

Integrating Cytogenetics and Gene Expression Profiling in the Molecular Analysis of Multiple Myeloma

John D. Shaughnessy and Bart Barlogie

Donna D. and Donald M. Lambert Laboratory of Myeloma Genetics, Myeloma Institute for Research and Therapy, University of Arkansas for Medical Sciences, Little Rock, AR, USA

Abstract

Multiple Myeloma (MM), is a currently incurable malignancy of a terminally differentiated antibody secreting plasma cell (PC) that can be controlled with high dose therapy and stem cell support. Conventional cytogenetic analysis has revealed a genomic instability that sets MM apart from the other blood cell cancers. In spite of this “genomic chaos” recurrent structural rearrangements and ploidy changes have aided the identification of important molecular mechanisms of disease etiology and that have also proved to be useful clinical landmarks. Yet, abnormal cytogenetics, present in only 30% of cases, combined with other clinical parameters, only account for 20% of the variability in clinical outcome, which can range from 2 months to >10 years. Thus, the genomic chaos may mask a unifying chromosome abnormality, e.g. Philadelphia chromosome in CML, or may indicate that MM is a broad descriptor of constellation of many distinct clinical and molecular entities. The advent of microarray profiling of global gene expression patterns is providing evidence that both of these possibilities may be true. We will discuss how the integration of conventional and molecular cytogenetics with gene expression profiling has confirmed past observations and, yet fundamentally changed the way we view the disease.

1. Introduction

Multiple Myeloma (MM) is an enigmatic tumor of terminally differentiated plasma cells (PC) that home to and expand in the bone marrow. MM causes a constellation of disease manifestations including a paraproteinaemia that can lead to organ damage, immunosuppression and anemia due suppression of normal hematopoietic stem cell function, or spinal cord compression and bone fractures caused by osteoclast activated diffuse osteoporosis and osteolytic lesions (Barlogie et al., 1999). MM is unique within the blood cell cancers in that, unlike the leukemias and lymphomas, which typically present with specific chromosomal abnormalities, MM presents with, on average, 7 distinct genetic abnormalities. The genomic instability present in MM involves both ploidy and structural rearrangements as evidenced by conventional cytogenetics (Dewald et al., 1985, Sawyer et al.,

1995) and more recently by comparative genomic hybridization CGH (Cigudosa et al., 1998) and spectral karyotyping (Sawyer et al., 1998; Sawyer et al., 2001). Although this instability has revealed a “genomic chaos”, several recurrent abnormalities have been identified.

Like many tumors of the B-cell lineage MM shows recurrent rearrangements of the *IGH* locus on 14q32 (for review see Kuehl & Bergsagel, 2002). The t(11;14)(q13;q32) translocation, common to B-cell leukemia and lymphomas, is present in approximately 20% of primary MM. Molecular evidence of transcriptional activation of cyclin D1 (*CCND1*) as a result of this translocation came from studies in MM cell lines (Chesi et al., 1996) and has been subsequently confirmed in primary tumors (Zhan et al., 2002). We recently discovered that cyclin D3 (*CCND3*) is activated by a recurrent t(6;14)(p21;q32) translocation (Shaughnessy et al., 2001), confirming the importance of the deregulated expression of the cyclin

D family in MM growth and/or development. The first MM specific *IGH* associated translocation to be molecularly cloned and characterized was the t(4;14)(p16.3;q32.3) that results in the activation of fibroblast growth factor receptor 3 (*FGFR3*) (Chesi et al., 1997). Chesi and colleagues later showed this translocation to also result in the production of a novel fusion transcript between *MMSET* and *IGH* (Chesi et al., 1998a). Stewart and colleagues have shown ectopic expression of *FGFR3* promotes myeloma cell proliferation and prevents apoptosis (Plowright et al., 2000) and is transforming in hematopoietic cells (Li et al., 2001). Using spectral karyotyping we were able to identify several recurring chromosome translocations not previously reported in MM including the t(14;16)(q32;q22), the t(9;14)(p13;q32), typically associated with Waldenstrom's macroglobulinemia and lymphoplasmacytoid lymphoma, and a t(6;8)(p10;q10) translocation (Sawyer et al., 1998). Chesi and colleagues went on to show that the t(14;16)(q32;q22) translocation results in activation of the *MAF* oncogene (Chesi et al., 1998b). Although translocations involving the *IGH* locus on chromosome 14q32 are frequent in MM (73%), recent FISH studies have shown that the translocation partners in 44% of the 14q32 rearrangements are not known (Avet-Loiseau et al., 2002). There is growing evidence that the known translocations are important genetic markers of specific biological characteristics or clinical outcome (Chesi et al., 2000, Avet-Loiseau et al., 2002, Fonseca et al., 2002a).

