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Clinical and Biological Implications of MYC Activation: A common difference between MGUS and newly diagnosed multiple myeloma

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Abstract

Events mediating transformation from the pre-malignant monoclonal gammopathy of undetermined significance (MGUS) to multiple myeloma (MM) are unknown. We analyzed a gene expression datasets generated on the Affymetrix U133 platform from 22 MGUS and 101 MM patients using gene-set enrichment analysis. Genes over-expressed in MM were enriched for cell cycle, proliferation and MYC activation gene-sets. Upon dissecting the relationship between MYC and cell cycle genesets, we identified and validated a MYC activation signature dissociated from proliferation. Applying this signature, MYC is activated in 67% of myeloma, but not in MGUS. This was further confirmed by immunohistochemistry using membrane CD138 and

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Authorship Contributions

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Ng SB – Perform IHC analysis

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Bergsagel PL – Design study and approve final manuscript

Fonseca R – Designed study, provide TMAs and approve final manuscript

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nuclear MYC double staining. We also showed that almost all tumors with RAS mutations expressed the MYC activation signature, and multiple mechanisms may be involved in activating MYC. MYC activation, whether assessed by gene expression signature or immunohistochemistry is associated with hyperdiploid MM, and shorter survival even in tumors that are not proliferative. Bortezomib treatment is able to overcome the survival disadvantage in patients with MYC activation.

Keywords

Myeloma; MYC; Bortezomib; MGUS; Hyperdiploid

Introduction

Multiple myeloma is a B-cell malignancy characterized by an accumulation of neoplastic plasma cells that usually secrete monoclonal proteins. Recent studies have shown that most if not all patients with myeloma have a preceding monoclonal gammopathy of undetermined significance phase^{1, 2}.

The primary genetic events involved in disease initiation have been well established in myeloma. Translocations involving maf genes, t(4;14), t(11;14), and hyperdiploidy, with its typical pattern of trisomies of chromosome 3, 5, 7, 9, 11, 15, 19, 21, are present in about 90% of all myeloma³. These abnormalities are already present in MGUS, often times in patients without transformation to MM for many years⁴⁻⁶. Large-scale epidemiological studies showed that MGUS progress to MM at the rate of 1% per year⁷. These data suggest that secondary or cooperating genetic events are probably required for MGUS to MM transition. However, these secondary genetic events have not been identified. The best characterized genetic difference between MGUS and MM is RAS mutations which is extremely rare in MGUS and present in about 20-30% of newly diagnosed MM^{8, 9}. An initial gene expression analysis showed that the expression profile of MM and MGUS is very similar¹⁰. A more recent analysis using a denser chip and larger number of patients identified some genes that are differentially expressed but the functional implications of these genes are unknown¹¹.

In this study, we derived and validated a MYC activation signature that is dissociated from cell cycle / proliferation and showed that this signature is activated in about two-thirds of MM but not MGUS. In addition, MYC activation is associated with unique genetic subtypes, is functionally relevant and identifies patient with poorer diagnosis, who responds better to bortezomib and has improved outcome compared to dexamethasone.

Materials and Methods

Primary Gene expression dataset

Gene expression was performed on CD138⁺ selected bone marrow PCs using the Affymetrix U133A chip from 15 healthy donors, 22 MGUS, and 101 MM from the Mayo Clinic (GEP data are available from GEO, accession #GSE6477). RNA isolation, purification, and microarray hybridization have previously been reported¹². Samples were

collected after informed consent was obtained in accordance with the Declaration of Helsinki, and the study was approved by the Mayo Clinic Institutional Review Board.

Gene expression analysis

Gene expression intensity values were generated using the Affymetrix MAS 5.0 software, median-centered, and analyzed using GeneSpring 7.3.1 (Agilent Technologies, Palo Alto, CA). To examine the biological and functional relevance of the differentially expressed genes between MM and MGUS, we performed gene set enrichment analysis (GSEA), which has been described elsewhere ¹³.

To define the enrichment of MYC pathway genes and the other correlated pathways or functional program at the individual sample level, we made a modification to the GSEA algorithm, called iGSEA (individual sample GSEA) that allow us to generate enrichment scores for different genesets for individual samples. Using a method analogous to leading edge analysis, several core gene signatures were derived from highly correlated genesets that may represent similar biological processes or pathways. We further summarized the expression of genes constituting the core gene signatures into a single value using iPASA (see supplementary methods for details) so that each signature is represented by a number on a continuous scale.

IHC

The C-MYC and CD138 IHC double stain was carried out using the BondMax Autostainer (Leica Microsystems) which utilizes the Define Polymer technique. Five- μ m tissue section containing the tissue microarrays (TMA) was placed onto the BondMax with covertile positioned over the slide, which was deparaffinized and hydrated. Heat-induced epitope retrieval was accomplished for C-MYC using ER1 for 20 min. Endogenous peroxidase was blocked using hydrogen peroxide. For detection of C-MYC, we used a new anti-C-MYC antibody that has been validated using control experiments with genetically defined C-MYC expression ¹⁴. The slide was incubated in the first primary antibody, C-MYC (Epitomics, clone Y69, 1:50), for 30 mins followed by incubation in a post-primary DAB for 10 mins, polymer DAB for 10 mins and chromogen DAB for 10 mins (Bond Polymer Refine detection kit, catalogue no. DS9800). Subsequently, heat-induced epitope retrieval was performed for the second primary antibody, CD138, using ER1 for 20 min. This was followed by incubation with the second primary antibody, CD138 (DAKO, clone MI 15, 1:100) for 30 mins, application of the post-primary AP for 20 mins, polymer AP for 30 mins and Fast Red for 20 mins (Bond Polymer AP Red detection kit, catalogue no. DS9305). The nuclei were counterstained with hematoxylin and the slide dehydrated, cleared in xylene and coverslipped. Appropriate positive controls were used. The number of plasma cells expressing C-MYC was counted and scored as a percentage of the total CD138-positive plasma cell population

