UNIVERSITY TEKNOLOGI MARA

THE ANTIPROLIFERATIVE PROPERTIES OF TINOSPORA CRISPA ON TRIPLE NEGATIVE BREAST CANCER CELL LINES

REYADH RADHIAL-RASHIDI

Thesis submitted in fulfillment
of the requirements for the degree of
Master of Science

Faculty of Medicine

December 2013

ABSTRACT

Tinospora crispa is a traditional medicinal plant in Malaysia with anti-cancer properties as shown in recent studies. The main objective of this study was to determine the anti-proliferative effect of *T. crispa* methanol extract on triple negative breast cancer. MTT assay was performed to determine the cell viability of triple negative breast cancer cell lines (MDA-MB-231 and HCC1806) and normal breast cell line (MCF-lOA). The type of cell death was determined using flow cytometry and cellular DNA fragmentation ELISA while Comet assay was used to determine the genotoxicity. qPCR was used to investigate the mRNA expression levels of the caspases 3, 8, 9 and NF-KB. The present results showed that *T. crispa* decreased the cell viability in triple negative breast cancer cells in a dose dependent manner with an IC50 of 66±3|ag/ml and 60±4^g/ml in MDA-MB-231 and HCC1806 cells respectively. While for MCF-lOA, the IC_{50} was 248±4ug/ml. The type of cell death in MDA-MB-231, HCC1806 and MCF-lOA cells was mainly due to apoptosis. The comet assay data for 71 *crispa* did not detect any DNA damage on MDA-MB-231, HCC1806 and MCF-lOA cell lines. Our results also showed that cisplatin significantly up-regulated NF-KB gene expression. Many studies reported that the up-regulation of NF-KB increases the resistance of cancer cells to apoptosis. Unlike cisplatin, *T. crispa* did not show any significant change in the mRNA expression levels of NF-KB. Furthermore, when used in combination *T. crispa* and cisplatin, the combination significantly down-regulated the gene expression of NF-KB and significantly up-regulated the gene expression of caspases 3, 8 and 9 in the cancer cells compared to single usage indicating more apoptotic activity. In Conclusion, *T. crispa* showed anti-proliferative effect on triple negative breast cancer cells with less toxicity to the normal breast cells. The cell death was mainly due to apoptosis and the combination of *T. crispa* and cisplatin significantly down-regulated the NF-KB gene expression which in turn increased apoptosis in the cancer cell lines.

ACKNOWLEDGMENT

In the name of Allah, the All-merciful, the All-compassionate. All my praise goes to Allah, whom has given me strength and blessings. First and foremost, I would like to express my gratitude to my supervisor Assoc. Prof. Dr. Gabriele Anisah Froemming whom has effortlessly guided me throughout the completion of my thesis with her patience and knowledge. My sincere thanks also go to my co-supervisor Mr. Mohammad Johari Ibrahim whom without his valuable knowledge and assistance this study would not have been successful.

During my time in the IMMB/faculty of medicine, I had the chance to meet and to work with the best people, and I am grateful for their kindness and friendship. My warm thanks to all members of IMMB/faculty of medicine for a convivial place to work.

Finally, my deepest gratitude goes to my family for their constant support and love throughout my life.

TABLE OF CONTENTS

CHAPTER ONE
INTRODUCTION

1.1 OVERVIEW

Triple negative breast cancer (TNBC) is a subtype of breast cancer that accounts for approximately 15% - 20% of breast cancer cases. TNBC is negative for expression of progesterone receptor (PR), oestrogen receptor (ER) and erythroblastic leukaemia viral oncogene homolog 2 (ErbB2) [also known as human epidermal growth factor receptor 2 (HER2)]. It is characterized by its aggressive behaviour, unique molecular profile, lack of targeted therapies and distinct pattern of metastasis [1]. The pattern of metastasis of TNBC is more directed to an orderly progression from the primary site to the regional lymph node or the sentinel lymph node in the majority of cases with subsequent dissemination to the systemic sites. TNBC have higher tendency of visceral metastasis as compared to other types of breast cancer. This may be explained in part by altering the expression of Epidermal Growth Factor Receptor (EGFR) which in turn promotes aggressive pattern of metastasis in TNBC cells [2, 3].

Several studies showed the efficacy of cisplatin in treatment of TNBC [4-6]. Strategies to optimize the existing chemotherapy treatments have the potential to consolidate chemo-sensitivity and decrease their side effects. Recent studies suggested that a combined therapy using plant extracts and cisplatin can improve the anti-proliferative activity and reduce the side effects of cisplatin in several types of cancers [7-9]-

Cancer chemotherapeutic agents can often provide prolongation of life, temporary relief from symptoms and occasionally, cures. An efficient chemotherapeutic agent should incapacitate or kill cancerous cells without causing unnecessary damage to the normal cells. Inducing apoptosis in cancerous cells could accomplish this ideal condition since apoptosis only involves one cell and it will not lead to inflammation, destruction, or scarring and fibrosis of adjacent tissues. The existence of both cancer and normal cells is considerably affected by the rate of apoptosis [10]. Therefore, modulating apoptosis may be valuable in the prevention or healing of cancer.