A Transgenic Mouse Model of Plasma Cell Malignancy Shows Phenotypic, Cytogenetic, and Gene Expression Heterogeneity Similar to Human Multiple Myeloma

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Abstract
Multiple myeloma is an incurable plasma cell malignancy for which existing animal models are limited. We have previously shown that the targeted expression of the transgenes c-Myc and Bcl-XL in murine plasma cells produces malignancy that displays features of human myeloma, such as localization of tumor cells to the bone marrow and lytic bone lesions. We have isolated and characterized in vitro cultures and adoptive transfers of tumors from Bcl-xl/Myc transgenic mice. Tumors have a plasmablastic morphology and variable expression of CD138, CD45, CD38, and CD19. Spectral karyotyping analysis of metaphase chromosomes from primary tumor cell cultures shows that the Bcl-xl/Myc tumors contain a variety of chromosomal abnormalities, including trisomies, translocations, and deletions. The most frequently aberrant chromosomes are 12 and 16. Three sites for recurring translocations were also identified on chromosomes 4D, 12F, and 16C. Gene expression profiling was used to identify differences in gene expression between tumor cells and normal plasma cells (NPC) and to cluster the tumors into two groups (tumor groups C and D), with distinct gene expression profiles. Four hundred and ninety-five genes were significantly different expression between tumor cells and normal plasma cells (NPC) and to cluster the tumors into two groups (tumor groups C and D), with distinct gene expression profiles. Four hundred and ninety-five genes were significantly different between both tumor groups and NPCs, whereas 124 genes were uniquely different from NPCs in tumor group C and 204 genes were uniquely different from NPCs in tumor group D. Similar to human myeloma, the cyclin D genes are differentially dysregulated in the mouse tumor groups. These data suggest the Bcl-xl/Myc tumors are similar to a subset of plasmablastic human myelomas and provide insight into the specific genes and pathways underlying the human disease.

Introduction
Multiple myeloma is an incurable form of B-cell cancer characterized by a proliferation of malignant plasma cells in the bone marrow. A mouse model that has been developed for the study of multiple myeloma is the double transgenic line 3KE-Bcl-XL/igH-MycC (Bcl-xl/Myc; ref. 1). These mice have the oncogene c-Myc inserted into the genome under the control of the 3′-Cyclin IgH enhancer element, which activates expression in postswitch B cells and plasma cells. Transgenic Bcl-XL is regulated by the 3′- immunoglobulin light chain enhancer, which is also active in late B-cell development and plasma cells (2). Consequently, the Bcl-xl/Myc mice have an early expansion of nonmalignant plasma cells, which then develop into clonal plasma cell malignancies in multiple tissue types. These plasma cell tumors develop in 100% of the mice, all involving the bone marrow, and show close resemblance to human multiple myeloma.

A significant challenge in the treatment of multiple myeloma has been the genetic heterogeneity of disease among patients, which affects disease initiation, progression, and therapeutic response. Bcl-xl/Myc transgenic mice develop plasma cell tumors with variable tumor latency, suggesting that similar genetic heterogeneity may be required for tumor development. To better describe and characterize the Bcl-xl/Myc tumors, we established several primary tumor cell lines and compared their differences and similarities as seen by phenotypic markers, cytogentics, gene expression, and tumor progression by adoptive transfer into recipient mice.

Materials and Methods

Generation and analysis of mouse tumors. 3KE-Bcl-XL/igH-MycC transgenic mice (3) were mated to IgH-MycC+ BL6 mice (1). Litters were genotyped by PCR as described previously (1). Bcl-xl/Myc double transgenic animals were euthanized at first sign of tumor morbidity. Bone marrow was flushed from femurs with sterile PBS. Single-cell suspensions of spleen and lymph nodes were made by mincing tissue in PBS and then passing through a sterile cotton filter. Lymphocytes were partially purified by running over a gradient of Histopaque 1077 (Sigma-Aldrich) and then resuspended in RPMI 1640 containing 10% bovine growth serum (Hyclone), 50 units/mL each of penicillin and streptomycin, and 2 mmol/L L-glutamine. Cells were counted and cultured overnight at 2 × 106 to 5 × 106 cells per mL. For continued culture, cells were maintained in RPMI 1640 buffered with 25 mmol/L HEPES and supplemented with 15% FCS, 1 mmol/L sodium pyruvate, 50 units/mL each of penicillin and streptomycin, 2 mmol/L L-glutamine, 50 μmol/L β-mercaptoethanol, and 0.5 ng/mL interleukin (IL)-6 (R&D Systems). Medium was replaced every 3 to 4 days, keeping cell concentrations between 2 × 106 and 5 × 106 cells per mL.

