THE EXPRESSION PATTERN OF MATRIX METALLOPROTEINASES (MMP-1, MMP-8, MMP-13) IN RELATION TO CTCF AND YB-1 PROTEIN INTERACTIONS ON HUMAN CANCER CELL LINES

BY

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ABSTRACT

Collagenases, which are proteins from the matrix metalloproteinases family, have been implicated to play a crucial role in tumor invasion. Previous studies have also suggested that collagenases may have a significant influence in the expression of CTCF and YB-1 proteins, which have also been implicated in numerous cancer studies. However, the depth of its involvement in the fundamental molecular mechanisms of cancer remains to be poorly known. This study aims to determine the degree of MMP-1, MMP-8, MMP-13, YB-1 and CTCF protein expressions in breast, cervix and skin human cancer cell lines and to ascertain its probable involvement in cancer development. An ELISA assay was conducted in order to estimate the concentration of the proteins that could be found in the cells. The quantitative expressions of MMP-13 proteins in A375, MCF-7 and HeLa cells were evaluated through ELISA test. The results showed a marked expression of MMP-13 proteins in MCF-7 (12900 pg/mL), followed by HeLa (8109 pg/mL) and A375 (7515 pg/mL). This correlated to the Western blot assay where MCF-7 showed the strongest protein expression followed by HeLa and A375. The qualitative expressions of the proteins in cervix cancer (HeLa), breast cancer (MCF-7), skin melanoma (A375) and normal fibroblast (CCD1090Sk) cells were demonstrated through Western Blot analysis. The results showed that the strongest expression for MMP-1 was detected in HeLa cells at ~52 kDa, while MCF-7 and A375 cells produced very weak or almost non-detectable protein expressions. In contrast, MMP-8, MMP-13, CTCF and YB-1 proteins were highly expressed at varying degree of expressions in all cell lines. For MMP-8 analysis, multiple bands were detected at ~70 to 45 kDa, while MMP-13 only produced a single band at ~48 kDa. The results also showed the detection of CTCF protein bands ranging from ~180 to ~80 kDa and YB-1 protein expressions at ~55 kDa. In Co-Immunoprecipitation assay, results showed that most of the protein-protein interactions caused the expressions of most of the proteins to be highly up-regulated though some of the interactions also resulted in a decreased in protein expression. It was taken note that although the molecular mass of the proteins correlated to its respective Western blot assay results, their protein expressions were either highly expressed or were of less prominence. It was elucidated that the increase and decrease of the protein expression may have been caused by the interactions and binding of the proteins to its protein partners which then affect cellular regulation. Overall, although variations were found in the expression of the collagenase in various human cancer cell lines, the results depicted a prominent presence and the probable involvement of MMP-1, MMP-13, YB-1 and CTCF proteins in the development and metastasis of cancer. Thus, it is believed that obtaining a detailed understanding of collagenases and its interacting partners may be vital for future endeavours in respect to the possible design of therapeutic MMP strategies for prospective targeted human therapeutic treatments.

