Germline Lysine-Specific Demethylase 1 (LSD1/KDM1A) Mutations Confer Susceptibility to Multiple Myeloma

Xiaomu Wei1,2, M. Nieves Calvo-Vidal1, Siwei Chen2, Gang Wu3, Maria V. Revuelta1, Jian Sun1, Jinghui Zhang3, Michael F. Walsh4, Kim E. Nichols3, Vijai Joseph4, Carrie Snyder5, Celine M. Vachon6, James D. McKay7, Shu-Ping Wang8, David S. Jayabal1, Lauren M. Jacobs4, Dina Becirovic5, Rosalie G. Waller9, Mykyta Artomov10, Agnes Viale4, Jayeshkumar Patel1, Jude Phillip1, Selina Chen-Kiang1, Karen Curtin9, Mohamed Salama9, Djordje Atanackovic3, Ruben Niesvizky1, Ola Landgren4, Susan L. Slager6, Lucy A. Godley11, Jane Churpek11, Judy E. Garber12, Kenneth C. Anderson12, Mark J. Daly10, Robert G. Roeder8, Charles Dumontet7, Henry T. Lynch5, Charles G. Mullighan3, Nicola J. Camp9, Kenneth Ofit4, Robert J. Klein13, Haiyuan Yu2, Leandro Cerchietti1, and Steven M. Lipkin1

Abstract

Given the frequent and largely incurable occurrence of multiple myeloma, identification of germline genetic mutations that predispose cells to multiple myeloma may provide insight into disease etiology and the developmental mechanisms of its cell of origin, the plasma cell (PC). Here, we identified familial and early-onset multiple myeloma kindreds with truncating mutations in lysine-specific demethylase 1 (LSD1/KDM1A), an epigenetic transcriptional repressor that primarily demethylates histone H3 on lysine 4 and regulates hematopoietic stem cell self-renewal. In addition, we found higher rates of germline truncating and predicted deleterious missense KDM1A mutations in patients with multiple myeloma unselected for family history compared with controls. Both monoclonal gammapathy of undetermined significance (MGUS) and multiple myeloma cells have significantly lower KDM1A transcript levels compared with normal PCs. Transcriptome analysis of multiple myeloma cells from KDM1A mutation carriers shows enrichment of pathways and MYC target genes previously associated with myeloma pathogenesis. In mice, antigen challenge followed by pharmacologic inhibition of KDM1A promoted PC expansion, enhanced secondary immune response, elicited appearance of serum paraprotein, and mediated upregulation of MYC transcriptional targets. These changes are consistent with the development of MGUS. Collectively, our findings show that KDM1A is the first autosomal-dominant multiple myeloma germline predisposition gene providing new insights into its mechanistic roles as a tumor suppressor during post-germinal center B-cell differentiation.

Significance: KDM1A is the first germline autosomal dominant predisposition gene identified in multiple myeloma and provides new insights into multiple myeloma etiology and the mechanistic role of KDM1A as a tumor suppressor during post-germinal center B-cell differentiation. Cancer Res; 78(10); 2747–59. ©2018 AACR.

Introduction

In multiple myeloma, abnormal plasma cells (PC) derived from post-germinal center B cells abnormally proliferate and produce high amounts of immunoglobulin or paraprotein, which leads to lytic bone lesions, anemia, and renal failure (1). Multiple myeloma is preceded by a premalignant condition, monoclonal gammapathy of undetermined significance (MGUS). Consistent with genetic predisposition, having a first-degree relative with multiple myeloma elevates multiple myeloma risk 2- to 5-fold (2).

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerr.es.aacrjournals.org/).
Identifying multiple myeloma predisposition genes can provide mechanistic insights into MGUS, myeloma pathogenesis, and PC differentiation. Genome-wide association studies have identified 16 common variants at loci significantly associated with multiple myeloma risk (3–8). However, the role of rare multiple myeloma high-penetrance predisposing genes is poorly understood. To identify multiple myeloma predisposition genes, we performed germline whole-exome sequencing (WES) for familial multiple myeloma probands and identified kindreds that carry germline truncating mutations in lysine (K)-specific demethylase 1 (KDM1A, also called LSD1; ref. 9). KDM1A is an epigenetic transcriptional repressor that primarily demethylates monomethylated and dimethylated histone H3 on lysine 4 (H3K4me1/me2) to repress target gene promoters and enhancers (10–12).

We used CRISPR to introduce a ‘second hit’ mutation in lymphoblastoid B cells from a germline KDM1A mutation carrier, which increased H3K4me1 levels. MGUS and multiple myeloma cells have significantly lower KDM1A transcript levels compared with normal PCs, and may be particularly sensitive to KDM1A mutations causing loss of function or haploinsufficiency. We also performed mutation burden test analysis of patients with multiple myeloma unselected for family history and controls, which showed higher rates of germline KDM1A mutations in patients with multiple myeloma. Mice treated with a KDM1A small-molecule inhibitor, GSK-LSD1, have enhanced secondary immune response with expansion of PCs, increased immunoglobulin production, and appearance of serum paraprotein. DNA sequencing and ran on Illumina HiSeq 2000s/2500s. FASTQ files were aligned to human reference genome (GRCh37) to generate BAM files using BWA v0.7.12. Picard tools were used for quality metric calculation and marking duplicate reads. GATK version 3.5-0-g36282e4 was used for variant calling using the haplotype caller algorithm. Variant quality score recalibration (VQSR) data were used for filtering variants. Variant-level and interval-level annotations used SNP@R, ANNOVAR, and CAVA programs. Downstream analysis consisted of filtering out low-quality variant calls and common variants. Average coverage depth was 80×–100×. Variants with read depth (DP) of 10 or greater and a genotype quality (GQ) score of 20 or greater were included in analyses. Variant-, exon-, and gene-level data were obtained using information from the 1000 Genomes Project, NHBLI GO Exome Sequencing Project Exome Variant Server (EVS), Exome Aggregation Consortium (ExAC), and the combined annotation dependent depletion (CADD) server (13). Deleterious variants were defined as loss-of-function (frameshift insertion or deletion, stop-gain, splice-site change) or missense variants with CADD score >15. We performed segregation analysis using either exomes from family members or targeted Sanger sequencing. Co-segregating qualifying variants in Family 1 (Fig. 1A) shared by exomes are listed in Supplementary Table S2. Exome sequencing data can be accessed at NCBI (NCBI SRR5641111).

