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Advances in Biology and Therapy of Multiple Myeloma

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Advances in Biology and Therapy of Multiple Myeloma

To my parents Gulab and Chandravadan Munshi who have inspired me and taught me what I know

and

To my wife Medha and sons Vidit and Manit whose love, support, and sacrifice helped me become who I am

Nikhil C. Munshi

To my mentors who sparked my scientific and clinical interest in myeloma

To my fellow researchers and caregivers, with whom I have been privileged to work

To my patients, who are my true heroes and inspire all that I do

And to Cynthia, Emily, David, and Peter for their loving support

Kenneth C. Anderson

Preface

Multiple myeloma has evolved from an incurable disease with no therapeutic options 5 decades ago to a readily treatable disease, based upon increased understanding of its biology and pathogenesis. Nonetheless, myeloma remains a complex disease driven by both genomic and epigenetic alterations. Moreover, interaction of tumor cells with the bone marrow microenvironment confers additional tumor cell growth, and survival advantage, and drug resistance. Advances in our understanding of the pathobiology of the disease have also translated to improved diagnostic and prognostic methods including high-throughput genomics, serum-free light chain, MRI, and PET scanning. Notably, proteasome inhibitors, immunomodulatory agents, as well as other targeted agents, when used singly or in combination, have transformed myeloma therapy and now achieve unprecedented frequency and extent of response. These rapid advances highlight the need for a state-of-the-art resource focused on the biology of myeloma and its clinical application. Our book describes the basic advances in our understanding of the disease biology and delineates molecular mechanisms mediating tumor growth and progression, as well as bone disease and organ dysfunction. Importantly, it provides the preclinical rationale for and clinical efficacy of single and combination targeted therapies directed at the tumor cell in its bone marrow milieu. With an eye toward the future, we update the recent advances using high-density, high-throughput genomic technologies to integrate both DNA and transcriptional changes for improved molecular classification and personalized therapeutic options. Finally, since studies are already reporting prolonged disease-free survival in myeloma, our book highlights the fact that we are now at the threshold of curative outcome in this disease.

Boston, MA, USA

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Chapter 1
Genomic Strategies Determining Progression from MGUS to Multiple Myeloma

Esteban Braggio and Rafael Fonseca

Abstract In the past years we have learned much about the genetics and biology of multiple myeloma (MM) and plasma cell disorders. It is now clear that (nearly) all MM cases are preceded by a benign phase of expansion of monoclonal PCs known as monoclonal gammopathy of undetermined significance (MGUS). It is also known that MGUS is a common condition that increases in prevalence with advancing age. Trying to couple the understanding we have of the genetics of the disease with the specific risk of progression from MGUS to MM could be of importance in determining different risk of progression with associated management strategies. Currently there is limited information regarding the specific factors that drive the progression from MGUS to MM and the risk associated with different genetics classes of the disease. A better understanding of the biologic steps that are needed for progression from a benign stage to a malignant form of the disease is needed, as well as better markers to provide a more dynamic monitoring for incipient disease evolution. In this chapter we discuss some of the background knowledge and basic biology of the disease, and some future strategies for a better surveillance of patients with MGUS.

1.1 Myeloma

Multiple myeloma (MM) is a malignancy that affects nearly 20,000 new cases per year in the USA, and at any given time, there are ~60,000 people living with this disease [1]. Despite many advances in therapeutic options offered, the disease

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remains lethal for the majority of patients. Recent studies have shown at least doubling, if not tripling, of the expected median survival time for patients with MM with the advent of novel therapeutics such as bortezomib and lenalidomide, alone or in combination with other agents such as thalidomide, alkylators, and corticosteroids [2–12]. Better approaches for the early detection of MM as well as for the generation of strategies to prevent the progression from MGUS to MM are needed as they would reduce the morbidity associated with disease progression and would allow for a better quality of life with early treatment of those at imminent risk for disease progression.

MM is characterized by the expansion of monoclonal plasma cells (PCs), mostly restricted to the bone marrow (BM) microenvironment for their survival. In most patients, the presence of circulating monoclonal PCs is at very low numbers, thus not detectable through the standard clinical testing (e.g., hemogram). Plasma cells are detected at high numbers in the blood only in cases of very aggressive MM such as in plasma cell leukemia (PCL).

Monoclonal PCs are characterized by the production of monoclonal immunoglobulins [13]. In all cases, PCs produce at least a light chain (kappa more commonly than lambda) and frequently a heavy chain (mainly IgG and in decreasing proportion IgA, IgD, and IgE). The heavy chain is secreted from the cells into the BM interstitial fluid and ultimately reaches the circulation and allows for monitoring for the disease as well as responsiveness to treatment. The test that has been classically used for the detection of monoclonal immunoglobulins is the serum protein electrophoresis and is widely available as a routine clinical test, often leading also to the diagnosis of MGUS. Multiple additional assays exist for the determination and measurement of these monoclonal proteins [immunofixation, quantitative immunoglobulins, serum free light chains (FLCs), and urine tests] that will not be further discussed in this chapter [14].

