**LETTER TO THE EDITOR**

**WDR26 and MTF2 are therapeutic targets in multiple myeloma**

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**Abstract**

Unbiased genetic forward screening using retroviral insertional mutagenesis in a genetically engineered mouse model of human multiple myeloma may further our understanding of the genetic pathways that govern neoplastic plasma cell development. To evaluate this hypothesis, we performed a tumor induction study in MYC-transgenic mice infected as neonates with the Moloney-derived murine leukemia virus, MOL4070LTR. Next-generation DNA sequencing of proviral genomic integration sites yielded rank-ordered candidate tumor progression genes that accelerated plasma cell neoplasia in mice. Rigorous clinical and biological validation of these genes led to the discovery of two novel myeloma genes: **WDR26** (WD repeat-containing protein 26) and **MTF2** (metal response element binding transcription factor 2). WDR26, a core component of the carboxy-terminal to LisH (CTLH) complex, is overexpressed or mutated in solid cancers. MTF2, an ancillary subunit of the polycomb repressive complex 2 (PRC2), is a close functional relative of PHD finger protein 19 (PHF19) which is currently emerging as an important driver of myeloma. These findings underline the utility of genetic forward screens in mice for uncovering novel blood cancer genes and suggest that WDR26-CTLH and MTF2-PRC2 are promising molecular targets for new approaches to myeloma treatment and prevention.

**Keywords:** Forward genetic screen, Moloney murine leukemia virus, Plasma cell neoplasia, Carboxy-terminal to LisH (CTLH) complex, Polycomb repressive complex 2 (PRC2)

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To the Editor,

Multiple myeloma (MM) is a common blood cancer derived from terminally differentiated B-lymphocytes called plasma cells (PCs). Despite recent advancements in treatment options, MM remains incurable in the great majority of cases, with no more than half of patients surviving past 5 years [1]. Reasons for poor outcome include tumor heterogeneity and severe limitations in our knowledge base on genetic pathways that drive neoplastic PC development from an early progenitor stage to frank malignancy. Unbiased genetic forward screening using proviral insertional mutagenesis [2] in a dedicated mouse model of human myeloma may lend itself to attacking this knowledge gap. Here, we employ this approach, for the first time, to discover two candidate genes that may yield new opportunities for molecularly targeted myeloma treatments: **WDR26** (WD repeat-containing protein 26) and **MTF2** (metal response element binding transcription factor 2).

Our experimental strategy for detecting presumptive therapeutic targets in MM is depicted in Fig. 1a. The first step was a tumor induction study in iMyc<sup>Cre<sup>Eyu</sup></sup> mice, a gene-insertion model of the chromosomal T(12;15) translocation that results in deregulated expression of Myc in B-lineage cells [3]. Because T(12;15) is a tumor-initiating event in mouse plasmacytoma [4] and
upregulation of MYC is a well-established mechanism of tumor progression in human myeloma [5], the iMycΔEµ transgene served as an ideal “sensitizer” for skewing the oncogenic potency of the murine leukemia virus (MuLV), MOL4070LTR, to plasmablasts and PCs. MOL4070LTR is a modified Moloney-MuLV that contains the LTR U3 enhancer region from the amphotropic MuLV, 4070A [6]. Infection of newborn iMycΔEµ mice with MOL4070LTR resulted in accelerated tumor development (Fig. 1b): 51 of 68 (75%) virus-treated mice developed tumors by 210 days of age, whereas less than a quarter of untreated mice demonstrated malignant growth by 505 days. Histopathological tumor classification relied on immunostaining for T cell (CD3), B cell (Pax5, B220) and PC (CD138) markers to assign tumor-bearing mice with virus-accelerated neoplasms to the B-lineage (31%) or T-lineage (44%). A quarter of mice (25%) contained both B- and T-cell tumors (Fig. 1c). From all mice carrying B-lineage tumors (n = 21), eight individual tumor samples (spleen plus peripheral and deep lymph nodes) were collected on average. Most tumors were categorized as plasmacytoma (Fig. 1d, left) or plasmablastic lymphoma (Fig. 1d, right) in accordance with the Bethesda proposal of lymphoid tumors in mice [7]. A total of 168 tumor specimens were analyzed for common retroviral insertion sites (CIS) as depicted in Additional file 1: Fig. S1. From nearly half a million mapped sequence reads, approximately 45 thousand proviral integration events were extracted. To unequivocally identify CIS, we used a biocomputational algorithm based on Monte Carlo statistics that considered both the number of independent integration sites in a given DNA window and the distance between the sites. We defined a CIS as the minimum genomic region in which 5 to 7 unique insertions were found to be significant at p < 0.05, provided that no more than two insertions were derived from the same tumor. CIS windows ranged from 10 to 40 kb, corresponding to the size of the transcriptional unit of the average mouse gene (~30 kb). A total of 171 CIS-tagged candidate genes were identified and rank ordered according to proviral insertion frequency. The top 100 genes are shown in Fig. 1e. Included are many genes one might have expected in a forward genetic screen of neoplastic PC development; e.g., Ccnd2 on Chr 6, Hras on Chr 7 and Myc on Chr 15.

