Meeting Report

Genetics and Cytogenetics of Multiple Myeloma: A Workshop Report

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Abstract

Much has been learned regarding the biology and clinical implications of genetic abnormalities in multiple myeloma. Because of recent advances in the field, an International Workshop was held in Paris in February 2003. This summary describes the consensus recommendations arising from that meeting with special emphasis on novel genetic observations. For instance, it is increasingly clear that translocations involving the immunoglobulin heavy-chain locus are important for the pathogenesis of one-half of patients. As a corollary, it also clear that the remaining patients, lacking IgH translocations, have hyperdiploidy as the hallmark of their disease. Several important genetic markers are associated with a shortened survival such as chromosome 13 monosomy, hypodiploidy, and others. The events leading the transformation of the monoclonal gammopathy of undetermined significance (MGUS) to myeloma are still unclear. One of the few differential genetic lesions between myeloma and MGUS is the presence of ras mutations in the latter. Gene expression platforms are capable of detecting many of the genetic aberrations found in the clonal cells of myeloma. Areas in need of further study were identified. The study of the genetic aberrations will likely form the platform for targeted therapy for the disease.

Introduction

Multiple and complex chromosomal abnormalities are present in the clonal plasma cells (PCs) of multiple myeloma (MM; Refs. 1–3). Using interphase fluorescence in situ hybridization (FISH), we now know that chromosomal abnormalities are nearly universal and are early events in the PC neoplasms (2–7). The biology associated with these abnormalities and reported recurrent prevalence favors their participation in the process of disease pathogenesis (8, 9). To review the present status of our knowledge of the genetic basis of MM and to identify areas in need of further study, a meeting was held in Paris, France, in February 2003. This article reports on the discussions held at this meeting.

The rekindled interest in the role of specific genetic aberrations in the outcome of MM was sparked by reports showing clinical implications for karyotype abnormalities (1, 10–13). Much has been learned, but a major challenge for the future will be the integration of the available genetic information into a coherent model of disease initiation and progression.

Biology of the Stages of the PC Neoplasms

MGUS. Monoclonal gammopathy of undetermined significance (MGUS) is a stable premalignant PC tumor that can progress to frankly malignant MM at a rate of 0.5–3% per year, depending on the level of monoclonal immunoglobulin (14). Several clinical definitions for MGUS have been proposed, but not one is universally accepted (14). A recent publication has reached a clinical consensus definition for these disorders (15). Although some definitions have inherent clinical usefulness, much discussion occurred with regard to the best biological definition of MGUS. It is easier to discern the clinical differences (tumor mass, secondary effects) between MGUS and MM than the intrinsic biological differences between the two states. That being said, there are intrinsic properties (myc abnormalities, ras mutations, p53 mutations) that appear to distinguish advanced MM, so that what is missing are intrinsic differences that distinguish the tumor cells of MGUS and early stages of MM. The detection of an abnormal karyotype in MM is highly correlated with an elevated PC labeling index and tumor burden, reflective of a higher mitotic rate, and also with tumor burden as manifested by a higher bone marrow plasma-cytosis (16).

MGUS is state of discrete expansion of a monoclonal population of PCs. It has been accepted that this expansion should not exceed 10% of the marrow cellularity, although significant variability of bone marrow sampling exists (14, 17). It is generally accepted that a serum monoclonal protein should be at a concentration of ≥3 g/dl (14). Although this upper limit could be considered as too high for patients with IgA MGUS, it was accepted that using a 3-g/dl for all patients is inappropriate. There is no minimal concentration currently required to make the diagnosis of MGUS, and this has been traditionally dictated by the sensitivity of the screening tests (i.e., electrophoresis versus immunofixation, and so forth). Recent work has shown that the risk of progression form MGUS to MM increases with increasing concentration of the monoclonal proteins (14).

Both MGUS and MM result from clonal proliferation and expansion of PCs. It has been accepted that at least 5 × 109 need be present for a monoclonal protein to be detectable in the serum, thus requiring at least 30 cell doublings (18, 19). A 2log or 2–3log further expansion is usually lethal (1–5 × 1012 cells). Therefore, considerable clonal expansion occurs in both MGUS and MM, the former stabilizing before producing clinical symptoms.

It was also recognized that more sensitive means to detect MM complications could have clinical utility but will not be required to include patients in biology studies of MGUS. For instance, although magnetic resonance imaging may detect otherwise occult bone les-
sions, this is usually not necessary for routine testing of patients. It was also recognized that the best test for an accurate diagnosis of MGUS (as compared with MM) is disease stability over a period of time after detection (6–12 months; Refs. 7, 14, 17, 20–23). By applying this criterion, a very small percentage of true MGUS with progression to MM would be missed (<2%), but most cases of early MM would be eliminated. The problem is that this criterion can only be applied retrospectively, but having such information in relevant publications would be of use for the interpretation of the data.

It is of critical importance to differentiate IgM-MGUS from other isotype MGUS (non-IgM-MGUS; Refs. 14, 24). The former is only rarely involved in transformation to MM and more commonly is associated with Waldenström macroglobulinemia and other lymphoproliferative disorders. In contrast, patients with IgG and IgA MGUS are those at risk of evolution to MM (14). In studies addressing the biology of MGUS and its relation to MM it is imperative to study only those with non-IgM MGUS.

Asymptomatic MM or SMM. Smoldering MM (SMM) is an intermediate entity between MGUS and active MM (25). Scant information is available with regard to the biology and natural history of SMM (17, 26, 27). Likewise, the biological differences between MGUS and SMM are not well defined. It was agreed at the meeting that SMM represents a modest proliferation of monoclonal PCs, but that this proliferation does not interfere with normal bodily functions of the patient. Commonly accepted criteria include having a bone marrow plasmacytosis in excess of 10% but, in most cases, not exceeding 30%. Most patients with SMM have a serum monoclonal protein >3 g/dl, but the rate of immunoglobulin synthesis is variable and not necessarily predictive of the marrow plasmacytosis (25). Patients with SMM should not have any of the after complications ascribed to the clonal PC proliferation: anemia, renal failure, bone destruction, or hypercalcemia. There is a spectrum of patients with SMM. Some patients probably represent early active MM and require therapy shortly after diagnosis is made. On the contrary, other patients can have prolonged periods of stability without treatment despite moderate expansion of the monoclonal PCs.

