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Multiple Myeloma and Other Plasma Cell Neoplasms

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Epidemiology and Pathophysiology of Multiple Myeloma

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Malin Hultcrantz, Gareth J. Morgan, and Ola Landgren

1.1 Introduction

Multiple myeloma is characterized by an abnormal plasma cell proliferation and in the majority of patients, production of monoclonal immunoglobulin heavy chains (M-protein) or light chains (Morgan et al. 2012). Findings of an M-protein in the blood of asymptomatic patients were first described in 1960 by Professor Jan Waldenström who called this condition "essential hypergammaglobulinemia" (Waldenstrom 1960). Kyle later observed that patients with monoclonal gammopathies were at a higher risk of developing plasma cell malignancies primarily multiple myeloma and thus concluded that this gammopathy was not always benign. They therefore coined the term monoclonal gammopathy of undetermined significance (MGUS) (Kyle 1978). More recent studies on sequential serum samples by Landgren et al. revealed that multiple myeloma is consistently preceded by MGUS (Landgren et al. 2009a). In a recent large screened study, the overall prevalence of MGUS was 2.4% with the highest prevalence observed in the African-American population (Landgren et al. 2014).

Myeloma has traditionally been associated with a poor outcome; however, the median survival has improved across all age groups after the introduction of novel agents more than 15 years old(Kristinsson et al. 2014). Importantly, survival has continued to improve with the subsequent development of second and third generations of the proteasome inhibitors and immunomodulatory drugs as well as new treatment options such as monoclonal antibodies (Kristinsson et al. 2014).

Genetically, multiple myeloma is a complex disease including multiple genetic hits and branching disease evolution. During progression from MGUS to multiple myeloma, plasma cells acquire a number of genetic hits and the ability to evade the immune system. The techniques to detect genetic aberrations and functional changes are becoming increasingly sensitive and precise. With the use of massive parallel sequencing, we have gained important insights on disease evolution during the recent years. In addition to cytogenetic changes, somatic mutations affecting various cellular mechanisms have been identified in myeloma (Manier et al. 2017). Furthermore, in myeloma there is an intense interplay with the bone marrow microenvironment and immune system acting in various ways to promote disease progression. Here we

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describe the epidemiology and pathophysiology including the genetic landscape and the role of the bone marrow microenvironment of multiple myeloma.

1.2 Epidemiology

Multiple myeloma is the second most common hematological malignancy in adults in Western countries with an age-adjusted incidence of 5/100,000 individuals in Western countries (Velez et al. 2016; Siegel et al. 2016). Myeloma is more common in the elderly population; the median age at diagnosis is 69–70 years (Kristinsson et al. 2007). Myeloma is consistently preceded by the precursor state monoclonal gammopathy of undetermined significance (MGUS) (Landgren et al. 2009a). The disease trajectory spans from MGUS which can progress to smoldering multiple myeloma and to multiple myeloma requiring therapy (Rajkumar et al. 2014). The rate of progression from MGUS to myeloma is 0.5–1% per year (Kyle et al. 2010; Turesson et al. 2014).

The etiology of MGUS and myeloma is not fully understood, but a number of host factors as well as external factors are of importance for disease evolution. Host factors include age where older individuals have a higher risk of developing myeloma. MGUS and myeloma are more common in men, and there are racial disparities in regard to incidence; MGUS and multiple myeloma are more common in African-American and African blacks compared to whites and Mexican Americans (Landgren et al. 2014; Landgren et al. 2007; Waxman et al. 2010). In a recent population-based screening study, the prevalence of MGUS was 3.7% in African-American blacks, 2.3% in whites, and 1.8% in Mexican Americans (Landgren et al. 2014). Genome-wide association studies have identified several single nucleotide polymorphisms associated with myeloma development indicating an inherited susceptibility (Morgan et al. 2014). Furthermore, exposure to certain pesticides and herbicides including Agent Orange has been correlated to an increased risk of developing MGUS (Landgren et al. 2009b; Landgren et al. 2015).

1.3 Genetic Landscape of Multiple Myeloma

Genomic instability plays a major role in the pathogenesis of multiple myeloma and the disease including translocations, copy number abnormalities, as well as somatic mutations (Bianchi and Ghobrial 2014). The disease is heterogeneous and includes a number of subclones which evolve in a branching pattern similar to Darwinian evolution (Bolli et al. 2014; Walker et al. 2014). Initial genomic analyses captured mainly gross anatomical aberrations, while more modern techniques have rendered new insight to disease pathogenesis and individual disease patterns. The myeloma genome was first assessed using metaphase cytogenetics which is of limited value in myeloma due to limited sensitivity and the low proliferation of terminally differentiated plasma cells. Fluorescence in situ hybridization (FISH) is widely used in clinical praxis to assess translocations and copy number variations. Interphase FISH can capture also cryptic aberrations; however, FISH is hampered by several limitations; it detects only known genetic aberrations, the sensitivity is limited, and the analyses are labor intensive. Gene expression profiling was developed as a prognostic model that can be used within certain given therapies. More recently, massive parallel sequencing techniques with high-throughput sequencing of DNA have revolutionized genomic analyses. Using whole genome, whole exome, as well as targeted sequencing, great insights have been gained into the genomic landscape of multiple myeloma (Bolli et al. 2014; Chapman et al. 2011; Lohr et al. 2014; Walker et al. 2015a). Sequencing techniques have also been used to detect IgH translocations and hyperdiploidy; the modern techniques tended to be more sensitive compared to interphase FISH (Bolli et al. 2016). Here, we describe the emerging field of genomics in myeloma from cytogenetics and FISH to gene expression

profiling and next-generation sequencing techniques.

