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

Genomic screening studies recently revealed that mutations in ribosomal protein (RP) genes represent a novel class of defects in cancer. In T-cell acute lymphoblastic leukemia (T-ALL), 20% of children harbor acquired mutations and deletions in RPL10 (uL16 in the new nomenclature[1]), RPL5 (uL18) and RPL22 (eL22), 3 proteins of the large 60S ribosomal subunit.[2,3] Strikingly, 7.9% of pediatric T-ALL patients carried the same RPL10 R98S missense mutation.[2] Somatic mutations in RPs are not confined to T-ALL. *RPL5* is mutated in 11-34% of glioblastoma, melanoma and breast cancer samples, and 10-20% of chronic lymphocytic leukemia samples have *RPS15* mutations.[4,5,6] The plasma cell malignancy multiple myeloma (MM) is an attractive candidate for harboring RP mutations: initial genome sequencing revealed that half of the patients carry mutations in genes that may be functionally linked to protein translation, and we recently described that *RPL5* is in a 58 kb minimal deleted region on 1p22 that is deleted in ≥20% of MM cases.[7,8] In the study herein, the integration of published sequencing data, targeted resequencing of all 81 RP genes in a cohort of 37 MM cases and Sanger sequencing of *RPL10* in 141 MM cases revealed rare somatic defects in *RPL5* and *RPL10*. Interestingly, the *RPL10* mutations clustered in a different region as compared to the described mutational hotspot in T-ALL.[2]

Initiating events of MM consist of translocations involving the IgG locus or hyperdiploidy of uneven chro-

Table 1. RP mutations across different cohorts.

Gene status	Sample	Genomic mutation	AA change	Sanger	Somatic	VAF	Known variant	SIFT score	PolyPhen score	Patient status
RPL10 mutants										
UZ Leuven cohort										
RPL10	MM10	g.chrX:153628163T>G	p.I70M	Confirmed	Yes	20%	No	deleterious (0)	probably damaging (0.97)	Diagnostic
RPL10	T44	g.chrX:153627842A>G	p.I33V	Confirmed	Yes	NA	No	deleterious (0.05)	benign (0.02)	Diagnostic
Chapman cohort										
RPL10	MM-0282	g.chrX:153628150A>G	p.E66G	NA	Yes	76%	No	deleterious (0)	possibly damaging (0.8)	Treated
RPL10	MM-0347	g.chrX:153628161A>C	p.I70L	NA	Yes	93%	COSM3034228	deleterious (0)	possibly damaging (0.5)	Diagnostic
