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Widespread Genetic Heterogeneity in Multiple Myeloma: Implications for Targeted Therapy

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SUMMARY

We performed massively parallel sequencing of paired tumor/normal samples from 203 multiple myeloma (MM) patients and identified significantly mutated genes and copy number alterations and discovered putative tumor suppressor genes by determining homozygous deletions and loss of heterozygosity. We observed frequent mutations in *KRAS* (particularly in previously treated patients), *NRAS*, *BRAF*, *FAM46C, TP53*, and *DIS3* (particularly in nonhyperdiploid MM). Mutations were often present in subclonal populations, and multiple mutations within the same pathway (e.g., *KRAS*, *NRAS*, and *BRAF*) were observed in the same patient. In vitro modeling predicts only partial treatment efficacy of targeting subclonal mutations, and even growth promotion of nonmutated subclones in some cases. These results emphasize the importance of heterogeneity analysis for treatment decisions.

INTRODUCTION

We previously reported the sequencing of 38 matched tumor/normal multiple myeloma (MM) pairs, and that report of the genomic landscape of MM pointed to a number of recurrently mutated genes (e.g., *FAM46C* and *DIS3*) that are likely causal drivers of the disease (Chapman et al., 2011). However, that study design was only powered to detect *commonly* mutated genes, not less commonly mutated genes, due to the weak statistical power provided by the small sample size. It also did not examine copy number alterations, leading to homozygous deletions or loss of heterozygosity (LOH), or clonal heterogeneity due to the modest sequence coverage (~30×) of those whole genome sequences.

The identification of driver mutations in MM holds great promise for personalized medicine, whereby patients with particular mutations would be treated with the appropriate targeted therapy (Fonseca et al., 2009; Mahindra et al., 2012; Palumbo and Anderson, 2011). However, if the mutation is present in only a fraction of the cells, one might doubt whether such targeted

Significance

A vision for precision cancer medicine calls for the deployment of molecularly targeted therapeutics in genetically defined patient populations. A first step in that process involves a description of the genetic landscape of cancer. We describe here a more comprehensive characterization of the MM genome, identifying recurrently mutated genes, copy number alterations, and signaling pathways. We find evidence for extensive clonal heterogeneity in the disease, a finding that may complicate the interpretation of genome-inspired clinical trials for MM. More generally, our findings indicate a need for the delineation of clonal heterogeneity in genome-based diagnostic approaches to cancer.

therapy would be clinically efficacious. Recent studies have documented the existence of clonal heterogeneity in solid tumors and acute myeloid leukemia, albeit in small numbers of patients (Campbell et al., 2010; Carter et al., 2012; Ding et al., 2012; Gerlinger et al., 2012; Nik-Zainal et al., 2012; Shah et al., 2012; Walter et al., 2012). These studies demonstrated how acquisition of genetic alterations over time leads to clonal evolution. Systemic treatment with chemotherapy may affect the fitness of some subclones more than others, and thus may alter the tumor composition by promoting particular subclones (Landau et al., 2013b). Consequently, the full breadth of tumor heterogeneity, particularly in solid malignancies, may not be captured in a single biopsy, which represents a challenge for cancer therapy (Gerlinger et al., 2012). Clonal heterogeneity and clonal evolution have also been observed in MM by either whole-exome sequencing or array comparative genomic hybridization (CGH), albeit in a modest number of patients (Egan et al., 2012; Keats et al., 2012; Walker et al., 2012).

We therefore sought to estimate the extent of clonal heterogeneity in MM in a large-scale MM genome sequencing data set capturing a breadth of untreated and previously treated patients and to infer the timing of genetic events in MM. In the work presented here, we address several important questions: (1) can we identify significantly mutated genes by integrating evidence from both point mutations and copy number analysis, (2) how do the mutation profile and the clonal and subclonal composition of MM differ between hyperdiploid and nonhyperdiploid and between treated and untreated MM, and (3) can the contribution of subclones in a patient be reconstructed from a single biopsy to inform targeted therapy?