1.4 Chromosomal Abnormalities

Myeloma can broadly be divided into two groups based on chromosomal aberrations; translocations involving IgH on chromosome 14 and hyperdiploidy. These events are considered initiating or primary events indicating that evolution to myeloma can follow at least two distinct pathways (Stella et al. 2015). However, these events by themselves do not seem to be sufficient for myeloma development as they are found already at the MGUS stage (Fonseca et al. 2002). IgH translocations are found in 45% of patients and hyperdiploidy in 50% of patients with myeloma (Manier et al. 2017). Approximately 10% of myeloma patients harbor both an IgH translocation and hyperdiploidy, while in 5%, neither IgH translocations nor hyperdiploidy can be detected. In addition to these primary cytogenetic events, a number of chromosomal gains and losses as well as somatic mutations are found in myeloma and can offer additional prognostic information (Stella et al. 2015).

1.4.1 IgH Translocations

Translocations occur when double-stranded DNA breaks and is aberrantly rejoined (Walker et al. 2013). During the maturation process of B-lymphocytes in the germinal center of the lymph nodes, there is genetic editing in the immunoglobulin heavy chain (IgH) gene to enhance the affinity of the antibody. First, there is a rearrangement of the hypervariable region (V-D-J) in a process called *somatic hypermutation*. Later, the cell undergoes *class-switch recombination* which results in antibodies of different isotypes (Nutt et al. 2015). Both somatic hypermutation and class-switch recombination infer double-stranded DNA breaks in the immunoglobulin locus (14q32) and require expression of activation-induced deaminase (AID). Despite rigorous control mechanisms, this genetic editing may result in aberrant rejoining and thus chromosomal translocations (Morgan et al. 2012; Manier et al. 2017; Walker et al. 2015b). The majority of IgH translocations occur during class-switch recombination or somatic hypermutation, but translocations can also occur at various stages during B-cell development including early stages of pro-B-lymphocytes (Walker et al. 2013).

The most common IgH translocations in myeloma are *t*(4;14), *t*(6;14), *t*(11;14), *t*(14;16), and *t*(14;20), all resulting in an oncogene being placed under the strong IgH enhancer and are thus overexpressed. The net effect in the majority of these translocations is promotion of cyclin D proteins resulting in propagation of the cell cycle from G1 to S phase and a selective advance for the clone in question (Walker et al. 2013). Furthermore, the translocation partner gene is mutated in 10–25% of cases (Walker et al. 2015b). Translocations including IgH have different implications for disease prognosis and assessment for IgH rearrangement is recommended in the workup of myeloma patients (Rajkumar et al. 2014).

Translocation (11;14) between chromosome 11q13 (*CCND1*) and chromosome 14q32 is the most common translocation and is found in 15–20% of myeloma patients (Manier et al. 2017). The translocation results in the upregulation of *CCND1* and promotion of the cell cycle. Translocations between chromosome 11 and 14 are found also in mantle cell lymphoma, however, with a different breakpoints, and in 50% of patients with AL amyloidosis (REF). There is an ambiguous information on the prognostic information of *t*(11;14) in myeloma. Overall, it is considered to have a neutral impact, but there are indications that combination of *t*(11;14) translocation and *CCND1* mutations is associated with a poor prognosis (Bolli et al. 2014; Walker et al. 2015b). Concomitant *t*(11;14) translocations and CCND1 mutations are found in 10% of patients arising through a mechanism called *kataegis* (Bolli et al. 2014). Furthermore, the *t*(11;14) translocation often occurs early in B-cell development, already at the pro-B-lymphocyte stage (Walker et al. 2013), which may be an explanation behind the lymphoma-like phenotype

observed in some cases. Patients with *t*(11;14) translocations can have a lymphoplasmacytic differentiation, CD20 overexpression, and light-chain restriction. These patients may not respond as well to traditional myeloma drugs, and recently phase I/II studies indicate that these patients may respond better to treatment with novel drugs developed primarily for lymphoma (Sonneveld et al. 2016; Kumar et al. 2016; Moreau et al. 2016).

The *t*(4;14) translocation is cryptic and is not detected by traditional metaphase cytogenetics. Therefore, FISH or polymerase chain reaction (PCR) must be performed for detection (Stella et al. 2015). Translocation (4;14) juxtaposes the genes *MMSET* and *FGFR3* from chromosome 4p16 to IgH enhancers whereby these genes are overexpressed (Sonneveld et al. 2016). The breakpoint on chromosome 4 falls between the two genes, and MMSET remains on der(4), and FGFR3 is translocated to der(14) (Walker et al. 2013). *MMSET*, which affects epigenetic regulation through histone modification, is expressed in 100% of these translocations, while sustained expression of *FGFR3*, which is an oncogenic receptor tyrosine kinase, is detected in 75% (Stella et al. 2015; Lawasut et al. 2013). There is recent data indicating that both genes are important for initial transformation but that sustained expression of *FGFR3* is not essential and this part of the der(14) is deleted in 25–30% of cases with *t*(4;14) (Walker et al. 2013). In fact, a recent study on gene expression revealed that myeloma patients who have gene expression signature similar to those with the *t*(4;14) translocation, i.e., *MMSET*-like signatures, have an equally poor prognosis even though they are lacking the actual translocation (Wu et al. 2016). Translocation *t*(4;14) is associated with a poor outcome, both in regard to progression-free survival and overall survival (Sonneveld et al. 2016; Chng et al. 2014). Treatment with bortezomib and carfilzomib seems to at least partly overcome the adverse outcome in patients with *t*(4;14) (Sonneveld et al. 2016).

