Global Gene Expression Profiling in the Study of Multiple Myeloma

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Abstract

Multiple myeloma (MM) is a rare but uniformly fatal malignancy of antibody-secreting plasma cells. Although several key molecular events in disease initiation or progression have been confirmed (eg, FGFR3/MMSET activation) or implicated (eg, chromosome 13 deletion), the mechanisms of MM development remain enigmatic. Although it is generally indistinguishable morphologically, MM importantly exhibits a tremendous degree of variability in its clinical course, with some patients surviving only months and others for many years. However, measures of current laboratory parameters can account for no more than 20% of this outcome variability. Furthermore, the means by which current drugs impart their anti-MM effect are mostly unknown. The development of serious comorbidities, such as osteopenia and/or focal lytic lesions of bone, is also poorly understood. Finally, very little knowledge exists concerning the molecular triggers for the conversion of benign monoclonal gammopathy of undetermined significance (MGUS) to overt MM. Given that abnormal gene expression lies at the heart of most if not all cancers, high-throughput global gene expression profiling has become a powerful tool for investigating the molecular biology and clinical behaviors. Here I discuss recent progress made in addressing many of these issues through the molecular dissection of the transcriptome of normal plasma cells, MGUS, and MM.

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1. Global Gene Expression in Cancer

In the mid 1990s, Brown and colleagues developed a system of monitoring the expression levels of thousands of genes simultaneously [1,2]. These so-called microarrays can be viewed as a reverse Northern blot in that cloned DNA fragments (called the probe) are immobilized on a solid matrix; then, messenger RNA (mRNA) of the tumor or other tissue of interest is labeled with a fluorescent dye (Cy5), RNA from a normal control source is labeled with another dye (Cy3), and both are hybridized simultaneously. So-called high-density oligonucleotide microarrays were developed by using photolithography and solid-phase chemistry to produce arrays containing hundreds of thousands of oligonucleotide probes packed at extremely high densities [3]. The probes are designed to maximize sensitivity, specificity, and reproducibility, allowing consistent discrimination between specific and background signals and between closely related target sequences [4]. Affymetrix has now developed high-density oligonucleotide microarrays that can monitor the expression of nearly all 35,000 human genes simultaneously. Microarray technology was first used in 1996 to study cancer [5] and now has been used to develop predictors of (1) disease class in cancers that are morphologically indistinguishable [6-9] and (2) cancer response to therapeutic interventions [10,11] and metastatic potential [12].

2. Myeloma

Multiple myeloma (MM) is a plasma cell (PC) dyscrasia that accounts for 10% of all hematologic malignancies and is the second most frequently occurring hematologic cancer in the United States after non-Hodgkin's lymphoma [13,14]. At any one time, the prevalence of MM is nearly 50,000. Nearly 15,000 new cases are currently diagnosed, and 11,000
die from the disease each year. Although MM is uniformly fatal, long remissions can be achieved with high-dose chemotherapy and stem cell support. The clinical spectrum of disease presentations of PC dyscrasias is wide and covers a presumptive precursor condition termed monoclonal gammopathy of undetermined significance (MGUS) with an annual progression to overt MM of 1% [15], solitary plasmacytoma of bone and soft tissue, smoldering MM (representing a more advanced MGUS), and truly overt and symptomatic MM. Symptoms of advanced disease consist of anemia, discrete lytic or diffuse bone lesions with hypercalcemia, renal failure, and recurrent infection because of profound immunosuppression.

In spite of many recent advances in our understanding of the genetic complexities in MM [16], a “genomic chaos” unique to this disease within the hematopoietic lineage has limited the identification of distinct molecular entities. Although much of the genetic heterogeneity in MM is thought to represent noise, it is possible that undefined discrete genetic entities that are suspected to account for the variable clinical course of MM may exist beneath this cloud of genomic chaos. Thus, with the use of more powerful genomic analyses such as microarray profiling, it is possible that MM will be discovered to represent a broad descriptor of several distinct clinical entities.

