Symposium article

Recurrent immunoglobulin gene translocations identify distinct molecular subtypes of myeloma

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Summary

Background: Chromosome translocations involving the immunoglobulin heavy chain gene (IgH) on 14q32 are a seminal event in the pathogenesis of many B-cell malignancies. Since myeloma is a post-germinal center tumor of mature, isotype switched plasma cells, we hypothesized that 14q32 translocations would usually involve IgH switch regions.

Materials and methods: We analyzed a panel of 21 human myeloma cell lines using a Southern blot assay to detect illegitimate rearrangements involving the switch regions. We then cloned the breakpoints, developed probes for FISH analysis, and characterized the oncogenes dysregulated by the translocations.

Results: Only half of the cell lines demonstrated a 14q32 abnormality by conventional karyotypic analysis, but we were able to identify translocations involving IgH switch regions in 15 of 21 lines, including all of the lines in which a 14q32 translocation was not identified by conventional karyotypic analysis. Six cell lines have an Ig translocation involving 1q13 with overexpression of cyclin D1. Six cell lines have an Ig translocation involving 16q23 with overexpression of c-maf. Five cell lines have an Ig translocations involving 4p16 with overexpression of FGFR3 and a novel gene, MMSET. The 4p16 breakpoints occur within the 5' introns of MMSET, and are associated with IgH-MMSET hybrid mRNA transcripts. The remaining five cell lines have translocations involving other loci, including: 6p25 (MUM1), 8q24 (c-myc), and 21q22 (AML1).

Conclusions: Recurrent Ig translocations identify at least three distinct molecular subtypes of myeloma. Our long-term goal is to determine if there are phenotypic, prognostic and therapeutic differences associated with these molecular subtypes.

Key words: chromosomal translocations, cyclin D1, FGFR3, MMSET, multiple myeloma

Introduction

According to the SEER cancer statistic review, the mortality rate in the USA from B-cell neoplasms is 11 per 100,000 (1992–1996). As shown in the diagram in Figure 1 multiple myeloma (MM) accounts for a large portion of these deaths (27%). MM is preceded by a common pre-malignant monoclonal expansion of plasma cells called monoclonal gammopathy of undetermined significance (MGUS) that is present in 1% of the adult population, and that progresses to MM at a rate of 1% per year [1]. Despite intense efforts over the last thirty years, there have been few advances in the treatment of MM, and there is no known way to identify those who will progress from MGUS to MM or to delay or prevent this progression. Clearly a better understanding of the molecular pathogenesis of these conditions is fundamental to developing more effective prognostic, treatment and prevention approaches.

Chromosomal translocations into the immunoglobulin heavy chain (IgH) locus on chromosome 14q32 are the hallmark of many B cell malignancies and their characterization has led to the identification of critical dysregulated oncogenes (e.g., c-myc, BCL-2, cyclin D1) that play a key role in the pathogenesis of these diseases [2]. In contrast, conventional cytogenticists failed to identify recurrent translocations in MM, mainly due to the low proliferative index of MM cells [3, 4]. Since myeloma is almost exclusively a tumor of plasma cells that have undergone the processes of somatic hypermutation of the immunoglobulin genes, and isotype switch recombination, we hypothesized that errors during the physiologic process of switch recombination may occur, leading to chromosome translocation into the immunoglobulin genes, with the consequent dysregulation of oncogenes on the juxtaposed partner chromosomes. Therefore, we developed a Southern blot assay to identify illegitimate switch rearrangements, most of which represent breakpoints of chromosome translocations into the immunoglobulin locus [5]. In the past three years, we have determined that IgH translocations are present universally in MM cell lines, and that they occur mainly as a result of errors during the physiologic switch recombination process. Three chromosome loci are frequently involved and account for 75% of myelomas, thereby dividing them into three molecularly distinct subtypes: myelomas with a translocation to 1q13 that overexpress cyclin D1, to 16q23 that overexpress c-maf, and to 4p16 that overexpress FGFR3 and MMSET [6–9].
Mortality from B-cell malignancies Mortality rates (per 100,000) reported in the SEER Cancer Statitstics Review for 1992–1996. The total mortality rate for all B-cell malignancies is 11 per 100,000. The percent contribution for the commonest B-cell malignancies is summarized in this pie chart. Abbreviations: HD – Hodgkin’s disease; DLCL – diffuse large-cell lymphoma; FL – follicular lymphoma, MCL – mantle-cell lymphoma; MALT – mucosal associated lymphoid tumors; ALL – acute lymphocytic leukemia; CLL – chronic lymphocytic leukemia; MM – multiple myeloma.

