEs are identified by having a larger size and increased levels of *MED1*, *BRD4*, and H3K27Ac. Stretch enhancers (STRE), which can be weak or strong enhancers but have a size of at least 3 kb, often are tissue-specific and overlap locus control regions [57]. SEs have been identified in the MM.1S MMCL and the GM12878 lymphoblastoid cell line, which is phenotypically similar to MM.1S. STRE have been identified in GM12878 but not in MM.1S.

Table 8.2 Characteristics of enhancers juxtaposed to MYC in multiple myeloma

	Super (Loven)	Super (Hnisz)	Stretch (Parker)	Conventional
Marks	MED1, BRD4	H3K27Ac	6 ChIP marks[a]	–
Clustering	Stitched (<12.5 kb gaps)	Stitched	No	No
Median size	19 kb	8.7 kb	>3 kb (no gaps)	~1 kb
Number in MM.1S (MMCL)	308	640	ND	~10,000
Number in GM12878 (LCL)		257	10,615 (2318>6 kb)	10,000 (Hnisz) 100,000 (Parker)[b]
Rearranged near MYC >> MYCN > MYCL in MM	IGH, IGL, IGK, IGJ, TXNDC5, FAM46C, FOXO3, PRDM1, SNX5, ANKRD55, FAM188A	NSMCE2	MTSS1, AHNAK, TRIB1, PVT1	

[a]CTCF, H3K4me3, H3K4me, H3K27ac, H3K27me3, H3K36me3
[b]Parker data includes weak conventional enhancers

MYC:*IG* rearrangements in MMCL and MM tumours have been characterized by a combination of FISH, CGH, mate-pair sequences, cloned sequences, and whole genome sequencing [18, 20, 27, 29, 40, 58–60]. Although some of the *MYC*:*IG* rearrangements are simple reciprocal translocations, most of them are complex translocations or insertions (Fig. 8.1).

The major reason for complexity may be related to aberrant mechanisms that are responsible for karyotypic complexity in most kinds of tumours, including MM. However, in some cases the complexity seems to have a more direct role in the process of dysregulating *MYC*, a conclusion supported by three examples: (1) in the Karpas 620 MMCL, a der(14) t(11;14) with dysregulation of *CCND1* undergoes a rearrangement with chr8 that results in a der(8)t(8;14;11) and a der(14)t(8;14;11) that share ~1,700 kb of sequences from chromosomes 8,11, and 14, including *MYC* and the 3′*IGH* SEs [60]; (2) in the RPMI 8226 MMCL, the insertion of *MYC* at a der(16)t(16;22) breakpoint is associated with a duplication of the 3′*IGL* SE so that both *MAF* and *MYC* are dysregulated by different 3′E.*IGL* SE sequences (WMK&PLB, unpublished); and (3) in the LP1 MMCL, a variant *IGH* translocation, i.e. der(8) t(8;14), includes an inversion on chromosome 14 so that the insulator sequences that normally are centromeric to the 3′*IGH* SE are not positioned between the SE and *MYC*, which is located centromeric to the SE for this translocation [29].

Recently the first molecular details about non-*IG MYC* locus rearrangements in MMCLs or MM tumours have been reported [29, 60]. The structures of these rearrangements involving *MYC* (or *MYCL*) were deduced from a combination of FISH, CGH, mate-pair sequences, cloned sequences, and whole genome sequences of 14 MMCLs and 11 MMRC MM tumours. In 18 of these 25 non-*IG MYC* locus rearrangements, *MYC* is repositioned near a nonrandom group of MM.1S SEs (*NSMCE2* [8q24.13], *TXNDC5* [6p24.3], *FAM46C* [1p12], *FOXO3* [6q21], *PRDM1* [6q21], *IGJ* [4q13.3], *SNX5* [20p11.2], *ANKRD55* [5q11.2], *FAM188A* [10p13]) or GM12878 STREs (*AHNAK* [11q12.3], *MTSS1* [8q24.13], *TRIB1* [8q24.13]) (Table 8.2).

Several of the SEs were recurrent (*NSMCE2, TXNDC5, FAM46C, FOXO3, SNX5*). Moreover, samples with centromeric CNAs appear to be mostly deletions or inversions that reposition *MYC* relatively close to SEs in intron 4 of *NSMCE2*, which is ~2,500 kb upstream of *MYC*, or to STREs associated with *TRIB1*, which is ~2,300 kb upstream of *MYC*. Six of the other seven non-*IG MYC* locus rearrangements position *MYC* near conventional enhancers (two), centromeric to *NBEA* [13q13.3] but not close to any obvious enhancer (two), or with large tandem duplications that are located 350–500 kb downstream of *MYC* (two).

