UNIVERSITI TEKNOLOGI MARA

ASSESSMENTS OF TWO-PORE CHANNEL 2 IN THE HUMAN MDA-MB-231 BREAST CANCER CELL LINE: FROM THE ASPECTS OF BIBLIOMETRIC, PROLIFERATION, APOPTOSIS, AND CHEMOSENSITIVITY

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

Faculty of Pharmacy

June 2022

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 Post Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

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ABSTRACT

Two-pore channel 2 (TPC2) is an endolysosomal calcium channel that governs physiological functions in cellular and organism levels such as angiogenesis, pH regulation, and autophagy. Recent findings demonstrated the implication of TPC2 signalling in cancer hallmarks and chemoresistance. TPC2 is recognised as a cancer driver and played role in the different cancer-related processes. However, the role of TPC2 in breast cancer and its ability to modulate the sensitivity of cancer cells to chemotherapy remains to be elucidated. Further, although TPC2 research is becoming more popular around the world, no studies using bibliometric methodologies to assess the overall characteristics of TPC2 research have been conducted. Generally, this study was designed to assess TPC2 from two different views; the bibliometrics perspective and the in vitro characterisation. From the bibliometric aspect, this study aimed to find out the global research on TPC2 via bibliometric analysis. The Scopus database, **VOSviewer, Harzing's Publish or Perish and Openrefine** tools were used for this aim. For the in vitro characterisation, this study intended to determine the roles of TPC2 on the proliferation and apoptosis of MDA-MB-231 cells, an in vitro model of triple-negative breast cancer (TNBC). The functional roles of TPC2 in MDA-MB-231 cells were explored using siRNA-mediated gene silencing. MTS assay was used to evaluate the effect of TPC2 silencing on the proliferation of MDA-MB-231 cells, and flow cytometry was employed to measure apoptosis. Furthermore, this study designed to elucidate the effect of TPC2 silencing on doxorubicin-induced cytotoxicity in MDA-MB-231 cells. Prior to doxorubicin treatment, expression of TPC2 in MDA-MB-231 cells was silenced. This was followed by an MTS assay to evaluate the effect of TPC2 silencing on doxorubicin's cytotoxic activity in MDA-MB-231 cells and Annexin V/PI flow cytometry for apoptosis assay. Moreover, to probe for the mechanisms of apoptosis, the expression of several apoptotic markers namely Bcl2, Tp53, and Bax were evaluated by qPCR. The bibliometric findings indicated that the total publication on TPC2 was increasing in 2021. The United Kingdom (UK) and the University of Oxford were the leading country and institution in TPC2 research. The in vitro results showed that TPC2 suppression did not influence the proliferation and apoptosis of MDA-MB-231 cells ($p>0.05$). Likewise, its inhibition did not have any effect on the chemosensitivity of doxorubicin in MDA-MB-231 cells ($p>0.05$). However, the MTS assay's results displayed that doxorubicin significantly reduced the proliferation of MDA-MB-231 cells. Following this, the Annexin V/PI flow cytometry's findings confirmed the results of the MTS assay which represented that doxorubicin treatment associates with a high number of apoptotic, and necrotic cells ($p<0.05$). Moreover, gene expression assay data exhibited the molecular mechanisms involved in cell death. The findings indicated that cell death was possibly related to a significant reduction of the Bcl2 level, and the significant enhancement of the Bax ($p<0.05$). Overall, our findings add to our knowledge of the TPC2 research worldwide. It opens up new opportunities for future research, particularly in the fields of oncology, autophagy and COVID-19. Furthermore, our discoveries shed light on TPC2 role in proliferation, apoptosis, and chemosensitivity in MDA-MB-231 cells. Future research on TPC2 in MDA-MB231 cells may want to apply a longer timeline and focusing on other cancer hallmarks apart from what have been assessed in the current study.

ACKNOWLEDGEMENT

First and foremost, I want to express my gratitude to Allah for providing me with the chance to pursue my dream of my Master's degree and for completing this long and interesting path at UiTM, Malaysia. Alhamdulillah.

Dr Aisyah Hasyila Jahidin, my supervisor, deserves the most of my gratitude and thanks who has sincerely and friendly helped me with every single step of my journey. Due to her kindly directions in this period, I always have her and her family in my prayers and wish to have another research journey with her in my life.

I want to express my gratitude to my family particularly my father and my mother and my friends particularly Miss Lutfia Ilsahaida Abdul Malik, and Miss Sze Min, for their assistance with this endeavour.

In addition, I would like to thank the faculty of pharmacy, UiTM, particularly the department of pharmacology and toxicology, for their unwavering support, which enabled me to complete my research on time.

I would like to acknowledge the Ministry of Higher Education Malaysia (MoHE) and Universiti Teknologi MARA (UiTM) for funding this research through the Fundamental Research Grant Scheme (FRGS) (600-IRMI/FRGS 5/3 (315/2019)) and Lestari (600-IRMI/FRGS 5/3/LESTARI (014/2019)). Further, I would like to acknowledge the Higher Education Development Program (HEDP) of the Ministry of Higher Education of Afghanistan and the Faculty of Pharmacy, Kabul University for supporting this study.

TABLE OF CONTENTS

CONFIRMATION BY PANEL OF EXAMINERS

AUTHOR'S DECLARATION

ABSTRACT

ACKNOWLEDGEMENT

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

CHAPTER ONE INTRODUCTION

1.1 Research Background

1.2 Problem Statement

1.3 Objectives

1.4 Significance of Study

1.5 Scope and Limitations

CHAPTER TWO LITERATURE REVIEW

2.1 Introduction

2.2 Cancer

2.3 Breast Cancer

2.3.1 Therapeutic strategies for management of breast cancer

2.4 Calcium signalling

2.4.1 The Endolysosomal Ca^{2+} Store and calcium signalling

2.5 Two - Pore Channels

2.5.1 Functions and role of TPCs

2.5.2 Pharmacological Modulators of TPCs

2.6 Cancer and calcium signalling

2.6.1 Calcium signalling and cancer cell proliferation

2.6.2 Calcium signalling and cancer cell death