Lohr cohort										
RPL10	MM-0191	g.chrX:153628161A>C	p.I70L	NA	Yes	3%	COSM3034228	deleterious (0)	possibly damaging (0.5)	Treated
RPL10	MM-0516	g.chrX:153628163T>G	p.I70M	NA	Yes	7%	No	deleterious (0)	probably damaging (0.97)	Treated
RPL10	MM-0524	g.chrX:153627842A>G	p.I33V	NA	Yes	9%	No	deleterious (0.05)	benign (0.02)	Treated
Other RP mutants										
UZ Leuven cohort										
RPLP0	MM07	g.chr12:120637269G>A	p.P25L	Confirmed	Yes	53%	No	tolerated (0.8)	possibly damaging (0.9)	Diagnostic
RPL5	MM09	g.chr1:93297677G>C	Splice Site	Confirmed	Yes	48%	rs200628272, CS100830	NA	NA	Diagnostic
RPL3L	MM14	g.chr16:1995913C>T	p.G324R	Confirmed	Yes	90%	rs375754739	deleterious (0)	probably damaging (0.9)	Diagnostic
RPL29	MM14	g.chr3:52027797G>C	p.R150G	NA	NA	20%	rs754268159, COSM340672	tolerated (1)	benign (0)	Diagnostic
RPL29	MM15	g.chr3:52027781G>T	p.T155K	NA	NA	23%	No	tolerated (0.06)	possibly damaging (0.8)	Diagnostic
Lohr cohort										
RPL5	MM-0465	g.chr1:93298955A>G	p.K5E	NA	Yes	41%	COSM2153192, COSM3493393	deleterious (0)	benign (0.3)	Diagnostic
RPL26L1	MM-0512	g.chr5:172395544G>A	p.R84Q	NA	Yes	15%	rs375645667	tolerated (0.05)	benign (0.02)	NA
RPL27	MM-0624	g.chr17:41151975G>A	p.R36H	NA	Yes	39%	rs776186138, COSM979717	tolerated (0.3)	benign (0.003)	Treated
RPL36	MM-0571	g.chr19:5691445T>G	p.L70R	NA	Yes	29%	No	tolerated (0.2)	probably damaging (0.98)	Diagnostic
RPL36AL	MM-0329	g.chr14:50085527C>T	p.R99K	NA	Yes	5%	No	tolerated (0.3)	benign (0.006)	Treated
RPL3L	MM-0423	g.chr16:2002974A>C	p.V89G	NA	Yes	5%	No	damaging (0)	probably damaging (0.98)	Treated
RPL4	MM-0533	g.chr15:66791990G>T	p.H347N	NA	Yes	72%	No	tolerated (0.5)	benign (0.006)	Diagnostic
RPL6	MM-0528	g.chr12:112844637_112844638insT	p.K131fs	NA	Yes	25%	No	NA	NA	Diagnostic
RPS11	MM-0499	g.chr19:50000463delT	p.Y10fs	NA	Yes	36%	No	NA	NA	Treated
RPS12	MM-0508	g.chr6:133137642G>T	p.E58D	NA	Yes	8%	rs767922042	deleterious (0.02)	benign (0.01)	Treated
RPS16	MM-0485	g.chr19:39924389C>T	p.V55I	NA	Yes	11%	No	tolerated (0.7)	benign (0)	Treated
RPS24	MM-0571	g.chr10:79795145G>A	p.M13I	NA	Yes	40%	No	tolerated (0.2)	benign (0.2)	Diagnostic
RPSA	MM-0637	g.chr3:39452456G>A	p.R155H	NA	Yes	32%	No	tolerated (0.3)	benign (0.007)	Diagnostic