We and others have demonstrated that chromosome 13 deletion, present in over 50% of MM cases, represents one of the most important adverse prognostic variables in MM (Tricot et al., 1995; Seong et al., 1998; Perez-Simon et al., 1998; Desikan et al., 2000; Zojer et al., 2000; Facon et al., 2001; Fonseca et al., 2002b; Tricot et al., 2002; Shaughnessy et al., in press). Chromosome 13 deletions are also seen in monoclonal gammopathy of undetermined significance (MGUS) (Konigsberg et al., 2000) and are associated with the transition to MGUS to overt MM (Avet-Loiseau et al., 1999). We are currently using a combination of global gene expression profiling, multi-probe FISH analysis, candidate gene and positional cloning techniques to clone and characterize this putative tumor suppressor gene (Shaughnessy et al., 2000; Zhan et al. 2001; Tian et al., 2001).

Even with tremendous recent advances in molecular characterization of MM, the genetic complexity of this disease has limited risk stratification and therapeutic advances. Thus, more powerful and comprehensive approaches to studying the genetics of MM are warranted. Inasmuch as all cancer mutations appear to directly or indirectly affect gene transcription, the ability to follow these changes represents a powerful way in which to classify and study the molecular biology of the disease. Changes in gene expression can be quantitatively monitored based on complementary base pairing of nucleic acids. In so called northern hybridizations, messenger RNA is isolated from cells of interests (tumor or normal), immobilized on nitrocellulose (referred to as tar-

get) and hybridized with a DNA fragment corresponding to a cloned gene of interest that has been labeled by incorporation of a reporter nucleotide (referred to as probe). In the mid 1990s Brown and colleagues developed a system, called cDNA microarrays, that allowed the simultaneous monitoring of the expression level of thousands of genes (Schena et al., 1995, Schena et al., 1996). This microarray technique can be viewed as a reverse northern blot, in that cloned cDNA gene fragments are immobilized on a solid matrix (probe) and the messenger RNA from the experimental source, e.g. cancer tissue, drug treated cells, is labeled with a nucleotide tagged with a fluorescent marker (Cy5) and RNA from a control, e.g. normal tissue, untreated cells, labeled with a Cy3-tagged nucleotide and the two labeled cRNAs hybridized simultaneously to the microarray. After washing and scanning, the ratio of the signals from the two colors indicate the over- or under-expression of a given gene in the experimental sample. So called high density oligonucleotide microarrays (HDA) were developed by exploiting technologies adapted from the semi-conductor industry using photolithography and solid-phase chemistry to produce arrays containing hundreds of thousands of oligonucleotide probes packed at extremely high densities (Fodor et al., 1991). The probes are designed to maximize sensitivity, specificity, and reproducibility, allowing consistent discrimination between specific and background signals and between closely related target sequences (Lipshultz et al., 1999). HDA have now been developed such that the expression of nearly all-35,000 human genes can be monitored simultaneously (<http://www.affymetrix.com/index.affx>). Microarray technology was first used to study cancer in 1996 (DeRisi et al., 1996) and now has been used to develop predictors of class in morphologically indistinguishable cancers (Golub et al., 1999, Alizadeh et al., 2000; Singh et al., 2002; Zhan et al., 2002), and predictors of response to therapeutic interventions (Shipp et al., 2002, Yeoh et al., 2002).

Immunomagnetic sorting of PC from bone marrow aspirates, allowing enrichment to >95% from as low as <1%, has permitted a precise determination of the gene expression differences that distinguish this malignancy from its normal counterpart (Zhan et al., 2002). We have now conducted microarray studies on more than 500 cases of MM, normal bone marrow PC, and MGUS. From these studies we have been able to demonstrate that: 1) MM can be differentiated from normal PC by approximately 120 genes that may represent fundamental genetic changes in the initiation or progression of MM; 2) MGUS, a benign dyscrasia, has GEP indistinguishable from MM 3) and 4) distinct subgroups of MM, with clinically relevant correlations, can be identified.

