Study of Translation in RNH1 Knock-Out Haematopoietic and non-Haematopoietic Cell Lines

Christoph, Liechti, BMA 18-21 B
College of Higher Education
Educational Program for Biomedical Scientists
Department for BioMedical Research (DBMR), University of Berne

1. Conclusion

This research aimed to identify the role of Ribonuclease inhibitor 1 (RNH1) in the translation regulation of neuronal cells. A *RNH1* knock-out neuronal cell model was established by CRISPR-Cas9 using lipofection. RNH1 is not involved in regulating global translation, based on polysome profiling and luciferase reporter assay with a non-structured 5'UTR, whereas it might facilitate mRNA specific translation in neuronal cells as well as in other non-hematopoetic cell models. To better understand the implications of these results, future studies could address RNA-Sequencing to determine which mRNAs are more or less translated. In general, the results correlate with previous data from other non-haematopoietic *RNH1* knock-out cell models and supports the overall hypothesis of the research group.

2. Introduction

Ribonuclease inhibitor (RNH1) is a mostly cytosolic expressed protein and known for the ability to inhibit pancreatic-type ribonucleases, horseshoe like shape as illustrated in Figure 1 [1, 2]. A recent discovery identified RNH1 as ribosomal-associated protein in hematopoietic cells. The binding enhances the translation of a subset of mRNAs. Absence of RNH1 in haemopoietic cells resulted in a decrease of global translation by decreased polysome formation, leading *in vivo* to anaemia in untero and abort. This indicates the involment in regulating erythropoiesis at translation level [3].

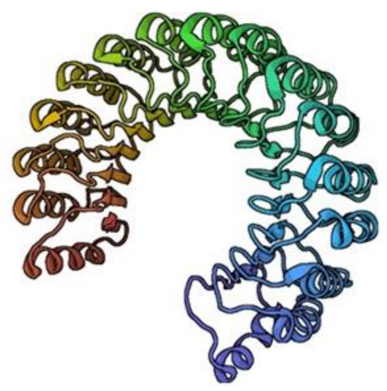

Fig. 1 Structure of Ribonuclease inhibitor 1. Visualization of the structure of RNH1 determined by cryo bio-crystallography of porcine RNH1 [5]. Created with BioRender.com (Liechti, 2021)

Surprisingly, the global translation of non-heamatopoetic cells was not changed in contrary to specific translation which was changed by RNH1-defiecncy [4]. Consequently, the question namely arose if RNH1 is involved in regulating translation of neuronal cells as well.

3. Hypothesises and Aim

Previous performed polysome profiles und luciferase assays among a variety of RNH1 knock-out cell types stated that hematopoietic and non-haematoipoetic cell translation is differently affected by RNH1-defiecncy. Neuronal cells are expected to maintain their global translation but exhibit a difference in specific translation as non-haematoipoetic cells.

To prove the hypothesis a *RNH1* knock-out neuronal cell model (SH-SY5Y) had to be established prior.

4. Material and Method

Human neuronal SH-SY5Y cells were cultured in DMEM with 10% FCS at 37 °C. The cell model was established by cationic lipofection of a plasmid encoding CRISPR-Cas9 and the gRNA against exon 2 of *RNH1*. Transfected cells were selected for GFP-expression and plated as single-cells for the expansion of monoclonal populations. The phenotype was validated by western blot.

Polysome profiling was performed with cycloheximide-treated cell lysate and separated by a 10-50% sucrose gradient at 39'000 rpm for 2.45 hours. Polysome profiles were acquired by continuously measuring the absorbance of the ribosomal proteins at 254 nm.

Luciferase assay was performed by cationic lipofection of the 5'UTR GAPDH modified *Luc+* pGL3-Promoter vector from Promega. Luminescence was measured after 72-hour of incubation.

5. Results

In line with the hypothesis RNH1 is not regulating the global translation of neuronal cells as indicated by the polysome profiling in Figure 2. Interestingly, the luciferase reporter assay in Figure 2 showed an alteration in specific translation of RNH1-deficient neuronal cells as expected.