Sanger sequencing

For the KDM1A-Sanger sequencing EA validation cohort, all coding regions of KDM1A (NM_015013) were amplified using primers available upon request. PCR amplicons were sequenced in the Applied Biosystems 3730 DNA (Applied Biosystems) Analyzer and analyzed using Mutation Surveyor (SoftGenetics) and Mutation Analyzer and analyzed using Mutation Surveyor (SoftGenetics). KDM1A mutations in probands and indicated family members (Fig. 1A) were verified by Sanger sequencing.

Cell culture

Cell culture methods used are described in Supplementary Data S1.

KDM1A knockout by CRISPR/Cas9

To generate biallelic KDM1A truncating mutations in lymphoblastoid (LCL) cells that carry the KDM1A c.805_806delAG (p.Arg269Aspfs7) heterozygous mutation, CRISPR/Cas9 gene editing sgRNA sequences were designed to target genomic sites near KDM1A c.805_806delAG (p.Arg269Aspfs7). pX458_sgRNA (5’-CACCCTCAACTTCCGATCTCATATAAG-3’, 5’-AACCTTATAGATGCAGAAGACTGAC-3’) was transfected into LCLs (Amax Nucleofector II device). GFP-expressing clones were seeded as single-cell clones in 96-well plates using flow cytometry and screened for mutations by PCR amplification, target sequencing, and Western blot analysis.

Western blot analysis and antibodies

LCL cells were harvested in lysis buffer containing complete Protease inhibitors (Roche, 4693159001). Whole-cell extracts were subjected to SDS-PAGE. Antibodies used include anti-H3K4me1 (Abcam ab8895, 1:500 dilution), anti-H3K4me2 (Abcam ab32356, 1:500 dilution), anti-H3K9me1 (Abcam ab9045, 1:500 dilution), anti-H3K9me2 (Abcam ab1220, 1:500 dilution), anti-Histone H3 (Abcam ab1791, 1:1000 dilution), anti-α-tubulin (Sigma T5192), and anti-KDM1A (Santa Cruz Biotechnology sc-398794, 1:200 dilution).

Digital droplet PCR

A Bio-Rad assay specific for detection of the KDM1A frameshift mutation was designed (forward primer: 5’-CGTCTAGTGGTCTTATCAACTT3’, reverse primer: 5’-AGAGG-TCCCTACTGTTGTT3’;
Wt probe: 5'CGGCATCTTAgGAGATAAAAACC3', mutation probe: 5'CGGCCATCTTAgGAGATAAAAACC3'. Cycling conditions were optimized for annealing/extension temperature and separation of positive from empty droplets on a QX200 ddPCR system (Bio-Rad). Technical duplicates were run. PCR reactions contain primers/probes, DNA, and digital PCR Supermix for probes (no dUTP). Reactions were partitioned into median approximately 16,000 droplets/well using QX200 droplet generator. Emulsified PCR reactions were run using cycling conditions (95°C 10 minutes; 40 cycles of 94°C 30 minutes 55°C 1 minute, 98°C 10 minutes, 4°C hold). Plates were read and analyzed with QuantaSoft software to assess the number of droplets positive for mutant DNA, wild-type (wt) DNA, both, or neither. Assay threshold sensitivity was set at 2 mutant droplets.

**Mutation burden analysis**
Exome data from 879 patients with sporadic multiple myeloma (dbGaP phs000748 and phs000348) and 2389 control

