

Investigating the role of Ribonuclease inhibitor (RNH1) in Toll-like receptor 7 (TLR7)-mediated interferon production

Celine Tran, BMA 23-26

Bildungsgang Biomedizinische Analytik HF

Department of BioMedical Research, University of Bern, Switzerland / Department of Hematology, Inselspital, Bern University Hospital, University of Bern, Switzerland

1. Abstract

RNH1 has several functions, but whether it is involved in interferon (IFN) signaling is still unknown. Therefore, the TLR7-mediated interferon production was investigated. To accomplish this, CAL-1 wild-type (WT) and RNH1-knockout (RNH1-KO) CAL-1 cells were stimulated with Resiquimod (R848) or RNA40, and the difference in IFN-β secretion was determined by ELISA. Furthermore, Co-Immunoprecipitation (Co-IP) experiments were performed to verify whether RNH1 interacts with ribonuclease T2 (RNase T2). This enzyme cleaves viral ribonucleic acids (RNAs) to generate TLR7-activating ligands. The increased IFN-β release in RNH1-KO cells and the interaction between RNH1 and RNase T2 suggest that RNH1 is involved in this pathway.

2. Introduction

The TLR7-mediated interferon production is activated by viral RNA. After the infection, their genome is cleaved by endolysosomal RNase T2 to produce immune-stimulatory RNA ligands upstream of TLR7 [1]. This transmembrane receptor is expressed in the endolysosomal compartment of innate immunity cells, particularly in plasmacytoid dendritic cells [1, 2, 3]. Its binding pockets have a specificity for degradation products of viral RNAs [4]. When an agonist binds, a signaling-competent receptor is formed, and the subsequent signaling results in a fast production and secretion of type I interferons (IFN-I). In humans, these are mainly IFN-α and IFN-β [1, 5].

The protein of interest in this study is RNH1. It is known for its function to bind and inhibit pancreatic-type RNases and therefore protecting RNAs from degradation. The research group aims to better understand the biological functions of it and has already published its negative regulatory role in other inflammatory pathways [6]. However, whether it is also involved in interferon signaling is still unknown. In addition, a previous experiment indicated that RNH1 not only binds to pancreatic-type RNases, but also to RNase T2.

3. Aims and Leading Questions

Determination of the difference of interferon-β secretion after stimulation with R848 or RNA40 in CAL-1 wild-type and RNH1-deficient CAL-1 cells.

* *How does RNH1 deficiency in CAL-1 cells influence the interferon-β secretion after stimulation with R848 or RNA40 in comparison to CAL-1 wild-type cells?*

Verification of whether RNH1 interacts with RNase T2 in CAL-1 cells using Co-Immunoprecipitation.

* *Can Co-Immunoprecipitation demonstrate an interaction between RNH1 and RNase T2 in CAL-1 cells?*

4. Methods and Material

CAL-1, a plasmacytoid dendritic cell line [7], was used for the experiments. To determine the IFN-β secretion, CAL-1 WT and RNH1-KO CAL-1 cell populations were generated. These cells were either stimulated with R848, a direct TLR7 agonist [1] or with RNA40, a synthetic TLR7-stimulating ligand [8]. The concentrations of the released IFN-β in the supernatants were then determined by ELISA.

To verify whether RNH1 interacts with RNase T2, cell lysates of original CAL-1 and R848-stimulated CAL-1 were used for Co-IP experiments. Magnetic beads coated with anti-RNH1 antibodies captured RNH1 and all proteins bound to it. Beads with anti-VSV-G antibodies served as negative control. The analysis was done by Western blot, and the evaluation was based on visual assessment of the membrane.

5. Results

Figure 1 shows the ELISA results. All unstimulated cells (control) did not produce IFN-β. In both stimulations, there is a trend that the KO cells released more IFN-β than the WT ones.

