Review

Input of DNA Microarrays to Identify Novel Mechanisms in Multiple Myeloma Biology and Therapeutic Applications

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Abstract   Multiple myeloma is a B-cell neoplasia characterized by the proliferation of a clone of malignant plasma cells in the bone marrow. We review here the input of gene expression profiling of myeloma cells and of their tumor microenvironment to develop new tumor classifiers, to better understand the biology of myeloma cells, to identify some mechanisms of drug sensitivity and resistance, to identify new myeloma growth factors, and to depict the complex interactions between tumor cells and their microenvironment. We discuss how these findings may improve the clinical outcome of this still incurable disease.

Multiple myeloma (MM) is a B-cell neoplasia that affects 15,000 new patients per year in Europe and 15,000 in the United States. It is characterized by the accumulation of a clone of malignant plasma cells in the bone marrow. Myeloma cells are strongly dependent on their microenvironment, which produces cell communication signals, in particular myeloma growth factors. One key finding to understand the biology of myeloma has been the identification of interleukin-6 (IL-6) as a major survival and proliferation factor for myeloma cells in 1988 (1, 2). However, although IL-6 is essential, it is not sufficient and additional factors, produced by the microenvironment or the tumor cells themselves, are required together with IL-6 to promote tumor growth (3). We and others have identified such factors, as for example insulin-like growth factor-I (4, 5), IFN-α (6), IL-10 (7), hepatocyte growth factor (8), Wnt family (9), and more recently members of the epidermal growth factor (EGF) family (10) and B-cell activating factor/a proliferation-inducing ligand (BAFF/APRIL; ref. 11). MM is still an incurable disease with an average 5-year survival after high-dose chemotherapy and autologous stem cell transplantation (12). A recent study has pointed out that allografting with human leukocyte antigen–identical sibling stem cells and immune cells of patients with newly diagnosed MM may considerably increase patients' outcome (13). New drugs, such as thalidomide or bortezomib, have also improved MM patient treatment (14). By understanding the biology of MM and defining important potential therapeutic targets, effective new treatments can be developed to improve patient outcome in MM. We review here the major inputs of DNA microarrays in the understanding of MM disease and their clinical applications.

New Molecular Classifications of MM

Genetic abnormalities and prognosis in MM.   In MM, genetic abnormalities led to overexpression of (putative) oncogenes such as cyclin D1-t(11;14) and trisomy 11-, cyclin D3-t(6;14)-, MMSET/FGFR3-t(4;14)-, or c-MAF-t(14;16)-. In addition, monoallelic deletions of chromosome 13q14 [del(13)] involving the *retinoblastoma* gene, or chromosome 17p13 [del(17)] involving *P53* are found in 50% and 10% to 30% of patients with MM, respectively (15). The t(4;14) translocation confers a bad prognosis, even after high-dose chemotherapy and autologous stem cell transplantation (16). Del(13) or del(17) have also been reported to be associated with poor prognosis (16, 17), however, the prognostic value of del(13) would be related to the t(4;14) and del(17) that are frequently associated with del(13) (ref. 18). Chromosome 1q21 amplification is found in 43% and 72% of patients with newly diagnosed MM or relapsed MM, respectively, and it is associated with a poor prognosis and a shortened postrelapse survival (19). Thus, cytogenetics is a powerful technique to identify some major chromosome aberrations and their clinical implications in myeloma. In the following, we will review the input of DNA microarrays to further identify myeloma disease heterogeneity and the mechanisms targeted by these gene abnormalities.

Gene expression profile–based classifications of newly diagnosed patients with MM.   Microarrays profiling has helped to establish relevant MM subclassifications. For example, gene expression profile (GEP) analysis of primary myeloma cells from 92 patients was analyzed according to their immunoglobulin types and light chain subtypes, revealing that several clusters of genes involved in various biological functions could