---

Figure 1.
Identification of germline KDM1A mutations in patients with familial and early-onset multiple myeloma. A, Pedigree of familial myeloma kindred carrying germline KDM1A c.805_806delAG (p.Arg269Aspfs*7) mutation. B, Pedigree of early-onset myeloma proband carrying KDM1A c.707delA (p.Gln236Hisfs*3) mutation. Last known ages, ages of death if applicable, affected status with different malignancies, and ages at diagnosis are indicated. MM, multiple myeloma; AML, acute myeloid leukemia; Colon, colon cancer; Pancreas, pancreatic cancer; Prostate, prostate cancer; +, wild-type; M1 and M2, mutations. For kindred shown in B, only the proband had a biospecimen available for testing. For this kindred, ages of diagnosis for colon, kidney, and prostate cancers in family members could not be rigorously confirmed and so are not included. C, Somatic LOH in germline and myeloma cells from probands III:6 and II:4 in family carrying KDM1A c.805_806delAG (p.Arg269Aspfs*7) using digital droplet PCR. Ratio of KDM1A c.805_806delAG (p.Arg269Aspfs*7) mutant:wild-type allele for germline and myeloma cells is indicated with mean. *, P < 0.01 by Mann–Whitney test. N = 3 and 4, respectively. Mean and SEM are shown. D and E, Western blot and densitometry analysis showing KDM1A null (-/-) LCLs (generated from family member III:6 from the kindred shown in Fig. 1A) have undetectable KDM1A and increased H3K4 monomethylation (H3K4me1:H3 ratio). H3 dimethylated isoform (H3K4me2), H3, and tubulin are shown for comparison. Heterozygote and compound heterozygote LCLs were made from Family 1 III:6 (lanes 1 and 2) and wild-type LCLs were made from Family 1 II:3, III:4 and III:5 (lanes 3–5). Densitometry represents three independent measurements from each of the LCL cell lines. Mean and SEM are shown. *, P = 0.015 by two-tailed Student t test. LCL, B lymphoblastoid cell line.
subjects (phs000179, phs000276, phs000403, phs000687, and phs000806) were simultaneously processed and variants jointly called. Exomes from cases/controls were required to have \( \geq 75\% \) of the 33.27 Mbps of CCDS with \( > 10 \) -fold coverage and \( < 3\% \) contamination using VerifyBamID (14). Exomes from cryptically related individuals were removed using KING algorithm (15).

Principal component analysis (PCA) was performed using SMARTPCA (16) to identify EA. EA exomes meeting these quality control metrics were selected (733 patients with sporadic multiple myeloma and 1,480 controls). We compared the sum of (i) predicted deleterious mutation or (ii) neutral synonymous coding variant qualifying carriers for each gene in multiple myeloma cases and controls using Fisher exact test (statistical function scipy.stats.fisher_exact in Scipy). We compared the sum of (i) predicted deleterious mutation or (ii) neutral synonymous coding variant qualifying carriers for each gene in multiple myeloma cases and controls using Fisher exact test (statistical function scipy.stats.fisher_exact in Scipy).

Multiple myeloma organoids

Peg-Mal (polyethylene glycol-malate; AA peptides) hydrogels with REDW (AA peptides) peptide ligands were impregnated with 30,000 U266 myeloma cells and 5,000 HSS nonproliferating (mitomycin-treated) stromal cells, or 30,000 mononucleated cells from a primary bone marrow biopsy sample as previously described (17) with the addition of VLA-4/Integrin \( \alpha 4 \) ligand fibronectin (18). After gelation, hydrogel 3D organoids were rehydrated with media and cultured as described previously (17, 19). A detailed protocol for multiple myeloma organoid culture is available upon request. U266 organoids untreated or treated with 1 \( \mu \)mol/L LSD1 inhibitor (GSK-LSD1) were followed by microscopy. Cells were harvested and counted by measuring ATP content/well (CellTiter Glo, Promega) at days 0, 1, 3, 5, and 7. Three to five replications were used per organoid treatment condition. For the primary bone marrow biopsy sample, after 6 days in culture, 2 organoids per experimental condition were mechanically digested and single cells assessed for PC markers CD138 and p63 using flow cytometry, and growth assessed at the same time point for 3 organoid datapoints per treatment condition using CellTiter Glo. To assess proliferation in a bone marrow sample of a patient with multiple myeloma in remission, mononuclear cells were preloaded with CellTrace Violet (Thermo Fisher Scientific).

RNA-seq analysis

For mouse PCs, polyadenylated single-read RNA-seq was performed using standard Illumina Truseq protocols and run at 3/ lane on HiSeq 2500. Reads were trimmed of adapters with Flexbar then aligned to mm10 mouse genome (20). Uniquely mapped reads were used for read counts. Raw read counts were processed in R with DESeq2 (21) and accessed at NCBI (NCBI GEO GSE85956). Gene set enrichment analysis (GSEA) scores were calculated as the negative \( \log _{10} \) of the false discovery rate (FDR) multiplied by the sign of the log, fold change (log FC). Scores were run against preranked GSEA Hallmarks (weighted score type, 1,000 permutations; ref. 22) or Boylan multiple myeloma gene sets (23). Differentially expressed genes (DEG) were grabbed as a volcano plot in R.

For analysis of MMRF CoMMpass RNA-seq multiple myeloma transcriptomes from KDM1A mutation carriers and patients with wt multiple myeloma, raw counts (19) were analyzed in R with DESeq2 (20). Multiple myeloma RNA-seq data were available from 4 KDM1A mutation carriers (MMRF_1201, 1730, 2293, and 2068). RNA-seq data from these 4 tumors were compared against 4 randomly picked multiple myeloma tumors from the 799 nonmutation carriers and run for 1,000 iterations. Significantly up- or downregulated genes \((P_{adj} < 0.05)\) were compared against mouse GSK-LSD1–treated PCs. Outputs with >5% frequency were input for pathway enrichment in ClueGO (23), using GO Biological Process, Kyoto Encyclopedia of Genes and Genomes, REACTOME, and Wikipathways ontologies. Two-sided hypergeometric test was used to calculate \( P \) values, followed by Bonferroni correction. Pathways with \( P_{adj} < 0.05 \) were considered significant. The complete lists of genes and pathways are given in Supplementary Table S3.

For analysis of hypodiploid acute lymphoblastic leukemia (ALL) gene expression data (24), we compared samples with truncating KDM1A mutations (SHYP0021 and SHYP0032) to 50 KDM1A wt hypodiploid ALL tumors and used GSEA \((n = 1,000 \) permutations) to test for enrichment of HoxA9 and LSK gene sets (25).