As a consequence of the growth of monoclonal PCs in the BM, once the disease becomes malignant, patients will have a reduction in normal hematopoiesis resulting in anemia and other cytopenias [15–18]. Thrombocytopenia is uncommon except in cases with very advanced plasmacytosis and extensive replacement of the BM by clonal PCs. As discussed elsewhere in this book, the clonal PCs produce substances that affect the normal bone metabolism resulting in bone loss, manifested as osteoporosis, lytic bone lesions, and the associated consequences including bone fractures [19]. Additionally, patients can also have other organs damaged, particularly renal insufficiency, as a consequence of the clonal growth and the production of monoclonal immunoglobulin [15–17, 19, 20]. The circulating light chains are small enough that they can be filtered into the tubular structures of the kidney, causing cast nephropathy and renal failure in a subset of patients with MM. The treatment of the disease can prevent further deterioration of bone lesions, but rarely do these lesions completely heal once they have been established. Patients can have improvements in bone stability as a consequence of sclerosis of the rims of those lesions as well as healing associated with some of those fractures; however, loss of natural function is common after a fracture secondary to MM. One of the complications that can be irreversible in patients with MM is the development of renal failure. While the failure

can be reversible if treated soon after it is established, patients who present with more protracted pictures will have difficulty in reversing the renal function and frequently will ultimately require hemodialysis. Conversely, the development of anemia or hypercalcemia can usually be reversed after medical interventions such as transfusions, intravenous fluids, or the use of steroids.

It is the presence of these bone lesions and renal failure that are dreaded complications of MGUS progressing to MM and that would be desirable to prevent at the preceding stage. As will be discussed below, however, it is currently difficult to know which patients are at risk for progression from MGUS to MM. A future where patients can be readily identified when they are at imminent risk of progression would be ideal, given that early treatment potentially could delay the development of MM and certainly would be useful in preventing organ damage, such as bone lesions and renal failure. Better management strategies are needed for the monitoring and surveillance of these patients.

MGUS is characterized by the clonal expansion of PCs inside the BM but without harm to the patient. The difference with MM is that this clonal expansion is discreet enough such that the PCs did not cause negative effects on the surrounding hematopoietic cells and patients therefore have no anemia. Likewise, patients have no evidence of lytic bone deletion or bone destruction and usually the protein production is low enough that the patients will not have renal failure [21]. MGUS is present in ~3% of the population over the age of 50, and the prevalence increases with advancing age [21, 22]. For practical purposes, it is important to differentiate those MGUS of the IgM type versus those that are not IgM. The IgM-type MGUS is derived from lymphoplasmacytic cells instead of from PCs and put the person at risk for the development of lymphoid disorders such as Waldenström's macroglobulinemia but not MM [21]. Relevant to this chapter are those gammopathies that are IgA, IgG, or light chain only. The group at Mayo Clinic in Rochester has recently described the presence of an MGUS variant characterized exclusively by the presence of circulating light chains with no heavy chain attached [23]. Biologically, these cases are likely to be variants of other PC clonal expansion processes and will also likely include the similar genetic abnormalities in those cells.

Clinically, the management of patients with MGUS includes routine surveillance to detect early signs of disease progression. However, the criteria that determine progression remain largely subjective and are often fraught with clinical nuances that make it difficult to determine which patients are in need of therapy versus those that are not. Extreme clinical situations are easily identified such as a patient with no evidence of complications, whatsoever, versus a patient who has clear need for treatment. However, frequently the clinician may encounter patients who have intermediate stages of a mild anemia or mild renal insufficiency where a subjective determination has to be made regarding the need for therapy. In addition, it is conceivable that some patients that present with a light chain that is particularly damaging to the kidney will result in a phenotype of renal failure despite a clonal process that is rather quiescent. It is then that the clinical classification of MM complications does not necessarily fully correspond to the stages of clonal evolution present inside the BM.

Patients are usually reassured of the benign nature of the disease when they receive a diagnosis of MGUS. These patients will be counseled to have at least a yearly determination of their monoclonal protein as well as measuring hemoglobin, calcium, and creatinine levels. However, the optimal strategy for determining the patient at imminent risk for progression or detection of early progression is not well defined. Recent studies have identified that patients with high concentration of monoclonal protein, serum FLCs, and ultimately indirect markers of increased tumor bulk are at greater risk for disease progression [24, 25]. Results showed that the risk of progression in patients with abnormal FLC κ/λ ratios was significantly higher than that in patients with normal ratios. Furthermore, the risk of progression increased as κ/λ ratios became more extreme. It is our hypothesis that this greater risk of progression results from a higher probability of acquiring a secondary genetic abnormality (stochastic) since there are a greater number of cells at risk for disease progression.

