

REVIEW ARTICLE

ISOTYPE CLASS SWITCHING AND THE PATHOGENESIS OF MULTIPLE MYELOMA

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SUMMARY

Translocations at the immunoglobulin heavy chain locus (14q32) are now considered the commonest karyotypic change in multiple myeloma. These translocations are thought to be intimately involved in the pathogenesis of this disease. The heavy chain locus is strongly transcriptionally active in B and plasma cells and transfer of a potential oncogene to 14q32 would result in its dysregulation. Molecular characterization suggests that the majority of these breakpoints cluster in switch regions within the heavy chain locus. Switch regions are normally involved in the regulated process of isotype switching so that in myeloma the rearrangements are believed to be a result of so-called illegitimate (aberrant) switch recombination and are likely to be an early event in myeloma development. A legitimate switch recombination event occurs between two switch regions producing a hybrid switch; this is necessary for class switching to proceed on a productive allele. In this review we describe the process of isotype switching and how illegitimate class switching may be related to the pathogenesis of multiple myeloma. Copyright © 2001 John Wiley & Sons, Ltd.

KEY WORDS: multiple myeloma; isotype switching; chromosomal translocations

INTRODUCTION

B cell development

B lymphocytes are the cells responsible for the production of immunoglobulins (Igs). Throughout life B cells derive from rare multipotential hemopoietic stem cells, which are responsible for the development of all the different blood-cell lineages. Hemopoietic stem cells are defined as cells with self-renewing capacity and are capable of generating all hemopoietic lineages.

The basic structural unit of Ig antibodies, produced by B cells consist of four polypeptide chains, two identical heavy (H) polypeptide chains and two identical light polypeptide (L) chains. The molecule is composed of two identical halves, with both light and heavy chains possessing amino-terminal end domains. These domains are variable from one Ig to another and it is these variable (V) region domains which cooperate to form the antigen-binding surface by which antibodies recognize the target they bind to. The tail or carboxy-terminal end of the molecule is termed the constant (C) region and determines the class and functional properties of the antibody. Thus the antigen-binding immunoglobulins produced by B cells are classified according to the heavy chain C region expressed. Humans possess five main classes of Igs namely IgM, IgD, IgG, IgE and IgA, including four subclasses of IgG (IgG1–4), and two

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subclasses of IgA (IgA1–2). Higher vertebrates also have two types of light chains, κ and λ , and each antibody may have either one of these (but not both).

For both types of IgL and the IgH chains there are separate, specific pools of gene segments from which polypeptide chains are eventually synthesized. These gene segments for the heavy chain are located on chromosome 14. In cells not committed to Ig synthesis (i.e. non-B cells) these gene segments remain in their separate germline state.

Normal B cell development is characterized by a large number of consecutive genetic and phenotypic changes. The V region of the heavy chain is encoded by three distinct gene segments, namely the variable (V_H), diversity (D) and joining (J_H) region. The IgH locus on chromosome 14q32 contains 51 functional V_H genes, 27 D segments and six J_H segments. The V_H genes belong to seven families (V_{H1} – V_{H7}), where an homology in sequence of more than 80% defines the members of each V_H family.

The first Ig gene rearrangements involve the heavy chain where D–J recombinations are followed by V–D–J recombination, if these are successful then subsequent rearrangement of the IgL κ and λ genes can occur. All of the above processes occur in bone marrow-derived precursor B cells and result in the formation of immature B cells expressing both IgM and IgD. To continue developing, the immature B cells leave the bone marrow and circulate in the blood. Eventually a very small minority of these B cells may locate themselves in a germinal centre where, if they express a recognizable Ig on their cell surface undergo a second round of genetic and phenotypic changes on exposure to antigen. This includes the process of somatic hypermutation, which subtly alters the sequence encoding the rearranged V_H genes. Approximately simultaneously, but independently, the process of isotype class switching may occur at the C region genes thereby altering the Ig expressed from IgM to IgD, IgG, IgA, or IgE (for extensive review of all the above non-class switching genetic rearrangements in B cells see reference 1).