significantly discriminate those different types of myeloma (20). Moreover, we and others have confirmed that patients whose myeloma cells have t(4;14) and t(11;14) translocations can be pointed out by DNA microarrays through an over-expression of *FGFR3*, *cyclin D1*, or *MAF* genes, respectively (21). Based on the hypothesis that the major genetic abnormalities can be picked up by DNA microarrays, Bergsagel et al. (22) proposed a GEP-based molecular classification of MM taking into account the unifying expression of cyclins. They pointed out a high expression of one of the three *cyclin D* genes as a general feature of MM and proposed a classification of patients within eight TC (translocation/cyclin D) groups (22). More recently, using Affymetrix GEP of 414 newly diagnosed patients, Zhan et al. (23) proposed a classification of MM in seven groups that are driven by the presence of translocations or hyperdiploidy. These groups are characterized by (*a*) an overexpression of genes involved in cell cycle and proliferation (PR group for proliferation); (*b*) a lower expression of genes involved in bone disease, like the wnt signaling antagonists Dickkopf 1 (*DKK1*) and Frizzled B, and a lower number of magnetic resonance imaging–defined focal lesions (LB group for "low bone disease"); (*c*) an overexpression of *FGFR3* and *MMSET* genes (MS group for MMSET); (*d*) a hyperdiploid signature (HY group); (*e-f*) an overexpression of *cyclin D1* and *cyclin D3* genes (CD-1 and CD-2 groups); (*g*) an overexpression of *MAF* and *MAFB* genes (MF group). With a 36-month median follow-up on the training group, HY, CD-1, CD-2, and LB groups had a higher event-free survival and overall survival compared with the PR, MS, and MF groups. On multivariate analysis, this seven-group GEP classification is a significant and independent predictor for survival (23). Using the same series of Affymetrix GEP data, Shaughnessy's group identified 70 genes whose up-regulation or down-regulation was linked with a bad prognosis in a subset of 13% of newly diagnosed patients (24). Seventeen of the 70 genes are sufficient to predict for this bad prognosis subset. A high-risk score based on the $\log_2$ of average of up-regulated gene minus the $\log_2$ of average of down-regulated genes has a strong independent prognostic value, in particular eliminating the prognostic value of the International Staging System stage. Thirty percent of the 70 genes are located on chromosome 1, mainly 1q. One major up-regulated gene is *CKSB1*. CKSB1 is a member of the Cks/Suc1 family of small proteins that bind the catalytic subunit of cyclin-dependent protein kinases and modulate their function. In particular, CKSB1 promote the ubiquitin-mediated $P27_{Kip1}$ degradation, resulting in cell cycle progression. Shaughnessy's group has shown that knockout of *CKSB1* in myeloma cells with a short hairpin RNA lentivirus results in P27 accumulation and apoptosis emphasizing the major role of *CKSB1* up-regulation in MM disease aggressivity (25).

Combining analysis of expression changes by GEP and cytogenetic aberrations by iFISH in highly purified myeloma cells, we could classify 128 newly diagnosed patients in four groups noted EC for "expression-cytogenetic" (26). EC1 groups are characterized by cyclin D1 overexpression and either additional copy of 11q13 (EC1-1) or t(11;14) translocations (EC1-2). EC2 groups are defined by cyclin D2 overexpression, either without 11q13+, t(11;14) and t(4;14) (EC2-1), or with t(4;14) and FGFR3 up-regulation (EC2-2). The EC2.1 group comprises several rare translocations that indirectly increase cyclin D2 expression, like the t(14;16), indicated by a spiked

MAF expression. Patients of the EC1.1 group are hyperdiploid, whereas those in EC1.2 and EC2.2 groups are mostly non-hyperdiploid. Highly significant differences in event-free survival were found between the four groups of patients treated with high-dose therapy and peripheral blood stem cell transplantation. In agreement with the published data for the t(4;14) translocation, patients of the EC2.2 group had the shortest median event-free survival. Patients of the EC1.2 group had the longest event-free survival, those of EC1.1 and EC2.1 groups represented an intermediate risk.

Frequent nuclear factor-κB pathway activation through recurrent genetic abnormalities. Using GEP and aCGH arrays, two groups recently identified a promiscuous array of genetic alterations yielding to activation of nuclear factor-κB (NF-κB) pathway in at ~20% of MM patients and 41% of myeloma cell lines (27, 28). They include overexpression and/or gain-of-functions of *NIK*, *NFKB2*, *NFKB1*, *CD40*, *LTBR*, and *TACI*, all these genes coding for proteins activating the NF-κB pathway. They also include inactivating abnormalities of *TRAF3*, *cIAP1*, *cIAP2*, *CYLD*, and *TRAF2*, which are negative regulators of NF-κB. These two studies disagree on whether the canonical or the noncanonical NF-κB pathways are mainly deregulated. Of note, Annunziata et al. (28) clearly showed that activation of NIK affected both the canonical and noncanonical pathways, emphasizing the interest of IKK2 inhibitors in patients (29). In addition, Keats et al. (27) reported that dexamethasone was poorly efficient compared with bortezomib in patients with NF-κB alteration, whereas they were both efficient in other patients. In normal plasma cells, NK-κB pathway is highly activated, likely in part due to the activation of TACI and BCMA by BAFF/APRIL (30) that are produced by bone marrow cells, in particular osteoclasts (31).