Mouse immunization and GSK-LSD1 treatment

CS7BL/6 male mice aged 11 weeks \((n = 18)\) were immunized intraperitoneally with 100 \( \mu \)g NP-CGG in alum on day 0 and day 21. On day 1, intraperitoneal treatment with either vehicle (PBS) or GSK-LSD1 (Cayman Chemical \#16439; 0.5 mg/kg/day) started and continued until day 45. On days 10 and 35, mice were bled. On day 45, mice were sacrificed, and heart blood, femurs, and spleens were collected and analyzed.

Cell sorting and FACS

Mouse spleens were processed into single-cell suspensions, pooled in groups of 3–4, and washed before resuspending Ficoll-isolated mononuclear cells in PBS containing 0.5% BSA and antibodies B220-PECy7 (eBioscience, #25-0452-82) and CD138-APC (BD Pharmingen #558626). After one wash, 20 million cells/ml were resuspended and DAPI (1 \( \mu \)g/mL) added to exclude dead cells. Cells collected were B220" CD138" (B cells) and CD138" (PCs), and were >95% pure. Bone marrow samples extracted from mouse femurs and spleen mononuclear cells were also analyzed by FACS with B220, CD138, CD3, CD11b, and Gr1 antibodies.

NP-immunoglobulin ELISA and serum electrophoresis

Day 35 retro-orbital blood sera were serially diluted, incubated overnight in 96-well plates precoated with NP (4)-CGG or NP (27)-CGG (Biosearch, #N-5055A and N-5055C), and preblocked with PBS with 1% BSA. After three washes with 0.05% Tween-20 PBS, horseradish peroxidase–conjugated antibodies against IgG1, IgG2a, IgG2b, IgG3, IgM, or IgA (SouthernBiotech,
Figure 2.
KDM1A germline mutations in patients with multiple myeloma. A, Schematic of KDM1A protein. Germline mutations from patients with multiple myeloma are shown on top. B and C, Quantile–quantile plots of gene-based burden tests for rare neutral synonymous variants (B) and rare predicted deleterious mutations (C). D, Quantile–quantile plot of multiple myeloma case and control common variants. Lambda statistics are shown. E, KDM1A mRNA levels in normal plasma cells (NPC), MGUS, and multiple myeloma cells. *, $P = 7.45 \times 10^{-5}$ (NPC-MGUS) and $1.70 \times 10^{-5}$ (NPC-MM), respectively, moderated $t$ test. Mean and SEM bars are indicated.
KDM1A bands. This identifies multiple myeloma early-onset (II:4 and III:6) of the kindred carrying the truncating mutation. Three were multiple myeloma affected (II:4, III:6, and III:7), one (III:3) with acute myelogenous leukemia (AML) and MGUS, and one (III:2) with MGUS. One sibling was affected with colorectal cancer, a half-sibling with renal cell cancer, and one uncle with prostate cancer.

CADD scoring is a widely used computational tool to predict whether germline variants are benign or deleterious (13). The higher a CADD score, the higher the likelihood that a genetic variant is deleterious. Using CADD >15 threshold we identified two predicted rare deleterious KDM1A missense mutations in the Tower and AO domains, (KDM1A c.1424T>C [p.Leu475Pro] and c.2003G>C [p.Arg692Pro]) in patients with multiple myeloma with the first-degree relatives also affected with multiple myeloma/MGUS. Collectively, these data confirm the presence of rare truncating and predicted deleterious missense KDM1A mutations in familial and patients with early-onset multiple myeloma.

To understand the prevalence of KDM1A truncating mutations in controls, we examined the ExAC database. Including all ethnicities only 9 predicted truncating and splice-site loss-of-function (LoF) variants were identified, carried by 10 individuals (10/60,694; LoF constraint metric pLI = 0.99). Seven of these are of EA. All KDM1A truncating mutations had mean allele frequencies <1.6 × 10⁻⁵ and no homozygotes were identified. Joint analysis of LoF mutations in familial and early-onset multiple myeloma cases (2/450) and ExAC EA participants as controls (7/36,664), showed enrichment of KDM1A LoF mutations among patients with familial and early-onset myeloma (P = 0.005, Fisher exact test; RR, 23.28; 95% confidence interval (CI), 4.85–111.75). Overall, despite possible small mismatch in mean age and the precise definitions of our multiple myeloma probands and ExAC database assignments of EA, our data provide evidence of KDM1A LoF mutation enrichment in familial and early-onset multiple myeloma.

Results
Germline truncating KDM1A mutations in familial multiple myeloma

Germline exome sequencing of 50 familial multiple myeloma kindreds (Supplementary Table S1) revealed EA family carrying a N-terminal truncating KDM1A mutation (c.805_806delAG [p.Arg269Aspfs1]; Fig. 1A) absent in ClinVar or Exome Aggregation Consortium (ExAC) databases. This was the only kindred with a qualifying truncating mutation (Supplementary Table S2). Therefore, we focused follow-up on this family. Sanger sequencing identified five relatives carrying this KDM1A mutation. Three were multiple myeloma affected (II:4, III:6, and II:7), one (II:3) with both acute myelogenous leukemia (AML) and MGUS, and one unaffected at age 48 (III:1; Fig. 1A). Three additional family members were diagnosed with pancreatic II:3, II:2, and colon cancers II:1).

KDM1A is composed of three major domains: SWIRM and Tower, which mediate protein–protein interactions, and a C-terminal amine oxidase (AO) domain (26). The truncating KDM1A c.805_806delAG [p.Arg269Aspfs1] mutation eliminates the AO and Tower domains. Western blot analysis of LCL derived from patient III:6 showed normal levels of full-length KDM1A and no truncated protein. RT-PCR and Sanger sequencing of cDNA revealed the absence of KDM1A-mutant transcripts.

