

Pathogenesis of Monoclonal Gammopathy of Undetermined Significance and Progression to Multiple Myeloma

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Monoclonal gammopathy of undetermined significance (MGUS), including immunoglobulin light chain only MGUS, is an age-dependent premalignant tumor that is present in about 4% of Caucasian individuals over the age of 50 years. It is comprised of two different kinds of tumors: about 15% lymphoid or lymphoplasmacytoid MGUS and the remainder plasma cell MGUS. Plasma cell MGUS is stable but can sporadically progress to multiple myeloma (MM) at an average rate of about 1% per year. Most, if not all, MM tumors are preceded by plasma cell MGUS, which shares four partially overlapping oncogenic features with MM. It presently is not possible to unequivocally distinguish an MGUS tumor cell from an MM tumor cell. However, two models based on clinical laboratory tests indicate that it is possible to stratify MGUS tumors into groups that have average rates of progression as low as 0.26% per year and as high as 12% per year. *Semin Hematol* 48:4–12. Published by Elsevier Inc.

Multiple myeloma (MM), the second most common hematological malignancy in the United States, presently is a mostly incurable disease, with an incidence of about 20,000 cases per year and an overall median survival of 4 to 7 years.¹ It usually is characterized by accumulation of malignant plasma cells throughout the bone marrow compartment, and is associated with the presence of secondary clinical manifestations, including skeletal lytic lesions, anemia, immunodeficiency, renal failure, and hypercalcemia.

Plasma cell monoclonal gammopathy of undetermined significance (MGUS) and smoldering multiple myeloma (SMM) are premalignant precursor tumors of MM that are stable and not associated with the presence of the secondary clinical manifestations listed above.^{2,3} They are derived from long-lived, post-germinal center B cells, which are activated B cells that have undergone several rounds of somatic hypermutation and antigen selection in germinal centers, and then immunoglobulin heavy chain (IgH) switch recombina-

tion before differentiating into plasmablasts. Plasmablasts from the germinal center typically migrate back to the bone marrow where they become terminally differentiated long-lived plasma cells.^{4–6} In fact, MGUS, SMM, and MM are monoclonal tumors that retain many of the phenotypic properties of healthy plasmablasts/plasma cells. However, in contrast to their normal counterpart they maintain low proliferation rates that can increase markedly in late stages of MM.

MGUS is present in about 4% of Caucasians over the age of 50 years, with a 1% average annual risk of progression to malignant MM.^{7–9} Typically, MGUS is asymptomatic apart from infrequent patients that develop primary amyloidosis as a result of the pathological accumulation of monoclonal immunoglobulin (M-Ig), ie, mostly Ig light chains, in various organs.¹⁰ Lymphoplasmacytoid or lymphoid MGUS tumors, comprising about 10% to 15% of patients with MGUS, usually secrete IgM, and can progress to lymphoma or Waldenström's macroglobulinemia.¹¹ The remaining plasma cell MGUS tumors only rarely (~1%) secrete a monoclonal IgM but instead usually secrete an intact non-IgM M-Ig (IgG>IgA> IgD>IgE). The plasma cell MGUS tumors that express an intact Ig previously have been shown to progress to MM or related plasma cell tumors that express an intact Ig with the same clonotype and same isotype.¹¹ Until recently, it was unclear if the approximately 20% of MM tumors that secrete only Ig light chain are generated from MGUS tumors that express intact Ig but lose IgH expression during progression from MGUS to MM, or from MGUS tumors

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that have an Ig light chain only phenotype. However, a recent study used a combination of an abnormal serum-free light chain ratio, an increased concentration of the relevant serum-free light chain, and absence of IgH by immunofixation to identify Ig light chain only MGUS tumors that secrete a monoclonal kappa or lambda Ig light chain but no IgH.⁹ The prevalence of the light chain only MGUS tumors is 0.8%, ie, about 20% of MGUS tumors, suggesting that light chain only MM is generated from light chain only MGUS, a result that has been confirmed in some studies.¹² Throughout the remainder of this review, the term MGUS will be used to denote plasma cell MGUS.