خلاصة البحث

ولقد تم استخدام طريقة الاليزا المختبرية للكشف عن الفائدة النوعية لاختبارات الوستيرن بلوتنك والكو اي بي ومن ثم مقارنتها بالنتائج النوعيه والكمية باستخدام طريقة الاليزا.ولقد تم تقييم التعبير البروتيني الكمي لبروتين الام ام بي ثلاثة عشر في خلايا سرطان البشرة وسرطان الثدي وسرطان عنق الرحم باستخدام طريقة الاليزا واظهرت النتائج ان هناك انتاج كميات ملحوظه من بروتين الام ام بي ثلاثه عشر في خلايا سرطان الثدي وبكميات اقل في سرطان عنق الرحم وسرطان البشرة ولقد تطابقت هذه النتائج مع نتائج الوستيرن بلوتينك حيث اظهر سرطان الثدي كميات اكثر من البروتينات بالمقارنه مع سرطان عنق الرحم وسرطان البشرة. ولقد تم قياس مستوى التعبير البروتيني لمختلف البروتينات في خلايا سرطان عنق الرحم وسرطان الثدي وسرطان البشرة وخلايا البشرة الطبيعيه باستخدام طريقة الويستيرن بلوتينك المختبريه وقد اظهرت النتائج ان اقوى تمثيل لبروتين الام ام بي واحد كان في خلايا سرطان عنق الرحم عند 52 كيلودالتون بينما كان انتاج هذا البروتين قليل جدا او غير ملحوظ في خلايا سرطان الثدي وسرطان البشرة. ومن الناحية الاخرى كانت كميات بروتينات الام ام بي ثمانية والام ام بي ثلاثة عشر وبروتين السي تي سي اف والواي بي وان موجودة بشكل ملحوظ وبمستويات مختلفه. بالنسبة للام ام بي ثمانيه اظهرت النتائج عدة طبقات عند 70 والى 45 كيلودالتون بينما كانت للام ام بي ثلاثة عشر طبقة واحده عند 48 كيلو دالتون. اظهرت النتائج ايضا ان طبقات بروتين السي تي سي اف المكتشفه كانت تتراوح بين 180 و 80 كيلودالتون بينما كان مستوى بروتين الواي بي وان عند 55 كيلو اما نتائج الكو ميونو بريسبتيشن او عملية الترسيب المناعي ولقد تم الاخذ بالاعتبار دالتون. من الناحية الجزيئية للبروتينات وبالمقارنه مع نتائجها في الويستيرن بلوتينك ان مستوى التمثيل البروتيني كان اما اكثر او اقل وظوحا ولقد تم اعزاء ذالك بسبب التفاعلات والارتباطات بين مختلف البروتينات مع شريكاتها والذي اثر على عملية التنظيم الخلوي .

APPROVAL PAGE

I certify that I have read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Health Sciences.

.....
Mohammad Syaiful Bahari Abdull Rasad
Supervisor

I certify that I have read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Health Sciences.

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Ridhwan Abdul Wahab
Examiner

I certify that I have supervised and read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Health Sciences.

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This thesis was submitted to the Kulliyyah of Allied Health Sciences and is accepted as a fulfilment of the requirement for the degree of Master of Health Sciences.

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Wan Azdie Mohd Abu Bakar
Dean, Kulliyyah of Allied Health Sciences

DECLARATION

I hereby declare that this thesis is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

Nur Aizura Binti Mat Alewi

Signature …… Date ……..

INTERNATIONAL ISLAMIC UNIVERSITY MALAYSIA

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Affirmed by Nur Aizura Binti Mat Alewi

……..……..…… ……..

Signature Date

To my beloved family, may they always be blessed by Allah S.W.T.

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In the name of Allah the Most Beneficent and the Most Merciful

Alhamdulillah, praise to Allah S.W.T. as because of His Mercy and Guidance I was able to complete this thesis within the duration given to me, as well as blessing me the joy of accomplishment after the years spent in sweat and tears to finish this thesis.

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I would like to take this opportunity to express my heartfelt appreciation to my family for the moral and financial support as well as their earnest encouragement on which I assemble my confidence on. Their love and patience did not falter during my long term of absence due to my full commitment towards this study. Thank you for all of your prayers. Not forgotten, to my fellow postgraduate friends and colleagues for their assistance as well as their fair share of contribution toward the effort on my project.

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Lastly, may Allah S.W.T. accept this endeavour as '*amal jariyah*' and may He forgive any faults or shortcomings that may be found in this study, for which I admit sole responsibility.

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LIST OF ABBREVIATIONS

AP-1	Activator protein 1
APS	Ammonium pershulphate
ATCC	American Type Cell Collection
BRCA1	Breast cancer 1, early onset
BRCA2	Breast cancer 2, early onset
BST	Biohazard safety cabinet
C_2H_2	Ethyne
C_2HC	Cys-Cys-His-Cys
CAPS	3-[cyclohexylamino]-1-propanesulfic acid
CBE	Clinical breast examination
cDNA	Complementary DNA
ChIP	Chromatin-Immunoprecipitation assay
CO_2	Carbon dioxide
Co-IP	Co-immunoprecipitation
CSD	Cold shock domain
CTSs	CTCF target sequences
CTCF	11-zinc finger protein or CCCTC-binding factor
DMEM	Dulbecco's modified eagle's medium
DMSO	Dimethylsulphoxide
DNA	Deoxyribonucleic acid
E.g.	Example given
ECM	Extracellular matrix
EGF	Epidermal growth factor
ELISA	Enzyme-linked immunosorbent assay
EMEM	Eagle's minimum essential medium
ERK	Extracellular signal related kinase
FBS	Fetal bovine serum
FW	Formula weight
G	Guanine base
H_2O	Water
HCl	Hydrochloric acid
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HGF	Hepatocyte growth factor
HPV	*Human papillomavirus*
IARC	The International Agency for Research on Cancer
IFN	Interferon
IGF-1	Insulin-like growth factor-1
IgG	Immunoglobulin G
IL	Interleukin
iP	Isoelectric point
JNK	c-Jun N-terminal kinase
KCl	Potassium chloride
kDa	kilodaltons

