Multiple myeloma (MM) is a heterogeneous disease. In this issue of Blood, Broyl and colleagues present a comprehensive analysis of gene expression profiles that leaves an overall impression consistent with previous molecular classifications and highlights 3 additional molecular subtypes of the disease.

A useful molecular classification forms the basis for identifying patients with shared biology, prognosis, and response to treatment. Although the use of a molecular classification is firmly established in leukemia and lymphoma, it has been only slowly adopted for myeloma. In part this is because, unlike in the former 2 diseases, the molecular subtypes of myeloma are not associated with very distinct morphologic or histologic appearances. They do, however, have distinct prognoses and response to treatment, which is leading to the more widespread use of molecular markers in the management of myeloma.

Myeloma can be thought of as having primary and secondary genetic events. The primary events include recurrent, nonoverlapping, immunoglobulin gene chromosome translocations involving 4p16 (FGFR3 and MMSET), 6p21 (CCND3), 11q13 (CCND1), 16q23 (MAF), and 20q11 (MAFB). The patients lacking 1 of these translocations are primarily characterized by hyperdiploidy, with trisomies of chromosomes 3, 5, 7, 9, 11, 15, 19, and 21. A unifying feature appears to be dysregulation of a D-type cyclin, and, to a large extent, the primary genetic events can be considered disease-defining events that will not change during the course of a given patient’s disease. Secondary genetic events include mutations that activate RAS and the NFKB pathway, inactivating mutations of p53 and KDM6A (UTX), rearrangements that dysregulate MYC, and closely associated 1p deletion/1q amplification. Although not disease-defining, the secondary genetic events may have important implications for prognosis and response to treatment, and be subject to modulation by effective therapy.

Some of these genetic events are associated with very distinctive gene expression profiles, most notably the MAF translocations, which are characterized by the unique and high level of coexpression of a number genes, many of which are thought to be direct targets of the MAF transcription factors. Similarly, activation of the NFKB pathway results in the coexpression of a set of characteristic NFKB target genes. Hyperdiploid MM is characterized primarily by the low level overexpression of a very large set of genes located on the chromosomes involved in the trisomies. Finally, some genetic events are not correlated with any patterns of gene expression, most notably RAS mutations.

Conversely, there are some distinctive patterns of gene expression that are not associated with known underlying genetic events. These include the coexpression of myeloid lineage genes (often interpreted to represent contamination of the CD138-selected plasma cells with myeloid cells), the coexpression of genes associated with proliferation, the coexpression of a number of cancer testis antigens (CTA), and, as noted by Broyl and colleagues, the coexpression of a set of genes that include PTP4A3 (PRL3), PTPRZ1, and SOCS3.

To take an unbiased approach to the molecular characterization of MM, the authors have analyzed the gene expression profiles of a new cohort of untreated MM patients, and using somewhat different analytical methods, repeated
the basic analysis first reported by Zhan et al from the University of Arkansas for Medical Sciences (UAMS). Most importantly they reproduced the unbiased identification of the 8 main subgroups (CD1, CD2, MF, MS, PR, HY, LB, MY) and identified 3 additional subgroups (NFKB, CTA, and PRL–3). Although the impression for the entire cohort was the same, at the individual patient level there were some important differences. In contrast to the previous report, only a third of the CD1 had a t(11;14), and there was a 38% discordance with the UAMS CD1. In addition, there was a discordance of 40% for the PR subgroup, 30% for the NFKB, and 18% for both the CD-2 and HY. There are some technical aspects that confound the analysis, most notably the divergent handling of the MY samples, the lack of an NFKB subgroup in the original UAMS classification, and the apparent use of the original translocation and Cyclin D (TC) classification based on the Affymetrix Hu95A GeneChip, as opposed to the one updated for the HU133Plus2 GeneChip actually used.

