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Mutagenesis Analysis of *ABCB4* Gene Promoter of *Danio rerio*

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ABSTRACT

Zebrafish *abcb4* gene (ortholog to human *ABCB1* gene) serves primarily in multidrug resistance (MDR) mechanism by effluxing chemotherapeutic agents, chemicals, xenobiotics, and numerous anti-cancer drugs out of the cells. This study aims to identify the specific transcription factor binding sites (TFBS) within the promoter region of zebrafish *abcb4* gene and determine the functional roles of these factors in *abcb4* gene expression regulation via mutagenesis analysis. First, primers were designed to target and amplify the promoter region of zebrafish *abcb4* gene through gradient PCR. The zebrafish *abcb4* gene promoter was then cloned into pGL3.0 vector and sent for sequencing. The sequencing results revealed high similarity to zebrafish DNA sequence from clone DKEY-24I24 in linkage group 16, indicating a successful cloning of targeted gene. Thereafter, consensus sequence of zebrafish *abcb4* gene promoter was generated with the length of 1,392 bp which was close to its expected size during primer design (1,500 bp). Using MATCH tool, 155 TFBSs were found within zebrafish *abcb4* gene promoter region. Activator protein 1 (AP-1) TFBS at 1,255 bp was chosen to be mutated through site-directed mutagenesis. Mutagenic primers (forward primer: 5' GGG CAA GGC AGT ATA AAC GTG 3' and reverse primer: 5' TTA TGT TTC TAG GGA TTA CGT CAC 3') were designed to substitute AGT with GGG to remove the AP-1 TFBS. By mutating the zebrafish *abcb4* gene promoter, the MDR phenomenon driven by zebrafish *abcb4* gene can be elucidated and this might provide clues to the development of tumor and malignancy in human. The results from this study may enrich the knowledge in chemotherapy and cancer treatments.

Keywords: ABC transporters gene family, multidrug resistance, site-directed mutagenesis, promoter, xenobiotics, transcription factor

INTRODUCTION

Tumor occurs very commonly in humans, some mammals and even in plants. Generally, tumor can be classified into three main types, namely benign, premalignant and malignant tumors. Azizah *et al.* (2019) reported that the mortality rate among the cancer (malignancy) patients is relatively high in Malaysia despite the vast availability of modern health facilities and medical chemotherapy treatments. This is probably due to the resistance of cancer cells to the chemotherapeutic agents over time.

The resistance to chemotherapeutic agents and some other anti-cancer drugs is driven by the ATP-binding cassette (ABC) transporters in multidrug resistance mechanism. Ferreira, Costa, and Reis-Henriques (2014) discovered the role of ABC proteins in multidrug resistance (MDR) for chemotherapeutic treatments. According to Annilo *et al.* (2006), all the ABC protein subfamilies (except for ABCH subfamily) found in zebrafish correspond to their respective human counterparts. For instance, the zebrafish *abcb4* gene is orthologous to that of the human *ABCB1* gene. Ergo, zebrafish *abcb4* gene is functionally similar to human *ABCB1* gene and they are important in cellular resistance to chemicals (Fischer *et al.*, 2013).

Previously, the *abcb4* gene of zebrafish and Sarawak rasbora (a close family member to zebrafish) has been functionally characterized by Fisher *et al.* (2013) as well as Lim *et al.* (2018a), respectively, and they had come to the same consensus that this gene has significant expressions in the intestinal tract of both species. In addition, the transcriptional analysis performed by Hamli (2019) as well as Lim and Chung (in press) on *abcb4* promoter of zebrafish showed that the zebrafish *abcb4* regulatory region is mainly controlled by SORY, HOMF, BRNF and HOXF. However, available knowledge and findings about zebrafish *abcb4* gene regulation is still