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Mutagenesis Analysis of *ABCG2* Gene Promoter of Zebrafish (*Danio Rerio)*

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ABSTRACT

Breast cancer is the commonest cancer among women worldwide and the probability of a woman dying from breast cancer is high (about 1 in 38 of total human population (2.6%)).The main factor for mortality is due to the resistance of this particular disease to chemotherapeutic agents. One of the most well-known proteins to be found to correlate significantly with breast cancer resistance to chemotherapeutic agent is the ATP-binding cassette super-family G member 2 (ABCG2). Knowledge on *ABCG2* gene regulation is still lacking in terms of how the increased cytotoxic levels are closely related to induce a hype in gene transcript levels and ultimately cause of the reduction in chemotherapeutic agents. The approach taken in this study is through mutational analysis of selected transcription factor governing the expression of *ABCG2*. In order to achieve this, a previously cloned *ABCG2* promoter which has been isolated (around 1500 bp in size) from *Danio rerio* and inserted into pGL3.0 plasmid, was subjected to site-directed mutagenesis. Selected transcription factor which is AP-1 was successfully mutated by deletion of 5'- TGACGCG -3' sequence at position 1113 bp from TSS+1 where it would bind in order to define their role in *ABCG2* physiological function. Sequencing result after site-directed mutagenesis shows high similarities about 98% with *ABCG2* gene of *Danio rerio*. Upon validation, it was found that the intended AP-1 binding site has been mutated. In future work, the mutated clone here will be subjected to transfection analysis where dual-luciferase assay will be conducted to verify the loss of activity from the *ABCG2* promoter upon mutation of the targeted AP-1 site. Hence, the mutagenesis analysis of *ABCG2* promoter are able to provide information on the involvement of AP-1 transcription factor in multidrug resistance mechanism of breast cancer and thus will be a potential target for chemotherapeutic agent.

Keywords: *Danio rerio*, *ABCG2* promoter, site-directed mutagenesis, transcription factor, xenobiotics

INTRODUCTION

About one in 20 women in the Malaysia suffers from breast cancer and the disease rate varied across the three main races, the Malays, the Chinese and the Indians (Yip, Bhoo, & Teo, 2014). ATP-binding cassette super-family G member 2 (*ABCG2*) gene expression showing correlation with grade of tumor advancement and high *ABCG2* gene expression level is associated with poor survival in early stage breast cancer patients (Maciejczyk *et al*., 2012). ABCG2 protein is well known as one of the ATP-binding cassette transporters (ABC transporters) which is capable to act as multidrug resistance because of its potential role in protecting the breast cancer stem cells (Mo & Zhang, 2012).

According to Hu *et al*. (2020), ABCG2 protein may produce resistance to chemotherapeutic agents. ABCG2 protein is responsible to control the movement of harmful and beneficial substrates such as flavonoids and phytoestrogens across the intestinal cells into the intestinal lumen. Therefore, inhibition of carcinogen substrate presence in living tissue will reduce the absorption of carcinogen substrates from the diet (Andersen *et al*., 2015). At the same time, ABCG2 function in transferring the chemotherapeutic drugs out of the cells and keeping the intracellular drug compound below the toxic level (Sukowati, 2012).

The functional characterization of *ABCG2* gene has been reported before on members of Danioninae like Sarawak rasbora and zebrafish (Kobayashi *et al*., 2008; Lim *et al*., 2018a). In addition, an *in vivo* spatiotemporal expression analysis has been conducted lately by Lim and Chung (in press) on *ABCG2* gene promoters in zebrafish