Translocations *t*(14;16) and *t*(14;20) affect the *c-MAF* proto-oncogene and the *MAFB* oncogene, respectively, and result in their overexpression (Sonneveld et al. 2016). These in turn affect *CCND2* which also promotes proliferation by affecting the regulation of the G1/S phases of the cell cycle (Stella et al. 2015). Both *t*(14;16) and *t*(14;20) are associated with a poor outcome (Sonneveld et al. 2016). In addition to upregulation of *c-MAF*, the chromosome 16 breakpoints in *t*(14;16) falls within the last intron of *WWOX*, a known tumor suppressor gene, resulting in the disruption of *WWOX* (Walker et al. 2013).

More rare translocations are *t*(6;14)(q21;q32) and *t*(12;14)(p13;q32) involving *CCND3* and *CCND2*, respectively, and also leading to upregulation of these cyclin D proteins and an overall promotion of the cell cycle. An alternative translocation also involving chromosome 6 is *t*(6;14)(p25;q32) where *IRF4* is juxtaposed to IgH on chromosome 14 (Stella et al. 2015). There is limited information on the impact of the latter translocation on outcome in myeloma patients.

1.4.2 Hyperdiploidy

Patients with hyperdiploidy have gains of odd numbers of chromosomes, 3, 5, 7, 9, 11, 15, 19, and 21, and the cells harbor in total between 48 and 75 chromosomes. The mechanism behind hyperdiploidy is less clear, but the leading hypothesis is that all chromosome gains occur during one unsuccessful mitosis rather than consecutive gain of one chromosome at a time (Manier et al. 2017). Patients with hyperdiploidy are a heterogeneous group, but overall, they have a better prognosis compared to patients with IgH translocations (Stella et al. 2015; Avet-Loiseau et al. 2009). Hyperdiploidy is more often associated with IgG kappa myeloma, and patients are overall older compared to patients with IgH translocations (Stella et al. 2015). In addition to the gains of odd number of chromosomes as a probable initiating hit, these patients often have additional translocations as secondary hits. The most common are del1p, +1q, del17p, and translocations and amplifications including the *MYC* locus on 8q24.

1.4.3 Secondary Translocations in Myeloma

In addition to IgH translocations and hyperdiploidy, gains and losses of chromosomal material are seen at diagnosis and then increasingly as the disease progresses. Translocations including *MYC* on 8q24 are frequently seen in myeloma, up to 18% of newly diagnosed patients and as many as 50% of patients in the relapse setting (Stella et al. 2015; Walker et al. 2015b). *MYC* has a number of translocation partners, and *MYC* rearrangements, leading to *MYC* upregulation, are associated with a poor outcome. The most common translocation partners were the immunoglobulin heavy- and light-chain genes *IGH*, *IGL*, and *IGK* as well as additional genes frequently involved in myeloma, e.g., *FAM46C* (Walker et al. 2015b). In addition, similar to *t*(11;14) and *CCDN1* mutations, there is also evidence of *kataegis* where *MYC* translocations are combined with mutations in *MYC* (Manier et al. 2017).

1.4.4 Copy Number Variations

Gains or amplifications of 1q21 are associated with a poor overall survival and are more frequent in relapse and posttreatment samples. The minimally amplified region contains 679 genes, of which several oncogenes such as *CKS1B* and *ANP23E* have been identified. *CKS1B* encodes for a cell cycle-regulating protein which activates cyclin-dependent kinases and induces ubiquitination of inhibitory proteins, thus promoting cell proliferation (Stella et al. 2015).

Thirty percent of myeloma patients harbor deletions of the short arm of chromosome 1. Deletion 1p is associated with an adverse prognosis and can involve primarily two regions: 1p12, 1p32, or both. The first, 1p21, harbors the tumor suppressor gene *FAM46C* whose function is of importance for protein translation. Moreover, 1p32 harbors *CDKN2C* and *FAF1*. *CDKN2C* inhibits cell cycling and preserved the cell in the G1 phase. Deletion of *CDKN2C* thus results in more rapid cell cycling (Stella et al. 2015). *FAF1* encodes for a protein involved in initiation and promotion of apoptosis (Manier et al. 2016a).

Deletion 17p is associated with a poor prognosis in myeloma as in many other hematological malignancies. *TP53*, an important DNA repair and tumor suppressor gene, is situated on 17p13, which is always included in the minimally deleted region on 17p. 17p deletions are seen in 10% of newly diagnosed myeloma patients and up to 80% of patients in later disease stages (Manier et al. 2017). Biallelic deletions of 17p or 17p deletion combined with *TP53* mutation on the remaining allele are common and associated with poor outcome (Weinhold et al. 2016a). Liu et al. recently reported from a mouse model study that 17p13 deletions were associated with a worse prognosis compared to *TP53* mutations. Their results indicated that there may be additional loci on 17p13 contributing to tumor progression through mechanisms independent of *TP53* (Liu et al. 2016).

Del13q is present in 40–50% of myeloma patients and is more common in IgH-translocated myelomas. In the majority of cases, the whole long arm of chromosome 13 is deleted. The minimally deleted region includes the tumor suppressor gene *Rb1* which has a role in cell cycle regulation. *DIS3* which is often mutated or deleted in myeloma is also located on the long arm of chromosome 13, however, not in the minimally deleted region (Manier et al. 2017). Historically, del13q has been associated with a poor prognosis; however, the majority of patients with 13q also harbor *t*(4;14) translocations. Therefore, it is currently not obvious whether del13q has a prognostic implication independent of *t*(4;14) translocations (Tables 1.1 and 1.2) (Manier et al. 2016a).