Should one be concerned at this high level of discordance? I do not think so, as it is not that important, and in fact quite predictable. The first part of the analysis identifies the 5% most variable genes, and uses these to perform hierarchical clustering. As one varies the number and identity of this list of genes (eg, 1% most variable, 10% most variable, etc), patients move between clusters, new clusters are identified, and old clusters split. Three different patients may express a variable number of genes characteristic of hyperdiploidy, proliferation, and NFKB (see figure). As one varies the number of genes from each subgroup within the list used for clustering, a given patient may be classified differently. In part it is a problem of taking multidimensional data and reducing it to a single dimension. An alternative would be to keep all of the dimensions, starting with the first dimension—the primary genetic event (captured to a great extent by the TC classification), and adding additional dimensions to represent the dominant secondary transcriptional signatures (PR, LB, NFKB, CTA, PRL–3).

So which molecular classification (TC, UAMS, IH65) should be used going forward? In my opinion, whichever one works best for the question being addressed, given the inherent limitations of all such classification. In the future we will have molecularly targeted therapies (eg, NFKB inhibitors, STAT3 inhibitors, FGFR3 inhibitors, CTA immunotherapy, antiproliferatives) and the molecular classification used will follow logically from the therapy being considered.

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REFERENCES


Comment on Weng et al, page 2579

PTEN: not just a tumor suppressor

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In this issue of Blood, Weng and colleagues provide genetic evidence for the PI3K (phosphatidylinositol-3-OH kinase)–dependent and –independent regulatory role of PTEN (phosphatase and tensin homologue deleted from chromosome 10) during collagen-induced platelet activation.

During vascular injury, the monolayer of endothelial cells that line blood vessel walls becomes disrupted, thereby exposing subendothelial adhesive proteins such as collagen. Circulating platelets attach to exposed collagen through integrin αIIβ3 and glycoprotein VI (GPVI). Collagen binding to the receptor GPVI induces a complex signaling cascade within the platelet that leads to a rise in intracellular calcium levels, cytoskeletal reorganization, and activation of integrin αIIβ3, the platelet fibrinogen receptor. One of the early events of this signaling pathway involves generation of inositol-1,4,5-trisphosphate (IP3). Phosphatidylinositol (PI) is a membrane phospholipid that can be phosphorylated at the 3, 4, and 5 position of the inositol ring to produce PI-3-phosphate, PI-4-phosphate, PI-5-phosphate, PI-3,4-biphosphate, PI-3,5-biphosphate, PI-4,5-biphosphate (PIP2), and PI-3,4,5-triphosphate (PIP3). Lipid kinases and phosphatases can rapidly interconvert phosphoinositide species to dynamically regulate their levels. Importantly, PI3K phosphorylates PIP2 to produce PIP3, whereas PTEN converts it back to PIP2. In addition, phospholipase C (PLC) hydrolyzes PIP2 to generate second messengers IP3 and diacylglycerol.
PI3K recruits serine/threonine kinase AKT to the plasma membrane where it is activated by a variety of activators such as the mammalian target of rapamycin, integrin-linked kinase, and 3-phosphoinositide-dependent protein kinase 1. PI3K activity in AKT activation is counterbalanced by PTEN. Although the roles of PI3K and AKT are well recognized in regulating platelet activation, the function of PTEN has not been studied. Weng and colleagues, for the first time, provide convincing evidence about the negative regulatory role of PTEN in collagen-induced platelet activation. They show that PTEN regulates collagen signaling in both a PI3K/AKT-dependent and -independent manner.

Because nonspecific ablation of PTEN is embryonically lethal, Weng and colleagues used inducible and hematopoietic tissue–specific deleted mice and found that PTEN suppresses collagen-induced platelet activation. PTEN deficiency significantly reduced bleeding time and increased responsiveness of platelets to collagen. Deficiency of PTEN enhanced collagen-induced activation of AKT. Surprisingly, inhibition of PI3K prevented the aggregation of wild-type platelets, but not PTEN-deficient platelets, suggesting that PTEN may regulate collagen-induced signaling through PI3K/AKT in both a dependent and independent manner.