Detection of RAS Mutation

RAS mutation was detected as previously described ⁸. Briefly, polymerase chain reaction (PCR) primers were designed to amplify DNA segments of K-RAS and NRAS codon 12, 13 and 61. The radiolabeled amplicons were run through 15% mild-denaturing 0.4 mm

polyacrylamide gel for 4 hours at 40 watts. The gel was dried and placed on a phosphorimager screen for analysis. Abnormal banding patterns were subsequently directly sequenced.

Statistical Analysis

The Student's test was used for comparison of continuous variables. Categorical variables were compared using the chi-square or Fisher's exact test. The distribution for progression-free survival (PFS) and overall survival (OS) was estimated using the method of Kaplan and Meier. The log-rank test was used to test for differences in survival between groups. $P < 0.05$ was considered significant.

Results

Derivation and Validation of Proliferation Dissociated MYC signature

We have previously shown that MYC and cell cycle related genesets are enriched in MM compared to MGUS¹⁵. It is unclear whether the enrichment of MYC genesets are due to greater proliferation of MM cells or implicated actual activation of the MYC pathways. To delineate this, we examined the genes that were enriched in the different MYC genesets and cell cycle genesets using the leading edge analysis tool in GSEA. Although some of the enriched genes in the MYC genesets were also enriched in the cell cycle genesets, there was a cluster of genes that was consistently enriched in the different MYC genesets but not in cell cycle / proliferation genesets (**Figure 1a**). These genes are involved in different functions not necessarily related to cell cycle, most of them have been validated as DNA binding target of MYC and all of them are upregulated by MYC (<http://www.mycancergene.org/>, supplementary table 1), suggesting that this will constitute a good gene signature for MYC activation that is dissociated from cell cycle and proliferation.

To further validate this signature, we examined the expression of these genes in 2 independent dataset where the objective of the original studies was to overexpress different oncogenes in either mouse fibroblast (GEO Accession GSE3151)¹⁶ or human mammary cell lines (GEO Accession GSE3158)¹⁷ to identify oncogene specific gene expression signatures. Our MYC activation signature was over-expressed in the cell lines transfected with MYC in both datasets but not those cells lines with RB knocked out or over-expressing E2F, which were also expected to be proliferative. A consistent observation was the expression of this signature in cell lines expressing activating RAS mutation in both datasets. This may suggest a relationship between activating RAS mutations and MYC activation (**Figure 1b and 1c**).

MYC Activation Signature is expressed in MM but not MGUS

Having defined and validated a MYC activation signature that is dissociated from cell cycle or proliferation, we assessed this signature in human myeloma dataset, together with other cell types for comparison: normal B-cells, normal plasma cells, Burkitt's lymphoma, chronic lymphocytic leukemia and Waldenstroms macroglobulinemia¹⁸. As expected, the signature was expressed highly in Burkitt's lymphoma, which harbours IgH-MYC translocations. The signature was not expressed in normal plasma cells or MGUS but was expressed in about

67% of myeloma (**Figure 2a**). When the MYC activation signature was summarized as the MYC Activation Index (MAI) using the median expression of the genes constituting the signature, the MAI was similar between new MM and relapse MM but significantly higher than normal plasma cells and MGUS ($p < 0.001$) (**Figure 2b**). It was also apparent that some tumors with low proliferation and low expression of *MYC* also have a high MAI. This suggests that there may be different mechanisms that lead to MYC activation. As we had observed an association between activating *RAS* mutation and expression of the MYC signature in the different validation datasets, we assessed our patient dataset for such an association. Indeed, almost all cases with *RAS* mutations (there is no difference between the percentages of K- and N-*RAS* mutations in those with high and low MAI, so both are analyzed together as *RAS* mutants) had a high MAI, confirming our previous observation. Of note, some MM have very high *MYC* mRNA expression that are possibly driven by IgH-*MYC* translocations but do not have *RAS* mutations and vice versa (**Figure 2c**).

Other Pathways Enriched in MM Compared to MGUS

Using iGSEA and leading edge analysis of correlated genesets, a number of core signatures were identified. Besides MYC and proliferation, proteasome, tRNA, metabolic pathway and interferon (IFN) pathway genesets were also enriched. In addition, there is a close correlation between MYC activation, proliferation, tRNA synthesis, increase proteasome subunits and metabolic activity. On the other hand, a subgroup of MM has mainly enrichment of IFN genes without enrichment of MYC and the other correlated signatures (**Figure 3a**). Similar pattern and relation was seen in a separate MM GEP dataset from UAMS (Supplementary Figure 1).