The immunoglobulin heavy chain (IgH) constant (C) gene locus (Figure 1) is situated within a 300-kb region of chromosome 14q32 located 3' of the J segments.¹ The human constant region appears to have duplicated, during evolution, at the 3' end into two sections of γ – γ – ϵ – α sequences downstream of the μ and δ constant region genes, i.e. $\gamma 3$ – $\gamma 1$ – $\psi\epsilon$ – $\alpha 1$ followed by $\gamma 2$ – $\gamma 4$ – ϵ – $\alpha 2$ (Figure 1). One of the duplicated ϵ sequences ($\psi\epsilon$) is a pseudogene where exons have

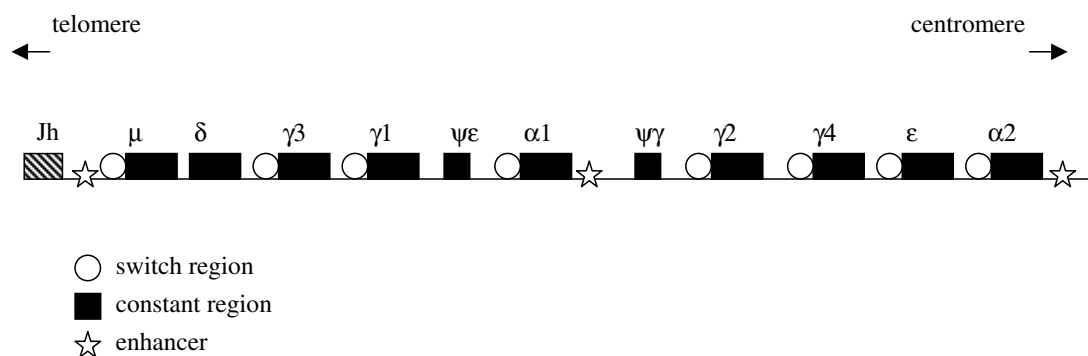


Figure 1. Schematic representation of the immunoglobulin heavy chain (IgH) locus constant region. The germline configuration shown is downstream of Joining (J_H) segments of the variable region. The relative positions of the appropriate switch regions (open circles), constant regions (black blocks) and enhancers (asterisks) are shown for the approximate 300 kb of the constant region (not to scale)

been deleted, whilst there is also a γ pseudogene ($\psi\gamma$), located between the two γ - γ - ϵ - α segments.²

Legitimate class switching

Newly developed B cells first express IgM often together with IgD as a result of differential splicing of mRNA. Alteration in antibody class expressed by B cells is mediated by the DNA deletion–recombination event termed class switch recombination.¹ This process occurs in the germinal centres of lymphoid follicles during antigen stimulated B cell proliferation. Studies of DNA extracted from multiple myeloma (MM) clones producing a functional heavy chain have shown that the recombination was located very close to, or within, internally repetitive DNA sequences, the so-called switch (S) regions. S regions are situated upstream of μ , $\gamma 3$, $\gamma 1$, $\alpha 1$, $\gamma 2$, $\gamma 4$, ϵ and $\alpha 2$ constant regions respectively (Figure 1). Switch regions are non-coding sequences ranging between 1 and 3 kb in length in humans, and are made up of GC-rich repetitive sequence elements in the form of multiple pentameric repeats (GAGCT and GGGGT most frequently and others including ACCAG, GGAGC, TGAGC and (C/T)AGGTTG), which can form secondary structures such as stem loops. There is a high degree of homology between the S regions of mice and humans, which suggests a biologically significant role for these sequences, but they are highly polymorphic between individuals.

There are strong enhancer complexes located downstream of both α constant region genes³ and an $E\mu$ enhancer located about 0.5 kb from the 3' most downstream J_H region and spread over about 0.3 kb. The $E\mu$ segment is located upstream of $S\mu$, this is of particular relevance since $E\mu$ is retained when exogenous coding sequences are moved into these regions, in addition binding sites for nuclear proteins have been identified.

A legitimate switch recombination event occurs between two S regions producing a hybrid S region (Figure 2). Recombination between the upstream $S\mu$ and the downstream S region ($S\gamma 2$, in this case) results in the deletion of the intervening DNA, conventionally thought of as a process of looping out in the form of a 'switch' circle.⁴ The result on the remaining chromosome is that the variable encoding VDJ region, which in the normal course of B cell development will have already been rearranged, is repositioned in front of the 'new' constant region. Therefore all heavy chain genes 5' to the one being expressed are deleted. If the recombination event has occurred correctly then the B cell in question will now produce functional IgG2 heavy chain in its secreted immunoglobulin. The isotype switching event is believed to generally occur in cis, i.e. intrachromosomally, however there is evidence that trans-chromosomal recombination can occur at a frequency of 7% within the IgH locus of B lymphocytes in normal individuals.⁵

There are no conventional switch regions upstream of δ , $\psi\epsilon$ or $\psi\gamma$. Isotype switching to δ can proceed by homologous recombination between duplicated 442-bp sequences ($\sigma\mu$) and ($\sigma\delta$) which are located upstream of $S\mu$ and the first δ exon⁶.