Table 1.1 High-risk and standard-risk cytogenetic aberrations (Sonneveld et al. 2016)

High-risk cytogenetic aberrations	Standard-risk cytogenetic aberrations
t(4;14)	*t*(11;14)
t(14;16)	*t*(6;14)
t(14;20)	
del(17/17p)	
gain(1q)	
Non-hyperdiploidy	

Table 1.2 Most common cytogenetic aberrations in myeloma and the genes involved

Chromosomal aberration	Genes involved
t(4;14)(p16;q32)	MMSET/FGFR3-IGH
t(6;14)(p25;q32)	IRF4/IGH
t(6;14)(p21;q32)	CCND3/IGH
t(11;14)(q13;q32)	CCND1/IGH
t(14;16)(q32;q23)	IGH/c-MAF. WWOX disrupted
t(14;20)(q32;q11)	MAFB/IGH
8q24	MYC
del(17/17p13)	TP53
gain(1q)	CSK1B
del(13q)	Rb1, DIS3

1.5 Somatic Mutations

Through massive parallel sequencing, a number of recurrent somatic mutations have been identified in multiple myeloma by using next-generation sequencing (Bolli et al. 2014; Chapman et al. 2011; Lohr et al. 2014; Walker et al. 2015a). So far, no unique disease-specific gene mutation has been identified, but a number of the recurrently mutated driver genes have been described. The frequently mutated genes affect various cellular functions including the MAPK and NFKB signaling pathways as well as DNA repair, RNA editing, and cell cycling.

Mutations in *KRAS* and *NRAS* are observed in 50% of patients and are in the majority of cases mutually exclusive (Bolli et al. 2014; Lohr et al. 2014; Walker et al. 2015a). *KRAS* and *NRAS* are oncogenes which are mutated in a large spectrum of tumors and affect intracellular signaling through the RAS/MAPK pathway. Activation of the RAS/MAPK pathway alters gene expression ultimately affecting cell differentiation, proliferation, and survival. *BRAF* is also part of this signaling pathway and is mutated in 10% of myeloma patients. *BRAF* mutations are primarily found in codon 600 (V600E), same as in hairy cell leukemia, but additional mutations in *BRAF* have also been observed (Walker et al. 2015a). Walker et al. reported the mean clonal cancer fraction of *KRAS*, *NRAS*, and *BRAF* mutations to be around 30% suggesting that these mutations are secondary subclonal events associated with progression rather than being founder mutations (Walker et al. 2015a). The NFKB pathway is upregulated in myeloma cells leading to gene transcription and cell proliferation. This signaling pathway is important in myeloma cells which also is reflected in frequent mutations in a number of genes involved in this signaling pathway, e.g., *TRAF3*, *CYLD*, *MAP3K14*, *BIRC2*, *BIRC3*, *IKBKB*, and more (Lohr et al. 2014; Walker et al. 2015a).

DNA repair mechanisms are altered by somatic mutations and gene deletions of *TP53* and deletions of the short arm of 17p. Mutations and deletions affecting the 17p region become more frequent as the disease progresses and are associated with a poor prognosis (Manier et al. 2017). Mutations and deletions in *ATM* and *ATR*, which are part of the same DNA repair mechanism as TP53, are also commonly observed in myeloma (Walker et al. 2015a). Mutations involving genes associated with regulation of RNA editing and protein translation are common in myeloma. FAM46C and DIS3, both involved in RNA regulation and protein translation, are affected by inactivating mutations and/or deletions (Bianchi and Ghobrial 2014; Bolli et al. 2014; Lohr et al. 2014; Walker et al. 2015a).

In addition to several translocations and mutations affecting the cyclin D proteins, cell cycle regulation is affected through events resulting in the loss of function of negative cell cycle regulatory genes such as *CSKN2C*, *CDKN2A*, and *RB1*. These genomic events can be inactivating mutations, gene deletions, or a combination of both (Weinhold et al. 2016a). The most frequently mutated genes in myeloma are listed in Table 1.3.

Weinhold et al. recently observed that biallelic inactivating events are common in myeloma. These include deletions and/or inactivating mutations in known tumor suppressor genes such as *TP53*, *FAM46C*, *TRAF3*, *CYLD*, and more (Weinhold et al. 2016a). Biallelic events were more common in the relapse setting compared to newly diagnosed patients and are associated with adverse gene expression profiling signatures. Especially biallelic events including 17p deletion

Table 1.3 Most frequent genetic mutations in multiple myeloma

Gene	Frequency (%) (Bolli et al. 2014; Lohr et al. 2014; Walker et al. 2015a; Kortuem et al. 2016)
KRAS	20–23
NRAS	19–20
BRAF	6–12
FAM46C	6–11
TP53	3–12
DIS3	1–11
PRDM1	5
EGR1	2–6
SP140	4–6
TRAF3	2–5
CCND1	2–4
ATM	2–4
HISTH1E	3
CYLD	1–5
LTB	1–4
RB1	2–3
IRF4	3
STAT3	3
MAX	1–3
ATR	1–2

and *TP53* mutations were associated with a poor prognosis (Weinhold et al. 2016a).

1.5.1 Clonal Evolution

Chromosomal translocations are necessary but not sufficient for developing myeloma. MGUS and smoldering myeloma are similar to myeloma in regard to translocations, but myeloma is more genetically complex and has a higher mutational load (Walker et al. 2014; Malek et al. 2016). There is so far limited information on the genomic landscape and clonal evolution during transition from MGUS to smoldering myeloma to myeloma. Progression from MGUS to myeloma may be caused by acquisition of additional genetic events or the expansion of pre-existing clones already present at the MGUS stage. In the myeloma stage, there are often multiple disease clones present at diagnosis. Lohr et al. reported that most myeloma patients have at least three subclones and many patients had up to seven clones (Lohr et al. 2014). Their study was able to detect subclones that were at least 10% of the tumor sample, while in reality, the number of subclones per myeloma patients is likely far greater (Lohr et al. 2014). Furthermore, some mutations in myeloma tend to be clonal, e.g., *RB1*, *CCND1*, and *TP53*, while others are more often subclonal, e.g., *KRAS/NRAS* and *FAM46C*, indicating early vs later acquisition (Manier et al. 2017; Walker et al. 2015a).

