Low frequency mutations in ribosomal proteins RPL10 and RPL5 in multiple myeloma

Isabel J.F. Hofman,[1] Stephanie Patchett,[2] Mark van Duin,[3] Ellen Geerdens,[4,5] Jelle Verbeeck,[1] Lucienne Michaux,[6] Michel Delforge,[7] Pieter Sonneveld,[3] Arlen W. Johnson[2] and Kim De Keersmaecker[1]

[1]Department of Oncology, KU Leuven - University of Leuven, LKI - Leuven Cancer Institute, Belgium; [2]Department of Molecular Biosciences, The University of Texas at Austin, TX, USA; [3]Department of Hematology, Erasmus MC Cancer Institute, Rotterdam, the Netherlands; [4]Center for Cancer Biology, VIB, Leuven, Belgium; [5]Center for Human Genetics, KU Leuven - University of Leuven, LKI - Leuven Cancer Institute, Belgium; [6]Center for Human Genetics, KU Leuven - University Hospitals Leuven, Belgium and [7]LKI - Leuven Cancer Institute, Department of Development and Regeneration, KU Leuven - University Hospitals Leuven, Belgium

Correspondence: kim.dekeersmaecker@med.kuleuven.be
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SUPPLEMENTARY MATERIAL

LETTER TO THE EDITOR

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Isabel JF Hofman[1], Stephanie Patchett[2], Mark van Duin[3], Ellen Geerdens[4,5], Jelle Verbeeck[1], Lucienne Michaux[6], Michel Delforge[7], Pieter Sonneveld[3] , Arlen W. Johnson[2], Kim De Keersmaecker[1]

[1] Department of Oncology, KU Leuven - University of Leuven, LKI - Leuven Cancer Institute, Leuven, Belgium

[2] Department of Molecular Biosciences, The University of Texas at Austin, Austin, TX, USA

[3] Department of Hematology, Erasmus MC Cancer Institute, Rotterdam, The Netherlands

[4] Center for Cancer Biology, VIB, Leuven, Belgium

[5] Center for Human Genetics, KU Leuven - University of Leuven, LKI - Leuven Cancer Institute, Leuven, Belgium

[6] Center for Human Genetics, KU Leuven - University Hospitals Leuven, Leuven, Belgium

[7] LKI - Leuven Cancer Institute, Department of Development and Regeneration, KU Leuven - University Hospitals Leuven, Leuven, Belgium

SUPPLEMENTAL MATERIALS AND METHODS

Patient samples

We studied 75 diagnostic MM bone marrow samples from UZ Leuven (Leuven, Belgium) and 66 diagnostic MM bone marrow samples from the Erasmus Medical Center (Erasmus MC) (Rotterdam, The Netherlands). All analyzed samples contained at least 70% plasma cells (UZ Leuven range: 70-100%, median 79%; EMC Rotterdam range: 78-100%, median 92%; Supplementary table S1). For the UZ Leuven cohort, no sample purification was done and purity was determined by morphological assessment of a bone marrow aspirate. Diagnostic samples from the EMC Rotterdam cohort were purified using CD138 magnetic microbeads (Miltenyi Biotec). Purity was analyzed by performing flow cytometry for the CD138 marker (CD138-PE; Beckman Coulter). Percentages of CD138 cells in analyzed samples are reported in supplementary Table 1. This study was approved by the ethics committees of the institutes involved and informed consent was obtained from the participants. Samples and clinical data were stored in accordance with the declaration of Helsinki. The public cohorts we included in our analysis ("Chapman cohort"[1] and "Lohr cohort"[2]) were a mix of diagnostic and treated patients.

Haloplex resequencing

We analyzed 37 cases using a custom-designed Haloplex (Agilent technologies) sequence enrichment assay that captures the coding sequences of the 81 ribosomal genes as well as a set of leukemia implicated genes followed by Illumina massive parallel sequencing of the library (Supplementary table S2). Sequencing data are available at EGA (accession number EGAS00001002405). Data analysis was performed using NextGENe software (v2.2.1, Softgenetics, State College, PA, USA), performing the following steps: (i) the fastQ output file was converted into a FASTA file to eliminate reads that were not "paired" and that did not meet the criteria of the default settings; (ii) reads from the converted unique FASTA file were aligned to the reference genome (Human_v37.2). After alignment a *.pjt file was created and opened in the NextGENe Viewer; (iii) a mutation report was created using the coordinates from the targeted enrichment kit as a *.bed file to enable calling of single nucleotide variants and small insertion/deletions (indels) in the regions of interest. For variant calling, we required a minimum read depth of 20 and an allele frequency of at least 3%. Detected variants were confirmed by Sanger sequencing on diagnostic material and were tested for their somatic origin on germline DNA if available.

