Published OnlineFirst April 27, 2018; DOI: 10.1158/0008-5472.CAN-17-3115

Review

Cancer Research

From MGUS to Multiple Myeloma, a Paradigm for Clonal Evolution of Premalignant Cells

Niels van Nieuwenhuijzen[1,2], Ingrid Spaan[1], Reinier Raymakers[2], and Victor Peperzak[1]

Abstract

Multiple myeloma (MM) is a treatable, but incurable, malignancy of plasma cells (PC) in the bone marrow (BM). It represents the final stage in a continuum of PC dyscrasias and is consistently preceded by a premalignant phase termed monoclonal gammopathy of undetermined significance (MGUS). The existence of this well-defined premalignant phase provides the opportunity to study clonal evolution of a premalignant condition into overt cancer. Unraveling the mechanisms of malignant transformation of PC could enable early identification of MGUS patients at high risk of progression and may point to novel therapeutic targets, thereby possibly delaying or preventing malignant transformation. The MGUS-to-MM progression requires multiple genomic events and the establishment of a permissive BM microenvironment, although it is generally not clear if the various microenvironmental events are causes or consequences of disease progression. Advances in gene-sequencing techniques and the use of serial paired analyses have allowed for a more specific identification of driver lesions. The challenge in cancer biology is to identify and target those lesions that confer selective advantage and thereby drive evolution of a premalignant clone. Here, we review recent advances in the understanding of malignant transformation of MGUS to MM. *Cancer Res; 78(10); 2449–56. ©2018 AACR.*

Introduction

Multiple myeloma (MM) is a malignant growth of clonal plasma cells (PC) primarily located in the bone marrow (BM) and is the second most common hematologic malignancy (1). Survival improved with the introduction of immunomodulatory drugs and proteasome inhibitors in the previous decade, but the current 5-year survival rate does not exceed 50% (2). MM represents the most important clinical manifestation in a spectrum of PC dyscrasias, and it is unique in that it is consistently preceded by a premalignant phase, termed monoclonal gammopathy of undetermined significance (MGUS; refs. 3, 4). MGUS is defined as the presence of monoclonal immunoglobulin (Ig) in blood or urine (M protein), less than 10% clonal PC in the BM, and the absence of myeloma-related end-organ damage (4, 5). MGUS is found in 3% of the population above the age of 50, and its prevalence increases with age (5). The rate of progression from MGUS to MM is approximately 1% of patients per year, which means that the majority of MGUS patients neither diagnosed nor progressed to a symptomatic malignancy (3, 5). Some patients develop an intermediate disease stage between MGUS and MM, termed smoldering MM (SMM). SMM patients have an M protein level of more than 30 g/L and over 10% clonal PC in the BM, but are asymptomatic with regard to myeloma-related end-organ damage. Ten percent of SMM patients progress to MM during the first 5 years after diagnosis, after which the rate of progression declines (6). In the final stages of the disease, MM cells can acquire the ability to grow outside the BM, which is referred to as extramedullary MM or PC leukemia (Fig. 1).

Malignant transformation of a healthy cell into a cancer cell is a multistep and multifaceted process. Advances in cancer biology have stipulated that tumors are genetically heterogeneous and that clonal evolution drives tumor progression (7). The existence of a well-defined clinical spectrum of premalignant states that defines MM provides the rare opportunity to study premalignant cells in their clonal evolution, much like the progression of colorectal adenomas into colorectal carcinomas has served as a model for the malignant transformation of epithelial cells (8). However, predicting progression of MGUS/SMM to MM remains a challenge. Unraveling the mechanisms of malignant transformation of PC might enable early identification of MGUS patients at a high risk of progression and may point to novel early and more precise therapeutic targets. Here, we review recent advances in the understanding of MGUS-to-MM progression, as these represent two ends on the spectrum between a benign premalignant condition and an overt cancer. We show that multiple mechanisms of MGUS-to-MM progression are universal principles in malignant evolution.

From Plasma Cell to MGUS

MGUS is believed to arise from post-germinal center (GC) PC that have regained their capacity for proliferation. Two mostly nonoverlapping modes of pathogenesis can be discriminated that are thought to initiate PC proliferation. First, approximately half of both MGUS and MM cases are hyperdiploid, usually with extra copies of the odd-numbered chromosomes (typically 3, 5, 7, 9, 11, 15, 19, 21; ref. 9). Second, most nonhyperdiploid MGUS/MM cases are characterized by a primary translocation involving the Ig heavy-chain gene at 14q32 (4, 10). The majority of translocations

[1]Laboratory of Translational Immunology, University Medical Center, Utrecht, the Netherlands. [2]Department of Hematology, University Medical Center, Utrecht, the Netherlands.

Corresponding Author: Victor Peperzak, University Medical Center Utrecht, Heidelberglaan 100, Utrecht 3584 CX, the Netherlands. Phone: 31-88-7567391; E-mail: v.peperzak@umcutrecht.nl

