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EPIGENOME EDITING OF TGF-BETA RECEPTORS USING DCAS9-KRAB SYSTEM TO ALTER MESENCHYMAL STEM CELL DIFFERENTIATION POTENTIAL

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ABSTRACT

Degenerative disc disease is a major cause of workplace disability and costs the US $100 billion annually. A tissue donation of an intervertebral disc (IVD) is able to restore disc height, function, and mobility in a human patient. However, this solution relies on tissue donations, which are scarce, and has drawbacks like rejection by the host. This has led to significant interest in generating a tissue-engineered IVD. Despite significant progress, current tissue-engineered solutions have been unable to generate the tissue gradients in the IVD that contribute to its unique mechanical properties. We seek to explore techniques to generate these gradients using the lentiviral dCas9-KRAB system, a genetic engineering tool that modifies a cell's genome to express dCas9-KRAB and a single guide RNA to epigenetically downregulate target genes. We aim to utilize the dCas9-KRAB system to alter the differentiation potential of human adipose-derived mesenchymal stem cells by downregulating the transforming growth factor β receptors TGFBR1 and TGFBR2. We were able to attain up to 55% downregulation of target genes. This shows that we can regulate expression of key cell receptors, which should allow us to selectively modulate the differentiation potential of stem cells for the generation of cellular gradients in the creation of a tissue-engineered IVD.