The prevalence of both MGUS and MM increases with age, and is two- to threefold higher in African-Americans than Caucasians, whereas there is no difference in the frequency of progression to MM in these two populations of MGUS patients.¹³ There is also an increased prevalence of MGUS and MM in other family members when an individual has been diagnosed with either of these tumors.¹⁴ Two recent studies have confirmed that almost all cases of MM are preceded by MGUS.^{12,15} The prospective PLCO (Prostate, Lung, Colorectal, and Ovarian) Cancer Screening Trial, which banked serum samples, identified 71 MM patients with a median age of 70 years, 4% of whom were African-Americans; more than 95% of these MM patients had MGUS 2 or more years prior to the diagnosis of MM.¹⁵ The second study analyzed 30 MM patients treated at Walter Reed Army Medical Center using sera stored by the Department of Defense Serum Repository years before any clinical signs of MM. The median age of patients was 48 years and 47% were African-Americans. The results from this study demonstrated the presence of the MGUS at least two years prior to the diagnosis of MM in 27/30 patients (90%).¹² Of the three patients without demonstrable MGUS diagnosis, two had IgD MM (characterized by the frequent lack of an M-component; the rate of catabolism of IgD is 26% per day, compared with 3%–6% per day for IgG, and 10%–15% per day for IgA), and one patient had only a single serum sample available 9 years prior to diagnosis of MM. Thus, in the military study, all cases of non-IgD MM with serum available within 4 years of diagnosis had evidence of prior MGUS.

In contrast to MGUS, SMM is much more likely to progress to MM, with an average rate of progression of about 10% per year.^{16,17} Based on the diagnostic criteria developed by the International Myeloma Workshop to differentiate monoclonal gammopathies, plasma cell MGUS is defined by a serum M-Ig concentration lower than 3 g/dL, plasma cells less than 10% of the mononuclear cells in the bone marrow, and absence of end-organ damage (excepting amyloidosis). SMM is defined as either M-protein greater than 3 g/dL and/or plasma cells greater than 10% of bone marrow mononuclear cells but also in the absence of end-organ damage.¹⁸ It

seems likely that SMM is not a single biological entity but rather a spectrum of different stages of disease, including at least three distinct possibilities: (1) MGUS with an increased but stable number of tumor cells; (2) minimally progressive MM without end-organ damage; or (3) moderately progressive MM but with end-organ damage that is not yet detectable.¹⁹ Depending on whether one looks from a perspective of symptomatology, molecular profiles, or fluorescence in situ hybridization (FISH) analyses, SMM overlaps with MGUS and early MM.

Again, the above definitions of MGUS and SMM can include primary amyloidosis, which typically is an MGUS—or perhaps sometimes a SMM—tumor that is associated with deposition of M-Ig light chain in various tissues, often despite a barely detectable M-Ig and the presence of a very small number of bone marrow tumor cells.¹⁰

MGUS/MM CELLS DISTINGUISHED FROM PLASMA CELLS BUT NOT EACH OTHER

Immunophenotypic analyses can distinguish healthy plasma cells (CD138⁺CD19⁺CD45⁺CD56⁺) from MGUS, SMM, and MM tumor cells, which are CD138⁺, but usually have an abnormal immunophenotype that can include any or all of the following: CD19⁺, CD45⁺, CD56⁺.^{20,21} However, there are no immunophenotypic markers that can distinguish these different kinds of tumor cells.

Initial studies in which gene expression profiles were obtained on purified CD138⁺ MM, MGUS, and healthy plasma cells showed that the expression patterns distinguished MM and MGUS tumor cells from healthy plasma cells but not from one another.²² However, unlike MGUS tumors, a small fraction of MM tumors had gene expression profiles that resulted in their clustering with MM cell lines that are generated mostly from plasma cell leukemia or an advanced, extramedullary stage of MM. In a subsequent gene expression profile study of CD138-selected cells derived from 351 patients with MM, 44 with MGUS or SMM, and 20 healthy donors, 52 differentially expressed genes were identified. Again, MM and MGUS/SMM could be distinguished from healthy plasma cells but not from one another.²³ However, when these and additional samples were analyzed and clustered, they identified four groups of samples: MGUS-like MM, comprising about one quarter of MM tumors; non-MGUS-like MM; MGUS-like MGUS; and non-MGUS-like MGUS (~20% of MGUS tumors).²⁴ The MGUS-like MM signature was associated with favorable clinical features and longer survival than a non-MGUS-like MM signature. They speculated that non-MGUS-like MGUS would be more likely to progress than MGUS-like MGUS, but there were not enough data to test this hypothesis. Curiously, these four groups were not reproduced by others using the same data set.²⁵ In any case, it seems clear that with the very large number of MM

tumors but only a limited number of MGUS tumors that have been analyzed, there is a substantial overlap, so that presently it is not possible to distinguish MGUS from MM by gene expression profile.