doi: 10.1158/0008-5472.CAN-17-3115

Published OnlineFirst April 27, 2018; DOI: 10.1158/0008-5472.CAN-17-3115

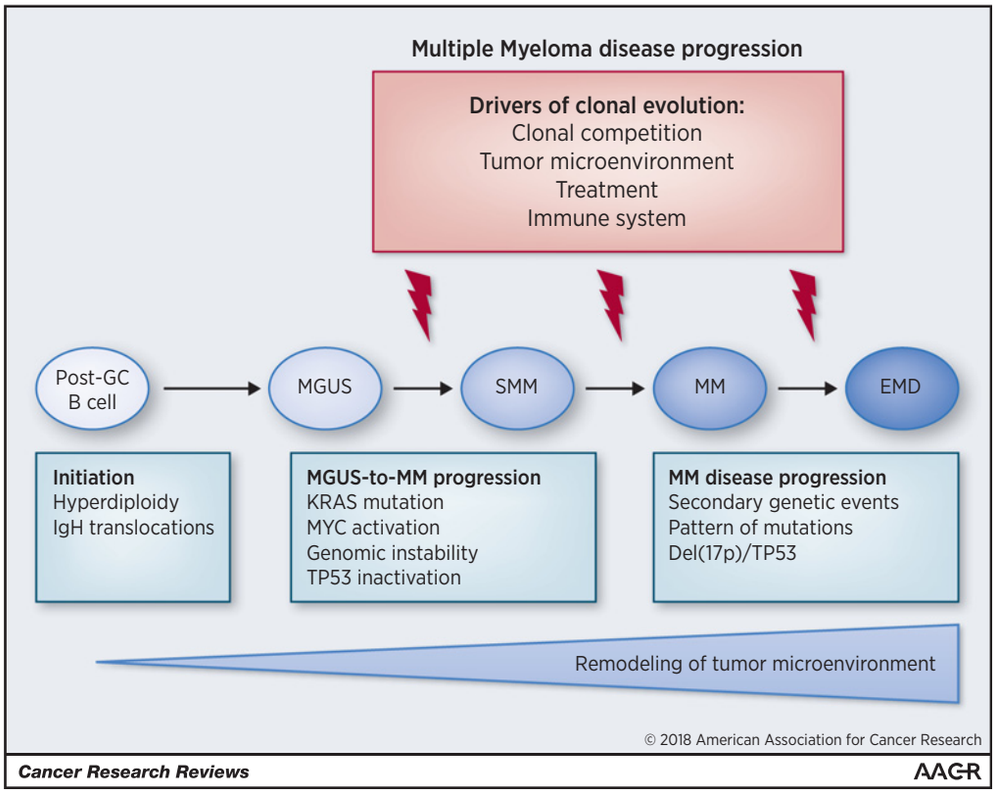

Figure 1.

Clonal evolution of plasma cell dyscrasias. The malignant transformation of a post-GC B cell or plasma cell into MGUS and subsequently MM requires both an initiating event and multiple secondary genetic events. Initiating events are broadly subdivided into IgH-translocations or hyperdiploidy. CNVs, mutations, and epigenetic changes are secondary genetic events that characterize progression. In the continuum of disease stages, genetic lesions accumulate in the tumor clone. Progression of MGUS to MM is promoted by a remodeling of the BM microenvironment. Clonal evolution is driven by clonal competition, the tumor microenvironment, immune cells, and therapy regimens.

go unnoticed, except when an oncogene is juxtaposed near the potent Ig-enhancers, most often involving cyclin D genes, *MAF* transcription factors, or *NSD2/FGFR3* (10). Less than 10% of patients are nonhyperdiploid and are negative for known translocations (11). Dysregulation of the G_1–S cell-cycle transition via overexpression of a cyclin D gene is present in both hyperdiploid and nonhyperdiploid MM and is thought to be a common and early initiating event in MM pathogenesis (12). Overexpression of cyclin D genes has been reported in various solid tumors, including breast cancer and melanoma, but overexpression is especially implicated in the pathogenesis of lymphomas and is for example considered to be the molecular hallmark of mantle cell lymphoma (13, 14).

Because the development of tumors generally requires multiple hits, overexpression of cyclin D alone, either due to hyperdiploidy or IgH translocations, is not sufficient for progression from MGUS to MM (4). However, these primary genetic events in myelomagenesis do have prognostic relevance upon diagnosis of MM. Generally, hyperdiploid MM is associated with a better prognosis than MM with a primary Ig translocation. Translocations t(14;16) and t(4;14) especially confer a high risk of fulminant disease (Table 1). Within the premalignant PC clone, secondary translocations, copy-number variants (CNV), oncogenic mutations, epigenetic alterations, and microenvironmental changes drive clonal evolution from MGUS to MM. Together, primary and secondary events produce the cancer phenotype and are implicated in a differential disease course, prognosis, and therapy response (4).

Copy-Number Variants

The number of mutations in cancer varies from only several to many hundreds, whereby a hematopoietic malignancy generally has less mutations than a solid tumor. Most of the genetic events that are present in cancer are neutral mutations that have arisen from genetic instability—so-called passenger lesions. The challenge is to identify and consequently target those genetic changes that confer a selective advantage to the cancer clone and thereby drive malignant evolution. A genetic event is considered a driver lesion when (i) its associated gene(s) are recognized to play a role in malignant pathophysiology, (ii) the lesion itself has been associated with clonal expansion, and (iii) the frequency of the event exceeds the normal background mutation rate (15). Results from knockdown and overexpression studies of genes in animal and *in vitro* models confer extra weight to the status of potential driver lesions. Most of the studies discussed here compare genetics of PC from healthy donors and MGUS, SMM, or MM patients, occasionally accompanied by animal studies. A more reliable method to identify driver lesions is the use of sequential, paired

Published OnlineFirst April 27, 2018; DOI: 10.1158/0008-5472.CAN-17-3115

Table 1. Intrinsic drivers of malignant progression from MGUS-to-MM and their prognosis in MM

Type of event	Potential oncogenes	Frequency in MGUS (%)	Frequency in MM (%)	Prognosis in newly diagnosed MM
Initiating events				
t(11;14)	*CCND1*	12	19	[c]
t(12;14)	*CCND2*	<1	<1	0
t(6;14)	*CCND3*	0	1	0
t(14;16)	*MAF*	3	4	[d]
t(14;20)	*MAFB*	3	1	[e]
t(4;14)	*NSD2/FGFR3*	9	13	[d]
Hyperdiploidy	[a]	50	55	[c]
Secondary cytogenetic aberrations				
Amp 1q	*CKS1B, ILF2*	25	50	[d]
Del(1p)	*CDKN2C, FAM46C*	6	40	[d]
Del(13)	*RB1*	30[b]	70	[f]
Del(17p)	*TP53*	1	12	[d]
Translocations 8q24	*MYC*	3–4	20	[d]
Oncogenic pathways				
MAPK activation	*NRAS*	36	33	
	KRAS	<1	33	
	BRAF	27	19	
MYC dysregulation	*MYC*	<1	67	[d]
Constitutive NFκB activation	*TRAF6, CYLD*	<1	20	

[a]The exact oncogenic mechanism of hyperdiploidy remains to be elucidated, but cyclin D1 is consistently overexpressed.
[b]The frequency of del(13) in MGUS is dependent on concomitant presence of specific primary IgH translocations. It is high in patients with t(4;14), t(14;16), and t(14;20), but low in t(11;14) and t(6;14).
[c]Denotes a positive impact on survival and 0 denotes no reported impact on survival. Source refs. 18, 36, 37, 41, 46.
[d]Denotes a negative impact on survival.
[e]t(14;20) is associated with poor prognosis in MM, but correlates with quiescence in MGUS.
[f]The prognosis of del(13) depends on the presence of specific IgH translocations.

