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Case Study 1

Question/Biological Problem (10 points)

Age-related macular degeneration is a progressive disease that attacks the macula, the eye's retinal region, and can cause blindness in adults 65 and older. The most severe symptoms recorded include extreme visual impairment to complete blindness (loss of central vision).. The retinal region is a highly packed, active, dense neural network, metabolizing quickly and thus, need high blood flow to maintain proper vascular structure (choroid structure). This structure normally maintains a steady flow of oxygen, blood, and nutrients in healthy aging patients. Several physiological factors related to this regulation will be further discussed and investigated as a potential therapeutic/cure for AMD, using the CRISPR/cas9 minicafé variant. There is no cure for AMD, however, the use of monoclonal antibody treatments as a therapeutic is currently active

New developments to the CRISPR-mediated gene system, the widely known therapeutic gene editor, resulted in the 'minicafe' variant, a CRISPR/cas9 based compact and potent transcriptional activator. The smaller variant can activate endogenous target genes just like its predecessor, however, its advantage is its small size, where normal cas9 exceeds the packaging capacity of most common viral vectors - this smaller cas9 will not. Minicafe contains an engineered nuclease-null cas9 from camp. Jejuni, along with potent transcriptional activators to make for robust gene expression in human and mice cells. Therefore, this compact model carries great therapeutic potential against human diseases like AMD.

Biological Question:

My aim is to understand and investigate the role of up-regulated and down-regulated genes in patients with AMD using a suitable human cell line, using the CRISPR/cas9 mini variant and cell culture studies.

Research Model:

My aim is to investigate two physiological mechanisms of action that cause AMD:

- The upregulation of VEGF, or vascular endothelial growth factor
- The under expression of the HTRA1 gene, within chromosome 10, by the retinal pigmented epithelium region of the eye

Using the cas9 compact model described as way to limit or control expression of these genes/molecules in AMD.

Research Plan:

- To engineer a gene of interest using an engineered mini CRISPR/cas9 system ortholog that targets/binds gDNA to enable specified and controlled expression critical to AMD
- To see how over-expression of VEGF affects normal human cell lines and investigate what proteins or signals are at play during over-expression
- To see how down-regulation of the HTRA1 gene affects human cell line and investigate what proteins or signals are at play during down regulation
- Find a GOI or use engineered minicafe with GOI and transfect it into a control cell line suitable as a vector.
- Investigate several routes of drug delivery optimal for AMD and this research study

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Sources

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