MM. MM is clinically defined when a PC neoplasm results in clinical complications (28). Most patients have evidence of marrow infiltration (almost always >10% and more commonly ≥30% of the bone marrow cellularity). MM is a heterogeneous disorder, and the designation MM may encompass several related entities that share the common characteristic of being composed of monoclonal PCs.

De novo MM has been proposed as a state in which patients have MM without a known antecedent history of MGUS (29). In the majority of cases, no screening before a diagnosis of MM has been done and it is difficult to know at present whether only a fraction or the majority of MM cases has a preexisting MGUS. However, some epidemiology studies suggest that, in most cases with MM, a preexisting MGUS is almost always present (30). This is in need of further study because it has clear implications for the full biological understanding of the disease.

Bone Marrow Examination. A serious limitation for the clinical care and understanding of MGUS and SMM has been the infrequent collection of bone marrow samples in these patients. Because of the imprecise correlation of the monoclonal protein concentration and the clone burden of the bone marrow, it is difficult at present to determine what is the natural history of patients with SMM. It is likely that at least some historical patients diagnosed with MGUS who did not have a bone marrow examination may in fact have SMM. It is possible, then, that the actual rate of progression of true MGUS to MM may be lower if one excludes patients with a >10% plasmacytosis of the bone marrow. In some cases, the discontinuous nature of the infiltrates may under (or over)-estimate the actual plasmacytosis. In other cases, the clonal cells produce small amounts of the monoclonal protein, and extensive marrow infiltrates are possible in patients with lower concentration monoclonal proteins. We suspect that progression from MGUS to MM is not merely a stochastic process, but one for which there is differential risk of progression, depending on the specific molecular genetic abnormalities. We recommended obtaining bone marrow examinations in all patients with suspected PC neoplasms for their accurate diagnostic classification.

Methodologies

Methods of Sorting. Although multiple methods of sorting are available to enrich PC populations, the most practical and widely available is the one using CD138 magnetic microbeads (31). This method appears to be reliable, although significant interuser variability exists with regard to purity and yield. This method does not distinguish normal and tumor PC. Although in some MM cases, cell subpopulations may be negative for the surface marker CD138 (likely apoptotic cells), the group accepted the positive sort fraction as highly representative of the clone, and suitable for most genetic studies (32). In slide-based assays, the immune-fluorescent detection of immunoglobulin (usually light-chain)-restricted cells provides a useful way of distinguishing tumor and normal PC from other kinds of cells (e.g., as in clq-FISH or FICTION; Ref. 33).

Conventional Cytogenetics. Understanding the biology of MM genetics lagged behind other hematological malignancies because of the low yield for karyotype abnormalities from MM bone marrow samples (34). In most cases (50–70%), the karyotype reveals normal metaphases that originate from the myeloid elements (1, 11, 12, 16, 34–38).

Even among patients with abnormal karyotypes, some of the most important aberrations may be cryptic (39–41). For instance, the t(4;14)(p16.3;q32) was not recognized as present in MM karyotypes, even when it is seen in 15–20% of patients (39, 40). Likewise, the t(14;16)(q32;q23) may be difficult to detect using G-banding without the use of specialized techniques.

In contrast, the karyotype will be highly sensitive for the detection of numerical chromosomal abnormalities (1, 11, 12, 34–38). According to this detection, patients can be accurately classified into the specific ploidy categories (1, 11, 12, 34–38). The karyotype information has been thought to be reliable for the detection of chromosome 13 abnormalities (Δ13) including interstitial deletion and monosomy. The karyotype is limited in that it cannot describe possible heterogeneity within a population of clonal cells.

Metaphase Spectral Karyotype Imaging/Multicolor-FISH (SKY/M-FISH) and CGH. In an effort to improve on the accuracy of conventional karyotype analysis, other methods of investigation have been attempted. Multicolor metaphase FISH has been used to provide greater details of complex karyotypes (41–43). This technique is still limited by the need of the cell to undergo mitosis. Another interphase technique is comparative genomic hybridization (CGH). However, CGH allows only for the detection of net DNA gain or loss and cannot detect balanced structural abnormalities, and it needs to be done with purified cell populations. CGH has been used to study MM, and several areas of recurrence have been identified (44–47).

FISH. MM has also been successfully studied by interphase FISH, because this is an assay that can be done in nondividing cells (2–6, 48–52). FISH has been applied for the study of trisomies/monosomies, Δ13 and 17p13.1 and translocations involving the immuno-

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globulin heavy-chain locus (IgH) and immunoglobulin λ-light chain locus (IgL-λ). FISH signals are generally of lower intensity in the clonal PCs as compared with other cellular elements of the bone marrow.

Estimating the prevalence of chromosomal abnormalities, interphase FISH is best done in purified cells (e.g., clonal PCs as compared with other cellular elements of the bone marrow rearranged IgH and immunoglobulin light-chain locus (IgL)). In 15–30% of patients, this means that 13 monosomy by karyotype is usually observed in 7–15% of patients. This number has been derived from samples that are appropriately collected and shipped to reference laboratories (31). The scoring criteria have been variable but a "fused" strategy that detects any IgH translocation using probes that localize to the IgH allele can result in weak FISH signals that may be difficult to detect. Translocations can also be detected by a "fusion" strategy that results in colocalization of FISH signals with probes for the regions of interest (4, 7, 20, 49, 53, 54, 56). In this strategy, probes localize to chromosomes involved in translocations (e.g., 11q13 or 16q23) are cohybridized with probes that localize to 14q32 and are labeled with a different fluorophore. The fusion of these probes is considered indicative of a translocation. Because of the recently recognized unbalanced nature of immunoglobulin translocations in MM it is desirable to have FISH probes that enable a double fusion strategy (60, 61).

The scoring criteria have been variable but a "cutoff" value of 10% abnormal cells has been used by most groups, and FISH is regarded as sensitive and highly specific for most assays. This number has been derived from the repeated observations that the mean plus three SDs toward the extremes when scoring FISH tests. This is, that once observers start seeing a common pattern for the FISH signal (normal or abnormal), they are more likely to confidently ascribe subsequent scored cells in the same diagnostic category. All FISH probes must be validated using positive and negative controls. By using FISH, a primary event should be detectable in nearly 100% of sorted cells.