MGUS–MM or SMM–MM samples from the same patient that has progressed from one disease stage to another. We have tried to include these studies when possible; however, the number of completed studies and included patients is limited. In addition, MGUS and SMM patients are often pooled. The advent of liquid biopsies will allow for more convenient sequential sampling in the near future (16).

Variations of gene-copy numbers are common to both solid and hematologic cancers and are believed to contribute to tumor growth. Examining several thousand cancer copy-number profiles revealed 158 regions of somatic CNVs that are altered at significant frequency across multiple cancer types (17). CNVs are more frequently found in MM than MGUS, and there is a greater median number of CNVs in each MM versus MGUS patient (18). Although CNVs can be merely passenger events, some of them have an effect on MGUS progression and MM prognosis (11). In addition, sequential sequencing of MGUS/SMM patients that progressed to MM and MGUS/SMM patients that did not progress to MM within follow-up revealed a greater number of CNVs at baseline in patients that progressed to MM (19). Consistently, LOH was much more frequent at baseline in patients that were about to progress. This study suggests that the degree of genomic instability is a driver of MGUS-to-MM progression itself. CNVs are generally more frequent in patients with nonhyperdiploid MM, contributing to the worse prognosis of these patients (11).

Amplification of the chromosomal region 1q21 is the most common chromosomal gain reported in MM, often occurring concomitantly with the deletion of 1p. Gain of 1q21 is more frequent in MM (40%) than in MGUS (25%; Table 1; ref. 18). It is associated with a higher risk of progression to MM in MGUS patients and with a poor prognosis in MM patients (11). Similarly, the transformation from a myeloproliferative neoplasm to acute myeloid leukemia is associated with amplification of chromosome 1q (20). Remarkably, 30% of the GEP-70 gene set that has been shown to predict high-risk disease in MM maps to chromosome 1 (21). Despite its high prevalence and relation with high-risk disease, the oncogenes on 1q21 responsible for malignant transformation are subject to debate. *CKS1B* was originally proposed to be involved in disease progression, although a more recent study found no association between *CKS1B* expression and clinical parameters (22). Recently, *ILF2* was identified as a potential oncogene in 1q21 amplification (23). *ILF2* is involved in DNA damage repair, and its overexpression enables genomic instability, thereby enhancing MM cell survival and drug resistance. In line, inhibition of ILF2 resulted in an increased frequency of apoptosis in MM cells with a 1q21 amplification, designating ILF2 as potential therapeutic target (23). In addition, multiple other candidates are located on 1q21 that may contribute to disease progression, including *MCL1* and *IL6R* that are both known to play a role in MM cell survival (4).

The frequency of 1p deletions in MM is approximately 30%, opposed to only 6% in MGUS (Table 1; ref. 18). A majority of patients have interstitial deletions, but removal of the entire short arm has also been observed. Two tumor-suppressor genes linked to the pathogenesis of del(1p) are *CDKN2C* and *FAM46C* (24). Deletion of 1p32.3 (*CDKN2C*) increases from MGUS (5%) to MM (15%) and is associated with adverse overall survival (25). 1p12 (*FAM46C*) was found to be deleted in 19% of MM patients and also confers an impaired risk of survival. Its frequency of deletion in MGUS is unknown (24).

Half of MM patients show loss of the complete chromosome 13, but it is more common in nonhyperdiploid MM (66%) than in hyperdiploid MM (34%; Table 1; ref. 26). Its frequency in MGUS is dependent on the concomitant presence of specific IgH-translocations. Del(13) is almost equally frequent in MGUS and MM with t(4;14) and t(14;16) translocations, suggesting that it is

Published OnlineFirst April 27, 2018; DOI: 10.1158/0008-5472.CAN-17-3115

an early event in these patients (26). In contrast, del(13) is practically absent in MGUS with t(6;14) and t(11;14) translocations but common in MM patients carrying either translocation (40% and 67%, respectively), implicating del(13) in MGUS-to-MM progression (26). The retinoblastoma (RB1) tumor-suppressor gene is located on chromosome 13. Inactivation of *RB1* is associated with both initiation and progression of many solid and hematopoietic cancers, including the progression to invasive growth in prostate and bladder cancer and the progression to a blast crisis in chronic myeloid leukemia (27). Experiments demonstrated that complete loss of *RB1* increased proliferation in both MM cell lines and murine GC B cells, but was unable to initiate malignant transformation by itself (28). Therefore, LOH of *RB1* in case of del(13) could potentially contribute to MGUS-to-MM progression, although other genes located on chromosome 13 may also be involved.

Deletions of the short arm of chromosome 17 are uncommon in MGUS, but 12% prevalence was reported in untreated MM (Table 1; ref. 18). Multiple studies have confirmed that del(17p) in MM is associated with extramedullary disease and with very poor prognosis (11). The prototypical tumor-suppressor gene *TP53* is located on the short arm of chromosome 17 and functions to halt cell-cycle progression and/or induce apoptosis in case of intracellular stress following DNA damage. In addition, more recent studies have recognized that p53 modulates its tumor-suppressing effects via other mechanisms, including regulation of metabolism and autophagy (29). Approximately half of all malignancies are affected by a *TP53* mutation, making it the most common genetic change in human cancers (29). Mutations of *TP53* have been reported in 37% of MM patients with del(17p), but are absent in cases without the deletion. This suggests that haploinsufficiency of *TP53* may be important for disease progression directly, or that it increases the probability for loss or mutation of the remaining allele (30).