Identification of germline truncating KDM1A mutations in early-onset multiple myeloma

We next sequenced KDM1A exons in 400 additional EA multiple myeloma early-onset (<age 60) or familial myeloma probands. This identified another EA patient with early-onset multiple myeloma (age 59) and a truncating KDM1A mutation (KDM1A c.707delA [p.Gln236Hisfs3]) in the SWIRM domain (Fig. 1B). No other family members were diagnosed with multiple myeloma/MGUS. One sibling was affected with colorectal cancer, a half-sibling with renal cell cancer, and one uncle with prostate cancer.

CADD scoring is a widely used computational tool to predict whether germline variants are benign or deleterious (13). The higher a CADD score, the higher the likelihood that a genetic variant is deleterious. Using CADD >15 threshold we identified two predicted rare deleterious KDM1A missense mutations in the Tower and AO domains, (KDM1A c.1424T>C [p.Leu475Pro] and c.2003G>C [p.Arg692Pro]) in patients with multiple myeloma with the first-degree relatives also affected with multiple myeloma/MGUS. Collectively, these data confirm the presence of rare truncating and predicted deleterious missense KDM1A mutations in familial and patients with early-onset multiple myeloma.

Digital droplet PCR analysis of germline KDM1A-mutant multiple myeloma cells

Myeloma cells from bone marrow biopsies were available for two members (II:4 and III:6) of the kindred carrying the KDM1A c.805_806delAG (p.Arg269Aspfs1) mutation. Digital droplet PCR (ddPCR) was performed on DNA isolated from multiple myeloma cells and normal bone marrow. The KDM1A mutation was enriched in multiple myeloma cells from deletion of the wt allele compared with germline bone marrow cells carrying the heterozygous germline mutation in both family members tested (both P < 0.01, Mann–Whitney test [Fig. 1C]). Next, we generated LCLs from members of this kindred carrying KDM1A heterozygous or wt genotypes. We used CRISPR/Cas9 gene editing technology to introduce a second hit on the wt allele and an LCL cell line was derived carrying biallelic KDM1A truncating mutations [[c.805_806delAG (p.Arg269Aspfs1)] and c.801_802insT (p.Lys268*)]. Western blot analysis of KDM1A biallelic (−/−), heterozygous (+/−), and wt (+/+)) LCLs revealed that heterozygous and wt LCLs had similar KDM1A protein levels, but that biallelic mutant LCLs had no detectable KDM1A protein (Fig. 1D). Accordingly, KDM1A−/− LCLs have significantly higher overall cellular levels of H3K4me1, whereas total H3K4me2, H3K9me1, and H3K9me2 levels were not significantly changed (Fig. 1D and E).

Increased KDM1A mutations in sporadic patients with multiple myeloma

We performed gene burden tests using jointly called germline exomes from EA patients with multiple myeloma unselected for
family history and EA controls (Fig. 2A). Exomes were matched for quality metrics as before (27, 28), and KING algorithm used to exclude individuals with cryptic relatedness (15). PCA showed no apparent population stratification between EA multiple myeloma cases/controls (Supplementary Fig. S1). The distribution of neutral synonymous coding variants showed no significant difference in the number of genes carrying neutral synonymous coding variants in multiple myeloma cases (N = 733; yellow, average 17.55 ± 0.26/gene) and controls (N = 1480; blue, average 17.52 ± 0.18/gene; Mann–Whitney U test, P = 0.91; Supplementary Fig. S1). We used Fisher exact test (28, 29) to generate a quantile plot comparing neutral synonymous coding variant and predicted deleterious coding mutation gene burden tests. We also used Fisher exact test (28, 29) to generate a quantile–quantile plot comparing multiple myeloma case and control common variant (MAF ≥ 5%) (Table 1). The rate of predicted deleterious missense mutations and LoF/truncating mutations (P = 1.27 × 10^{-3}) in patients with multiple myeloma compared with controls (1.97 × 0.14%; Table 1).

Among patients with multiple myeloma, we detected another patient (56 years old) with early-onset multiple myeloma with an exon 2 splice-donor LoF/truncation mutation KDM1A c.517+1G>A, p.(Glu19Trpfs’15), but no LoF/truncation mutation in controls. The myeloma tumor from this patient carried a somatic deletion of encompassing the entire KDM1A locus (chr1:830,800-120,666,600). In addition, screening for predicted deleterious KDM1A missense mutations with CADD scores >15 identified 8 patients with multiple myeloma with heterozygous-predicted deleterious missense mutations and 2 non-multiple myeloma control subjects (1.23% LoF/misssense mutations in patients with multiple myeloma and 0.14% LoF/miissense mutations in controls; RR, 9.08; 95% CI, 1.97–41.95; Table 1). The rate of predicted KDM1A LoF and missense mutations in EA controls is similar to the ExAC database (0.22%; 81/36,664). However, none of the 7 tumors from patients with multiple myeloma carrying KDM1A missense mutations whose tumor somatic mutation data were available demonstrated loss of heterozygosity (LOH; refs. 30).