Most recently, microRNA (miRNA) expression was determined in 49 MM cell lines, and CD138-selected plasma cells from 16 MM tumors, six MGUS tumors, and six healthy individuals. Several miRNAs were overexpressed in the MM cell lines, and also in the MGUS and MM tumors compared to healthy plasma cells. Other miRNAs were overexpressed in the MM cell lines and MM tumors but not in MGUS tumors.²⁶⁻²⁸ It is clear that many more samples will need to be analyzed before we can conclude that miRNA expression patterns can effectively distinguish MGUS and MM tumors.

There are many studies on the analysis of methylation of specific genes in MGUS and MM. In general, the frequency of methylation of specific genes is highest in MM cell lines and extramedullary MM tumors/plasma cell leukemia, lower in MM, and lowest in MGUS. However, these are mostly quantitative differences that are not sufficient for determining if individual tumor samples are MGUS or MM. Moreover, this approach has not been able to convincingly identify genes for which expression is altered by methylation, and that then contribute specifically to the pathogenesis of MGUS/MM.^{29,30} The difficulty of drawing clear

conclusions from this approach is exemplified by studies on methylation of p16INK4a, for which methylation is present in 20% to 30% of both MGUS and MM tumors, and more than 90% of extramedullary MM tumors and MM cell lines. Yet p16INK4a does not appear to be expressed at significant levels in most MM tumors regardless of whether or not the gene is methylated, which suggests that methylation of p16INK4a is likely to be an epiphenomenon that does not determine expression.³¹⁻³⁵

RISK STRATIFICATION MODELS TO PREDICT TUMOR PROGRESSION

The lack of unequivocal genetic or phenotypic markers to distinguish MGUS from SMM or MM tumor makes it difficult to predict if and when an MGUS tumor will progress to MM. However, by univariate analysis, several clinical parameters are associated with an increased rate of progression of MGUS to MM: increased M-Ig levels, increased bone marrow plasmacytosis, an abnormal free light chain ratio, non-IgG isotype, immunoparesis, abnormal plasma cells greater than 95%, and aneuploidy detected by flow cytometric analysis of DNA content. By multivariate analysis using combinations of these parameters, two models of risk stratification for progression of MGUS or SMM have been proposed (Table 1).

Table 1. Progression of MGUS and SMM to Symptomatic MM

	% per Year*	No. of Risk Factors	No. of Patients (%)	Overall % per Year†
Spanish cohorts and stratification				
%/5 years ³⁷				
2	0.40	MGUS.0	127 (46.0)	MGUS, 1.91%
10	2.11	MGUS.1	133 (48.2)	
46	12.32	MGUS.2	16 (5.8)	
4	0.82	SMM.0	28 (26.4)	SMM, 14.12%
46	12.32	SMM.1	39 (36.8)	
72	25.46	SMM.2	39 (36.8)	
Mayo cohorts and stratification				
%/20 years ^{16,36}				
5	0.26	MGUS.0	449 (39.1)	MGUS, 1.15%
21	1.18	MGUS.1	420 (36.6)	
37	2.31	MGUS.2	226 (19.7)	
58	4.34	MGUS.3	53 (4.6)	
%/5 years ^{16,36}				
25	5.75	SMM.1	82 (30.0)	SMM, 15.71%
51	14.27	SMM.2	115 (42.0)	
76	28.54	SMM.3	76 (28.0)	

*Assumes that progression rate is stochastic and constant so that $\ln(X_0/X_t) = kt$, but for the Mayo SMM cohort the rate of progression decreases with time.

†Overall rate of progression of all MGUS or SMM patients in each study is estimated from the original data. The apparently different overall rates of progression of MGUS patients in the two studies should be noted.