Adoptive transfers were done by injecting 5 × 106 to 8 × 106 cells in a volume of 100 to 500 μL of PBS into the tail veins of wild-type syngeneic FVB/N × BL6. Recipient mice were monitored and euthanized at signs of hind limb paralysis, enlarged lymph nodes, and/or severe lethargy.

Flow cytometry was done on a FACScalibur machine (BD Pharmingen) using CellQuest Pro software (BD Pharmingen). Antibodies were from BD Pharmingen. Similar results were obtained for CD45 using either CD45R/ B220 or a pan CD45 antibody. Giemsa stains were done using a Giemsa Stain kit (IMEB, Inc.).

Reverse transcription-PCR. RNA was isolated from cells using a RNaseasy kit (Qiagen) and treated with DNase I on-column (Qiagen). cDNA was

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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generated using SuperScript III First-Strand Synthesis SuperMix kit (Invitrogen), and template cDNA was amplified using the following primers: Ezh2, 5'GTGGGACATCGAAGGCAGT-3' (forward) and 5'TCCTCCGCTGTTCCACT-3' (reverse); Dusp6, 5'GGCGAGAAACACCGGGTT-3' (forward) and 5'TGGTCGGCAGTCCATCAG-3' (reverse); Galk1, 5'CTGGGATCTATTCCAGA-3' (forward) and 5'CCCGGATAGGATAAACCAC-3' (reverse); Etv5, 5'GCTCCACCTCCCACCAAG-3' (forward) and 5'GAGGACCAGGCATCCCAG-3' (reverse); Blimp1, 5'TGGCGGATCTATTCCAGA-3' (forward) and 5'CCCGGATAGGATAAACCAC-3' (reverse); Pax5, 5'GACCGCGTGTTTGAGAGA-3' (forward) and 5'CAGGCACCATCCCTGTC-3' (reverse); and Xbp1, 5'CTGGGATCTATTCCAGA-3' (forward) and 5'GAGGACCAGGCATCCCAG-3' (reverse). For each primer set, cycle number was determined to find the linear amplification range, and relative expression was determined by normalizing to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression: 5'-GGGCGCCTGGTCACCAGGGCTG-3' (forward) and 5'-GGGGCCATCCACAGTCTTCTG-3' (reverse).

Cytogenetic analysis. G-band cytogenetic analysis and spectral karyotyping (SKY) were done on metaphase cells harvested from mouse cell lines and primary tumor cells. Cells were exposed to colcemid (Irvine Scientific) for 1.5 h and harvested using standard cytogenetic protocols for suspension cultures.

Slides for SKY and G-band analyses were prepared from the fixed cell suspensions. SKY slides were stored at 4°C. The G-banded slides were aged in a 90°C oven for 2 h and stained using Wright’s/Giemsa stain.

Mouse SkyPaint probe (Applied Spectral Imaging) was applied to metaphase chromosomes according to the manufacturer’s protocol. Metaphases were visualized using a SKY filter and then captured and karyotyped using an interferometer-based cooled coupled device camera and SkyView software (Applied Spectral Imaging). Seven to 20 metaphase cells were examined by SKY depending on cellularity of the sample.

Microarray labeling and analysis. RNA from 5 million cultured mouse tumor cells, frozen in RNAlater (Ambion) according to the manufacturer’s instructions, was isolated with Trizol (Invitrogen) and isopropanol precipitated using glycogen as a carrier. RNA was DNase treated and concentrated with a MinElute column (Qiagen). Double-stranded cDNA was made using the GeneChip Expression 3' Amplification One Cycle cDNA kit (Affymetrix) according to the manufacturer’s instructions. Labeled cRNA was prepared from double-stranded cDNA with the BioArray High Yield RNA Transcript Labeling kit (Enzo Life Sciences, Inc.), also according to the manufacturer’s instructions, except that the labeling reaction was incubated overnight at 37°C. cRNA was hybridized to Affymetrix Murine 430_2.0 GeneChip Microarrays and scanned with the GeneChip 3000 scanner according to the manufacturer’s instructions. Normal plasma cells (NPC) were isolated from lymph node cells of P-selectin–deficient and E-selectin–deficient mice (4). Expression data (CEL files) from normal mouse plasma cells and Bcl-xl/Myc tumor cells were normalized using Lowess normalization. Data were analyzed using Genedata Expressionist Pro (v1.0) software.
Results