Myeloma evolution has been shown to proceed according to a branching disease evolution driven by competing subclones (Morgan et al. 2012). Bolli et al. observed four different patterns of disease progression in patients where they had sequential samples. These included lineal evolution, branching evolution where there was a different dominant subclone at relapse, a new subclone had emerged in parallel with the original dominant clone, or the emergence of a new subclone while the original clone was not detectable (Bolli et al. 2014).

1.5.2 Prognostic Impact

So far, approximately 900 patients in four published studies have been sequenced using whole exome or targeted sequencing. Thus, there is currently no robust information on the prognostic effect of specific gene mutations. In these studies, mutations in *TP53*, *KRAS*, *STAT3*, *PTPN11*, *PRDM1*, *CXCR4*, *IRF4*, *MAFB*, *ZFHX4*, *NCKAP5*, and *SP140* were associated with a shorter overall and/or progression-free survival (Bolli et al. 2014; Lohr et al. 2014; Walker et al. 2015a; Kortuem et al. 2016). *TRAF3* was on the other hand associated with a longer progression-free survival (Kortuem et al. 2016); however, as mentioned, there is so far limited data to support these findings.

1.5.3 Relapse

Regarding chromosomal aberrations, high-risk features such as gain1q, del 17p, and genetic events involving MYC are more common in

relapse samples (Walker et al. 2015b; Kortum et al. 2016). Moreover, Weinhold et al. described higher frequencies of del(1p) and loss of heterozygosity at 6q and 16q (Weinhold et al. 2016a). Furthermore, mutations affecting specific treatment pathways such as cereblon, the target of immunomodulatory drugs (IMiDs), are more common in relapse samples compared to samples analyzed at diagnosis. In a recent study on 50 heavily pretreated myeloma patients, Kortuem et al. found cereblon-associated mutation in 25% of the relapse patients. All of these patients were refractory to IMIDs. These mutations included *CRBN*, *CUL4B*, *IRF4*, and *IKZF1*. In some of these, pretreatment samples were available for comparison. None of the pretreatment samples harbored the *CRBN*-associated mutations even with increased sequencing depth supporting that the resistance was indeed acquired over the course of the disease (Kortum et al. 2016).

In addition to *CRBN*, mutations were also found in the proteasome 19S subunit in patients that were refractory to proteasome inhibitors and immunomodulatory drugs. Kortum et al. also reported mutations in genes coding for proteasome subunits, i.e., *PSMB8* and *PSMD1*. In addition, mutations in *XBP1*, also found in one patient, have also been associated with PI resistance (Kortum et al. 2016). In the study by Kortuem et al., the majority of patients with *CRBN* mutation were found to be refractory to IMIDs (Kortum et al. 2016).

Weinhold et al. recently reported on sequential sequencing at diagnosis and relapse of 33 myeloma patients. The majority of the relapse samples showed a pattern of branching disease evolution. In the relapse samples, there were increasing proportions of 17p deletions, *TP53* mutations, as well as *MYC* translocations. There was also a higher mutational load in the relapse samples compared to the diagnostic samples, on average 43 nonsynonymous somatic mutations at presentation versus 60 at relapse. Furthermore, there were more biallelic events in tumor suppressor genes, e.g., *TP53*, *FAM46C*, and *TRAF3*, at relapse. No increase in *CRBN* mutations was observed (Weinhold et al. 2016a). These 33 patients were all treated on the total therapy protocols with a combination of alkylating agents and proteasome inhibitors. Weinhold et al. did observe any *CRBN* mutations; however, none of the patients were reported to be IMiDs refractory (Weinhold et al. 2016a).

1.6 Gene Expression

The first molecular classification in myeloma was performed using gene expression profiling. Assessing gene expression through microarray has provided a tool for prognostication that can contribute with additional information to conventional risk stratification using FISH. These analyses have revealed over- as well as underexpression of various genes including oncogenes, tumor suppressor genes, and cell signaling and transcription factor genes. The Arkansas group, the IFM group, and the HOVON group have all published gene expression signature that can predict favorable versus unfavorable outcome (Decaux et al. 2008; Kuiper et al. 2012; Shaughnessy et al. 2007).

Initially, Shaughnessy et al. within the Arkansas group identified a 70-gene signature (GEP70) based on myeloma patients treated within the total therapy protocols (Shaughnessy et al. 2007). Depending on the level of expression of these 70 genes, patients were classified into seven separate subgroups with high or low risk of disease progression. These seven subtypes largely corresponded to the most common chromosomal translocations and hyperdiploidy (Shaughnessy et al. 2007; Zhan et al. 2006). The seven subtypes were abbreviated MS, reflecting the activation of *MMSET* in the *t*(4;14) translocation, MF reflecting translocations *t*(14;16) and *t*(14;20) and activations of *c-MAF* and *MAFB*, CD-1 corresponding to *t*(11;14) and *CCND1* activation and CD-2 corresponding to *t*(6;14) translocation and activation of *CCDN3*, HY corresponding to the hyperdiploid karyotype, PR reflecting a subset of patients with a high disease proliferation, and LB which includes patients with a low prevalence of bone disease (Shaughnessy et al. 2007). In the newly diagnosed setting, around 10–15% had high-risk

signatures, while in the relapse setting, a significantly higher proportion of patients had gene expression profiles associated with a high risk of progression (Weinhold et al. 2016a; Shaughnessy et al. 2007; Weinhold et al. 2016b). The most common upregulated genes are found on 1q, and the majority of the downregulated genes are located on 1p (Shaughnessy et al. 2007; Zhan et al. 2006). The GEP70 model was able to predict outcome independently of the International Staging System (Shaughnessy et al. 2007).