Examination of germline S region sequences in comparison with myeloma-derived composite S sequences appears to show that there is no obvious specific site in any switch region where recombination always occurs, but rather less specific regions to which the event can be localized. In the case of $S\mu$ 40% of the recombination breakpoints occur 5' of the actual switch region. Inversion of switch sequences and sequential switching can also occur, although sequential switching is not a required pathway and its role is not understood. Detailed examination of composite switch region sequences has shown that there are mutations in the DNA around the recombination breakpoint when compared to the appropriate germline sequences.^{7–9} Such mutations involving deletions and substitutions have been linked to the theory that error-prone DNA replication is implicated in the switch recombination process.¹⁰

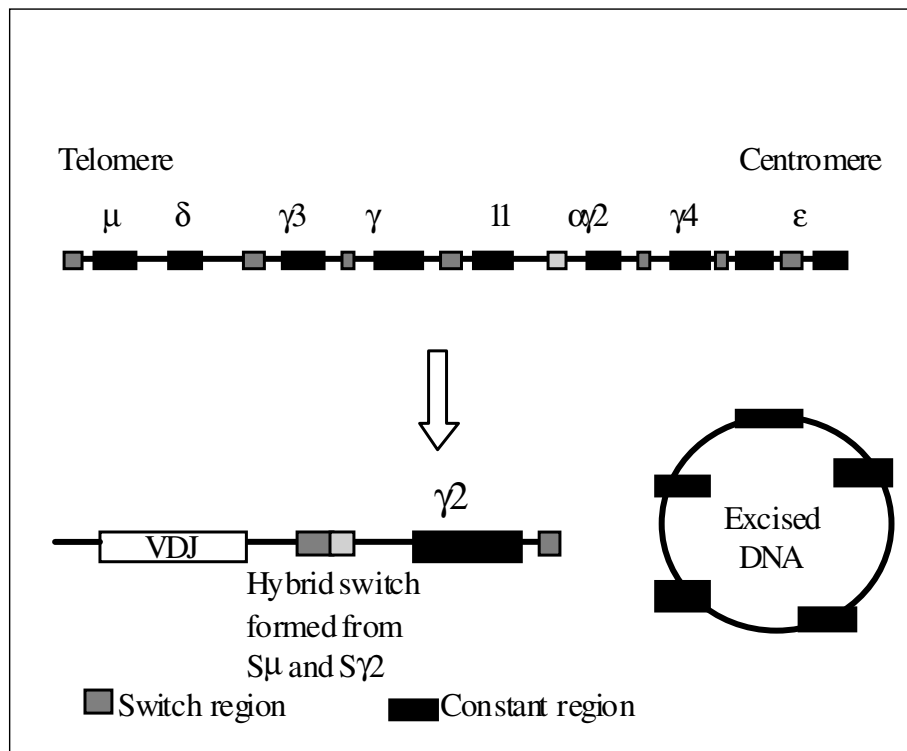


Figure 2. Model of isotype switch recombination leading to altered expression of the heavy chain from IgM to IgG2. DNA recombination occurs between S_{μ} and $S_{\gamma 2}$, producing a rearranged IgH gene that includes VDJ and $S_{\gamma 2}$, separated by a recombined $S_{\mu}/S_{\gamma 2}$ intron. The DNA 3' of the S_{μ} recombination site and 5' to the $S_{\gamma 2}$ site, including the μ , δ , $\gamma 3$, $\gamma 1$ and $\alpha 1$ heavy chain genes is lost, here represented by a 'looping out' circle. Any RNA transcript of the rearranged gene is modified to delete out the recombined switch region

