UNIVERSITI TEKNOLOGI MARA

EXPRESSION ANALYSIS OF GASTRIC CANCER ASSOCIATED GENES IN *BPIFB2* STABLE CELL LINES

NUR AMIRA BINTI ZULKIFLI

MSc

December 2021

AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the results of my own work, unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

I, hereby, acknowledge that I have been supplied with the Academic Rules and Regulations for Postgraduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

Name of Student	:	Nur Amira Binti Zulkifli
Student I.D. No.	:	2015625086
Programme	:	Master of Science (Applied Biology) – AS751
Faculty	:	Applied Sciences
Thesis Title	:	Expression Analysis of Gastric Cancer Associated Genes in BPIFB2 Stable Cell Lines
Signature of Student	:	 ……..
Date	:	21 December 2021

ABSTRACT

Gastric cancer (GC) is one of the leading causes of cancer morbidity worldwide. Most GC cases are detected at a later stage with poor prognosis and much of the molecular mechanism involved is poorly understood. Bactericidal/Permeability-Increasing Fold containing family B member 2 (*BPIFB2*) belongs to the lipid transfer/lipopolysaccharide binding protein where the gene and protein mainly express in the oral cavity, nasopharyngeal region, and stomach. Currently, limited data is available on *BPIFB2*. Differential expression of *BPIFB2* was reported in diseases such as mucoepidermoid carcinoma and oral squamous cell carcinoma and its exact function and role in GC has never been investigated. However, preliminary gene expression study showed that the gene is differentially expressed in GC tissue. This study aims to investigate the role of *BPIFB2* in GC as well as its association with other GC-related genes by using gene expression analysis and to generate GC cell lines stably overexpressing *BPIFB2* as *in vitro* models for GC research. Generation of *BPIFB2* expression vector construct (designated MEX6BP2) was carried out using pcDNA™6.2/cLumio™-DEST plasmid and the GC cell lines (AGS, HGC-27, MKN45) were transfected with MEX6BP2 using Turbofect™ transfection reagent. *BPIFB2* stable cell lines of AGS, HGC-27 and MKN45 cells were successfully generated whereby the expression vector was integrated into the genome of the cells. Subsequently, fluorescence and confocal microscopy were carried out to determine the localisation of *BPIFB2* in the GC cells, the Lumio-tagged *BPIFB2* protein were mostly found localised in the cytoplasm. By using real-time QPCR, the baseline and differential expression levels of *BPIFB2* in relation to other GC-associated genes were measured. QPCR analysis showed significant increase of *BPIFB2* level in the transfected GC cell lines (all having p=.001*) which then caused differential expression of the GC-associated genes. In AGS cells, *BPIFB2* overexpression significantly downregulated expression levels of the GC-associated genes, whereby *BPIFB1, CDH1, CDH2, SNAI1,* and *VIM* have a p-value of p=.001* while *MUC5AC* has p=.049*. In HGC-27 cells, upregulation of *BPIFB1* (p=.708)*, CDH2* (p=.075)*,* and *SNAI1* (p=.085)*,* were not significant, only *CDH1* (p=.015*) upregulation together with *VIM* (p=.027*) and *MUC5AC* (p=.001*) downregulation were significant. In MKN45 cells, *BPIFB1* (p=.002*)*, CDH1* (p=.001*)*, SNAI1* (p=.001*)*,* and *VIM* (p=.001*) expression levels were increased significantly except for *CDH2* (p=.106*) while *MUC5AC* (p=.001*) was significantly downregulated. In conclusion, we have successfully generated AGS, HGC-27, and MKN45 cell lines stably overexpressing *BPIFB2* to be used as *in vitro* models for GC research. We also demonstrated molecular cross-talking between *BPIFB2* and the GC-associated genes in these GC cells and these findings may help in elucidating the exact role and function of *BPIFB2* in GC.

ACKNOWLEDGEMENT

All praise and thanks be to Allah S.W.T.

I am eternally grateful to Allah the Almighty for giving me this precious opportunity to embark on my MSc research and for the successful completion of this challenging journey. I convey my greatest gratitude and appreciation to my supervisor Dr. Maslinda Musa for all her valuable guidance, insightful comments, and for being an exemplary mentor throughout the duration of this study. I sincerely want to thank my co-supervisors, Associate Professor Dr. Siti Hamimah Sheikh Abdul Kadir, Dr. Zeti Rahayu Abdul Karim, and Associate Professor Dr. Khalilah Khalil for all their constructive guidance and suggestions.

My acknowledgement and appreciation goes to all the dedicated staff of the Institute of Medical Molecular Biotechnology (IMMB), the Faculty of Medicine and the Faculty of Applied Sciences whose crucial roles have provided the facilities, equipment, and assistance that are necessary for my research work. Special thanks to my colleagues and fellow lab mates for generously sharing their knowledge and for their assistance during this project.

It is impossible to extend enough thanks and appreciation to my family and loved ones, especially my loving parents, Hamidah Abdul Hamid Ayub and Zulkifli Din for their prayers, love, understanding, and sacrifices. My endless gratitude goes as well to my beloved sisters, Nur Afniza, Nur Amalina, Nur Athira, Nur Aisya Humaira, my brother-in-laws Jasmin Brutus, Gusdhofir Muchyidin, Osama Hassan Imam, and my cherished friends for their consistent encouragement and support. Finally, to my wonderful and dearest best friend Paulius Mažrimas, thank you for being with me through all the ups and downs of this journey.

I dedicate this thesis, my small victory to all of you. Alhamdulillah.

TABLE OF CONTENTS