A CRISPR-Based Base-Editing Screen for the Functional Assessment of BRCA1 Variants

Introduction

BRCA1 is a tumor suppressor gene crucial for maintaining genome integrity (Mehrgou & Akouchekian, 2016). Mutations associated with this gene lead to the development of various tumor types, such as prostate and breast cancers (Kweon et al., 2020). The development of the CRISPR-based base-editing system is one of the most revolutionary aspects of scientific research. It offers a high-throughput analysis of the genome under study by inducing guided mutations on its constituting genes (Kweon et al., 2020). This allows us to understand the functional impacts of gene variants, including the BRCA1 gene.
This study aims to evaluate the likelihood of functional assessment of BRCA1 in the HAP1 cell line and find which mutations have the most significant consequences. To achieve this aim, the study aims to calculate and report indel and substitution rates in transfected HAP1 cell lines through deep sequencing. The significance of this study lies in its potential to present the HAP1 cell line as a viable system for studying BRCA1 function and its role in cancer development.

Methods

CRISPR-mediated mutagenesis was done by transfecting HAP1 cell lines with gRNAs targeting the BRCA1 gene. HAP1 cells were infected with lentiviral particles of Cas 9 and BE3 to form HAP1- Cas 9 and HAP1- BE3 cell lines. Two BRCA1–targeting gRNA KO# 1 ACTGTGAAGGCCCTTTCTTC and KO#2 AACTTCTCAACCAGAAGAAA were introduced into the HAP1-Cas9 cell line. The CCR5-targeting gRNA TGACATCAATTATTATACAT was used as control. The mutated genes were measured through deep frequency. Further, HAP1-BE3 was used to induce pathogenic mutation to alter the function of BRCA1. Two gRNAs were used i) CCAGACTAGCAGGGTAGGGG (81-1G>A) and ii) TTACATAAAGGACACTGTGA (191G>A). The gRNA TTGCTCGCTTTGGACCTTGG (5252G>A) was used as a control. After deep sequencing, the FASTQ reads were taken through quality checks using FASTQC, and trimming was done using FASTP. The files were then processed using cas-analyzer http://www.rgenome.net/cas-analyzer/#! using the BRCA1 transcript NG_005905.2 as the reference sequence. The indel rate and substitution rate were obtained by dividing the number of insertions and deletions (substitutions in the case of substitution rate) by the total reads. Subsequent data analysis and plotting were done using R.

Results

The relative indel frequency decreased relatively with time where there were knockouts compared to controls (Figure 1b). Further, for the BE3 system, we showed that the pathogenic variants were decreased while the benign variant was retained as evidenced by a decrease in substitution rate (Figure 1c). Through the BE3 system, we show that 81-1G>A and 191G>A mutations are pathogenic.

Discussion

This study proves that by using CRISPR tools (Cas 9 and BE3), the HAP1 cell line can be used in the functional assessment of BRCA1. This agrees with other studies like Brown et al. (2021) who also demonstrated that HAP1 cell lines can be CRISPR-targeted to induce mutations. However, the current study provides proof that this system can be used to study the pathogenic effects of BRCA1 mutations and that 191G>A and 81-1G>A are pathogenic (Kweon et al., 2020). In this study, the 191G>A had the most significant consequences among the pathogenic mutations.

References

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Figures

- ➔ Project workflow
- ➔ Figure 1a
- ➔ Figure 1b
- ➔ Figure 1c