UNIVERSITI PUTRA MALAYSIA

MODULATION OF C5A RECEPTOR IN MAMMARY GLAND TUMOUR BY EP54 AND PMX205 PEPTIDES

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

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June 2015

Chairman: Mohd Hezmee Mohd Noor, PhD
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Drug resistance has become the main issue in cancer therapy field. This situation causes the increasing number of cancer related disease in the world. The usage of complement 5a has become a new method of therapy against cancer by following agonist-antagonist treatment. This project was mainly about the agonist (EP54) and antagonist (PMX205) modulate the expression of C5aR causing the regression of mouse mammary gland tumour. The objectives of this project were to determine the expression of C5a receptor on 4T1 cell line, to determine the mechanism of mouse mammary gland tumour cell death after treatment with respective peptides, determine the effect of the peptides on mouse mammary gland tumour cell, and to determine the effect of EP54 and PMX205 on the liver and kidneys of mice with 4T1-induced mammary gland tumour. Several methods were conducted such as immunofluorescence staining, PCR, ELISA (TNF-α, VEGF, Caspase 3 and C5a), acridine orange and propidium iodide double staining and serum biochemical analysis. The results showed that the presence of C5a receptor on 4T1 cell line was based on the immunofluorescence staining and PCR. The presence of the receptor showed that the 4T1 cell was suitable to be used with those peptides. The mechanism was determined by using ELISA. Based on ELISA results, it showed that the apoptosis becomes the underlying pathway that is used in mammary gland tumour regression for both environments in vitro and in vivo. These findings showed that the apoptosis is an important process involved in most organisms for survival. In order to validate the findings, acridine orange/propidium iodide staining (AO/PI) and cell viability assay were conducted. Besides, tumour measurements also were used as to validate the mechanism proposed. Both peptides showed capability to present apoptosis based on the AO/PI result. While in the cell viability assay (Alamar Blue & MTT) in which it represents data in vitro, it showed that PMX205 showed greater potential in treating the cancer compared to EP54 group. In Alamar Blue assay, the result showed that the absorbance PMX205 was lower compared to EP54 group. Similar trend could also be found from the MTT assay. Tumour measurement recorded from the in vivo experiment, shows that the size of tumour decreased in EP54 group whereas the PMX205 group, the tumour maintain its own size. In the serum biochemical analysis, no significant effects were obtained on the liver and kidney of the animal. Based on these results, it showed that EP54 and PMX205 could modulate the expression of
C5aR causing the regression of mouse mammary gland tumour. Apoptosis was the underlying mechanism involved during the treatment and the treatment did not produce significant effects on the organ of the animal.
analisis biokimia serum, tiada kesan yang penting telah diperolehi pada hati dan buah pinggang haiwan. Berdasarkan keputusan ini, ia menunjukkan bahawa EP54 dan PMX205 boleh memodulasi ungkapan C5aR menyebabkan regresi tumour kelenjar mama tikus. Apoptosis adalah mekanisme asas yang terlibat dalam rawatan dan daripada rawatan tersebut ia tidak menghasilkan kesan yang besar ke atas organ haiwan.
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“In the name of Allah, Most Gracious, Most Merciful”
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LIST OF ABBREVIATIONS

bp           base pair
BCA        Bicinchoninic acid
CO2           Carbon dioxide
Caspase cysente-dependent aspartate-directed proteases
dH2O         distilled water
DEPC        DiethylenePyrocarbonate
DMSO        Dimethyl sulfoxide
DNA        Deoxyribonucleic acid
FBS        Fetal bovine serum
µg/ml           microgram per milliliter
g            gram
GAPDH         Glyceraldehyde 3-phosphate dehydrogenase
h            hour
HCL          Hydrochloric acid
IBS        Institute of Bioscience
IC50      half maximal inhibitory concentration
kDa        Kilodalton
L            Litre
M            molar
min          Minute
mins         Minutes
ml          Mililiter
mM          Milimolar
MTT          Methylthiazol Tetrazolium
NaCl          Sodium Chloride
NaOH        Sodium Hydorxide
NCR        National Cancer Registry
PBS        Phosphate Buffer Saline
PCR        Polymerase Chain Reaction
PI        Propidium Iodide
RNA        ribonucleic acid
rpm    rotated per minute
RPMI          Roswell Park Memorial Institute
RT-PCR    Reverse Transcriptase Polymerase Chain Reaction
Sec          Seconds
TNF-α        Tumour Necrosis Factor
UPM          Universiti Putra Malaysia
µl            microliter
mg/ml        milligram per milliliter
v/v          volume over volume
w/v          weight per volume
˚C           Degree Celcius
CHAPTER 1

