Plasma Cells Expression from Smouldering Myeloma to Myeloma Reveals the Importance of the PRC2 Complex, Cell Cycle progression, and the Divergent Evolutionary Pathways within the Different Molecular Subgroups

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Letter to Leukemia

Words: 1485 (1,500 words);

Tables and figures: 2 (max 2)

Reference: 15 (max 15)

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Abstract

Sequencing studies have shed some light on the pathogenesis of progression from smouldering multiple myeloma (SMM) and symptomatic multiple myeloma (MM). Given the scarcity of smouldering samples, little data are available to determine which translational programs are dysregulated and whether the mechanisms of progression are uniform across the main molecular subgroups. In this work, we investigated 223 SMM and 1348 MM samples from the University of Arkansas for Medical Sciences (UAMS) for which we had gene expression profiling (GEP). Patients were analysed by TC-7 subgroup for gene expression changes between SMM and MM. Among the commonly dysregulated genes in each subgroup, *PHF19* and *EZH2* highlight the importance of the PRC2.1 complex. We show that subgroup specific differences exist even at the SMM stage of disease with different biological features driving progression within each TC molecular subgroup. These data suggest that MMSET SMM has already transformed, but that the other precursor diseases are distinct clinical entities from their symptomatic counterpart.

To the editor,

In the last decade, sequencing studies have shed some light on the pathogenesis of progression from smouldering multiple myeloma (SMM) and symptomatic multiple myeloma (MM) highlighting the importance of factors such as *MYC* rearrangements,[1] the MAPK pathway,[2,3] and the APOBEC mutational processes.[3] Given the scarcity of samples, little data are available to determine which translational programs are dysregulated and whether the mechanisms of progression are uniform across the main molecular subgroups.

To answer these questions, we investigated 223 SMM and 1348 MM samples from the University of Arkansas for Medical Sciences (UAMS) for which we had gene expression profiling (GEP). We analysed previously published data from MM patients who were recruited onto one of the Total Therapy trials[4] (**Supplemental-Figure 1**) and SMM patients who were either recruited to the S-0120 (NCT00900263- IRB:7417) or M-0120 studies (M0120 is 2011-61 IRB:136962) studies.[5,6] GEP of CD138+ plasma cells using U133 Plus 2.0 arrays (Affymetrix) were obtained. Raw data were MAS5 normalized and the TC-7 subgroups derived, as previously reported.[7] SMM and MM samples were analysed by TC-7 subgroup. The TC-7 classification identifies seven major subtypes (D1-HRD, D2, CCND1-11q13, CCND3-6p21, MMSET, MAF and MAFB) and has been validated against cytogenetic subgroup. Log2 fold-change (FC) and t-statistics were computed. Expression was compared using an ANOVA method and corrected for multiple testing. A cut-off of q<0.05 were considered significant. Volcano plots were generated using EnhancedVolcano[8] and pathway analysis performed using FGSEA. The Kaplan–Meier estimator was used to calculate time-to-event distributions and compared using the logrank test.

For SMM patients, although shorter for the MMSET subgroup, the time to therapy did not differ significantly between TC subgroups, **Figure 1A**. The breakdown of the different TC subgroups was similar between SMM and MM except for fewer MMSET (9% vs 13%) and D1-HRD patients (27% vs 33%) in SMM, **Figure 1B**. Due to a low number of samples and the infrequency of some subgroups, particularly in SMM, we did not study the CCND3-6p21 and MAFB groups further.

There were 47 significantly upregulated genes between SMM and MM in all translocated groups, **Supplemental-Figure 2A,** and 45 upregulated in all five groups considered, **Supplemental-Figure 2B**. Among them, there were two members of the PRC2.1 complex, *EZH2* and *PHF19*. *EZH2* expression is normally repressed by the p53/RB pathway, consistent with its over-expression in MM. *PHF19* has previously been associated with high-risk MM.[9] PHF19 modulates the catalytic activity and recruitment of the PRC2 complex to key genes involved in cell cycle. Cell cycle progression requires expression upregulation of *EZH2* and *E2F8* by *E2F1*, a key regulator of G1-to-S phase transition. *E2F8,* was also upregulated in all subgroups suggesting cooperation to enable cell cycle progression in MM. Another regulator, *ANLN*, was also upregulated and has been associated with *EZH2* upregulation and promotion of pancreatic cancer progression. Other genes were implicated in cell cycle regulation (such as *CCNB1/2*, or *AURKA*), DNA replication (including *TYMS*, *RRM2*, *CDC45*, *GMNN*), mitosis (*CDK1*, *CCNB1*, *MAF2L1*, *CENPA/E/K/N/W*) and apoptotic response (such as *TPX2*, *CDK1*, *BUB1B*, *ESPL1*, *BUB1*, *MELK*). Three *MYC* targets were present (*CCNB1, CKS2, TYMS*) but not *MYC* itself. Interestingly the top GEP4 gene,[5] *RRM2,* was upregulated in all groups and the second, *DTL*, in all but the MMSET subgroup (FC=1, t=3.7, q=0.054). Twenty genes were significantly down-regulated in the translocation groups including tumour suppressors such as *IGF1* and *DENND2D*, **Supplemental-Figure 3A**. There was only one gene, *CD36*, consistently downregulated across all five groups, **Supplemental-Figure 3B**. This gene, encoding for the thrombospondin receptor, has previously been associated with favourable outcome in MM.[10] Overall, these data highlight, the importance for the PRC2.1 complex as a common marker of progression from SMM to MM, and suggest that high-risk MM features (*PHF19, RRM2*) are associated with MM and low-risk MM features (*CD36*) with the precursor condition.