Recurrent Mutations and Cell Signaling Pathways

With the advent of gene expression profiling (GEP), molecular subclasses were described for breast cancer based on distinctive interpatient expression of gene clusters. Subsequently, molecular subgroups were recognized in other cancers, including MM (31). These molecular subgroups of MM were found to be already present in MGUS (32), which is in line with the finding that genetic differences between MGUS and MM are smaller than the differential gene expression between healthy PC and MGUS cells (33). Similarly, serial whole-exome sequencing (WES) analyses of paired MGUS–MM or SMM–MM samples demonstrated that most somatic mutations are present before the onset of clinical MM (19, 34, 35). Nevertheless, the genetic complexity increases as MGUS progresses to MM, and the mutational load itself is associated with poor prognosis (36, 37). Eight driver genes that are recurrently mutated on progression from MGUS to MM have been identified using next-generation sequencing (NGS): *KRAS*, *NRAS*, *BRAF*, *TP53*, *CCND1*, *FAM46C*, *IRF4*, and *LTB* (37). A study using WES reported the additional involvement of *HIST1H1E* and *EGR1*, confirming the genetic heterogeneity of driver genes in MM (36). These results correspond with studies showing that all tumors have a variable and increasing clonal heterogeneity during development (7). In contrast, a more recent study does mention that most frequent mutations including *NRAS*, *KRAS*, and *HIS1TH1E* in MM are in fact already present in MGUS (35). Oddly, in 20% of MM patients, no mutations in any of the aforementioned driver genes were found, suggesting that currently unknown mechanisms play a role in MM pathogenesis in these patients (37). Recent WES studies of paired MGUS–MM or SMM–MM patient samples confirmed the widespread intraclonal heterogeneity of MM (38). In 10 patients, 82 different genes were gained or lost during progression of MGUS to MM. Beyond the previously identified driver genes, further potential genetic events of MGUS-to-MM progression that were identified in this study include *ICAM5*, *DUSP27*, *HERPUD1*, *NOD2*, *and TOP2A* (38). Surprisingly, the comparison of samples from MGUS/SMM patients that progressed to MM with samples from patients that did not progress revealed no difference in mutational load (19). In addition, *de novo* acquired mutations at progression to MM were rare in studies using paired SMM–MM samples specifically (34, 35). Although preliminary, these results indicate that the specific pattern of mutations drives MM disease progression, especially from SMM to MM. However, it needs to be mentioned that purification of PC using markers CD138 (and CD38) in abovementioned studies does not discriminate between healthy and (pre)malignant PC. Because healthy PC can constitute up to 2% of total leukocytes in the BM and MGUS never exceeds 10%, there can be a substantial contamination of healthy PC in the MGUS fraction.

An important feature of malignant cells is their acquired independence from mitogenic signaling for cell proliferation, which is often achieved by constitutive activation of one of the cell signaling pathways. Whole-genome sequencing studies showed that 40% to 60% of MM patients have mutations in genes involved in the MAPK pathway, making it the most frequently mutated pathway in MM (37). Recent studies using NGS have shown that RAS protein family mutations accumulate during disease progression, which is in line with earlier GEP results that demonstrated increased expression of RAS proteins in MM compared with healthy PC. *NRAS* and *BRAF* mutations that result in their constitutive activation are found in both MGUS and MM cells, whereas for *KRAS*, this is only the case in MM cells. Interestingly, it was reported that only *KRAS* mutations are associated with downstream pathway activation in MM, whereas NRAS mutations were not (Table 1; ref. 39). These findings with respect to the MAPK pathway were recently confirmed by the aforementioned paired MGUS–MM WES. Combined, these results suggest a critical role for MAPK pathway signaling—and specifically KRAS—in MGUS-to-MM progression.

Expression of *MYC* induces pleiotropic downstream effects that drive cell proliferation and is under strict regulation in healthy cells. The activation of *MYC* in cancer generally results from either constitutive activation of one of the pathways regulating *MYC* expression, or through chromosomal amplifications or translocations, where the latter is more commonly seen in hematopoietic malignancies (40). *MYC* rearrangements involving chromosome 8q24 were detected by FISH in 3% of MGUS and 15% of newly diagnosed MM patients, although a more recent study using comparative genomic hybridization found these rearrangements in almost 50% of MM cases (41). GEP of *MYC* showed *MYC* activation in the majority of MM (67%), whereas little to no activation was demonstrated in healthy controls and MGUS (Table 1; ref. 42). Transgenic mice with constitutive overexpression of *MYC* in B cells develop post-GC PC tumors similar to human MM (42). Suppression of the *MYC*-activating LIN28B/let-7 axis significantly reduced tumor growth and prolonged survival in a xenograft mouse model, thereby exposing a novel

Published OnlineFirst April 27, 2018; DOI: 10.1158/0008-5472.CAN-17-3115

therapeutic target (43). Most MYC-driven MM mouse models are generated on the C57BL/6 genetic background, and MYC activation is generally believed to be less important in mice with a different genetic background. Nevertheless, enforced expression of *MYC*, together with IL6, does result in the outgrowth of malignant PC in BALB/c mice (44). However, it is questionable whether the level of MYC expression in these mouse models is comparable with the level of *MYC* expressed in MGUS/MM patients.

The NFκB pathway regulates expression of many genes involved in inflammatory and immune responses. NFκB activation in cancer is common and can result either from intrinsic mutations of NFκB pathway–related genes or from extrinsic signals from the tumor microenvironment. Intrinsic activation is more commonly found in hematopoietic cancers, whereas activation of NFκB by the microenvironment is required as an antiapoptotic survival factor for various types of solid cancers that arise from chronic inflammation (45). Ordinarily, the NFκB pathway is activated by extrinsic signals from BM stromal cells in healthy PC, MGUS, and most MM cells. However, 17% of untreated MM have mutations that constitutively activate part of the NFκB pathway (Table 1; ref. 46). Intrinsic activation of NFκB makes MM cells less dependent on the BM microenvironment and thereby facilitates extramedullary progression. A recent study demonstrated that TRAF6, implicated in regulating NFκB and MAPK signaling, is significantly overexpressed in patients with active MM compared with MGUS (47). Inhibition of TRAF6 using a TRAF6-dominant–negative peptide decreased NFκB–related signaling, induced apoptosis of MM cells, and reduced MM growth (47).