GENERAL INTRODUCTION

Breast cancer is a combination of the most notorious cancers on earth (about 22% of all type of cancers), followed by a malady of the prostate, colon, lung and ovaries, accordingly. According to Parkin et al. (2005), mammary gland disease is also associated with a 14% from cases of all deaths from cancer among women worldwide, and also known as the most common cancer for women in both developing and developed countries. Evidence in 2003 from the National Cancer Registry of Malaysia recorded that about 3738 new cases associated with breast cancer were recorded to the registry on that year, producing an age standardized incidence rate (ASR) of 46.2 per 100,000 women. This mechanism focuses 1 in 20 women in Malaysia purposefulness transport breast cancer in their lifetime (Yip et al., 2006).

There are different types of treatment that can be used to treat breast cancer such as surgery, radiation therapy, hormone therapy, chemotherapy and targeted therapy. Each of these treatments has its own positive or negative effects. For example, in chemotherapy, several kinds of drugs are used during a session. The problem is when certain kind of drugs was introduced to the cancer cell, it will cause some genetic alterations in the cancer cell (Gottesman, 2002), which later causes failure of the respective drugs to work against cancer cells. This situation is known as drug resistance which has also become one of the most common problems that usually occur in cancer therapy.

The study on possible mechanism of tumour regression especially in malignant mammary tumour has gained some focus lately, as it is capable of promoting the development of new drugs or peptides that are useful to be used for cancer therapy as well as yielding a wealth of information about complement therapies in treating cancer diseases. From past to present, it is recorded that the resistance of certain type of tumour towards commercial cancer therapy medicine has become a major problem (Dexter and Leith, 1986). The resistance of tumour towards drugs occurred due to few factors such as host factor and genetic alterations in cancer cells (Gottesman, 2002). Both of these factors contributed towards the failure of cancer therapy.

The complement C5a system has become a potential treatment to be applied in cancer therapy based on its involvement in immune defence mechanism, where it acts as a protector for an organism against the presence of any foreign substances inside an organism. In addition, the expression of complement C5a receptor is not just restricted on myeloid cells such as macrophages (McCarthy and Henson, 1979), basophils and neutrophils (Hook et al., 1975) and eosinophils (Kay et al., 1973), but it is also expressed on non-myeloid cells such as epithelial, endothelial and smooth muscle cells in the human liver and lung (Zwirner et al., 1999). The widespread expression of C5a receptor suggested more of its general and systemic functionality.
The experiment was planned to observe the expression of complement C5a receptor on malignant type of breast cancer model cell that is known as 4T1 cells. Further experiments were constructed to see the effects or interactions between complement peptides, agonist (EP54) and antagonist (PMX205) with 4T1 cell line in vitro and in vivo.

The hypothesis of this study was agonist (EP54) and antagonist (PMX205) modulate expression of C5aR causing regression of mouse mammary gland tumour. The objectives of this study were to determine the:

1) expression of C5a receptor on 4T1 cell line.
2) mechanism of mouse mammary gland tumour cell death after treatment with EP54 and PMX205 peptides.
3) effect of EP54 and PMX205 peptides on mouse mammary gland tumour cell.
4) effect of EP54 and PMX205 on the liver and kidney of mice with 4T1-induced mammary gland tumour.
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