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1083-8791/06/1201-0114$32.00/0
doi:10.1016/j.bbmt.2005.10.002

Using Genomics to Identify High-Risk Myeloma after Autologous Stem Cell Transplantation

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ABSTRACT

Multiple myeloma is a malignancy of antibody-secreting plasma cells that expand in the bone marrow. Although high-dose therapy/autologous stem cell transplantation has become the standard of care for patients with multiple myeloma, survival is highly variable and can range from a few years to >10 years after diagnosis. Application of high-throughput genomics on a large uniformly untreated cohort of patients has revealed that activation of 1 of the 3 cyclin D genes is a universal initiating event in this disease and that acquisition of abnormalities of chromosome 1 leads to activation of *CKS1B*, a regulator of p27Kip1 degradation. Synergy between cyclin D2 and CKS1B, but not cyclin D1 and CKS1B, may lead to early treatment failure.

KEY WORDS

Multiple myeloma • Autologous stem cell transplantation • High-dose therapy • Survival

Multiple myeloma (MM) is a malignancy of antibody-secreting plasma cells that expand in the bone marrow and cause severe osteolytic bone destruction, hypercalcemia, immunosuppression, anemia, and kidney failure [1]. High-dose therapy/autologous stem cell transplantation has become the standard of care in the treatment of MM. Although outcome has been greatly improved, survival is still highly variable, with patients surviving several years to >10 years [2-4]. Aggressive disease, with increased proliferation and a higher frequency of abnormal metaphase karyotypes, increased lactate dehydrogenase, and extramedullary manifestations, seen in approximately 20% of newly diagnosed patients, inevitably appears in all cases. The molecular mechanisms leading to this aggressive conversion are not understood. In this review, we describe the most common genetic lesions in myeloma and our current attempts to identify the genetics of high risk.

Recurrent nonrandom genetic lesions have been identified in myeloma, and these have been related to clinical course and response to therapy [5]. At the genetic level, myelomas can be broadly separated into hyperdiploid and nonhyperdiploid diseases [6]. Nonhyperdiploid myelomas, which typically harbor immunoglobulin-mediated translocations that lead to transcriptional activation of normally silent proto-oncogenes, account for approximately 50% of newly diagnosed cases. The recurrent translocations are t(11;14), which activates *CCND1*, in approximately 17%, followed by t(4;14), which activates the *FGFR3* and *MMSET* genes, in another approximately 17%; t(14;16), which activates *MAF*, in approximately 6%; t(14:20), which activates *MAFB*, in another 6%; and t(6;14), which activates *CCND3*, in no more than approximately 3% [7-10]. The remaining 50% of myelomas are hyperdiploid, with aneuploidy resulting from trisomies of chromosomes 3, 5, 7, 9, 11, 15, 19, and 21 [11-14].

Gene expression studies have revealed that virtually all myelomas, regardless of ploidy status, exhibit deregulated expression of 1 of the 3 cyclin D genes, thus suggesting that cyclin D activation may be an initiating genetic event in this malignancy [12]. Myelomas with translocations that result in activation of *CCND1* are typically diploid and have a more favorable prognosis than those with translocations that activate *MAF* or *FGFR3/MMSET* [15-18]. Deletion of chromosome 13q14, which is strongly linked to *IGH*-mediated translocations; chromosome 17p; and hypodiploidy are associated with a poor prognosis [18-22]. Hyperdiploid tumors are thought to be more dependent on interactions with the bone marrow microenvironment, as evidenced by higher levels of

DKK1 expression, increased incidence of lytic bone lesions, and their conspicuous absence in myeloma cell lines [12,23,24]. Virtually all of the recurrent genetic lesions seen in myeloma are also observed in the benign plasma cell dyscrasia monoclonal gammopathy of undetermined significance [25-28], and this suggests that additional, uncharacterized gene mutations may be required for progression of this condition.

