MYC dysregulation in the progression of multiple myeloma

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Multiple myeloma (MM) is a plasma cell malignancy preceded by a premalignant stage, named monoclonal gammopathy of undetermined significance (MGUS), and often a smoldering phase (SMM).1,2 Primary events, which include recurrent translocations of the IgH locus and hyperdiploidy, occur early in pathogenesis, and are followed by the acquisition of secondary genetic events such as MYC structural variants (SV), mutations that activate the RAS or NFκB pathways, mutations of DIS3 or FAM46C that drive precursor stages of disease toward MM.3–6 Whole exome sequencing (WES) studies comparing serial MGUS/SMM and MM samples indicate clonal stability, and no significant increase in mutational load in patients that progress rapidly to MM.7 In contrast, in 33 unselected MGUS patients single-nucleotide variants (SNVs) were less frequent, and no MYC translocations identified.8

To study the role of MYC in myeloma we performed an integrated genomic analysis of 612 newly diagnosed myeloma (NDMM) patients enrolled in the CoMMpass study, as well as...
targeted sequencing of 23 patients with MGUS and 90 patients with SMM. We identified MYC SV in 42% of NDMM, including the majority of HRD (57%), and a quarter of MM with primary IgH translocations. The majority of these rearrangements resulted in juxtaposition of a super-enhancer (SE) and/or stretch enhancer adjacent to MYC, with one third involving an Ig super-enhancer, one third involving another recurrent super/stretch enhancer and the remaining third split between non-recurrent super/stretch enhancers, no identified super/stretch enhancer, or rearrangements wholly confined to the region telomeric to MYC, frequently duplications with no exogenous sequences present (Table S1A–B, Table S2–4). The IgH MYC rearrangements often were complex - sometimes involving duplications and 3 or more chromosomes - and the IgH breakpoints were often within or near the 3’ SE regions, suggesting a different timing or mechanism than the primary IgH translocations.

By using an informative group of patients in which we were able to identify germline polymorphisms within the exons of MYC (n=147), we found 66/69 (96%) NDMM with elevated mono-allelic MYC expression have a MYC SV, whereas in 69/77 (90%) with variable levels of biallelic MYC expression no MYC SV was identified (Table S5). This highlights the functional significance of MYC SV, suggesting that our analysis is neither missing nor overcalling the presence of many MYC SVs, and that the primary mechanism of cis-dysregulation of MYC is by SV. The level of expression of MYC is higher in samples with rearrangements compared to those without (Figure 1A, B), with similar levels whether an Ig or non-Ig enhancer is involved (p-value > 0.10), but intermediate levels for samples with a wholly confined telomeric rearrangement.

While in patients with MYC SV there was no correlation between MYC expression and NFkB index (Figure 1C), in patients lacking MYC SV, there was a strong linear correlation (Figure 1D), identifying coordinate dysregulation of MYC associated with both constitutive and ligand-dependent NFkB pathway activation. Unlike many cancers, we did not find a correlation between the presence of MYC SV, or the level of MYC expression, and proliferation, as measured using a gene expression index (data not shown). As recently noted9 MAX mutations or inactivation correlate with extremely low levels of MYC expression, and we found that aberrations in these genes rarely occur together (Figure 1B). (Table S6). This data suggests that as reported for small cell lung cancer10 and oligodendroglial tumors11, aberrancies of MYC and its heterodimeric partner MAX12, operate in a mutually exclusive fashion.

Taken altogether, MYC SV, MAX inactivation and NFkB pathway mutation, identify a genetic mutation associated with MYC/MAX pathway dysregulation in two-thirds of NDMM (261+22+ 127/612, Table S1A). In 86 of the remaining patients (14% of the total) there is ligand dependent NFkB activation associated with increased MYC expression. The overwhelming majority of the remaining patients, representing 14% of the total (86/612), have a mutation activating the MAPK pathway (RAS/BRAF/FGFR3). Only one in twenty (30/612) tumors lacks evidence of dysregulation of the MYC/MAX, NFkB or MAPK pathway. In contrast, there is no correlation between MYC dysregulation and mutations of the MAPK pathway, which are instead inversely correlated with NFkB activation, particularly in patients without MYC SV (Figure 1E and 1F, Table S7, S8).