RP: ribosomal protein; SIFT: sorting intolerant from tolerant algorithm; AA change: amino acid change; VAF: variant allele frequency; PolyPhen: polymorphism phenotyping.

mosomes. Further malignant progression is driven by NFkB and MAPK/ERK signaling.[9,10] Until a few years ago, the mutational landscape for MM was largely unknown. The first whole genome sequencing study uncovered only 10 significantly mutated genes, 2 of which were the previously identified *NRAS* and *KRAS*. One of the novel findings in that study was that nearly half of the patients carry mutations in genes with a function that may be linked to protein translation. Of interest are the mutations in *FAM46C* in 13% of MM cases. *FAM46C* expres-

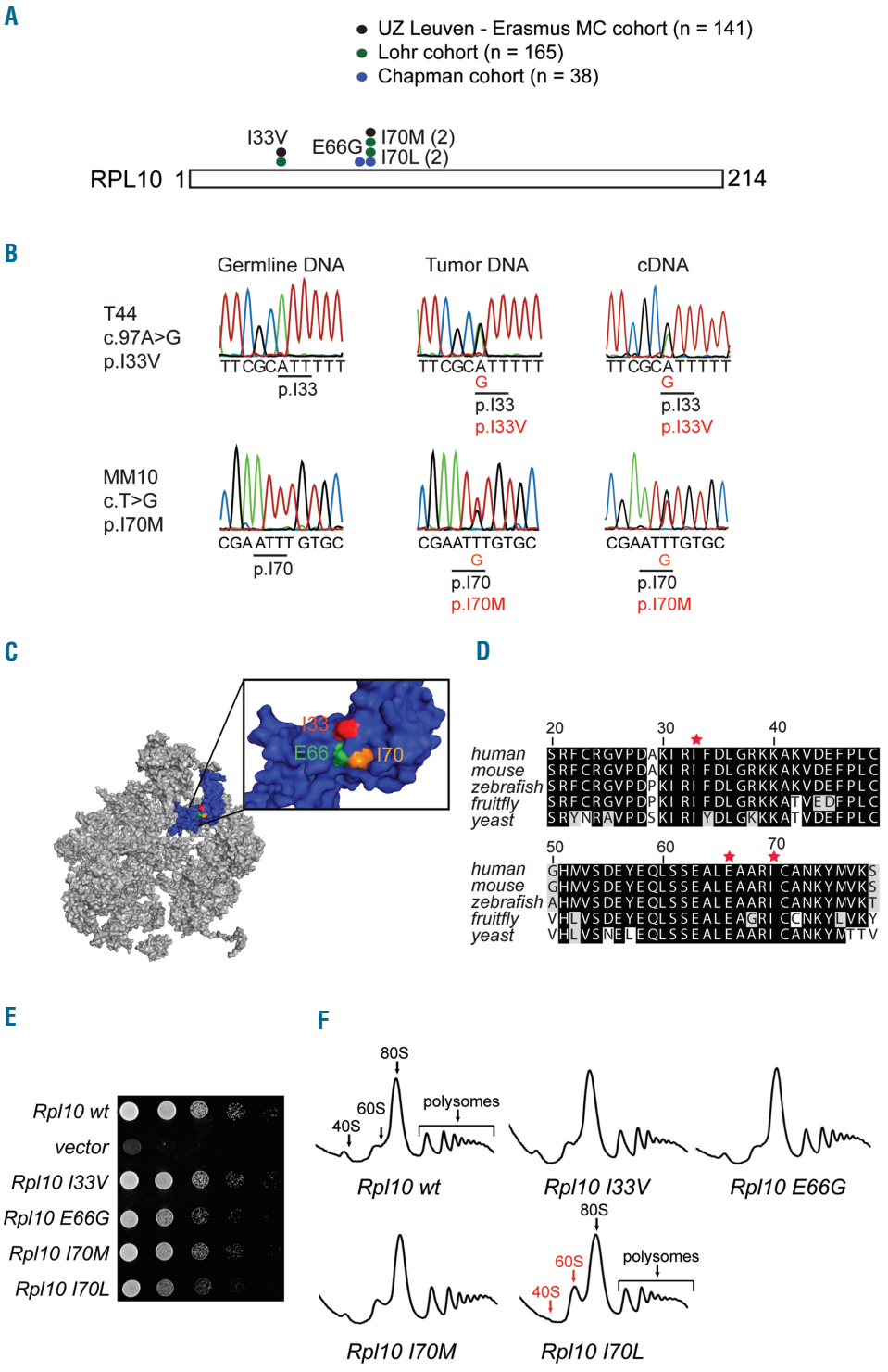

Figure 1. *RPL10* mutations in MM. (A) Linear view of the RPL10 protein with mutations from different cohorts indicated. (B) Chromatograms of mutations found by Sanger sequencing. Germline DNA control, tumor DNA, and tumor cDNA are shown. (C) 3D model of the 60S subunit of the ribosome with RPL10 in blue. In the blow up, the mutant residues I33, E66 and I70 are colored red, green and orange, respectively. (D) Alignment of the RPL10 protein sequence in different species with mutant residues indicated by red stars. (E-F) Functional studies in yeast, testing the effect of the different mutants in Rpl10 on proliferation (E) and polysome profiles (F).

sion correlates with that of RPs and translational initiation and elongation factors.[7]