3. Gene Expression Profiling in Multiple Myeloma

Klein and colleagues were the first to use gene expression profiling (GEP) to study MM [17]. In their work, the investigators used human MM cell lines and small-scale, filter-based, complementary DNA arrays to identify key intercellular signaling genes expressed in malignant PCs. In a more recent report, Stewart and colleagues used a combination of high-throughput DNA sequencing and microarray hybridization of RNA derived from cells pooled from several PC leukemia cases to establish a comprehensive list of genes expressed in MM [18]. Because MM cell lines and PC leukemias represent terminal stages of the disease, it is important to put GEP data involving the use of these cells in the context of newly diagnosed disease so that gene expression events associated with initiation and those associated with progression can be more adequately defined. These studies also demonstrated the need to establish gene expression profiles of normal PCs. Given that PCs typically make up less than 5% of the cells in normal human bone marrow, isolation of a sufficient number of cells for GEP without resorting to RNA amplification techniques required specialized methodologies. Two different and complementary techniques have been used to accomplish this objective. We have employed automated immunomagnetic bead sorting of PCs from large-volume bone marrow aspirates by using a monoclonal antibody, BB4, raised against syndecan-1/CD138 [9]. This technique has now been used to isolate highly homogeneous populations of normal PCs from both bone marrow and tonsil [19]. The need to isolate sufficient numbers of PCs from normal human marrow for large-scale GEP experiments makes this technique an impractical endeavor for most laboratories. Thus, to create a source of polyclonal PCs from healthy donors, Tarte and colleagues developed a method for the in vitro differentiation of peripheral blood B-cells [20]. Global expression profiling of polyclonal PCs and normal bone marrow PCs derived via immunomagnetic sorting has revealed strong similarities, but also distinct differences, between the 2 PC populations and MM [20] (K. Tarte et al, unpublished data).

In an attempt to develop a comprehensive picture of the gene expression changes associated with the normal development and neoplastic transformation of human PCs, my laboratory began in March of 2000 to profile CD138-enriched PCs from the bone marrow of healthy donors, patients with newly diagnosed and end-stage MM, as well as patients with other PC dyscrasias, including MGUS, smoldering MM, and Waldenström macroglobulinemia. As of this writing, we have performed GEP on more than 800 cases. In an initial study investigating the expression of 6800 genes in 74 patients with newly diagnosed MM, 7 with MGUS, and 31 normal bone marrow PC [9], we were able to show the following: (1) short-term serial GEP reveals little intrasample variability, suggesting that changes observed between GEP at diagnosis and GEP at fulminate relapse may reveal clues to the mechanisms of resistance; (2) microarray-derived gene expression levels and protein levels as determined by fluorescence-activated cell sorter analysis are tightly correlated; (3) the spiked expression of CCND1, CCND3, MAF, and FGF3/ MSSET reflects the presence of 14q32 translocations into these loci; (4) MM can be significantly differentiated (P < .0001) from normal bone marrow PCs by approximately 120 of 6800 genes, possibly reflecting fundamental genetic changes that are involved in or reflect neoplastic transformation; (5) GEP can accurately distinguish normal PCs from MM, but MGUS is indistinguishable from MM; and (6) 4 distinct molecular subgroups of MM can be identified via the use of unsupervised hierarchical clustering, with the so-called MM4 subgroup being related to highly proliferative human myeloma cell lines (HMCL) and the MM1 subgroup being more similar to MGUS. As expected, the most significant expression differences between MM1 and MM4 were found to be of highly expressed genes in MM4 that are related to the control of cell cycle and DNA metabolism. The MM4 group also exhibits a significant increase in the incidence of karyotypic abnormalities and elevated levels of serum β2-microglobulin compared with the other 3 subgroups. We have concluded from these data that the MM4 subgroup represents a high-risk entity. However, long-term follow-up of these patients will be required to realize these predictions. Importantly, these data revealed that a subgroup of newly diagnosed MM cases has GEP features of HMCL, implying that HMCL represent an appropriate model system for studying the biology of the disease and evaluating preclinical drug efficacy, at least for the HMCL-like subgroup of the disease.

Here I describe some of the major observations derived from our recent progress in GEP studies of MM.

4. Determining the Molecular Switches in Human PC Differentiation

Knowledge of the gene expression patterns associated with B-cell differentiation should provide a deeper under-