As depicted in the figure, PI3K converts PIP2 to PIP3, which activates AKT, whereas PTEN opposes this process. One would therefore predict that PTEN deletion would increase AKT activation. As expected, PTEN-deficient platelets show enhanced AKT activation. Furthermore, PTEN helps replenish the pool of PIP2, which is important for granular secretion. Thus, decreased secretion due to reduced PIP2 availability was expected in PTEN-deficient platelets. Contrary to the prediction, collagen-induced granular secretion is increased in the absence of PTEN. Furthermore, inhibition of PI3K in wild-type platelets abrogates collagen-induced AKT activation and platelet aggregation, suggesting that collagen-induced platelet activation is entirely PI3K/AKT-dependent.

Surprisingly, in PTEN-deficient platelets, inhibition of PI3K failed to inhibit collagen-induced aggregation. These results suggest that in addition to dephosphorylation of PIP3, PTEN exerts an inhibitory effect on granular secretion and/or integrin activation, which is PI3K/AKT independent. One possibility is that PTEN may dephosphorylate signaling proteins downstream of protein kinase C (PKC). This is conceivable, considering the protein phosphatase activity of PTEN. In addition to being a phosphatidylinositol–3-phosphatase, PTEN is also a dual specificity protein phosphatase. It is also well established that PTEN is a tumor suppressor. PTEN’s role in tumor suppression has been extended beyond simply down-regulation of the PI3K/AKT pathway. It has been shown to regulate phospholipase D and PLC. Furthermore, genetically modified mice confirm that mutation in PTEN results in tumorigenesis; however, activated AKT transgenic lines do not develop tumors, suggesting that AKT-independent mechanisms contribute to PTEN tumorigenesis. Another exciting possibility is that deletion of PTEN together with inhibition of PI3K may alter PIP2 levels or its interaction with downstream signaling proteins. For example, PIP2 has been shown to regulate talin, a key activator of integrin αIIbβ3.

Further experimentation will be needed to identify the PI3K/AKT-independent inhibitory mechanism of collagen–induced platelet activation by PTEN. Nevertheless, the finding reported by Weng and colleagues not only identifies PTEN as a novel regulator of collagen signaling, but also opens new avenues for investigation of its role beyond PIP2 production in platelets.

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REFERENCES


Iron, bone, and marrow

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Osteoporosis is a common problem in disorders characterized by iron overload, such as the thalassemias and hereditary hemochromatosis. The exact role of iron in the development of osteoporosis in these disorders is not established. In this issue of Blood, Tsay et al1 suggest that increased bone resorption caused by increased iron-associative oxidative stress may be a significant pathogenetic mechanism.

Iron overload was associated with increased bone resorption (see figure), increased oxidative stress, and evidence of systemic inflammation. Significantly, they were prevented by treatment with the antioxidant N-acetyl-L-cysteine, supporting the hypothesis that oxidative stress is involved in their pathogenesis. Osteoblast
The relation between iron overload and increased oxidative stress: oxidative stress in turn induces inflammation, offering carefully collected data supporting the claim that the bone defect associated with iron overload is caused by increased osteoclastic activity. The clear prevention of iron-associated bone abnormalities by the antioxidant N-acetylcysteine is strong evidence supporting the role of ROS in the observed bone abnormalities. By contrast, the experiments involving inflammatory cytokines are less convincing. This is not because of the correlations observed, but the lack of evidence supporting a mechanistic cause-and-effect relation.

Despite these caveats and the need for further studies to validate these concepts in the clinical settings, the above observations may have significant implications for the management of clinical complications encountered in patients with chronic iron overload.

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REFERENCES
linking proteins, ankyrin and protein 4.1R, is responsible for the remarkable flexibility, mechanical stability, and cohesion of the normal red cell membrane. Various inherited red cell membrane disorders, such as hereditary elliptocytosis, ovalocytosis, and spherocytosis, result from either a misassembled skeletal network and/or defects in linkage of the network to the membrane. Over the past 3 decades, identification of mutations in genes encoding the actin-capping proteins may account for the 10% to 15% of cases of human hereditary spherocytosis and elliptocytosis in which the underlying molecular defect has yet to be defined. The study of Moyer and colleagues begins to provide some answers to these questions by showing that tropomodulin does play a role in regulating actin filament length and that absence of tropomodulin leads to assembly of a disordered membrane skeleton.