The basic mechanism of isotype switch recombination has still to be elucidated despite extensive studies. Indeed at the time of writing no proteins with potential cutting activity to cleave switch regions have been isolated. This is in sharp contrast to the process of VDJ recombination where cleavage proteins such as RAG1 and RAG2 have been identified. In addition the VDJ recombination process involves precise signal recognition sequences and involves coding DNA, which is not apparently the case in isotype switch recombination. It is postulated that switch recombination could be initiated by a double strand break. If this were the case then the fact that the DNA repair proteins Ku70¹¹ and Ku80¹² and DNA-dependent kinase (DNA-PK)¹³ are necessary for switch recombination to occur, provides credence for such a mechanism. Both Ku70 and Ku86 are members of the Ku complex which binds to DNA double strand breaks and is required for DNA double strand repair, they are also implicated in VDJ recombination. Recently Tai *et al.* have shown that myeloma cells from 12 of 14 myeloma patients express a 69-kD variant of Ku86 that lacks a C-terminus.¹⁴ Their studies suggest that this Ku86 variant accounts for decreased double-stranded DNA repair in multiple myeloma cells.

What are the candidate proteins that have been postulated as having a specific role in class

switch recombination? A recent review considering class switching in the context of DNA repair¹⁵ has suggested ATM, a component of the cellular network for sensing DNA damage. This putative protein kinase is deficient (i.e. mutated) in ataxia telangiectasia, a genetic condition where immunodeficiency of both T and B cells is evident as one of the characteristics. Borgreffe *et al.* characterized a multiprotein complex designated SWAP with a recombination activity that is specific for switching B cells and prefers S region substrates.¹⁶ They identified four of the proteins in the SWAP complex: B23 (nucleophosmin), C23 (nucleolin), poly (ADP-ribose) polymerase (PARP) and SWAP-70.

What is known is that for switch recombination to occur, both S regions that will be involved in the recombination are simultaneously transcribed to produce germline transcripts, this has been extensively reviewed.¹⁷ Germline transcripts are RNA transcribed from non-rearranged IgH constant region genes and are induced before switch recombination has occurred. Each S region is associated with its own upstream promoter exon (termed I region). All of the constant region genes are organized into transcription units in which a specific promoter and a short, non-coding exon lie upstream of each switch region with germline transcription being initiated from these I-region promoters and termination being located downstream of the specific IgH constant region gene. Germline transcripts are not translated. Deletion of the specific promoter and therefore the ability to initiate transcription has been shown to abolish the ability of the S region to recombine. Additionally the 3' enhancer, or locus control regions, which are present in the IgH locus are influential in the class switching process.¹⁸ Studies have utilized gene-targeting techniques in mice to investigate the mechanistic role of transcription in switch recombination.¹⁹ Review of the results of such studies suggests that on its own transcription does not activate class switching, but does allude to the fact that processing of the transcript via RNA splicing is required within the class switch process. This implies that either RNA splicing machinery or the processed transcripts are mechanistically important in switch recombination. Alternatively the germline transcripts themselves have no function but direct switch recombination by creating areas of accessible DNA.²⁰ When B cells are treated with activators and cytokines, or transcription factor expression is altered, switch recombination can be affected despite no observed alteration in germline transcript expression.¹⁷ A number of cytokines (TGF β , IL-4, IL-10 and IFN γ) have been shown to be important in inducing this transcription and directing switching to particular isotypes. Stimulation of B cell proliferation stimulates class switching and inhibition of DNA synthesis prevents switching, suggesting that switch recombination requires DNA synthesis.

Multiple myeloma and illegitimate isotype switching

Errors in the process of isotype switch recombination can in rare cases lead to the occurrence of chromosomal translocations. As has been discussed, isotype switch recombination is a mechanism whereby discontinuous sequences from a chromosome are joined together whilst the intervening sequence are excised.¹⁰ If this process of splicing and reassembling the DNA goes awry it is possible that foreign DNA from another chromosome can become involved and a translocation result (Figure 3). Such an event is termed illegitimate (or aberrant) isotype switching; multiple myeloma (MM) results from the accumulation of malignant plasma cells in the bone marrow and accounts for about 10% of all hematological malignancies. The plasma cells associated with MM are by convention termed post-germinal centre B cells since they have undergone immunoglobulin gene somatic hypermutation, VDJ recombination, antigen selection and (usually) isotype switch recombination. Cytogenetic techniques such as FISH have shown that 95–100% of cases show abnormal karyotypes, usually with highly complex