Among the CCND1-11q13 (SMM=41 and MM=262) patients, 3798 genes were differentially expressed (q<0.05). Genes of interest include the upregulation of *APOBEC3B (*FC=1.18, t=7.6, q=$1.08.10^{-7}$) which was not seen in the other groups and *NEK2*[11] (FC=0.99, t=6, q=$9.6.10^{-6}$) which was also seen in the MAF group. Additionally, *MYC* (t=2.6, q=0.04) and several *MYC* targets were noted (*BAX, EIF3C, KAP1, RCC1, MRTO4, TK1, UBE2C, CCT5, PHB, CBX3, CDC25A, CDC25C*).[12] Among the genes

that were downregulated in MM compared to SMM, we note the mineralocorticoid receptor *NR3C2 (*FC=-1.3, t=-8.9, q=7.2.10^{-10}) and *CTSW (*FC=-2.6, t=-4.9, q=0.0004) that has been associated with favourable outcome in endometrial cancer[13], and breast cancer[14], **Figure 2A**. In terms of pathways analysis, pathways relating to cell division (chromosome segregation, nuclear division, DNA replication, centromere assembly, DNA replication, cell division, spindle organization, checkpoints and cycle transition) together with amino acid metabolic processes were the most upregulated. Pathways related to normal plasma cell function such as defence response, chemotaxis and inflammation response were downregulated in symptomatic myeloma, **Figure 2A**.

In contrast, in the MMSET subgroup (SMM=20, MM=181), there are fewer dysregulated genes (n=577) than in the other subgroup (**Supplemental-Figure 4**) consistent with the idea that the MMSET SMM has already transformed to MM. *PARP15* (FC=-1.7, t=-6.6, q=0.0005) usually designated as B aggressive lymphoma proteins[15] and *PRKG1* (FC=-2.1, t=-5.3, q=0.007) a protein kinase, were downregulated in MM compared to SMM. Only two *MYC* targets were noted (*HSPE1, TXN*). These results support previous hypotheses that this group is rapidly driven to a symptomatic disease phenotype and that even at the SMM stage they have features of aggressive disease and as such could be considered at high-risk of rapidly transforming to MM, **Figure 2B.** These results support the decision made by the IMWG where the t(4;14) is considered a high-risk subgroup.

The MAF group differs from the MMSET group in that while it is considered high-risk in MM paradoxically in MGUS it is a good prognostic feature. There were more dysregulated genes (n=1801) in the MAF group than in the MMSET group, but fewer than in the CCND1-11q13 group. In terms of genes, *SLAMF1* expression was higher in SMM (FC=-2.4, t=-7.7, q=2.6x10^{e-6}), despite being on 1q23, whereas *NEK2* (FC=1.8, t=4.7, q=0.008) [11], was associated with MM, **Figure 2C**. *MYC* (t=4.0, q=0.01) and several *MYC* targets were also noted such as (*CCT5, PCNA, SNRPB,UBE2C, NPM1, CDC25C, NAP1L1, MSH2, CDC25A, PSMG1, HSPD1*).[12] In terms of pathways, we show that cell division was upregulated and normal plasma cell function was

downregulated at the transition to MM. Interestingly, the DNA repair pathway was upregulated in MM, **Figure 2C**.

