New developments and treatment in multiple myeloma: new insights on molecular biology

J. Drach & H. Kaufmann

University of Vienna, Department of Internal Medicine I, Clinical Division of Oncology, Vienna, Austria

Introduction

Multiple myeloma (MM) is a malignancy of differentiated B-lymphocytes characterized by accumulation of clonal plasma cells in the bone marrow (BM), presence of a monoclonal immunoglobulin (Ig) in the serum and/or urine, and osteolytic bone lesions. Despite advances in systemic and supportive therapies, MM has remained an incurable malignancy. It is our hope that a better understanding of the biology and molecular pathology of the disease will provide the background for novel therapeutic strategies targeting the myeloma cell and its BM microenvironment in a more specific and efficacious fashion. This review summarizes recent advances in our understanding of MM biology, with a focus on clinically relevant findings.

The malignant clone

In MM, malignant plasma cells exhibit important features of normal long-lived plasma cells after stimulation with antigen, i.e. somatic hypermutation of rearranged immunoglobulin (Ig) genes and isotype switch recombination. It is assumed that during these physiological processes of B-cell maturation, errors may occur that lead to chromosomal translocations frequently involving Ig genes, in particular the Ig heavy-chain (IgH) locus on chromosome 14q32 [1]. The presumed myeloma precursor cell is likely to be a B-lymphocyte that has already migrated through the follicle center of lymph nodes and subsequently homes to the BM, where it gives rise to further clonal expansion [2]. In agreement with this model, a subset of peripheral blood B-lymphocytes has been shown to be part of the malignant clone in MM.

Chromosomal instability

Metaphase cytogenetic analyses of MM cells are difficult to perform due to the low proliferative activity of myelomatous plasma cells in vitro. Abnormal metaphases can be obtained in about 30–40% of patients with newly diagnosed MM, usually showing a complex pattern of numerical and structural abnormalities. Frequent numerical abnormalities include monosomy 13 and gains of chromosomes 3, 5, 7, 9, 11, 15 and 19. Common structural aberrations involve chromosome 1 (both the p-arm and the q-arm, with no apparent locus specificity), 6q, 11q and 14q32 (IgH locus).

Based upon molecular cytogenetic studies, which do not only rely on the presence of actively dividing cells, it has been established that virtually all cases of MM are characterized by chromosomal aberrations [3]. Studies utilizing interphase fluorescence in situ hybridization (FISH) have shown that specific chromosomal abnormalities in MM are generally two to three times more common than previously reported by metaphase cytogenetic investigations. In this context, it is worth noting that reliable detection of specific aberrations (in particular deletion of 13q) is clinically relevant due to prognostic implications (see below). FISH studies have also demonstrated that chromosomal aneuploidy is a common finding already at the level of monoclonal gammopathy of undetermined significance (MGUS): plasma cells from individuals with MGUS may exhibit not only numerical changes (e.g. trisomies of chromosomes 3, 7, 9 and 11) [4, 5], but also structural aberrations such as 14q translocations and deletions of 13q [6, 7]. From these studies, as well as from findings summarized below, one can conclude that critical chromosomal abnormalities leading to karyotypic instability already occur in MGUS plasma cells, and that additional genetic events take place during evolution of MM and further progression to advanced stages of the disease [2].

Deletion of chromosome 13q

In recent years, the importance of cytogenetic abnormalities for MM biology and prognosis has been recognized. Presence of a hypodiploid karyotype in general represents an unfavorable prognostic factor in MM [8, 9], but partial or complete deletion of chromosome 13q has been identified as the single most important specific abnormality associated with poor outcome in MM. This correlation was initially observed in MM patients treated with high-dose chemotherapy whose BM cells, when studied by classical metaphase analysis, revealed chromosome 13q abnormalities in about 15% of MM patients at diagnosis [10]. Interphase FISH studies of chromosome 13q, which were performed with DNA probes for distinct loci at chromosomal band 13q14, have shown a higher frequency of 13q deletions in MM, occurring in 39–54% of newly
dysregulation of a second gene [myeloma overexpressed gene] as a result of this heterogeneity, a t(11;14) may also result in scattered over a relatively large genomic region [22]. Probably in contrast to mantle cell lymphoma, breakpoints on 11q13 in MM some 14 and location results in dysregulated expression of two genes, 4p16.3 and 16q23 being most commonly involved in 14q32 translocations in MM. As a consequence of these translocations, genes that may function as oncogenes, growth factors or transcription factors may be dysregulated.

11q13

A t(11;14)(q13;q32) can be found in 15–20% of patients with MM and leads to overexpression of cyclin D1 [20, 21]. In contrast to mantle cell lymphoma, breakpoints on 11q13 in MM are not clustered in the Major Translocation Cluster, but are scattered over a relatively large genomic region [22]. Probably as a result of this heterogeneity, a t(11;14) may also result in dysregulation of a second gene [myeloma overexpressed gene (myeov)], which is located centromeric to cyclin D1 [23].