To better understand the potential use of gene expression profiling (GEP) in the clinical management of MM and to gain better insight into the mechanisms of disease, we have been integrating GEP with conventional and molecular cytogenetics. We will present results of studies showing that:

- GEP reflects changes in ploidy of some, but not all chromosomes in MM
- Co-expressed cell cycle genes are up-regulated in MM with abnormal cytogenetics
- Loss of expression of specific genes can be used to accurately predict deletion of chromosome 13
- GEP can identify known and suspected gene activating 14q32 translocations in MM

2. Methods

2.1. FISH Analysis of Chromosome 13

Triple Color Interphase FISH methods for chromosome 13 deletion detection in clonotypic PC have been described (Shaughnessy et al., 2000).

2.2. Cell Procurement, Cell Purification and RNA Isolation

Bone marrow aspirates from subjects with MM and normal donors (normal bone marrow PCs) taken after written informed consent was obtained in keeping with institutional policies. PC isolation from mononuclear cell fraction was performed by immunomagnetic bead selection with monoclonal mouse anti-human CD138 antibodies using the AutoMACs automated separation system (Miltenyi-Biotec, Auburn, CA). PC purity of >95% homogeneity was confirmed by two-color flow cytometry using CD138⁺/CD45 and CD38⁺/CD45 criteria (Becton Dickinson, San Jose, CA), immunocytochemistry for cytoplasmic light chain immunoglobulin, and morphology by Wright-Giemsa staining. RNA was isolated with RNAeasy kit (Qiagen, Carlsbad, CA).

2.3. Conversion of total RNA to labeled cRNA and Hybridization to Microarray

Double-stranded cDNA was synthesized with a Superscript cDNA Synthesis System Synthesis Kit (Life Technologies, Gaithersburg, MD) using a T7-oligo (dT) 24 primer (Genset, La Jolla, CA). The cDNA were transcribed by T7 polymerase using biotinylated nucleotides using the Enzo BioArray kit (Affymetrix, Santa Clara, CA). A total of 20×g of cRNA in buffer (40 mM Tris, acetate [pH 8.1], 100 mM potassium acetate, 30 mM magnesium acetate) was fragmented at 94°C for 35 minutes. cRNA were mixed with 0.1 mg/ml Herring Sperm DNA (Promega, Madison, WI) and 4 bacteria and bacteriophage control cRNAs (1.5 pM BioB, 5 pM BioC, 25 pM Bio D, and 100 pM CreX). Aliquots of fragmented human cRNA mixtures (15×g cRNA in 250 µl hybridization mix) were hybridized to the HuGeneFL or U95av2 GeneChip[®] microarrays. Each array was washed and scanned according to procedures developed by the manufacturer (Affymetrix, Santa Clara, CA). Scanned output files were visually inspected for hybridization artifacts and then analyzed with GeneChip[®] 3.3 or 5.0.1 software (Affymetrix). Arrays were scaled to an average intensity of 1,500 and analyzed indepen-

dently.

2.4. GeneChip[®] Data Analysis

A quantitative measurement of gene expression was produced as (1) "Average Difference" (AD) (Affymetrix 3.3) or "Signal" (Affymetrix 5.0), which is a value derived from the ratio between the intensities of the sequence-specific perfect match probe set and the mismatch probe set; or (2) as an absolute call (AC) of whether the transcript is present or absent as determined by the Affymetrix 5.0 algorithm. Signal was transformed by the natural log or log base2. Statistical analysis of the data was performed with software packages SPSS 10.0 (SPSS, Chicago, IL); S-Plus 2000 (Insightful Corp., Seattle, WA);

The chi-square (χ^2) or the Fisher exact test were used to find significant differences between two groups using the absolute call and the Wilcoxin rank sum test of the quantitative expression levels. Some analyses were also performed with the Significance Analysis of Microarray algorithm (SAM) (Usher et al., 2001). Hierarchical clustering of average linkage clustering with the centered correlation metric was employed using Gene Cluster/Treeview (Eisen et al., 1998).