Sanger sequencing

The entire coding sequence of the RPL10 gene was PCR amplified and Sanger sequenced in diagnostic material from all 75 UZ Leuven cases and on whole genome amplified material from the 66 cases from Erasmus MC. Analysis of Sanger chromatograms was performed using CLC Main Workbench (CLC Bio, Aarhus, Denmark). Detected variants were confirmed on original, non-amplified material and were tested for their somatic origin on germline DNA if available. Primers used for Sanger sequencing are listed in Supplementary table S3.

Yeast experiments

Appropriate codons of yeast (*Saccharomyces cerevisiae*) Rpl10 in the centromeric LEU2 vector pAJ2522 were changed by site-specific mutagenesis. Wild-type and mutant were then introduced into the rpl10 deletion strain AJY1437 (MATα rpl10::KanMX lysΔ0 met15Δ0 his3Δ0 leu2Δ0 ura3Δ0 pAJ392 - RPL10 URA3 CEN) by plasmid shuffle and assayed for growth by plating ten-fold serial dilutions onto selective medium. Polysome profiles were analyzed as described. (Klinge, Science, 2011) Wild-type and mutant RPL10 were introduced into AJY1837, containing a glucose repressible RPL10 gene (GAL-RPL10), the leptomycin-B-sensitive allele of CRM1-T539C and NMD3-GFP, and AJY2766, containing GAL-RPL10 and TIF6-GFP. Cultures were grown in selective medium containing galactose. Glucose was added to repress expression of wild-type genomic RPL10 for 2 hours. Images were captured using a Nikon E800 microscope fitted with a 100X Plan Apo objective and a Photometrics CoolSNAP ES camera controlled by NIS-Elements AR 2.10 software. Images were prepared using Adobe Photoshop 7.0.

Table S1 Patient information

UZ Leuven cohort (no CD138 purification)				
MM	**Date**	**Plasma cells in BM (%)**	**Translocation**	**Hyperdiploidy**
MM01	29/02/2012	70	t(11;14)	No
MM02	26/08/2011	70	No	No
MM03	4/01/2012	90	t(11;14)	No
MM04	22/04/2011	77	t(11;14)	No
MM05	8/11/2011	80	t(11;14)	No
MM06	21/09/2011	75	Anomaly at 14q32 but no confirmed translocation	No
MM07	30/03/2011	75	No	Yes
MM08	11/03/2011	80	No	Yes
MM09	3/02/2011	74	t(11;14)	No
MM10	22/02/2011	83	t(11;14)	No
MM11	25/05/2011	84	No	Yes
MM12	27/05/2011	81	No	Yes
MM13	1/06/2011	70	t(11;14)	No
MM14	15/06/2011	77	t(11;14)	Yes
MM15	19/10/2011	76	No	No
MM16	31/10/2011	71	t(14;20)	No
MM17	21/02/2012	86	No	Yes
MM18	15/03/2012	91	t(6;14)	No
MM19	9/02/2012	70	No	Yes
MM20	9/02/2012	79	t(11;14)	No
MM21	7/12/2011	72	No	No
MM22	10/11/2011	72	t(14q32) IGH translocation with unknown partner	No
MM23	12/10/2010	95	t(14;16)	No
MM24	15/01/2010	80	No	Yes
MM26	8/01/2010	79	t(14;16)	No
MM27	18/11/2009	99	No	Yes
MM28	8/12/2011	100	No	Yes
MM29	22/04/2010	85	t(4;14)	No
MM30	22/12/2009	78	No	Yes
MM31	24/06/2010	82	t(11;14)	No
MM32	4/01/2010	87	t(11;14)	No
MM33	23/11/2010	81	No	Yes
MM34	10/11/2009	80	No	Yes
MM35	23/12/2011	78	No	Yes
MM36	16/11/2010	78	No	Yes
MM37	11/06/2010	70	No	Yes
MM38	19/08/2010	75	t(11;14)	No

UZ Leuven cohort (no CD138 purification)	
Minimal plasma cell % in BM	70
Maximal plasma cell % in BM	100
Median plasma cell % in BM	79
EMC Rotterdam (CD138 purified)	
Minimal plasma cell % in purified sample	78
Maximal plasma cell % in purified sample	100
Median plasma cell % in purified sample	92