Understanding of the Biology of Myeloma Cells

Comparison of the GEP of myeloma cells, of plasma cells from healthy individuals, and of plasma cells from patients with monoclonal gammopathy of undetermined significance

Several analysis comparing the GEP of malignant plasma cells to that of their normal counterpart have provided new insights to understand the molecular mechanisms of the multistep pathogenesis of MM. Comparing the GEP of purified myeloma cells and myeloma cell lines to that of B cells (32) or *in vitro* generated plasmablastic cells (33), we identified genes that were statistically significantly overexpressed in myeloma, including oncogenes and genes coding for tumor antigens or intercellular communication signals (growth factors, chemo-kines, and proapoptotic and antiapoptotic proteins; ref. 34). A quarter of those genes were also identified by Zhan et al. (35), like *MYC* and *cyclin D1*, using purified bone marrow plasma cells from healthy individuals as a normal counterpart of myeloma cells. The use of either mature plasma cells or immature plasmablasts may explain the partial overlap of the gene lists described in these two studies providing comple-mentary information. In 2004, Munshi et al. (36) analyzed the GEP of normal and malignant plasma cells purified from the bone marrow of genetically identical twins; that is, from an identical genetic background. Among genes overexpressed in myeloma cells versus plasma cells of the healthy twin, they found oncogenes/transcription factors (*FGFR3*, *Jun-D*, *v-fos*)
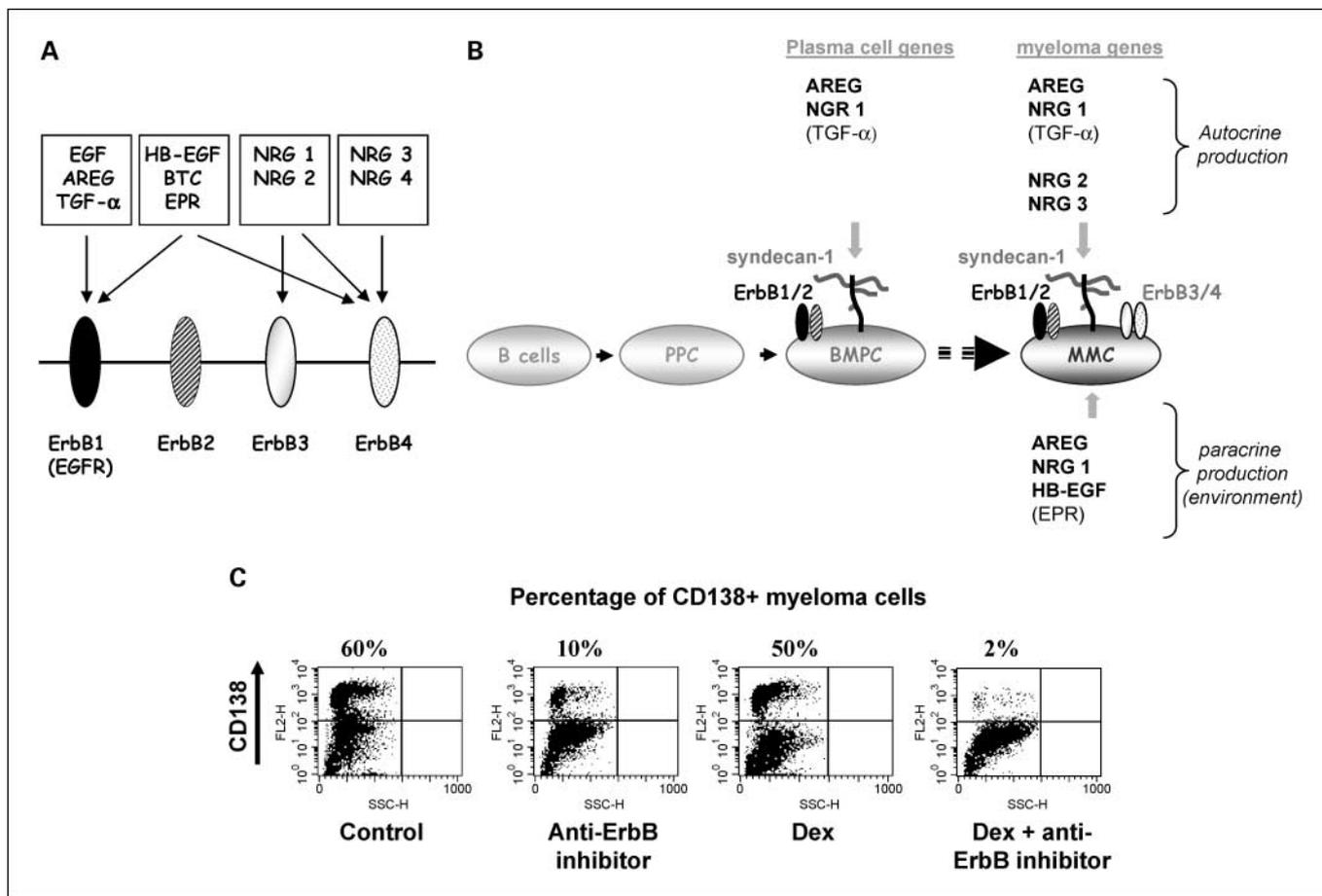
Fig. 1. Syndecan-1, ErbB receptors, and EGF ligand expression throughout plasma cell differentiation and malignant transformation. *A,* the 10 ligands can be subdivided into four groups according to their specificity (*arrows*) for ErbB receptors. *B,* late plasma cell differentiation is associated with the development of a possible functional autocrine loop involving ErbB1 and ErbB2, their ligands, and their coreceptor syndecan-1. Malignant transformation is associated with the development of additional possible autocrine loop involving ErbB3 and ErbB4, neuregulins and syndecan-1, and paracrine expression of EGF-ligands. TGF-α and epiregulin (*in parentheses*) do not have a heparan sulfate–binding domain (i.e., they are not able to promote myeloma cell growth). *C,* total mononuclear cells from patients with MM were cultured for 5 d with either the PD169540 ErbB inhibitor or dexamethasone (*Dex*), or a combination of both. Cells were stained with an anti-CD138-phycoerythrin antibody. PPC, polyclonal plasmablastic cells; BMPC, bone marrow plasma cells; MMC, MM cells; AREG, amphiregulin; TGF-α, tumor necrosis factor-α; HB-EGF, heparin-binding EGF-like growth factor; BTC, betacellulin; EPR, epiregulin; NRG, neuregulin.