Establishment of Bcl-xl/Myc tumor cell lines. To characterize the Bcl-xl/Myc tumors and to set up both an in vitro and an in vivo experimental system, several Bcl-xl/Myc primary tumor cell lines were established after creating single-cell suspensions and transferring cells to culture at time of necropsy (Supplementary Table S1). Tumor cells derived from spleen, bone marrow, and lymph node cells from mice with primary tumors, as well as tumors that were adoptively transferred to control mice, were cultured in vitro and analyzed by flow cytometry, Giemsa stain, and reverse transcription-PCR (RT-PCR) for tumor latency (time from adoptive transfer until death; Fig. 1). The majority of cells were found to be CD45+/CD138+CD38+CD19+ by flow cytometry, with few exceptions. For example, the spleen and lymph node cells taken from mouse DP3(AT4) were both CD38+CD19+CD138+; however, the lymph node cells were CD45+, whereas the spleen cells were CD45− (Fig. 1A and B). Cell lines established from these primary tumor cells were found to be clonally related based on a common immunoglobulin gene rearrangement (data not shown). The comparison of DP42A and DP24A cells exemplifies different phenotypes arising in cells from the same tissue (ascites fluid) but different mice. Both cell lines are CD138+, but DP42A is CD45+CD38+CD19+, whereas DP24A is CD45−CD38− with a mixed population of CD19+ and CD19− cells. Thus, although all mice develop malignancy, there is heterogeneity among tumor populations, an observation also noted among human myeloma cell comparisons (5).

Cells were also analyzed for tumor aggressiveness and any corresponding change in phenotype as they were passaged through successive adoptive transfers (Fig. 1A). Typically, related tumor cell lines had similar degrees of aggressiveness as seen by similar tumor latency after injection of tumor cells into recipient mice. However, for DP3(AT4), spleen cells (CD45+) were more aggressive than lymph node cells (CD45−), causing tumors to form in recipient mice in an average of 1.7 weeks compared with an average of 3.5 weeks for lymph node cells. In a second example, DP42A (CD45+) cells quickly formed tumors in recipient mice, but DP24A (CD45−) cells either formed tumors very slowly (more than 41 weeks) or not at all. These data suggest that expression of CD45 is related to the in vivo proliferation rate as has been shown in the 5T mouse model (6). The mouse plasmacytoma cell line S194 was analyzed as well as expression of the B-cell–associated genes Pax5 and Xbp1 were analyzed (Fig. 1D). Relative expression levels of plasma cell–associated genes Blimp1 and Xbp1 were analyzed as well as expression of the B-cell–associated gene Pax5. The mouse plasmacytoma cell line S194 was used as a positive control for plasma cell gene expression (Blimp1Xbp1Pax5), and all expression levels were normalized to their Gapdh expression. Most Bcl-xl/Myc tumor cell lines showed high level expression of both Blimp1 and Pax5, which is interesting, considering that Pax5 expression is directly downregulated by Blimp1 during NPC development (7).

Bcl-xl/Myc tumors display chromosomal instability. To determine if the tumors developing in double transgenic animals have chromosomal aberrations similar to those seen in human myeloma, we did SKY on primary tumor cell lines and cell lines derived from Bcl-xl/Myc tumors. We found a large number of abnormalities, both numerical and structural, in seven of eight independent karyotypes analyzed (Fig. 2; Supplementary Table S2). Trisomies were commonly observed, particularly for chromosomes 12 and 16. In contrast, deletions were only occasionally seen, once each for chromosomes 2, 3, 9, and 14. Additionally, we saw three recurrent sites for translocations: at 4D/E, 12F (the region of the murine IgH locus), and 16C3-C4. Two samples had increased ploidy, in addition to other abnormalities. Within one tumor (DP74SP), we observed three related abnormal cell lines, suggesting a progression of karyotypic changes within a single tumor. Common to all DP74SP cells was trisomy for chromosome 12 (four cells had +12 as the sole abnormality) followed by an additional copy of chromosome 16 (seven cells). The third cell line observed in this sample also contained a trisomy for chromosome 16; however, the long arms of these chromosomes formed isochromosomes (six cells). A second tumor, DP54(AT3)LN, showed the presence of two unrelated or divergent clones, again both containing a trisomy for chromosome 12. In addition to trisomy 12, 30% of the cells had an additional chromosome 8, whereas 70% of the cells had an additional chromosome 16. Finally, we observed that karyotypes became increasingly complex in tumors that were adoptively
transferred to littermate controls. DP42(AT2)LN had several additional trisomies (+17, +18) over the source tumor cells DP42, and DP54(AT4a)LN has a complex rearrangement involving chromosomes 1, 12, and 16 that was not present in the previous adoptive transfer tumor [DP54(AT3)LN]. From the results of the karyotype analysis, we conclude that, although no single abnormality is required for tumor formation, the tumors found in Bcl-xl/Myc transgenic animals maintain genomic instability. Similar observations have been made in tumor progression in myeloma patients (8).