Similarly, the EMC92 is based on a 92-gene signature, and the IFM model is based on a 15-gene signature. Of note, there is a little overlap in the genes included in the different models. Even though the gene expression profiles are powerful prognostic tools, the signature models have been developed in specific patient cohorts that were uniformly treated with clinical trials. The models perform well within their respective patient population, but overall, not all gene expression profiling models have held true when cross-validated between patient cohorts. In addition, gene expression profiling generates large amounts of data and requires complex analyses. This, together with issues getting sufficient RNA for the microarrays, has hampered the implementation of gene expression profiling in the general clinical praxis (van Laar et al. 2014).

Recently, a simplified subgroup classification was presented, which is based on gene expression profiles as well as DNA sequencing data in a subset of patients. The new classification includes five translocation cyclin (TC) subgroups identified as the name implies through translocations and deregulation of cyclin D (Stein et al. 2016). There were clear associations between chromosomal aberrations (TC subtypes), somatic mutations, and RNA expression. Interestingly, activation of the NFKB pathway and MAPK pathway was inversely associated, and activation of these pathways was different between the TC subtypes (Stein et al. 2016).

Looking forward, gene expression is being assessed using high-throughput RNA sequencing of bone marrow samples and single-cell analyses of circulating tumor cells in the peripheral blood (Lohr et al. 2016). Additionally, RNA assessment can identify subsets of patients with gene expression profiles mimicking those in the high-risk groups, such as the MMSET-like profile leading to a poor prognosis similar to patients who harbor the actual *t*(4;14) translocation as mentioned earlier (Wu et al. 2016). Studies including analyses of transcriptome modifiers such as alternative splicing, microRNAs, and epigenetic profiles are also ongoing (Szalat and Munshi 2015). Gene expression, particularly using RNA sequencing in combination with DNA sequencing will be of great interest to further delineate myeloma pathogenesis.

1.7 Bone Marrow Microenvironment

In addition to genomic aberrations and changes in gene expression, there is growing evidence that the bone marrow environment plays an important role in the pathogenesis of myeloma. There are multiple interactions, e.g., through direct cell–cell interactions and adhesion molecules, secretion of cytokines and chemokines as well as exosomes with miRNA, between the bone marrow niche and the malignant plasma cells. These interactions result in the promotion of tumor cells survival and proliferation. The bone marrow environment consists of a cellular component including hematopoietic and non-hematopoietic cells and a noncellular component including the extracellular matrix, liquid milieu, and oxygen level. There is a dense interplay including multiple feedback loops between all compartments with an overall effect of promoting malignant plasma cell growth and survival (Landgren 2013).

The hematopoietic cells within the cellular component are hematopoietic stem cells, myeloid cells, B- and T-lymphocytes, natural killer (NK) cells, dendritic cells, and macrophages. Several of these cells have an altered function in myeloma resulting in either immunosuppressive effects allowing the malignant plasma cell to evade the immune system or various mechanisms to support growth and survival of the myeloma clone (Manier et al. 2016b; Balakumaran et al. 2010).

The immunosuppressive mechanisms, often induced by the tumor cells, are mediated through expansion of regulatory/inhibitory immune cells, primarily myeloid-derived stem cells (MDSCs) and regulatory T-cells (Tregs). MDSCs are immature cells that under normal circumstances develop into granulocytes, macrophages, and dendritic cells. In myeloma, however, they remain in this early form with immunosuppressive properties and may enable immune escape and inhibit the T-cell response and thus facilitate myeloma cell growth (Malek et al. 2016; Gorgun et al. 2013; Kawano et al. 2015). Through bidirectional interaction, MDSCs assist in protecting the MM cells against chemotherapy and promote angiogenesis and metastasis (Malek et al. 2016). In addition, the MDSCs can contribute to bone destruction in myeloma by directly serving as osteoclast precursors (Kawano et al. 2015; Zhuang et al. 2012). IMiDs and bortezomib both act on myeloma cells and on the bone marrow microenvironment, however, they have not been shown to be effective in reversing the immunosuppressive effect of MDSCs (Gorgun et al. 2013; Kawano et al. 2015). Tregs are CD4+ T-cells characterized by the expression of the transcription factor FOXP3. In myeloma, Tregs accumulate in the blood and bone marrow, and an increasing number of Tregs have been associated with a poorer prognosis. Like MDSCs, Tregs also suppress an effective anti-myeloma immune response; the effect is mediated through inhibiting the function of normal antigen-presenting cells and effector T-cells either by direct contact or through cytokine secretion (Moschetta et al. 2016). In addition, dendritic cells, which promote either immunity or tolerance, and natural killer cells (NK cells) are observed to be functionally defective in myeloma further aiding myeloma cells to proliferate and evade the immune system (Kawano et al. 2015). In addition, NK cells expresses PD-1 which binds to PDL-1 on myeloma cells, not on normal plasma cells, thereby suppressing the antitumoral effect of NK cells in myeloma (Manier et al. 2016b; Moschetta et al. 2016).

Taken together, these effects result in immune escape and tumor growth through the direct stimulation and loss of effective antigen presentation, effector cell dysfunction, deletion of myeloma-specific T-cells, and increasing presence of inhibitory cells (Tregs and MDSCs).