2.5. Multivariate Stepwise Discriminant Analysis

Multivariate analysis, based on multivariate stepwise discriminant analysis (MSDA) with the Wilk's lambda criterion, were used to select the best combination of genes that differentiate two groups using the top 100 differentially expressed genes using the χ^2 , WRS, and SAM methods. After MSDA and resulting linear discriminant function between two groups is established, both forward and backward variable selections were performed. The choice to enter or remove variables was based on minimizing the within-group variability with respect to the total variability across all the samples.

3. Results and Discussion

3.1. GEP Reflects Changes In Ploidy Of Some, But Not All Chromosomes

Abnormal G-banding karyotypes on 212 newly diagnosed patients treated with tandem transplants from 1989 to 1998 were analyzed for gains and losses of each of the autosomes and the X chromosome. The most frequent gains were of 3, 5, 7, 9, 11, 15, 19, and 21 whereas the most frequent losses were of chromosomes 13, 14, 16 and 22. To determine if gene expression patterns could be linked to changes in ploidy in malignant PC, we performed GEP on PC from 194 MM and 33 normal bone marrow donors interrogating ~ 6,800 genes. A comparison of the two groups using c2 and WRS revealed 1714 genes were differentially expressed ($P < .01$). A total of 894 genes were lower and 820 genes were higher in MM compared to normal bone marrow PC. The chromosome map position of

these genes was determined and the percentage of abnormally expressed genes from each autosome and sex chromosomes determined. Approximately ~25 to 40% of all genes on each chromosome were altered, with chromosomes 19 (40%) and 13 (38%) having the greatest number of altered genes. When the total was broken down into up- and down-regulated genes, more dramatic shifts were evident. Although chromosome 19 harbored the most altered number of genes, there was an almost even split of up- and down-regulated genes on this chromosome. This was in contrast to chromosome 13 in which over 90% of all altered genes were down-regulated in MM. Chromosomes 5, 15, and 21 harbored the most up-regulated genes whereas chromosomes 12, 13, 14, 16 and 22 contained the most down-regulated genes. Links between gene expression profiles and ploidy were marked for chromosomes 5, 13, 14, 15, 16, 19, 21, and 22. A correlation of chromosome abnormalities and clinical outcome has shown links between abnormalities in chromosome 5, 13, 14, 15, and 21 and short survival (our unpublished data). Thus, these data indicate that changes in ploidy affect virtually every chromosome in MM and that global gene expression patterns reflect this ubiquitous phenomenon. However, some chromosomes are more frequently gained and lost in MM and a concomitant up-regulation or down-regulation of genes mapping to a subset of these chromosomes can be observed. Several of these chromosomes showing correlation between ploidy and gene expression patterns have been linked to outcome suggesting that copy number gain or loss of gene(s) from these chromosomes may have a direct role in the pathogenesis of MM. This hypothesis is reinforced by the observation of chromosome 13 loss being linked to loss of gene expression and the unambiguous data linking loss of this chromosome with poor outcome in this disease. Efforts to pinpoint the culpable gene(s) on this chromosome are presented below.

3.2. Co-expressed Cell Cycle Genes are Up-regulated in MM with Abnormal Cytogenetics

Given the importance of abnormal cytogenetics as a negative prognostic variable in MM (Dewald et al., 1985; Seong et al., 1998; Tricot et al., 2002), we sought to identify genes that distinguish this particular clinical subgroup. We performed GEP on PC from 149 newly diagnosed MM cases interrogating ~12,000 genes. Cytogenetic evaluations were also performed on all patients. In total, 93 were found to have CA and 56 to have no evidence of CA. We used *c2*, Wilcoxin Rank, and SAM statistical analyses to identify the most significantly differentially expressed genes in the comparison of the two groups ($P < 0.01$; range 0.01 to 0.000001). Pearson correlation analysis showed that all genes were up-regulated in the CA group. The most significant expression changes were in two nucleolar genes. However, genes associated with cell cycle and DNA metabolism composed, by far, the largest group of genes. Interestingly, many of the genes in this group were also found as having highly correlated expression

in unsupervised hierarchical cluster analysis. Unsupervised hierarchical cluster analysis was performed on 150 patients with 9,220 genes; 23 genes associated with DNA metabolism and cell cycle control were highly correlated. We recognized that expression of these genes was extremely high in a subset of patients and suggested that these patients may have a distinct disease subtype. Hierarchical cluster analysis with 11 of the most highly correlated 23 genes on 143 of the 150 newly diagnosed patients stratified MM into two major and two minor groups. Support for the hypothesis that these groups represent clinically relevant groups was derived from strong correlations between these expression groups and the presence of cytogenetic abnormalities (CA) such that 73% of those in the "HIGHEST" group had CA whereas only 15% of those in the "LOWEST" group had CA.