Transcriptional silencing of genes by DNA methylation is an important epigenetic method to regulate gene expression. Alterations in DNA methylation profiles are known to affect oncogenic pathways and are thought to play a role in tumorigenesis. Genome-wide hypomethylation was found to occur at the transition from MGUS to MM, accompanied by hypermethylation of specific tumor-suppressor genes (48). This aberrant methylation profile was shown to be a universal characteristic for many types of cancer (49). Global hypomethylation is believed to result in genomic instability and thereby facilitates chromosomal rearrangements, whereas hypermethylation of tumor-suppressor genes has been associated with aberrant activation of Wnt and JAK/STAT3 signaling pathways in MM (50). One study reported that methylation status regulates expression of only a few genes, challenging clinical relevance of DNA methylation in MM (51). However, another study has shown that changes in methylation status of 195 tumor-suppressor genes are significantly associated with adverse survival (52).

Clinical Predictors of Progression

A number of clinical risk factors are recognized that allow stratification of the risk of MGUS-to-MM progression. These parameters do not provide an account for underlying causes of malignant progression, but have proven useful for predicting risk of progression in individual patients (53). Important and easy to determine parameters are based on the size of the MGUS clone: both the percentage of BM PC and the baseline level and rate of increased serum M-protein level predict progression to MM (54, 55). Further risk factors include the heavy-chain isotype—whereby the risk of progression is most prevalent for IgD and greater for IgA/IgM MGUS than for IgG MGUS—serum free light-chain (FLC) ratio, detection of focal lesions by MRI, and Bence Jones proteinuria (53). Combining these parameters has led to the development of models predicting progression of MGUS to MM. The first model that was proposed uses the M-protein level, heavy-chain isotype, and serum FLC ratio to stratify MGUS patients in four groups from low risk to high risk of progression over a 20-year disease course (56). Two other models are based on the percentage of aberrant PC in the BM and either DNA aneuploidy or development of M-protein level, both stratifying MGUS patients in three risk groups at 5 and 7 years after diagnosis, respectively (54, 57). Similar models to predict risk of progression have been developed for SMM (32).

Many of the genetic events that have been discussed above impart an influence on an MGUS patient's risk of progression to MM (Fig. 1). It was found that the aforementioned GEP-70 gene set not only predicts high-risk disease in MM, but also independently signifies a higher risk of MGUS-to-MM progression (32). The combination of conventional clinical risk factors with genomic predictors of progression, such as the GEP-70 risk score, was used to identify new subsets of high-risk SMM patients that require earlier therapy (32). Incorporation of genomic data in prediction models of MGUS progression should lead to more accurate stratification of high-risk MGUS patients in the near future. This may allow for specific and early treatment and may thereby delay or prevent clonal evolution of a premalignant lesion.

Tumor Microenvironment in Malignant Progression

It has become increasingly clear that reciprocal interaction between tumor cells and the tumor microenvironment plays an essential role in the development of cancer (7). In solid tumors, the establishment of tumor-associated stroma facilitates not only tumor growth and progression, but also invasive and metastatic growth (58). Similar to healthy PC, MM cells initially depend on signals from the BM microenvironment for their survival (4). Intricate interactions between MM cells and cells from the BM microenvironment play an important role in MM proliferation, survival, migration, and drug resistance (4, 59). The nature and relevance of the microenvironment in MM have been described extensively elsewhere, including the important role of bone and immune cells in MM pathophysiology (60, 61). This is nicely illustrated by recent studies where MGUS cells were shown capable of progressive growth in mouse models, and that immune surveillance and extrinsic restraints from the endosteal niche are involved in MGUS dormancy (62, 63). Here, we will primarily discuss differences that have been found in BM stroma when comparing MGUS and MM patients. It is believed that malignant evolution of MGUS is mediated by structural and functional alterations of the tumor-associated stromal cells, making the BM microenvironment an active participant in malignant transformation and thus an interesting target for therapy in early disease stages (Fig. 1; refs. 4, 59–61, 64).

Tumor-associated stromal cells are active participants in structural and functional remodeling and constitutive activation of angiogenesis in a cancer microenvironment (58). Endothelial cells (EC) in the microenvironment of solid tumors overexpress genes related to the extracellular matrix (ECM), proliferation, migration, and especially angiogenesis (58). Similarly, GEP of BM EC in MGUS and MM patients revealed differential expression of 22 genes involved in resistance to apoptosis, ECM formation, bone remodeling, cell adhesion, and angiogenesis, and thus

Published OnlineFirst April 27, 2018; DOI: 10.1158/0008-5472.CAN-17-3115

implicates a functional transformation of BM EC in MGUS-to-MM progression (65).

A proteomic analysis of fibroblast-like cells and ECM demonstrated that ECM proteins, ECM receptors, and ECM-modulating enzymes are progressively upregulated from MGUS to MM (59). Two proteins, Annexin A2 (ANXA2) and Galectin-2 (LGALS2), were identified in the BM ECM of MM but were absent in BM from healthy donors or MGUS patients (59). Remarkably, high expression of these proteins was associated with decreased overall survival, suggesting that remodeling of BM ECM contributes to a permissive tumor microenvironment (59).

Angiogenesis results in tumor growth via increased blood flow and a greater supply of nutrients to tumor cells and is widely recognized as vital to cancer progression (58). Angiogenesis in the BM microenvironment was shown to increase during progression from MGUS to MM and is associated with poor prognosis and therapy resistance (66). Endothelial progenitor cells (EPC) mediate angiogenesis in the BM microenvironment. It has been shown that levels of circulating EPC are significantly higher in MM compared with healthy subjects and MGUS patients (64). Targeting EPC with a VEGFR2 antibody in mice effectively delayed MM growth only during early disease progression. In addition, the BM microvessel density (MVD) in mice with spontaneous MM was twice as high as in mice with MGUS and strongly correlated with the level of monoclonal Ig in blood (67). In line with these findings, it was found that MVD in BM of MGUS patients who showed progression to MM at follow-up significantly increased compared with MVD of patients with MGUS that remained quiescent (67). Since the FDA approval of bevacizumab in 2004, many angiogenesis inhibitors, alone or in combination with other drugs, have been introduced for the treatment of a range of tumors. However, antiangiogenic therapy, monotherapy especially, proves not to be as effective as was predicted (68). Aforementioned results illustrate the need to further investigate antiangiogenic drugs during early stages of malignant transformation.

