Mapping the role of hRNase P individual protein subunits via CRISPR/Cas9

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Introduction

Ribonuclease P is a ribonucleoprotein important for 5' maturation of pre-tRNAs. Human RNase P consists of the H1 RNA and 10 core protein subunits, contrary to 1 found in prokaryotes[1]. Two important subunits are Rpp21, which is not shared with RNase MRP, and Rpp29. Rpp21 and Rpp29 can form a heterodimer and together with Rpp38 form the wrist module of RNase P[2]. Moreover, both are involved in homology directed-repair of double-strand breaks[3]. To get new insights on the biological significance and the possible additional roles of Rpp21 or Rpp29 we screened HeLa cells using CRISPR/Cas9.

1 Generation of *RPP21* & *RPP29* knockout HeLa cells

The 2nd and 3rd exon of *RPP21* and *RPP29/POP4* genes respectively, were targeted for cleavage by Cas9. *RPP21* edited cells used in this study code for two premature terminated peptides and one with 2 aa substitution. Whereas *RPP29* edited cells code for two peptides, one with 4 substitutions and 1 deletion and another with 16 aa deletion.

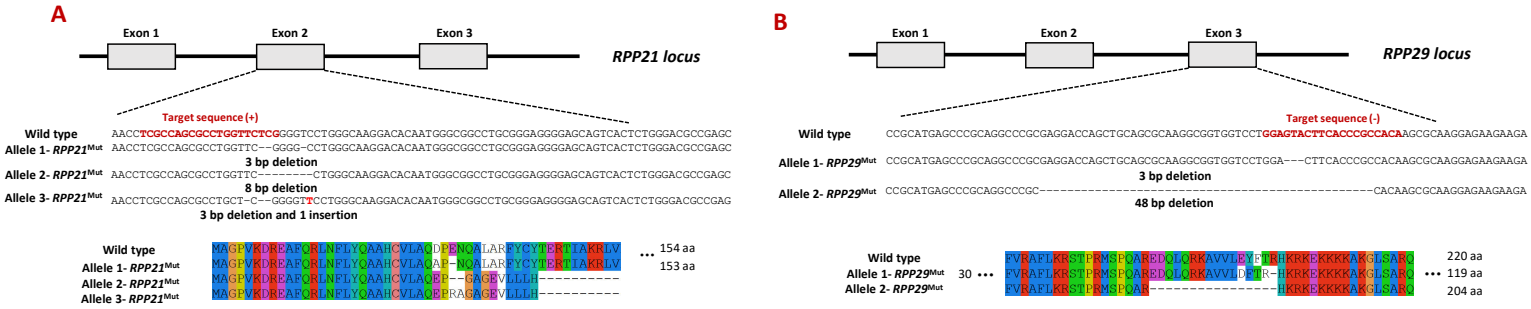

Figure 1. Sequence analysis of *RPP21* **(A)** and *RPP29* **(B)** edited cells**.** The expression of the corresponding targeted gene was examined, *via* qRT-PCR, in clones exhibiting an apparent different phenotype. The locus targeted of possible candidates was amplified, sequenced and analyzed in CRISP-ID[4].

2 *RPP21* & *RPP29* edited HeLa cells have altered morphology and exhibit lower growth rate

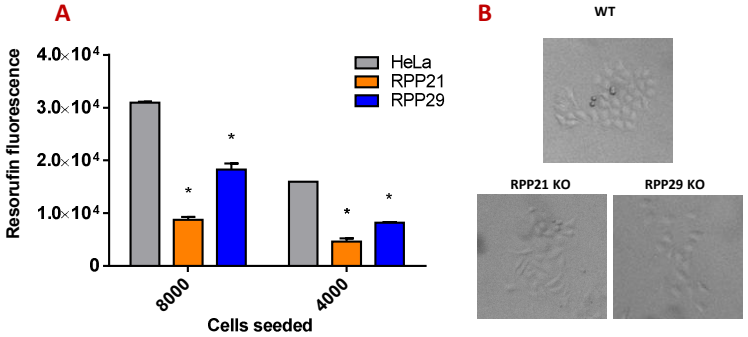

Figure 2. **(A)** Resazurin proliferation assay as measured 24h after seeding the cells and **(B)** representative bright field images of the HeLa clones used in this study.