Microarray Technology (RNA Gene Expression). The advent of gene expression profiling has revolutionized the study of genetic abnormalities associated with disease pathogenesis and clinical implications (62–65). Gene expression profiling has been used for the molecular classification of MM and to study its relation to preexisting benign disorders. Most investigators now use fluorescent or radioactive labeled cDNA or oligonucleotide arrays that enable the investigator to simultaneously detect thousands of genes (62–66). The study of gene expression profiling has initially focused on purified PC populations but is quickly expanding to obtain a complete picture of the bone marrow microenvironment (62–65). Recent studies have suggested that for some studies adequate RNA samples may be obtained from samples that are appropriately collected and shipped to reference laboratories (31). Gene expression profiling carries the possibility that many of the chromosome abnormalities currently detected only by other methodologies may also be detected using profiling methods. This will need validation by the many research centers engaged in the study of MM genetics. Although there currently are no specific clinical implications associated with the use of gene expression profiling, it is quite likely that, with additional follow-up information, specific prognostic features will be identified (62–65).

Aneuploidy

Prevalence. MM is characterized by the frequent occurrence of aneuploidy (1–3, 5, 6, 34, 35, 37, 38, 67). It was initially believed that trisomies were more common than monosomies in MM, but the opposite is true. The most common trisomies are 3, 5, 7, 9, 11, 15, 19, and 21. The most common monosomies are 13, 14, 16, and 22. No specific numerical chromosomal abnormality is constant or predictive of disease progression. The prevalence of aneuploidy is independent of stage (i.e., MGUS versus MM; Refs. 5, 21, 22, 36, 58, 68–70). Globally, aneuploidy analysis segregates patients into four subcategories: hypodiploid, pseudodiploid, hyperdiploid, and near tetraploid (the last also referred to as the hypotetraploid; Refs. 11, 12, 38, 71). The cutoff values for the different categories have varied but can include the following: hypodiploid up to 44–45 chromosomes, pseudodiploid 44/45 to 46/47 chromosomes, hyperdiploid greater than 46/47 chromosomes, and near tetraploid as 75 or more chromosomes (11, 12, 38, 71). With the DNA index as marker of ploidy, the following cutoff values have been used: hypodiploid <0.95, pseudodiploid 0.95 to 1.05, hyperdiploid >1.05, and near-tetraploid as close to 2.0 but most commonly as >1.75 (67, 72–74).

Hyperdiploid versus Nonhyperdiploid MM. A detailed analysis of numerical chromosome abnormalities in MM reveals specific patterns of association (11, 12, 38). The near-tetraploid appear to represent 4N duplications that are often present with cells having pseudo-
diploid or hypodiploid karyotypes (11, 12, 38). Because of this, the near tetraploid have been classified together with the hypodiploid and pseudodiploid MM as nonhyperdiploid MM (11, 12, 38). The nonhyperdiploid MM is characterized by a very high prevalence of IgH translocations (>85%) whereas IgH translocations are less common in the hyperdiploid MM (<30%); Refs. 38, 75, 76. The association is primarily evident for the three main recurrent IgH translocations (4p16.3, 11q13, and 16q23). Likewise, chromosome 13 monosity is more common in patients with nonhyperdiploid karyotype (38, 76). In addition to IgH rearrangements, the hyperdiploid MM harbor, in general, a lower prevalence of structural chromosome abnormalities (median, 4.7 breaks per karyotype as compared with 8.7 breaks per karyotype in the hypodiploid patients; Ref. 76).

What Occurs First: Aneuploidy or Translocations? It is not clear whether IgH translocations or aneuploidy occurs first in the PC neoplasms. Both genetic aberrations are seen in the very early stages of the PC disorders and no clear pattern is evident. The high prevalence of 13 among patients with the t(4;14)(p16.3;q32) and t(14;16)(q32;q23) suggests primacy for 13, but it is also possible that these IgH translocations allow 13 to be “tolerated” by the cell (4, 51).

Patterns of Involvement. The percentage of PCs harboring trisomies at any given time is variable and reflects ongoing genomic instability (5, 22, 58, 69). Several studies have shown this in MM, but additional work is needed for MGUS (5, 22, 58, 69). Preliminary evidence suggests that greater heterogeneity is seen in MGUS and may be reflective of less stability at this stage. Little information is available with serial testing of patients with MM or MGUS. However, two studies have shown the nonhyperdiploid and hyperdiploid categories are stable over time, although the various percentages of specific trisomies suggest that individual chromosomes may drift (67, 74).

Clinical Implications of Ploidy (Excluding 13). In some studies, the presence of certain trisomies (6, 9, and 17) was associated with an improved survival (77), likely because of their association with the hyperdiploid MM. Several groups have shown that ploidy category has a significant impact on the prognostication of patients. Hypodiploid MM is associated with a shorter survival (11, 38, 72, 78–80). Hypodiploidy has been found to supersede 13 as a prognostic marker in some studies but not in others (11, 38, 80). When DNA ploidy has been determined by DNA content, the prognostic implications are not seen because of the inability of the DNA index to discriminate hypodiploid MM from pseudodiploid MM, and in most cytogenetic studies, these two groups are pooled. Because the high-risk IgH translocations seem to be overrepresented in patients with hypodiploid MM, this may imply that some of the negative prognostic effect of hypodiploidy in MM may be caused by the presence of these adverse cytogenetic features (75, 76).

The finding of chromosome aberrations seen in myelodysplasia in MM samples, within the PC clone, has been found to be associated with a shorter survival. This is predominantly seen in patients with prior therapy and might be therapy-induced

Chromosome 13 Abnormalities (∆13)

Prevalence and Biology. 13 are highly prevalent in MM and other PC disorders (20, 81, 82). 13 were originally detected in ~50% of patients with abnormal karyotypes, so that it was detectable in 10–20% of all patients (37, 41, 42). The prevalence of 13 is the same when these abnormal karyotypes have been studied by multicolor metaphase FISH (e.g., SKY; Refs. 37, 41, 42). 13 were originally not described in MGUS or SMM, probably because of the difficulty in obtaining clonal metaphases.