We analyzed published transcriptome databases containing gene expression from normal PCs, MGUS, and multiple myeloma (Supplementary Table S3; refs. 34–37). KDM1A inhibition also promoted IgM to IgG2b isotype class-switching, a process where B cells activated upon antigen encounter are costimulated by Th cells (Fig. 3D). The increase in immunoglobulin production could be detected by serum electrophoresis as an increased gamma globulin fraction (Fig. 3E). These findings are consonant with a model whereby KDM1A activity is required to control abnormal generation and/or expansion of PC.

KDM1A pharmacologic inhibition promotes expansion of multiple myeloma and PCs

To test a role of KDM1A in post-germinal center B-cell differentiation, we conducted antigen-driven B-cell differentiation in vivo experiments. To induce differentiation of B cells into PCs, we challenged immunologically mature C57BL/6 mice with T-cell–dependent antigens on day 1 and boosted at day 21. In one group of mice, we used sheep red blood cells (SRBC) as antigen and in another group NP-chicken globulin (NP-CCG), which allows quantification of total and high-affinity immunoglobulins. KDM1A was inhibited with GSK-LSD1, an irreversible KDM1A inhibitor (Fig. 3A) with IC_{50} of 16 nmol/L that shows >1,000 × selectivity for KDM1A over closely related FAD utilizing enzymes including LSD2, MAOA, and B. Mice were randomized to receive vehicle (PBS) or GSK-LSD1 0.5 mg/kg daily by intraperitoneal injection (Fig. 3B). Mice were monitored for primary (day 10) and secondary (day 35) antibody immune responses, and sacrificed (day 45).

Phenocopying the Kdm1a knockout mouse models (25, 32), GSK-LSD1 significantly decreased neutrophils and increased monocytosis in bone marrow (Fig. 3C). Antigen-challenged mice treated with GSK-LSD1 showed increased splenic PCs (Fig. 3C). Analysis of serum total and high-affinity anti-NP antibody production revealed that KDM1A inhibition promoted secondary immune response to the T-cell–dependent antigen NP-CCG (Fig. 3D). KDM1A inhibition also promoted IgM to IgG2b isotype class-switching, a process where B cells activated upon antigen encounter are costimulated by Th cells (Fig. 3D). The increase in immunoglobulin production could be detected by serum electrophoresis as an increased gamma globulin fraction (Fig. 3E). These findings are consonant with a model whereby KDM1A activity is required to control abnormal generation and/or expansion of PC.

KDM1A inhibition promotes in vivo PC upregulation of MYC transcriptional targets

To characterize the transcriptional consequences of KDM1A inhibition in PCs, we performed RNA-sequencing of DEG in sorted PC (CD138^-) from GSK-LSD1 treated and control mice. To functionally categorize these genes, we conducted pathway analysis using GSEA (33) and identified "MYC targets (v1)" as a significant pathway associated with upregulated genes (Fig. 4A, P = 0.0, FDR = 0.0). To evaluate mouse genes dysregulated by Kdm1a inhibition in PCs with development of multiple myeloma, we compared DEG in PCs from GSK-LSD1 treated versus control mice with Bcl-xL/Myc transgenic mouse multiple myeloma cells (23). GSEA showed enrichment of genes upregulated in Bcl-xL/Myc transgenic mouse multiple myeloma cells in GSK-LSD1–treated PC DEG (normalized enrichment score 1.64, P = 0.0, FDR 0.008; Fig. 4A), including Myc-driven multiple myeloma oncogenes Ccnd1 and Ccnd2 (Supplementary Table S3; refs. 34–36). Thus, Kdm1a pharmacologic inhibition enriched for upregulated MYC transcriptional targets including Ccnd1 and Ccnd2 as potential drivers of PC proliferation.

Table 1. Predicted deleterious KDM1A mutation frequencies in multiple myeloma probands and control subjects

<table>
<thead>
<tr>
<th>Probands</th>
<th>Deleterious</th>
<th>Benign</th>
<th>Total</th>
<th>Mutation burden test (Fisher exact test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple myeloma</td>
<td>9 (1.23%)</td>
<td>724 (98.77%)</td>
<td>733</td>
<td>P = 1.27 × 10^{-3}</td>
</tr>
<tr>
<td>Control subjects</td>
<td>2 (0.14%)</td>
<td>1,478 (99.86%)</td>
<td>1,480</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>2,202</td>
<td>2,213</td>
<td></td>
</tr>
</tbody>
</table>
KDM1A pharmacologic inhibition in mice promotes secondary immune response, immunoglobulin class switching, and serum paraprotein. 

A, Chemical structure of KDM1A inhibitor GSK-LSD1. 

B, Schedule of administration to mice and immune response elicited by antigen NP-CGG. 

C, Kdm1a inhibition recapitulates Kdm1a−/− mouse phenotype, with increased monocytes and reduced neutrophils in bone marrow (BM; left), and causes increased splenic PCs in the secondary immune response (right). At least $n = 9$ per condition. *, $P < 0.05$; **, $P < 0.01$, Mann–Whitney test. Mean and SEM are shown. 

D, Kdm1a inhibition promotes NP-specific IgG2b antibodies production (high-affinity antibodies that detect the less haptenated antigen NP-4) and total antibodies that detect the highly haptenated antigen NP-27). At least $n = 5$ per condition. *, $P < 0.05$; **, $P < 0.01$, Mann–Whitney test. Mean and SEM are shown. 

