UNIVERSITI PUTRA MALAYSIA

EFFECTS OF VELOGENIC NEWCASTLE DISEASE VIRUS STRAIN AFF2240 ON THE ONCOGENES AND MORPHOLOGICAL CHANGES OF 4T1 BREAST CANCER CELL IN BALB/c MICE

ZOLKAPLI ESHAK

FPSK(p) 2011 23

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By

ZOLKAPLI ESHAK

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

MAY 2011

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 beloved wife, who has put up with me during the writing of this thesis; and to my sons and daughters, who bring joy and happiness to my heart.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

EFFECTS OF VELOGENIC NEWCASTLE DISEASE VIRUS STRAIN AFF2240 ON THE ONCOGENES AND MORPHOLOGICAL CHANGES OF 4T1 BREAST CANCER CELL IN BALB/c MICE

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Chairman : Professor Fauziah Othman, PhD

Faculty : Medicine and Health Sciences

The need in finding alternative treatments in breast cancer has become increasingly important as conventional treatment of breast cancer usually have severe side effects. The usage of viruses as an anti-cancer agent has been discovered and studied for decades. The present study was conducted to find a new anti-cancer agent for the treatment of breast cancer. The AF-2240 strain of NDV was propagated in the allantoic fluid of 11-days-old embryonated eggs for 72 hours. The virus was harvested, purified and stored at -80^{0}C. The haemagglutination (HA) test conducted on the purified virus showed that the virus obtained was 64 HA unit. The induction of breast cancer was done on the axillary region of female inbred BALB/c mice by using 1 X 10^4 4T1 breast cancer cells. The mice were then grouped into two big groups; tumour induced and normal control. Out of 10 groups induced with cancer, the cancer only developed in 4 groups and therefore, mice were regrouped into two; tumor -bearing group which

consist of groups named cancer treated (CT), cancer control (CC), cancer treated with 0.5 ug/ml of tamoxifen and 32 HA of NDV (CTND32) and cancer control treated with 0.5ug/ml of tamoxifen and 64 HA of NDV (CTND64); and tumor- free groups which consist of groups named normal control (NC), normal treated with 8 HA of NDV (NND8), Normal treated with 16 HA of NDV (NND16), normal treated with 32 HA of NDV (NND32), normal treated with 64 HA of NDV (NND64), cancer treated with 8 HA of NDV (CND8), cancer treated with 16 HA of NDV (CND16), cancer treated with 32 HA of NDV (CND32), cancer treated with 64 HA of NDV (CND64), cancer treated with 0.5 ug/ml of tamoxifen and 8 HA of NDV (CTND8) and cancer treated with 0.5 ug/ml of tamoxifen and 16 HA of NDV (CTND16). Preliminary results showed that NDV-AF2240 alone or in combination with tamoxifen not exceeding 16 HA unit of NDV have an anti-cancer effect. The 4T1 breast cancer models were further evaluated by mean weight of the mice, tumor mass and volume, transmission electron microscopy for localization of the virus, apoptotic peroxidase staining and comet assay for detection of apoptotic cells and RT-PCR for c-myc, c-erbB2 and c-fos oncogenes expression. The mean tumor mass and volume proved that there was no evidence of tumor regression instead; the pattern showed exponential growth of the tumor along with time. The treatment given and the condition of the animals have no effect in term of body weight as there were no significant difference being noticed between tumor-bearing mice and tumor-free mice ($p>0.05$). It is believed that tumour weight have contributed indirectly to the overall bodyweight in the tumor bearing group. The effectiveness of the treatments was later translated by observing the number of apoptotic cells. All tumors samples exhibited apoptotic features analyzed by using apoptotic peroxidase staining and comet assay. The analysis showed that combination treatments using NDV and