With the use of interphase FISH, it is now clear that 13 will occur in all stages of the PC neoplasms including MGUS and SMM (7, 20, 83, 84). The reported prevalence in MM is 30–55% when interphase FISH has been applied (7, 20, 83, 84). The prevalence of 13 in MM reported by the different investigators is by necessity linked to the method of detection. Recent information suggests 13 are seen in 50% of patients (50).

Conflicting reports exist with regard to the actual prevalence of the abnormality in MGUS (7, 20, 57, 85). Some report a substantially lower incidence in MGUS as compared with MM (~25%; Ref. 20), whereas others show near identical prevalence (~50%; Refs. 7, 57). The former prevalence would imply that 13 may be involved in the progression of MGUS to MM, whereas the latter strongly suggests that 13 are initiation and not progression events. One group suggested that the presence of 13 in MM may be related to whether MM is de novo MGUS or associated with a transition from MGUS (29). Detailed studies to resolve these discrepancies are needed.

Area of Deletion. The minimal area of deletion has not been precisely mapped for chromosome 13 (52, 55, 86). In most cases these abnormalities present monosity of chromosome 13 (52, 55). However, available karyotypic data suggest that interstitial deletions, predominantly involving band 13q14 are also observed (1, 37, 42, 86). Two detailed FISH studies show that, in most cases (80–90%), interphase FISH-detected 13 is representative of chromosome 13 monosity, whereas the remaining 15% of cases have interstitial deletions (52, 55). Bi-allelic deletions seem to be rare. Most of these studies have not ruled out the possibility that other loci may be involved in deletions in MM, even bi-allelic ones.

Percentage of Abnormal Cells in Patients with 13 (Heterogeneity?). The percent of abnormal PCs with 13 when tested by FISH has shown various degrees of involvement according to the different reported studies (52, 55, 83, 86). In some studies, the median percentage of abnormal PCs has been close to 90%, whereas in some others it is closer to 75% (52, 55, 83, 86). It seems possible that these interstudy differences reflect technical limitations. In any case, this abnormality is present in most, perhaps all, of the tumor cells in most of the MM tumors having this abnormality. Nonetheless, it remains to be determined whether there are tumors with significant heterogeneity that reflects ongoing clonal evolution. Clearly, additional studies, including serial analyses, are needed to resolve this issue.

The situation is less clear in MGUS, in which the correct identification of the clonal PCs is more difficult than in MM because of the low fraction of PCs and the coexistence of the clonal tumor cells with normal polyclonal PCs (7, 20, 57). Probably because of technical reasons, the actual percentage of clonal PCs with 13 in MGUS is not clearly defined.

Is it an Important Chromosome or Is it a Marker? Several pieces of evidence suggest that 13 is not merely a marker of hypodiploid variant MM but that rather its loss confers unique biological features to the cells, supporting their clonal expansion. Some of the supporting facts include the following. Gene expression profiling of patients with and without 13 is associated with very specific patterns of genetic alterations (mostly down-regulation). At this time, 13 have been reported by several groups as recurrent and in similar proportions of patients (albeit with the aforementioned discrepancies to be resolved; Table 1 and Refs. 52, 55, 83, 86). Chromosome 13 is associated with very specific clinical implications (50, 83, 84, 87–89). Although still being resolved, it appears there is evidence for clonal selection of cells with 13. Lastly, chromosome 13 trisomy is exceedingly rare (1, 3, 42, 52, 55).

In contrast, other facts argue for the possibility that 13 may be merely a surrogate marker of hypodiploid MM. Hypodiploid MM and 13 are associated, but the associations are not absolute, and up to
Translocations Involving the Immunoglobulin Locus

Studies indicate that the prevalence of IgL-cells. There is less information for IgL translocations, but limited translocations when a sample contains only a low percentage of tumor valid despite the technical difficulties of accurately detecting IgH MGUS and SMM suggests that the result for MGUS is likely to be Refs. 4, 53, 95). The similar prevalence of IgH translocations in leukemia tumors, and nearly 90% of human MM cell lines (HMCLs; 70% of intramedullary MM tumors, 80% of primary PC leukemia. Two (sometimes even three or four) independent immunoglobulin translocations occur at a substantially higher prevalence in HMCLs (9). It is unclear to what extent the increased prevalence of single or multiple immunoglobulin translocations with disease stage reflects an accumulation of secondary immunoglobulin translocations during progression versus selective progression of tumors that have immunoglobulin translocations.

Clinical and Pathological Implications Including Prognosis. A seminal observation in MM genetics was that deletions/monosomy of chromosome 13 (Δ13) detected by karyotype were associated with a shorter survival (10, 87, 90). Independent of the mode of treatment (standard- versus high-dose chemotherapy) and the mode of detection (karyotype versus FISH), Δ13 are associated with shorter survival and lower response rates to treatment (13, 50, 77, 83, 84, 87, 88). The net effect of Δ13 on prognosis, when Δ13 is viewed as a prognostic factor, is greater when Δ13 is detected by karyotype than when it is detected by interphase FISH (13, 50, 77, 83, 84, 87, 88). This is because of the additive effects (to that already imposed by Δ13) on prognosis contributed by the observation of abnormal metaphases, which indicate a larger tumor burden and a more proliferative clone.

To determine the biological implications of a loss of chromosome 13, several groups have investigated the association of Δ13 detected by FISH and standard clinical parameters. Δ13 are more frequently associated with λ-type light-chain, higher proliferation (PC labeling index) and MM with lower serum monoclonal serum spike concentrations (50, 84, 91). They have also been associated with increased angiogenesis in some studies (92) but not in others (50).

The retrospective comparison between the data published by the Eastern Cooperative Oncology Group and the Intergroupe Francophone du Myelome suggest that the net benefit of high-dose chemotherapy is much greater for patients who do not have Δ13 (detected by FISH; Refs. 50, 84). In a single study, the administration of IFN-α to patients with Δ13 resulted in shorter survival (50).