Concluding Remarks

The 5-year survival rate of MM patients does not exceed 50%, notwithstanding recent therapeutic advances (2). Treatment of MGUS patients before progression to MM is currently not considered beneficial. The high degree of genetic and molecular heterogeneity makes it difficult to identify those patients who are at imminent risk of progression and to decide on a choice of therapy that outweighs the risks and costs. In an ongoing phase I clinical trial, high-risk MGUS patients are being treated with anti-CD38 monoclonal antibody daratumumab, which, in contrast to chemotherapy, is believed to be nonmutagenic (69). Further delineating the molecular mechanisms that drive malignant transformation may allow for a more precise definition of high-risk MGUS and may lead to prevention or delay of MM development by targeting specific signaling pathways involved in disease progression. The efficacy of targeting actionable mutations is hindered by clonal heterogeneity, highlighting further difficulties in finding appropriate therapeutic regimens.

Existence of the well-defined spectrum of disease stages that marks MM allows for research on the transformation of premalignant cells. Sequential paired MGUS–MM sequencing is an elegant method to study the genetics of MGUS-to-MM progression and should become more convenient with liquid biopsies in the near future, yielding more precise data on the link between genomic events and malignant evolution (16). Ideally, this could be extended to monitoring virtually any premalignant lesion in its progression toward overt cancer. Simultaneously, there is a need to further characterize remodeling events of the tumor microenvironment during early stages of cancer. Novel and precise targeting of the BM microenvironment in MGUS should abrogate the effect of stromal cells on tumor growth, survival, and resistance to therapy, thereby preventing progression from MGUS to MM. Rapid advances of genomic techniques to study premalignant cells in their progression should enable identification and subsequent targeting of driver events during clonal evolution in MM and cancer in general.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

This work was supported by a Bas Mulder Award from the Dutch Cancer Foundation (KWF)/Alpe d'HuZes Foundation (Number UU 2015-7663) to V. Peperzak and a project grant from the Dutch Cancer Foundation (KWF)/Alpe d'HuZes Foundation (Number 11108) to V. Peperzak.

Received October 10, 2017; revised January 16, 2018; accepted March 16, 2018; published first April 27, 2018.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. CA Cancer J Clin 2017;67:7–30.
2. Howlader N, Noone A, Krapcho M, Miller D, Bishop K, Altekruse SF, et al. SEER cancer statistics review, 1975–2013. Bethesda, MD: National Cancer Institute; 2016.
3. Landgren O, Kyle RA, Pfeiffer RM, Katzmann JA, Caporaso NE, Hayes RB, et al. Monoclonal gammopathy of undetermined significance (MGUS) consistently precedes multiple myeloma: a prospective study. Blood 2009;113:5412–7.
4. Pawlyn C, Morgan GJ. Evolutionary biology of high-risk multiple myeloma. Nat Rev Cancer 2017;17:543–56.
5. Kyle RA, Therneau TM, Rajkumar SV, Larson DR, Plevak MF, Offord JR, et al. Prevalence of monoclonal gammopathy of undetermined significance. N Engl J Med 2006;354:1362–9.
6. Kyle RA, Remstein ED, Therneau TM, Dispenzieri A, Kurtin PJ, Hodnefield JM, et al. Clinical course and prognosis of smoldering (asymptomatic) multiple myeloma. N Engl J Med 2007;356:2582–90.
7. Gupta RG, Somer RA. Intratumor heterogeneity: novel approaches for resolving genomic architecture and clonal evolution. Mol Cancer Res 2017;15:1127–37.
8. Strum WB. Colorectal Adenomas. N Engl J Med 2016;375:389–90.
9. Smadja NV, Fruchart C, Isnard F, Louvet C, Dutel JL, Cheron N, et al. Chromosomal analysis in multiple myeloma: cytogenetic evidence of two different diseases. Leukemia 1998;12:960–9.
10. Fonseca R, Debes-Marun CS, Picken EB, Dewald GW, Bryant SC, Winkler JM, et al. The recurrent IgH translocations are highly associated with nonhyperdiploid variant multiple myeloma. Blood 2003;102:2562–7.
11. Rajan AM, Rajkumar SV. Interpretation of cytogenetic results in multiple myeloma for clinical practice. Blood Cancer J 2015;5:e365.
12. Bergsagel PL, Kuehl WM, Zhan F, Sawyer J, Barlogie B, Shaughnessy J. Cyclin D dysregulation: an early and unifying pathogenic event in multiple myeloma. Blood 2005;106:296–303.
13. Vose JM. Mantle cell lymphoma: 2015 update on diagnosis, risk-stratification, and clinical management. Am J Hematol 2015;90:739–45.