KH_2PO_4	Potassium dihydrogen phosphate
MMP	Matrix metalloproteinase
MRI	Magnetic resonance imaging
MT1-MMP	Membrane-type metalloproteinase
Na_2HPO_4	Sodium hydrogen phosphate
NaCl	Sodium chloride
NaOH	Sodium hydroxide
NP-40	Nonyl phenoxypolyethoxylethanol
PBS	Phosphate buffer saline
PDGF	Platelet-derived growth factor
PenStrep	Penicillin-streptomycin
PKC	Protein kinase C
PMN	Polymorphonuclear leukocyte
PMSF	Phenylmethylsulfonyl fluoride
PVDF	Polyvinylidene fluoride
PYK	Praline-rich tyrosine kinase
RNA	Ribonucleic acid
S.D.	Significant difference
S.E.M.	Standard error of the mean
SDS	Sodium dedocyl sulphate
SDS-PAGE	Sodium dedocyl sulphate-Polyacrylamide Gel Electrophoresis
SPF	Sun protection factor
T25	25 cm^2 tissue culture flask
T75	75 cm^2 tissue culture flask
TBS	Tris Buffered Saline
TEMED	Tetramethylethylenediamine
TGF	Transforming growth factor
TGF-α	Transforming growth factor-alpha
TIMP	Tissue inhibitor
TNF	Tumor necrosis factor
Tris-HCl	Tris- Hydrochloric acid
TrypLE	TrypLE™ Express
UK	United Kingdom
USA	United States of America
UV	Ultraviolet
v/v	Volume/volume
w/v	Weight/volume
WHO	World Health Organization
YB-1	Y-box binding protein
ZF	Zinc finger

CHAPTER ONE

INTRODUCTION

1.1 SUMMARIZATION OF CHAPTERS

There are many achievements which were made possible through the development and advancement of science and technology in the area of cancer research. Nowadays, the technologies available to mankind are far more sophisticated and advanced. Most assuredly, these technologies have, and will make, a great impact on the life of mankind and their health as far more in depth searches and understanding could be attempted in finding the cure for cancer. Consequently, this thesis experimented and discussed on the expression of collagenases in different cancer cells as well as attempting to identify MMP-interacting protein partners in order to elucidate biological consequences of MMPs binding to different targets. To dissect the many steps and factors that signify the importance of this thesis in the matter of cancer research, the thesis was divided into several chapters.

In Chapter 1 and Chapter 2, an in depth explanation of each aspects that composed this thesis was elucidated in detail. From the great impact of cancer towards health and the various approaches made by man to treat it, to the protein-cellular components of the MMPs (MMP-1, MMP-8 and MMP-13) and transcription factors (CTCF and YB-1) utilized in this study, every aspect was comprehensively described so as to better understand their impact in life. Through the facts presented, the objectives of the study were also highlighted so that its importance and relevance in the promising field of molecular science was clarified.

This was followed by Chapter 3 that focused upon the basic requirements and protocols that were necessary for the purpose of setting up and implementing the various procedures required for cellular, proteomic and molecular experimentations that were relevant to the study. Concise explanations for every procedure, such as Western blotting and co-immunoprecipitation (Co-IP) assay, were illustrated so that a step-by-step methodology of the study could be outlined. Furthermore, the importance and connections of each procedure to one another was also demonstrated. Hence, a perceptive on how the various procedures applied in this study were constructed and integrated into a single methodology in order to attained the required results were established.