Translocations Involving the Immunoglobulin Locus

Prevalence in MGUS, MM, and HMCLs. Translocations that involve both the immunoglobulin heavy-chain (IgH) and light-chain (IgL) genes have been implicated as seminal events in the pathogenesis of some MM (93, 94). Studies using FISH indicate that IgH translocations are detectable in nearly 50% of MGUS or SMM tumors, 55–70% of intramedullary MM tumors, 80% of primary PC leukemia tumors, and nearly 90% of human MM cell lines (HMCLs; Refs. 4, 53, 95). The similar prevalence of IgH translocations in MGUS and SMM suggests that the result for MGUS is likely to be valid despite the technical difficulties of accurately detecting IgH translocations when a sample contains only a low percentage of tumor cells. There is less information for IgL translocations, but limited studies indicate that the prevalence of IgL translocations is ~10% in MGUS/SMM and ~20% of advanced intramedullary MM tumors and HMCLs (7). Translocations involving the IgL-κ locus are quite rare, occurring in only a small percentage of intramedullary MM tumors. Two independent translocations that involve IgH and/or IgL loci seem to occur only infrequently in MGUS, SMM, or intramedullary MM tumors, but seem to be present in 5–10% of advanced MM tumors and primary PC leukemia. Two (sometimes even three or four) independent immunoglobulin translocations occur at a substantially higher prevalence in HMCLs (9). It is unclear to what extent the increased prevalence of single or multiple immunoglobulin translocations with disease stage reflects an accumulation of secondary immunoglobulin translocations during progression versus selective progression of tumors that have immunoglobulin translocations.

Diversity of Chromosomal Loci Involved in Immunoglobulin Translocations. Unlike other B-cell tumors, for MM there is a marked diversity of chromosomal loci involved in immunoglobulin translocations (Table 2). Apart from 8q24 (c-myc), each of four loci (oncogenes) are involved recurrently in 3% or more of MM tumors: 11q13 (cyclin D1), ~15%; 4p16 (FGFR3 and MMSET), ~15%; 16q23 (c-maf), ~6%; and 6p21 (cyclin D3), ~4%. Although the actual prevalence varies in different studies, together these loci are involved in immunoglobulin translocations in ~40% of MM tumors. Other recurrent loci, including 20q11 (mafB) and 6p25 (IRF-4/MUM-1), occur less frequently, and have not been well characterized in large studies. Approximately 20–30% of MM tumors have translocations that involve other chromosome partners that occur at a prevalence of 1% or less (4, 53). Whether these represent primary translocations, secondary translocations, or secondary rearrangements of primary translocations is unknown. It will be important to determine the nature of these chromosomal partners even though most will be nonrearranged. It is possible that the IgH locus, even after having sustained a translocation, remains fundamentally unstable and prone to undergoing additional chromosomal translocations.

IgH Translocations in MGUS. IgH translocations are early genetic lesions because the prevalence in MGUS is nearly as high as in MM (7, 20). The reported prevalence of the specific partners of IgH translocations in MGUS and SMM, as compared with MM, is still being elucidated (4, 53, 95). However, two principles seem apparent. First, it appears that all IgH partners detected in MM are also seen in MGUS. Second, the prevalence of specific partners may differ for MGUS and MM, although this is an issue that is not yet fully resolved (7, 53, 60, 91). The significance of these differences remains to be determined.

Primary versus Secondary Immunoglobulin Translocations. IgH translocations may be primary genetic events but some variants will likely be progression events (i.e., secondary translocations; Ref. 8). It has been proposed that most primary immunoglobulin translocations result from errors in B-cell-specific DNA modification processes, mostly IgH switch recombination or less often somatic hypermutation, and rarely, if ever, VDJ recombination. These translocations are then predicted mostly to have translocation breakpoints within, or very near, IgH switch or J regions. By contrast, secondary translo-
tions would not involve B-cell-specific DNA modification processes, which are not active in normal or tumor PCs. Translocations that involve c-myc provide a paradigm for secondary immunoglobulin translocations that occur late in pathogenesis (96, 97). In fact, the c-myc translocations (which need not actually involve an immunoglobulin locus) often include a number of the following distinguishing features: absence in MGUS, heterogeneity within the tumor cell population, karyotypic complexity manifested by unbalanced translocations or insertions, and translocation breakpoints that are not within or near IgH switch or JH regions. Although not yet documented, it remains possible that some secondary translocations may occur relatively early in pathogenesis and may, thus, be present homogeneously even within most MGUS tumors. The chromosomal structural properties, translocation breakpoint sites, and mechanisms responsible for involvement of the partner chromosomes in primary and secondary immunoglobulin translocations remain unknown. Specifically, the relation of these structural abnormalities with specific sites such as fragile sites and telomeric ends of chromosomes remains to be elucidated. Breakpoints at 16q23 appeared to be occurring in chromosomal fragile sites (FRA16D; Refs. 98, 99).

Biological Significance of IgH Translocations. IgH translocations form plausible pathogenic founder lesions in a multitude of other B-cell neoplasms (100, 101). Likewise IgH translocations are seen as likely culprits in the pathogenesis of some MMcs because (a) they are seen in most, if not all, clonal PCs in most MM and MGUS tumors; (b) they are recurrent and have been reported at a similar prevalence by several groups (4, 49, 51, 54); (c) they result in the juxtaposition of the IgH enhancers next to oncogenes (8); and (d) IgH translocations apparently are highly conserved during the process of disease evolution, although little information is available. They appear to extend over a long range. Oncogenes located hundreds of kilobases away from the enhancers have been found under the influence of cis transcriptional up-regulation (40). This is particularly evident in the case of the cell line KMS-11, in which cis up-regulation of c-maf is present, even when the enhancer is close to 1 Mb away from the maf gene (40). Because IgH enhancers are segregated into the two derivative chromosomes, one or more oncogenes in each derivative chromosome can be up-regulated (8).

Is There a Fundamental Difference for Tumors with and without Immunoglobulin Translocations? Despite the similar characteristics of MM tumors with and without immunoglobulin translocations, recent published and unpublished results now show a striking association of the three major recurrent IgH translocations (4p16, 11q13, and 16q23) and nonhyperdiploid MM (75, 76). The prevalence of these three recurrent translocations is low in hyperdiploid MM. By contrast, immunoglobulin translocations not involving these three recurrent partners occur with a similar, or even a somewhat higher, prevalence in hyperdiploid compared with nonhyperdiploid MM. This result implies two different mechanisms in the initial pathogenesis of the disease. For most nonhyperdiploid tumors, IgH translocations may represent the primary immortalizing event. By contrast, for most hyperdiploid tumors, it is assumed that there is some other, yet-to-be-defined immortalizing event, but possibly somehow related to the acquisition of hyperdiploidy per se (11, 38).