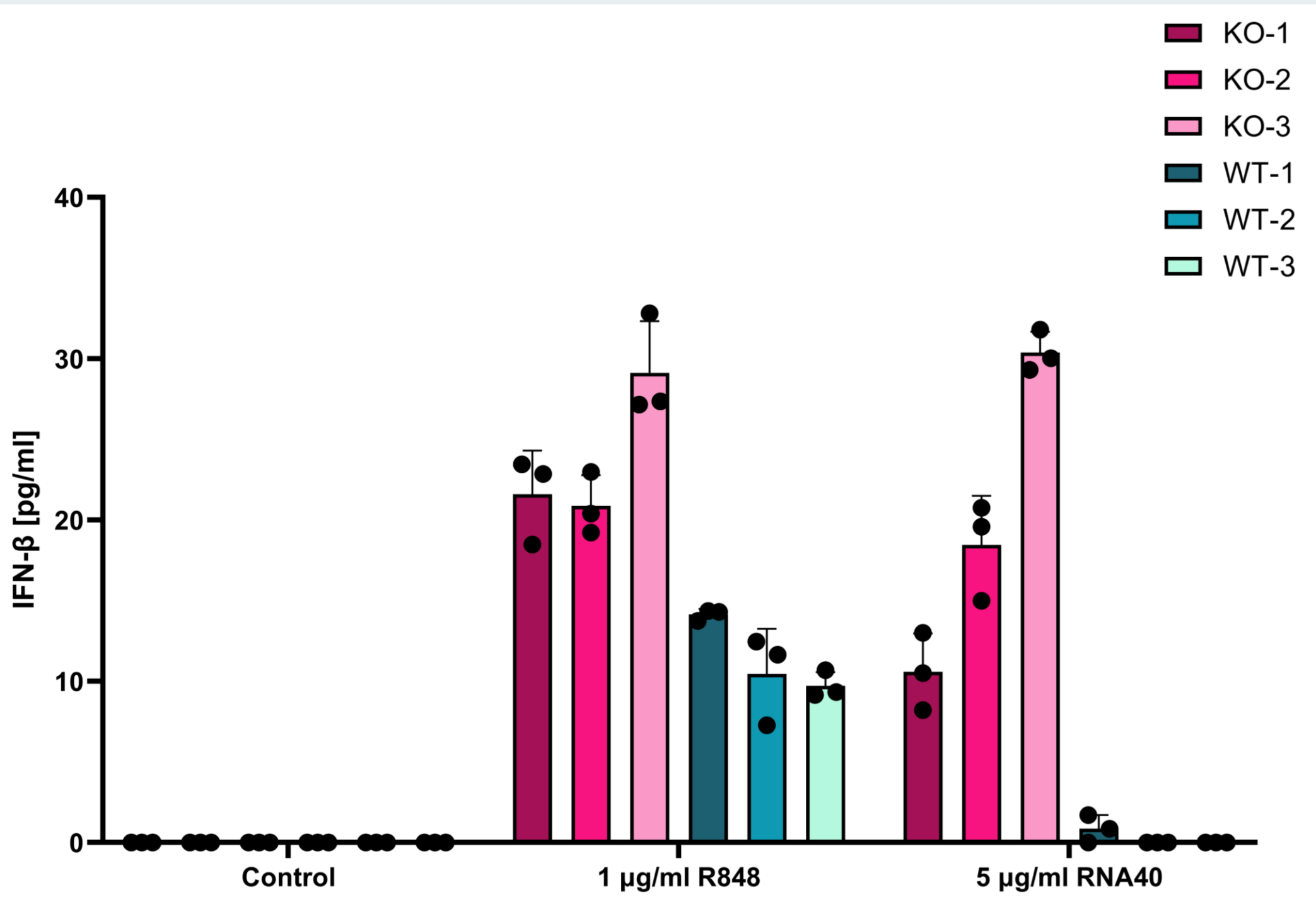


Figure 1: Result of IFN-β ELISA with CAL-1 RNH1-KO and -WT cells after stimulation with R848 (1 µg/ml) or RNA40 (5 µg/ml) for 16 h (Tran, 2026)

Figure 2 presents the Western blot results of the Co-IP experiment. The Ponceau stain shows all proteins that were transferred from the gel to the membrane. The whole cell lysates (WCL) are in the first three positions, and the Co-IP samples are in the last three. All WCLs and all samples, where the Co-IP was performed using magnetic beads coated with anti-RNH1 antibodies, showed RNH1 and RNase T2 bands. In addition, the stimulation with R848 increased the binding capacity between RNH1 and RNase T2. The negative control showed no bands.