Table S2 Haloplex design

Target regions	1976
Total size	404921 bp
Coverage	99,10%

Gene	**Source**
ABL1	CCDS35165.1, CCDS35166.1
AKAP6	CCDS9644.1
AKT1	CCDS9994.1
ARPP21	NM_001025068, NM_001025069, NM_016300, NM_198399
BCL11B	CCDS9949.1, CCDS9950.1
BMS1	CCDS7199.1
BRAF	CCDS5863.1
CDKN2A	CCDS6510.1, CCDS6511.1, CCDS34998.1
CDKN2B	CCDS6512.1, CCDS6513.1
CNOT3	CCDS12880.1
CT47B1	CCDS48161.1
CTCF	CCDS10841.1
DCLRE1C	CCDS31149.1, CCDS7105.1, CCDS31150.1
DNM2	CCDS32908.1, CCDS45968.1, CCDS32907.1, CCDS45969.1
DNMT3A	CCDS1718.2, CCDS33157.1, CCDS46232.1
DRG1	CCDS13897.1
DUSP12	CCDS1234.1
ECT2L	CCDS43508.1
EED	CCDS8274.1, CCDS8273.1
EFTUD1	CCDS42070.1, CCDS42071.1
EIF2A	CCDS46935.1
EIF6	CCDS13250.1, CCDS13249.1
EP300	CCDS14010.1
EPDR1	CCDS5454.1
ETV6	CCDS8643.1
EZH2	CCDS5892.1, CCDS5891.1
FAT1	CCDS47177.1
FAT2	CCDS4317.1
FAT3	CCDS44706.1
FAT4	CCDS3732.3
FAU	CCDS8095.1
FBXW7	CCDS3778.1, CCDS34078.1, CCDS3777.1
FLT3	CCDS31953.1
GATA3	CCDS7083.1, CCDS31143.1
GRID2	CCDS3637.1
GTPBP4	CCDS31132.1
HIST1H1B	CCDS4635.1
HMCN1	CCDS30956.1
HNRNPA1	CCDS44909.1, CCDS41793.1
HNRNPR	CCDS232.1, CCDS44085.1
IDH1	CCDS2381.1

IDH2	CCDS10359.1
IGF1R	CCDS10378.1
IKZF1	NM_006060
IL7R	CCDS3911.1
JAK1	CCDS41346.1
JAK2	CCDS6457.1
JAK3	CCDS12366.1
JAKMIP2	CCDS4285.1
KDM6A	CCDS14265.1
KRAS	CCDS8702.1, CCDS8703.1
LCK	CCDS359.1
LEF1	CCDS47122.1, CCDS3679.1, CCDS47123.1
LPHN2	CCDS689.1
LSG1	CCDS33922.1
MAGEC3	CCDS14676.1, CCDS14677.1
MLH3	CCDS32123.1, CCDS9837.1
MRTO4	CCDS191.1
MTMR8	CCDS14379.1
MYB	CCDS5174.1, CCDS47482.1, CCDS47481.1
NIP7	CCDS10877.1
NMD3	CCDS3194.1
NOTCH1	CCDS43905.1
NPM1	CCDS4376.1, CCDS43399.1, CCDS4377.1
NRAS	CCDS877.1
ODZ2	NM_001122679
PAX5	CCDS6607.1
PHF6	CCDS14639.1, CCDS14640.1
PIK3CA	CCDS43171.1
PKHD1L1	CCDS47911.1
PTCH1	CCDS47995.1, CCDS43851.1, CCDS47996.1, CCDS6714.1
PTEN	NM_000314
PTPN11	CCDS9163.1
PTPN2	CCDS11864.1, CCDS11863.1, CCDS11865.1
PTPRC	CCDS1397.1, CCDS1399.1, CCDS1398.1, CCDS44291.1
RB1	CCDS31973.1
RELN	CCDS47680.1, CCDS34722.1
RPL10	CCDS14746.1
RPL10A	CCDS4806.1
RPL10L	CCDS32071.1
RPL11	CCDS238.1
RPL12	CCDS6872.1
RPL13	CCDS10979.1
RPL13A	CCDS12768.1
RPL14	CCDS33739.1, CCDS43070.1
RPL15	CCDS2640.1
RPL17	CCDS45865.1
RPL18	CCDS12726.1
RPL18A	CCDS12367.1