Taken together these data suggest that over-expression of specific subset genes associated with cell cycle and DNA metabolism is linked to CA, that expression of these genes is highly correlated, and that these genes may be co-regulated. Given the negative impact of CA and by inference the expression of the genes identified here, we hypothesize that use of drugs that target specific components of the cell-cycle apparatus may be a valid treatment approach for this GEP subgroup. For example, the topoisomerase 2 gene (*TOP2*) is over-expressed in this group, and thus it is possible that VP-16 or etoposide may show a higher efficacy in patients with *TOP2* over-expression than is appreciated in MM as a whole. Elevated expression of a cyclin-ubiquitin-conjugating enzyme and two mitotic cyclins was seen in both tests. The regulated destruction of mitotic cyclins near the end of mitosis is essential for the inactivation of their partner kinase CDC2 and exit from mitosis into G1 of the next cell cycle. This inactivation requires proteolysis mediated by enzymes of the ubiquitin pathway, such as E1, E2, and E3. We suspect that if we could interfere with the ubiquitin ligation of the mitotic cyclins or prevent their destruction by the proteasome, that we might be able prevent these cells from entering mitosis. Recent studies have also shown that a novel anti-tumor agent, XK469, induces mitotic arrest and is correlated with cyclin B1 ubiquitination (Lin et al., 2002).

3.3. Loss of Expression of Specific Genes Can be Used to Accurately Predict Deletion of Chromosome 13

A comprehensive interphase FISH deletion analysis of chromosome 13 ploidy has demonstrated that monosomy is present in ~50-60%, 13q14 is a deletion hotspot, that FISH deletion is linked to poor prognosis (Shaughnessy et al., 2000; Shaughnessy et al., 2002, in press). In an effort to determine if GEP could be used to predict chromosome 13 deletion we combined FISH deletion analysis with GEP of primary MM. FISH analysis with four probes spanning the long arm of chromosome 13 was performed on 112 patients as described (Shaughnessy et al., 2000). 74 MM were determined to lack

deletion of chromosome 13 (FISH13-) and 38 patients were found to have monosomy of all four probes in > 50% of the clonotypic PCs (FISH13+). PC from all patients were subjected to GEP and a comparison of gene expression between FISH13- and FISH13+ MM showed that 46 genes were differentially expressed (WRS $P < 10^{-4}$). A comparison between the profiles of normal bone marrow PC and MMN13 showed that a subset of 17 of these genes were also significantly different, suggesting that deregulated expression of these genes was not linked to deletion of chromosome 13. Surprisingly, the top 14 differentially expressed genes, with P values ranging from 5.89×10^{-5} to 6.98×10^{-11} , map to chromosome 13. Although 84 genes mapping to chromosome 13 were interrogated 34 were not expressed in any sample tested and 28 genes that were expressed showed no change in expression in any comparison. The 22 significantly altered genes were clustered at 13q12, 13q14 or 13q32. The 14 most significantly differentially expressed genes were used in a linear discriminate analysis to predict the presence of chromosome 13 deletion in an unknown set of MM samples. The 114 patients with known deletion status were used as a training group and 22 MM patients and 8 MM cell lines with known, but blinded, chromosome 13 FISH deletion status, were used to validate the model. The deletion status of the validation group was predicted with 100% accuracy. An MSDA of the 14 genes showed that expression of 7 genes could predict deletion with 100% accuracy. Overall these data indicate that 1) deletion of chromosome 13 results in loss or reduced expression of only specific genes on the chromosome and 2) profiles of these genes can be used to accurately predict chromosome 13 deletion in primary MM. Given the profound influence of chromosome 13 deletion on MM outcome, we are currently determining whether expression of any one or combination of these genes is correlated with inferior outcome.

