UNIVERSITI TEKNOLOGI MARA

GENE EXPRESSION ANALYSIS OF *CDH1* AND *MUC5AC* IN SELECTED *BPIFB1* TRANSFECTED AND UNTRANSFECTED GASTRIC CANCER CELL LINES

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Thesis submitted in fulfillment
of the requirements for the degree of
Master of Science
(Biomolecular Science)

Faculty of Applied Sciences

January 2020

ABSTRACT

The human Bactericidal/Permeability-Increasing Fold containing family B, member 1 (BPIFB1), or also known as LPLUNC1, C20orf114, is a member of the BPI/LBP/PLUNC protein family. *BPIFB1* differential expression patterns have been reported in several cancers such as nasopharyngeal carcinoma (NPC) and oral mucoepidermoid carcinoma (MEC), in gastric cancer (GC) the role of *BPIFB1* is not known, but the expression of the gene has been reported in the stomach co-expressing E-cadherin (*CDH1*) together with making 5, tracheobronchial/gastric (*MUC5AC)*, the two genes that had been implicated in GC. To investigate the role of *BPIFB1* in GC development and its interactions with *CDH1* and *MUC5AC*, overexpression of the gene was induced in in-vitro using three different GC cell lines; AGS, HGC-27 and MKN-45. Human *BPIFB1* expression construct was first generated using Gateway cloning technology followed by transfection into the cell lines for generation of stable GC cell lines overexpressing *BPIFB1*. The localization of *BPIFB1* was done through Lumio green and Hoechst staining in transfected GC cell lines. The present of *BPIFB1* protein in a transfected GC cell line was detected through in gel Lumio Green Detection. To quantify the expression level of *BPIFB1* in each of gastric carcinoma, expression of mRNA level of *BPIFB1*, *CDH1* and *MUC5AC* were then performed via RT-PCR and quantitative Real-time PCR (qPCR) for the *BPIFB1* transfected and non-transfected GC cells followed by statistical analysis. *β -ACTIN*, a housekeeping gene was always included in both RT-PCR and qPCR reaction. For *BPIFB1* mRNA level, the expression increased in the transfected cells with the highest in AGS with the p=.003* followed by MKN-45 with the p=.009* and HGC-27 with the p=.015*. The expression of *MUC5AC* is upregulated in *BPIFB1* transfected GC cells whereby the highest expression is observed in *MKN-45* with the p=.05* followed by AGS with the p=.001* and HGC-27 with the p=.026*. *CDH1* expression is downregulated in all *BPIFB1* transfected GC cells with the lowest expression found in HGC-27 with the p=.001* followed by AGS with the p=.003* and MKN-45 with the p=.004*. Thus, induction of *BPIFB1* overexpression in vitro in GC cells contributes to the decreased in *CDH1* expression and increased in *MUC5AC* expression suggesting that *BPIFB1* other than co-expressed in the gastric gland with the two genes also regulates gene expression. By inducing an over-expression of the *BPIFB1* in the GC cell lines we have generated an in-vitro model to elucidate the role of *BPIFB1* in GC and its interactions with *CDH1* and *MUC5AC* the two genes implicated in GC. We found that the overexpression of *BPIFB1* downregulated the expression of *CDH1* and upregulated *MUC5AC* gene expression. This finding was supported by both RT-PCR and qPCR. Work at the protein level and in-vivo needs to be done to support and further validate these findings. Our findings suggest that *BPIFB*1 is differentially expressed in GC cell lines and regulate the expression of *CDH1* and *MUC5AC*.

ACKNOWLEDGEMENT

Firstly, I wish to thank God for giving me the opportunity to embark on my Msc and for completing this long and challenging journey successfully. My gratitude and thanks go to my supervisor Dr. Maslinda Binti Musa and co-supervisor, Associate Professor Dr. Siti Hamimah Binti Sheikh Abdul Kadir, Dr. Zeti Rahayu Binti A. Karim and Associate Professor Dr. Khalilah Binti Abdul Khalil

My appreciation goes to the staff of the Institute of Medical Molecular Biotechnology (IMMB) and Faculty of Applied Science who provided the facilities and assistance during lab work. Special thanks to my colleagues and friends for helping me with this project.

Finally, this thesis is dedicated to the loving memory of my very dear late father (Abdul Aziz Bin Mahasan) and mother husband (Captain Mohamad Faridzal) and daughter (Qisya Nur Qistina) for the vision and determination to educate me. This piece of victory is dedicated to all of you. Alhamdulillah.

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CHAPTER ONE
INTRODUCTION

1.1 Research Background

Cancer was described a disease that caused by an uncontrolled cell growth and addition of metastasis properties (American Cancer Society, 2016). The fatality and incurability cancer make it as a leading cause of death across the world (American Cancer Society, 2016). The most common diagnosed cancer in men were prostate lung, stomach, colorectal and liver cancer (Park, Forman, Waskito, Yamaoka, & Crabtree, 2018). The most common types of cancer in women were cervix, breast, colorectal, and stomach cancer (American Cancer Society, 2016).

Gastric cancer (GC) or also known as a stomach cancer ranked as the third most common cause of cancer related death worldwide and fifth most common malignancy (Park *et al.*, 2018). GC is a disease developed from the lining of the stomach through multiple genetic and epigenetic alteration (Hu *et al.*, 2017). GC known to often occur in men compared to women (American Cancer Society, 2016). GC remains difficult to cure and mostly diagnosed at advanced stage due to lack of early associated symptoms and their prognosis is very poor (Qu *et al.*,2013). GC was also resistant toward a combination of treatment such as surgery, chemotherapy and radiotherapy (Qu *et al.*, 2013). There are many risk factors associated in the development of GC (Park *et al.*, 2018). It may cause by association with *Helicobacter pylori* (*H. pylori*) infection, environmental, dietary factors, genetic susceptibility, and chronic inflammation (Hu *et al.*, 2012). These factors may cause the progression of GC by alter or damage some of the genes or called as mutation. However, how these factors cause the cells in stomach to become a cancerous cell is the thing need to be investigated.

Some studies have verified that several genes, known as oncogenes or tumour suppressors genes (TSGs), were related to the initiation and progression of cancer (Sarkar *et al.*, 2013). The loss function of TSGs and activation of oncogene is an important point in cancer including GC. It is because the TSGs play a role in apoptosis, programmed cell death, repair DNA mistakes and slowly down cell division while oncogenes help the cells to grow (Sarkar *et al.*, 2013). Hence, mutation