The first model, proposed by a group at the Mayo Clinic, is based on abnormalities of the serum M-Ig.^{16,36} Three risk factors are considered in the progression of MGUS to MM: (1) serum level of M-Ig greater than 1.5 g/dL; (2) abnormal free light chain ratio (<0.26 or >1.65); and (3) non-IgG M-Ig isotype. In a 20-year follow-up study, the absolute risks of progression for MGUS patients with 0, 1, 2, or 3 of these factors were 5%, 21%, 37%, and 58%, respectively. For patients with SMM the three risk factors are: (1) M-Ig level greater than 3 g/dL; (2) bone marrow plasma cells greater than 10%; and (3) abnormal free light chain ratio (<0.125 or >8), with either of the first two risk factors being required for a diagnosis of SMM. At 5 years from the diagnosis the risks of progression in patients with 1, 2, or 3 risk factors were 25%, 51%, and 76%, respectively.^{9,16,36}

The second model, proposed by a Spanish group, introduces novel prognostic criteria for MGUS and SMM based on the flow cytometric immunophenotypic profile of bone marrow plasma cells.^{37,38} This includes (1) abnormal plasma cells greater than 95%, and (2) DNA aneuploidy for MGUS, so that patients with 0, 1, or 2 risk factors have a risk of progression at 5 years of 2%, 10%, and 46%, respectively. For SMM patients the risk factors are greater than 95% abnormal plasma cells and immunoparesis of one or more polyclonal Igs. Patients with a score of 0, 1, or 2 have, respectively, a 4%, 46% and 72% probability to progress to MM in 5 years.⁷ Table 1 summarizes the results for these two

models, and includes an approximate yearly rate of progression for easier comparison of the four groups. The two models demonstrate that MGUS and SMM patients can be stratified into different risk subgroups that partially overlap, indicating the need for the development of better diagnostic and/or risk stratification criteria.

Both models apply only to conventional MGUS tumors that express an intact Ig since light chain only MGUS had not been well documented at the time these models were developed. However, the light chain only MGUS tumors fall mostly into the lowest risk group since their overall rate of progression to MM was found to be about 0.3% per year.⁹

MOLECULAR GENETIC EVENTS SHARED BY MGUS AND MM TUMORS

There are four very early and partially overlapping molecular pathogenic events that are shared by MGUS and MM tumors: IgH translocations, hyperdiploidy, chromosome 13 deletion, and dysregulation of a *CYCLIN D* gene (Figure 1).^{25,39-42}

Interphase FISH analyses have identified IgH translocations in about 40% to 50% of MGUS tumors, 50% to 70% of MM tumors, 80% to 85% of plasma cell leukemia tumors, and 90% of MM cell lines.^{40,43} There appear to be two types of IgH translocations: primary and secondary.^{25,44,45} Primary IgH translocations generally have