and genes involved in cell survival pathway (*MCL-1*). Recently, using the U133 Affymetrix microarrays to analyze a larger cohort of patients, we found that *CD200*—a highly conserved type I transmembrane glycoprotein expressed by thymocytes, B and T cells, dendritic cells, endothelial cells, and neurons (37)—was significantly overexpressed in myeloma cells compared with normal plasma cells, plasmablastic cells, and B cells, and was a bad prognostic factor independent of International Staging System stage or β_2-microglobulin, for newly diagnosed patients (38). CD200 is a potent immunosuppressive molecule, down-regulating activation of T cells that express CD200 receptor (39). Antibodies to CD200 can block the immunosuppression induced by CD200$^+$ Burkitt cell lines and promote tumor rejection in severe combined immunodeficient mice receiving the tumor cell lines and allogenic human T cells (40). This emphasizes that CD200 may a potential therapeutic target in MM.

Concerning monoclonal gammopathy of undetermined significance (MGUS) patients, it was first reported that whereas MGUS and myeloma could be distinguished from normal plasma cells, those two conditions were very difficult to differentiate from each other (41, 42). Using whole-genome

microarrays on larger cohorts of patients, Zhan et al. (43) recently identified genes differentially expressed between plasma cells from normal donors, patients with MGUS, and patients with MM. Unsupervised hierarchical clustering of the genes differentially expressed between MGUS and MM identified a subset of MGUS-like MM, which had favorable clinical features and a longer survival. This MGUS-like signature was found in the majority of the patients surviving more than 10 years after initiation of total therapy (43).