**Gene expression profiles of Bcl-xl/Myc tumors.** To understand the genetic heterogeneity of the Bcl-xl/Myc tumors in more detail, we looked at the global gene expression profiles of tumor cells in comparison with normal mouse plasma cells using Affymetrix Murine 430_2.0 microarrays. Profiles from normal, nontransformed plasma cells were derived from the polyclonal plasma cell populations in selectin-deficient mice, described in Underhill et al. (9). Principal component analysis (PCA) was used as a data reduction tool and to identify components of the total genetic variation between tumors and NPCs that are associated with tumor formation. PCA describes the variance in a data set and assigns a coefficient (eigenscore) to each gene as it is associated with the component of variation (10). Each gene contributes variably and independently to each component, allowing functional information to be identified based on the correlation coefficients of the genes for each of the components (11). The use of PCA to extract functional information from microarray data has been shown for the classification of tumor cell lines (12) and for stages of B-cell differentiation (13). Analysis of the principal components of gene expression for 28,748 genes for which valid expression values were obtained identified 23 components of variation within the data. Figure 3A shows the genetic differences between tumors and NPCs as described by principal components 1 (PCOMP1) and 3 (PCOMP3). The difference between the Bcl-xl/Myc tumors and NPCs is best described by PCOMP1. Of the top 200 genes associated with PCOMP1, it can be seen that the majority of genes are down-regulated in tumors compared with NPCs. The gene with the highest positive association to component 1 (expressed in NPCs) corresponds to the immunoglobulin light chain variable region. In fact, 35 of the top 200 genes contributing to PCOMP1 correspond to genes related to the immunoglobulin heavy or light chain genes (Supplementary Table S3). The overrepresentation of Ig genes in NPCs was also noted in gene expression profiles for human myeloma cells compared with normal controls (4, 14). Only 18 of the top 200 genes from PCOMP1 represent genes that are up-regulated in tumors. The gene with the highest negative association to PCOMP1 (expressed in tumors) is Bcl-XL, one of the transgenes that drives tumor development in our mouse model. Other PCOMP1 genes up-regulated in tumors include the Cd27-binding protein Siva [a direct transcriptional target of p53 (15)] and the cell cycle regulator polo-like kinase 2 (Plk2).

PCOMP3 describes the genetic variation among the different mouse tumors (Fig. 3A; Supplementary Table S4). Genes with a positive correlation to PCOMP3 are expressed in some tumors, whereas genes with a negative correlation score are expressed in others. For the top 200 genes from PC3, the gene with the highest positive correlation to PCOMP3 is fatty acid–binding protein 5, which shows significant overexpression in prostate cancer (16) and has been identified as a direct target of the transcription factor Myc (17). The gene with the highest negative correlation to PCOMP3 is IL-7 receptor, a gene involved in the regulation of cell survival pathways and B-cell development (18, 19).

**Tumors cluster into two groups.** We used unsupervised hierarchical clustering to identify tumors with similar gene expression patterns using the top 874 positive and negative scoring genes in the tumor variation vector (PCOMP3). Tumors clustered into two groups: tumor group C and tumor group D (Fig. 3B). Within the tumor groups, we observed that tumors related by adoptive transfer generally clustered tightly together. A detailed comparison of differences in gene expression between tumor groups can be found in Supplementary Fig. S1.

![Figure 3](cancerres.aacrjournals.org)
To determine if genetic heterogeneity within the tumors results in different pathways to malignancy, we compared the tumor groups C and D to NPCs. Using the 1,754 genes that contribute most to the variation between tumors and normal (PCOMP1), and among tumors (PCOMP3), tumor groups were separately compared with NPCs using a Welch t-test. Genes significantly dysregulated (P < 0.005; fold change >2) for each group were then compared by Venn diagram to identify common genes as well as genes unique to each tumor group. This analysis revealed 495 genes that were dysregulated in both tumor groups, whereas 124 genes were dysregulated only in tumor group C and 204 genes were uniquely dysregulated in tumor group D (Fig. 4; Supplementary Table S5).