What are the implications of these findings? One is that tropomodulin—like other protein components of the red cell membrane skeleton that interact with actin such as adducin, dematin, and protein 4.1R—is a key regulator of red cell membrane stability. Hence, deficiency of tropomodulin in mouse red cells results in spherocytic elliptocytosis. These findings, along with similar findings from adducin- and dematin-deficient mouse red cells, raise the possibility that mutations in genes encoding the actin-capping proteins may account for the 10% to 15% of cases of human hereditary spherocytosis and elliptocytosis in which the underlying molecular defect has yet to be defined. But in the broader context, the reported findings could lead to obtaining new insights into the assembly during terminal erythroid differentiation of the unique and highly ordered spectrin-actin–based red cell membrane skeleton, which is critical to ensure the remarkable durability of the mature erythrocyte during its 120-day life span in circulation.

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REFERENCES

 Comment on Cooley et al., page 2411

Donor selection for AML: do the KIR

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How to select the donor when there is a choice of several HLA-matched donors? In this issue of Blood, Cooley and colleagues describe that matched donors with a favorable KIR gene content confer a significantly reduced risk of posttransplantation relapse in patients with AML.

A lllogeneic hematopoietic stem cell transplantation (HSCT) was developed to cure otherwise incurable leukemia. Whereas HSCT was initially restricted to patients who had a human leukocyte antigen (HLA)—matched sibling donor, more and more patients with otherwise incurable leukemia benefit from HSCT by the expansion of the donor pool through the recruitment of matched unrelated volunteer donors. HLA matching for...
HLA class I and II alleles is still the most important criterion for donor selection. Beyond the eradication of leukemic cells by the myeloablative regimen and the replacement of defective bone marrow by the allogeneic graft, the success of HSCT depends to a large extent, on the balance between the donors’ alloreactive T lymphocytes against the recipients’ tissues (graft-versus-host disease [GVHD]) and the favorable reaction of the donors’ T lymphocytes toward the leukemic cells (graft-versus-leukemia effect [GVL]).

More recently, it has also been shown that the recipients’ innate donor immune system also contributes significantly to the eradication of residual leukemic cells. Natural killer (NK) cells, as key members of the innate immune system, seem to play an especially important role in leukemia control after HSCT. The role of NK cells in leukemia control after HSCT is of particular interest since NK cells are the first and most abundant infiltrating cells in the bone marrow in the early stages of acute myeloid leukemia (AML) and ALL, and the development of NK cells is believed to be derived from hematopoietic stem cells. NK cells also play a critical role in the eradication of leukemic cells by the allogeneic graft, and the favorable reaction of the donors’ alloreactive T lymphocytes against the recipients’ reactive T lymphocytes against the recipients’ alloreactive T lymphocytes toward the leukemic cells (graft-versus-leukemia effect [GVL]).

The present work confirms and extends previous findings and raises several important issues: Besides the classical HLA matching for class I and II, there is with the KIR gene family an independent second immunogenetic system that has a significant influence on the outcome of allogeneic HSCT. It is not clear why this effect is seen only in adult patients with AML but not with ALL. In pediatric patients with ALL, an influence of the donor KIR genotype or phenotype has been described in haploidentical transplantation with T cell–depleted grafts. There might be significant differences in the expression of various inhibitory or activatory KIR ligands on AML and ALL targets might reveal such differences, and the antileukemic effects of the donor KIR genotype might also be harnessed in patients with ALL, for example by manipulating the HLA/KIR interactions.

Inexpensive methods for KIR genotyping are available and can be easily applied concomitantly to HLA genotyping. Based on the
already published data, the detailed analysis in this article, and the only positive but not negative effects of donor KIR genotypes on the transplantation outcome, the time has come to add KIR genotyping to the confirmatory HLA genotyping during the donor search for patients with AML and to select among different HLA-matched donors for a given patient the one donor predictive for the most effective relapse protection based on his KIR B gene content score as described by Cooley and colleagues.

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REFERENCES

Impressions of the myeloma landscape

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