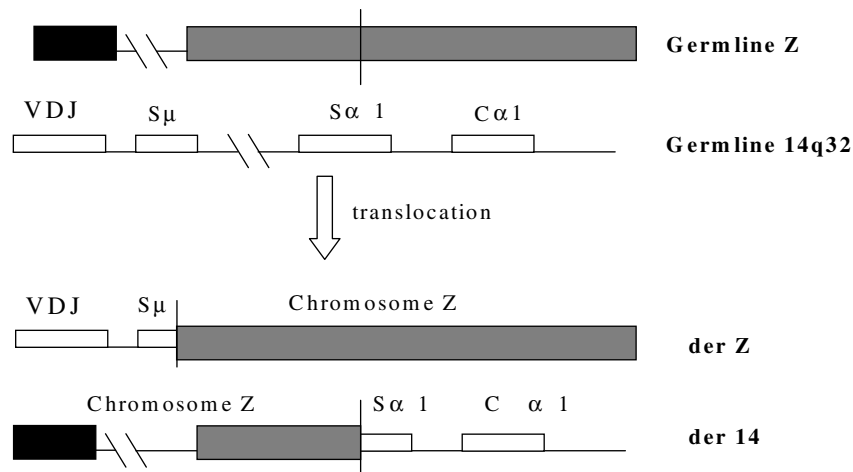


Figure 3. Illegitimate isotype switch recombination events can lead to translocations in MM. Instead of a DNA recombination event occurring between two switch regions, another chromosome (here termed 'Z') becomes involved. This leads to the formation of two derivative chromosomes, der 14 and der Z. In the above model the telomeric region of 14q32 up to $S\mu$ has been translocated to the partner chromosome Z with a reciprocal translocation partner resulting in DNA from chromosome Z being juxtaposed to a 3' $S\alpha 1$ locus. The intervening DNA from 14q32 i.e. from 3' $S\mu$ to 5' $S\alpha 1$ (including the μ , δ , $\gamma 3$, $\gamma 1$ heavy chain genes) has been deleted, possibly by a 'looping out' circle mechanism, during this illegitimate isotype switching event

changes. In particular rearrangements of the immunoglobulin heavy chain (IgH) locus at 14q32 can occur in up to 74% of cases.²¹ It has been hypothesized that translocations to the IgH loci provide one of the initial immortalizing events in the molecular pathogenesis of myeloma.²² The IgH locus is strongly transcriptionally active in B cells and transfer of an oncogene to this region can result in dysregulation of the gene in question. This is due to the presence of the IgH enhancers, which are defined as DNA fragments which activate transcription in an orientation-dependent manner, a function which is independent of distance. For example in Burkitt's lymphoma, cytogenetically characterized by t(8;14) (q24;q32.3), the oncogene *c-myc* (located on 8q24) is translocated to the IgH locus.²³ This results in overexpression of *c-myc* since the oncogene is now under the influence of the strong IgH enhancers. Similarly in cases of Mantle cell lymphoma associated with t(11;14) (q13;q32), the proto-oncogene cyclin D1, a regulator of cell division, is translocated to the IgH locus and as a result overexpressed.²⁴ In both of the former conditions, these translocations are generally associated with aberrant VDJ recombination, therefore rearrangements on 14q32 are located in the S regions. MM plasma cells generally express a functional heavy chain as a result of a legitimate switch recombination event, therefore any illegitimate recombination event logically occurs on the other, non-productive, allele to produce the 14q32 rearrangements associated with MM.

Recently, recurrent chromosomal translocations in multiple myeloma have been identified in MM cell lines including t(11;14)(q13;q32), t(4;14) (p16;q32), t(14;16) (q32;q23) and t(6;14) (p25;q32). Conventionally these translocations have been associated with the dysregulation of specific genes, namely cyclin D1,²⁵ *FGFR3*,^{26,27} *c-maf*²⁸ and *MUM1/IRF4*²⁹ respectively. Since these findings were the result of studies of MM cell lines, representative data of the spectrum of

genes deregulated *in vivo* is still lacking. Avet-Loiseau *et al.*³⁰ identified the t(11;14) translocation using FISH in 16% (23 of 141 patients) and the t(4;14) translocation in 12% (17 of 141) of patients with plasma cell malignancies. More recently Finelli *et al.*³¹ using double-colour FISH identified 5/30 patients with the t(4;14) translocation. It is becoming clear from studies, of both patient and cell line samples, that a promiscuous array of partner loci are implicated in translocations to the IgH locus. More than 20 different chromosomal partner regions have so far been reported, making karyotypic 14q32 translocations the most frequently observed genetic abnormality in MM,²¹ (for a summary see Table 1). It is clear that cytogenetic techniques such as routine banding and FISH, using IgH probes, will not pick up all the translocations associated with the IgH locus, especially if these events are located at the extreme telomeric regions of chromosomes. There is also known to be a major limitation of cytogenetics to obtain metaphases from this indolent slow-growing tumour. However despite studies utilizing new cytogenetic techniques such as spectral karyotyping (SKY),^{32,33} the identity and recurring frequency of all the partner chromosomes and the identity of associated oncogenes has still to be elucidated.