Ploidy Category and IgH Translocations in MM. In Fig. 1, two major groups of patients can be observed, but they have some overlapping features. The overlap is composed of patients with IgH translocations and variable ploidy. Given the strong association of the recurrent (primary) IgH translocations and the nonhyperdiploid variety, it is believed that this group is composed of patients with the non-recurrent IgH translocations (secondary).

IgH Translocations and Bone Marrow Microenvironment Independence. IgH translocations are highly prevalent in the human MM cell lines, because >90% of them harbor such abnormalities (8, 94). IgH translocations are likewise nearly universal in patients with primary PC leukemia (94, 102). The increased prevalence of IgH translocations in each case seems related to an increased prevalence of the t(11;14);t(4;14) and t(14;16) translocations. By contrast, the third of MM tumors that ectopically express cyclin D1 without a t(11;14)(q13;q32) are not represented by any HMCL. Virtually all HMCLs are generated from primary PC leukemia or intramedullary tumors that have progressed to an extramedullary phase. Together, these observations suggest that IgH translocations can provide a survival or proliferative effect that facilitates progression of MM to a stromal independent, extramedullary phase.

Mechanisms of Oncogene Action: A Unique Mechanism or an As-Yet-Undefined Common Mechanism? Although most translocations up-regulate different kinds of oncogenes, it is possible that all translocations converge in a pathway that ultimately leads to a block in differentiation, increased cell survival, and increased cell proliferation. There is no common mechanism apparent from the possible consequences of oncogenes up-regulated by the translocations. However, recent analyses derived from the gene expression profiling suggest that, in virtually all MM tumors, including those with or without an immunoglobulin translocation, at least one of the cyclin D genes is expressed at a high level compared with normal PCs (103). As a direct consequence of the immunoglobulin translocation, cyclin D1 mRNA is expressed at very high levels in association with t(11;14)(q13;q32), and cyclin D3 mRNA is expressed at very high levels in association with the t(6;14)(p21;q32) (104) As an indirect consequence of the immunoglobulin translocation, there is increased expression of cyclin D2 mRNA in tumors with either t(4;14)(p16.3;q32) or t(14;16)(q32;q23). In more than one-third of MM tumors, there is ectopic expression of cyclin D1 mRNA despite the apparent absence of a t(11;14)(q13;q32). Because cyclin D1 is not expressed in normal lymphoid cells, it is reasonable to conclude that there is dysregulation of cyclin D1 expression, although we do not understand the mechanism. Most of the remaining tumors express relatively high levels of cyclin D2 mRNA, and only a small percentage of tumors express no cyclin D1 and levels of cyclin D2 and/or cyclin D3 that are comparable with normal PCs. It seems likely that the latter three groups of tumors mostly are members of the hyperdiploid group of tumors.

Fig. 1. Ploidy category and IgH translocations in multiple myeloma (MM). MGUS, monoclonal gammopathy of undetermined significance.
**Cytogenetics versus RNA Arrays for Diagnosis of Immunoglobulin Translocations.** Traditional methods of detecting IgH translocations include a karyotype analysis for the t(11;14)(q13;q32) and interphase FISH methods for detecting t(4;14)(p16.3;q32) and t(14;16)(q32;q23) or reverse transcription-PCR (105). Recent experiments suggest that the majority of the recurrent IgH translocations will also be detectable by gene expression profiling (62), with spiked patterns of gene expression indicating the presence of a putative translocation. It is important to remember that a sizable fraction of IgH translocations (up to 50% but still being defined) may represent unbalanced IgH translocations. It is, therefore, imperative to consider spiked patterns of genes involved in the two derivative chromosomes so as not to miss the loss of a derivative chromosome (53, 60, 61).

**t(11;14)(q13;q32)**

**Biology and Prevalence.** The t(11;14)(q13;q32) results in ectopic expression of *cyclin D1* (106). The use of interphase FISH has now conclusively established the prevalence of the abnormality at 15–20% (4, 49). The t(11;14)(q13;q32) is readily detectable in the karyotype (in patients with informative karyotypes; Ref. 107), as well as by FISH and gene expression profiles (62). The t(11;14)(q13;q32) is also seen in MGUS (15–30%) and light-chain amyloidosis (<50%: 7, 20, 48, 108). The biological consequences of t(11;14)(q13;q32) remain unknown, although it has been shown that the MMs presenting this translocation are unexpectedly less proliferative than others. The slightly higher prevalence of the t(11;14)(q13;q32) in MGUS and light-chain as reported by some groups, but not others, would suggest its negative selection for evolution to MM (7, 48), but other data suggest no difference (20).

**Breakpoints and Mechanisms.** The breakpoints at 11q13 are scattered over at least 700 kb (109–113). In MM cell lines, high levels of *cyclin D1 cis* expression are tightly linked to the presence of t(11;14)(q13;q32), but positivity by immunohistochemistry may be detected in up to 30% of patients (112, 114, 115). Up to 30% of patients with the t(11;14)(q13;q32) may be negative for *cyclin D1* by immunohistochemistry, indicating the possible loss of the der11 (11, 14, 113). Similar observations have recently been made in patients with mantle cell lymphoma who lack expression of *cyclin D1* (by RNA analysis) despite having the molecular gene expression signature of the disease (114, 116). Although most IgH translocations in MM occur at the time of isotype switching, the t(11;14)(q13;q32) may involve nonswitch IgH loci and could be mediated via other B-cell-specific mechanisms such as somatic hypermutation, and may even be a secondary translocation in some cases (9).