4p16.3

A t(4;14)(p16;q32), which can only be detected by molecular cytogentic studies due to the telomeric breakpoint on chromosome 4, is present in about 15% of MM cases [21, 24]. This translocation results in dysregulated expression of two genes, fgfr3 (fibroblast growth factor receptor 3) on the derivative chromosome 14 and mmset (multiple myeloma SET domain) on the derivative chromosome 4 [24–26], fgfr3, which is not expressed by normal plasma cells, is overexpressed as a consequence of the translocation, and in some cases, activating mutations have also been found on the translocated allele.

Observations that expression of fgfr3 promotes myeloma cell proliferation and prevents apoptosis, and that activation of fgfr3 is a transforming event in hematopoietic cells further substantiate an oncogenic role for fgfr3 in MM [27–29]. Similar to fgfr3, mmset overexpression only occurs in the presence of a t(4;14), but the contribution of mmset to the pathogenesis of MM is still unknown.

16q23

The t(14;16)(q32;q23), which is present in 5–10% of patients with MM, results in expression of c-maf in MM cells at a high level [30, 31]. c-maf has been identified as a transcription factor in lymphoid cells involved in regulation of expression of interleukin-4 [32], but its role in the molecular pathology of MM still needs to be determined.

Other translocation partners

There are several other chromosomal regions that have been reported as partner loci for 14q32 translocations in MM, but each of them appears to occur in less than 5% of patients with MM. In some instances, breakpoints have been cloned and information on dysregulated genes is available. This includes t(6;14)(p21;q32) leading to overexpression of cyclin D3 [33] and t(6;14)(p25;q32) with the IRF-4 gene being expressed at high levels in the MM cell lines carrying the translocation.

Clinical implications of IgH translocations in MM

Whether or not specific 14q32 translocations carry any prognostic information in MM is currently being investigated. Preliminary data indicate that approximately 25% of MM patients without evidence of a 14q32 translocation mostly do not have a deletion of 13q, suggesting that they belong to a rather favorable prognostic group. In contrast, presence of a t(4;14) and t(14;16) is frequently associated with other indicators of poor prognosis, in particular deletion of 13q and elevated serum levels of β2-microglobulin. Presence of a t(11;14) does not appear to have a significant impact on prognosis [15]. Clearly, more information is necessary to clarify whether or not clinically relevant MM subtypes can be identified based on distinct 14q32 translocations.

Late oncogenic events in MM

Translocations involving c-myc

Chromosomal aberrations of 8q24 (c-myc gene locus) have only rarely been reported by classical karyotypic analyses of MM. Similarly, a t(8;14)(q24;q32) as studied by interphase FISH was present in only three of 140 primary MM tumors [6]. However, multicolor FISH studies of metaphase chromosomes obtained from patients with advanced MM indicated that complex translocations involving c-myc occur in about 40% of the 38 cases investigated [34]. In contrast to Burkitt’s...
lymphoma, where c-myc is rearranged with Ig gene loci as a primary molecular event, translocations with c-myc in MM mostly involve non-Ig gene sequences and represent a late event in the pathogenesis of MM.

Mutations of N- and K-ras

Whereas mutations of N- and K-ras are absent in MGUS and solitary plasmacytoma, they may be present in about 30% of patients with MM at diagnosis and at even greater frequency in MM at an advanced and terminal stage. Activating mutations of ras can contribute to growth factor-independent proliferation of myeloma cells [35]. Multiple myeloma cell lines carrying a t(4;14) without an activating mutation of fgr3 may have mutations of N- and K-ras [28], providing further evidence for a role of such mutations during progression of MM.

Abnormalities of p53

p53 mutations are a rare event in MM at diagnosis, but may be found with increasing frequency in patients with relapsed disease and plasma cell leukemia [36]. Deletions of 17p13 including the p53 gene may also be present in MM at diagnosis, but since these abnormalities are often small interstitial deletions, molecular cytogenetic techniques are required for their detection [37].

Role of the BM microenvironment in the pathogenesis of MM

Several aspects highlight the importance of the BM microenvironment for growth and survival of the myeloma clone.

Cytokines

It has been well established that a complex network of cytokines regulates myeloma cell proliferation and differentiation [38]. Interleukin-6 (IL-6) represents a major growth and survival factor for MM cells and is predominantly produced by BM stroma cells. Binding of MM cells to BM stroma cells up-regulates IL-6 transcription and secretion within stroma cells, a process that is further enhanced by cytokines secreted by MM cells (in particular transforming growth factor β) [39]. On MM cells, IL-6 activates specific signal transduction pathways (most notably the JAK2/STAT3 pathway), thereby inducing proliferation and inhibiting apoptosis [40]. Thus, such interactions between MM cells and the BM microenvironment may be important determinants of responsiveness of MM cells to cytotoxic drugs.

