UNIVERSITI PUTRA MALAYSIA

MOLECULAR AND CYTOSKELETAL CHANGES IN BREAST CANCER CELL LINES TREATED WITH VELOGENIC NEWCASTLE DISEASE VIRUS STRAIN AF2240

ZOLKAPLI ESHAK

IB 2006 16

MOLECULAR AND CYTOSKELETAL CHANGES IN BREAST CANCER CELL LINES TREATED WITH VELOGENIC NEWCASTLE DISEASE VIRUS STRAIN AF2240

By

ZOLKAPLI ESHAK

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

Mac 2006

I would like to dedicate this thesis to my parents, I thank you for the unconditional love and sacrifices you made for me; to my wife, who has put up with me during the writing of this thesis; and to my sons and daughter who bring joy and happiness to my heart.

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

MOLECULAR AND CYTOSKELETAL CHANGES IN BREAST CANCER CELL LINES TREATED WITH NEWCASTLE DISEASE VIRUS STRAIN AF2240

By

ZOLKAPLI BIN ESHAK

March 2006

Chairman : Associate Professor Fauziah Othman, PhD

Institute : Bioscience

The study was carried out to investigate the oncolytic effect of Newcastle disease virus (NDV) strain AF2240 on the MCF-7, MDA-MB-231 breast cancer cell lines and 3T3 fibroblast. Studies were conducted to investigate the cytoskeletal protein structure and the molecular changes of the oncogenes. The AF2240 strain of NDV was propagated in 11 days old embryonated eggs for 72 hours. The virus in the allantoic fluid was harvested, purified and stored at -80^{o}C. The haemagglutination (HA) test was conducted on the purified virus to determine the HA titre of the NDV strain AF2240 which was 16384 HA units. The inhibition concentration of AF2240 towards several types of breast cancer cell lines was carried out using microculture tetrazolium (MTT) assay via two methods; monolayer and co-culture techniques to determine the inhibition concentration (IC_{50}) value. The IC_{50} values for MDA-MB-231 breast cancer cell

lines treated with NDV strain AF2240 were 8 and 2 HA units for the monolayer and co-culture techniques respectively, whereas the IC_{50} value for MCF-7 was 2 HA units for both techniques. NDV strain AF2240 has no oncolytic effect towards 3T3 mouse fibroblast. Further on confocal microscopy was carried out to observe the localization of the virus in the cells. For detection of the virus, polyclonal antibody and anti-chicken conjugated with fluorescein isothiocynate (FITC) were used. The virus particles were detected in the cytoplasm of both breast cancer cell lines after 24 and 48 hours post treatment. Budding-off of the virus was detected after 72 hours post treatment. Further study using TdT-mediated dUTP nick-end labelling (TUNEL) assay was conducted to label and quantify the percentage of apoptotic cells. By using independent t-test, the analysis revealed that NDV strain AF2240 works better towards MDA-MB-231 cells compared to MCF-7 ($p \leq 0.05$). These methods confirmed that NDV causes cell death to the breast cancer cells via apoptosis. The finding also suggesting that NDV react better towards MDA-MB-231 cells compared to MCF-7 cell ($P \leq 0.05$). The immunolabelling of the cytoskeletal proteins, namely, microfilaments, microtubules and intermediate filaments was conducted by using FL Phallicidin, monoclonal anti-α-tubulin FITC and monoclonal anti-vimentin Cy3. The cytoskeletal proteins of the cell lines were disrupted after 72 hours post treatment. However, the number of cells with disrupted cytoskeletal proteins was much higher in MDA-MB-231 cells compared to MCF-7 cells. The study of oncogenes was conducted by using reverse transcriptase polymerase

chain reaction (RT-PCR) method. The expressions of c-myc, c-erb-2 and c-fos oncogenes were detected at pre and post-treatment in the MCF-7 and MDA-MB-231 breast cancer cell lines. These results prove that cells which had undergone apoptosis due to NDV strain AF2240 treatment did not suppress the oncogenes. This study concluded that even though strain AF2240 of NDV have significant cytotoxic effect towards MCF-7 breast cancer cell lines, the number of apoptotic cells were higher in MDA-MB-231 cell line and therefore, further study is needed to understand the underlying mechanism in making the NDV strain of AF2240 as an anti-cancer agent.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PERUBAHAN MOLEKULAR DAN SITOSKELETAL SEL KANSER PAYUDARA YANG DI RAWAT DENGAN VIRUS PENYAKIT NEWCASTLE STRAIN AF2240

Oleh

ZOLKAPLI ESHAK

Mac 2006

Pengerusi : Profesor Madya Fauziah Othman, PhD

Institut : Biosains

Kajian ini dijalankan untuk menyelidik kesan onkolitik virus penyebab Newcastle disease (NDV) strain AF2240 ke atas sel kanser payudara MCF-7, MDA-MB-231 dan 3T3 fibroblast. Kajian ini dijalankan untuk menyelidik perubahan strukur protein sitoskeletal dan perubahan molekular onkogen. Propagasi NDV telah dilakukan di dalam telur ayam berembrio berusia 11 hari selama 72 jam. Virus yang terdapat dalam cecair alantoik kemudiannya dikumpul, ditulenkan dan disimpan pada -80^{o}C. Ujian penggumpalan (HA) telah dijalankan ke atas virus yang telah ditulenkan untuk menentukan titer HA virus NDV strain AF2240 yang mana HA unitnya ialah 16384. Kesan NDV AF2240 ke atas sel kanser payudara telah dilakukan mengguna kaedah mikrokultur tetrazolium (MTT) esei melalui dua cara; teknik monolayer dan ko-kultur untuk menentukan nilai $IC_{50.}$ Nilai IC_{50} untuk sel payudara MDA-MB-231 yang dirawat dengan NDV strain AF2240 adalah masing-masing 8 dan 2 HA