The focus of Chapter 4 was on the results that were obtained and the approaches used in order to discuss their significance with relevance to the objectives of the study. Descriptions and explanations of each important fact were written at great length though emphasis was put upon the interactions between the collagenases, CTCF and YB-1 proteins that could have affected the cell growth. Thus, through this chapter, an understanding on the mechanisms and significance of the proteins in the development of cancer was achieved with reflection upon accomplishing the objectives set earlier in the study. In addition, a brief outlook on the Islamic perspective of this study was also discussed which act as not only an optional necessity for the University's requirement, but also as a platform of ideas for Muslims researchers to incorporate Islamic concepts and laws into their scientific researches. By incorporating scientific innovations with respect to Islamic boundaries and perceptions, Islam can move forward to achieve betterment in both intellectual and spiritual aspects of life as is encouraged in Islam.

Finally, in Chapter 5, a conclusion was constructed by correlating the objectives of the study to that of the results that were discussed. Overall, the study achieved its aims and was able to elucidate the biological significance of the interaction between MMP-1, MMP-8, MMP-13, CTCF and YB-1 in cancer.

1.2 PROBLEM STATEMENT

At a worldwide scale, 14.1 million cancer cases were estimated for the year 2012 and of these cases 7.4 million were recorded in men, while 6.7 million were in women (Ferlay, Soerjomataram, Ervik, Dikshit, Eser, Mathers, Rebelo, Parkin, Forman and Bray, 2014). In Malaysia, the incidence of cancer that were reported in 2012 were estimated to be around 37,426 cases, where 21,678 of those cancer cases resulted in death (Ferlay et al., 2014). Hence, it could clearly be perceived that cancer is among the most life-threatening disease which is not only affecting human life, but also the economical state of the world.

Cancer, since it has existed for decades, is a disease known to be as old as the beginning of life itself. According to the International Agency for Research on Cancer (IARC), cancer is now the world's largest killer as the estimated amount of cases in the years to come are approximated to constantly increase (Ferlay et al., 2014). Even with the recent developments in science and technology (American Cancer Society, 2013a, 2013b and 2013c), the number of cancer cases continue to rise as the survival rate of cancer becomes lower and lower. Therefore, it has been suggested that in order to understand the mechanism and initiate the development of the treatment for cancer, researchers should point their studies to the more basic aspects that constitute cancer.

Many studies, both *in vitro* and *in vivo*, have attempted to prove the correlation of collagenases, CTCF and YB-1 protein interaction and expression profiles with the development of cancers (Noel, Jost and Maquoi, 2008; Egeblad & Werb, 2002; Chernukhin, Shamsuddin, Robinson, Carne, Paul, El-Kady, Lobanenkov and Klenova, 2001), and based on previous studies, common trends have emerged. Consequently, implications of MMPs, CTCF and YB-1 in early stages of tumor evolution, including stimulation of cell proliferation and modulation of angiogenesis, and metastasis, have been widely recorded (Chernukhin et al., 2001; Hidalgo & Eckhardt, 2001). It is a known fact that the existing treatments and screening procedures for cancer have shown low survival rates of cancer patients as well as causing unwanted side-effects to them. Hence, the approach of understanding the basic mechanisms of cancer development through its protein interactions could be seen as a more effective and safer approach towards finding a cure for cancer.

1.3 SIGNIFICANCE OF STUDY

Recognition on the desperate search of a more permanent and effective treatment for cancer is increasing among the worldwide population due to the unrelenting increase in death toll of cancer patients. Recent research interests have been directed towards exploring and understanding the more molecular aspects of cancer development as the more intrusive treatments such as surgery and radiations have not shown much promising results and have shown to cause undue side-effects to the patients.

Consequently, in this study, an attempt was made in order to assess and correlate the expression of collagenases in different cancer cells as well as to identify MMP-interacting protein partners which should sequentially elucidate the biological consequences of MMP binding to different targets. These correlations could possibly

be applied as potential predictive and/or prognostic markers to help determine the best course of treatment in human cancer. The determination of their relative contributions in the context of tumor progression is critical since evidence has suggested that the same MMP can have opposing effects based upon the cell type in which it is expressed (Martin & Matrisian, 2007).