Bioinformatics analysis of the top 100 genes using STRING (string-db.org) demonstrated their tight association with the oncogenic MYC network (Fig. 1f). KEGG analysis (www.kegg.jp) revealed significant enrichment in cancer-relevant pathways including blood cancers such as AML and CML (Fig. 1g). GO analysis of biological processes (geneontology.org) demonstrated strong enrichment in pathways of hematopoiesis, hematopoietic or lymphoid organ development, and regulation of leukocyte differentiation (Fig. 1h). These results underscored the relevance of the top 100 genes for MM and encouraged us to narrow them down to the most promising candidates. This process began with two steps denoted “Filter 1” in Fig. 1a, top right. The first step asked the question whether upregulation of the human orthologs of the top 100 mouse genes predicted to be upregulated by proviral insertion might be associated with inferior survival in human myeloma. We chose the MMRF CoMMpass study to test for associations of gene expression and survival because this study evaluates outcomes in over

**Fig. 1** Discovery of WDR26 and MTF2 in unbiased genetic forward screen using Myc-transgenic mice. a Schema of workflow that led to the nomination of WDR26 and MTF2 as candidate myeloma genes. Filters used to pare down the list of 100 input genes to 2 candidate genes are indicated on the right. b Accelerated tumor development in iMycΔEµ gene-insertion mice treated with MOL4070LTR (mean tumor onset 178 ± 94 days; range 46–348 days) compared to mice not infected with virus (mean tumor onset 384 ± 86 days; range 245–505 days). Virus was injected IP (5 × 10⁴ colony forming units/10 µL) using a 30-gauge needle. c Tumor pattern in virus-infected mice from a stained according to hematoxylin and eosin (H&E) and immunostained using antibody to CD138, respectively. d Ideogrammatic representation of mouse autosomes plus chromosome X indicating the genomic location of the top 100 candidate B cell and plasma cell tumor genes detected. Genes that passed Filters 1, 2 and 3 in a are labeled using orange, green and red dots, respectively. Thin red or blue lines denote whether CRIS mapping predicts increased or decreased gene expression due to proviral insertion. f Network of MYC-interacting proteins visualized by STRING (Search Tool for the Retrieval of Interacting Genes). Proteins (n = 27) that interact with MYC directly or indirectly are depicted in red or blue, respectively. The minimum required interaction score was 0.5. Red and black lines within the network circle denote direct and indirect interactions with MYC, respectively. The symbols for WDR26 and MTF2 are enlarged for enhanced visibility. Network visualization relied on Cytoscape 3.8.2. g KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway analysis of the top 100 candidate genes. Enrichment scores are denoted by ovals which indicate both the number of pathway genes involved (count) and level of statistical significance (blue saturation). h GO (Gene Ontology) term enrichment analysis of biological processes using the top 100 candidate genes from a as input. i Magnitude of RNAi-dependent knockdown (KD) of WDR26 and MTF2 expression in three different HMCLs relative to HMCLs transfected with scrambled message (control). j Programmed cell death in HMCLs exhibiting low WDR26 or MTF2 expression (KD) compared to HMCLs cells containing normal message levels (control). k Growth inhibition (PrestoBlue™) of HMCLs harboring low levels of WDR26 or MTF2 message. Genes were knocked down using Mission EHU esiRNAs 150,671 (WDR26) and 042,951 (MTF2).
Infection of newborn mice with MOL4070LTR