Macrophages interact with malignant plasma cells through contact as well as non-contact mechanisms, thereby stimulating cell growth and tumor cell invasion as well as protecting myeloma cells from therapy-induced apoptosis (Kawano et al. 2015; Moschetta et al. 2016). Macrophages secrete several pro-angiogenic cytokines including vascular endothelial growth factor (VEGF), interleukin-8 (IL-8), fibroblast growth factor, as well as the cytokines IL-1b, IL-10, TNFa, and IL-6 with net effects of promoting angiogenesis and myeloma cell growth (Figs. 1.1 and 1.2) (Kawano et al. 2015).

The cells within the non-hematopoietic cellular compartment are stromal cells including mesenchymal stem cells, fibroblasts, bone marrow adipocytes, osteoclasts, osteoblasts, and endothelial cells. Bone marrow stromal cells (BMSCs) bind closely to the plasma cells through various adhesion molecules such as intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1). This adhesion triggers signaling through a number of pathways in the plasma cells, e.g., the RAS/MAPK, NFKB, and PI3K signaling pathways, resulting in cell proliferation and drug resistance (Manier et al. 2016b). The BMSCs secrete cytokines, e.g., IL-6, which is a key cytokine in myeloma as it promotes proliferation and survival of myeloma cells (Kawano et al. 2015). The plasma cells in turn secrete growth factors such as VEGF, fibroblast growth factor, and many more to stimulate proliferation of BMSCs, endothelial cells, and neoangiogenesis (Kawano et al. 2015). This creates a loop of cytokine secretion between the bone marrow plasma cells and the bone marrow niche which is essential for the survival of the myeloma cells (Manier et al. 2016b). Furthermore, BMSCs secrete stromal cell-derived factor 1 (SDF-1) belonging to the CRCX4 axis which is critical for stromal-myeloma interaction in the bone marrow niche and for dissemination of myeloma cells within the bone marrow as well as to extramedullary sites (Manier et al. 2016b;