**Clinical Implications.** Heterogeneous clinical implications for the t(11;14)(q13;q32) had been reported until the advent of interphase FISH (10, 107, 117). The t(11;14)(q13;q32) is associated with an improved survival, which is especially apparent in patients treated with high-dose chemotherapy and stem cell support (49, 91). There is an association of the t(11;14)(q13;q32) with oligosecretory variant MM, CD20 expression, and lymphoplasmacytic morphology (49, 91, 114, 118). It has been found in high prevalence in AL and IgM MM and nonspecific MM (48, 108, 119). Because of the overrepresenta-

**t(4;14)(p16.3;q32)**

**Biology and Prevalence.** The t(4;14)(p16.3;q32) is karyotypically cryptic and was first detected because of the cloning experiments in the human MM cell lines (39, 120). The translocation is seen in 15–20% of primary MM samples and in 25% of human MM cell lines (39, 51, 91, 120, 121). The t(4;14)(p16.3;q32) can be detected by interphase FISH or by reverse transcription-PCR detection of the IgH-MMSET hybrid transcript (39, 120). In a minority of human MM cell lines with t(4;14)(p16.3;q32), activating mutations of FGFR3, akin to those of thanatophoric dwarfism, are detected (122–124). The t(4;14)(p16.3;q32) has been detected in 10% of MGUS cases by some investigators (7, 105, 125), whereas others have failed to observe the abnormality in MGUS but did observe it among SMM patients (4, 60). Other investigators have noted the abnormality in patients (15%) with light-chain using the reverse transcription-PCR strategy (125). When described in MGUS, the abnormality has been found as insufficient for further progression to MM, because patients may stay in stable MGUS phase for years (7, 105).

In almost all patients with the t(4;14)(p16.3;q32), there is coexistence of Δ13, both in MM and MGUS (4, 51). It has been postulated that because of this strong association, at least in some cases, Δ13 precede the translocation. However, it is also possible that the t(4;14)(p16.3;q32) is permissive to the loss of chromosome 13, and that the t(4;14)(p16.3;q32) antedates Δ13.

**Breakpoints and Mechanisms.** The breakpoints at 4p16.3 are centromeric to FGFR3 and dispersed over 200 kb, usually within the 5′ introns of MMSET (39, 120). All of the cloned IgH translocation breakpoints for the t(4;14)(p16.3;q32) are into switch regions (9). In 25% of MM with a t(4;14)(p16.3;q32), the translocation may be unbalanced but apparently always at the expense of the loss of the der (14) or, in some cases, retention of der (14) but with loss of FGFR3 expression (60, 61). The consistent persistence of MMSET suggests that this may be the most important oncogene dysregulated by the t(4;14)(p16.3;q32), even though the role of MMSET deregulation remains unknown. On the other hand, there is no doubt that dysregulation of FGFR3 becomes important if it acquires an activating mutation late in tumorigenesis, and it is certainly possible that dysregulation of both FGFR3 and MMSET are important initiating events.

**Clinical Implications.** The t(4;14)(p16.3;q32) is an unfavorable prognostic factor for MM patients treated with either conventional or high-dose chemotherapy (53, 60, 91). There is no difference in survival between t(4;14)+ patients with or without FGFR3 overexpression (60). There is an association between the t(4;14)(p16.3;q32) and more aggressive clinical features, but also with an IgA isotype and λ-light chain use (53, 91). There are available compounds that may target the oncogenes involved in the IgH translocations in MM, and one such compound being tested is PD173074. This agent inhibits FGFR3 and is capable of blocking proliferation and inducing differentiation plus apoptosis in some human MM cell lines with the t(4;14)(p16.3;q32) that express FGFR3. The development of these agents will pave the way for true targeted therapy of MM, and indicate that additional avenues of therapy targeting MM IgH translocations should be explored.

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17. P. Leif Bergsagel, unpublished data.
t(14;16)(q32;q23) and Other maf Translocations

**Biology and Prevalence.** The t(14;16)(q32;q23) is detectable in 2–10% of patients and in about 25% of MM cell lines (4, 40, 53). *Cis* up-regulation of *maf* is transcriptionally up-regulated as a result of this translocation (40). The t(14;16)(q32;q23) is difficult to detect by conventional karyotypes but has been reported in 7% of MM patients with abnormal metaphases studied by SKY (41). Other studies have reported a very low prevalence of the abnormality in newly diagnosed patients. Although some of the differences in the reported prevalence exist and may be technical in nature, it is clear that, at least in a small fraction of patients, the abnormality is seen at the time of diagnosis. As is the case with the t(4;14)(p16.3;q32), the t(14;16)(q32;q23) has also been described in MGUS by some, whereas others have failed to observe it, and it is usually associated with Δ13 (7, 20).

Variant c-*maf* translocations also include the Ig*λ*-locus in the human MM cell line S226 (9, 40). In addition to c-*maf* a small fraction of patients and two human MM cell lines harbor translocations that involve b-*maf* (126).

**Clinical Implications.** In one series of patients the t(14;16)(q32; q23) was associated with a shorter survival among patients treated with conventional chemotherapy (53).

**Other IgH Translocations.** An array of other chromosomal partners has been detected in human MM cases (see above). One of these other partners is cyclin D3 [t(6;14)(p21;q32); Ref. 104]. This translocation, which is present in about 3–4% of MM tumors, is also detectable by interphase FISH, conventional cytogenetics, multicolor metaphase FISH, and gene expression profile (42). There is no known clinical or prognostic information for this translocation or as to whether it is also seen in MGUS. Another IgH translocation that involves chromosome 6 is the t(6;14)(p25;q32). It has been described in MM by some, whereas others have failed to observe it, and it is usually associated with Δ13 (7, 20).

**Ras Mutations.** Activating Ras mutations have been noted in 35–50% of MM (129, 130) patients, and a similar fraction of HMCLs (123). The prevalence of the mutations may increase somewhat with advancing stages of the disease (129, 131–134). Most mutations involve K- and N-ras at codons 12,13 and 61, but an atypical activating mutation of H-ras was identified in a fraction of cells from one HMCL (135). With rare exceptions, it appears that a mutation occurs in only one N- or K-ras allele in a single tumor cell, although there is some evidence that subpopulations of MM tumor cells within an individual can have different Ras mutations (129, 130). Tumors and HMCLs with t(4;14)(p16.3;q32) can have activating mutations of FGFR3 or Ras, but these events appear to be mutually exclusive, suggesting that activating mutations of Ras or FGFR3 have a similar effect (123). Ras mutations appear to be rare in MGUS, suggesting that this is a molecular marker if not causative in the progression from MGUS to MM for some tumors (130, 132). There is no available information regarding the prevalence of ras mutations in SMM. Mutations of K-ras, but not of N-ras, have been associated with shorter survival (129). Treatment strategies addressing the constitutive activation of ras are being explored.