C.iMycΔEµ

Tumor induction and classification

Mapping of CIS in B cell & PC tumors

Oncogenomic analysis of candidate genes

Biological validation of candidate genes

WDR26 & MTF2

n = 100

n = 22

n = 8

n = 2

Infection

Infected with MuLV

n = 67

Not infected

n = 68

T and B cell

25%

T cell

44%

B cell

31%

Fig. 1 (See legend on previous page.)
Clinical and biological validation of WDR26 and MTF2 in myeloma. (A) WDR26 (top) and MTF2 (bottom) expression levels (GSE 2658 and 5900 datasets) in bone marrow plasma cells from healthy individuals (BMPC, n = 22) or patients with monoclonal gammopathy of undetermined significance (MGUS, n = 44), smoldering myeloma (SMM, n = 12) or frank myeloma (FM, n = 559). MM data are from GSE2658, all others from GSE5900. (B) Comparison of mean mRNA levels of WDR26 (top) or MTF2 (bottom) in patients with standard-risk myeloma (SR, n = 690) or high-risk myeloma (HR, n = 287), using data from the Multiple Myeloma DREAM Challenge study. (C) Overall survival (OS) of patients with myeloma in the MMRF CoMMpass study stratified according to WDR26 (top) or MTF2 (bottom) message levels in malignant plasma cells. The top quartile (n = 194, red) and bottom quartile (n = 194, blue) are compared. HR, hazard ratio. (D) Western analysis of WDR26 (left) or MTF2 (right) in normal (N) or gene-targeted (KO) myeloma cells, OPM2, H929 and MM1.S. KO protocols including gRNA sequences are available upon request. (E) Growth of HMCLs in bulk suspension culture. Cells deficient in WDR26 (blue) or MTF2 (red) are compared to parental cells (black) used as control. F) Clonogenic growth of OPM2 (top), H929 (center) and MM1.S cells (bottom) lacking WDR26 (blue) or MTF2 (red) or containing the proteins (black). Representative images of soft-agar plates are shown to the left. The bar diagram to the right displays mean colony numbers ± SD based on three independent experiments. G) Representative flow cytometric scatter plots of apoptotic death (red, labeled rectangles) of WDR26 or MTF2 deficient HMCLs compared to normal cells. H) Mean values of apoptosis based on three independent measurements. Standard deviations are indicated by short vertical lines (**p < 0.001). I) Bioluminescence images of NSG mice on days 10, 20, 30 and 40 following challenge with OPM2 cells (upper panel) or H929 cells (lower panel) deficient of WDR26 (center column) or MTF2 (right column). Parental cells proficient of these proteins served as control (left column). J) Quantitative analysis of bioluminescence signal strength in mice from h. K) Kaplan–Meier survival curves of mice depicted in h. L) Representative images of secondary tumors in foot pads of CD45.1 host mice xenografted with WDR26- or MTF2-deficient H929 cells (center) or wild type H929 cells (right). M) Growth of HMCLs in xenografts in SCID mice xenografted with WDR26- or MTF2-deficient OPM2 cells (blue) or parental OPM2 cells (red) as control. N) Growth of HMCLs in multicellular tumor spheroids. The bar graph to the right displays mean colony numbers ± SD based on three independent experiments. O) Bioluminescence images of NSG mice on days 10, 20, 30 and 40 following challenge with OPM2 cells (upper panel) or H929 cells (lower panel) deficient of WDR26 (center column) or MTF2 (right column). Parental cells proficient of these proteins served as control (left column). P) Flow cytometry histogram distinguishing GFP-expressing tumor cells in the bone marrow of xenotransplanted mice from h (smaller peaks, right) from bone marrow cells not expressing GFP (larger peaks, left). Q) Abundance of GFP-expressing tumor cells in the bone marrow of mice from h.
Fig. 2 (See legend on previous page.)
Abbreviations
AAD: 7-Aminoactinomycin D; AML: Acute myeloid leukemia; CML: Chronic myeloid leukemia; CI: Common retrieval insertion site; CTH: Carboxy-terminal to LisH; GFP: Green fluorescent protein; HMCL(s): Human myeloma cell line(s); LTR: Long terminal repeat; MM: Multiple myeloma; MMRF: Multiple Myeloma Research Foundation; MOL4070LTR: Moloney derived MuLV; MT2F: Metal response element binding transcription factor 2; MuLV: Murine leukemia virus; NGS: Next-generation sequencing; PC(s): Plasma cell(s); PRC2: Polycomb repressive complex 2; WDR26: WD repeat-containing protein 26.

Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s13045-021-01217-9.

Additional file 1. Fig. S1. Identification of proviral integration sites and candidate driver genes. Genomic DNA was extracted from malignant tissues harvested from MOL4070LTR-infected mice. Approximately 1 μg of genomic DNA was then digested using either Msel or Nalll. Next, 200 ng of digested DNA was ligated to double-stranded adaptors (Nalll linker: 5’-GTA ATA CGA CTC ACT AGG CGG CTC CGG TTA AGG GAC CAT GTG-3’ and 5’-Phos-GTC CCT TAA GCG GAG-3’; Msel linker: 5’-GTA ATA CGA CTC ACT AGG CGG CTC CGG TTA AGG GAC CAT GTG-3’) and the proviral LTR (5’-GCT AGC TTG CCA AAC AAC CAG GTG G3’). PCR products were diluted 1:50 in sterile water. Two microliters of diluted PCR product was re-amplified (secondary PCR) using nested primers annealing to the adaptor (5’-AGG GCT CCG CTT AAG GGA C-3’) and the proviral LTR (5’-GCT AGC TTG CCA AAC AAC CAG GTG CG3’). Amplicons from the second round of PCR were purified to remove unincorporated primers and nucleotides and directly sequenced on an Illumina platform. Raw sequences were trimmed to remove adaptors and viral sequences and mapped to the mouse reference genome. Candidate driver genes were identified using Monte Carlo simulation as previously described (PMID: 21931803).

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Authors’ contributions
FS and YC performed in vitro and in vivo studies, carried out bioinformatic and statistical analyses, designed the figures, and wrote the manuscript. AD and JDR performed NGS studies and determined CIS. WD performed the tumor induction study, harvested and shipped tissue specimens, and took care of animal husbandry. MP performed PubMed searches and edited the manuscript. JD contributed to experimental procedures and data analytical approaches. BM and FZ provided infrastructure support and understanding of genetic and biological pathways of mouse plasmacytoma and human myeloma. PH provided infrastructure support and insights into myeloma treatment and prevention. SJ conceived and supervised the study and edited the figures and the manuscript. All authors contributed to reading and approved the final version of the manuscript.

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Availability of data and materials
Detailed information on materials and methods used, including KD and KO primer sequences, are available from the corresponding author upon request.

Declarations
Ethics approval and consent to participate
Studies involving laboratory mice were approved by the institutional IACUC and performed in accordance with prevailing guidelines for the welfare and use of animals in cancer research. Because analysis of clinical data did not contain any personally identifiable information from any sources and did not require approval of the Institutional Review Board, informed consent is not applicable.

Consent for publication
Not applicable.

Competing interest
The authors declare no conflict of interest.