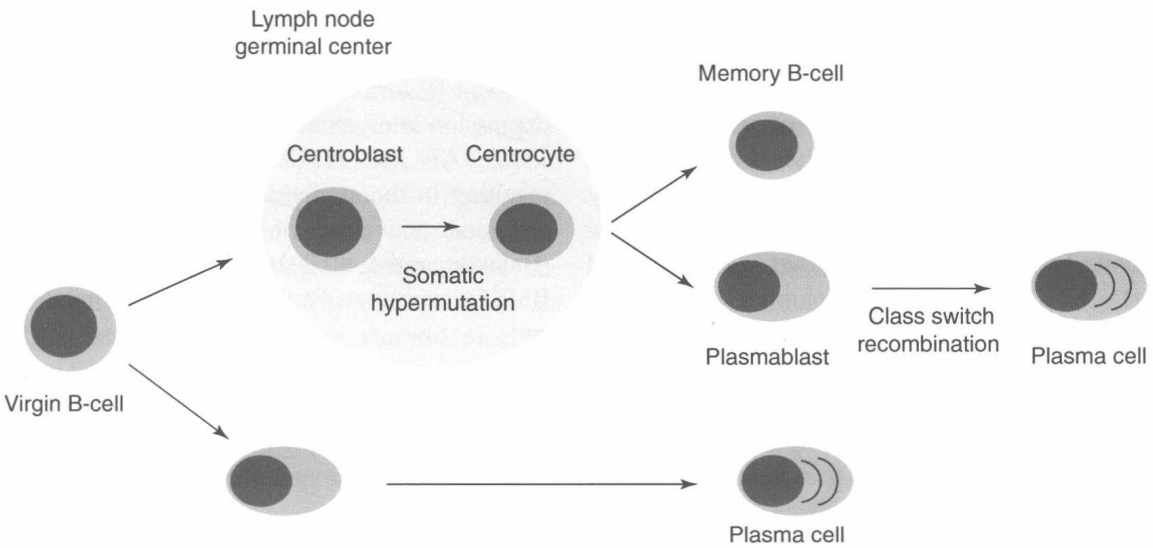

Fig. 1.1 Origin of malignant plasma cell

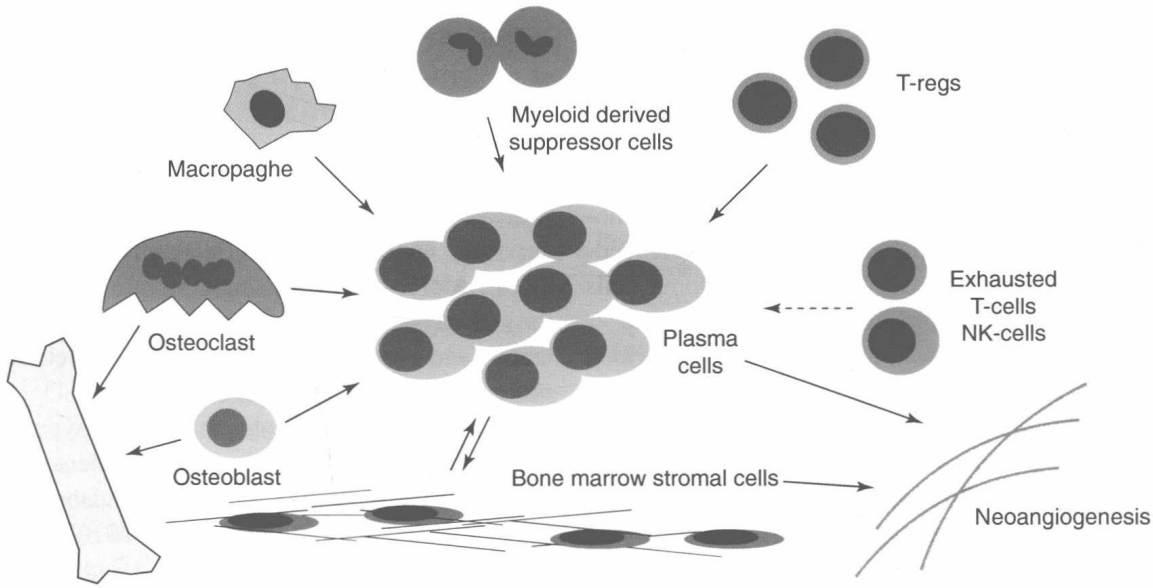

Fig. 1.2 Interactions of myeloma cells with marrow stromal

Kuiper et al. 2012; Stein et al. 2016; Lohr et al. 2016; Szalat and Munshi 2015). In addition, BMSCs release exosome with miRNA and specific proteins that are taken up by the plasma cells and have the potential to affect gene expression and tumor growth (Kawano et al. 2015). Bortezomib can reverse many of the interactions between myeloma and stromal cell interactions as well as inhibit cytokine production and secretion (Manier et al. 2016b).

In patients with myeloma, there is an ongoing neovascularization within the bone marrow. This process is gradually increased from MGUS to smoldering myeloma to multiple myeloma, and elevated microvascular density has been correlated to a worse prognosis (Rajkumar et al. 2002). Within the bone marrow, myeloma cells secrete VEGF and stimulating endothelial cells which in turn secrete IL-6 resulting in simultaneous proliferation of both myeloma cells and neoangiogenesis

(Munshi and Wilson 2001; Rajkumar and Kyle 2001). Treatment with IMiDs has a negative effect on angiogenesis (Kawano et al. 2015).

In myeloma, the balance between bone formation and bone resorption is altered favoring bone resorption and suppression of osteoblast activity. Osteoblasts, which normally are responsible for bone formation, are suppressed via Dickkopf-1 (DKK1), a Wnt signaling inhibitor, contributing to lytic lesions. Osteoblasts also secrete IL-6 and osteoprotegerin blocking TRAIL-mediated programmed cell death MM by secreting (Manier et al. 2016b). In myeloma, the balance is tipped toward osteoclast activation leading to lytic lesions. Myeloma cells produce receptor activator of nuclear factor kappa-B ligand (RANKL), macrophage inflammatory protein 1a (MIP-1a), IL-3, and IL-6, all contributing to an increased osteoclast activity. RANKL is in the TNF family and plays a major role in osteoclast activation in myeloma. Blocking RANKL with the monoclonal antibody denosumab, which is a soluble form of RANK, has been shown to modulate bone loss and improve overall survival in in vivo models (Manier et al. 2016b). Furthermore, bisphosphonates can inhibit osteoclasts but also target feedback loop with osteoclasts and myeloma cells (Manier et al. 2016b).

The noncellular compartment can be divided into the extracellular matrix component and the soluble component. The extracellular matrix consists of fibrous proteins including collagenous proteins to 90%; the remaining 10% is made up of proteoglycans, glycosaminoglycans, and small integrin-binding ligand N-linked glycoproteins (SIBLINGs) (Balakumaran et al. 2010). These proteins constitute a supporting structure for bone marrow cells but also interact with myeloma cells directly promoting cell proliferation (Balakumaran et al. 2010). Remodeling of the extracellular matrix by BMSC may be important in the progression from MGUS to myeloma (Slany et al. 2014).

The soluble component includes a variety of cytokines, growth factors, and adhesion molecules produced by the myeloma cells and nontumor cells in the bone marrow (Balakumaran et al. 2010). As mentioned, IL-6 is primarily produced by BMSCs and osteoblasts and is a key growth factor in myeloma cell growth. IL-6 stimulates osteoclasts formation as well as affects gene expression in myeloma cells through the MAPK, JAK/STAT, and PI3K/Akt signaling pathways resulting in the expression of transcription factors and activation of antiapoptotic proteins (Balakumaran et al. 2010). SDF-1α produced by BMSCs upregulates adhesion between myeloma cells to fibronectin and VCAM-1 resulting in proliferation, migration, and protection against drug-induced apoptosis. SDF-1 also affects BMSCs leading to upregulated secretion of IL-6 and VEGF. TNFα and members of the TNF superfamily including CD40L, BAFF, and APRIL all mediate myeloma cell growth, through either direct mechanisms or upregulation of IL-6. RANKL, also a member of the TNF family, as mentioned increases osteoclastogenesis through binding to RANK on the osteoclasts (Balakumaran et al. 2010). Additional growth factors include VEGF from myeloma cells stimulating endothelial cells and angiogenesis. Insulin-like growth factor-1 (IGF-1) which also is found in the liquid milieu of myeloma patients promotes cell growth, survival, and migration (Balakumaran et al. 2010). Moreover, matrix metalloproteinases act through growth factors resulting in neovascularization and osteoclast activity leading to myeloma progression (Balakumaran et al. 2010).

The liquid milieu is physiologically hypoxic and organized with varying oxygen content near the trabecular bone and near the vascular niche near sinusoids (Moschetta et al. 2016). The hypoxia of the endosteal niche supports myeloma cells primarily mediated through HIF-1 and HIF-2. In addition to promoting myeloma clone growth, hypoxia also decreases CD138 expression and induces a more immature and stem cell-like expression program in myeloma cells (Moschetta et al. 2016).

1.8 Future Perspective

The field of genomic assessment in multiple myeloma has over a short period of time gone from gross anatomical assessment using cytogenetics