*Clin. Lab. Haem.*
2002, **24,** 259–269

**REVIEW**

# Chromosomal and genetic abnormalities in myeloma

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**Summary** Chromosomal translocations are a hallmark of lymphoid tumours. Multiple myeloma (MM) is a tumour of the plasma cell, the terminally differentiated B lymphoid cell. In recent years, a large number of chromosomal and genetic abnormalities have been detected in myeloma, the most prominent being chromosome 13q deletions and translocations affecting the immunoglobulin heavy chain (IgH) locus on chromosome 14q32. The latter involve a large array of chromosomal partners, from which multiple oncogenes have been proposed as candidates for dysregulation. In addition, a wide variety of changes including numerical aberrations, translocations involving loci other than the immunoglobulin genes, and aberrations of known oncogenes such as N-*ras* mutations, have been found. With the refinement of molecular cytogenetic techniques, the sensitivity of detecting these molecular abnormalities is continuing to increase. However, with the exception of 13q deletions which have been consistently associated with an adverse prognosis, the role of the other changes in the pathogenesis of MM, and their effect on disease behaviour and prognosis are still being clarified. In this review, we will discuss the most common molecular abnormalities found in primary MM and cell lines, and consider the available evidence for a pathogenic role in MM.

**Keywords** Myeloma, MGUS, chromosomal translocation, karyotype, oncogenesis

## Introduction

Multiple myeloma (MM) is a malignancy of the plasma cell, the terminally differentiated B cell. Early investigations of the chromosomal abnormalities of MM were based on conventional karyotype analysis, with the detection of several recurrent abnormalities such as monosomy 13 and the t(11; 14) translocation, the nature of which has been elucidated by molecular techniques. In more recent years, developments in molecular cytogenetics ranging from metaphase and interphase fluorescent *in situ* hybidization (FISH) to multicolor spectral karyotyping (SKY) and comparative genomic hybridization (CGH) have vastly increased the scope of detection of cytogenetic abnormalities. The clinical and prognostic implications of these changes are still to be fully elucidated, while specific genetic aberrations have increased our understanding of the pathogenesis of this malignancy.

Accepted for publication 13 May 2002
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## I. Monosomy 13

Monosomy 13 was one of the first recurrent chromosomal abnormalities found in MM, detectable in many cases by conventional karyotype, and for which adverse prognostic significance has been consistently established (Tricot *et al.*, 1995; Perez-Castro *et al.*, 1997; Zojer *et al.*, 2000). This has been confirmed by interphase FISH studies, in which monosomy 13 was found to be the most powerful predictor of survival (Königsberg *et al.*, 2000; Facon *et al.*, 2001), especially in combination with $\beta_2$ microglobulin (Facon *et al.*, 2001). Because of the adverse prognostic impact of monosomy 13 shown in these studies, it has been postulated to be a late determinant of disease progression rather than an oncogenic factor. However, with the development of molecular cytogenetics, the frequency of detection of monosomy 13 has also risen

from approximately 15–20% by conventional cytogenetics (Lai *et al.*, 1995; Seong *et al.*, 1998; Desikan *et al.*, 2000) to approximately 50% by FISH (Avet-Louseau *et al.*, 2000; Fonseca, Oken & Greipp, 2001) and SKY (Sawyer *et al.*, 2001), with an incidence of 30–45% also found in monoclonal gammopathy of unknown significance (MGUS) (Königsberg *et al.*, 2000; Fonseca *et al.*, 2001). Moreover, in the molecular cytogenetic studies, monosomy 13 was often found in conjunction with a large variety of other chromosomal aberrations, and the prognostic significance of monosomy 13 alone was more difficult to define. For example, it was observed that while all patients carrying translocations involving the IgH locus such as t(4;14) and t(14;16) were found to have del 13, the reverse was not true (Sawyer *et al.*, 1998; Fonseca *et al.*, 2001). This suggests the possibility that del 13 occurred before the IgH translocation events, possibly as an early oncogenic change. A role for monosomy 13 in the transformation of MGUS to MM has also been proposed, supported by a higher incidence of monosomy 13 in MM cases with pre-existing MGUS (70%) compared with those cases without such a history (31%) (Avet-Loiseau *et al.*, 1999a). In addition, while monosomy 13 occurs in the majority of plasma cells in a MM tumour, in MGUS it is only present in subpopulations (Avet-Loiseau *et al.*, 1999b).