Genes that were significantly overexpressed in both tumor groups compared with NPCs might be considered to play a general role in tumor development. These include genes for transcription factors (Sox4 and Bcl11a) and genes that regulate transcription or modulate chromatin structure (Supt16h and Nap111). Sox4 is a member of the SRY-related high-mobility group box family of transcription factors, which is overexpressed in several different types of cancer (20, 21) and was recently shown to be an oncogene in prostate cancer (22). The genes for molecules involved in signaling pathways that are generally overexpressed in the mouse tumors are protein kinase inhibitor γ (Pkgk), Rhoh, and T2bp. The overexpression of Pkgk suggests the tumors have reduced protein kinase A signaling (23). Rhoh, a GTP-binding protein expressed exclusively in hematopoietic cells, has also been shown to inhibit the signaling function of other Rho GTPases (24). In contrast, T2bp, a Traf2-binding protein, can activate NFκB and AP-1 (25). The overexpressed genes that play a role in apoptosis, in addition to transcription factors and signaling molecules, include genes for proteins involved in the intrinsic and extrinsic apoptotic pathways, such as Bcl-xl, Fabp5, and Myc.

Figure 4. Tumor groups C and D have similar and unique gene expression changes from NPCs. A, Venn diagram of genes with significantly different (P > 0.005) expression from NPCs for tumor group C (TC) and tumor group D (TD). Table lists genes with increased expression in the different tumor groups. B, tile plot of gene expression for genes with significantly different expression from tumor group C, tumor group D, and NPCs. Side bars, highlight genes from tumor group C (red), tumor group D (blue), or both tumor groups (purple) with differences in gene expression from NPC.
to Bcl-XL, are Siva and Bag2. Siva has been shown to bind to CD27, and its overexpression can induce apoptosis (26). Siva also binds to Bcl-X, protein and inhibits its antiapoptotic effect (27). The opposing effects of Siva and Bcl-X, on apoptosis suggest that the overexpression of Bcl-X, in the Bcl-xl/Myc tumors can overcome the proapoptotic effects of Siva expression or that the antiapoptotic function of Bcl-X, is required for tumor initiation but not maintenance. Interestingly, no genes for cell adhesion molecules were up-regulated in both tumor groups, but the expression of several adhesion molecules was significantly down-regulated in tumors (Itgal, Tgfb1, Cdhn7, Alcam, and Itgb5) compared with normal mouse plasma cells. Similarly, genes involved in immune function (17 genes) and inflammatory or defense responses (2 and 11 genes, respectively) were only down-regulated in tumors as were genes involved in bone formation (11 genes, respectively). We have more fully characterized the Bcl-xl/Myc mouse model of myeloma and found that tumors express a range of cell surface phenotypes and have varied expression levels of plasma cell and cyclin D genes have been proposed to be a unifying pathogenic event (39). Similarly, we see dysregulation of cyclin D genes in our mouse tumors (Fig. 5). Compared with normal mouse plasma cells, all of the Bcl-xl/Myc tumors have significantly reduced expression of cyclin D2 and increased expression of cyclin D1. When Bcl-xl/Myc tumors are compared with each other, we also see differences in cyclin D expression. Tumors in group C have increased expression of cyclin D3, whereas tumors in group D express higher levels of cyclin D1. The differential expression of cyclin D genes in the Bcl-xl/Myc tumors suggests that, in addition to the dysregulation of the Bcl-X and Myc genes, dysregulation of a cyclin D gene may also be required for tumorigenesis or disease progression.

**Discussion**

We have more fully characterized the Bcl-xl/Myc mouse model of myeloma and found that tumors express a range of cell surface phenotypes and have varied expression levels of plasma cell and
B-cell markers as well as multiple myeloma–associated genes. The length of time from adoptive transfer of malignant cells to tumor formation also differs among tumors. The data suggest that the Bcl-xl/Myc cells are more similar to human plasmablastic and proliferative myeloma tumors than to homogeneous slow-growing plasma cell tumors. It has been shown that tumors with a high percentage of plasmablastic cells associate with more aggressive multiple myeloma, as well as decreased survival rates, both for patients treated with chemotherapy and those receiving autologous stem cell transplants (40–42). However, this group of human myelomas remains understudied due to a lack of plasmablastic cell lines and mouse models.

Bcl-xl/Myc tumors showed variable expression of cell surface markers, especially CD45. Expression of the cell surface marker CD45 is typical of all lymphocytes and is gradually lost in mature B cells as they develop into plasma cells (43). CD38 is highly up-regulated at the plasma cell switch, along with increasing expression of CD138 (43). However, recently, a subset of highly proliferative and aggressive human multiple myeloma cells was characterized by a CD45 CD11a Bcl-2low phenotype (44). It has also been previously shown that expression of CD38 and CD45 correlates with extensive tumor infiltration of the bone marrow and lower remission in patients, suggesting that cells expressing CD38 and CD45 are involved in the generation of new malignant bone marrow plasma cells (45). This supports the data we have collected on our Bcl-xl/Myc primary tumor cell lines, in which expression of CD45 correlates with shorter adoptive transfer time (more aggressive tumors).