The supplements of this first MM sequencing paper also described *RPL10* mutations (E66G and I70L) in 2/38 patients analyzed (Figure 1A and Table 1, Chapman cohort).[7] The same group later expanded this cohort and identified 3 additional *RPL10* mutations (I70L, I70M and I33V) out of 165 new patients (Figure 1A and Table 1, Lohr cohort). Intriguingly, 2 out of 3 mutations again affected residue 70 of RPL10. The supplements of this paper also included rare mutations in several other RPs (Table 1).[11] Given these observations, we explored the spectrum of mutations in ribosomal protein genes in MM in more detail. We started by Sanger sequencing the entire *RPL10* coding region in 141 MM samples. This uncovered 2 *RPL10* mutations (I70M and I33V), which were expressed at the ribonucleic acid (RNA) level and absent in the germline DNA of the patients, confirming their somatic nature (Figure 1A-B; UZ Leuven-Erasmus MC cohort). Putting these results together with those in the published genome sequencing studies,[7,11] there are 7 mutations in *RPL10* in 344 patients, or a mutation frequency of 2%. On a linear view of the RPL10 protein, 3 variants lie close to one another, with I33V located more towards the N-terminus of the protein (Figure 1A). Interestingly, in a 3D conformational view of the protein, the mutations cluster in a region that is distinct from the mutational hotspot described in T-ALL (Figure 1C).[2] The mutated residues are conserved (Figure 1D), and the Sorting Intolerant from Tolerant algorithm (SIFT) predictions for all these mutations are deleterious, suggesting a damaging effect on protein function (Table 1). PolyPhen (Polymorphism Phenotyping) scores are more conservative, with possibly/probably damaging predictions for mutants E66G, I70L and I70M but a benign prediction for mutant I33V. To further test whether the identified RPL10 mutations could alter RPL10 function, we engineered yeast cells expressing wild-type (wt) Rpl10 or the identified Rpl10 mutants as the sole copy of Rpl10, similar to the experiments we previously conducted for the T-ALL associated R98S mutation.[2] In yeast proliferation assays, the I33V mutant did not show any difference from wt Rpl10 expressing yeast, whereas the remaining three Rpl10 mutants showed a decrease in proliferation, which was most pronounced in the I70L mutant (Figure 1E). To investigate the effect of the mutants on ribosome biogenesis, polysome profiling was used to measure the relative abundance of the 60S and 40S subunits, mature 80S ribosomes, and actively translating ribosomes associated with messenger (m)RNA (polysomes). Only the I70L mutant showed a pronounced phenotype, with an increase in 60S subunit abundance and absence of 40S subunit signal (Figure 1F). While further research is needed to clarify the effect of these mutants in the cell and their role in carcinogenesis, it is conceivable that mutants in RPL10, which reaches into the catalytic center of the ribosome, could differentially alter the translation of certain transcripts. Further studies with these mutants in human MM cell lines would be required to validate this hypothesis.

The ribosome is composed of 81 ribosomal proteins. We suspected that defects in other ribosomal proteins, besides RPL10, might also occur in MM. To explore this, we ran a custom-designed HaloPlex targeted capture assay covering all exonic regions of the 81 ribosomal genes, followed by next-generation sequencing on 37 UZ Leuven MM samples. We identified 5 variants targeting 4 different RP genes in 5 MM patients (Table 1). All variants for which Sanger sequencing could be performed were confirmed in diagnostic material, and when available, the somatic nature of the variant was tested by Sanger sequencing of germline material. One somatic variant (in *RPLP0*) has previously never been reported in SNP databases or in disease-associated databases such as the Catalogue Of Somatic Mutations In Cancer (COSMIC). Two other somatic variants (in *RPL5* and *RPL3L* [uL3]) have been described before as very rare SNPs (Multiple Allele Frequency (MAF) ≤0.001). The variant in *RPL5* is interesting as deletion of this gene is recurrent in MM, and because the same variant has also been described in ribosomopathy Diamond-Blackfan anemia (DBA), a congenital disease caused by mutations in RP genes such as *RPL5*.[8,12] Additionally, 2 mutations were found that could not be tested by Sanger sequencing (both in *RPL29* [eL29]). One of the RPL29 variants (R150G) is described both as a rare SNP (MAF <0.001) and as a mutation identified in lung cancer (COSM340672), while the other is a novel variant (T155K). The supplements of the extended sequencing study (Lohr cohort) included another 13 variants in RP genes (Table 1). Interestingly, one of these variants again targets RPL5, while all others affect RPs distinct from those picked up in our HaloPlex assay. It thus seems that *RPL5* and *RPL10* are the only RP genes recurrently mutated in MM.

RPL10 is mutated at a low frequency at what might be a MM-specific hotspot. Although the mutations did not show a significant ribosome biogenesis defect in yeast, their modest growth defect suggests an impact on Rpl10 function. Moreover, the somatic nature of the mutations, conservation of affected residues, and clustering in a mutational hotspot argue against them being passengers. We can only speculate why the MM hotspot is different from the one in T-ALL. The R98 residue mutated in T-ALL is close to the catalytic center of the ribosome, while the identified mutations in MM occur in a distinct region that could differentially impact ligand binding to the ribosome.