Although the majority (92%) of chromosome 13 abnormalities are monosomies, partial deletions are also recognized (Avet-Loiseau *et al.*, 2000). In the latter, the region of deletion has been localized to 13q14–21, overlapping the minimal region of deletion in chronic lymphocytic leukaemia (Cigudosa *et al.*, 1998; Avet-Loiseau *et al.*, 2000). The retinoblastoma gene (Rb) is located on chromosome 13q and its role has been examined in MM. Despite the frequency of monosomy 13 and monoallelic Rb loss (up to 60% of tumours and cell lines), abnormalities in Rb expression are rare and not correlated with monosomy 13, Rb gene rearrangements or mutations (Zandecki *et al.*, 1995). It is therefore unlikely that monosomy 13 acts solely through Rb.

## II. Immunoglobulin heavy chain (IgH) switch translocations

### *A. Isotype switch recombination*

During B cell maturation, the IgH genes undergo the processes of variable region (VDJ) recombination, followed by isotype switching and somatic hypermutation after exposure to antigen. Isotype switching is the process by which a given Ig variable region can be associated with different isotype classes which confer different physiological functions. Its particular relevance in MM lies in the fact that the majority of translocations affecting the IgH locus on chromosome 14q32 occur at the switch regions where normal isotype switch recombinations occur, and are known as switch translocations (STs) (see Figure 1) (Bergsagel *et al.* 1996). The IgH genes are arranged in the order of 5′ – Cμ, Cδ, Cγ3, Cγ1, Cψε, Cα1, Cγ2, Cγ4, Cε, Cα 2–3′. Switch regions, consisting of repeat sequences, are located in front of each constant region gene except Cδ. The first heavy chain isotype IgM is expressed by RNA splicing from the end of the rearranged variable region to the Cμ gene. During isotype switch recombination the μ isotype changes to another isotype by recombination of the respective switch regions. For instance, Sμ recombines with Sα for the μ to α switch, with the deletion of the intervening DNA in an excision circle. Switch recombination mostly involves switch regions on the same chromosome (in *cis*), while *trans*-switching involving switch regions on both alleles may also occur more rarely. The fact that switch regions are physiological recombination hotspots may account for their propensity to be involved in chrosomomal translocations. The retention of Ig production by the myeloma plasma cell would imply that the STs are most likely to occur on the nonproductive allele.

### *B. Switch translocations in myeloma*

Apart from monosomy 13, the most common cytogenetic abnormality in MM is chromosomal translocation into the switch regions of the IgH genes on chromosome 14q32. These translocations were initially found by conventional cytogenetics at a rate of 20–40% (Taniwaki *et al.*, 1994; Lai *et al.*, 1995; Taniwaki *et al.*, 1996). The partner chromosome was often not elicited, and the karyotypic abnormality was denoted 14q+. Using a southern blot technique to look for illegitimate switch recombinations (ISRs) as candidates for switch translocations (STs) in human myeloma cell lines (HMCLs), it became evident that STs were much more common than previously suspected (Bergsagel *et al.*, 1996). It is estimated that approximately 90% of HMCLs have either IgH or IgL (immunoglobulin light chain) translocations. Cloning of breakpoints and molecular cytogenetic studies have revealed a large array of chromosomal partners for the STs, the most common being chromosomes 11q, 4p, 16q and 6p, with candidate oncogenes characterized on each partner. The translocation breakpoints are mostly centromeric to the candidate oncogenes, which are translocated to der(14) and placed under the control of one of the