tamoxifen have no significant effect toward the breast cancer cells. Only CT group which were treated with tamoxifen showed significant ($p<0.05$) higher number of apoptotic cells compared to the rest of the groups. Like any other types of *paramyxovirus*, NDV-AF2240 was found to be localized in the cytoplasm of the breast cancer cells observed by using transmission electron microscope. Further analysis of the c-myc, c-erbB2 and c-fos oncogenes revealed that the presences of the oncogenes in all tumor bearing mice group regardless of treatment given. In conclusion, NDV-AF2240 has the potential as an anti-cancer agent if it is used alone or at low HA titre if in combination with tamoxifen. The use of the virus at high HA titre and in combination with tamoxifen has to be monitored with cautioun as it has an antagonist effect.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

KESAN VIRUS PENYAKIT NEWCASTLE STRAIN AF2240 VELOGENIK TERHADAP ONKOGENE DAN PERUBAHAN MORFOLOGI SEL KANSER PAYUDARA 4T1 DI DALAM MENCIT BALB/C.

Oleh

ZOLKAPLI ESHAK

MEI 2011

Pengerusi : Profesor Fauziah Othman, PhD

Fakulti : Perubatan dan Sains Kesihatan

Keperluan dalam mencari rawatan alternatif terhadap kanser payu dara telah menjadi bertambah penting kerana rawatan konvensional kanser payu dara biasanya mempunyai banyak kesan sampingan yang tidak diingini. Penggunaan virus sebagai agen anti-kanser telah ditemui dan dikaji sejak berdekad. Kajian ini dijalankan untuk mencari agen anti-kanser yang baru untuk merawat kanser payudara. Strain NDV-AF2240 telah dikembang biakkan di dalam cecair allantoik telur berembrio berusia 11 hari selama 72 jam. Virus itu kemudiannya dituai, ditulenkan dan disimpan di -80^{0}C. Ujian penggumpalan darah yang dilakukan ke atas virus yang telah di tulenkan menunjukkan virus yang didapati adalah pada 64 HA unit. Pengaruhan kanser payudara telah dilakukan pada bahagian axillary mencit biak baka dalam BALB/c betina dengan menggunakan 1 X 10^4 4T1 sel kanser payudara. Tikus itu kemudiannya di bahagikan kepada dua kumpulan; kanser teraruh dan kawalan normal. Daripada 10 kumpulan yang diaruh, kanser hanya terbentuk

dalam 4 kumpulan dan dengan itu kumpulan mencit telah di kumpulkan semula kepada dua kumpulan; kumpulan yang mempunyai tumor yang terdiri daripada kumpulan kanser dirawat (CT), kanser kawalan (CC), kanser dirawat dengan 0.5 ug/ml tamoxifen dan 32 HA NDV (CTND32) dan kanser dirawat dengan 0.5ug/ml tamoxifen dan 64 HA NDV (CTND64); dan kumpulan bebas tumor yang terdiri daripada kumpulan kanser kawalan (NC), normal dirawat dengan 8 HA NDV (NND8), normal diwrawat dengan 16 HA NDV (NND16), normal dirawat dengan 32 HA NDV (NND32), normal dirawat dengan 64 HA NDV (NND64), kanser dirawat dengan 8 HA NDV (CND8), kanser dirawat dengan 16 HA NDV (CND16), kanser dirawat dengan 32 HA NDV (CND32), kanser dirawat dengan 64 HA NDV (CND64), kanser dirawat dengan 0.5ug/ml tamoxifen dan 8 HA NDV (CTND8) dan kanser dirawat dengan 0.5ug/ml tamoxifen dan 16 HA NDV (CTND16). Keputusan awal menunjukkan bahawa NDV-AF2240 sendiri atau dengan gabungan tamoxifen tidak melebihi 16 HA unit NDV mempunyai kesan anti-kanser. Model 4T1 kanser payu dara seterusnya dinilai dari segi purata berat mencit, jisim tumor, isipadu, kajian transmisi elektron mikroskop untuk mengesan kedudukan virus tersebut, pewarnaan apoptotik-peroksidase dan juga asai komet untuk mengesan sel apoptotik dan juga RT-PCR untuk ekspresi onkogen c-myc, c-erbB2 dan c-fos. Rawatan yang telah diberikan dan keadaan haiwan terbabit tidak memberi kesan dari segi berat badan memandangkan tiada perubahan ketara diantara mencit yang mempunyai tumor dan mencit bebas tumor ($p>0.05$). Adalah dipercayai bahawa berat tumor itu sendiri menyumbang kepada berat badan secara keseluruhan. Keberkesanan rawatan yang telah diberikan kemudiannya diterjemahkan dengan memerhati jumlah sel apoptotik yang hadir. Semua sampel tumor menunjukkan kehadiran sel apoptotik yang dianalisa menggunakan pewarnaan apoptotik peroksida dan asai komet. Analisis tersebut