In the HRD-D1 (SMM=61 and MM=446) and HRD-D2 (SMM=72 and MM=347) groups, 4244 and 2311 genes were differentially expressed (q<0.05), respectively. When looking at individual subgroups, *NUF2,* a member of the kinetochore complex (FC=1.33, t=5.2, q=2.85^{e-8}), *DKK1*, the Wnt signalling inhibitor (FC=1.2, t=7.4, q=3.3^{e-5}), and *MYC* (t=3.6, q=0.004) and multiple *MYC* targets (*HK2, HSPD1, MGST1, TK1, CDC25A, CDC25C, CBX3, NBN, EIF3C, PAX3, UBE2C, CTSC, CCND2, CCT5, HNRNPA1, MRTO4*)[12] were significantly upregulated in MM D1-HRD. In D2-HRD, *CD44* (FC=1.3, t=3.8, q=0.004) and a few *MYC* targets (*CBX3, CDC25A, CDC25C, TK1, UBE2C*) were significantly upregulated but not *MYC* (t=0.7, q=0.5). No downregulated pathways were identified in neither D1-HRD nor D2-HRD subgroups. In the D1-HRD subgroup, pathways related to cell cycle and DNA repair were upregulated and in the D2-HRD cell cycle and protein ubiquitination pathways, **Figure 1C and 1D.**

When analysing the overlap between subgroups, *PHF19* and *EZH2* appear as prime candidates to elucidate disease progression highlighting the importance of the PRC2.1 complex, **Supplemental-Figure 5**. Close attention should be given to the PRC2.1 complex as a mean of identifying high-risk SMM cases and as a potential therapeutic target. Consistent with previous data identifying different oncogene dependencies in MM. we show that subgroup specific differences exist even at the SMM stage with different biological features driving progression within each TC molecular subgroup thus highlighting the importance of MYC in some sub-groups such as the CCND1, MAF, and HRD-D1 as a late event but not others such as MMSET and HRD-D2. These data were in keeping with a comparison between our previous work showing, that in HRD patients, the frequency of MYC translocations does not differ between MM (187/569=33%)[12] and SMM (15/46=33%)[3] but when considering the t(11;14) and t(14;16), 13% (29/227) and 25% (11/44) of MM patients had a *MYC* translocation versus none at the SMM stage, consistent with the idea that *MYC* translocations in the t(11;14) or t(14;16) are a late event associated with the transition from SMM to MM or alternatively a shorter SMM disease phase. Further biochemical

analysis unravelling the mechanisms by which PRC2.1 and MYC drives progression remains to be ascertained. These data suggest that MMSET SMM has already transformed, thus contributing to the argument favouring their treatment, but that the other precursor diseases are distinct clinical entities from their symptomatic counterpart highlighting the divergent evolutionary pathways between SMM and MM within the different molecular subgroups. Importantly, if intervention strategies are developed, they should potentially be subtype specific or at least subtype specific therapeutic effects may be expected, therefore confirming the importance of adequate biological characterisation of SMM patients.

Figure 1: Distribution of TC subgroups, impact on outcome, and patterns of expression changes between SMM and MM. A. Outcome of patients depending on their TC subgroup suggesting there are no difference in terms of time to progression between the different groups B. Proportion of TC groups in the different disease states (centre SMM, periphery MM) suggesting there are fewer MMSET and D1-HRD subsets in SMM C. Volcano plot highlighting the differentially expressed genes between D1-HRD SMM and MM. Lollipop plot highlighting the pathways enriched in the D1-HRD group between SMM and MM. D. Volcano plot highlighting the differentially expressed genes between D2-HRD SMM and MM. Lollipop plot highlighting the pathways enriched in the D2-HRD group between SMM and MM.

Figure 2: Patterns of expression changes differ among cytogenetic subgroups. A. Volcano plot highlighting the differentially expressed genes between CCND1 SMM and MM. Lollipop plot highlighting the pathways enriched in the CCND1 group between SMM and MM. B. Volcano plot highlighting the differentially expressed genes between MMSET SMM and MM. Lollipop plot highlighting the pathways enriched in the MMSET group between SMM and MM. C. Volcano plot highlighting the differentially expressed genes between MAF SMM and MM. Lollipop plot highlighting the pathways enriched in the MAF group between SMM and MM.

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Disclosures

The authors have no conflict of interest to disclose.

Acknowledgments

Authorship

Designed the study: EMB, BAW, GJM, FVR, Analysed the data: EMB, AR, AH, Interpreted the data: EMB, BAW, FED, HG, YuW, GJM Acquired the data: PF, MR, CA, MB, SKJ, CPW, YanW, CDS, ST, MZ, BB, MVD, FVR, GLM, FED, Wrote the manuscript: EMB, FED, BW, Reviewed the manuscript: all authors