3 Gene expression of transcription and translation regulators is altered in knockout cells

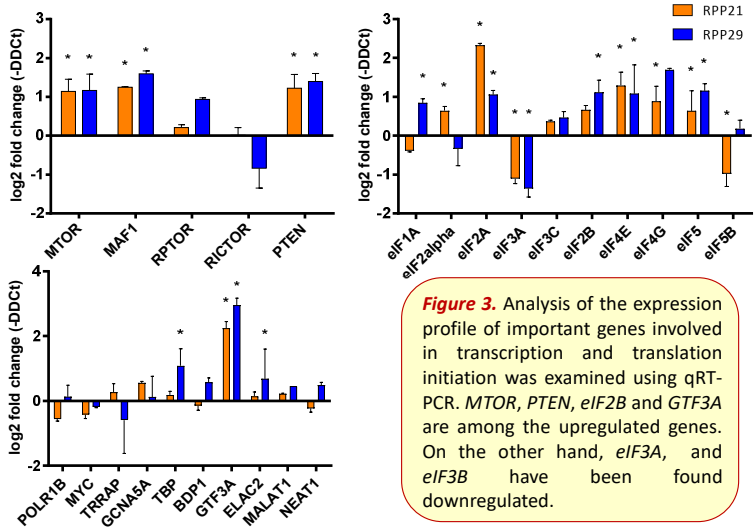

Figure 3. Analysis of the expression profile of important genes involved in transcription and translation initiation was examined using qRT-PCR. *MTOR*, *PTEN*, *eIF2B* and *GTF3A* are among the upregulated genes. On the other hand, *eIF3A*, and *eIF3B* have been found downregulated.

4 Important translation regulators are downregulated at the protein level in Rpp21 and Rpp29 deficient cells

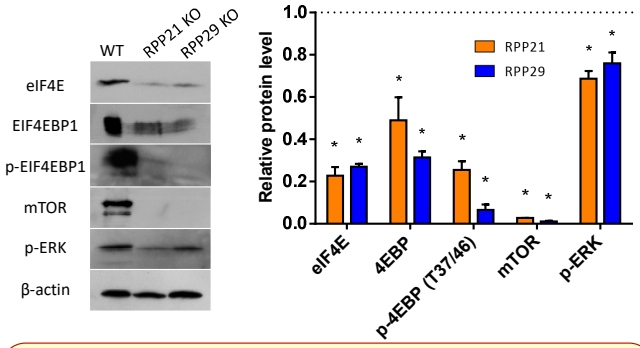

Figure 4. Protein levels of important translation regulators were verified through Western blot analysis. Contrary to their transcript levels, eIF4E and mTOR proteins are downregulated suggesting regulation at the translational or post-translational level. The combined effect of these observations possibly leads to overall reduced translation rates.

Conclusions

- Lower growth rate was observed after *RPP21* & *RPP29* knockout in HeLa cells indicating reduction of cell proliferation.
- Both mutant cell lines exhibit alterations in mRNA and protein levels of important genes implicated in transcription and translation regulation.
- Further analysis of these cell lines will provide new insights on the possible redundancy of these subunits in pre-tRNA maturation and their role beyond the RNase P complex.

References

1. Jarrous N. (2017) *Trends Genet.* 594-603
2. Wu J. et al. (2018) *Cell* 1393-14043
3. Abu-Zhayia ER et al. (2017) *Sci Rep.* 1002
4. Dehairs, J. et al. (2016) *Sci Rep.* 28973

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