CRISPR-Cas9 makes vitiliGO : Revolutionary CRISPR therapy for treating vitiligo

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Abstract

Clustered Regularly Interspaced Short Palindromic Repeat, popularly known as CRISPR is a unique technology that can edit parts of the genome by removing, adding, or altering sections of the DNA Sequence. It is currently the simplest, most versatile, and precise method of genetic manipulation. Cas9 nuclease is used for its ability to target gene efficiently. Cas9 endonuclease is directed by guide RNA (gRNA) which is made up of target genomic region-specific single guide RNA (sgRNA) and tracrRNA. After reaching the targeted point, double strand break. It will be followed by non-homologous end joining (NHEJ) or Homology Directed Repair (HDR) DNA repairing systems. In HMB311 CRISPR lab, Green Fluorescence Protein (GFP) was produced by changing a single codon coding for Hys66, responsible for BFP, into Tyr66. gRNA was produced by in vitro transcription (IVT). Two options were selected for optimal transfection of CRISPR-Cas9; half of the class did: Cas9 mRNA + gRNA + HDR template (ssODN) and the other half did: Cas9 protein + gRNA + HDR template (ssODN). For both transfection methods, non-viral delivery mode e.g., lipofectamine was used. At last, the results obtained from Cas9 mRNA and protein was compared and they pertained to the fact that delivery by Cas9 mRNA showed more effective results in eBFP -> eGFP. Using CRISPR-Cas9 was successful in changing a single base pair turning a significant percentage (about 22%) of cells from blue to green which was keenly speculated under high tech inverted fluorescent microscopy and measured by countess. These results were very encouraging for proposing an ex vivo CRISPR mediated gene editing treatment for Vitiligo, an autoimmune disease which results in skin depigmentation.

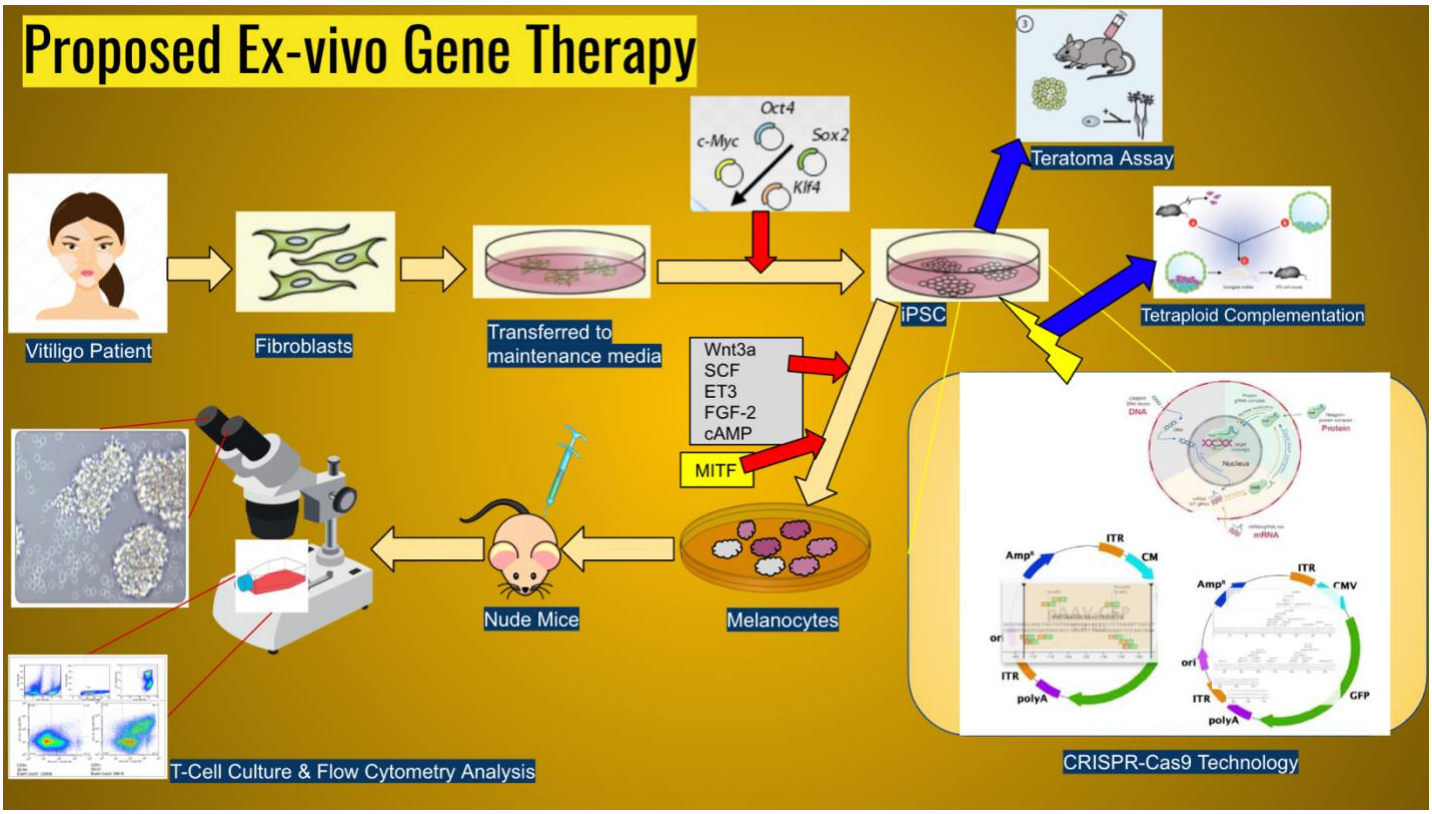


Fig: Proposed Ex-vivo Gene Therapy for treating vitiligo

Biography

Ahir Roy achieved Honors Bachelor of Science degree from the University of Toronto in 2019, majoring in genetics. She is very much interested in conducting research with CRISPR technology for different therapeutical approach. Her research works in genetics and other human biology field have encouraged her to propose the ex-vivo gene therapy for treating vitiligo.

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