Although dysregulation of candidate oncogenes is conventionally associated with a certain translocation, e.g. cyclin D1 overexpression with t(11;14) (q13;q32), there is now increasing evidence that other events also occur. For example, the t(11;14) translocation is now being associated with dysregulation of other genes located at 11q13,^{34,35} indeed one of these studies has identified the putative oncogene *myeov*.³⁴ DNA fibre FISH studies mapped *myeov* to a locus 360 kb centromeric of the cyclin D1 oncogene on 11q13. In three of seven MM cell lines with a characterized t(11;14)(q13;q32) and cyclin D1 overexpression, Northern blot analysis also revealed overexpression of *myeov*.³⁵ The relevance of activation of both genes in the clinical context remains to be elucidated.

Overexpression of cyclin D1 due to an insertional event has been shown in the U266 myeloma cell line³⁶ where a portion of excised IgH switch intervening sequences containing the 3' α -1 enhancer, formed during a IgH μ - ϵ switch recombination, is inserted into chromosome 11q13. The t(11;14)(q13;q32) translocation is thought to be associated with a more aggressive

Table 1. Summary of partner loci associated with translocations to IgH locus at 14q32 in multiple myeloma. Genes which have been implicated as being affected by each specific translocation are listed (along with a relevant reference)

Partner locus	Gene(s) implicated	Reference
1q21	MUM2/MUM3	48
4p16	FGFR3 (MMSET, TACC3)	26, 27 39, 40, 41
6p21	Cyclin D3	49
6p25	MUM1/IRF4	29
8q24	c-myc	22
9p13	PAX5	50
11q13	cyclin D1 (<i>myeov</i>)	25 35
16q23	c-maf (WWOX)	28 51
18q21	BCL2	22
Other rare partner loci for which no gene partners have been implicated: 1p13, 3p11, 6p25, 7q11, 7q32, 11q23, 12p11, 12p12, 12q24, 13q22, 16q24, 21q22, 22q21, Xq28		

disease but there is a lack of data assessing the prognostic significance of other 14q32 translocations in multiple myeloma.

The t(4;14) (p16.3;q32.3) translocation was primarily associated with overexpression of FGFR3 on der(4). FGFR3 is a receptor tyrosine kinase, which in human germline can contain activating mutations associated with various forms of dwarfism.³⁷ Some of these mutations have been implicated in the FGFR3 genes translocated into the IgH switch regions of MM plasma cell DNA.²⁶ The implications of translocated FGFR3 (either wild-type or constitutively activated) with respect to the development or maintenance of MM plasma cells is still unclear. A very recent study has demonstrated that IL-6-dependent murine cells transduced with retroviruses containing functional wild-type FGFR3 show enhanced proliferation and survival in comparison to control cells.³⁸ Interestingly they also demonstrated that when the same cells overexpress mutant FGFR3 there was also resultant IL-6 independence and enhanced proliferation in response to IL-6. The t(4;14) translocation has also been shown to merge a candidate gene, WHSC1/MMSET, with the IgH locus resulting in hybrid transcripts from the reciprocal der(4) chromosome and consequentially upregulation of WHSC1/MMSET.^{39,40} In addition a novel gene, transforming acidic coiled coil-containing gene (TACC3), has been found adjacent to the FGFR3 gene and may have a role in the control of cell growth and differentiation since it is upregulated in various cancer cell lines and in embryonic development in mice.⁴¹ TACC3 is one of a family of three genes, TACC1, 2 and 3 which appear to be linked to FGFR1, FGFR2 and FGFR3 respectively, strongly suggesting that the FGFR and TACC genes arose from a physically linked ancestral pair. The proximity of TACC3 to FGFR3, and consequently to the breakpoints in t(4;14) raises the possibility that this translocation may also dysregulate the expression of TACC3.