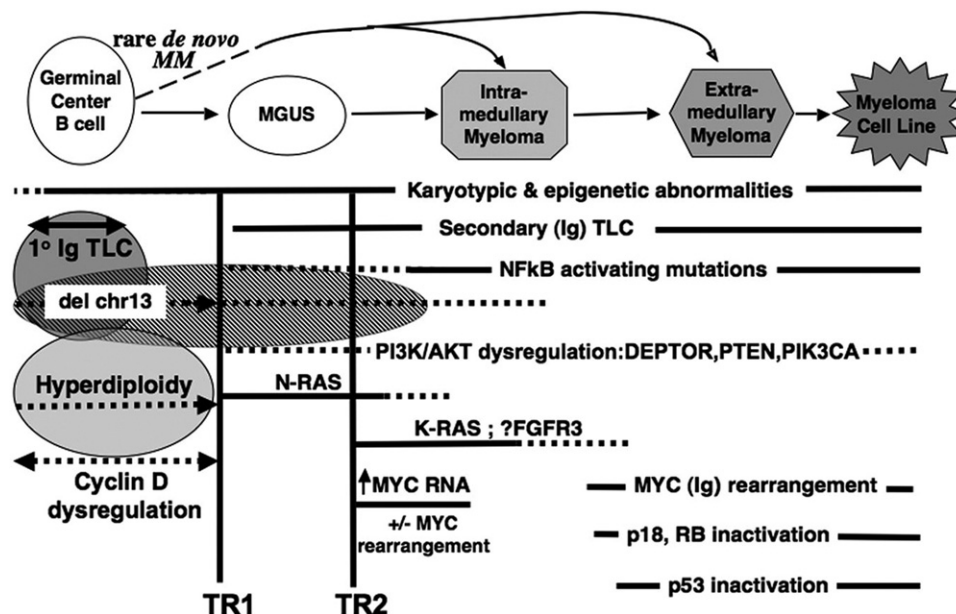


Figure 1. Molecular pathogenesis of MGUS and multiple myeloma. Four early and partially overlapping events are shared by MGUS and MM tumors, but it is not clear what other events are necessary for the transition (TR1) to a pre-malignant MGUS tumor. Some events (eg, karyotypic abnormalities) probably can occur at any stage of pathogenesis, whereas other events (eg, *p53* inactivation) might occur mainly at late stages of tumor progression. Two events (deletion chr13 and activating *K-RAS* mutations) may be associated with the transition (TR2) from MGUS to MM for some tumors, whereas a third event (increased *MYC* expression and sometimes *MYC* locus rearrangements) may be more universally involved in this transition. Note that *MYC*(Ig) rearrangements can also occur as a late progression event. See text for additional details.

the following characteristics: (1) for any given tumor they are present in all MGUS or MM tumor cells; (2) they are simple reciprocal translocations, although sometimes one of the derivative chromosomes is not present, eg, der (14) in some MM tumors with t(4;14) (see below); (3) the translocation breakpoints mainly occur near or within IgH switch regions and less often near or within JH regions, suggesting errors, respectively, in switch recombination and somatic hypermutation as B cells pass through the GC; (4) translocation breakpoints in JH regions result in cosegregation of the intronic $E\mu$ and two 3' IgH enhancers to der (14), whereas breakpoints in switch regions dissociate the intronic $E\mu$ enhancer from one or both 3' IgH enhancers so that an oncogene can be dysregulated on either derivative chromosome, eg, MMSET by $E\mu$ on der (4) and FGFR3 by a 3' IgH enhancer on der (14); and (5) oncogenes can be dysregulated even if located 1 Mb or more distant from the 3' IgH enhancer. Presently, there are seven recurrent chromosomal loci (oncogenes) that appear to be involved in primary IgH translocations, all of which have been found in MM, and (excluding 12p13 and 8q24.3) in MGUS tumors. They comprise three primary translocation groups (with approximate prevalence cited for MM)^{24,39,40,46}: *CYCLIN D*: 11q13 (*CYCLIN D1*), 15%; 12p13 (*CYCLIN D2*), <1%; 6p21 (*CYCLIN D3*), 2%; *MAF*: 16q23 (*MAF*), 5%; 20q12 (*MAFB*), 2%; 8q24.3 (*MAFA*), <1%; and *MMSET/FGFR3*: 4p16 (*MMSET* and *FGFR3*), 15% [loss of der (14)(*FGFR3*) in 20%].

The prevalence of the t(4;14) translocation is substantially lower (~3%–4%) in MGUS tumors but has a similar prevalence in SMM and MM, which suggests the unproven possibility that this translocation results in a higher rate of progression of MGUS to SMM or MM.^{39,43} It also has been shown that there is a marked increased prevalence of the t(11;14) translocation in Ig light chain only MM (~30%), and also in primary amyloidosis (30%–55% in different studies) that mostly secretes only a M-Ig light chain; currently there is no satisfactory explanation for this observation.^{43,47–50} Rarely, translocations from two of these groups (all combinations) are observed in the same tumor, with one of the translocations representing a secondary translocation that is found in only a subset of tumor cells.⁵¹

Secondary IgH translocations have the following characteristics: (1) sometimes present in only a subset of MGUS or MM tumor cells; (2) complex rearrangements that can involve three different chromosomes or insertions, both of which can also have associated duplication, inversions, deletions, etc; and (3) breakpoints generally do not involve IgH switch regions or JH regions, consistent with the apparent lack of switch recombination or somatic hypermutation in plasma cells and plasma cell tumors. At least for MM cell lines, for which secondary IgH translocations have been best characterized, it appears that most IgH translocations

involving *MYC* or a non-recurrent partner are secondary translocations, although rarely one of the recurrent translocation partners can be involved in a secondary translocation (see above).⁵¹ In addition, it has been proposed that translocations involving kappa (2p11) or lambda (22q11) light chain Ig loci and most *MYC* translocations are secondary translocation events. The involvement of *MYC* in IgH translocations occurs in about 3% of newly diagnosed MM tumors but has not been detected in MGUS or SMM.⁵² It is curious that the prevalence of IgH translocations not involving one of the recurrent partners appears to be approximately 10% in MGUS and about 15% in MM, which seems somewhat surprising if these are secondary translocations.³⁹ Clearly, it is unfortunate that we do not know more about the structures and chromosomal partners involved in IgH translocations not involving the seven recurrent partners in both MGUS and SMM, but at present it seems reasonable to believe that these are secondary translocations.

