UNIVERSITI TEKNOLOGI MARA

THE GENERATION OF CYP2C9 KNOCKED-DOWN HUMAN HEPATOCYTE LIBRARY: A UTILIZATION OF CRISPR/CAS9 SYSTEM

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ABSTRACT

There is an issue of inconsistencies of working with hepatocytes obtained from different human liver donors in drug metabolism studies. CRISPR type II (Cas9) is the most recent development in biotechnology that could potentially resolve this limitation by precise and efficient alteration of genetic sequences. It works in almost any kind of living cells and became possible after the recent discovery of genome editing technologies. Clustered regularly interspaced short palindromic repeats (CRISPR) – associated nucleus (Cas), allows for small changes to a known, targeted location on the DNA sequence efficiently at a faster rate, and ease of use. This study aims to construct guide RNAs into PX461 containing Cas9 and the sgRNA scaffold specifically to target the gene *CYP2C9*. This CRISPR/Cas9 vector will then be tested for its ability to down regulate *CYP2C9* expression. A 20-nucleotide guide RNA was designed to target the gene of interest. The mutant plasmid was derived from *Streptococcus pyogenes* with enhanced green fluorescent protein (EGFP), and cloning backbone for sgRNA produces a Cas9 D10A mutant nickase (Cas9n). This study provides an optimization of CRISPR/Cas9 methods development to knock-down the metabolizing CYP2C9, starting from selection of target, evaluation of cleavage efficiency and finally analysis of the target activity. The genetic modification steps in this study were validated at each stage, using standardised experimental guidelines. Result from this study showed that the CRISPR/Cas9 structure was observed to introduce a single break (nick) within the *CYP2C9* gene. In summary, the designed CRISPR/Cas9 system developed have successfully lowered the expression of rifampicin-induced CYP2C9 enzyme by two-fold in Hep-G2 cell line, and more than five-fold reduction in Huh-7 cell line.

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CHAPTER 1

INTRODUCTION

1.1 Research Background

The study of the effects of genetic mutations through *in vitro* approach is now widely used for studies on drug metabolism. This approach is especially paramount to study drug biotransformation due to the limitations of genotype manipulation technology on complex living hosts. The *in vitro* study is an approach used to filter the mechanism of drug metabolism, a drug biotransformation pathway that subsequently will be used for further in-depth, whole organism studies. However, there are limitations to an *in vitro* study, one of which is the difficulty in getting a complete picture of how drugs work within the complexity of a whole organism or *in vivo*. Additionally, hepatocytes and microsomes may provide inconsistent harvest results between individuals requiring more time to optimize a standard workflow, making experiments generally expensive and studies on metabolic ability more challenging.

The aim of this study is to create a modifiable drug metabolism assay from human hepatocytes using CRISPR/Cas9 system to silence targeted or identified metabolism enzymes without compromising the cellular activity of the hepatocytes. This will allow for better measurement of metabolic activity for each desired enzyme, by limiting co-metabolism of the same drug by other enzyme (Gómez-Lechón et al., 2014). Generating an in-house method to manipulate drug metabolizing enzymes may result in a more economical experimental tool for drug metabolism related studies. To test this hypothesis, this study attempted to establish a working method using CRISPR/Cas9 to manipulate the expression of a specific cytochrome enzyme in metabolism of human hepatocytes. Cytochrome P450 family 2 subfamily C member 9 (*CYP2C9*) is the gene of choice due to its extensively studied and well documented characteristic. The long-term outcome of this study is to create a CYP modifiable hepatocyte library, to allow for better measurement of metabolic activity for each desired enzyme, by limiting co-metabolism of the same drug by other enzymes. This study is the beginning of an exploration to modify (enhance the expression or reduce the expression) the expression of Cytochrome P450 (CYP450) enzymes. The first step