In all of the above examples the genes which are deregulated as a result of the various IgH translocation events appear to come under the influence of the Ig enhancers. Translocations involving switch regions, as observed in myeloma, will separate the E μ and E α enhancers, the consequences of this mean that it is possible to activate different genes on the two reciprocal chromosomes simultaneously.³⁶ For example, it has been observed in two t(11;14) (q13;q23)-positive MM cell lines that that cyclin D1 is juxtaposed to the most centromeric E α -enhancer (E α 2) and myc to the E μ enhancer.³⁵ These workers also observed that in one additional t(11;14) MM cell line the E α 2 and E α 1 enhancers were juxtaposed to cyclin D1 and myc, respectively. Similarly for the t(4;14) (p16;q32) translocation in MM patients the FGFR3 and WHSC1/MMSET genes fall under the influence of the E α and E μ respectively.^{39,40}

There is now increasing evidence that illegitimate isotype switching is involved in the pathogenesis of other hematological malignancies, in addition to myeloma. For example, mucosa-associated lymphoma tissues (MALT) can undergo DNA rearrangements within or near switch regions.⁴² The latter study discovered one high grade case where there was a deletion of the IgH intronic enhancer (E μ) as well as a 336-base pair insertion into S μ of a gene normally located at 15q24. There is also limited evidence that sporadic Burkitt's lymphoma have IgH/myc breakpoints located in either the first intron or 5' of myc and within IgH S regions.⁴³

Hematological malignancies which show translocations, apparently as a result of illegitimate class switching, can obviously be contrasted with conditions such as lymphomas where a translocation is located in the J_H region, presumed to be the result of illegitimate VDJ recombination. Conventionally it has been considered that such translocations situated in the J_H region occur in a B-cell precursor cell, within the bone marrow. By the same convention a translocation within an S region would be thought of as occurring as a later event, in a more developed B cell. However in most cases of IgH translocations in lymphomas,

targeting of oncogenes to the J_H region appears to occur in mature B cells after antigen is encountered and IgH class switching has occurred, presumably due to reactivation of recombination activation genes RAG1 and RAG2, in germinal centre B cells.⁴⁴

Interestingly there are also reports of a very rare sub-class of IgM-secreting myeloma, such cases have not apparently undergone any class switching events.⁴⁵ Analysis of the V_H region genes of these few cases showed a high level of somatic mutation, which indicates that the cell of origin has traversed a germinal centre. In addition the presence of clonally related IgM and IgG transcripts where tumour VDJ was joined to both γ 1 and γ 2 transcripts, led the authors to speculate that such IgM myelomas have developed from a cell of origin arrested at a IgM to IgG switch point. Such data, and speculation, highlights the fact that the identity of a neoplastic cell of origin in myeloma is contentious. A study of IgG- and IgA-expressing myeloma cases where bone marrow plasma cells were analysed, showed as expected, that rearranged a V_H region specific to each myeloma was expressed in a transcript associated with the appropriate C γ or C α gene sequence.⁴⁶ But in addition it was also shown that there were transcripts where the specific V_H region was still joined to the C μ gene, suggesting the presence of 'pre-switch' B cells in the bone marrow. But this data is not conclusive for the presence of such precursor cells and it should be noted that it is possible that there could be alternative splicing of a large Ig RNA transcript from B cells co-expressing μ , and γ or α genes, as has been observed in memory B cells.

Approximately 20% of MM cases show no expression of IgH, only Ig light chain is detected in serum or urine giving light chain (or Bence-Jones) disease. There is also an even rarer form occurring in <1% MM, called non-producer MM, where there are no detectable IgH and Ig light chains. Together such cases can make up a subgroup termed IgH⁻ MM; a study of such cases showed that 9/12 patients were unable to produce IgH protein due to DNA defects which were related to either illegitimate IgH class switching or deletion of the functional VDJ allele.⁴⁷ Such defects could conceivably occur during, or after, any malignant transformation process.

It is clear that the process of illegitimate isotype switching is heavily implicated in the pathogenic development of neoplastic B cells in MM. Although well studied, until the process of normal legitimate class switching at 14q32 in normal B cells has been elucidated, it is going to be very difficult to deduce the mechanisms involved in producing the IgH translocations associated with MM. In addition, although the characterized 14q32 rearrangements constitute the most frequent cytogenetic abnormality in MM, it has yet to be observed in 100% of patients. Such an observation will require improved cytogenetic and molecular techniques to conclusively prove that this is indeed the case, as well as leading to the deduction of the identity and recurring frequency of all the translocation partner chromosomes and thus elucidation of any associated oncogenes.

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