To further investigate the relationship between these pathways, we assessed their expression in the P493-6 cell line model system (GEO Accession GSE 19703)¹⁹ using iPASA. The P493-6 cells were established from primary peripheral blood B cells^{20, 21}. This cell line was immortalized by an Epstein-Barr viral (EBV) genome that was complemented with an Epstein-Barr Nuclear Antigen-Estrogen Receptor (EBNA2-ER) fusion protein and a tetracycline repressible MYC transgene. It is therefore possible to manipulate the cells to achieve at least three states of MYC activation²². Using this model system, we showed that all of these core signatures except the IFN signature were induced upon MYC activation (**Figure 3b**), similar to the pattern observe in the tumors expressing the MYC activation signature in the clinical datasets.

MYC Activation and IRF4 Activity

A recent study suggests that MM cells are addicted to aberrant IRF4 activation and that IRF4 forms a positive auto-regulatory loop with MYC in MM²³. When we assess the IRF4 index (calculated from 35 IRF4 target genes²³ using iPASA) in our dataset, MM with high MAI have higher IRF4 Index (mean±SD, 2.43±0.29 versus 1.14±0.23, p-value = 0.005). This provides further evidence that the MAI is identifying functionally relevant MYC activation.

MYC activation and the NFKB pathway

The activity of the NFKB pathway can be modulated by MYC and vice versa²⁴⁻²⁷. Indeed, the tumors with MYC activation (higher MAI) had lower expression of NFKB gene signature (mean NFKB Index, calculated as median expression of genes constituting the NFKB signature, 0.94 ± 0.6 versus 1.35 ± 0.9 arbitrary units, t-test p -value = 0.01). This relation was also seen upon modulation of MYC expression in the P493-6 cell line system (**Figure 3b**).

MYC staining on IHC correlates with MYC activation signature

Using double staining for surface CD138 and nuclear MYC by IHC in 48 MM cases (36 newly diagnosed and 12 relapse) and 8 MGUS (**Figure 4**), MYC activation was not detected in MGUS but was identified in 29 out of 48 (60%) MM (p -value = 0.07) (**Table 1**). This confirms that MYC is activated in a substantial proportion of myeloma but not in the premalignant MGUS.

The percentage of plasma cells with MYC expression is variable. As the distribution of the clinical parameters and the percentage of MYC staining cells was not statistically different between the newly diagnosed and relapse MM in our TMA dataset (supplementary table 2), we grouped them together for analysis. The percentage of MYC staining cells increases with increasing MAI (**Figure 4e**).

Genetic and clinical association of MYC expression

High MYC expression (>20% PCs) was significantly more common in tumors with t(4;14) although numbers were small while MYC expression (<20%) was significantly more common in tumors over-expressing CCND1 with or without CCND2 (**Table 1**). As most tumor over-expressing CCND1 are hyperdiploid, we sought to assess if a high MAI was more common in hyperdiploid MM. Indeed, in the Mayo dataset where ploidy was assessed using FISH, MAI > 1 was found in 81% (46 of 57) of patients with hyperdiploid MM and only 62% (26 of 42) of patients with non-hyperdiploid MM (2-sided Fisher's Exact test p -value = 0.04).

We also assessed the association of MAI with clinical parameters such as PCLI, beta-2 microglobulin, and ISS stage. Patients with MAI > 1 had higher PCLI (median 1 versus 0.25, $p=0.007$) and ISS III disease (90% ISS III versus 62% ISSI/II have high MAI, $p=0.03$) although there is no difference in beta-2 microglobulin level (median 3.89 versus 2.6 mg/dL, $p=0.15$).

In order to investigate the prognostic impact of MYC expression separate from proliferation, we excluded tumors with PCLI greater than one from the survival analysis. Of the remaining tumors, the PCLI of tumors with or without MYC staining was similar (*data not shown*), yet patients with MYC expressing tumors still had significantly shorter survival suggesting that the prognostic impact of MYC expression is independent of proliferation (**Figure 5a**). As there is an association between MYC activation by IHC and the gene expression based MAI, we assessed the prognostic impact of MAI on survival. Indeed, amongst new diagnosed MM

patients, patients with MAI >1 have significantly shorter survival, even when only patients with lowly proliferative tumors were assessed (**Figure 5b**).

Recent studies have shown that bortezomib-induced apoptosis is via tumor specific upregulation of NOXA in a MYC dependent manner²⁸. We therefore hypothesize that tumors with MYC activation may be more sensitive to bortezomib treatment. We analyzed a gene expression dataset of MM patients entered into a randomized study of bortezomib versus dexamethasone to determine if there was any relationship between the MAI and outcome with bortezomib. Although single agent bortezomib is rarely used in clinical practice now, this dataset is ideal for testing if bortezomib treatment itself may have an impact on MYC expressing tumors. Consistent with our hypothesis, patients with MAI > 1 had significantly better response (57% versus 27%, *p-value* = 0.03) to bortezomib whereas response rates to bortezomib and dexamethasone were similar for patients with MAI = 1 (51% versus 48%, *p-value* = 0.83). This was translated into better PFS and OS for patients with MAI >1 when treated with bortezomib compared to dexamethasone. On the other hand, this benefit was not observed in patients with MAI = 1 (**Figure 5c and 5d** and supplementary figure 2).