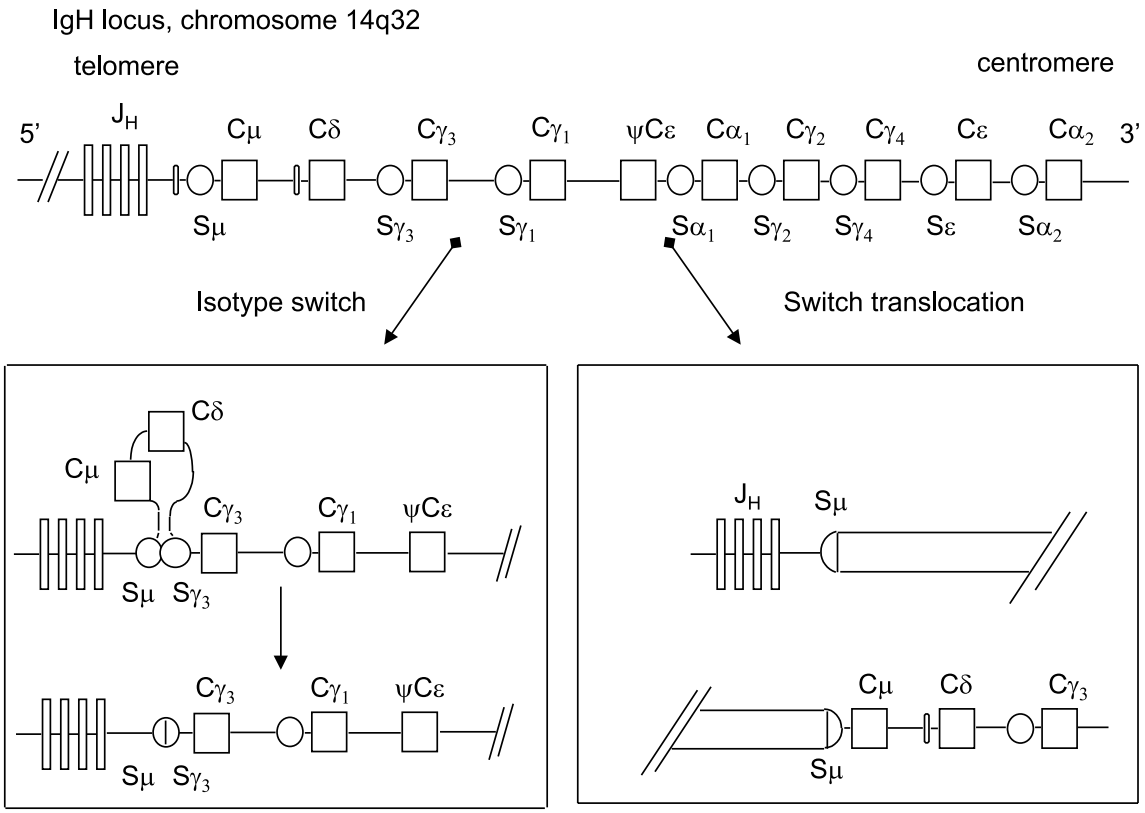


**Figure 1.** Switch translocations occur at the IgH Constant gene Switch regions, sites of physiological isotype switch recombination. The structure of the IgH locus with the arrangement of the Constant genes is shown on the top. The Constant genes are represented by squares and denoted 'C', the Switch regions are represented by circles and denoted 'S'. The small rectangles upstream of Sμ and Cδ represent the σμ and σδ sequences which are involved in the μ to δ switch. Physiological isotype recombination is shown in the lower left panel with recombination of the Switch regions and deletion of the intervening DNA sequence. In contrast, switch translocations are shown in the lower right panel, with a non-Ig sequence translocated to the IgH Switch regions which are recombination 'hotspots'.

3′ IgH enhancers (Eα1 or Eα2). In addition, in cases such as chromosome 4p, a second putative oncogene is also overexpressed on der(4), presumably dysregulated by the 5′ intronic enhancer (Eμ) translocated to der(4). The role of these candidate genes in the pathogenesis of myeloma has been examined by assessing their level of expression and their oncogenicity in both *in vitro* and *in vivo* models.

### C. Translocation partner chromosomes

The most common switch translocation partners and the associated candidate oncogenes are listed in Table 1.

#### 1. Chromosome 11q

Chromosome 11q has been identified as a partner chromosome in 15–20% of MM (Avet-Loiseau *et al.,* 1998; Fonseca *et al.,* 1998), with translocations located at 11q13. Early studies suggested that this translocation was associated with a poor prognosis (Tricot *et al.,* 1995; Fonseca *et al.,* 1998). However, these results were obtained by conventional cytogenetics which require metaphases from actively dividing cells, possibly 'selecting' for more aggressive disease. Subsequent studies by interphase FISH have not confirmed the prognostic relationship (Avet-Loiseau *et al.,* 1998).

Chromosome 11 ST breakpoints are not as telomeric compared with other partner chromosomes such as chromosome 4p, and are therefore more amenable to detection by conventional cytogenetics. Although mantle cell lymphoma (MCL) also bears a characteristic t(11;14)(q13;q32) translocation, the majority of MCL breakpoints are located within the major translocation cluster (MTC) region 110 kb upstream of *Cyclin D1* (*CD1*, candidate oncogene for both MCL and MM). In contrast, the breakpoints in MM are more widely scattered over a region 100–330 kb centromeric to *CD1*. In MM, except for several breakpoints in the $J_H$ region, most of the known IgH breakpoints occur in the switch regions. In contrast, in MCL they are all located in the $J_H$ region, indicating the involvement of the VDJ recombination mechanism at an earlier stage of B cell development.

**Table 1.** Common chromosomal partners and candidate oncogenes in switch translocations in myeloma

| | Candidate Oncogenes | | | |
|---|---|---|---|---|
| Chromosomal partner | Candidate Oncogene | Localization | Distance between breakpoints & telomeric oncogenes | Function |
| 11q13 | 1. *Cyclin D1* | der(14) | 100–330 kb | Cell cycle regulator |
| | 2. *myeov* | der(11) | | unknown |
| 4p16 | 1. *FGFR3* | der(14) | 50–100 kb | Growth factor receptor tyrosine kinase |
| | 2. *MMSET/WHSC1* | der(4) | | Epigenetic regulator of transcription (chromatin remodelling) |
| 16q32 | *c-maf* | der(14) | 550–1350 kb | Transcription factor |
| 6p21 | *Cyclin D3* | der(14) | 65 kb | Cell cycle regulator |
| 6p25 | *MUM1/IRF4* | der(14) | Immediately adjacent | Transcriptional regulator of IFN & IFN-stimulated genes |

IFN: interferon.