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References
1 van de Donk N, Pawlyn C, Yong KL. Multiple myeloma. Lancet. 2021;397:410–27.
2 Ranzani M, Annunziato S, Adams DJ, Montini E. Cancer gene discovery: exploiting insertional mutagenesis. Mol Cancer Res. 2013;11:141–58.
3 Duncan K, Roseant TR, Tompkins VS, Olivier A, Sompallae R, Zhan F, Tricot G, Acevedo MR, Ponto LL, Walsh SA, et al. (18)F-FDG-PET/CT imaging in an IL-6- and MYC-driven mouse model of human multiple myeloma affords objective evaluation of plasma cell tumor progression and therapeutic response to the proteasome inhibitor bortezomib. Blood Cancer J. 2013;3:e165.
4 Janz S, Muller J, Shaugnessy N, Potter M. Detection of recombination between c-myc and immunoglobulin switch alpha in murine plasma cell tumors and preneoplastic lesions by polymerase chain reaction. Proc Natl Acad Sci USA. 1993;90:7361–5.
5 Misund K, Keane N, Stein CK, Asmann YW, Day G, Welsh S, Van Wier SA, Riggs DL, Ahmann G, Chesnari M, et al. MYC disreulgation in the progression of multiple myeloma. Leukemia. 2020;34:322–6.
6 Wolff L, Roller R, Hu X, Anver MR. A Moloney murine leukemia virus-based retrovirus with 4070A long terminal repeat sequences induces a high incidence of myeloid as well as lymphoid neoplasms. J Virol. 2003;77:4965–71.
7 Morse HC 3rd, Anver MR, Firkedson TN, Haines DC, Harris AW, Harris NL, Jaffe ES, Kogan SC, MacLennan IC, Pattengale PK, Ward JM. Bethesda proposals for classification of lymphoid neoplasms in mice. Blood. 2002;100:246–58.
8 Mason MJ, Schinke C, Eng CLP, Towfic F, Gruber F, Dervan A, White BS, Pratapa A, Guan Y, Chen H, et al. Multiple Myeloma DREAM-Challenge reveals epigenetic regulator PHF19 as marker of aggressive disease. Leukemia. 2020;34:1866–74.
9. Huffman N, Palmieri D, Coppola V. The CTLH complex in cancer cell plasticity. J Oncol. 2019;2019:4216750.

10. Napoli M, Li X, Ackerman HD, Deshpande AA, Barannikov I, Pisegna MA, Bedrosian I, Mitsch J, Quirlian P, Thompson A, et al. Pan-cancer analysis reveals TAp63-regulated oncogenic lncRNAs that promote cancer progression through AKT activation. Nat Commun. 2020;11:5156.

11. Piunti A, Shilatifard A. The roles of Polycomb repressive complexes in mammalian development and cancer. Nat Rev Mol Cell Biol. 2021;22:326–45.

12. Maganti HB, Jirade H, Cafariello C, Manias-Rothberg JL, Porter CJ, Yockell-Lelieevre J, Battaion HL, Khan ST, Howard JP, Li Y, et al. Targeting the MTF2-MDM2 axis sensitizes refractory acute myeloid leukemia to chemotherapy. Cancer Discov. 2018;8:1376–89.

13. Ren Z, Ahn JH, Liu H, Tsai YH, Bhanu NV, Koss B, Allison DF, Ma A, Storey AJ, Wang P, et al. PHF19 promotes multiple myeloma tumorigenicity through PRC2 activation and broad H3K27me3 domain formation. Blood. 2019;134:1176–89.

14. Boyde EM, Rosenthal A, Ghamlouch H, Wang Y, Farmer P, Rutherford M, Ashby C, Bauer M, Johnson SK, Wardell CP, et al. 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. Leukemia. 2021 (Online ahead of print).

15. Schinke CD, Bird JT, Qu P, Yaccoybo S, Lyzogubov VV, Shelton R, Ling W, Boyde EM, Deshpande S, Byrum SD, et al. PHF19 inhibition as a therapeutic target in multiple myeloma. Curr Res Transl Med. 2021;69:103290.

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