Like MM, MGUS is more common in individuals of African origin. Population-based studies have shown that MM appears to be at least two times more common in individuals of African origin than in individuals of Caucasian descent [26, 27]. A recent study conducted in Ghana showed that patients there have twice the prevalence of MGUS as do patients in the USA, indicating that the higher prevalence of MM in Africans is not due to a higher rate of disease progression, but rather due to a higher propensity for developing MGUS [27]. Likewise, another study looking at serially collected sera from two patient populations has conclusively shown that all MM patients are preceded by an MGUS stage [28, 29]. It is then that we understand that MGUS is of paramount importance to understanding MM. Furthermore, the majority of genetic abnormalities observed in MM are also observed in MGUS, and the specific prevalence and implications will be discussed below. By performing comparative longitudinal genetic analysis between the clonal cells of MGUS and MM, we would expect to be able to elucidate those genetic factors that result in more rapid cell growth or ineffective apoptosis ultimately leading to clonal expansion and a malignant phenotype.

1.2 The Genetics of Plasma Cell Disorders

Over the past 15 years, much progress has been made in understanding the genetic basis associated with the clonal expansion of PCs. At the very top level, two major genetic subtypes of MM can be found: the so-called hyperdiploid MM (H-MM) and the non-hyperdiploid MM (NH-MM) [30–32]. The H-MM variant is characterized by the presence of multiple trisomies, particularly affecting the odd-numbered chromosomes 3, 5, 7, 9, 11, 15, 19, and 21 [31, 33–36]. The recurrent pattern observed suggests that the presence of extra copies of these specific chromosomes somehow results in a favorable environment for the clone, allowing further expansion and proliferation. Patients with H-MM can have a number of other genetic abnormalities such as gains of 1q and deletions of 1p, 13, 17p, as well as *IgH*

translocations. However, the overall majority of patients with H-MM will not have *IgH* translocations, which are the hallmark of the NH-MM as will be discussed below. Indeed, if the H-MM have *IgH* translocations, they are likely to belong to either the t(4;14) group or patients with the less common forms of the *IgH* translocations. Several studies have shown that the presence of H-MM is an early event in the clonal expansion of PCs and can be readily detectable in cases of MGUS [37–39]. Furthermore, the presence of H-MM has been associated with a more favorable outcome among MM patients [40–42]. Patients with H-MM have a propensity to have a higher extent of bone disease and tend to be older than those patients without H-MM. It is currently unknown whether there is a negative or a positive effect on the likelihood of progression from MGUS to MM in patients who have H-MM.

The other main genetic subgroup is the NH-MM. This genetic subtype of the disease, which comprises approximately half of patients, is mainly characterized by the presence of *IgH* translocations. These translocations are thought to be seminal events in the pathogenesis of the disease and involve an array of recurring chromosomal partners. The most common chromosomal translocations include the t(4;14) and the t(11;14) present in about 15% of patients each, and the t(14;16) present in ~5% of patients. In lower frequency are found the t(6;14) and the t(14;20) present in 3–5% of patients each. There are a number of other translocations, which are less common [43–45]. These translocations are present since the early stages of the disease and when present are detectable in a large majority of clonal PCs [42, 46]. In fact, those clonal cells without the translocation likely are not displaying it due to technical issues. Nevertheless, a recent paper suggested that the *IgH* translocations could be present in a lower percentage of clonal cells, and if indeed confirmed, this would have profound implications regarding the importance of chromosomal translocations in the pathogenesis of the disease [47]. Nevertheless, the vast majority of clinical studies published so far shows that the majority of clonal cells harbor these chromosomal translocations [42, 46, 48].

1.2.1 Translocation t(11;14)

The t(11;14) results in the upregulation of the *CCND1* gene and, in general, results in a more favorable outcome [42, 46, 49–51]. Nevertheless, there are certain subtypes of MM with the t(11;14) that display a more aggressive behavior. Interestingly, the cells that harbor this translocation frequently display lymphoplasmacytic morphology with scant cytoplasm [52, 53]. It is also notable that other malignancies affecting late-B cell and PCs are also enriched for this translocation. In particular, a large fraction of patients with primary PCL can harbor the t(11;14) as well as patients with IgM-variant MM [54]. It is also remarkable that nearly half of patients with light chain-associated amyloidosis, a disease state that can result from a minimal plasmacytosis, will harbor this translocation [55]. The translocation is found in patients with remarkable genomic stability showing few other changes on top of the t(11;14).