The finding of *CD1* up-regulation in MM tumours and cell lines bearing t(11; 14) supports its role as a candidate oncogene in MM (Chesi *et al.,* 1996; Hoyer *et al.*, 2000; Pruneri *et al.*, 2000). The Cyclins are a family of cell cycle regulators, of which the members *CD2* and *CD3* are normally expressed in lymphoid cells. The expression of *CD1* is therefore ectopic in t(11; 14) bearing MM cells. All the Cyclin D proteins interact with CD-dependent kinases, phosphorylating and inactivating the retinoblastoma protein (Rb), thus promoting the G1/S phase transition (Sherr, 2000).

A second candidate oncogene named *myeov* has been characterized on chromosome 11q, 360 kb centromeric to *Cyclin D1*, with all the known MM breakpoints situated in the region between *CD1* and *myeov* (Janssen *et al.*, 2000). *Myeov* was originally isolated from gastric carcinoma by a tumorigenicity assay. In MM, *myeov* was up-regulated in 3 of 7 cell lines carrying t(11; 14), indicating that not all myeloma lines carrying the translocation overexpressed *myeov*. The variation is presumably related to the site of the breakpoint, the orientation of the translocation and the placement of enhancers. Surprisingly, some cell lines not carrying t(11; 14) express moderate levels of *myeov* (Janssen *et al.*, 2000). Hence the role of *myeov* in MM is still not clear.

*2. Chromosome 4p*

The t(4; 14)(p16;q32) translocation is difficult to detect by conventional cytogenetics or SKY (Rao *et al.,* 1998; Sawyer *et al.,* 1998; Sawyer *et al.*, 2001), due to the extreme telomeric localization of the breakpoints. The cloned chromosome 4 breakpoints were located 50–100 kb centromeric of *FGFR3*, one of the 2 candidate oncogenes (Chesi *et al.,* 1997; Richelda *et al.,* 1997). The translocation can be detected by FISH, with the reported incidence varying from 12% (Avet-Loiseau *et al.,* 1998) to 17% (Finelli *et al.,* 1999) in MM. It is also detected in 2–6% in MGUS (Avet-Loiseau *et al.,* 1999a; Malgeri *et al.*, 2000), the latter estimate was obtained by the demonstration of a fusion RNA transcript derived from chromosomes 4 and 14 (see below).

*FGFR3* is one of a family of fibroblast growth factor receptor tyrosine kinases, mutations of which cause several forms of dwarfism including thanatropic dysplasia (TD) (Naski *et al.,* 1996; Webster *et al.,* 1996). *FGFR3* can be dysregulated in MM by translocation to the vicinity of IgH enhancers, activating mutations, or both. It is overexpressed in several MM cell lines and tumours, with selective expression of the mutant allele in the cell lines carrying *FGFR3* mutations (Chesi *et al.*, 2001). The frequency and role of *FGFR3* mutations in primary MM tumour is much debated, due to reports of a low incidence in primary tumour (Fracchiolla *et al.,* 1998; Intini *et al.*, 2001). In *FGFR3* genomic DNA, a single nucleotide change was found in one of 80 primary MM tumours (Fracchiolla *et al.,* 1998). A low incidence was also detected in cDNA from the expressed allele – only one of 11 cases overexpressing *FGFR3* demonstrated an activating mutation (Intini *et al.,* 2001). It was concluded from these studies that the incidence of activating mutations of *FGFR3* in MM was 10% in cases bearing t(4; 14), which constitutes only 2% of all MM primary tumours.

Possible mechanisms of action of *FGFR3* in MM have been extensively investigated. In TD, *FGFR3* mutations were shown to cause constitutive phosphorlyation of the receptor, leading to ligand-independent activation (Webster *et al.,* 1996). By retroviral transduction of wild-type

and mutant *FGFR3* into murine IL6-dependent B9 plasmacytoma cell line, increased levels of *FGFR3* expression (both wild-type and mutant) increased cellular proliferation and survival, with an enhanced response to IL6 and IL6-independence (Plowright *et al.*, 2000). Mutant *FGFR3*-transduced cells also exhibited independence of ligand (FGF). However, the increased cellular proliferation and survival from up-regulated expression of wild-type *FGFR3* would appear to be dependent on the level of *FGFR3* expression and not specifically on the mutation. IL6 has both mitogenic and anti-apoptotic effects on MM cells, the former is promoted primarily by the mitogen-activated protein kinase (MAPK) pathway, and the latter by the JAK-STAT (Janus kinase-signal transducer and activator of transcription) pathway with up-regulation of bcl-$X_L$ through gp130 as an intermediary (Schwarze & Hawley, 1995). Plowright *et al.* (2000) found that in the absence of gp130 and IL6, up-regulated *FGFR3* increased phosphorylation of STAT3, which then up-regulated bcl$X_L$ to reduce apoptosis. There was no evidence of MAPK involvement as an explanation for *FGFR3*-induced IL6-responsive cellular proliferation.