It appears that the pathogenesis of plasma cell MGUS and MM involves two pathways defined by chromosome content: hyperdiploid and non-hyperdiploid.^{53–55} The hyperdiploid tumors are characterized by the presence of 48 to 75 (mostly 49–58) chromosomes, with extra copies (usually trisomies but sometimes tetrasomies) of two or more of eight odd-numbered chromosomes: 3,5,7,9,11,15,19, and 21. The non-hyperdiploid tumors are characterized by the presence of less than 48 and/or greater than 75 chromosomes, including hypodiploid, pseudodiploid, and subtetraploid tumors. Currently, several groups have developed interphase FISH assays that measure the number of copies of three of the odd-numbered chromosomes, and have shown that extra copies of two of the three chromosomes provides a reliable method of detecting hyperdiploid tumors.^{39,56} The prevalence of hyperdiploid tumors is about 50% to 60% in MM and SMM but lower in MGUS (reported as 35%–42% but possibly 47% if one excludes MGUS tumors that had no FISH abnormalities and could represent inadequate samples that contain only healthy plasma cells).³⁹ Primary IgH translocations are present in 60% to 70% of non-hyperdiploid MM tumors but only about 15% of hyperdiploid MM tumors.^{53–55} A recent study shows that the five most common primary translocations were strongly associated with a non-hyperdiploid genotype, ie, 94%, 83%, and 73%, respectively, of MGUS, SMM, and MM tumors.³⁹ The different results for MGUS versus MM tumors might be that non-hyperdiploid MGUS tumors occasionally become hyperdiploid MM tumors, which could explain the lower prevalence of hyperdiploid tumors in MGUS, and the increased likelihood that primary IgH translocations are found in hyperdiploid tumors in MM compared to MGUS. In contrast to primary IgH translocations, it appears that secondary translocations have a similar prevalence in hyperdip-

loid and non-hyperdiploid tumors, consistent with the hypothesis that these translocations are progression events for both types of tumors.^{39,51}

Deletion of chromosome 13—usually the entire chromosome—has been reported to be present in about 25% to 50% of MGUS tumors by different groups, whereas all groups agree that this abnormality is present in approximately 50% of MM tumors.^{40,43,57} However, a recent study on a large number of MGUS (n = 189), SMM (n = 127), and MM (n = 400) tumors showed that the respective prevalences of chromosome 13 deletion were 25%, 34%, and 47%. Moreover, they showed that there was a high and similar prevalence of chromosome 13 deletion affecting virtually all cells in MGUS, SMM, and MM tumors with t(4;14) (~90%) and t(14;16) (~70%).^{39,40} By contrast, the prevalence of chromosome 13 deletion was less than 5%, approximately 15%, and approximately 40%, respectively, in MGUS, SMM, and MM tumors that had t(11;14) translocations.³⁹ Significantly, for t(11;14) tumors, they found that often only a subset of cells within a tumor had deleted one copy of chromosome 13. Therefore they concluded that chromosome 13 deletion is an early event in tumors with t(4;14) and t(14;16) but a progression event that occurs at or beyond the MGUS to MM transition in tumors with t(11;14) or t(6;14). Unfortunately, we still do not understand what gene or genes are targeted by chromosome 13 deletion, even though there has been continuing speculation but no convincing evidence that RB-1 haploinsufficiency may be involved.