**Inactivation of p53 (17p13).** p53 inactivation by either deletion or mutation seems to be a rare event in MM, and is restricted mostly to the late stages of disease progression (136, 137). Deletions of 17p13 are detectable in 10% of patients and are associated with a shorter survival (53, 136). Mutations of p53 have also been observed, with a prevalence of 5% at diagnosis, 20–40% in advanced MM or PC leukemia, and in >60% of HMCLs (138–141). Deletions of 17p13 seem to be rare in MGUS, and no systematic studies of p53 mutations have been done in MGUS (142). There has been no systematic study of other genes involved in the p53 pathway (p14/ARF, MDM2), although one study reported an increased level of expression of the p53 negative regulator MDM2 (143).

**Inactivation of Tumor Suppressors in the Rb Pathway.** An altered germ-line allele and subsequent inactivation of p16INK4a contribute significantly to the generation of plasma cell tumors in BALB/c mice (144–146). Notably, MM tumor cells from one individual retained a defective germ-line p16INK4a allele but lost the normal allele, suggesting that this gene can function as a tumor suppressor in MM (147). Methylation and presumptive inactivation of p16INK4a is detectable in up to 40% of human MM, and seems to be more prevalent with advancing stages of the disease (148–153). However, other reports show a similar prevalence of methylation in MGUS and in MM (154). Methylation of p16 seems to be much more common in extramedullary tumor and in HMCLs (149). In one study, the presence of p16 methylation was associated with aggressive clonal features, including a higher S phase and a shorter survival (153). The p18INK4c gene seems to be critical for enabling cessation of plasmablast proliferation and terminal differentiation to PCs (155). Bilallelic deletion of p18INK4c occurs in about 30% of HMCLs, but preliminary results suggest that bi-allelic deletion of this gene is much less frequent in intramenedullary MM (156). Inactivation of the RB gene by mutation or deletion seems to be a rare, late event in MM (157, 158).

**PTEN Mutations.** Inactivation of PTEN, which negatively regulates the phosphatidylinositol 3-kinase (PI3K)-mediated phosphorylation of AKT and BAD, inhibits apoptosis. Inactivating mutations of PTEN were identified in two of eight HMCLs, and transfection of normal PTEN into one of these HMCLs inhibited tumor formation in mice (159, 160).

**Myc.** Because of the universal presence of c-myc translocations involving IgH or IgL loci in the mouse plasmacytoma model, initially it was thought that c-myc translocations were likely to occur in human MM (161–166). However, careful analysis of abnormal karyotypes revealed that translocations involving c-myc and an immunoglobulin locus could be identified only in a small minority of patients (42). It is now known that complex abnormalities of c-myc correlate with advanced stages of the disease (96, 97). These complex karyotypic abnormalities of c-myc are present in about 15% of MM tumors by interphase FISH, and may be present in up to one-half of MM tumors that generate abnormal karyotypes, and >90% of HMCL, consistent with a late progression event. Some of the c-myc abnormalities are simple reciprocal translocations involving c-myc and immunoglobulin loci, most often IgH or IgL-λ. Other c-myc abnormalities, either with or without involvement of an immunoglobulin locus, involve complex translocations and insertions that are unbalanced, can involve more than two chromosomes, and can also include amplification, duplication, and inversion. Karyotypic abnormalities of L-myc (one HMCL) and N-myc (one MM tumor) have also been observed. Importantly, all informative HMCL express L-myc (U266 HMCL) or only one c-myc allele. In addition, although most MM tumors express c-myc, RNA microarray analyses show occasional tumors that express N-myc but no c-myc. Taken together, these data suggest the following hypothesis: (a) in MM and early stages of MM, there is bi-allelic expression of c-myc, which is regulated by transcription factors determined...
by intrinsic properties of the tumor cell and it responds to environmental factors such as interleukin 6; (b) secondary, complex translocations can dysregulate one allele of c- N-, or L-myc, and thereby contribute to more aggressive growth and perhaps stromal cell independence. The mono-allelic dysregulation of myc does not necessarily mean that the critical effect is higher levels of expression as opposed to unregulated expression, although this remains to be determined. The clinical and prognostic implications for myc abnormalities are unknown, but likely will be negative for overall survival, given that myc alterations are suspected of representing late progression events that are more frequent in the subset of tumors that generate abnormal karyotypes, have a higher PC labeling index, and a relatively poorer prognosis.

Conclusions and Recommendations

It is clear that, although we have learned much about the biology of MM, many questions remain. All of the available data suggest that IgH translocations are present in a majority (~50–60%) of tumors, yet are not sufficient to exert the full malignant potential of the clone. Although early dysregulation of cyclin D1, D2, or D3 may represent a unifying event, it seems likely that two distinct pathways exist in the pathogenesis of MM. One pathway involves an early IgH translocation that usually includes one of the four recurrent partners (11q13, 16q12, 6p21), and mostly is associated with a nonhyperdiploid chromosomal content. The second pathway infrequently, if ever, involves an early IgH translocation but mostly is associated with a hyperdiploid chromosomal content, perhaps a reflection of intrinsic genetic instability, although we have virtually no understanding of this pathway. The timing and nature of additional genetic events that are involved in early pathogenesis is unclear. In addition, apart from a possible role for ras mutations, the molecular events required for the transformation from MGUS to MM are also unclear. Finally, although a number of other abnormalities involving c-myc, p53, PTEN, and various components of the Rb pathway have been identified in some tumors, we still do not have a full picture of when these progression events occur, as well as what other kinds of genetic and epigenetic events occur during progression.

In addition to the aforementioned areas in need of further studies as highlighted above the committee made the following recommendations:

(a) a global integration of the existing genetic, epigenetic, cytogenetic, and gene expression data are needed to formulate a coherent model of disease initiation and progression;

(b) the generation of a comprehensive database that contains all of this information is highly desirable; the database should be readily accessible to all investigators with as much pertinent information as possible.

(c) the experimental study of MM biology needs to carefully consider that currently available human MM cell lines are derived from the subset of patients with nonhyperdiploid MM and high incidence of IgH translocations. There is a need to develop human MM cell lines representative of patients without IgH translocations. One such mechanism may be the perpetuation of cells in animal MM cell lines representative of patients without IgH translocations. Incidence of IgH translocations. There is a need to develop human from the subset of patients with nonhyperdiploid MM and high

References