E, Gamma globulin fraction in serum electrophoresis of same mice at day 45. $n = 10$ per condition. *, $P < 0.05$, Mann–Whitney test. Mean and SEM are shown.
Figure 4. KDM1A inhibition in mouse plasma cells and germline KDM1A mutations in patients with multiple myeloma alters gene expression. A, GSEA of differentially expressed genes in plasma cells from mice treated with GSK-LSD1 in MYC v1 MSigDB Hallmark (top) and Bcl-xL/Myc transgenic mouse multiple myeloma (bottom) gene sets. NES, normalized enrichment score. B, Volcano plot of differentially expressed genes in sorted plasma cells from mice treated with GSK-LSD1. Labels (human nomenclature) indicate genes differentially regulated in common for both GSK-LSD1-treated mouse PCs and multiple myeloma tumors from KDM1A germline predicted mutation carriers. C, ClueGO analysis pathways enriched in DEGs comparing multiple myeloma tumors from KDM1A germline mutation carriers to those from wild-type patients. Pathways with adjusted $P_{adj} < 0.05$ are shown. Circles, pathways. Size represents the percentage of genes shared with a pathway. Red, upregulated gene pathways; blue, downregulated gene pathways. Edges (lines) indicate shared genes between pathways.
Increased CCND2 and alterations in other pathways in multiple myeloma cells from KDM1A mutation carriers

To determine the impact of germline KDM1A mutations on patient multiple myeloma tumors, we compared multiple myeloma tumor transcriptomes from patients with KDM1A-predicted deleterious mutations to those from KDM1A wt patients. First, we evaluated shared DEG common to both (i) GSK-LSD1–treated PCs and (ii) multiple myeloma cells from KDM1A mutation carriers versus wt patients. Uregulated shared DEG included CCND2 (Fig. 4B). Among downregulated shared DEG were the transcription factor KLF4 that has tumor suppressor functions in multiple myeloma (37) and PRDM11, a tumor suppressor in MYC-driven B-lymphomagenesis (Fig. 4B; ref. 38). Thus, CCND2, KLF4, and PRDM11 are potential transcriptional targets downstream of KDM1A whose dysregulation promotes MGUS and multiple myeloma in patients.

We then analyzed DEG in multiple myeloma tumors from KDM1A mutation carriers compared with wt patients by ClueGO pathway analysis (Fig. 4C; ref. 39). Pathways related to myelomagenesis enriched in upregulated genes include Immunoglobulin Production, Antigen Processing, and Presentation (40), IL10 signaling (41), ERK Signaling Cascade (42). Vasculature...
Development (43), and Extracellular Matrix Organization (44), while in downregulated genes, the TGFβ signaling pathway was enriched (Fig. 4C). This included SMAD1, which suppresses MYC-dependent transcription in multiple myeloma cells (45). Altogether, these data reveal enrichment in pathways associated with both intrinsic multiple myeloma pathogenesis and multiple myeloma–bone marrow microenvironment interactions in KDM1A mutation carriers.

To directly test whether KDM1A affects myeloma cell proliferation, we treated human myeloma cells with GSK-LSD1 in 3D organoids with extracellular matrix–like biophysical properties that resemble the microenvironment of lymphoid tissues (17, 19). When U266 multiple myeloma cell line (Fig. 5A) or primary sporadic multiple myeloma patient cell (Fig. 5B and C) organoids were treated with GSK-LSD1, KDM1A inhibition significantly stimulated multiple myeloma cell number (P < 0.001 and <0.01, t test). In contrast, GSK-LSD1 treatment did not affect proliferation of nonmultiple myeloma CD138+ bone marrow cells as controls (Fig. 5D and E). These data support roles for KDM1A regulating PC differentiation to prevent MGUS and multiple myeloma development, and also to promote multiple myeloma proliferation.

Discussion

MGUS and multiple myeloma risk is increased in relatives of affected individuals (2). We used whole-exome sequencing of familial myeloma kindreds, mutation cosegregation in family members, somatic tumor studies, CRISPR-induced somatic second-hit mutations, targeted sequencing of additional patients with familial and early-onset multiple myeloma, mutation burden analysis in patients with multiple myeloma unselected for family history, multiple myeloma tumor transcriptome studies and functional studies of a KDM1A inhibitor on human multiple myeloma cells, such as increased CCND2 and reduced KLF4 and PRDM11 (37, 38). In line with multiple myeloma organoid findings, tumor transcriptomes from KDM1A-mutant patients are enriched in pathways associated with both intrinsic multiple myeloma pathogenesis and extrinsic multiple myeloma–bone marrow microenvironment interactions. These data are consistent with KDM1A inhibition/mutation acting at distinct steps to promote both PC immune response and multiple myeloma proliferation by dysregulating both cell-intrinsic (including MYC driven) and extrinsic microenvironment signaling pathways.

We identified both KDM1A germline truncating and missense mutation carriers (Fig. 2). Somatic multiple myeloma tumor analyses performed with two members of our index family carrying a KDM1A truncating mutation (Fig. 1) showed evidence of possible LOH. However, this finding may be by chance, as chromosome 1p deletion is frequent in myeloma (1, 42). Of seven additional KDM1A predicted germline mutation carriers with somatic multiple myeloma mutation data, only one patient with multiple myeloma tumor showed somatic LOH. The other 6 patients with multiple myeloma without tumor LOH carried germline missense mutations. KDM1A is a component of the NuRD and CoRest chromatin remodeling complexes, and participates in multiple protein–protein interactions with distinct histone demethylase activities (10–12). It is possible that KDM1A missense mutations act through a dominant negative mechanism in PC NuRD or CoRest complexes to promote MGUS/multiple myeloma predisposition. Alternatively, because MGUS and multiple myeloma cells both have significantly lower KDM1A levels compared with normal PCs (Fig. 2E), they may be particularly vulnerable to epigenetic dysregulation from KDM1A hypomorphic missense mutations and haploinsufficiency.

