MOOGENE MEDI

Ultrasound-Activated Particles as CRISPR/Cas9 Delivery System for Androgenic Alopecia Therapy

Traditional androgenic alopecia treatments like oral remedies or topical agents either incur various side effects or exhibit limited therapeutic efficacy. To overcome these limitations, we have developed a new genetic approach by CRISPR/Cas9 for androgenic alopecia therapy. Unlike common CRISPR/Cas9 modalities that use viruses as a delivery system, we have developed an ultrasound-activated microbubble-nanoliposomal system to deliver the genome editing proteins into the cells.

We selected a highly efficient sgRNA sequence that targets the mouse SRD5A2 gene. SRD5A2 is an enzyme that converts testosterone into dihydrotestosterone (DHT), a strong endogenous androgenic steroid which causes damages to the growth pathway of hair dermal papilla cells (DPC), causing them to remain in the telogen phase of cell growth, thereby inducing hair loss. By snipping this gene using our Cas9/sgRNA, we can reduce DHT and recover the anagen phase of DPC.

For seven weeks, we tested our system on ten treatment groups of ten male mice per group. All the mice had their dorsal side depilated with the hair follicles synchronized to the telogen phase. The treatments groups are as follows:

NL = Nanoliposome

MB = Microbubble

US = Ultrasound

Group 1: Control (No Treatment at all)

Group 2: Testosterone Only

Group 3: Ultrasound Only

Group 4: Cas9/sgRNA Only

Group 5: NL(Cas9/sgRNA) Only)

Group 6: NL(Cas9/sgRNA) with US (no MB)

Group 7: MB-NL(Cas9/sgRNA) (no US)

Group 8: MB-NL(empty) with US (no Cas9/sgRNA)

Group 9: MB-NL(scRNA) with US (RNA sequence is scrambled)

Group 10: MB-NL(Cas9/sgRNA) with US

Testosterone was topically applied daily for seven weeks to maintain telogen retention as an androgenic alopecia model. Mice from Group 2-9 which were treated with materials other than our MB-NL(Cas9/sgRNA) with US system maintained their telogen hair cycle throughout the seven weeks. No hair growth was observed as telogen is the resting phase of the hair follicle. Only the mice control group and Group 10 exhibited recovery of hair growth, showing that all components of our gene editing formulation are required to produce the desired effect of normal hair regrowth by cutting off the DHT pathway.

The proposed mechanism of action is that when ultrasound is used, the MB pops adjacent to the DPC which causes a small instance of cavitation that allows the nanoliposome-protect Cas9/sgRNA editing tools to enter the cells and edit the target DNA sequence. Microscopy confirmed that this method produces a deep level of cellular penetration, and the gene edit can recover the anagen phase of cell growth, as well as increase the amount of Vascular Endothelial Growth Factors (VEGF), which are cellular factors that promote angiogenesis to supply nutrients to hair follicles.

Our experiments showed that ultrasound-activated microbubble-nanoliposomal Cas9/sgRNA had notable gene editing efficiency (71.6% vs. Control, $p < 0.01$), and the SRD5A2 mRNA and protein that we aimed to suppress both showed 70% reduction ($p < 0.0001$). By downregulating SRD5A2, our method recovered anagen and showed up to 90% hair regeneration.

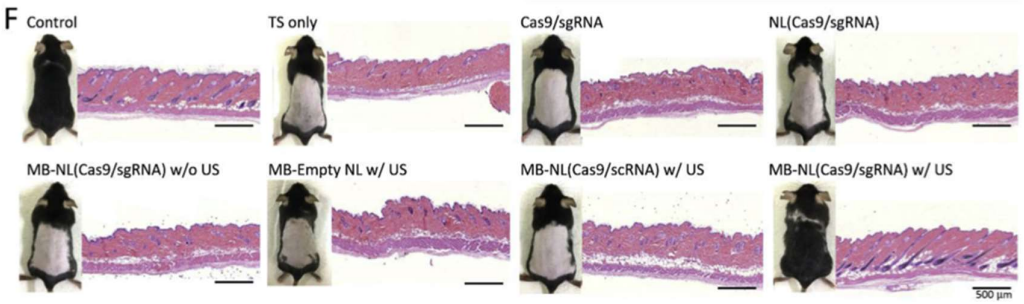

Fig. Hair growth progress of mice groups after 7 weeks

Reference: Ryu, J. Y., Won, E. J., Lee, H. A. R., Kim, J. H., Hui, E., Kim, H. P., & Yoon, T. J. (2020). Ultrasound-activated particles as CRISPR/Cas9 delivery system for androgenic alopecia therapy. Biomaterials, 232, 119736.