EXPRESSION OF RON TYROSIN KINASE RECEPTOR ON BREAST CANCER CELL LINES

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Special Dedicated to:

All my family whom give me Strength and Full Support

My Beloved Father

The Princess Mother

My Great Brothers
Hani, Majed and Ayman

My Best Friend Ever
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ABSTRACT

RON receptor is one member of the MET family of receptor tyrosine kinases. When it is activated by its own ligand, macrophage stimulating protein (MSP), RON initiates a downstream signaling pathways which result of some function in the cell such as dissociation, migration, and invasion. Several RON variants that arise through mRNA splicing or by an alternate translational start site have been identified, some with oncogenic potential. The purpose of the present project was to determine the expression of RON receptor in two breast cancer cell lines since it is the most common distribution cancer in women around the world. MDA-MB-213 and MCF-7 breast cancer cell lines and WRL-68 embryonic liver as a normal cell line were used in this experiment to characterize the expression. The expression of RON receptor on the cell surface, the concentration of mRNA and the RON protein were done by utilizing Immunofluorescent staining (IF), polymerase chain reaction (PCR) and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) respectively. Collectively, it was found that RON receptor was overexpressed in these two breast cancer cell lines comparing to the normal cell lines.

ABSTRAK

Reseptor RON adalah sebahagian ahli keluarga MET daripada reseptor kinases tyrosina. Apabila RON diaktifkan oleh ligannya sendiri, protein perangsang makrofaj (MSP), RON memulakan isyarat tapak jalan hiliran yang menyebabkan beberapa fungsi sel seperti penceraian, migrasi dan pencerobohan. Beberapa varian RON muncul hasil daripada proses penyuntingan mRNA atau oleh tapak permulaan translasi alternatif telah dikenal pasti, setengahnya berpotensi sebagai oncogenic. Tujuan projek ini adalah untuk menentukan ekpressi reseptor RON pada sel kanser payu dara kerana ia merupakan kanser yang paling biasa tersebar di kalangan wanita di seluruh dunia. MDA-MB-213 dan MCF-7 adalah sel kanser payudara dan WRL-68 adalah sel hati embrio sebagai sel normal telah digunakan dalam eksperimen ini untuk mencirikan ekspresi. Ekpresi reseptor RON pada permukaan sel, kepekatan mRNA dan protien RON telah dilakukan dengan menggunakan immunofluorescent (IF), tindak balas rantaian polimerase (PCR) dan natrium sulfat dodesil polyacrylamide gel elektroforesis (SDS-PAGE). Secara kolektifnya, telah didapati bahawa reseptor RON adalah terlebih ekspress dalam kedua-dua sel kanser payu dara berbanding dengan sel normal.

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LIST OF ABBREVIATIONS AND SYMBOLS

°C	-	Degree Celsius
%	-	Percent
cm	-	Centimeter
mm	-	Millimeter
g	-	Gram
V	-	Volt
mA	-	Mill amber
L	-	Liter
mL	-	Milliliter
M	-	Molar
mM	-	Milli molar
mg	-	Milligram
min	-	Minute
nm	-	Nano meter
µg	-	Micro gram
µL	-	Micro Liter
et al.,	-	And others
e.g.	-	Example
PBS	-	Phosphate buffered saline
BSA	-	Bovine Serum Albumin
CO_2	-	Carbon dioxide
HNO3	-	Nitric acid
v/v	-	Volume per volume
w/v	-	Weight per volume

DMEM	-	Dulbecco’s Modified Eagle Medium
RPMI	-	Roswell Park Memorial Institute medium
SDS	-	Sodium Dodecyl Sulfate
APS	-	Ammonium Persulfate
TEMED	-	N,N,N',N'-Tetramethylethylenediamine

CHAPTER 1

INTRODUCTION

1.1 General Introduction

Cancer is a group of several linked diseases. All kinds of cancer share similarity in the growth and extend of abnormal tissues and cells. Progression of cancer can be regulated by biomarkers including oncogenesis and tumor suppressor genes (American Cancer Society). RON receptor shows a role in cancer metastasis and progression by the enlargement of their expression between itself and its ligand named as Macrophage Stimulating Protein (MSP).

RON (Receptuer d'Origine Nantaise) is one of the members that belong to the RTKs (Receptors Tyrosine Kinase) family of Met proto-oncogene, which is hepatocyte growth factor receptor, containing c Met, c-Sea and Stk. The hepatocyte growth factor receptor (HGF; or scatter factor) is related with a lot of processes that connected to tumor development and cell survival.

All RTKs (Receptors Tyrosine Kinase) have quite similar architecture of the molecules, with ligand domains binding in the extracellular area, a single

transmembrane helix, and a cytoplasmic region that contains the protein tyrosine kinase (TK) domain with additional carboxy (C-) terminal and juxtamembrane regulatory regions (Lemmon and Schlessinger, 2010).

RON is involved in the tumor progression and metastasis. It is a protein of 180-kDa shaped as a heterodimer of two main chains: a 40-kDa α-chain and a 150-kDa β-chain (Camp *et al*., 2005).

RON is originally created as a precursor of a single-chain and pro-RON, and then it separates into the two active chains. The first chain (α-chain) is a wholly extracellular, and the second chain (β-chain) cut off into the cell membrane and has the intracellular tyrosine kinase and regulatory basic elements. RON and c- Met are considered to be the only RTKs (Receptors Tyrosine Kinase) that have a Sema domain in the protein extracellular part (a stretch of ~500 amino acids with several highly conserved cysteine residues). This domain has a conserved region of amino acid sequences, cysteine residues and a potential glycolsylation site. It intercedes a lot of signaling cascades that relate to the motility of cells, proliferation, adhesion, and apoptosis.

Macrophage Stimulating Protein (MSP), which is a RON ligand, was initially known as a serum protein that stimulate murine resident peritoneal, the chemotaxis of the macrophage in response to complement factor C5a. Moreover, MSP is a heterodimer of 80-kDa composed of a 53-kDa α-chain and a 30-kDa β-chain linked by a disulfide bond. MSP is also known as HGF-like protein (hepatocyte growth factor) or scatter factor that belongs to the family of plasminogen-prothrombin gene that contains plasminogen and HGF, with others (Camp *et al*., 2005).

Mature MSP plays on a group of cell types such as epithelia, macrophages, and hematopoietic cells. The actions of macrophages involve activation of movement,

creation of phagocytosis of the erythrocytes which is serum complement-coated, discouragement of the ability of incitement to synthase up-regulation by swelling activation, and stimulation of interleukin-6 (IL-6) secretions.

It was found that RON expression is not related with sex, age, or disease and also found to positively correlate with lymph node metastasis like perigastric lymph node metastasis. Previous studies have even publicized that RON expression attaches with the invasion of tumor cells (Zhou *et al*., 2008).

1.2 Problem Statement

Previous studies showed that the development of cancer cells associated with the expression of the RON receptor in cells. Therefore, determination of RON receptor and its expression can be used as an indicator for the progression of the cancer cells.

1.3 Research Objectives

The main purpose of this study is to determine the expression of RON receptor on breast cancer cell lines by using: Indirect Immunofluorescent Staining (IF), Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) and Polymerase Chain Reaction (PCR).

1.4 Scope of Study

This study is going to be focused on the RON receptor expression on the surface of breast cancer cell lines that are MDA-MB-231 and MCF-7. These cell lines

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