RPL19	CCDS42312.1
RPL21	CCDS9320.1
RPL22	CCDS58.1
RPL23	CCDS11330.1
RPL23A	CCDS11241.1
RPL24	CCDS33809.1
RPL26	CCDS11142.1
RPL26L1	CCDS4382.1
RPL27	CCDS11449.1
RPL27A	CCDS7790.1
RPL28	CCDS46189.1, CCDS46190.1, CCDS46192.1, CCDS46191.1, CCDS12924.1
RPL29	CCDS2845.1
RPL3	CCDS13988.1
RPL30	CCDS34928.1
RPL31	CCDS46374.1, CCDS2049.1, CCDS46373.1
RPL32	CCDS2614.1
RPL34	CCDS3680.1
RPL35	CCDS6858.1
RPL35A	CCDS33930.1
RPL36	CCDS12147.1
RPL36A	CCDS14483.1
RPL36AL	CCDS9689.1
RPL37	CCDS3934.1
RPL37A	CCDS2404.1
RPL38	CCDS11696.1
RPL39	CCDS14586.1
RPL39L	CCDS3286.1
RPL3L	CCDS10450.1
RPL4	CCDS10218.1
RPL41	CCDS44919.1
RPL5	CCDS741.1
RPL6	CCDS9162.1
RPL7	CCDS6212.1
RPL7A	CCDS6965.1
RPL7L1	CCDS4873.1
RPL8	CCDS6433.1
RPL9	CCDS3452.1
RPLP0	CCDS9193.1
RPLP1	CCDS10234.1, CCDS10233.1
RPLP2	CCDS7717.1
RPS10	CCDS4792.1
RPS11	CCDS12769.1
RPS12	CCDS5164.1
RPS13	CCDS7823.1
RPS14	CCDS4307.1
RPS15	CCDS12067.1
RPS15A	CCDS10571.1
RPS16	CCDS12535.1

RPS17	CCDS10320.1
RPS18	CCDS4771.1
RPS19	CCDS12588.1
RPS2	CCDS10452.1
RPS20	CCDS6163.1
RPS21	CCDS13497.1
RPS23	CCDS47241.1
RPS24	CCDS7355.1, CCDS44443.1, CCDS7356.1
RPS25	CCDS8406.1
RPS26	CCDS31832.1
RPS27	CCDS1059.1
RPS27A	CCDS33202.1
RPS27L	CCDS42048.1
RPS28	CCDS45953.1
RPS29	CCDS32072.1, CCDS9685.1
RPS3	CCDS8236.1
RPS3A	CCDS3775.1
RPS4X	CCDS14418.1
RPS4Y1	CCDS14773.1
RPS4Y2	CCDS44028.1
RPS5	CCDS12978.1
RPS6	CCDS6492.1
RPS7	CCDS1648.1
RPS8	CCDS513.1
RPS9	CCDS12884.1
RPSA	CCDS2686.1
RSL24D1	CCDS10152.1
RUNX1	CCDS13639.1, CCDS42922.1, CCDS46646.1
SBDS	CCDS5537.1
SETD2	CCDS2749.2
SH2B3	CCDS9153.1
SUZ12	CCDS11270.1
TBP	CCDS5315.1
TDRD6	CCDS34470.1
TET1	CCDS7281.1
TET2	CCDS47120.1, CCDS3666.1
TET3	CCDS46339.1
TLR1	CCDS33973.1
TP53	CCDS11118.1, CCDS45605.1, CCDS45606.1
TSR1	CCDS32525.1
UBA52	CCDS12382.1
UNC5D	CCDS6093.2
USP9X	CCDS43930.1
WT1	CCDS7877.2, CCDS7878.2, CCDS44562.1, CCDS44561.1

Table S3 Sanger sequencing primers

Gene	Exon	Forward	Reverse
RPL10	Exon 1	TTGCTGGTTCTCACACCTTTT	ATTGTTTTCAGCGGCCATAG
	Exon 2-3	AAAGTGCCTGTTGGGCTTT	AGTGTATGTGGGTGGGGTTG
	Exon 4	ACTCAGCCAACACAGTTCCC	CAGACCAAGCTCACCTGTCA
	Exon 5-6	AGTGTACGCAGCCTGTTGGT	GGGGCTGCAGCACTACATAC
RPLP0	Exon 7	CAGGAGGGTGGTGGGTAATA	AAGTTGGTTGCTTTTTGGTGA
RPL5	Exon 1	GACTTGGTCGAGGTGCAGT	CCGCACTCAGGCTGTCTAC
RPL3L	Exon 8	GGATCCCCACACTTGATGTT	AGCTCCAGCTCCCTACTCG

Supplementary references

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