Mutation analysis of all other ribosomal proteins did not uncover any other strikingly recurrent defects. However, RPL5 remains an interesting candidate in MM because it is deleted in 20-40% of MM cases and it appears to be the only other recurrently mutated ribosomal protein gene in MM, besides *RPL10*, when putting together multiple sequencing studies.[8,11] It is worth pointing out that another group likewise reported 1 missense and 1 splice site mutation in *RPL5*.[13] Overall, our data point to a low frequency of mutations in ribosomal proteins in MM, conforming with the observation of few recurrent mutations in the disease in general.[11,13,14] Other mechanisms besides deletions and mutations might influence the expression of RPs in MM. Regarding *RPL5*, we previously showed that some patients show a lowered expression in the absence of mutation or deletion.[8] Interestingly, Table 1 shows that 1 patient can carry multiple RP defects (MM14 and MM0571).

Although we failed to identify any RPs recurrently mutated at a high frequency, our results do support that the ribosome in general, and RPL10 and RPL5 in particular, are targets of mutation in MM. Together with the recurrent deletion of *RPL5* in ≥20% of MM and the observation that half of MM patients carry mutations in genes linked to translation,[7,8] it would appear that defects in the ribosome and in translation in general are a significant

class of defects in MM. In light of our recent finding that deletions in *RPL5* are associated with a better response to clinically used proteasome inhibitors, such as bortezomib in MM,[8] it will be of interest to determine whether this is also the case for these other lesions in the translation machinery.

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References

1. Ban N, Beckmann R, Cate JHD, et al. A new system for naming ribosomal proteins. Curr Opin Struct Biol. 2014;24:165–169.
2. De Keersmaecker K, Atak ZK, Li N, et al. Exome sequencing identifies mutation in CNOT3 and ribosomal genes RPL5 and RPL10 in T-cell acute lymphoblastic leukemia. Nat Genet. 2013;45(2):186–190.
3. Rao S, Lee S-Y, Gutierrez A, et al. Inactivation of ribosomal protein L22 promotes transformation by induction of the stemness factor, Lin28B. Blood. 2012;120(18):3764–3773.
4. Fancello L, Kampen KR, Hofman IJ, Verbeeck J, De Keersmaecker K. The ribosomal protein gene RPL5 is a haploinsufficient tumor suppressor in multiple cancer types. Oncotarget. 2017;8(9):14462-14478.
5. Ljungstrom V, Cortese D, Young E, et al. Whole-exome sequencing in relapsing chronic lymphocytic leukemia: clinical impact of recurrent RPS15 mutations. Blood. 2015;127(8):1007.
6. Landau DA, Tausch E, Taylor-Weiner AN, et al. Mutations driving CLL and their evolution in progression and relapse. Nature. 2015;526(7574):525.
7. Chapman MA, Lawrence MS, Keats JJ, et al. Initial genome sequencing and analysis of multiple myeloma. Nature. 2011;471(7339):467–472.
8. Hofman IJF, van Duin M, De Bruyne E, et al. RPL5 on 1p22.1 is recurrently deleted in multiple myeloma and its expression is linked to bortezomib response. Leukemia. 2017 Jan 3. [Epub ahead of print]
9. Morgan GJ, Walker BA, Davies FE. The genetic architecture of multiple myeloma. Nat Rev Cancer. 2012;12(5):335–348.
10. Kuehl WM, Bergsagel PL. Molecular pathogenesis of multiple myeloma and its premalignant precursor. J Clin Invest. 2012;122(10):3456–3463.
11. Lohr JG, Stojanov P, Carter SL, et al. Widespread Genetic Heterogeneity in Multiple Myeloma: Implications for Targeted Therapy. Cancer Cell. 2014;25(1):91–101.
12. Quarello P, Garelli E, Carando A, et al. Diamond-Blackfan anemia: genotype-phenotype correlations in Italian patients with RPL5 and RPL11 mutations. Haematologica. 2010;95(2):206–213.
13. Bolli N, Avet-Loiseau H, Wedge DC, et al. Heterogeneity of genomic evolution and mutational profiles in multiple myeloma. Nat Commun. 2014;5:2997.
14. Walker BA, Boyle EM, Wardell CP, et al. Mutational spectrum, copy number changes, and outcome: results of a sequencing study of patients with newly diagnosed myeloma. J Clin Oncol. 2015; 33(33):3911–3920.