Furthermore, as we had demonstrated similar advantage for bortezomib in patients with low TRAF3 expression previously²⁹, we wanted to assess the association between the MAI and TRAF3 level. Treating the normalized expression values as continuous variables, there was no correlation between the MAI and TRAF3 expression levels with a Pearson correlation coefficient of -0.07 (*p-value* = 0.38). When we categorized patient into those with MAI >1 (MYC activation) and TRAF3 < 0.6 (TRAF3 inactivation), there was again no association (Fisher's exact *p-value* = 0.56). When we assessed the interactions between TRAF3 expression levels and MAI on outcome following Bortezomib treatment compared to dexamethasone, only patients with MAI >1 and TRAF3 < 0.6 had significantly better PFS when treated with bortezomib compared to dexamethasone. In terms of OS, there was a trend towards better OS for patients with MAI >1 regardless of TRAF3 level when treated with bortezomib compared to dexamethasone. On the other hand, in patients with MAI = 1, even when TRAF3 < 0.6, the OS was similar between those treated with bortezomib and dexamethasone (**Table 2**).

Discussion

MYC abnormalities have been classically described as a late event in myeloma pathogenesis typically characterized by complex rearrangement involving multiple partners³⁰. These types of abnormalities are particularly prevalent in human myeloma cell lines³¹. In contrast, our study suggests that MYC activation is also likely to be an early event in myeloma pathogenesis. Indeed, MYC rearrangement has been reported to be present in about 15% of newly diagnosed myeloma³². We see a similar percentage of patients in our dataset with very high expression of MYC ('spike' expression), at levels seen in Burkitt's lymphoma, suggesting the presence of IgH-MYC or IgL-MYC translocations. However, this mechanism could not account for most of the cases with MYC activation in our dataset.

RAS mutation is present in about 30% of newly diagnosed myeloma and rarely seen in MGUS suggesting that it is a possible transforming event^{8,9}. In our analysis, the MYC activation signature is present in almost all cases of MM with RAS mutations, regardless of K- or N-RAS mutations, and in the validation datasets when activating mutations of RAS was expressed, suggesting an association between RAS mutations and MYC activation. Our observation is consistent with previous studies showing cooperation between RAS mutations and MYC activation in tumorigenesis and stabilization of the MYC protein by RAS mutants, prolonging the oncogenic activity of MYC^{33,34}. This suggests that RAS mutation is another mechanism leading to MYC activation in MM.

As RAS mutation and 'spiked' expression of MYC occur in a mutually exclusive manner, these aberrations would account for the majority of patients with MYC activation. The remaining mechanisms leading to MYC activation is currently unknown, but may include loss of miRNA that regulate MYC translation³⁵⁻³⁷, or cytokine-mediated via IL-6, an important survival factor for MM^{38,39}.

Our result is also consistent with a recent transgenic mouse model of myeloma based on sporadic activation of MYC in mice that developed background asymptomatic gammopathy, akin to MGUS¹⁵. It is also consistent with a recent analysis in a single case where rapid emergence of clonal cells with MYC rearrangements preceded progression from smoldering MM to MM⁴⁰. MYC activation as a cooperating event to cyclin D dysregulation⁴¹, a universal primary molecular event observed in MGUS, is also mechanistically consistent with the recently described non-transcriptional activity of MYC on DNA replication forks during S-phase of the cell cycle⁴². The cooperative deregulation of 2 different phases of the cell cycle, G₁M and S phase, may result in transformation into myeloma.

Other pathways may be involved in transformation. In our analysis, we found that proteasome signature, tRNA core signature, cell cycle signature, metabolic signature and interferon signature were enriched in MM compared to MGUS. In particular, the interferon signature is enriched in samples where the MYC activation signature is not present and vice versa. On the other hand, the other pathways are enriched in a correlated fashion with MYC activation. These results are consistent with published data that MYC activation lead to increase metabolic activity, protein synthesis and cell proliferation⁴³⁻⁴⁵ and suppression of interferon response²⁶. Furthermore, we showed that myeloma with MYC activation has lower expression of NFκB pathway signature. It has been shown that MYC activation suppresses NFκB pathways^{26,27} and NFκB is not needed by MYC induced tumorigenesis^{24,25}, consistent with the lack of expression of NFκB pathways genes in MYC-driven Burkitt's lymphoma⁴⁶. These correlations were further verified by the gene expression data derived from the P-493-6 cell model upon MYC induction. We also demonstrated a strong association between MYC activation and higher IRF4 activity in the patients, providing further verification of a recent study showing an auto-regulatory loop between IRF4 and MYC in MM⁴⁷. These observations strongly suggest that the predominant signatures seen in the gene expression analysis are due to functionally relevant MYC activation.

MYC activation was associated with protein synthesis (tRNA core signature) and hyperdiploid myeloma in our analysis. This is consistent with our previous observations from gene expression study that hyperdiploid myeloma over-express genes related to ribosome biogenesis and protein synthesis¹². It is intriguing that while the dichotomy between hyperdiploid and non-hyperdiploid myeloma occur at the earliest stages of pathogenesis, the secondary events also appear to be subtype specific with MYC activation preferred by hyperdiploid MM whereas chromosome 13 deletion with potentially RB haploinsufficiency more common in tumors with t(4;14) and translocations involving MAF family genes.