The transcription factor Pax5 is expressed throughout B-cell development and is repressed by Blimp1 at the plasma cell switch (7). Interestingly, most of our Bcl-xl/Myc tumor cells express both Blimp1 and Pax5, suggesting that they are suspended somewhere between late B-cell and plasma cell development. Examination of the expression of Pax5 isoforms in multiple myeloma patients and B-cell and plasma cell populations in healthy donors shows expression of multiple isoforms of Pax5 and also premature expression of Blimp1 in B cells of myeloma patients, but not in healthy donors, suggesting that the expression of Blimp1 causes proliferating B cells to prematurely differentiate into plasma cells in myeloma (46). Pax5 RNA and protein expression has also been detected in plasma cells of CD320 myelomas (47). A recent article by Zhan et al. (48) shows that a subgroup of human myeloma tumors that overexpress cyclin D3 also expresses Pax5.

Similar to human myeloma, tumors derived from the Bcl-xl/Myc mice accumulate cytogenetic abnormalities. The most commonly aberrant mouse chromosomes are 16, 12, 4, and 1. Mouse chromosome 1 is highly syntenic with human chromosome 1q, and gain of chromosome 1q in human myeloma has been associated with poor prognosis (49). Expression of Myc is higher in tumor group D than in tumor group C. Group D tumors have a high frequency of trisomy 12, which could account for the increased Myc expression in this group. Several regions of recurrent translocation were also observed in the mouse tumors at chromosome 12F, 4D/E, and 16C. 12F is the region of the mouse immunoglobulin heavy chain locus, a common region of translocation in myeloma and also the location of the Myc transgene in the Igh-MycC mouse. We have not observed any 12F translocation partners that mimic those common to myeloma. However, we do see increased expression of the Frap1 gene in tumors with translocations near the Frap1 locus on chromosome 4D. Frap1, or mTor, has been identified as a candidate gene for plasmacytoma susceptibility in mice (50) and is part of the phosphatidylinositol 3-kinase/AKT signaling pathway that is frequently dysregulated in myeloma patients and cell lines (51).

Numerous gene expression profiling studies have been done on plasma cells from human myeloma patients (14, 48, 52–56). In our analysis of global gene expression comparing NPCs with mouse plasma cell tumors resembling human myeloma, we identified more than 90 genes significantly different in the mouse tumors with homologues that have been identified as deregulated in human myeloma (Table 1; Supplementary Table S6). Genes identified as up-regulated in human myeloma that are also up-regulated in the Bcl-xl/Myc mouse tumors are Apc, Arl4, Cond1, Fas, Gart, c-Myc, Sck29a1, and Uhr1l. Conversely, Aim, Apos, Ela2, Entpd1, Len2, and S100a9 were all identified as significantly down-regulated in both the mouse and human tumors (14, 53). Additionally, several genes with significant down-regulation between monoclonal gammopathy of undetermined significance (MGUS) and myeloma plasma cells were also down-regulated in the mouse tumors (52). In human extramedullary plasma cell tumors, only genes with significant down-regulation were common to the mouse and human tumors, whereas several genes (Adam19 and Runx2) found to be up-regulated in human extramedullary plasma cell tumors were down-regulated in the mouse tumor cells (54).

Recently, Zhan et al. (48) used expression profiling to identify seven myeloma subgroups with common gene signatures. Several genes from six of the seven subgroups showed similar dysregulation in the mouse and human tumors. Sfmt1 and Tyns were overexpressed in both mouse and human tumors, whereas Alcam, Basp1, Cdb2, Ifj27, and Smad1 were down-regulated. Interestingly, two of the subgroups identified were associated with overexpression of cyclin D1 and cyclin D3. Within the cyclin D subgroups, STC2 and VPREB3 were overexpressed and ADAM19 and ANX2A were down-regulated similar to the mouse tumors. Several other genes associated with the cyclin D myeloma subgroups showed similar expression between the mouse and human tumors. Siva, Gata1, and Pkig genes associated with both cyclin D1 and cyclin D3 expression were overexpressed in the mouse tumors as were Stom (associated with the cyclin D1 subgroup) and Bcl11a (associated with cyclin D3 subgroup). No genes associated with the hyperdiploid myeloma subgroup (48) showed similar dysregulation in the mouse tumors.