Identification of myeloma growth factors

Based on GEP data, we have identified new families of proteins that play a major role in the biology of the MM, and that are therefore promising therapeutic targets in this pathology.

EGF family members and ErbB receptors. The EGF/EGF-receptor family comprises four receptors (ErbB1-4) and 10 ligands (see Fig. 1A; ref. 44). Expression and/or activation of ErbB receptors are altered in many epithelial tumors but this family of protein had never been involved in hematologic malignancies. Using Affymetrix microarrays, we have shown

that five genes (*AREG*, *TGF-α*, *NRG1*, *NRG2*, and *NRG3*) are expressed by primary myeloma cells. Among them, two—*NRG2* and *NRG3*—are "myeloma genes" (i.e., they are significantly overexpressed in myeloma cells compared with their normal counterpart), whereas other three genes—*AREG*, *TGF-α*, and *NRG1*—are "plasma cell genes" (i.e., they are expressed both in normal and malignant plasma cells but not in B cells and plasmablastic cells; ref. 10). We further showed that ErbB receptor expression is induced during plasma cell differentiation (ErbB1-2) and oncogenesis (ErbB3-4; Fig. 1B; ref. 45). The importance of ErbB receptor activation for myeloma cell survival has been shown by the finding that a pan-ErbB kinase inhibitor (PD169540, Pfizer), either used alone or combined with other drugs, induced a dramatic apoptosis of primary myeloma cells cultured *in vitro* for 5 days with their environment, in 10 of 14 patients (Fig. 1C). The ErbB inhibitor did not affect the viability of other bone marrow cells present in the culture (Fig. 1C; ref. 46). More recently, we have shown that syndecan-1, the main heparan sulfate proteoglycan present on plasma cells, plays a major role in the EGF/ErbB pathway, concentrating high levels of heparin-binding EGF ligands at the cell membrane, which likely facilitates ErbB activation (10). Altogether, these data indicate that ErbBs are excellent candidates for targeted therapy and that inhibition of the EGF family pathway may be useful in the treatment of MM.

BAFF, APRIL, and their receptors. BAFF is a tumor necrosis factor family member involved in the survival of normal and malignant B cells. APRIL is highly expressed in several tumor tissues where it can stimulate tumor cell growth (47). Using Affymetrix microarrays, we found that *TACI* and *BCMA*, each gene coding for a receptor of BAFF and APRIL, were overexpressed in malignant plasma cells compared with their normal counterparts. We and others have shown that BAFF and APRIL can support the growth of myeloma cell lines and, conversely, an inhibitor of BAFF and APRIL can induce apoptosis of primary myeloma cells (11, 48). BAFF and APRIL serum levels are increased in patients with MM compared with age-related healthy individuals (11). Using Affymetrix microarrays, real-time reverse transcription-PCR, and ELISA, we found that BAFF and APRIL are mainly produced by the tumor environment, in particular by monocytes and myeloid cells (31). Furthermore, GEP data of purified myeloma cells from 65 newly diagnosed patients were analyzed by supervised clustering of groups with higher (TACI^hi) versus lower (TACI^lo) *TACI* expression levels. TACI^hi group displayed a mature plasma cell gene signature, indicating dependence on the bone marrow environment. In contrast, the TACI^lo group had a plasmablastic gene signature, suggesting a weaker dependence on the microenvironment (31). In 2005, a phase I-II trial using a receptor TACI coupled to Fc fragments of immunoglobulin (TACI-Ig, which blocks the effect of BAFF and APRIL) has been initiated at the university hospital of Montpellier and Heidelberg. This is the first clinical trial using this molecule (30). One can anticipate that GEP may help in identifying groups of patients who might benefit most from treatment with BAFF/APRIL inhibitors.

