RaDiChal'21
FINAL

2021 Rare Disease Challenge

POSTER TITLE

Targeting NLRP3 Protein Degradation with Antisense Oligonucleotides via Liposomes for FMF Potential Treatment

TEAM NAME

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Abstract

Familial Mediterranean Fever (FMF) occurs due to mutations in the MEFV gene. The MEFV gene encodes the pyrin protein and as a result of mutation, the functioning of the pyrin protein is disrupted. The mutated MEFV gene cannot be phosphorylated via the PKN1/2 protein and the ASC and Pro-Casp-1 binds to cause inflammation (1). In this study, the degradation of NLRP3 protein, which enables the recruitment of ASC and Procaspase-1, is targeted. In this way, inflammation can be prevented. Antisense oligonucleotide (ASO) designed specifically for the target mRNA is used. The ASO is delivered to HEK293 T cells via liposomes. In order to examine the results, Western Blot is used. Modified cells are given to patients in vivo, if the results support the hypothesis. ASOs that bind to the target mRNA reduce the production of the NLRP3 protein by providing RNAseH activation.

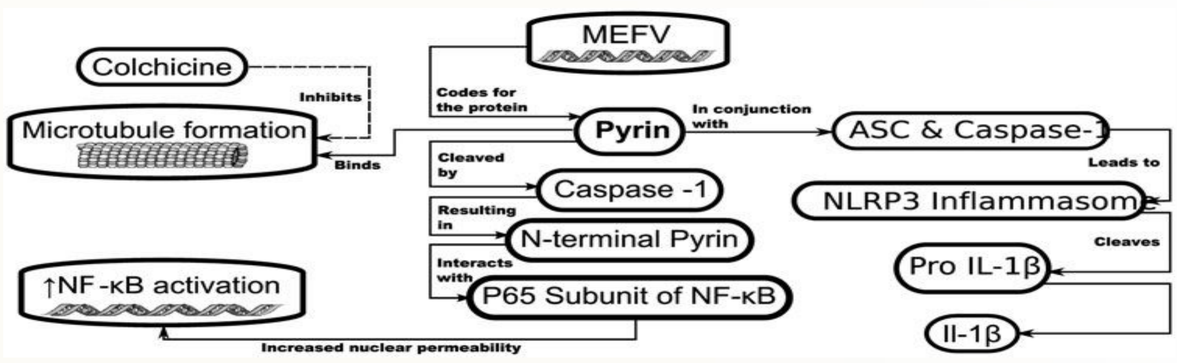

Figure 1: Association of pyrin domain on MEFV gene with NLRP3 inflammasome (2).

Projects

ASO was designed from the Kozak Sequence of the target protein, which functions as the protein translation initiation site in most eukaryotic mRNA transcripts (3).

One of the designed ASO sequence: **TTTCTTCCATGGCTCAG**

6-7 nucleotides before and after the NLRP3 kozak sequence were selected to specific binding. The antisense oligonucleotides were engineered via Eurogentec by selecting different modifications. The designed ASO was encapsulated with listeriolysin in order to assist its passage from the endocytic compartment to the cytosol (4). Liposome was formed with the kit (5). Encapsulated ASO was combined with the liposome. It was given to HEK293 T cells. HEK293 T cells were cultured to propagate the cells. The results will be analyzed using the Western Blot technique.

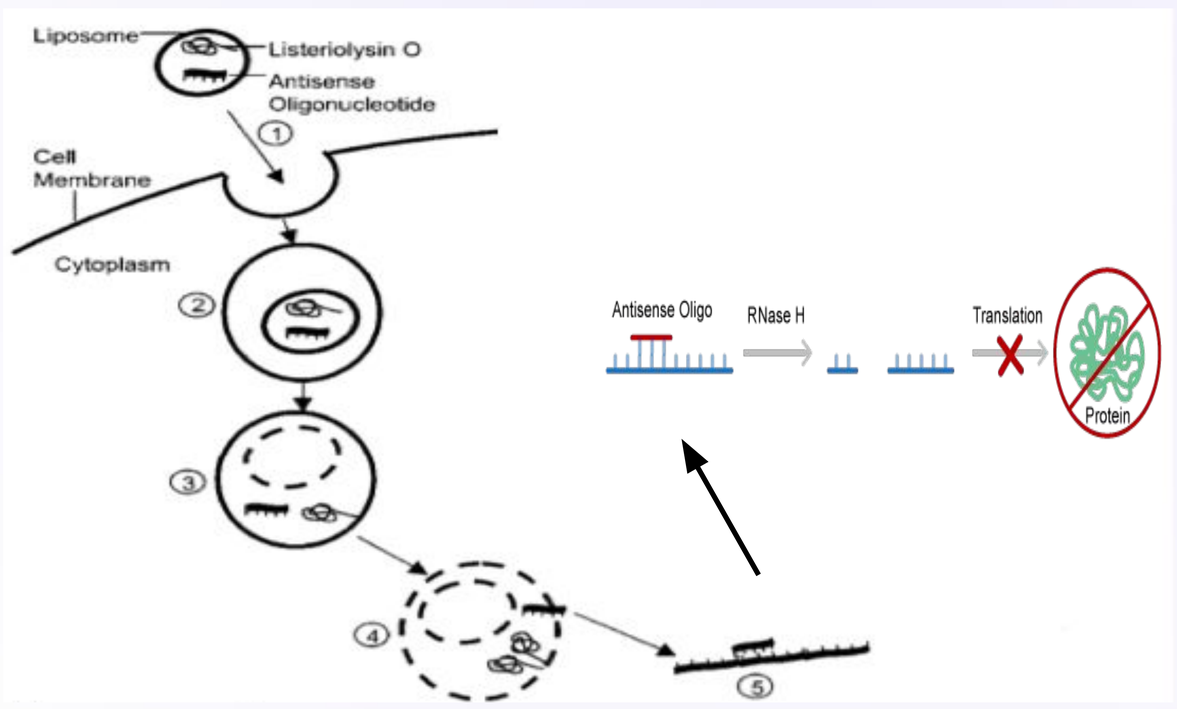

Figure 2 : Giving the ASO via liposomes to cell (4).

