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Investigating CRISPR-Cas9-mediated knock-in for PECAM-1 reporter line generation in HEK293T cells.

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Introduction: Platelet Endothelial Cell Adhesion Molecule (PECAM-1) is a transmembrane glycoprotein crucial for vascular endothelial cell (VEC) differentiation from precursor cells. Monitoring PECAM-1 expression within human induced pluripotent stem cell (hiPSC) models is essential for understanding VEC differentiation. Here, we aimed to demonstrate the utility of CRISPR Cas-9 in generating a PECAM-1 fluorescent reporter line, allowing spatiotemporal analyses of PECAM-1 expression.

Body: We designed a gRNA-p2Cas9-2A-EGFP plasmid targeting PECAM-1 for double strand breaks (DSB), and an EGFP-2A-PuroR PCR donor DNA cassette for stable integration into the PECAM-1 locus via homology-directed repair (HDR). Agarose gel electrophoresis and sequencing analysis of the gRNA-Cas9 plasmid validated the successful creation of both elements specific to the PECAM-1 genomic locus. To assess the functionality of the gRNA and donor DNA in human cells, these components were transfected into HEK293T cells, with EGFP expression subsequently analyzed using fluorescent microscopy. Fluorescent microscopy of transfected HEK293T cells revealed low EGFP expression in the gRNA plasmid and donor DNA template, suggesting poor transfection efficiency. Additionally, non-viability of HEK293T cells post-puromycin selection indicated donor DNA integration failure.

Conclusion: These experiments provided important insights into necessary optimizations for successful CRISPR-Cas9-mediated PECAM-1 fluorescent reporter line generation, including protocol modifications to improve transfection efficiency. Successful refinement of techniques will enable the transition of such reporter lines to more physiologically relevant models, such as hiPSC models and cardiac organoids. With the improvement of cardiac organoid technology, new prospects, such as advancements in drug testing and transplantation technology, may become a reality in the future.

Biography

I am a fourth-year medical student at King's College London with a dedicated interest in cardiology. I recently completed an intercalated BSc in Cardiovascular Medicine at King's College London (A BHF Centre for Excellence), for which I achieved First Class Honors. I undertook a project at the James Black Centre for Cardiology, focusing on cardiac organoid technology. This enhanced my understanding of genomics within cardiology and the pre-clinical underpinnings of cardiac intervention. Additionally, undertaking this Bsc furthered my clinical skills by engaging with echocardiography and point of care ultrasound. These experiences have strengthened my commitment to advancing in the field of cardiology.

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