In contrast, *FGFR3* dysregulation was found to affect the MAPK pathway in other experiments. Ronchetti *et al.* (2001) examined three cell lines containing 'native' mutant *FGFR3* and demonstrated phosphorylation of MAPK in all 3 lines, even though only two of them showed constitutive activation of *FGFR3*. Neither STAT1 or STAT3 were involved. Chesi *et al.*, (2001) showed that the stimulation of three t(4; 14)-bearing cell lines by FGF also resulted in rapid MAPK phosphorylation. The oncogenicity of mutant *FGFR3* was demonstrated by an *in vitro* focus formation assay and an *in vivo* animal model using a mutant FGFR3-transfected NIH 3T3 fibroblast cell line. As the MAPK pathway is known to be mediated by *ras* (Kanai *et al.*, 1997), a dominant negative form of *ras* was used to inhibit the *FGFR3* oncogenic effect, demonstrating involvement of both MAPK and *ras* as previously shown. Mutant FGFR3 has been found to cause transformation in haemopoietic cells, but whether up-regulated levels of nonmutated FGFR3 may have the same effect is presently unclear (Li *et al.*, 2001). *FGFR3* and *ras* mutations appeared to be mutually exclusive in MM, and may therefore have overlapping roles in myeloma pathogenesis (Chesi *et al.*, 2001). However, as mentioned earlier, the low incidence of *FGFR3* mutations in primary MM must be considered (Fracchiolla *et al.*, 1998; Intini *et al.*, 2001).

A second candidate gene for t(4;14) was localized on the reciprocal chromosome [der(4)]. The chromosome 4p breakpoints are telomeric to or within the 5′ introns of *MMSET*/*WHSC1*, characterized as a candidate gene for a multiple malformation syndrome known as Wolf-Hirschhorn Sydrome (Stec *et al.*, 1998). *MMSET* is a member of the trithorax nuclear proteins which includes MLL, one of the most common genes involved in human acute leukaemia. MLL is involved in the epigenetic regulation of gene expression through chromatin remodelling, and consists of PHD (zinc finger) and SET domains. The latter has been shown to cause transformation in fibroblasts (Cui *et al.*, 1998). In t(4;14) *MMSET* is placed in close proximity to and presumably up-regulated by the 5′ intron IgH enhancer. Hybrid transcripts of 5′IgH joined to *MMSET* are produced, initiating either from the $J_H$ or Iμ exons (Chesi *et al.*, 1998b; Malgeri *et al.*, 2000). However few of the fusion transcripts are expected to produce functional proteins due to premature stop codons, or the absence of the amino terminal as a result of splicing. The size of proteins could also vary according to the use of different promoters, alternative splicing, specific mRNA degradation and variation in the site of polyadenylation. Although it has been proposed that such variations may modulate the phenotypic diversity of MM (Stec *et al.*, 1998), so far there has been no evidence for a correlation with clinico-pathological characteristics.

*3. Chromosome 16q*

The t(14;16)(q32;q23) translocation has been found in approximately 5–12% of MM (Sawyer *et al.*, 1998; Sawyer *et al.*, 2001). The chromosome 16q23 breakpoints were localized to a region 550–1350 kb centromeric of the candidate oncogene c-*maf*, although a breakpoint telomeric to c-*maf* was also found in a t(16; 22) translocation (Chesi *et al.*, 1998a; Bergsagel & Kuehl, 2001). C-*maf* is a basic zipper transcription factor involved in cellular differentiation, proliferation and the IL-6 response, and is capable of oncogenic transformation in a model system (Kataoka *et al.*, 2001). Its role in myelomagenesis is supported by the finding of upregulated expression in five HMCLs carrying the t(14;16) translocation including the tumour of origin of one cell line, with selective expression of one c-*maf* allele in two informative lines (Chesi *et al.*, 1998a). The cloned breakpoints are located in a common fragile site (FRA16D) on chromosome 16, within the newly characterized *WWOX* gene, a candidate tumour suppressor gene in breast cancer (Bednarek *et al.*, 2000). Although this region is susceptible to allelic deletion in several non-haematological cancers, no mutations have been found in the remaining allele of *WWOX*, and its possible role as a tumour suppressor gene is not yet known. Similarly its role in MM is unclear, as breakpoints occurring within *WWOX* would inactivate only one of the two alleles.