Social Impact

Followers: 138
Likes: 43
Sharings: 14
Watchs: 335

Many posts on our Instagram account were shared to raise awareness of Familial Mediterranean Fever, which is a rare disease, and to inform the public.

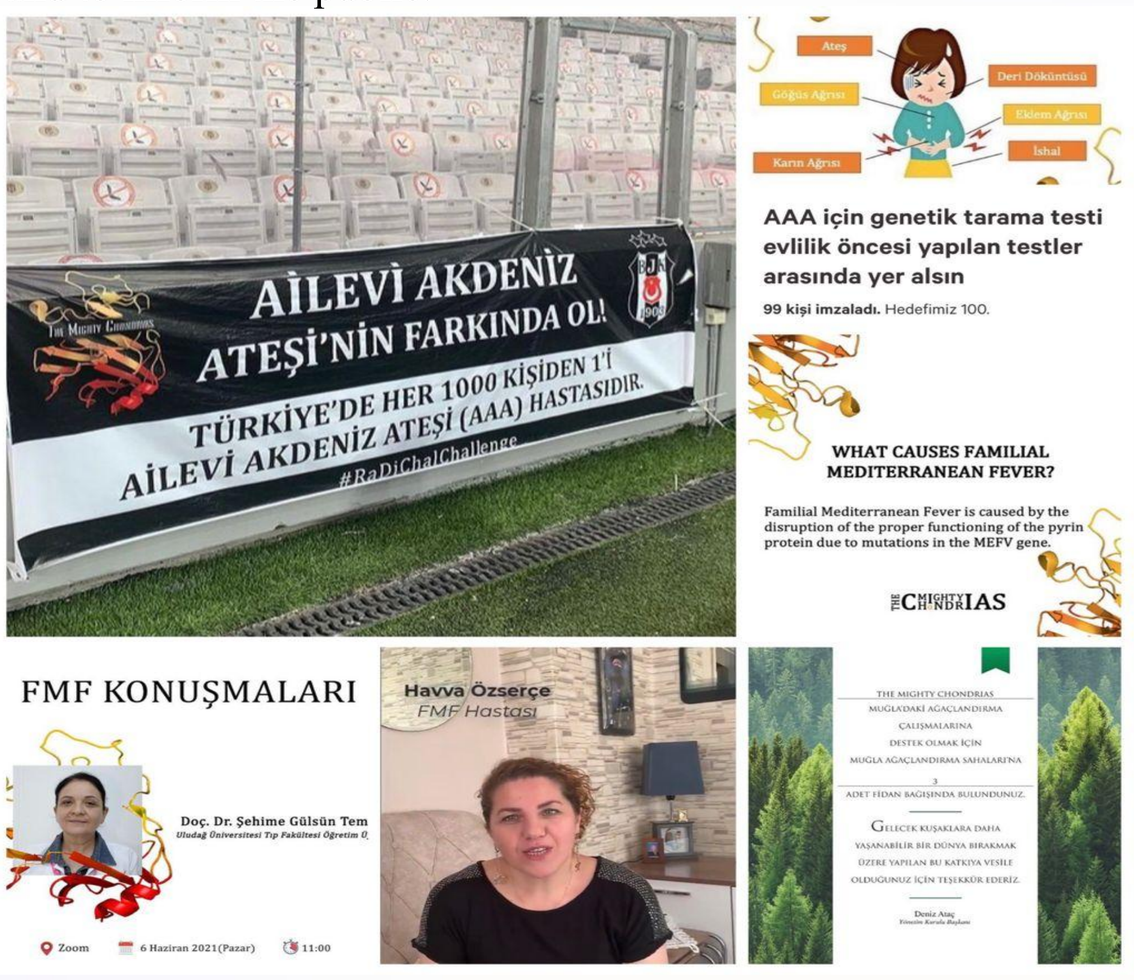

Figure 3: Social media posts on the Instagram account.

Conclusion

As a result, NLRP3 protein which leads to inflammation in FMF patients is reduced by using ASO and it is combined with liposomes to give the HEK293 T cells. If the hypothesis of the project is supported, the cells will be proliferated via cell culture and the treatment will be applied to patients with giving modified HEK 293 cells with in vivo method.

References

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4. Mathew, E., Hardee, G. E., Bennett, C. F., & Lee, K. D. (2003). Cytosolic delivery of antisense oligonucleotides by listeriolysin O-containing liposomes. *Gene therapy*, *10*(13), 1105-1115.

5. Liposomes: Protocol. (2016). Retrieved 27 September 2021, from https://inanobotdresden.github.io/liposome-protocol.html.