3.4. GEP Can Identify Known and Suspected MM Associated 14q32 Translocations

Like many tumors of the B-cell lineage MM shows recurrent rearrangements of the *IGH* locus on 14q32 (for review see Kuehl & Bergsagel, 2002). These translocations typically result in the activation of proto-oncogenes that are brought into juxtaposition with powerful *IGH* enhancers elements. Molecular studies have identified the *CCND1*, *CCND3*, *FGFR3* and *MAF* genes as targets of primary translocations and *MYC* as a target of secondary translocations. We have recently shown that GEP can be used to identify 14q32 translocations in MM (Zhan et al., 2002). Briefly, genes demonstrating so called "Spiked" expression are those that are not expressed in normal bone marrow PC and most MM, but expressed at very high levels in a subset of MM cases. *FGFR3* and *CCND1* spikes showed a 100% correlation with the presence of the t(11;14)(q13;q32) or t(4;14)(p16;q32), respectively. We also were able to use a similar strategy to identify the *CCND3* activating

t(6;14)(p21;q32) (Shaughnessy et al., 2001). GEPs of 145 newly diagnosed MM cases were screened for genes showing Spiked expression. Spiked Signal (> 10,000) for both *CCND1* and *FGFR3* was seen in 13%. *MAF* was spiked in 7.5% and *CCND3* in 4.1% of the cases. Although *CCND1* and *FGFR3* spikes did not overlap, 3 of the *MAF* spikes overlapped with a *FGFR3* and 1 with a *CCND1* spike and 1 *CCND3* spike overlapped with a *CCND1* spike, suggesting the possibility of dual activation in these cases. Thus, 34% of primary MM have a unique spike of one the four recurrent *IGH* translocation partners.

Recent FISH analysis of a large population of primary MM (n = 669) showed that 14q32 rearrangements are found in 73% of MM and that the translocation partners in 44% of these rearrangements are not known (Avet-Loiseau et al., 2002). The incidence of spikes (our study) and FISH translocations (Avet-Loiseau et al., 2002) of *CCND1* (13% versus 16%), *FGFR3* (13% versus 10%) and *MAF* (4.1% versus 2%) is reasonably consistent. A total of 21 genes, including the 4 common translocation partner genes, were spiked in 109 of 145 (75%) of the cases. This total spike incidence is also consistent with the data of Avet-Loiseau and colleagues showing that 14q32 rearrangements are observed in 73% of MM. We suspect that many if not all of the 17 unclassified spike genes identified in this analysis represent candidates for the unknown 14q32 translocation partner genes.

References

1. Alizadeh AA, Eisen MB, Davis RE, et al. Distinct types of diffuse large B-cell lymphoma identified by GEP. *Nature*. 2000;403:503-511.
2. Avet-Loiseau H, Facon T, Grosbois B, et al. 14q32 and 13q chromosomal abnormalities are not randomly distributed, but correlate with natural history, immunological features, and clinical presentation. *Blood*. 2002;1;99:2185-2191.
3. Avet-Loiseau H, Li JY, Morineau N, et al. Monosomy 13 is associated with the transition of monoclonal gammopathy of undetermined significance to MM. Intergroupe Francophone du Myelome. *Blood*. 1999;94:2583-2589.
4. Barlogie B, Shaughnessy J, Munshi N, Epstein J. Plasma cell myeloma. In: Beutler E, Lichtman M, Coller B, Kipps T (eds): *William's Hematology*. 6th Edition. New York: McGraw-Hill 199.
5. Chesi M, Bergsagel PL, Brents LA, Smith CM, Gerhard DS, Kuehl WM. Dysregulation of cyclin D1 by translocation into an IgH gamma switch region in two MM cell lines. *Blood*. 1996;88:674-681.
6. Chesi M, Bergsagel PL, Shonukan OO, et al. Frequent dysregulation of the c-maf proto-oncogene at 16q23 by translocation to an Ig locus in MM. *Blood*. 1998b;91:4457-4463.
7. Chesi M, Kuehl WM, Bergsagel PL. Recurrent immunoglobulin gene translocations identify distinct molecular subtypes of myeloma. *Ann Oncol*. 2000;11(suppl 1):131-135.
8. Chesi M, Nardini E, Brents LA, et al. Frequent translocation t(4;14)(p16.3;q32.3) in MM is associated with increased expression and activating mutations of fibroblast growth factor receptor 3. *Nat Genet*. 1997;16:260-264.
9. Chesi M, Nardini E, Lim RS, Smith KD, Kuehl WM, Bergsagel PL. The t(4;14) translocation in myeloma dysregulates both *FGFR3* and a novel gene, *MMSET*, resulting in *IgH/MMSET* hybrid transcripts. *Blood*. 1998a;92:3025-3034.