RESULTS

We first set out to create a MM genome data set that would be sufficiently powered to comprehensively assess the genetic diversity of the disease and the extent to which subclonal heterogeneity is observed within patients. Approval for this study was obtained as outlined in the Experimental Procedures, and a total of 203 tumor-normal pairs were analyzed: 177 by whole-exome sequencing and 26 by whole-genome sequencing (16 and 23, respectively, have been previously reported [Chapman et al., 2011]). The average depth of coverage for the whole exomes and whole genomes was 89× and 30×, respectively. To estimate the statistical significance of mutation frequency (as a measure of positive selection), we used a new version of the MutSig algorithm (MutSigCV) that compares observed mutation frequencies against sequence context-specific, tumor-specific, and gene-specific background mutation frequencies (Lawrence et al., 2013). Additionally, we developed analytical tools to further prioritize homozygous somatic single-nucleotide variants (SSNVs), or genes which harbor mutations that are positionally clustered or preferentially affecting highly conserved amino acids (see the Supplemental Experimental Procedures available online). Analysis of the 203 tumor-normal pairs showed that 11 genes were recurrently mutated using a standard significance threshold of $q < 0.1$ (Figures 1 and S1). The individual and combined p and q values for these prioritization procedures are shown in Tables S1 and S2. Mutation validation studies were performed on 140 mutations, with a validation rate of 90.4%, in line with other large-scale cancer genome sequencing studies (Table S2).

Among the 11 significantly mutated genes were five genes (*KRAS*, *NRAS*, *FAM46C*, *DIS3*, and *TP53*) previously identified as the most commonly mutated genes in our 38-patient pilot MM genome study (Chapman et al., 2011). An additional four genes (*BRAF*, *TRAF3*, *CYLD*, and *RB1*) have been implicated in the pathogenesis of MM (Annunziata et al., 2007; Chapman et al., 2011; Demchenko et al., 2010; Keats et al., 2007; Walker et al., 2012). *PRDM1* is a transcriptional repressor that is involved in plasmacytic differentiation, and it acts as a tumor suppressor gene in activated B cell-like diffuse large B cell lymphoma (DLBCL). Mutations that disrupt its function have been described in DLBCL (Mandelbaum et al., 2010), but are not known to play a role in MM. *PRDM1* has been shown to promote survival of transformed plasma cells (Lin et al., 2007), and transgenic mice prone to plasmacytoma development show reduced plasmacytoma incidence if one or two *PRDM1* alleles are knocked out (D'Costa et al., 2009). We find a recurrent missense mutation (S552C) in two patients, with two additional patients harboring closely clustered missense mutations (S605R and S606I), and an additional five patients with truncating frame shift or splice site mutations, supporting a role of *PRDM1* as a tumor suppressor (Figures 1 and S1; Tables S1 and S2).

Additionally, several recurrently mutated and biologically relevant genes fall just below the significance threshold (Table S1). For example, *EGR1* was previously shown to abrogate JUN-induced MM growth inhibition and cell death when knocked down in MM cells and has been reported as a mechanism of resistance to MM therapy (Chen et al., 2010). We found that *EGR1* mutations were clustered toward the 5′ end of the gene (Tables S1 and S2; Figure S1), a pattern of mutation often associated with somatic hypermutation (Pasqualucci et al., 2001). To further explore this possibility, we asked whether the observed mutations occurred within WRCY motifs known to be the targets of activation-induced cytidine deaminase (AID), a key enzyme that catalyzes somatic hypermutation. This analysis revealed that *EGR1* indeed had significant enrichment of mutations in WRCY motifs ($q < 0.1$; Table S3), consistent with a somatic hypermutation mechanism. Whether these mutations act as "drivers" and are positively selected or merely constitute "passengers" remains to be seen.

We also found four missense mutations in the interferon regulatory factor IRF4, with three of the mutations being identical (K123R) (Chapman et al., 2011), establishing K123R as a recurrent, "hot spot" mutation in *IRF4* (Figure S1; Table S2). *IRF4* has previously been reported as a MM survival factor, wherein a loss-of-function RNAi screen showed that *IRF4* inhibition results in loss of viability of MM cell lines (Shaffer et al., 2008). *SP140* is the lymphoid-restricted homolog of *SP100*, expressed in plasma cells, and a genome-wide association study identified *SP140* as a susceptibility locus for chronic lymphocytic leukemia (Di Bernardo et al., 2008), with risk alleles being associated with reduced levels of *SP140* mRNA. We identified missense, nonsense, frame shift, and splice site alterations in eight patients, with LOH observed for two of these alterations, consistent with its possible role as a tumor suppressor in MM.

The available clinical characteristics of the patients in the study are shown in Figure 2 and Table S4. Identification of