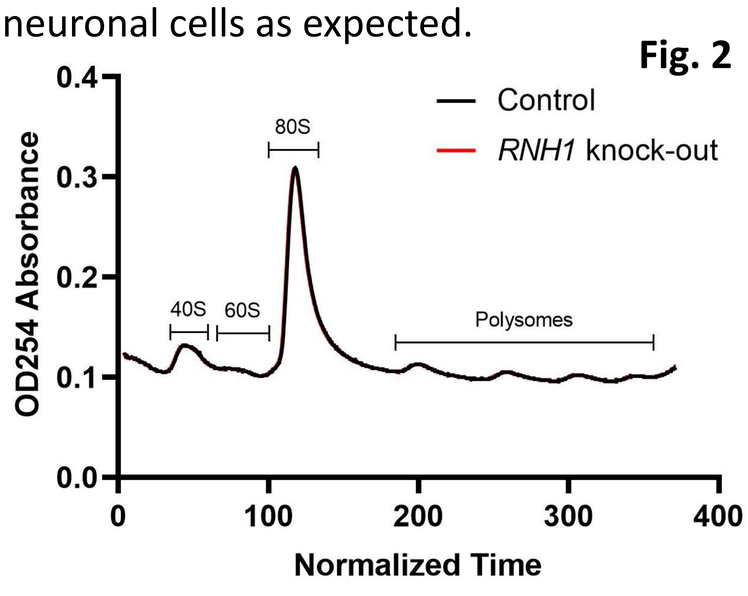

Fig. 2 Polysome profile analysis of RNH1-deficient human neuronal SH-SY5Y cells. Polysome profile of *RNH1* knock-out SH-SY5Y cells (red) and wild-type cells (black). Small ribosomal subunit (40S), large ribosomal subunit (60S), ribosome (80S) and polyribosomes (polysomes) are indicated. Density of sucrose gradient increases from 0 (low density) to 400 (high density) seconds (Liechti, 2021)

Fig. 3 Translation of 5'UTR GAPDH luciferase reporter plasmid in RNH1-deficient human neuronal SH-SY5Y and wildtype cells. Bar chart of *RNH1* knock-out SH-SY5Y cells (grey) and wild-type cells (white). A double-tailed unpaired t-test was performed. Data is shown as mean ±SD from three technical replicates (Liechti, 2021)

6. Discussion

In summary with the previous result, it is hypothesised that RNH1 might regulate mRNA specific translation in all three cell types. The data contributes to a clearer understanding of the functions of RNH1 in the global translation of different cell types. However, the mechanism of how RNH1 is regulating the translation in different cell types remains elusive and needs to be researched further.

Referenzen

[1]] Dickson, K. A., Marcia C. H., & Ronald T. R. (2005). Ribonuclease Inhibitor: Structure and Function. *Progress in Nucleic Acid Research and Molecular Biology*, Vol. 80, 349–74.
[2] Furia, A., Marco, M., Gaetano, C., Elio, P., Elena, C., Maria, R. A., Franca, E., Lucio, N., and Giuseppe, D A. (2011). The Ribonuclease/Angiogenin Inhibitor Is Also Present in Mitochondria and Nuclei. *FEBS Letters*, 585(4):613–17.
[3] Chennupati, V., Diogo, F. T. V., Kendle, M. M., Nicola, A., Aubry, T., Eric, C., Martina, S., Cedric, S., Michel, A. D., Manfredo, Q., Irene, R., Vijay, G. S., H. Robson, M.d, Nicolas, F., Anne, A., Pascal, S., Trang, H., Ramanjaneyulu, A. (2018). Ribonuclease Inhibitor 1 Regulates Erythropoiesis by Controlling GATA1 Translation. *Journal of Clinical Investigation*, 128(4), 1597–1614.
[4] Stilinović, M. (2020). Understanding the Role of Ribonuclease Inhibitor (RNH1) in Erythropoiesis and Translation (Dissertation). University of Bern, Bern.
[5] Kobe, B., Johann, D. (1996). Mechanism of Ribonuclease Inhibition by Ribonuclease Inhibitor Protein Based on the Crystal Structure of Its Complex with Ribonuclease A. *Journal of Molecular Biology*, 264(5):1028–43.

Abbildungen

Fig. 1 Liechti, C. (2021). *Structure of Ribonuclease inhibitor 1*. Berne: own figure
Fig. 2 Liechti, C. (2021). *Polysome profile analysis of RNH1-deficient human neuronal SH-SY5Y cells.* Berne: own figure
Fig. 3 Liechti, C. (2021). *Translation of 5'UTR GAPDH luciferase reporter plasmid in RNH1-deficient human neuronal SH-SY5Y and wildtype cells.* Berne: own figure