10. Cigudosa JC, Rao PH, Calasanz MJ, et al. Characterization of nonrandom chromosomal gains and losses in MM by comparative genomic hybridization. *Blood*. 1998;91:3007-3010.
11. DeRisi J, Penland L, Brown PO, et al. Use of a cDNA microarray to analyse gene expression patterns in human cancer. *Nat Genet*. 1996;14:457-460.
12. Desikan R, Barlogie B, Sawyer J, et al. Results of high-dose therapy for 1000 patients with MM: durable complete remissions and superior survival in the absence of chromosome 13 abnormalities. *Blood*. 2000;95:4008-4010.
13. Dewald GW, Kyle RA, Hicks GA, Greipp PR. The clinical significance of cytogenetic studies in 100 patients with MM, plasma cell leukemia, or amyloidosis. *Blood*. 1985;66:380-390.
14. Eisen MB, Spellman PT, Brown PO, Botstein D. Cluster analysis and display of genome-wide expression patterns. *Proc Natl Acad Sci USA*. 1998;95:14863-14868.
15. Facon T, Avet-Loiseau H, Guillemin G, et al. Chromosome 13 abnormalities identified by FISH analysis and serum beta2-microglobulin produce a powerful myeloma staging system for patients receiving high-dose therapy. *Blood*. 2001;97:1566-1571.
16. Fodor SP, Read JL, Pirrung MC, Stryer L, Lu AT, Sotgiu D. Light-directed, spatially addressable parallel chemical synthesis. *Science*. 1991;251:767-773.
17. Fonseca R, Blood EA, Oken MM, et al. Myeloma and the t(11;14)(q13;q32); evidence for a biologically defined unique subset of patients. *Blood*. 2002a;99:3735-3741.
18. Fonseca R, Harrington D, Oken MM, et al. Biological and prognostic significance of interphase fluorescence in situ hybridization detection of chromosome 13 abnormalities (delta 13) in MM: an Eastern Cooperative Oncology Group study. *Cancer Res*. 2002b;62:715-720.
19. Golub TR, Slonim DK, Tamayo P, et al. Molecular classification of cancer: class discovery and class prediction by gene expression monitoring. *Science*. 1999;286:531-537.
20. Konigsberg R, Ackermann J, Kaufmann H, et al. Deletions of chromosome 13q in monoclonal gammopathy of undetermined significance. *Leukemia*. 2000;14:1975-1979.
21. Kuehl WM, Bergsagel PL. MM: evolving genetic events and host interactions. *Nature Rev Cancer*. 2002;2:175-187.
22. Li Z, Zhu YX, Plowright EE, et al. The myeloma-associated oncogene fibroblast growth factor receptor 3 is transforming in hematopoietic cells. *Blood*. 2001;97:2413-2419.
23. Lin H, Liu XY, Subramanian B, Nakeff A, Valeriote F, Chen BD. Mitotic arrest induced by XK469, a novel antitumor agent, is correlated with the inhibition of cyclin B1 ubiquitination. *Int J Cancer*. 2002;97:12.
24. Lipshutz RJ, Fodor SP, Gingeras TR, Lockhart DJ. High density synthetic oligonucleotide arrays. *Nat Genet*. 1999;21(suppl):20-24.
25. Perez-Simon JA, Garcia-Sanz R, Tabernero MD, et al. Prognostic value of numerical chromosome aberrations in MM: a FISH analysis of 15 different chromosomes. *Blood*. 1998;91:3366-3371.
26. Plowright EE, Li Z, Bergsagel PL, et al. Ectopic expression of fibroblast growth factor receptor 3 promotes myeloma cell proliferation and prevents apoptosis. *Blood*. 2000;95:992-998.
27. Sawyer JR, Lukacs JL, Munshi N, et al. Identification of new nonrandom translocations in MM with multicolor spectral karyotyping. *Blood*. 1998;92:4269-4278.
28. Sawyer JR, Lukacs JL, Thomas EL, et al. Multicolour spectral karyotyping identifies new translocations and a recurring pathway for chromosome loss in MM. *Br J Haematol*. 2001;112:167-174.