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We further expanded our genomic analysis and included premalignant stages in MM development. First, we established a sequencing panel targeting regions surrounding $\text{IgH}$ (500 Kb), $\text{IgL}$ (100 Kb), $\text{IgK}$ (50 Kb) and $\text{MYC}$ (2 Mb) loci, in addition to detecting SNVs in 88 important MM genes\textsuperscript{13}. The robustness of the approach was tested by comparing with FISH data from 90 primary samples, and for 60 of these also with Mate Pair whole genome sequencing (WGS). Across all 90 samples, the Custom Capture approach was able to detect 93\% (39/42) of $\text{IgH}$ translocations and 86\% (19/22) of $\text{MYC}$ SVs previously detected by FISH and Mate Pair WGS, respectively (details in Supplementary Methods), and as such we slightly underestimate the incidence of $\text{MYC}$SV in MGUS and SMM compared to MM patients in the CoMMpass study.

We analyzed 23 unselected MGUS cases using the sequencing panel. Three patients had an N and/or K-RAS, two had NFkB pathway mutations (TRAF2 and CYLD) and none had rearrangements in the $\text{MYC}$ locus. However, canonical initiating events (HRD and $\text{IgH}$ translocations with $\text{MAF}$, $\text{MAFA}$, $\text{MMSET}$, $\text{CCND1}$, and $\text{CCND3}$) were observed in all but four samples, three with no clear initiating event and the other an $\text{IgH}$ rearrangement with $\text{UPK2}$ (Table S9, Table S10–S12). When analyzing 90 SMM samples with the sequencing panel, 22 cases were observed with $\text{MYC}$ SVs (24\%), including 5 $\text{IgH}$-$\text{MYC}$ and 1 $\text{IgL}$-$\text{MYC}$SV (Table S9). The time to progression (TTP) for SMM cases with non-$\text{Ig}$ $\text{MYC}$SVs was not significantly different than cases without any $\text{MYC}$SV (median TTP of 45 versus 61 months, $p$-value >0.10). However, the SMM cases with $\text{Ig}$ $\text{MYC}$SVs progressed rapidly to MM (all 6 cases progressed within 23 months of observation, Figure 2A). On multivariate analysis performed using Mayo Clinic criteria for high risk of progression $\text{Ig}$ $\text{MYC}$SVs retained significance (HR 4.59, $p$ 0.003) as an independent prognostic marker for rapid progression to MM (Table S13).

In an expanded analysis of potentially relevant genomic features within this SMM cohort, $\text{DIS3}$ mutations associated with rapid progression to MM along with $\text{Ig}$ $\text{MYC}$SVs (Table S14). Notably, many CNAs that are commonly aberrant and often associated with adverse prognosis in NDMM, such as gain of 1q or deletion of 13q, bore no significant association with progression to MM despite increased frequency with advancing disease stage (Figure 2C, Table S15, S16).