Ilq13 translocations

Normally B cells express cyclin D2 and cyclin D3. However, the hallmark of mantle-cell lymphoma (MCL) is a t(11;14)(q13;q32) translocation that results in overexpression of cyclin D1. The translocation breakpoints in pre-germinal center MCL are near J segments, consistent with their occurrence as a result of errors in VDJ recombination that occur at the earliest stages of B-cell differentiation. The majority of MCL breakpoints are clustered into the major translocation cluster (MTC) region, approximately 110 kb centromeric to the cyclin D1 gene at Ilq13 [10]. As a result of this translocation, the cyclin D1 gene is dysregulated by juxtaposition to two regulatory elements on the IgH locus, the intronic Eu and the 3’ Ea enhancers. Five myeloma cell lines (KMS12, SK-MM2, FLAM-76, H1112 and MM-M1) had been reported to have a t(11;14)(q13;q32) translocation by conventional cytogenetics techniques. Because of the localization of cyclin D1 in the middle of the q arm of chromosome 11 (Figure 2a), this is the only recurrent translocation in MM that has been identified previously by conventional cytogenetics. We cloned and characterized t(11;14) breakpoints in three lines (KMS12, SK-MM2 and MM-M1) [9, 11]. In contrast to MCL, the breakpoints in KMS-12 and SK-MM2 involve an IgH switch region (Figure 2b), whereas the MM-M1 breakpoint is located between J5 and J6. In addition, instead of being clustered into the MTC as for MCL, the translocation breakpoints in MM are dispersed over a 100–330 kb region centromeric to cyclin D1. We analyzed MM lines for cyclin D1 expression by Northern blot, and found that these five lines overexpress cyclin D1. The only other line overexpressing cyclin D1 is U266, which has does not have a t(11;14) translocation by conventional karyotypic analysis. Recently, however, we identified a novel mechanism of cyclin D1 dysregulation in U266 [12]. During the productive IgH mu–epsilon switch recombination event, a 100 kb segment of the excised IgH region containing the 3’Enhancer was not deleted from the cell, but instead inserted on chromosome Ilq13, resulting in overexpression of the cyclin D1 gene that is located 12 kb from the site of insertion. This result explains why the t(11;14) translocation had not been detected in the U266 MM cell line by conventional cytogenetics.
Figure 3. Dysregulation of c-maf by a t(14;16)(q32;q23) translocation. The positions of the IgH locus on 14q32.3, and c-maf on chromosome 16 are indicated on the diagrams of the chromosomes. The approximate positions of the translocation breakpoints cloned from four myeloma cell lines and of c-maf in a 500 kb region are shown to the right.

16q23 translocations

In contrast to 11q13 translocations, translocations affecting the 16q23 locus have never been reported before. This is explained by the localization of the breakpoints in the IgH locus at the telomere of chromosome 14, and near the telomere of the q arm of chromosome 16 (Figure 3). We cloned t(14;16) breakpoints in four MM lines (ANBL-6, KMS11, JNJ3 and MM.1-144) and mapped them in a region 100-500kb centromeric to c-maf (Figure 3a) [8]. This is the first example where the IgH 3'Enhancer is shown to be able to dysregulate the expression of a gene at such a distance. Using a PI clone containing the c-maf gene, we showed by FISH analysis that in another myeloma line, OCI-MY5, there is a t(14;16)(q32;q23) translocation. In addition, the myeloma line 8226 has a variant t(16;22) translocation affecting the IgL locus, with the breakpoint telomeric to c-maf. It has been shown in other tumors that IgH translocation breakpoints and variant translocation breakpoints bracket the dysregulated oncogenes (i.e., cyclin D1, BCL-2 and c-myc). In an analogous way, the translocation breakpoints in these six myeloma lines bracket c-maf.

A chromosome translocation typically results in cis dysregulation of the oncogene located on the partner chromosome involved in the translocation, whereas the allelic copy of the oncogene on the non-translocated chromosome is not dysregulated. In both informative cases we were able to demonstrate selective expression of one of the two c-maf alleles, consistent with the dysregulation of c-maf by translocation of one allele to an Ig locus.