Scales and colleagues recently reported a multiple myeloma exome mutation burden test study in 513 United Kingdom northern EA multiple myeloma cases from two clinical trials (48). While no individual gene reached genome-wide significance in this study, the authors nominated KIF18A as a promising multiple myeloma predisposition candidate. However, in our EA multiple myeloma mutation burden study, we did not see an association between KIF18A and multiple myeloma (Fisher exact test, P = 0.787). Furthermore, the study of Scales and colleagues did not nominate KDM1A as multiple myeloma predisposition gene. A likely reason for these different findings is that Scales and colleagues used variant MAF <1% while our study used MAF ≤0.05% to define rare candidate mutations. Thus, each study used different inputs for mutation burden testing. Additional potential reasons include methodologic differences in variant annotation and mutation burden testing pipelines, possible differences in EA population substructure (American vs. UK patients with multiple myeloma), and different rates of clinical covariates for patients with multiple myeloma and their tumor molecular subtypes.

Bolli and colleagues performed exome sequencing of a large, multiplex MM/MGUS kindred (49). The authors nominated EP300 and PDK1, but not KDM1A, as candidate MGUS/multiple myeloma predisposition genes. However, in contrast to the KDM1A mutation carrying kindreds we identified (Fig. 1A and B), this family did not have non-multiple myeloma/MGUS cancers. Thus, additional MGUS/multiple myeloma specific high-risk predisposition genes may exist.
Recently, recurrent somatic KDM1A mutations were identified in chronic myeloid leukemia (50). We also identified one patient with a germline truncating KDM1A mutation diagnosed with MGUS and AML (Fig. 1A). In mice, Kdm1a knockdown causes monocytosis (25, 32). However, KDM1A inhibition was found to induce terminal differentiation of AML cell lines and several primary AML cultures (51). On the basis of this, KDM1A small-molecule inhibitors are used in clinical trials for AML and other conditions. Our study of germline KDM1A mutations supports individuals being treated with KDM1A inhibitors being monitored for MGUS/multiple myeloma.

We also surveyed germline data from patients with pediatric hypodiploid ALL (24). This revealed a germline KDM1A truncating mutation c.1368_1371delAGAA (p.Lys456Asn*12) in a patient with near-haploid hypodiploid ALL whose tumor demonstrated LOH and another patient with near-haploid hypodiploid ALL, whose tumor harbored a somatic KDM1A truncating mutation c.1249C>T (p.Gln417*, MAF 83%). Mouse Kdm1a−/− LSK hematopoietic stem-progenitor cells have upregulated HOXA9 and LSK leukemia stem cell signatures (25). Reanalysis of KDM1A-mutant and wt hypodiploid ALL tumor transcriptomes revealed that KDM1A mutations enriched for these same leukemia stem cell signatures (Supplementary Fig. S2; ref. 25). In addition, query of germline sequence data from The Cancer Genome Atlas revealed germline truncating and splice-site KDM1A mutations in 3 patients with globlastoma multiforme (KDM1A c.237C>T [p.Gln791*]), glioma, and ovarian serous cystadenocarcinoma (both KDM1A c.1476+1_1476+2delGT [p. Met448_Lys492del]). Thus, our data are consistent with KDM1A mutations contributing to predisposition of other tumors in addition to multiple myeloma.

Disclosure of Potential Conflicts of Interest

K.E. Nichols reports receiving commercial research grants from Inbyte and Alpine Biosciences. L.A. Godley has provided expert testimony for UpToDate, Inc. J.E. Garber reports receiving commercial research support from Novartis Pharmaceuticals, is a consultant/advisory board member for Novartis Pharmaceuticals, CTC Pharmaceuticals, and Helix Genetics, and has provided expert testimony for OriC Pharmaceuticals. C.G. Mullighan reports receiving commercial research grants from Loxo Oncology, Pfizer, and Abbvie, has received speakers bureau honoraria from Amgen, and is a consultant/advisory board member for Genome Quebec and Cancer Science Institute. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions

Conception and design: X. Wei, M.N. Calvo-Vidal, V. Joseph, C.M. Vachon, S. Chen-Kiang, M. Salama, N.J. Camp, R.J. Klein, L. Cerchietti, S.M. Lipkin

Development of methodology: X. Wei, M.N. Calvo-Vidal, J. Zhang, M.F. Walsh, K. Curtin, R.J. Klein, H. Yu, L. Cerchietti


Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): X. Wei, S.-P. Wang, D.S. Jayabalavan, L.M. Jacobs, R.G. Waller, D. Atanackovic, O. Landgren, R.G. Roeder, N.J. Camp

Study supervision: X. Wei, N.J. Camp, H. Yu, L. Cerchietti, S.M. Lipkin

Acknowledgments

We acknowledge funding from R01 CA167824, R01 CA134646, R01 CA178765, T15 LM007124, R21 CA152336, L51 606-07, NCI P30 CA42041, HHSN261201000026C, the V Foundation (V2015-003), the Well-Cornell Program in Mendelian Genetics, the Utah Genome Project, Huntsman Cancer Institute, Utah Population Database (UPDB), the Utah Cancer Registry (IHC), Icahn School of Medicine at Mount Sinai Office of Research Infrastructure of the NIH award number 5R01DK110522, and a generous donation from Matthew Bell.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received July 5, 2017; revised November 7, 2017; accepted March 16, 2018; published first March 20, 2018.