Our analysis also showed that MYC activation detected either using the MYC activation signature or nuclear MYC immunoreactivity by IHC has prognostic importance. Although a previous study shows that MYC rearrangements as detected by FISH lack prognostic relevance³² but as mentioned, MYC activation in myeloma is likely mediated by different mechanisms other than genetic rearrangement. Of note, the prognostic relevance of MYC activation also applies to patients with nonproliferative tumors. Although it is not an independent prognostic factor on multivariate analysis (Supplementary Table 3), this nevertheless highlights the functional and clinical relevance of MYC activation in MM.

As MYC activation is seen in a substantial number of newly diagnosed MM and is of functional relevance, it may be potentially exploited for therapy. Indeed, we found that MM with MYC activation benefit from bortezomib treatment. Within the limitations of these being a subgroup analysis, these observations are interesting and should be confirmed in other bortezomib gene expression datasets as well as prospective studies. A previous report showed that induction of apoptosis in myeloma cells by bortezomib requires upregulation of NOXA via a MYC-dependent mechanism²⁸. In addition, in T-cell lymphoma, bortezomib can downregulate IRF4 and MYC⁴⁸. These are possible reasons for the greater efficacy for bortezomib seen in patients with MYC activation.

An important limitation is that even though we used CD138+ sorted cells from MGUS patients, a large amount of these will be the normal component compared to MM, resulting in potential bias. However, the corroboration of GEP results by IHC using a double staining method allowing for visualization of individual cells further supports our conclusion.

In summary, MYC activation may be a common transforming event in myeloma. This activation may be mediated by multiple mechanisms, and tumors with MYC activation have a more aggressive course associated with shorter survival. Targeting MYC either directly⁴⁹, or indirectly, through synthetic lethal targets, such as CDK1⁵⁰, may be useful therapeutic avenues in myeloma.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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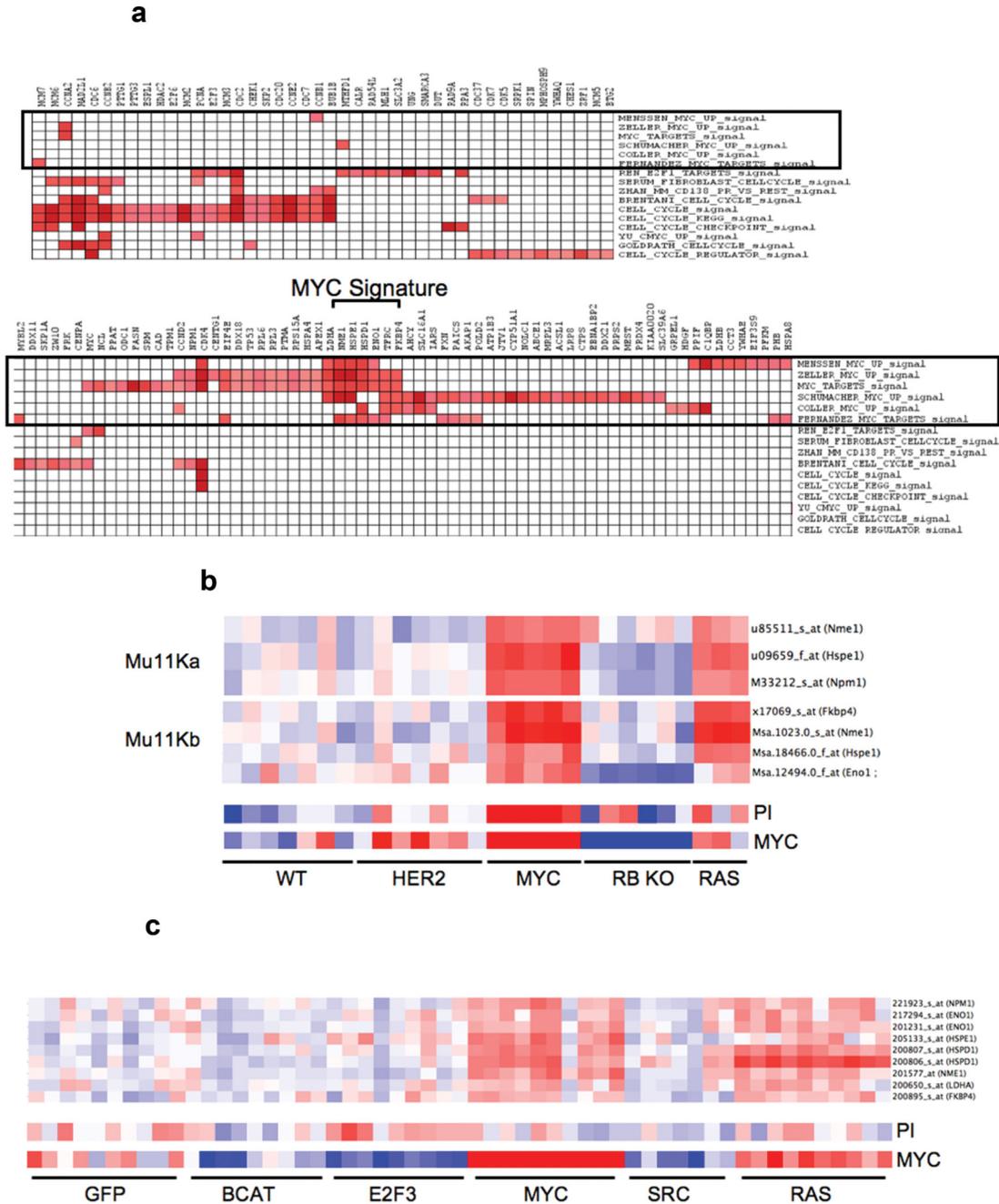


