A CRISPRi-Seq screen for functional assessment of BRCA1 mutants

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Introduction

For the proper functioning and stability of the genome, the BRCA1 gene is very essential in DNA repair. Mutations in this gene raise the risk of breast and ovarian cancer[1]. There are limitations to the variant pathogenicity assays currently in use. Although targeted genome editing is possible with CRISPR-Cas9, it is ineffective for large genes like BRCA1[2]. Precise nucleotide substitutions are made possible by Base Editor 3 (BE3), which facilitates functional assessment and helps detect the pathogenicity of unknown BRCA1 variants[3].

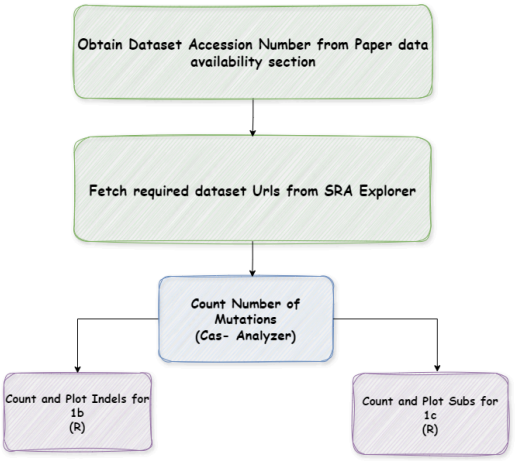
Significance

CRISPR-mediated base editing can aid in the functional analysis of BRCA1 variants, helping to identify novel variants and reclassify variants of uncertain significance. Utilising CRISPR technology for functional assessment provides a powerful tool for evaluating the pathogenicity of BRCA1 variants, potentially guiding personalised treatment strategies for patients with identified mutations. This study's findings can contribute to improving the accuracy of clinical genetic testing for BRCA1 mutations, enhancing the management of individuals at risk of breast and ovarian cancers.

Aim

This study aims to identify the CRISPR-induced mutations in the BRCA1 gene including insertions, deletions and substitutions.

Pipeline



Results

Available in the GitHub repository

Discussion

CRISPR is a gene-editing technology which involves two vital parts: a gRNA and a protein. This technology allows modifications to the genome [4], [5].The CRISPR-associated protein 9 (Cas9) and the deaminase apolipoprotein B mRNA editing enzyme catalytic subunit 1 (BE3) [6] were used in this study.

HAP1-Cas9 cells were used to disrupt BRCA1 with gRNAs, showing decreasing indel frequencies over time with olaparib treatment. HAP1-BE3 cells introduced pathogenic BRCA1 mutations (c.81-1G>A, c.191G>A) with reduced substitution frequencies, contrasting with a likely benign variant (c.5252G>A) retaining its frequency.

References

1. Roy R, Chun J, Powell SN. BRCA1 and BRCA2: different roles in a common pathway of genome protection. Nat Rev Cancer. 2011;12: 68–78.
2. Knott GJ, Doudna JA. CRISPR-Cas guides the future of genetic engineering. Science. 2018;361: 866–869.
3. Kweon J, Jang A-H, Shin HR, See J-E, Lee W, Lee JW, et al. A CRISPR-based base-editing screen for the functional assessment of BRCA1 variants. Oncogene. 2020;39: 30–35.
4. Asmamaw M, Zawdie B. Mechanism and Applications of CRISPR/Cas-9-Mediated Genome Editing. Biologics. 2021;15:353-361
5. Redman M, King A, Watson C, et al. What is CRISPR/Cas9? Archives of Disease in Childhood - Education and Practice 2016;101:213-215.
6. Zhang D, Hussain A, Manghwar H, et al. Genome editing with the CRISPR-Cas system: an art, ethics and global regulatory perspective. Plant Biotechnol J. 2020;18(8):1651-1669.