In an effort to identify genes linked to an aggressive clinical course, we applied RNA from highly purified plasma cells derived from 351 newly diagnosed patients with MM to Affymetrix (Santa Clara, CA) U133Plus2.0 microarrays. Expression extremes of approximately 54 000 probe sets were correlated with disease-related and overall survival after 2 cycles of high-dose melphalan and autologous stem cell transplantation. This analysis revealed a statistically significant overrepresentation of chromosome 1 genes in a group of 70 genes whose expression was linked to poor outcome. In particular, overexpression of *CKS1B*, which maps to an amplicon at 1q21 in myeloma [29-35] and regulates SCF^{Skp2}-mediated ubiquitination and proteolysis of the cyclin-dependent kinase (Cdk) inhibitor $p27^{Kip1}$ [36,37], was significantly overexpressed in patients with poor survival ($P < .001$). Increased expression of *CKS1B* was strongly correlated with gene amplification ($P < .0001$), which in turn was an independent predictor of unfavorable outcome in multivariate analyses ($P = .002$). *CKS1B* expression and amplification both increased at relapse and imparted a short postrelapse survival ($P < .005$).

Fourth-quartile *CKS1B* expression was observed in nearly two thirds of patients with *MAF* or *MAFB* activation, in one third each with *FGFR3/MMSET* and *CCND1* activation, and in only 18% of those who lacked such translocations ($P < .0001$). In the context of metaphase karyotypes, hyperdiploidy is associated with a more favorable prognosis than hypodiploidy [5]: *CKS1B* quartile 4 expression was observed in approximately 20% of cases with hyperdiploid or normal (uninformative) karyotypes but in nearly 50% of hypodiploid MM cases ($P = .0002$). Adjusting for these genetic subgroups in multivariate analyses, high *CKS1B* expression remained an independent adverse predictor. *MMSET*-, *MAF*-, and *MAFB*-activating translocations conferred inferior event-free survival ($P = .001$) but not inferior overall survival ($P = .164$). Consistent with the favorable implications of *CCND1* translocations, the superior event-free and overall survival of this subgroup was not negated by high *CKS1B* expression.

The eukaryotic cell cycle is controlled by Cdks, which are opposed by Cdk inhibitors [38]. Reduced protein levels of the Cdk inhibitor $p27^{Kip1}$, which regulates Cdk2/cyclin E activity and the late restriction point of the G_1 to S transition of the cell cycle, are associated with a poor prognosis in many cancers [39]. The absence of inactivating mutations in the *CDKN1B/p27*^{Kip1} gene has raised speculation that hyperactivation of SKP2 or CKS1B, representing the rate-limiting components of the $SCF^{Skp2\text{-}Cks1}$ ubiquitin ligase, may lead to inappropriate degradation of $p27^{Kip1}$ [39]. Recent studies have shown that loss of $p27^{Kip1}$ is associated with shortened survival in patients with myeloma [40]. On the basis of our current knowledge, we propose that increased degradation of $p27^{Kip1}$ and poor prognosis in myeloma may be caused in part by a gene dosage–related increase in *CKS1B* gene expression. In support of this concept, we also observed that *CKS1B* overexpression and amplification commonly surfaced at relapse in patients who lacked such features at diagnosis; this suggests that 1q21 rearrangements in MM may, in part, target the *CKS1B* gene.

Cyclin D dysregulation is a common event in cancer and contributes to tumorigenesis by promoting hyperphosphorylation of the RB1 protein, activation of E2F, and transition through the early G_1 to S phase of the cell cycle. We have recently reported that dysregulated expression of 1 of the 3 D-type cyclins may be initiating genetic lesions in MM. On the basis of our current knowledge, we propose that activation of a CyclinD gene, especially Cyclin D2, and CKS1B gene activation may cooperate to deregulate both early and late restriction points of the G_1 to S phase of the cell cycle.

CKS1B gene amplification, along with chromosome 13q14 deletion and abnormal metaphase cytogenetics, accounted for almost 40% of the observed survival variability in this analysis. This underscores that myeloma risk is best assessed by molecular and cellular genetic tests. Routine application of such studies, performed on a single bone marrow sample, is recommended for appropriate patient stratification in therapeutic trial design. The survival effect of new agents, such as bortezomib and thalidomide and its derivatives, will be profound if their clinical efficacy also extends to genetically defined high-risk myeloma, which to date has not been investigated. CKS1B function seems to directly or indirectly interact with ubiquitin ligases, the proteasome, or both to regulate cell-cycle progression [41]. New therapeutic strategies that directly target CKS1B or related pathways may represent novel, and more specific, means of treating de novo high-risk myeloma and may prevent secondary evolution.

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