Figure 1. Derivation and Validation of MYC signature dissociated from cell cycle

(a) Leading edge analysis of enriched MYC and cell cycle genesets identified a set of genes which are consistently enriched (red squares) across the different MYC genesets (in black box) that are not enriched in the cell cycle genesets. This set of genes constitutes our MYC signature that is dissociated from cell cycle and proliferation. In the grid, the columns represent genes that constitute the main enrichments (leading edge genes) in the different genesets (row). We then validated this signature in a (b) mouse fibroblast and (c) human mammary cell line gene expression dataset. In the heatmaps, each row represents a gene and

each column a sample. Red represents upregulation and blue downregulation of the respective genes.

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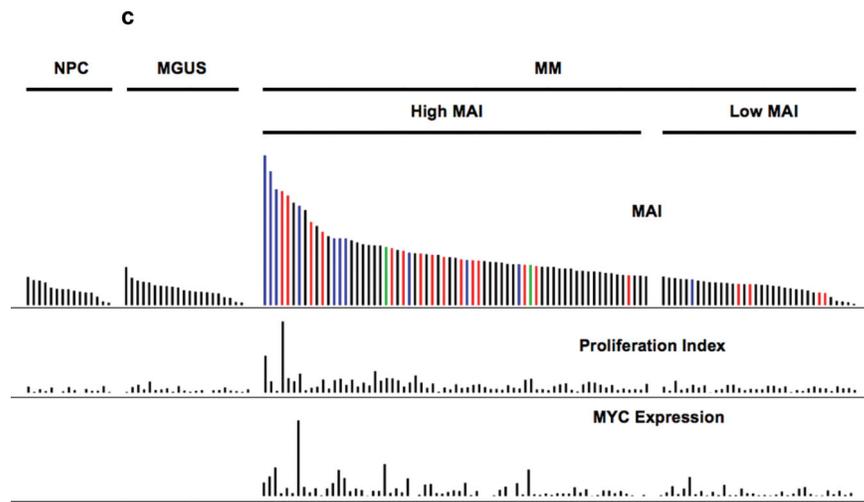
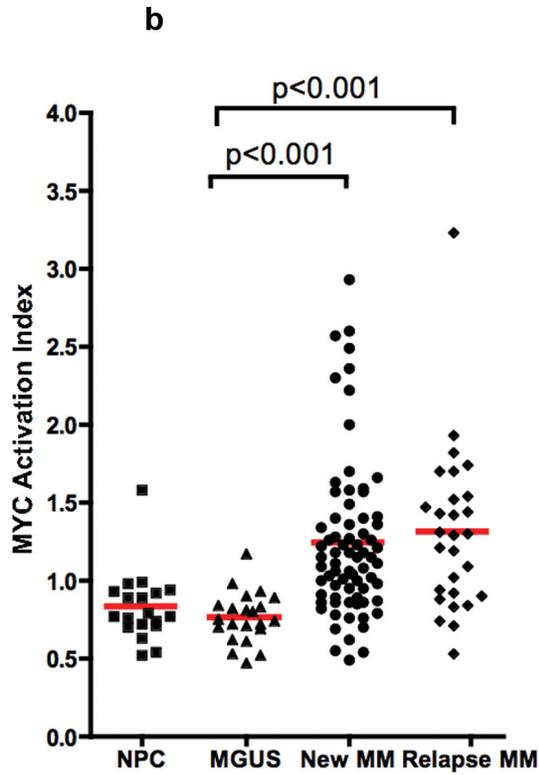
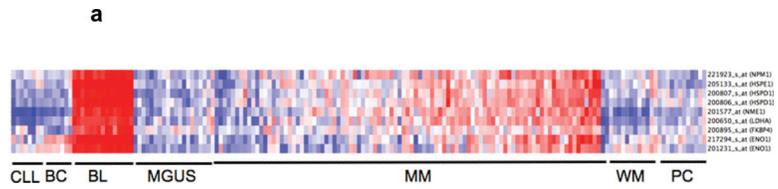


Figure 2. Expression of MYC activation signature in MM and not MGUS

(a) The derived MYC signature is highly expressed in Burkitt's lymphoma and also expressed in a substantial number of MM but not MGUS. (b) When the MYC activation signature is summarized as the MYC Activation Index (MAI), based on median expression

of genes constituting the signature, it is significantly higher in both newly diagnosed and relapsed MM compared to MGUS and normal plasma cells (NPC). The MAI is similar in both new and relapse MM. (c) The samples are arranged according to the MAI. Each row represents a different parameter, with MAI followed by proliferation index, and *MYC* mRNA expression. In the MAI row, the colored bars represent samples with *RAS* mutations (red), 'spiked' *MYC* expression (5 fold or greater expression than median) and both *RAS* mutations and 'spiked' *MYC* expression (green). It is clear that *RAS* mutations and 'spiked' *MYC* expression (resulting from IgH-*MYC* translocations) are generally mutually exclusive. Amongst the MM with high MAI, some of them have low proliferation (PI) or low expression of *MYC* itself or both.