While $\text{DIS3}$ mutation and $\text{Ig}$ $\text{MYC}$SVs were significantly associated with rapid progression to MM in SMM, $\text{Ig}$ $\text{MYC}$SVs alone only bordered on significance in NDMM (PFS $p$-value = 0.055) but did achieve significance when paired with $\text{DIS3}$ mutation (PFS $p$-value < 0.05, Table S17). In contrast to SMM, we observed that IgL, rather than IgH or IgK $\text{MYC}$SV, were associated with more rapid disease progression in NDMM (Figure S1)\textsuperscript{14}. In a more focused analysis comparing prognostic associations of $\text{MYC}$SV types (Ig, non-Ig, or none), we observed that HRD cases with a non-Ig $\text{MYC}$SV had uniquely beneficial prognosis with a significantly reduced rate of progression (82\% cases without PFS events at 2-years compared to 59\% in remaining cases, Figure 2B) while no difference in outcome was noted across $\text{MYC}$SV type in non-HRD MM (Figure S2, S3). Both the combination of non-Ig $\text{MYC}$SVs with HRD positivity and IgL $\text{MYC}$SV retained significant association with PFS in multivariate models including covariates for key genomic features ($\text{MMSET}$ or $\text{MAF}$ translocations, 1q gain, 13q loss, 17p loss, $\text{DIS3}$ mutation), treatment strategy, i.e. use of
combined therapy with Immunomodulatory drugs (Imids), and International Staging System (ISS) stage (Table S18). Whereas previous studies in lymphoma, and MM have shown MYC SVs to be an adverse prognostic factor \(^6\), \(^15\) we did not observe this in our studies of SMM or MM. This suggests that MYC may serve a somewhat different role in MM, less focused on proliferation and instead driving protein translation and metabolism to meet the demands of highly secretory plasma cells. Our findings require further investigation but provides preliminary evidence that outcome, and likely function, of MYC rearrangements is dependent upon partnered enhancer and genetic context. It is supported by the parallel observation of a much more rapid progression from MGUS through SMM to MM for patients with Ig MYC SV, but not non-Ig MYC SV.

Our analyses demonstrate that MM tumors rely for progression on a few signaling pathways (MYC, RAS, NFkB) that show functional redundancy and complementary activation, with at least one pathway activated in 95% of NDMM. In contrast to previous studies of serial samples,\(^7\) our analysis of MGUS cases showed a lack of key progression features, e.g., 0/23 with MYC SV and only 2/23 with a clonal NFkB or KRAS mutation. This discrepancy is likely due to not selecting samples known to progress to MM. Focusing on the same 3 progression pathways for SMM vs MM, MYC SV are 24 vs 43%, NFkB mutations 12 vs 32%, and RAS pathway mutations 46 vs 53%. Rapid progression of SMM to MM appears to be independently associated with Ig MYC SV and DIS3 mutations, and possibly with NFkB mutations.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Location of MYC breakpoints and variation in MYC expression
The exact location of breakpoints (black dots) at the MYC locus for the 260 NDMM cases with MYC SV in the CoMMpass cohort, shows that the breakpoints clustered within an approximately 2Mb region around MYC, with three breakpoint cluster regions: one centered on MYC, a less frequent one centromeric, and more common one telomeric to MYC. The level of MYC expression (log transform of Salmon TPM) is shown on the Y-axis, and shows that the breakpoints were associated with an increased expression of MYC, (A). The level of MYC expression is highest in cases with IG or Non-IG MYC SVs (median TPM 79; non-
significant one-sided Wilcoxon test between IG and Non-IG MYC SVs, p-value > 0.05) according to data from 612 NDMM CoMMpass cases. Cases with IG or non-IG MYC SVs have significantly higher MYC expression than those with wholly confined telomeric MYC SVs (median TPM 38, p-value < 0.001), who in turn have significantly higher expression than cases with NFkB aberrations (median TPM 26, p-value < 0.05), and cases with RAS or FGFR3 mutations (median TPM 15) have low expression of MYC, even lower than cases with NFkB aberrations (p-value < 0.001). MYC expression is lowest in cases harboring MAX aberrations (median TPM 1, B). Across patients with MYC SVs, there was no correlation between the level of expression of MYC and NFkB aberrations or index (C and E). However, in patients without MYC SV, there is a significant correlation between the level of MYC TPM and the NFkB index (D and F). Vertical line in plots C and D denotes the median NFkB1. Correlation triangles report Spearman correlations between variables when highly significant (p-value < 0.001) with negative correlation in blue, positive correlation in red, and size of circle associated with absolute value of correlation.

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Figure 2. Progression in SMM and NDMM and genomic copy number comparison of MGUS, SMM, and NDMM

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prevalent across disease stages, however gain of 1q and loss of 13q significantly increase in frequency with disease stage, more so than any other chromosomes. (C)