Cyclin D expression has recently emerged as a prominent feature of human myeloma (39, 57, 58), with nearly all tumors showing increased and mutually exclusive expression of cyclin D1, cyclin D2, or cyclin D3. We also observed cyclin D dysregulation in the Bcl-xl/Myc mouse tumors. In contrast to normal human plasma cells, which express only low levels of cyclin D2, expression of cyclin D2 in normal mouse plasma cells is high (9). Consequently, in the mouse tumors, we observed that the cyclin D2 expression was significantly reduced or absent. Tumors with reduced but moderate expression of cyclin D2 had significantly elevated cyclin D1 expression, whereas tumors with no expression of cyclin D2 overexpressed cyclin D3. Cyclin D2 overexpression in human myeloma patients is associated with up-regulation of MAFB and c-MAF. We observed low levels of Maf expression in the mouse tumors, suggesting either the activation of other Maf family members occurs in the mouse tumors, or perhaps the reduced expression of Maf and cyclin D2 reflects tumor progression. Mouse tumor group C is characterized by increased cyclin D3 expression compared with group D tumors and normal mouse plasma cells.
Table 1. Genes with similar dysregulation in Bcl-xl/Myc mouse and human plasma cell tumors

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Gene name</th>
<th>Function</th>
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<td><strong>Genes up-regulated in Bcl-xl/Myc tumors</strong></td>
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<td>Apc</td>
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<td>Wnt signaling</td>
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<td>ADP-ribosylation factor-like 4</td>
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<td>MM*&lt;sup&gt;a&lt;/sup&gt;,&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>Bcl11a</td>
<td>B-cell CLL/lymphoma 11A (zinc finger protein)</td>
<td>Transcription, B-cell differentiation</td>
<td>CD2&lt;sup&gt;c&lt;/sup&gt;,&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Notch signaling</td>
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<td>RNA processing, transcriptional activator</td>
<td>MM*</td>
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<td>CD2&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>Transcription, cell proliferation, apoptosis</td>
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<td>Nuclear autoantigenic sperm protein</td>
<td>DNA packaging, histone binding</td>
<td>MM,&lt;sup&gt;c&lt;/sup&gt; CD1&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vpreb3</td>
<td>Pre-B lymphocyte gene 3</td>
<td>Immune response, B-cell signaling</td>
<td>CD2&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Genes down-regulated in Bcl-xl/Myc tumors</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adam19</td>
<td>A disintegrin and metalloproteinase domain 19</td>
<td>Metalloproteinase activity</td>
<td>CD1&lt;sup&gt;d&lt;/sup&gt;, EM&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Aim1</td>
<td>Absent in melanoma 1</td>
<td>Unknown</td>
<td>MM*</td>
</tr>
<tr>
<td>Alcam</td>
<td>Activated leukocyte cell adhesion molecule</td>
<td>Cell adhesion, axon guidance</td>
<td>PR&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Anxa2</td>
<td>Annexin A2</td>
<td>Angiogenesis, skeletal development</td>
<td>CD2&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Apoe</td>
<td>Apolipoprotein E receptor binding</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basp1</td>
<td>Brain abundant, membrane signal protein 1</td>
<td>Regulation of transcription</td>
<td>LB&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cdb2</td>
<td>Cadherin 2</td>
<td>Cell adhesion</td>
<td>MS&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ctsg</td>
<td>Cathepsin G</td>
<td>Proteolysis</td>
<td>MG,&lt;sup&gt;k&lt;/sup&gt;,&lt;sup&gt;{b&lt;/sup&gt;,&lt;sup&gt;c&lt;/sup&gt;,&lt;sup&gt;*&lt;/sup&gt; EM&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td>Ela2</td>
<td>Elastase</td>
<td>Proteolysis</td>
<td>MG,&lt;sup&gt;k&lt;/sup&gt;,&lt;sup&gt;{b&lt;/sup&gt;,&lt;sup&gt;c&lt;/sup&gt;,&lt;sup&gt;*&lt;/sup&gt; EM&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Entpd1</td>
<td>Ectonucleoside triphosphate diphosphohydrolase 1</td>
<td>Cell adhesion, platelet activation</td>
<td>MM*</td>
</tr>
<tr>
<td>Fos</td>
<td>FBJ osteosarcoma-related oncogene</td>
<td>Regulation of transcription</td>
<td>EM&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>If27</td>
<td>IFN-α-inducible protein 27</td>
<td>Immunology</td>
<td>LB&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td>Klf6</td>
<td>Kruppel-like factor 6 (Copeb)</td>
<td>Transcription, B-cell differentiation</td>
<td>MM,&lt;sup&gt;c&lt;/sup&gt; EM&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lap3</td>
<td>LIM and SH3 protein 1</td>
<td>Focal adhesion organization, cell migration</td>
<td>MG&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td>Lcn2</td>
<td>Lipocalin 2</td>
<td>Regulation of apoptosis</td>
<td>MM,&lt;sup&gt;c&lt;/sup&gt; EM&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Marcks</td>
<td>Myristoylated alanine-rich protein kinase C substrate</td>
<td>Cell motility</td>
<td>MG&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Prkcq</td>
<td>Protein kinase C, δ</td>
<td>Protein kinase C activity</td>
<td>EM&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pipre</td>
<td>Protein tyrosine phosphatase, receptor, E</td>
<td>Receptor-like protein tyrosine phosphatase</td>
<td>EM&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Rgs1</td>
<td>Regulator of G protein signaling 1</td>
<td>B-cell activation</td>
<td>MG&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Runx2</td>
<td>Runx-related transcription factor 2</td>
<td>Transcription, skeletal development</td>
<td>EM&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>S100a8</td>
<td>S100 calcium-binding protein A8</td>
<td>Signaling, inflammatory response</td>
<td>MG&lt;sup&gt;k&lt;/sup&gt;&lt;sup&gt;a&lt;/sup&gt;,&lt;sup&gt;s&lt;/sup&gt;&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>S100a9</td>
<td>S100 calcium-binding protein A9</td>
<td>Signaling, inflammatory response</td>
<td>MM,&lt;sup&gt;c&lt;/sup&gt; EM&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sat1</td>
<td>Spermine:spermine N1-acetyltransferase 1</td>
<td>Spermine catabolism</td>
<td>MG&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sepp1</td>
<td>Selenoprotein P, plasma, 1</td>
<td>Selenium metabolism, antioxidant</td>
<td>MG&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Smad1</td>
<td>MAD homologue 1</td>
<td>BMP signaling pathway</td>
<td>LB&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Tgfbr1</td>
<td>Transforming growth factor, β induced</td>
<td>Cell adhesion</td>
<td>MG&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vcam1</td>
<td>Vascular cell adhesion molecule 1</td>
<td>Cell adhesion</td>
<td>MM*</td>
</tr>
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</table>