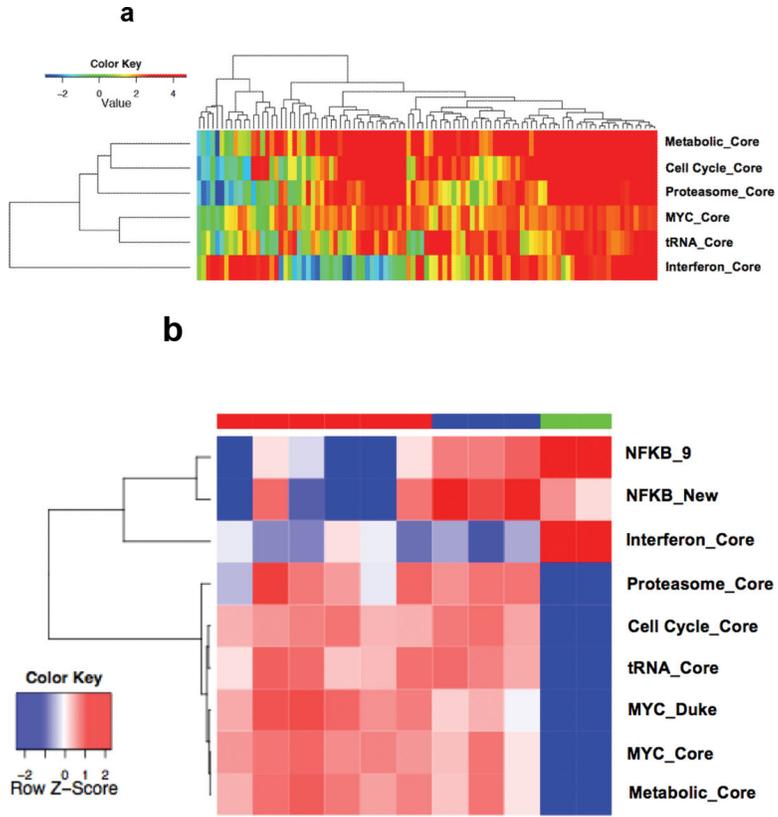


Figure 3. Core Signatures correlated with MYC activation signature

(a) In the Mayo MM dataset, enrichment for MYC, Cell cycle, proteasome, tRNA and metabolic genesets are highly correlated whereas the IFN pathway genesets are enriched in a subset of MM without MYC activation. (b) In the P493-6 cell line model where MYC expression can be manipulated and graduated. Similar patterns among these pathways are seen. In addition, increasing MYC expression and activation also lead to suppression of NFKB signature extracted from 2 recent studies designated as NFKB_9²⁹ and NFKB_New⁵¹.