unit untuk teknik monolayer dan ko-kultur, manakala nilai IC_{50} untuk MCF-7 adalah 2 HA unit untuk kedua-dua teknik. NDV strain AF2240 tidak mempunyai kesan onkolitik terhadap sel fibroblast 3T3. Kajian selanjutnya telah dilakukan dengan menggunakan mikroskop konfokal untuk melihat taburan virus tersebut di dalam sel. Untuk mengesan virus tersebut, antibodi poliklonal dan anti-ayam fluorescein isothiocynate (FITC) telah digunakan. Virus tersebut telah dikesan di dalam sitoplasma kedua-dua jenis sel payudara selepas dirawat selama 24 dan 48 jam. Walaubagaimanapun, pelepasan virus dari sel perumah selepas 72 jam dirawat hanya dapat dikesan di dalam sel kanser payudara. Kajian selanjutnya menggunakan asai TdT- mediated dUTP nick-end labelling (TUNEL) telah dijalankan untuk melabel dan mengira peratusan sel apoptotik. Dengan menggunakan ujian-t berdikari, analisis menunjukkan bahawa NDV strain AF2240 bertindak balas lebih baik terhadap sel MDA-MB-231 dibandingkan dengan MCF-7 ($p \leq 0.05$). Cara ini mengesahkan bahawa NDV menyebabkan sel payudara mati melalui apoptosis. Kajian ini juga mencadangkan bahawa NDV bertindak balas lebih baik terhadap sel MDA-MB-231 berbanding dengan MCF-7 ($P \leq 0.05$). Penglabelan imuno ke atas protein sitoskeletal seperti filamenmikro, tubulmikro dan filamen pertengahan telah dijalankan dengan menggunakan FL Phallicidin, monoklonal anti-α-tubulin FITC dan monoklonal anti-vimentin Cy3. Protin sitoskeletal sel rosak selepas 72 jam dirawat. Walau bagaimanapun, jumlah sel yang mengalami kerosakan protein sitoskeletal lebih tinggi di dalam sel MD-MB-231 berbanding sel MCF-7.

Kajian mengenai onkogen dilakukan menggunakan kaedah 'reverse transcriptase polymerase chain reaction' (RT-PCR). Ekpresi c-myc, c-erb-2 dan c-fos onkogen telah dikesan sebelum dan selepas rawatan di dalam sel MCF-7 dan MDA-MB-231. Keputusan ini menunjukkan bahawa apoptosis yang di sebabkan NDV strain AF2240 tidak menindas onkogen. Kajian ini menyimpulkan bahawa, walaupun NDV strain AF2240 mempunyai lebih kesan sitotoksik terhadap sel MCF-7, sel MDA-MB-231 mempunyai bilangan sel apoptotic yang lebih banyak dan dengan itu kajian yang lebih mendalam diperlukan untuk memahami mekanisma yang terselindung dalam menjadikan NDV strain AF2240 sebagai agen anti-kanser.

ACKNOWLEDGEMENTS

I would like to thank to all people who supported me and were involved in one way or another in the preparation of this thesis. I gratefully appreciate the guidance of my supervisor, Assoc. Prof. Dr. Fauziah Othman who inspired and provided me the purpose of my study. I am grateful to the support and mentoring of my co-supervisors, Prof. Dr. Aini Ideris, for her depth insightful knowledge of the virus and to Assoc. Prof. Dr. Asmah Rahmat, for her guidance in tissue culture and molecular works. Many special thanks to Assoc. Prof. Dr. Rozita Rosli from the Faculty of Medicine and Health Sciences and Assoc. Prof. Dr. Abdul Rahman Omar from Faculty of Veterinary Medicine, who gave me the chance to work on this topic in their lab.

I wish to thank Dr. Abdah Md Akim who mentored me on TUNEL assay and molecular work. I also want to thank Dessy Arisanty for her knowledge in analytical analysis, Tengku Shahrul for his invaluable lesson in computer software and Hadiyatul Hanim who is always there when help is needed. I would like to acknowledge the support of my lab mates and friends: Hernani, Mahani, Norhayati, Sally, and Phang who helped me with the editing of the current text. I also would like to thank the staff of Microscopy Imaging and Nanoscience Unit for helping me out with the microscopy imaging and their excellence services. With the biggest contribution to this thesis, I would like to thank Ministry of Science and Technology (MOSTE) and Majlis Kanser Nasional

(MAKNA) for sponsoring the research. Without their financial support, this research would be impossible.

Last but not the least; I want to thank my wife, Rohhannie, and my sons and daughter, Irfan Zunnurrain, Irfan Zikry and Aleeya for their understanding, support, and unconditional love. Their love and support has been and will continue to be my inspiration, and I am so blessed to have such a caring and supporting family.

I certify that an Examination Committee has met on 20th Mac 2006 to conduct the final examination of Zolkapli Eshak on his Master of Science thesis entitled "Molecular and Cytoskeletal Changes in Breast Cancer Cell Lines Treated With Newcastle Disease Virus Strain AF2240" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

Nasaruddin Abdul Aziz, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

Mohd Hair Bejo, PhD
Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Internal Examiner)

Azian Abd. Latiff, PhD
Lecturer
Faculty of Medicine
Universiti Kebangsaan Malaysia
(External Examiner)

_____
HASANAH MOHD GHAZALI, PhD
Professor/Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee are as follows:

Fauziah Othman, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

Aini Ideris, PhD
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

Asmah Rahmat, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

_____
AINI IDERIS, PhD
Professor/Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

_____

ZOLKAPLI ESHAK

Date:

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure **Page**