The present study will reveal further information on the characteristic of collagenases and CTCF/YB-1 proteins as well as contributing comprehensive molecular-cellular interactive relationship that could be added to the portfolio of studies on cancer development that would be useful as a guideline for the formulation and approaches of future studies. In addition, this study may influence the production and construction of safer and less intrusive approaches of cancer treatments or screenings that can be benefited by health organizations in helping cancer patients. Therefore, with further studies, better understanding of these proteins and its effects towards cancer progressions could be elucidated in the hopes of finding potentially therapeutic and diagnostic targets for the treatment and detection of human cancers.

1.4 OBJECTIVE

1.4.1 General Objective

The overall objective of this proposed research project is to investigate the characteristics of MMP-1, MMP-8, MMP-13, CTCF and YB-1 protein in human cancer cells.

1.4.2 Specific Objectives

1. To qualitatively detect and assess the expression of selected collagenases (MMP-1, MMP-8 and MMP-13), CTCF and YB-1 proteins in human cervical, skin and breast cancer cell lines.
2. To investigate the probable protein-protein interactions of MMP-1, MMP-8 MMP-13,CTCF and YB-1 with its selected interacting partners, through the qualitative analysis of *in vivo* co-immunoprecipitation assay.
3. To elucidate the potential correlation between collagenases (MMP-1, MMP-8 and MMP-13), CTCF and YB-1 proteins presence and expression in human cancer cells.

CHAPTER TWO

LITERATURE REVIEW

2.1 CANCER

In a normal human body, the organization of cell formation, maintenance and death requires balanced as well as a strict regulatory control of its division, differentiation and growth (Garden, Bradbury, Forsythe and Parks, 2012). Generally, when a group of cells that proceed towards a path of uncontrolled growth and migration, in which if the spreading is not controlled it can result in the death of an organism; it can be regarded as cancer (Wolpert, Tickle, Lawrence, Meyerowitz, Robertson, Jessell and Smith, 2011). These malformed cells serve no purpose and multiply in an atypical and unrestrained approach which later on causes the formation of benign or malignant cells (Garden et al., 2012). In brief, there are many kinds of cancer but they all start because of the out-of-control growth of abnormal cells.

The mechanisms by which cancer is induced are multifaceted and can be influenced by numerous factors. These factors can be either external factors, such as exposure to carcinogens and viruses (Garden et al., 2012), or internal factors that may include mutation of genes and proteins (Raven, Johnson, Mason, Losos and Singer, 2013). Table 2.1 shows a brief summary of major factors that increase the risk of cancer. It is the beliefs of many researchers that these causal factors may act in concert of one another or in succession for the initiation of cancer development.

Table 2.1
The major factors that increase the risk of cancer (Alters and Alters, 2006).

Factor	Examples of implicated cancer	Comments
Heredity	- Retinoblastoma (Childhood eye cancer). - Osteosarcoma (Childhood bone cancer).	Most cancers are not caused by heredity alone.
Tumor viruses	- Liver cancer. - Adult T cell leukemia/lymphoma. - Cervical cancer.	Five viruses are initiators of certain cancers.
Tobacco use	- Lung cancer. - Cancer of the oral cavity, esophagus and larynx. - Cancers of the kidney and bladder.	Cigarette smoking is responsible for approximately one-third of all cancers. Non-smokers have an increased risk of smoking-related cancers if they regularly breathe in side stream smoke.
Alcohol consumption	- Cancer of the oral cavity, esophagus and larynx. - Breast cancer.	The combined use of alcohol and tobacco leads to a greatly increased risk of these cancers.
Industrial hazards	- Lung cancer.	Certain fibers, such as asbestos, chemicals such as benzene and arsenic, and wood and coal dust are prominent industrial hazards.
Ultraviolet radiation from the sun	- Skin cancer.	Those at greatest risk are fair-skinned persons who burn easily. All types of UV radiation in tanning beds (UVA, UVB and UVC) are harmful and may lead to skin cancer.
Ionizing radiation	- Related to location and type of exposure.	Infants and children are particularly susceptible to the damaging effects of ionizing radiation.
Hormones (estrogen and possibly testosterone)	- Breast, cervical, ovarian and prostate cancers.	Estrogen-only and estrogen-progesterone hormone replacement therapies both increase the risk of breast cancer.
Diet	- Breast and prostate cancers (weak association with high-fat diets), stomach and esophageal cancers (nitrites).	Nitrites found in salt-cured, salt-pickled and smoked food increase the risk of cancer.