29. Sawyer JR, Waldron JA, Jagannath S, Barlogie B. Cytogenetic findings in 200 patients with MM. *Cancer Genet Cytogenet*. 1995;82:41-49.
30. Schena M, Shalon D, Davis RW, Brown PO. Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science*. 1995;270:467-470.
31. Schena M, Shalon D, Heller R, Chai A, Brown PO, Davis RW. Parallel human genome analysis: microarray-based expression monitoring of 1000 genes. *Proc Natl Acad Sci USA*. 1996;93:10614-10619.
32. Seong C, Delasalle K, Hayes K, et al. Prognostic value of cytogenetics in MM. *Br J Haematol*. 1998;101:189-194.
33. Shaughnessy J Jr, Gabrea A, Qi Y, et al. Cyclin D3 at 6p21 is dysregulated by recurrent chromosomal translocations to immunoglobulin loci in MM. *Blood*. 2001;98:217-223.
34. Shaughnessy J, Tian E, Sawyer J, et al. High incidence of chromosome 13 deletion in MM detected by multiprobe interphase FISH. *Blood*. 2000;96:1505-1511.
35. Shaughnessy J, Tian E, Sawyer J, et al. Prognostic impact of cytogenetic and interphase FISH defined chromosome 13 deletion in MM: early results of total therapy II. *Br J Haematol*. in press.
36. Shipp MA, Ross KN, Tamayo P, et al. Diffuse large B-cell lymphoma outcome prediction by gene-expression profiling and supervised machine learning. *Nat Med*. 2002;8:68-74.
37. Singh D, Febbo P, Ross K, et al. Gene expression correlates of clinical prostate cancer. *Cancer Cell Markers*. 2002;1:203-209.
38. Taparowsky E, Suard Y, Fasano O, Shimizu K, Goldfarb M, Wigler M. Activation of the T24 bladder carcinoma transforming gene is linked to a single amino acid change. *Nature*. 1982;300:762-765.
39. Tian E, Zhan F, Barlogie B, Shaughnessy J. The transcription repressor, TGF- stimulated clone (tsc-22), is a candidate for the 13q14 MM tumor suppressor gene. *Blood*. 2001;98:3221a.
40. Tricot G, Barlogie B, Jagannath S, et al. Poor prognosis in MM is associated only with partial or complete deletions of chromosome 13 or abnormalities involving 11q and not with other karyotype abnormalities. *Blood*. 1995;86:4250-4256.
41. Tricot G, Spencer T, Sawyer J, et al. Predicting long-term (5 years) event-free survival in MM patients following planned tandem autotransplants. *Br J Haematol*. 2002;116:211-217.
42. Tusher VG, Tibshirani R, Chu G. Significance analysis of microarrays applied to the ionizing radiation response. *Proc Natl Acad Sci USA*. 2001;98:5116-5121.
43. Yeoh EJ, Ross ME, Shurtleff SA, et al. Classification, subtype discovery, and prediction of outcome in pediatric acute lymphoblastic leukemia by GEP. *Cancer Cell Markers*. 2002;1:133-143.
44. Zhan F, Hardin J, Bumm K, et al. Global GEP can be used to accurately predict chromosome 13 deletion in MM. *Blood*. 2001;98:1553a.
45. Zhan F, Hardin J, Kordsmeier B, et al. Global GEP of MM, monoclonal gammopathy of undetermined significance, and normal bone marrow PC. *Blood*. 2002;99:1745-1757.
46. Zhan F, Tian E, Bumm K, Shaughnessy J. Global gene expression profiling reveals distinct transcript profiles associated with plasma cell differentiation. Submitted. 2002.
47. Zhan F, Tian E, Barlogie B, Shaughnessy J. Establishment of a B-cell-developmental stage-based classification of MM using global gene expression profiling. Submitted. 2002.
48. Zojer N, Konigsberg R, Ackermann J, et al. Deletion of 13q14 remains an independent adverse prognostic variable in MM despite its frequent detection by interphase fluorescence in situ hybridization. *Blood*. 2000;95:1925-1930.