*4. Chromosome 6p*

The t(6;14)(p25;q32) translocation was one of the first STs reported in a HMCL (Iida *et al.*, 1997), at which *MUM1/IRF4*, an interferon (IFN)-responsive factor (IRF), was proposed as a candidate oncogene. IRFs are involved in transcriptional control, can be rapidly induced by T and B receptor cross linking, and regulate the IFNs and IFN-stimulated genes. They therefore have a critical role in plasma cell development (Mittrucker *et al.*, 1997). *IRF4* was overexpressed in HMCLs and demonstrated transforming ability in fibroblasts (Iida *et al.*, 1997). Molecular cytogenetics have revealed an incidence of this ST of 18% in HMCLs (Yoshida *et al.*, 1999) but a low frequency of approximately 5% in primary MM (Sawyer *et al.*, 1998; Sawyer *et al.*, 2001), and its role in MM pathogenesis is as yet unclear.

More recently, a second translocation affecting chromosome 6p–t(6;14)(p21;q32) was identified. *Cyclin D3* (*CD3*), located approximately 65 kb telomeric of the breakpoint at 6p21, has been proposed as the candidate oncogene (Shaughnessy *et al.*, 2001). This translocation was originally found in one of 30 myeloma cell lines, and then in 4% of 150 primary tumours by metaphase FISH and SKY. *CD3* overexpression was further detected by microarray analysis in 3 of 53 primary tumours, in which t(6;14) breakpoints were subsequently confirmed, one other tumour having a variant t(6;22) translocation. As noted earlier, all the Cyclin D proteins are known to phosphorylate Rb, facilitating the G1/S phase transition. Unlike *CD1*, *CD3* is normally expressed in lymphoid cells and upregulated expression would be expected to promote cellular proliferation.

*5. Other chromosomal partners*

The c-*myc* on chr 8q24 is the oncogene characteristically dysregulated in murine plasmacytoma and human Burkitt's lymphoma. Reciprocal translocations involving chromosome 8q24 and the Ig genes account for only 25% of the c-*myc* rearrangements in MM (Avet-Loiseau *et al.*, 2001b), with the majority involving non-Ig loci. Hence c-*myc* rearrangements will be discussed below with the non-Ig translocations (see Section IIIB).

Apart from the more common STs already discussed, a large number of other chromosomal partners have been reported, many detected only once. Reciprocal translocations with chromosome 1q have been detected by SKY, accounting for up to 4–5% of primary tumours (Sawyer *et al.*, 1998). Possible candidate oncogenes on chromosome 1q are IRTA1 and 2, two novel B-cell surface receptors. IRTA2 has been found to be upregulated in t(1;14)-carrying tumour cell lines (Hatzivassiliou *et al.*, 2001). There are many other infrequent partners including 3q, 4q, 20q, 21q and 22q. Due to the nonrecurrent nature of some of these aberrations, their role in MM pathogenesis would be difficult to define.

### *D. Switch translocations – implications for pathogenesis?*

Whether STs are an early oncogenic change, a late 'trigger' of disease progression or a non-pathogenic marker of genetic instability has been an interesting question with important implications on our understanding of myeloma pathogenesis. If STs are oncogenic, their presence in 50–75% of primary MM tumours (Nishida *et al.*, 1997; Avet-Loiseau *et al.*, 1998; Sawyer *et al.*, 1998; Ho *et al.*, 2001) and the heterogeneity of chromosomal partners and partner oncogenes would imply that STs cannot be the only oncogenic factor, and a number of mechanisms must be involved. This heterogeneity was proposed as one of the arguments against STs being a unifying oncogenic change (Avet-Loiseau *et al.*, 1998). However, the 'final common pathway' of myeloma development from multiple mechanisms could simply reflect a single possible phenotype of the transformed plasma cell (Bergsagel & Kuehl, 2001).

The concept of primary and secondary translocations has been introduced to distinguish translocations that occur early in disease and may be oncogenic, from late changes which may play a role in disease progression (Bergsagel & Kuehl, 2001). Translocations such as STs which involve B cell recombination mechanisms, occur in some cases of MGUS, and demonstrate little heterogeneity within the myeloma cell population of each tumour, are more likely to represent primary changes. Conversely, changes which do not involve the Ig loci, are not present in MGUS and demonstrate heterogeneity within a tumour are more likely to represent late, secondary changes. These may include the complex c-*myc* translocations, which do not occur at the Ig recombination sites and demonstrate intratumour heterogeneity (see Section IIIB).