Published OnlineFirst April 27, 2018; DOI: 10.1158/0008-5472.CAN-17-3115

14. Musgrove EA, Caldon CE, Barraclough J, Stone A, Sutherland RL. Cyclin D as a therapeutic target in cancer. Nat Rev Cancer 2011;11:558–72.
15. Greaves M, Maley CC. Clonal evolution in cancer. Nature 2012;481: 306–13.
16. Mishima Y, Paiva B, Shi J, Park J, Manier S, Takagi S, et al. The mutational landscape of circulating tumor cells in multiple myeloma. Cell Rep 2017;19:218–24.
17. Beroukhim R, Mermel CH, Porter D, Wei G, Raychaudhuri S, Donovan J, et al. The landscape of somatic copy-number alteration across human cancers. Nature 2010;463:899–905.
18. Mikulasova A, Smetana J, Wayhelova M, Janyskova H, Sandecka V, Kufova Z, et al. Genomewide profiling of copy-number alteration in monoclonal gammopathy of undetermined significance. Eur J Haematol 2016;97:568–75.
19. Zhao S, Choi M, Heuck C, Mane S, Barlogie B, Lifton RP, et al. Serial exome analysis of disease progression in premalignant gammopathies. Leukemia 2014;28:1548–52.
20. Spivak JL. Myeloproliferative neoplasms. N Engl J Med 2017;376:2168–81.
21. Shaughnessy JD, Zhan F, Burington BE, Huang Y, Colla S, Hanamura I, et al. A validated gene expression model of high-risk multiple myeloma is defined by deregulated expression of genes mapping to chromosome 1. Blood 2007;109:2276–84.
22. Stella F, Pedrazzini E, Baialardo E, Fantl DB, Schutz N, Slavutsky I. Quantitative analysis of CKS1B mRNA expression and copy number gain in patients with plasma cell disorders. Blood Cells Mol Dis 2014; 53:110–7.
23. Marchesini M, Ogoti Y, Fiorini E, Aktas Samur A, Nezi L, D'Anca M, et al. ILF2 is a regulator of RNA splicing and DNA damage response in 1q21-amplified multiple myeloma. Cancer Cell 2017;32:88–100.e6.
24. Boyd KD, Ross FM, Walker BA, Wardell CP, Tapper WJ, Chiecchio L, et al. Mapping of chromosome 1p deletions in myeloma identifies FAM46C at 1p12 and CDKN2C at 1p32.3 as being genes in regions associated with adverse survival. Clin Cancer Res 2011;17:7776–84.
25. Leone PE, Walker BA, Jenner MW, Chiecchio L, Dagrada G, Protheroe RK, et al. Deletions of CDKN2C in multiple myeloma: biological and clinical implications. Clin Cancer Res 2008;14:6033–41.
26. Chiecchio L, Dagrada GP, Ibrahim AH, Dachs Cabanas E, Protheroe RK, Stockley DM, et al. Timing of acquisition of deletion 13 in plasma cell dyscrasias is dependent on genetic context. Haematologica 2009;94: 1708–13.
27. Burkhart DL, Sage J. Cellular mechanisms of tumour suppression by the retinoblastoma gene. Nat Rev Cancer 2008;8:671–82.
28. He Z, O'Neal J, Wilson WC, Mahajan N, Luo J, Wang Y, et al. Deletion of Rb1 induces both hyperproliferation and cell death in murine germinal center B cells. Exp Hematol 2016;44:161–5.e164.
29. Duffy MJ, Synnott NC, Crown J. Mutant p53 as a target for cancer treatment. Eur J Cancer 2017;83:258–65.
30. Lodé L, Eveillard M, Trichet V, Soussi T, Wuillème S, Richebourg S, et al. Mutations in TP53 are exclusively associated with del(17p) in multiple myeloma. Haematologica 2010;95:1973–6.
31. Zhan F, Huang Y, Colla S, Stewart JP, Hanamura I, Gupta S, et al. The molecular classification of multiple myeloma. Blood 2006;108: 2020–8.
32. Dhodapkar MV, Sexton R, Waheed S, Usmani S, Papanikolaou X, Nair B, et al. Clinical, genomic, and imaging predictors of myeloma progression from asymptomatic monoclonal gammopathies (SWOG S0120). Blood 2014;123:78–85.
33. Davies FE, Dring AM, Li C, Rawstron AC, Shammas MA, O'Connor SM, et al. Insights into the multistep transformation of MGUS to myeloma using microarray expression analysis. Blood 2003;102:4504–11.
34. Walker BA, Wardell CP, Melchor L, Brioli A, Johnson DC, Kaiser MF, et al. Intraclonal heterogeneity is a critical early event in the development of myeloma and precedes the development of clinical symptoms. Leukemia 2014;28:384–90.
35. Seckinger A, Jauch A, Emde M, Beck S, Mohr M, Granzow M, et al. Asymptomatic multiple myeloma - background of progression, evolution, and prognosis. Blood 2016;128:235.
36. Mikulasova A, Wardell CP, Murison A, Boyle EM, Jackson GH, Smetana J, et al. Somatic mutation spectrum in monoclonal gammopathy of undetermined significance indicates a less complex genomic landscape compared to multiple myeloma. Haematologica 2017;102:1617–25.
37. Rossi A, Voigtlaender M, Janjetovic S, Thiele B, Alawi M, März M, et al. Mutational landscape reflects the biological continuum of plasma cell dyscrasias. Blood Cancer J 2017;7:e537.
38. Dutta AK, Grady JP, Hewett DR, Bik To L, Fink L, Zannettino ACW. Whole exome sequencing of paired MGUS/SMM to MM patients reveals novel subclonal tumour evolution models in disease progression of multiple myeloma. Blood 2017;130:391.
39. Xu J, Pfarr N, Endris V, Mai EK, Md Hanafiah NH, Lehners N, et al. Molecular signaling in multiple myeloma: association of RAS/RAF mutations and MEK/ERK pathway activation. Oncogenesis 2017;6:e337.
40. Meyer N, Penn LZ. Reflecting on 25 years with MYC. Nat Rev Cancer 2008;8:976–90.
41. Affer M, Chesi M, Chen WD, Keats JJ, Demchenko YN, Roschke AV, et al. Promiscuous MYC locus rearrangements hijack enhancers but mostly super-enhancers to dysregulate MYC expression in multiple myeloma. Leukemia 2014;28:1725–35.
42. Chesi M, Robbiani DF, Sebag M, Chng WJ, Affer M, Tiedemann R, et al. AID-dependent activation of a MYC transgene induces multiple myeloma in a conditional mouse model of post-germinal center malignancies. Cancer Cell 2008;13:167–80.
43. Manier S, Powers JT, Sacco A, Glavey SV, Huynh D, Reagan MR, et al. , The LIN28B/let-7 axis is a novel therapeutic pathway in multiple myeloma. Leukemia 2017;31:853–60.
44. Rutsch S, Neppalli VT, Shin DM, DuBois W, Morse HC 3rd, Goldschmidt H, et al. IL-6 and MYC collaborate in plasma cell tumor formation in mice. Blood 2010;115:1746–54.
45. Hoesel B, Schmid JA. The complexity of NF-κB signaling in inflammation and cancer. Mol Cancer 2013;12:86.
46. Demchenko YN, Glebov OK, Zingone A, Keats JJ, Bergsagel PL, Kuehl WM. Classical and/or alternative NF-kappaB pathway activation in multiple myeloma. Blood 2010;115:3541–52.
47. Chen H, Li M, Sanchez E, Wang CS, Lee T, Soof CM, et al. Combined TRAF6 targeting and proteasome blockade has anti-myeloma and anti-bone resorptive effects. Mol Cancer Res 2017;15:598–609.
48. Walker BA, Wardell CP, Chiecchio L, Smith EM, Boyd KD, Neri A, et al. Aberrant global methylation patterns affect the molecular pathogenesis and prognosis of multiple myeloma. Blood 2011;117:553–62.
49. Wilson AS, Power BE, Molloy PL. DNA hypomethylation and human diseases. Biochim Biophys Acta 2007;1775:138–62.
50. Dimopoulos K, Gimsing P, Grønbæk K. The role of epigenetics in the biology of multiple myeloma. Blood Cancer J 2014;4:e207.
51. Jung S, Kim S, Gale M, Cherni I, Fonseca R, Carpten J, et al. DNA methylation in multiple myeloma is weakly associated with gene transcription. PLoS One 2012;7:e52626.
52. Kaiser MF, Johnson DC, Wu P, Walker BA, Brioli A, Mirabella F, et al. Global methylation analysis identifies prognostically important epigenetically inactivated tumor suppressor genes in multiple myeloma. Blood 2013;122:219–26.
53. van de Donk NW, Mutis T, Poddighe PJ, Lokhorst HM, Zweegman S. Diagnosis, risk stratification and management of monoclonal gammopathy of undetermined significance and smoldering multiple myeloma. Int J Lab Hematol 2016;38:110–22.
54. Pérez-Persona E, Vidriales MB, Mateo G, García-Sanz R, Mateos MV, de Coca AG, et al. New criteria to identify risk of progression in monoclonal gammopathy of uncertain significance and smoldering multiple myeloma based on multiparameter flow cytometry analysis of bone marrow plasma cells. Blood 2007;110:2586–92.
55. Rosiñol L, Cibeira MT, Montoto S, Rozman M, Esteve J, Filella X, et al. Monoclonal gammopathy of undetermined significance: predictors of malignant transformation and recognition of an evolving type characterized by a progressive increase in M protein size. Mayo Clin Proc 2007;82:428–34.
56. Rajkumar SV, Kyle RA, Therneau TM, Melton LJ 3rd, Bradwell AR, Clark RJ, et al. Serum free light chain ratio is an independent risk factor for progression in monoclonal gammopathy of undetermined significance. Blood 2005;106:812–7.
57. Pérez-Persona E, Mateo G, García-Sanz R, Mateos MV, de Las Heras N, de Coca AG, et al. Risk of progression in smouldering myeloma and monoclonal gammopathies of unknown significance: comparative analysis of the evolution of monoclonal component and multiparameter flow cytometry of bone marrow plasma cells. Br J Haematol 2010;148:110–4.