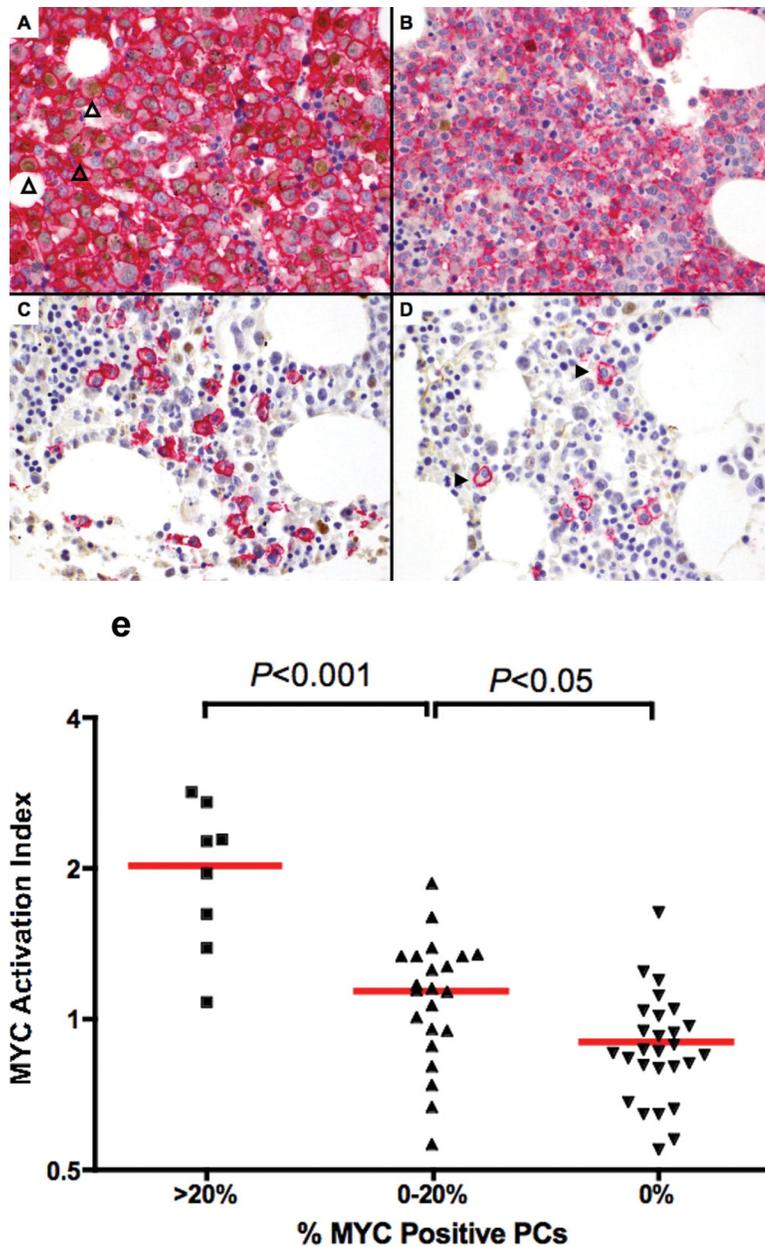


Figure 4. Expression of MYC is present in CD138 positive plasma cells in MM but not MGUS by IHC, and increase with increasing MAI

These bone marrow sections are double-stained with CD138 (red - membrane) and MYC (brown -nuclear). (A) MM with high CD138 infiltration showing positivity for nuclear MYC expression in CD138 positive cells (3 examples indicated by open arrowheads). However, there are MM cases with both (B) high or (C) low CD138 plasma cell infiltration that are negative for MYC. (D) CD138 positive plasma cells in MGUS are universally negative for nuclear MYC (2 examples indicated by closed arrowheads). (e) The MAI increase significantly across the categories of increasing percentage of MYC positive plasma cells.

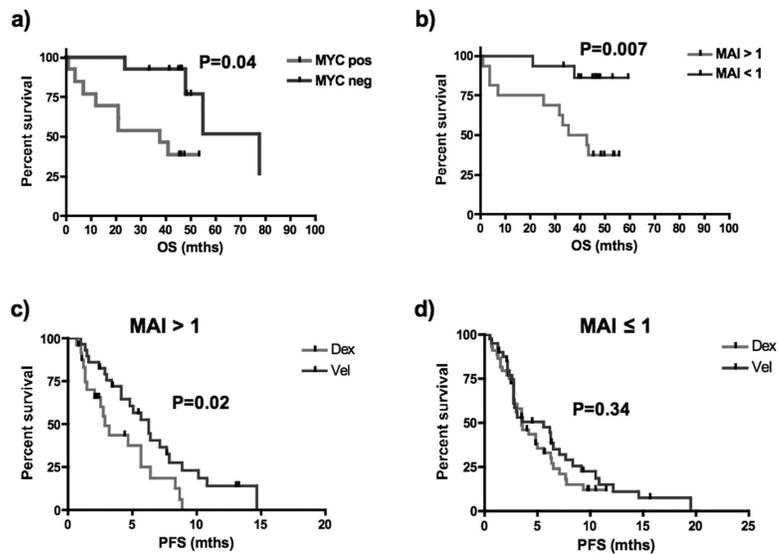


Figure 5. Survival according to the MAI and MYC staining by IHC

(a) When only newly diagnosed tumors with $PCLI < 1$ are analyzed, those with no MYC staining ($n=13$) still have significantly longer survival than those with MYC staining ($n=13$) (median survival 77.7 mths versus 37.9 months, log-rank p -value = 0.04). (b) Overall survival is significantly shorter in newly diagnosed MM patients with high MAI compared to those with low index even when only patients with low proliferation are considered (median survival 39.2 mths versus not yet reached; log-rank p -value 0.007). (c) In patients entered into a randomized study of bortezomib versus dexamethasone, those with $MAI > 1$ have significantly better PFS when treated with bortezomib compared to dexamethasone (6.3 months ($n=37$) compared to 2.9 months ($n=28$), log-rank p -value = 0.02). (d) In contrast, for those patients with $MAI \leq 1$, there is no difference in PFS with either treatment (5.6 months ($n=43$) versus 3.5 months ($n=48$), log-rank p -value = 0.34).

Table 1

Correlation of Genetic and Clinical Parameters with MYC staining

	%MYC Positive PCs			<i>p</i> -value
	0%	0-20%	>20%	
Stage				0.007
MM	19	21	8	
MGUS	8	0	0	
Ploidy				0.005
H	7	17	3	
NH	12	3	5	
13 Deletion				0.36
Yes	8	7	5	
No	11	14	3	
TC Class				
11q13+6p21	7	2	1	0.09
4p16	0	1	3	0.004
D1 & D1+D2	6	16	2	0.006
D2	4	0	1	0.09
Maf	1	2	1	0.8

Impact of MAI and TRAF3 expression on PFS and OS in MM patients treated with bortezomib or dexamethasone.

Table 2

MAI and TRAF3 combination	N	Progression Free Survival			Overall Survival		
		Bort, mths	Dex, mths	p-value	Bort, mths	Dex, mths	p-value
MAI>1, TRAF3<0.6	16	6.1	2.1	0.02	Not reached	12.1	0.10
MAI>1, TRAF3 0.6	49	6.3	4.7	0.35	19	9.6	0.12
MAI 1, TRAF3<0.6	18	7.2	4.6	0.21	25.5	26.2	0.95
MAI 1, TRAF3 0.6	73	3.1	4.2	0.96	23.1	31.9	0.26