Understanding signaling pathways involved in myeloma cell growth

Hallmarks of MM are the presence of genetic abnormalities, the requirement of the bone marrow environment for tumor cell survival/proliferation, and the major role of insulin-like growth factor-I and IL-6 as myeloma growth factors. To understand how different stimuli can affect myeloma cell growth, Cronquist and coworkers compared the GEP of the ANBL-6 human myeloma cell line, either treated with IL-6 or cocultured with bone marrow stromal cells, or carrying N-Ras activating mutation (i.e., independent on exogenous IL-6; ref. 49). They showed that the different activating signals share some genetic pathways, but they also affect myeloma cells distinctively through the regulation of specific gene signatures (49). It suggested that the GEP of myeloma cells is not only influenced by intrinsic oncogenic events but also by exogenous signals from the microenvironment. In another study, the antiapoptotic activity of IL-6 was evaluated on the INA-6 myeloma cell line. The authors showed that IL-6–dependent survival of INA-6 cells relies on an antiapoptotic pathway in which signal transducers and activators of transcription 3 plays a central role (50). Chatterjee et al. (51) have shown that in the presence of bone marrow stromal cells, the concomitant inhibition of both IL-6/signal transducers and activators of transcription 3 and Ras/MEK/extracellular signal-regulated kinase pathways is required to induce apoptosis of IL-6–dependent myeloma cells. To understand how those two pathways mediate their effect, the same team compared the GEP of the INA-6 HMCL cocultured with bone marrow stromal cells, and treated either with Sant7 or with a MEK inhibitor or with a combination of both inhibitors (52). They identified genes specifically down-regulated after the combined pathway blockade, among which heat shock proteins were highly represented. The authors focused on the two HSP90 genes, *HSP90α* and *HSP90β*, which were further shown to be critical for myeloma cell survival within the context of their microenvironment (52).