8.10 Characteristics of Secondary Immunoglobulin Rearrangements in Multiple Myeloma

Secondary rearrangements are not highly recurrent for partners other than *MYC* and, therefore, it has not been possible to compare the prevalence of these events in MGUS and MM. However, there are a few cases for which heterogeneity of a rearrangement in MM tumours suggests that a rearrangement is a secondary event. For example, the recently reported complex t(7;14) rearrangement, which is associated with ectopic expression of *EGFR*, was present in 85 % of MM tumour cells whereas other chromosome abnormalities were present in 97–99 % of tumour cells [61]. In the absence of more definitive proof, the following criteria, which are based on the analysis of *MYC* rearrangements, are suggestive that a rearrangement is secondary: (1) rearrangements are usually unbalanced translocations or insertions, often with involvement of three chromosomes, and sometimes with associated amplification, duplication, deletion, or inversion; and (2) rearrangement breakpoints usually are not within or very close to *IGH* switch regions or VDJ sequences even though these regions might sometimes be targeted by mechanisms other than the B cell specific DNA modification processes that are thought to be turned off in MGUS and MM tumours [18, 28]. Using these criteria, it is likely that rearrangements involving *IGK* and *IGL* are mostly secondary events, since they mostly are complex rearrangements that often involve *MYC* and rarely involve any of the seven primary *IGH* translocation partners [27]. Most *IGH* insertions and *IGH* rearrangements not involving one of the seven primary partners appear to be secondary events. However, the rare examples of MGUS or MM tumours with *IGH* rearrangements that target two of the primary partners suggest that one of the rearrangements is secondary [24, 27]; this is especially convincing for an MGUS tumour for which FISH identifies the t(4;14) in all tumour cells but the t(11;14) in ~30 % of tumour cells. In addition, *MAFB* expression is increased in the L363 MMCL, which has a complex rearrangement that positions *MAFB* near a *PDK1* SE (WMK&PLB, unpublished), and in the XG-2 MMCL, which has 3′ IGL sequences inserted near *MAFB* [27]. Therefore, it appears that *MAF* and *MAFB*, which are primary *IGH* translocation targets, may be particularly prone to be targets of secondary rearrangements. Secondary *IG* rearrangements have a similar prevalence in NHRD and HRD tumours, but *MYC* rearrangements not involving *IG* loci are significantly more frequent in HRD tumours than in NHRD tumours [20, 27, 29].

8.11 Concluding Thoughts and Unresolved Issues

Despite continued progress in our understanding of the roles of primary and secondary immunoglobulin rearrangements in the pathogenesis of MGUS and MM tumours, many important questions remain unanswered, some of which are briefly summarized below:

1. Are any of the primary translocations sufficient to cause MGUS? This does not seem to be the case for the t(11;14) translocations based on Eμ. CCND1 transgenic mice [45, 46] or for the t(4;14) since hybrid transcripts of *MMSET* are frequently found in healthy individuals (Marta Chesi, personal communication).
2. Primary *IGH* translocations target *CCND1>>CCND3>CCND2* both in MM and in mantle cell lymphoma despite the fact that B lineage cells express *CCND2* and *CCND3*, but little or no *CCND1*. There still is no compelling explanation for the more frequent involvement of *CCND1*.
3. Weinhold et al. [62] recently reported that the G allele for the rs603965 germline polymorphism(codon 870 G/A) is significantly associated (OR ~2, $P < 10^{-10}$) specifically with t(11;14) MGUS and MM but not t(11;14) mantle cell lymphoma. This is the first example of a specific translocation in MM that is associated with a germline polymorphism. It is unclear if the more efficient splicing of the G allele somehow enhances selection of cells with a t(11;14), or whether the G allele is more susceptible to acquiring a t(11;14).
4. Lopez-Corral et al. [63] have reported FISH studies showing that only a subclonal population of MGUS tumour cells derived from an individual patient have: t(4;14)[median 0.48]; t(11;14)[median 0.38]; or t(14;16)[0.30], with a higher fraction of smouldering MM having these translocations, and a still higher fraction having these translocations in symptomatic MM. They suggest that the t(4;14), t(11;14), and t(14;16) primary *IGH* translocations may actually be secondary events, with the tumour cell generated by the initial and unknown transforming events being outgrown by tumour cells that subsequently acquire one of these *IGH* translocations. This is a provocative result, which seems inconsistent with studies reported by others [20, 24, 64, 65] and, therefore, needs more rigorous validation of the hypothesis that they suggest.
5. Hebraud et al. [66] have reported that the t(4;14) can be present in only minor subclones of MM, and that there can be an apparent gain or loss of the t(4;14) during tumour progression. For most of the patients in this study, the observations were based on FISH studies that might not always detect the rearranged *MMSET* gene if the telomeric *IGH* sequences on the der(4) were lost during chromosome remodelling. In addition, it is unfortunate that the results reported in this study had very little experimental detail. In any case, this clearly is a provocative result that requires rigorous validation by other groups before their hypothesis can be accepted.
6. As indicated above, it remains to be rigorously confirmed that rearrangements mediated by apparent errors in D>J occur in pro-B cells and not at later stages of B cell development [43].

7. When do *MYC* rearrangements occur during pathogenesis? Interphase FISH studies suggest that they have a low prevalence (~3 %) in MGUS compared to MM (~16 %). Perhaps some of the MGUS tumours in that study were early MM or contained a combination of MGUS and MM cells. It is clear that some *MYC* rearrangements occur during progression of MM, but how often do *MYC* rearrangements occur in MGUS and drive the transition of MGUS to MM?
8. What is the full spectrum of super enhancers/stretch enhancers/conventional enhancers that are involved in *MYC* rearrangements? What is the basis for a nonrandom selection of these regulatory elements for *MYC* rearrangements?
9. Will the identification of recurrent super enhancers or stretch enhancers involved in *MYC* rearrangements lead to the identification of novel oncogenes that are dysregulated by juxtaposition to these regulatory elements in MM and other kinds of tumours?

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