An interesting consideration in the role of STs in the causation of MM is their occurrence in MGUS. Not all MGUS are premalignant, and there are presently no clearly defined molecular or other phenotypic features which enable us to predict the risk of MM development. If STs are an oncogenic change, the presence of STs may distinguish the benign and premalignant forms of MGUS. In the largest series of MGUS patients assessed by FISH so far (Avet-Loiseau *et al.*, 1999a), chromosome 14q32 translocations have been found to occur in 47%, with a

progression to 60% in intramedullary MM (stage III) and 70–80% in PCL (Avet-Loiseau *et al.*, 2001a).

The study of when STs occur in the ontogeny of the myeloma plasma cell can help to elucidate the cell of origin of the myeloma clone. Some studies have suggested that MM may originate in a pre-switch B cell or pre-plasma cell. These observations include the finding of pre-switch clonotypic cells, clonotypic IgM and nonclinical clonotypic isotypes (Corradini *et al.*, 1993; Bakkus *et al.*, 1994; Billadeau *et al.*, 1996; Szczepek *et al.*, 1998; Reiman *et al.*, 2001). As STs are commonly thought to occur during isotype switch recombination, the finding of pre-switch clonotypic cells raises the interesting question of whether these presumed myeloma precursors contain STs or not.

## III. Other chromosomal abnormalities in myeloma

It is evident in molecular cytogenetic studies of MM that there are multiple structural abnormalities which do not affect the Ig loci (Cigudosa *et al.*, 1998; Rao *et al.*, 1998; Sawyer *et al.*, 1998; Sawyer *et al.*, 2001). The nonrecurrent nature of such changes makes analysis of their role in the disease difficult, and it is possible that a substantial number may represent 'by-products' of genetic instability.

### *A. Numerical changes*

Numerical changes (monosomy, trisomy) have been a prominent finding in MM in conventional and molecular cytogenetic studies, detected in up to 80–90% cases (Drach *et al.*, 1995; Cigudosa *et al.*, 1998). The most common abnormality, monosomy 13, has already been discussed (Section I). Other recurrent aberrations include gains of chromsome 3, 5, 7, 9, 11 and 19. Comparative genomic hybridization (CGH) is a relatively new technique, by which tumour DNA is hybridized to normal metaphases and copy number changes are determined by comparison with reference DNA (Cigudosa *et al.*, 1998). One CGH study showed numerical changes in 70% of cases, the most common being a gain of chromosome 19, with deletions of chromosome 13 in 30%. Multiple other changes were detected including del 6q (13%) and 16q (17%), some of which could carry tumour suppressor genes. Two changes previously associated with poor prognosis – gain of 11q and deletion of 13q – were detectable by CGH (Cigudosa *et al.*, 1998).

### *B. Chromsome 8q and c-myc rearrangements*

As noted earlier, the majority of translocations affecting c-*myc* in MM involve non-Ig loci. Estimates of the incidence of c-*myc* dysregulation varies. Shou *et al.*, (2000) examined 20 myeloma cell lines by FISH for c-*myc* rearrangements (involving both Ig and non-Ig loci) and found a high incidence of 95%. Many were highly complex, with nonreciprocal translocations, multiple deletions and duplications. Dysregulated mono-allelic expression was demonstrated in all informative cell lines (when the 2 alleles could be distinguished). In contrast, Avet-Loiseau *et al.* (2001b) found a lower incidence (55%) of c-*myc* rearrangements in cell lines by FISH, and suggested that some of the previously described changes could be the result of *in vitro* propagation. In primary MM tumour, the reported incidences of c-*myc* rearrangements also varies from 15% in a large series of MM and primary PCL (Avet-Loiseau *et al.*, 2001b), to 50% in advanced MM (Shou *et al.*, 2000). In the former series, the rearrangements appeared to occur independently of disease stage, and there was no significant difference in incidence between patients at diagnosis (16%) and relapse (10%). Rearrangements involving Ig loci accounted for 25%. Due to the complexity of the chromosomal abnormalities and the involvement of non-B-cell specific mechanisms, c-*myc* rearrangements have been considered to represent late changes of disease progression. If this were the case, then c-*myc* rearrangements should correlate with disease stage. Although no such relationship was evident in the analysis of active MM cases so far, a correlation was found between c-*myc* dysregulation and $\beta_2$ microglobulin, a poor prognostic indicator (Avet-Loiseau *et al.*, 2001b). There was also intratumour heterogeneity in the occurrence of c-*myc* rearrangements, detected in 19% to 100% of plasma cells, supporting their role as secondary translocations. The incidence of c-*myc* rearrangements in MGUS (3%) and smouldering MM (4%) were also much lower than active MM (Avet-Loiseau *et al.*, 2001b).