1.2.2 Translocation t(4;14)

The t(4;14) results in the upregulation of the *FGFR3* and *MMSET* genes. This translocation has been associated with a more aggressive clinical behavior and has been associated with a shorter survival [42, 46, 50, 56, 57]. On the other hand, patients with this translocation have a lower prevalence of lytic bone lesions. These patients had a shorter progression-free survival when treated with standard chemotherapy followed by a single autologous stem cell transplant [46, 50, 58, 59]. It appears that this group of patients benefits from the addition of bortezomib as part of the first-line therapy of the disease, although when this translocation is used as a prognostic factor for groups treated with bortezomib, it still retains its prognostic significance [5, 60]. Early attempts at blocking the expression of *FGFR3* associated with this translocation have been unsuccessful, although elegant preclinical work has shown that targeting *FGFR3* may be of benefit in the management of MM patients with this specific abnormality [61, 62].

1.2.3 Translocation t(14;16) and Other MAF Translocations

The t(14;16) results in the upregulation of the *MAF* proto-oncogene [63]. This translocation is quite similar to the t(14;20), which results in the upregulation of *MAFB*. In several clinical series, the t(14;16) has been associated with a more aggressive behavior and shorter survival [42, 64]. A similar inferior outcome was also associated with t(14;20), affecting *MAFB* [65]. A recent study questioned the usefulness of t(14;16) as a prognostic marker, but at least four other studies have shown that patients with this abnormality have a shorter survival [66]. Even in the study that questioned its prognostic value, patients with t(14;16) had a higher number of circulating PCs, something that has been described as an adverse prognostic factor in MM. We have recently reported that patients with this translocation have absent expression of NCAM [67], something that could explain a higher propensity of PCs with the t(14;16) to be circulating in extramedullary sites and locations such as the peripheral blood [68]. No specific therapeutic strategies have been developed so far for the management of patients with t(14;16).

1.2.4 Deletion of Chromosome 13

Most cases with abnormalities in chromosome 13 are characterized by monosomy 13 (~85%), whereas the remaining 15% of cases have interstitial deletions [69, 70]. Deletion of chromosome 13 was initially associated with shorter survival in patients with MM [71–74]. It is now clear that this effect was the consequence of deletion 13 being an indirect marker for NH-MM. In particular, chromosome 13 is enriched in

patients with the t(4;14) and the t(14;16) and therefore preselected patients with aggressive variants of the chromosomal translocations [32, 46, 56]. Thus, at least 80% of patients with t(4;14) will also harbor deletion 13. It is no longer considered a high-risk genetic marker, although patients with this abnormality in general tend to have a shorter survival because of the association with the aforementioned translocations. Deletion 13 nevertheless appears to be important in the pathogenesis of the disease, as it is clonally selected and present in the majority of the clonal PCs [69]. The exact genes involved in the pathogenesis of MM associated with chromosome 13 have not been fully elucidated, although most genetic mapping studies continue to point at *RB1* as a suspect gene associated with deletion 13.

1.2.5 Deletion of 17p13

Deletions of the short arm of chromosome 17 are also thought to be important in the pathogenesis and progression of MM. Indeed, deletion of 17p13 remains the single most important genetic prognostic factor in MM [42, 46, 64, 75]. As in the case of deletion 13, the exact gene(s) have not been fully elucidated, although a body of data suggested *TP53* being the critical gene. Deletion of 17p13 is present in ~10% of patients but increases in prevalence with advancing stages of the disease [42, 76]. We have recently found that this abnormality is present in 20% of MM patients at the time of their first relapse and 30% of the patients at second relapse and later. Furthermore, we have shown that patients with PCL [76] as well as human myeloma cell lines (HMCLs) have a very high prevalence of chromosome 17 deletions and *TP53* mutations [77]. In contrast, deletion 17p13 appears to be quite rare in patients with MGUS and smoldering MM (SMM). In our series, we have found only one MGUS patient with deletion 17, and the prevalence in SMM disease has been estimated at 3%. While only longitudinal and prospective studies can fully address the question as to whether deletion 17 could be associated with the progression of the disease, the cross-sectional evaluation leaves no doubt that the acquisition of deletion 17 is a progression event, and therefore a patient in early stages of the disease with this abnormality should be considered at potential risk of earlier disease progression.

1.2.6 Chromosome 1

Copy number gain of 1q21 is among the most commonly reported genetic abnormalities seen in MM cases [31, 40, 78, 79]. A putative target of this amplification is *CKS1B*, which promotes the degradation of p27, an inhibitor of cell cycle progression. Previous studies suggest that gain of 1q21 is associated with both disease progression and poor prognosis [80]. It was shown that the prevalence of 1q abnormalities goes from 0% in MGUS to 43% in newly diagnosed MM and 72% of