Interestingly, we previously identified human c-maf from a subtractive c-DNA approach as a gene selectively expressed in myeloma but not lymphoblastoid cell lines [13]. We cloned, sequenced, and tested its expression in normal tissues and our panel of 21 myeloma cell lines. By Northern blot analysis, c-maf RNA is expressed at comparable levels in all the human tissues analyzed: spleen, thymus, prostate, testis, intestine, colon and peripheral blood lymphocytes. In myeloma, its expression is barely detectable, but in the presence of the 16q23 translocation c-maf becomes markedly overexpressed [8]. C-maf is a basic zipper transcription factor that is the cellular homologous to v-maf, a classical oncogene isolated from an avian retrovirus. It has been shown that the overexpression of the wild type c-maf is sufficient to induce cell transformation in cultured fibroblasts [14]. Along with c-myc and c-abl, c-maf is one of the three classical oncogenes to be involved in human tumors.
4p16.3 translocations

As with 16q23, translocations affecting the 4p16.3 locus have never been reported before. Again, this is explained by the telomeric localization of the IgH 4p16.3 breakpoints (Figure 4). We cloned and characterized 4p16 translocation breakpoints in five myeloma lines (OPM2, JIM3, H929, UTM2 and KMS11) and in a plasma cell leukemia patient. Similar to 11q13 and 16q23 breakpoints, the 4p16 translocation breakpoints are dispersed over a 30–100 kb region centromeric to the fibroblast receptor growth factor 3 (FGFR3) gene [7]. We showed that FGFR3 is ectopically expressed only in the cell lines and tumor samples carrying the t(4;14) translocation. FGFR3 is one of the four high affinity tyrosine kinase receptors for the FGF family of ligands. During development it is expressed in chondrocytes, where it regulates bone growth. In fact various kinase activating mutations in FGFR3 when inherited in the germline cause dwarfism, with the severity of phenotype directly proportional to the degree of activation of the receptor [15]. Interestingly we found that the same potent kinase activating mutations that cause thanatophoric dysplasia, the most severe (and lethal) form of dwarfism, are acquired by the myeloma cells during tumor progression [7].

Recently we have identified, cloned and sequenced another gene at the 4p16 locus, MMSET, and have shown that the t(4;14) translocation causes the concomitant dysregulation of both FGFR3 on der(14) and MMSET on der(4) [6]. This is the first example in any tumor of a chromosome translocation causing the dysregulation of two different genes on the two derivative chromosomes. The 4p16 breakpoints occur within the 5’ introns of MMSET with the consequent formation of hybrid mRNA transcripts, easily detectable clinically by RT-PCR, involving IgH or JH on chromosome 14 and splicing to the first coding MMSET exon on chromosome 4. MMSET is a new member of the trithorax group of nuclear proteins, one of which, ALL1/HRX/MLL1, is the oncogene involved in 11q23 translocations in acute leukemias [16]. Members of this family are thought to be involved in the epigenetic regulation of gene expression through chromatin remodeling. By Northern blot analysis, MMSET is most highly expressed in thymus and testis, with low levels of expression in all other tissues analyzed. In multiple myeloma it is expressed at a low level in all the cell lines analyzed, but it becomes highly expressed in the presence of t(4;14) translocation.

The dysregulation of MMSET in MM, together with its homology to the Hrx gene, suggests an oncogenic role for MMSET in MM tumors with t(4;14) translocation. The simultaneous dysregulation of FGFR3 by the same translocation may provide a survival or proliferative signal. Alternatively, it is possible that the overexpression of FGFR3 has no immediate effects on tumor formation, but that the occurrence of point mutations causing constitutive activation of FGFR3 in the absence of ligand contributes to tumor progression.

In summary we are developing a multi-step model for tumor initiation and progression for MM. The first step in the pathogenesis of myeloma is a premalignant condition, called MGUS. Immunoglobulin translocations have been detected in MGUS by other investigators using interphase FISH analysis, and by us using RT-PCR for IgH-MMSET hybrid transcripts (unpublished). Therefore the dysregulation of oncogenes, cyclin D1, c-maf, MMSET together with FGFR3, caused by immunoglobulin translocation is one of the earliest – perhaps the first – genetic alteration resulting in immortalization of the plasma cells. An immortalized plasma cell can then accumulate secondary genetic events, including ras and FGFR3 activating mutations, that lead to tumor progression. By understanding the different kinds of translocations in MM, we should be able to improve the diagnosis and treatment of molecular subtypes of this presently incurable malignancy.

References


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