Tumor necrosis factor α is another important cytokine in these interactions mediating up-regulation of adhesion molecules on MM cells (LFA-1, VLA-4) and BM stroma cells (ICAM-1, VCAM-1). This effect results in increased adhesion of MM cells to BM stroma cells thereby activating IL-6 secretion by the BM microenvironment [41].

BM angiogenesis

Increased BM microvessel density has been observed in patients with active MM [42, 43], which provides a background for exploring the role of antiangiogenic agents in the treatment of MM. Bone marrow neovascularization may be quite heterogeneous in MM, but particularly high levels of microvessel densities were found in patients with other adverse prognostic features such as enhanced proliferative capacity and presence of a chromosome 13q deletion [42, 44, 45]. Vascular endothelial growth factor (VEGF) is one of the important mediators leading to increased BM angiogenesis in MM. VEGF may be secreted by MM cells themselves [46], but up-regulation of VEGF secretion triggered by binding of MM cells to BM stroma cells is an important source of VEGF as well [47]. Other effects of VEGF in MM include enhancement of proliferation and migration of MM cells [48], suggesting that blocking of VEGF mediated events in MM could represent a novel therapeutic target.

Interactions of MM cells with BM stroma cells

In addition to the interactions described above, adhesion of MM cells to cells or other structures of the BM stroma may lead to activation of specific adhesion molecules. Specifically, binding of MM cells to fibronectin, which is mainly mediated by β1 integrin receptors, prevents CD95 (Fas) induced cell death in MM cells. This mechanism represents a novel form of drug resistance termed cell adhesion mediated drug resistance (CAM-DR): MM cells adhered to fibronectin were shown to be more resistant to melphalan compared with cells exposed to the drug in suspension [49].

Mediators of osteolysis in MM

Osteolytic lesions resulting from increased osteoclastic activity are a major clinical problem in MM. Recent findings suggest that an imbalance of two molecules, osteoprotegerin ligand (OPGL) and osteoprotegerin (OPG), is an important mechanism for the development of osteolytic lesions in MM [50, 51]. OPGL, also known as receptor activator of NF-κB ligand (RANKL) and TNF-related activation-induced cytokine (TRANCE), causes activation and differentiation of osteoclastic cells, whereas OPG is a naturally occurring inhibitor of OPGL. Thus, both molecules are important regulators of physiological bone resorption. In MM, however, OPGL expression by BM stroma cells is markedly enhanced, with a concomitant suppression of OPG production. This effect appears to be dependent on the interaction of MM cells with BM stroma cells and, at least in part, involves the integrin VLA-4.

Summary and conclusions

During the past decade, new insights into the molecular pathology of MM have been obtained recognizing the
importance of both the malignant clone and the BM microenvironment for disease evolution and propagation. It appears that progressive genetic changes are associated with the development of various stages of monoclonal gammopathies [2]. Translocations involving 14q32 with consecutive activation of an oncogene at one of the various translocation partners may be considered as a primary event leading to immortalization of a plasma cell clone and to the development of MGUS. Chromosomal instability becomes apparent and even more pronounced as soon as the disease progresses. Transformation to MM is associated with such additional chromosomal events, with a deletion of chromosome 13 occurring in about 50% of cases. Further expansion of the malignant clone is supported by factors provided by the BM microenvironment, which in turn is influenced by products of the malignant plasma cells. During this phase of the disease, MM cells remain growth factor-dependent and therefore are restricted to the BM itself. Late occurring genetic and molecular events (secondary translocations, dysregulation of additional oncogenes) characterize myeloma cell growth that is stroma independent, which clinically gives rise to a terminal, aggressive phase of the disease with development of extramedullary manifestations.

It is expected that the use of novel techniques, in particular global gene expression profiling, will further contribute to a molecular classification of MM [52]. Characterization of such critical events in the development of monoclonal gammopathies may be the basis for future therapeutic approaches. Agents targeting the BM microenvironment instead of the tumor cell (e.g. immunomodulatory drugs, proteasome inhibitors) are already being investigated in clinical trials and may be particularly active in combination with conventional cytotoxic drugs. Identification of MM subtypes with tumor-specific alterations, such as mutations of \( \text{fgfr}3 \), overexpression of cyclins and activation of specific kinases, provides the background for novel therapeutic targets. Delineation of the molecular pathway that causes bone destruction in MM will also improve our ability to treat and prevent osseous complications of the disease.

Acknowledgements

Supported in part by the Interdisciplinary Cooperation Project of the Austrian Federal Ministry for Education, Science and Culture.

References