Published OnlineFirst April 27, 2018; DOI: 10.1158/0008-5472.CAN-17-3115

58. Quail DF, Joyce JA. Microenvironmental regulation of tumor progression and metastasis. Nat Med 2013;19:1423–37.
59. Glavey SV, Naba A, Manier S, Clauser K, Tahri S, Park J, et al. Proteomic characterization of human multiple myeloma bone marrow extracellular matrix. Leukemia. 2017;31:2426–34.
60. Bianchi G, Munshi NC. Pathogenesis beyond the cancer clone(s) in multiple myeloma. Blood 2015;125:3049–58.
61. Ghobrial IM, Detappe A, Anderson KC, Steensma DP. The bone-marrow niche in MDS and MGUS: implications for AML and MM. Nat Rev Clin Oncol 2018;15:219–33.
62. Das R, Strowig T, Verma R, Koduru S, Hafemann A, Hopf S, et al. Microenvironment-dependent growth of preneoplastic and malignant plasma cells in humanized mice. Nat Med 2016;22:1351–7.
63. Lawson MA, McDonald MM, Kovacic N, Hua Khoo W, Terry RL, Down J, et al. Osteoclasts control reactivation of dormant myeloma cells by remodelling the endosteal niche. Nat Commun 2015;6:8983.
64. Moschetta M, Mishima Y, Kawano Y, Manier S, Paiva B, Palomera L, et al. , Targeting vasculogenesis to prevent progression in multiple myeloma. Leukemia 2016;30:1103–15.
65. Ria R, Todoerti K, Berardi S, Coluccia AM, De Luisi A, Mattioli M, et al. Gene expression profiling of bone marrow endothelial cells in patients with multiple myeloma. Clin Cancer Res 2009;15:5369–78.
66. Rajkumar SV, Mesa RA, Fonseca R, Schroeder G, Plevak MF, Dispenzieri A, et al. Bone marrow angiogenesis in 400 patients with monoclonal gammopathy of undetermined significance, multiple myeloma, and primary amyloidosis. Clin Cancer Res 2002;8:2210–16.
67. Calcinotto A, Ponzoni M, Ria R, Grioni M, Cattaneo E, Villa I, et al. Modifications of the mouse bone marrow microenvironment favor angiogenesis and correlate with disease progression from asymptomatic to symptomatic multiple myeloma. Oncoimmunology 2015;4:e1008850.
68. Rajabi M, Mousa SA. The role of angiogenesis in cancer treatment. Biomedicines 2017;5:34.
69. ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). Identifier NCT03236428, Phase II study of the CD38 antibody daratumumab in patients with high-risk MGUS and low-risk smoldering multiple myeloma; 2017. Available from: http://clinicaltrials.gov/ct2/show/NCT03236428.

Cancer Research
The Journal of Cancer Research (1916–1930) | The American Journal of Cancer (1931–1940)

AACR American Association for Cancer Research

From MGUS to Multiple Myeloma, a Paradigm for Clonal Evolution of Premalignant Cells

Niels van Nieuwenhuijzen, Ingrid Spaan, Reinier Raymakers, et al.

Cancer Res 2018;78:2449-2456. Published OnlineFirst April 27, 2018.