menunjukkan bahawa gabungan rawatan menggunakan NDV dan tamoxifen tidak mempunyai kesan yang signifikan terhadap sel kanser payu dara. Hanya kumpulan CT yang dirawat dengan tamoxifen menunjukkan bilangan sel apoptotik yang signifikan tinggi ($p<0.05$) berbanding dengan kumpulan yang lain. Sebagaimana dengan mana-mana *Paramyxovirus*, NDV-AF2240 telah dijumpai di dalam sitoplasma sel kanser payudara yang telah diperhatikan dengan menggunakan transmisi elektron mikroskop. Analisis selanjutnya terhadap c-myc, c-cerbB2 and c-fos onkegen mendedahkan kehadiran kesemua onkogen tersebut di dalam semua mencit yang mempunyai tumor tanpa mengambil kira rawatan yang diberikan. Kesimpulannya. NDV-AF2240 mempunyai potensi sebagai agen anti kanser jika digunakan sendiri atau pada HA titre yang rendah jika digunakan bersama tamoxifen. Penggunaan virus tersebut pada HA titre yang tinggi dan dengan kombinasi tamoxifen perlu di awasi secara teliti kerana ia mempunyai kesan antagonis.

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, 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. Datin Paduka Aini Ideris, for her depth insightful knowledge of the virus and to Prof. Dr. Asmah Rahmat, for her guidance in tissue culture and molecular work. Many special thanks to Assoc. Prof. Dr. Rozita Rosli from the Faculty of Medicine and Health Sciences who gave me the chance to work in her lab. I would also like to acknowledge and thank the staff of Laboratory of Vaccine, Institute of Bioscience for providing the animal house facility to make this study possible. I would like to extend my sincere thanks to Majlis Kanser Nasional (MAKNA), particularly to Yg. Berbahagia Dato' Mohd. Farid Ariffin, President of MAKNA for sponsoring my doctorate programme.

I wish to thank Dr. Normawati who mentored me on molecular works. I would like to acknowledge the support of my lab mates and friends: Gholamreza, Anushia, Juraimi, Siti Saleha, Norhayati, Maslina, Mun Yee and Lee Foong who helped me with the editing of the current text. I also would like to thank the staffs 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, Technology and Innovation (MOSTI) 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 parents, Eshak Yakob and Habibah Abu Bakar, my wife, Rohhannie, and my sons and daughters, Irfan Zunnurrain, Irfan Zikry, Nur Aleeya Safiyah and Nur Aina Dania 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 a Thesis Examination Committee has met on 27th of May 2011 to conduct the final examination of Zolkapli Eshak on his thesis entitled "Effects of Velogenic Newcastle Disease Virus Strain AF2240 On The Oncogenes and Morphological Changes of 4T1 Breast Cancer Cell in BALB/c Mice" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the candidate be awarded the Doctor of Philosophy.

Members of the Examination Committee are as follows:

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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DECLARATION

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

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ZOLKAPLI ESHAK

Date: 22 May 2011

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