Understanding Mechanisms of Drug-Induced Antitumor Activity and Drug Resistance

These studies were mainly conducted by K. Anderson's group comparing the GEP of myeloma cells treated or not with various inhibitors. They provided important insights to identify genes modulated in response to a given drug or genes that confer tumor chemoresistance. Chauhan et al. (53) determined the GEP of myeloma cells treated with dexamethasone using oligonucleotide arrays. They reported that transient activation of genes involved in DNA repair is followed by down-regulation of genes promoting growth and survival. In the same study, comparing the GEP of dexamethasone-sensitive (MM1S) and dexamethasone-resistant (MM1R) cells, they pinpointed genes differentially expressed between those cell lines that may confer dexamethasone resistance to MM1R. GEP analysis of primary myeloma cells validated those findings and confirmed their relevance *in vivo* (53). In a second study, the authors compared the GEP of MM1R and cells treated with 2-methoxyestradiol, an estrogenic derivative with antimyeloma activity. They showed that 2-methoxyestradiol down-regulates genes overexpressed in MM1R, suggesting mechanisms whereby 2-methoxyestradiol overcomes dexamethasone resistance (54). In further studies, oligonucleotide microarrays were used to characterize the transcriptional profile of myeloma cells treated with antitumor agents such as SAHA, a histone deacetylase inhibitor (55), or Velcade, a proteasome inhibitor (56). One of the interests of studying the molecular profile of tumor cells
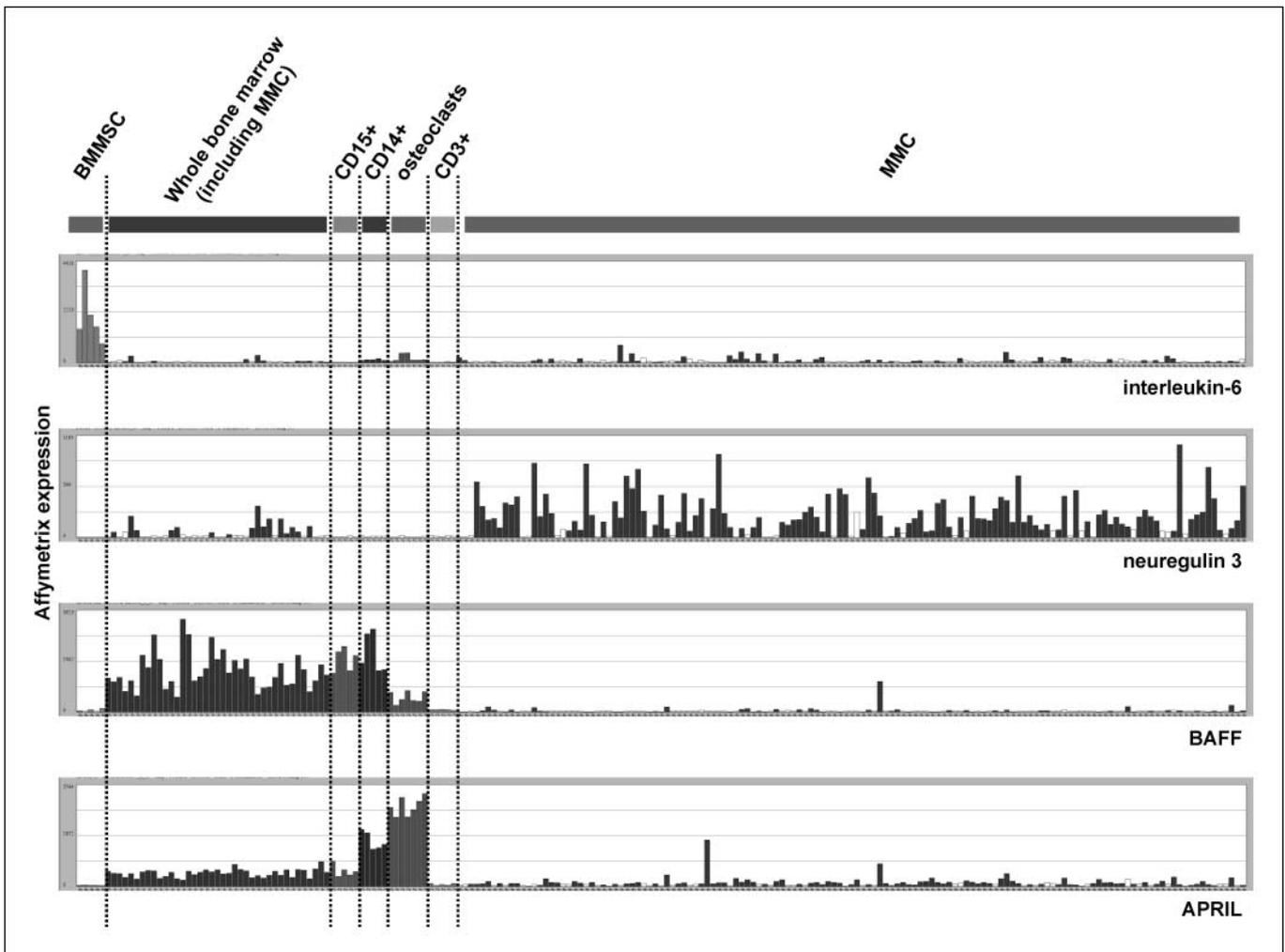
Fig. 2. Expression of growth factors in myeloma cells and in cells from the environment. Gene expression was measured by pan genomic U133 Plus2.0 Affymetrix oligonucleotide microarrays. The signal intensity for each gene is shown on the *Y* axis as arbitrary units determined by Affymetrix software. White boxes, the gene is not detected; that is, it has an "absent" detection call according to the definition of Affymetrix. Histograms were generated with our "Amazonia!" Web site (64). CD15+; polynuclear cells. CD14^{+}, monocytes; CD3^{+}, T cells. The bone marrow of patients with MM contains ~ 52% CD15^{+} polynuclear cells, 14% CD3^{+} T lymphocytes, and 10% CD14^{+} monocytes, with other cells being mainly erythroid cells (the mean percentages were determined on 22 consecutive samples; unpublished data).

treated with different drugs is to establish a framework for optimal design of combination therapies with conventional or targeted antitumor agents. Furthermore, a recent publication has provided the first description of the prospective development of a genomic classifier that predicts clinical response between myeloma patients treated with bortezomib or dexamethasone (57). Response and survival classifiers were developed and shown to be significantly associated with outcome via testing on independent data. Among gene sets significantly associated with response to bortezomib, the authors pointed out pathways that had already been described to be involved in bortezomib activity *in vitro* such as NF-κB activity or cell adhesion (57).

Using Microarrays to Analyze the Role of the Microenvironment in MM

Comparison of GEP of normal and MM bone marrow mesenchymal stem cells. In MM, it is now admitted that the bone marrow microenvironment plays a major role suggesting that the tumor may select a favorable microenvironment or that microenvironment cells could be abnormal. This hypothesis was recently shown by our group comparing the GEP of bone marrow mesenchymal stem cells (BMMSC) from normal donors to that of BMMSCs from patients with MM (58). An unsupervised analysis delineated two distinct homogeneous clusters of genes: a myeloma BMMSC cluster and a normal one. Interestingly, genes identified by a supervised analysis to be differentially expressed between normal and myeloma BMMSC were mainly classified in the "tumor microenvironment" category, according to Gene Ontology annotations. Genes overexpressed in myeloma BMMSCs included known myeloma growth factors (IL-6, amphiregulin, IL-1β), angiogenic factors, and proteins involved in bone disease (DKK1; ref. 58). In agreement with GEP data, normal and myeloma BMMSCs had functional differences as well. Myeloma BMMSCs showed a reduced matrix mineralization capability, and they could support the growth of the stroma-dependent Molp-6 cell line better than normal BMMSCs. Those data suggest that BMMSCs likely create a very efficient niche to support the survival and proliferation of myeloma cells.