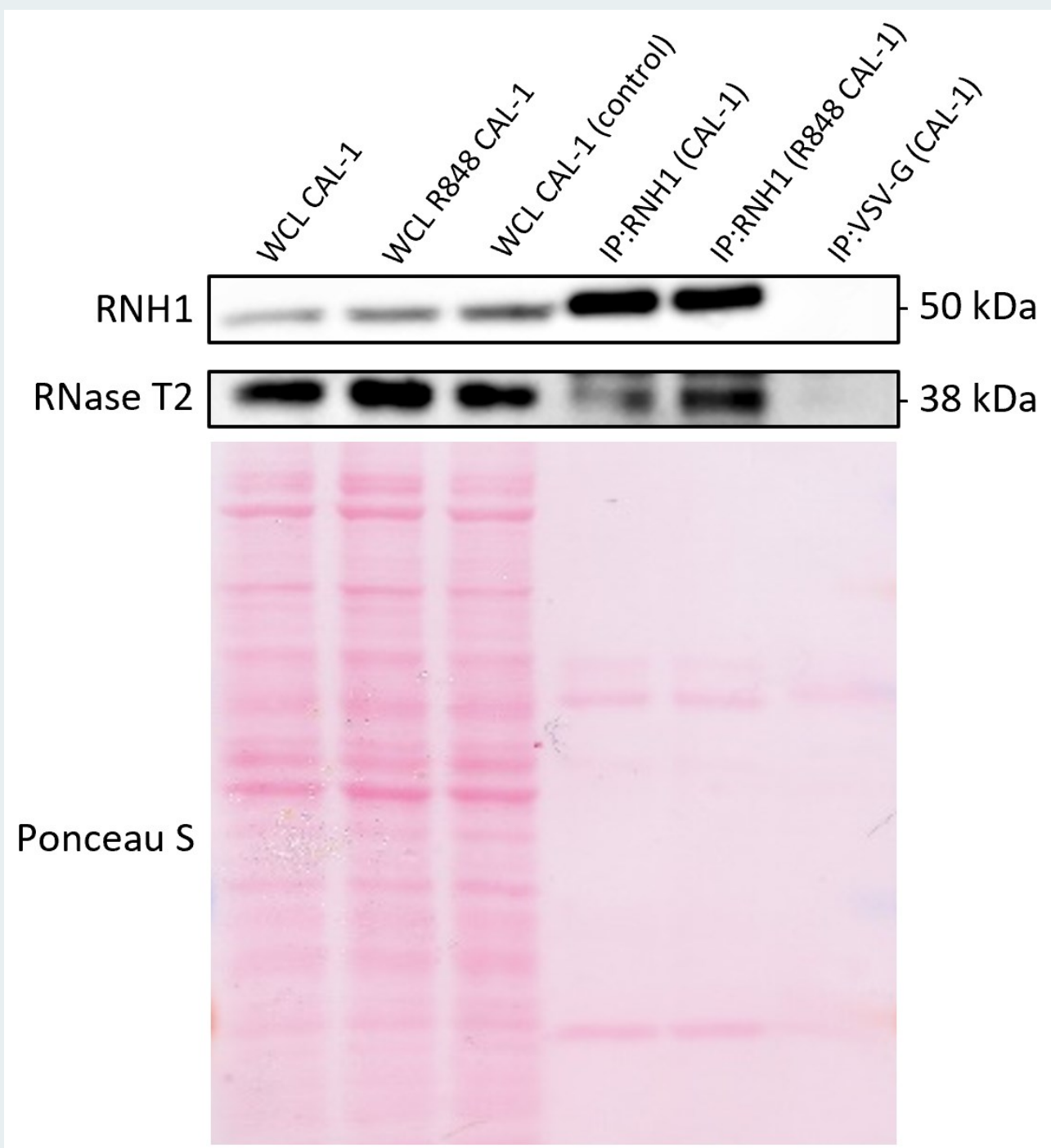


Figure 2: Result Co-IP with RNH1 in R848-stimulated and unstimulated CAL-1 cells (Tran, 2026)

6. Discussion and Conclusions

CAL-1 cells do not produce IFN-β without any reasons. The IFN-β release in WT and KO cells after the stimulation with R848 confirms that TLR7 was present and functional. In WT cells, RNH1 could either interfere with the recognition of R848 by TLR7 or with the downstream signaling, leading to less IFN-β production. RNase T2's ability to cleave RNA40 could be inhibited by its interaction with RNH1, resulting in the absence of TLR7 ligands to induce IFN-β production. However, a deficiency in RNH1 could allow unrestricted signal transmission.

Overall, this study suggests that RNH1 may play a role in the antiviral pathway by influencing the production and release of IFN-β, although further experiments are required to validate these findings.

List of references

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| [1] Bérouti et al., 2024, pp. 1482-1483 | [5] da Silva et al., 2021, pp. 1-2 |
| [2] Bérouti et al., 2025, p. 4880 | [6] Bombaci et al., 2022, pp. 1, 7, 9 |
| [3] Zhou et al., 2022, p. 1 | [7] Maeda et al., 2005, p. 148 |
| [4] Krieg, 2025, p. 7 | [8] Obermann et al., 2022, p. 1 |

Figures

Figure 1 Own illustration, Result ELISA, Tran, C. (2026)

Figure 2 Own illustration, Result Co-IP, Tran, C. (2026)