### *C. Other molecular aberrations not affecting the Immunoglobulin loci*

In a recent SKY study, a large number of rearrangements not involving the Ig loci were detected, such as chromosomes 11q and 8, as well as complex translocations involving 3 or more chromosomes and whole-arm translocations (Sawyer *et al.*, 2001). More than 50% of patients with complex karyotypes have coexistent chromosome 13 aberrations. Structural changes of chromosome 1q also feature prominently (Sawyer *et al.* 1995; Weh *et al.*, 1995), even though reciprocal translocations involving the IgH locus and chromosome 1 are relatively scarce.

Mutations of N-*ras* and K-*ras* have been reported in approximately 40% of MM patients (Neri *et al.,* 1989; Billadeau *et al.,* 1995). They occur rarely in MGUS, and are associated with disease progression and resistance to therapy (Neri *et al.,* 1989; Billadeau *et al.,* 1995). *Ras* mutations are therefore likely to represent late, secondary changes in MM pathogenesis. *In vitro* studies have demonstrated IL-6 independence of HMCLs bearing *ras* mutations (Billadeau *et al.,* 1995). Aberrations of p53, the tumour suppressor gene on chromosome 17p13, have also been postulated to play a role in MM pathogenesis. Although there are reports of p53 deletions as a predictor of poor survival, and del 17p13 has also been associated with poor prognosis (Drach *et al.,* 1998), most studies indicate a low incidence of both p53 mutations and deletions in MM, varying from 3 to 9% (Preudhomme *et al.,* 1992). The higher frequency of p53 mutations in PCL (20–40%) may indicate their role as a late molecular aberration in MM progression (Neri *et al.,* 1993).

## IV. Prognostic significance of switch translocations and other molecular abnormalities – an overview

By conventional cytogenetics, the most consistent chromosomal abnormality associated with poor prognosis is del 13, including both complete and partial deletions (Tricot *et al.,* 1995; Perez-Castro *et al.*, 1997; Zojer *et al.*, 2000). This finding has since been duplicated in some molecular cytogenetics series (Königsberg *et al.*, 2000; Facon *et al.*, 2001). Conventional karyotyping has also demonstrated a poor prognostic significance of chromsome 11q translocations including t(11;14) translocation and reciprocal translocations with chromosomes 8, 9 and 12 (Tricot *et al.,* 1995; Fonseca *et al.,* 1998). However, this was not confirmed by molecular cytogenetics (Avet-Loiseau *et al.,* 1998), and the possibility that conventional karyotypes have 'selected' out cases which are 'hyperproliferative' must be considered, especially when long-term cultures were used (Lai *et al.,* 1995). The combination of aberrations of both chromosomes 11 and 13 has been found to produce a dismal outcome (Tricot *et al.,* 1995).

In a study utilizing the detection of illegitimate switch recombinations (ISR) as an indication of STs in primary MM tumours, no relationship with parameters of disease activity or prognosis was found in patients with progressive disease (Ho *et al.*, 2001). It was concluded that as a single entity, STs were unlikely to be a feature of disease progression or have prognostic significance, but subgroup analysis according to translocation partner is obviously required. This has been most efficiently examined by molecular cytogenetics. No correlation was found between the presence of chromosome 14q32 translocations and disease stage or $\beta_2$ microglobulin in a study of 127 MM and 14 PCL patients (Avet-Loiseau *et al.,* 1998). In another analysis, Königsberg *et al.* (2000) divided 89 patients into three risk categories according to three chromosomal abnormalities – del 13q, abnormalities of chromosomes 11 and del 17q. When these factors were assessed separately, the only independent prognostic feature was del 13, with increased prognostic significance in conjunction with $\beta_2$ microglobulin. A recent study of PCL and late stage MM demonstrated a higher incidence of t(11;14), t(14;16) and monosomy 13 in patients with high tumour mass, but no difference in t(4;14). Paradoxically, a longer survival was observed in patients with t(11;14) in this group (Avet-Loiseau *et al.*, 2001a). Further investigation is obviously still required to elucidate the prognostic significance of ST subgroups.

## Conclusion

In conclusion, an overview of the molecular aberrations of MM is useful in illustrating the extremely complex abnormalities in this malignancy. The clear distinction of those changes which are oncogenic, from those which may promote tumour progression, and others which may be markers of genetic instability will be the subject of continued investigation, with important implications for our understanding of pathogenesis as well as management of the disease.

## Acknowledgements

I acknowledge the encouragement and support of Professors Douglas Joshua and Antony Basten, and all members of the Myeloma Research Unit, Royal Prince Alfred Hospital. P.J.H. is supported by the University of Sydney Cancer Research Fund and the Royal College of Pathologists of Australia.

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