Dysregulation of a *CYCLIN D* gene seems to be a unifying event for virtually all MGUS and MM tumors.^{25,42} In some cases this is caused directly (CYCLIN D group) or indirectly (MAF group since the MAF transcription factors target CYCLIN D2) by a primary IgH translocation. The mechanisms responsible for *CYCLIN D2* dysregulation with the t(4;14), or *CYCLIN D1* and/or *CYCLIN D2* dysregulation in hyperdiploid tumors has yet to be elucidated. Strikingly, a substantial fraction of the few percent of tumors that do not have increased expression of a *CYCLIN D* gene have inactivated RB-1, which should eliminate the need to express a *CYCLIN D* gene and further supports the hypothesis that disruption of the CYCLIN D/RB-1 pathway is a critical early event in the pathogenesis of MGUS and MM. Given that MGUS and MM tumors have a very low proliferation index, it is clear that dysregulation of a *CYCLIN D* gene cannot by itself drive proliferation but may render these tumor cells more susceptible to proliferative stimuli.

MOLECULAR GENETIC PROGRESSION EVENTS AT THE MGUS TO MM TRANSITION

Many somatic genetic abnormalities that are thought to represent progression events have been identified in

MM tumors (Figure 1).^{25,40} The precise timing and prevalence of these abnormalities at different stages of disease are not well understood. However, some of these abnormalities, eg, p18INK4c bi-allelic deletion, *p53* inactivation, and *MYC* rearrangement appear to represent late progression events associated with increased proliferation and decreased bone marrow stromal cell dependence in advanced MM tumors. Other abnormalities, eg, nuclear factor κ B (NF- κ B) mutations,^{58,59} secondary (Ig) translocations, and karyotypic abnormalities might occur at any stage of tumorigenesis. We do not know what somatic genetic abnormalities might contribute to the progression from MGUS to MM, but we will consider two specific possibilities in addition to the acquisition of chromosome 13 deletion in t(11;14) and t(6;14) tumors (see above).

First, it is significant that the prevalence of K- or N-RAS-activating mutations is approximately 15% each in MM tumors, whereas the prevalence of N-RAS mutations is 7% in MGUS tumors and K-RAS mutations have not been identified in MGUS tumors.^{60–62} Although K- and N-RAS-activating mutations were thought to have the same roles in tumorigenesis, it is increasingly apparent that K- and N-RAS-activating mutations are not equivalent.⁶³ In any case, although more data are needed for MGUS and SMM tumors, it seems possible that the acquisition of K-RAS mutations might be not only an early marker of MM but also a cause of the MGUS to MM transition for some MM tumors.

Second, it recently has become apparent that *MYC* expression is significantly increased in MM tumors compared to MGUS tumors. Importantly, a murine model using MGUS-prone mice generates MM tumors that are good phenocopies of human MM tumors when these mice contain a *MYC* transgene that can be activated by somatic hypermutation as B cells pass through a germinal center.⁶⁴ Therefore, this model supports the hypothesis that increased and dysregulated expression of *MYC* might be sufficient for the MGUS to MM transition. Previous results suggested that *MYC* rearrangements, which mostly are complex translocations and insertions that often, but not always, involve juxtaposition of *MYC* near an Ig enhancer, are a late progression event that is found rarely in MGUS or SMM tumors, but it is present in 15% of newly diagnosed MM tumors, nearly 50% of advanced MM tumors, and nearly 90% of MM cell lines.^{52,65} Given the new evidence cited above, it now appears that increased expression/dysregulation of *MYC* is important both for the MGUS to MM transition but also for late stages of tumor progression (Figure 1). The mechanism(s) responsible for early *MYC* dysregulation, and the relationship of early versus late *MYC* dysregulation remain to be clarified.

CONCLUSION

Despite the many advances that have been made in our understanding of the pathogenesis and biology of

MGUS, SMM, and MM, some of the many questions that remain to be answered include the following. First, is the progression of MGUS to SMM or MM mediated by the acquisition of somatic genetic abnormalities in the tumor cells and/or by non-tumor cell changes, eg, the bone marrow microenvironment or host immune function? Second, are there phenotypic or genetic markers that can distinguish MGUS and SMM tumor cells from each other and from MM tumor cells? Third, what determines the phenotype and progression of SMM tumors? Fourth, can we develop better stratification models to predict the probability that a given MGUS or SMM will progress to MM? Fifth, is progression to MM retarded by the presence of MGUS or SMM tumor cells that compete with the malignant clone, so that elimination of the premalignant cells can increase the rate at which the malignant cells expand? Finally, for an MGUS or SMM tumor that has a high probability of progression to MM, can we identify effective treatment protocols that will eliminate the tumor or significantly delay/prevent progression to MM?

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