NOTE: MM indicates genes dysregulated in myeloma compared with NPC, MG indicates genes dysregulated in multiple myeloma compared with MGUS, and EM indicates dysregulation in human extramedullary plasma cell tumors. CD1, CD2, LB, MF, MS, and PR indicate molecular classifications for human myeloma described by Zhan et al. (48). CD1, cyclin D1 group; CD2, cyclin D3 group; LB, low bone disease group; MB, MAF/MAFB group; MS, MMSET group; PR, proliferation group.

Abbreviation: CLL, chronic lymphocytic leukemia.

*aRef. 53.
*bRef. 56.
*cRef. 14.
*dRef. 48.
*eRef. 54.
*fRef. 52.
**Ref. 55.
similar to the human myeloma subgroup CD2, a subgroup identified by gene expression profiling and also characterized by increased cyclin D3 expression (48). Additionally, the human CD2 tumors have increased expression of VPREB3, an early B-cell marker, as well as the B-cell transcription factor PAx5. We also observed increased expression of Vpreb3 in mouse tumor group C; however, no valid values for Pax5 expression were recovered from the Affymetrix chip. Pax5 expression was observed in the mouse tumors by RT-PCR, although no obvious difference in Pax5 expression was visible between the tumor groups (Fig. 1D).

The expression of B-cell genes in the human myeloma CD2 subgroup suggests that these human tumors may have a plasmablastic phenotype similar to the Bcl-xl/Myc tumors.

Previous work from our laboratory has identified a unique gene expression signature for human myeloma cells containing an activating Ras mutation (59). This gene signature is composed of five genes, ETVS, DUSP6, SY2B, GALK1, and osteopontin (SPP1), induced to high levels in the presence of mutant NRAS. None of the Ras-induced genes were significantly overexpressed in the Bcl-xl/Myc mouse tumors, suggesting that activating Ras mutations do not play a role in the progression of the Bcl-xl/Myc tumors examined thus far. This is supported by Ras genotyping for several of the Bcl-xl/Myc tumor cell lines, which failed to identify activating mutations in N-ras or K-ras (data not shown).

In this study, we have shown genetic heterogeneity in a transgenic plasma cell tumor model that has similar features to human myelomas with plasmablastic phenotypes. The heterogeneity also is revealed in tumor aggressiveness in adoptive transfer studies. Significantly, this model system lends itself to further genetic manipulations and thus will be useful for dissecting the molecular mechanisms underlying different subtypes of myeloma as well as for identifying drug targets or testing new therapies.

Acknowledgments

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Kristin L.M. Boylan, Mary A. Gosse, Sarah E. Staggs, et al.


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