GEP analysis of bone marrow subpopulations of myeloma patients. To analyze comprehensively the various intercellular communication pathways involved in MM, we analyzed for the first time the GEP of whole bone marrow cells of 39 patients (including the tumor cells and environment cells) and that of myeloma cells purified from the bone marrow of those 39 patients. This makes it possible to determine the relative contribution of the environment and the tumor itself for the expression of a given gene. Using this approach, we could identify various genes that are mainly expressed by cells of the environment, as illustrated for BAFF in Fig. 2 (*third panel*). Another interesting example is provided by the gene encoding heparanase (*HPSE*), an enzyme that cleaves heparan sulfate chains and controls *syndecan-1* gene expression and shedding in myeloma cells (59, 60). In MM, HPSE stimulates angiogenesis and promotes metastasis of myeloma cells to bone in a murine model (61). Using Affymetrix microarrays, we found a high *HPSE* expression in the whole bone marrow samples, which was 7.6-fold higher than that found in the corresponding myeloma cells. Combining GEP and clinical data, we showed that *HPSE* expression in the whole bone marrow of patients with MM is an indicator of poor prognosis (59). Classifying patients according to *HPSE* expression in purified myeloma cells did not result in any significant differences in survival (59). This is of major interest as it is the first study showing that the expression of a gene mainly in the bone marrow environment (i.e., *HSPE*) has prognostic value in MM.

To dissect the role of each component of the bone marrow of patients with MM, we simultaneously purified four bone marrow subpopulations (polymorphonuclear cells, myeloma cells, T cells, and monocytes); then, we analyzed their GEP. Osteoclasts and BMMSCs that cannot be harvested *in vivo* were generated *in vitro* from MM patients' cells. This approach makes it possible to identify at a glance which cell component express a gene of interest. Four representative genes are shown in Fig. 2. We observed a high IL-6 expression in BMMSCs compared with other cell populations, indicating that those cells are the main source of

IL-6, in agreement with previous data (1). Neuregulin 3, an EGF family member, was exclusively expressed by myeloma cells. As it was not expressed by subpopulations of the environment, the neuregulin 3 expression found in some whole bone marrow samples is the reflection of its expression in myeloma cells. In contrast, BAFF was mainly expressed by cells from the environment, in particular by CD14$^+$ and CD15$^+$ cells. Osteoclasts are huge APRIL producer, emphasizing the importance of osteoclasts in promoting myeloma cell growth (62).

GEP and prediction of bone disease. Tian et al. compared the GEP of 137 myeloma patients with bone lesions to that of 36 patients in whom focal bone lesions could not be detected by magnetic resonance imaging. In this study, only four genes were shown to be significantly overexpressed by MM cells from patients with focal bone lesions (63). One of these genes was *DKK1*, a soluble inhibitor of the Wnt pathway. Consistently, recombinant DKK1 or bone marrow serum containing high levels of DKK1 inhibited osteoblastic differentiation from BMMSCs *in vitro* (63). Hence, DKK1 produced by MMC would shift the balance between bone formation by osteoblasts and bone destruction by osteoclasts in favor of osteoclasts, thereby enhancing bone resorption.

Conclusion

Measurement of the expression of thousands of genes in hundreds of patient samples using microarrays has revealed novel molecularly defined subclasses of tumor, some of them predicting clinical behavior. Furthermore, DNA microarrays proved to be very useful to improve our understanding of the myeloma pathogenesis, first, through a comparative analysis of tumor cells and their normal counterpart, and second, through a comprehensive analysis of the complex interactions between tumor cells and the bone marrow microenvironment. The challenge will be now to translate this fundamental knowledge into new prognostic, diagnostic, and therapeutic tools that will improve treatment and outcome of patients with MM.

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