UNIVERSITI PUTRA MALAYSIA

LOCALIZATION OF NEWCASTLE DISEASE VIRUS (NDV-AF2240) IN 4T1 XENOTRANSPLANT BREAST CANCER BALB/c MICE

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By

GHOLAMREZA MOTALLEB

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

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DEDICATION

With love and appreciation to:

My mother (Kobra Norouzi Ghotb abadi), Father (Nematollah Motalleb), My wife (Niloufar Nabi), My daughter (Mehrafarin), My son (Arian), My brother (Mohamadreza) and my sisters

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

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September 2009

Chairman : Professor Dr. Fauziah bt. Othman, PhD

Faculty : Medicine and Health Sciences

In situ reverse transcriptase polymerase chain reaction (*in situ* RT-PCR), polyclonal chicken antibody and goat anti-chicken antibody conjugated with fluorescein isothiocynate (FITC) using confocal laser scanning microscopy (CLSM) and negative staining transmission electron microscopy (NSTEM) were carried out to detect the NDV-AF2240 in tumor, liver, brain and lung during intratumural injection in 4T1 xenotransplant breast tumor in female BALB/c mice. A total of 300 female BALB/c mice were divided randomly into 15 groups (5 non cancerous groups, 10 cancerous groups) consisting 20 mice per group. The normal control (NC), normal treated with 8, 16, 32 and 64HA units of NDV-AF2240 respectively named as N/NDV8, N/NDV16, N/NDV32 and N/NDV64. The mice in cancerous groups were initially inoculated sub-cutaneously with 4T1 cells; co-culture either with NDV-

AF2240 or/and tamoxifen. Cancerous groups were divided into cancer control (CC), cancer treated with only 5 µg/ml tamoxifen citrate (CT), cancer treated with 8, 16, 32 and 64HA units of NDV-AF2240 without tamoxifen respectively named C/NDV8, C/NDV16, C/NDV32, C/NDV64, cancer treated with 8, 16, 32 and 64HA units of NDV-AF2240 with tamoxifen respectively named as CT/NDV8, CT/NDV16, CT/NDV32 and CT/NDV64 daily for four weeks. The normal mice treated with 8, 16, 32 and 64 HA unit of NDV-AF2240 did not affect its lifespan. All of the cancerous and non cancerous mice survived well and completed the 4-weeks treatment. Only 4 groups of mice developed tumor that was CC, CT, CT/CNDV32 and CT/NDV64, however these groups survived until end of the 4 weeks of treatment. Significant difference ($p < 0.05$) in mean body weight was found between N/NDV16, N/NDV64 and NC. Whereas, for the cancerous groups, mean body weight of the mice in CC group were significantly different ($p<0.05$) to compare with C/NDV8, C/NDV32, CT/NDV16, CT/NDV32 and CT/NDV64 groups. The mean tumour volume and mass of CT/NDV32 and CT/NDV64 were not significantly different ($p> 0.05$) to compare with each other and cancer control (CC), however, there was significant difference ($p <.05$) in the changes of tumour volume and mass over time. The CC and CT groups had a significantly ($p<0.05$) higher lung weight compared with the other groups. The CC group had a significantly ($p<0.05$) higher of liver weight compared with all groups. There was no significant ($p>0.05$) different in the brain weight between CC and all cancerous groups. To localize HN gene expression of NDV-AFF2240 in tissues, *in situ* RT-PCR was applied on formalin fixed paraffin-embedded (FFPE) sections that were positive by negative staining

transmission electron microscopy. The HN gene expression was detected in all the breast tumor cells. However, it was found mainly in the blood vessels of the brain, liver and lung. The intensity of the HN gene expression in all the organs within the same group is significantly similar except the breast tumor tissue. There was no significant different ($p>0.05$) in HN gene intensity between CT/NDV8 and CT/NDV16 groups, however, it was significantly different ($p<0.05$) compared to CT/NDV32 and CT/NDV64 groups. Virus dissemination seems to be determined by the infusion dose during intratumoral injection. β actin as internal control was expressed in breast cancer tissue, brain, lung and liver. *In situ* RT-PCR showed similar constant strong intensity of β actin gene expression in all mentioned tissues. Immunofluoresence and CLSM successfully detected the virus particles in tumor and all the organs of the cancerous groups during intratumoral injection. In tumor tissue the virus are found in the cells, whereas, in the lung, brain and liver are found mainly in the blood vessels. They are mainly found at the central vein (C.V.) and sinusoidal capillaries of the liver. This phenomenon was similar to results of *in situ* RT-PCR. Negative staining with transmission electron microscopy as a gold standard method was successfully used to detect the NDV-AF2240 at breast tumor, lung, liver and brain tissues during intratumoral injection in 4T1 xenotransplant breast cancer induced in mice. The results illustrated the presence of NDV-AF2240 in all organs of cancerous groups, but not in the normal groups treated with virus. The morphology of Newcastle disease virus was seen pleomorphic, spherical and ranging from 60-320 nm. The virion has an envelope and prominent surface projections. Occassionally, virions were seen to be rod in

shape. Besides observing the whole virus, nucleocapsids which is confined in the virion was frequently detected outside the virion and are also seen filamentous. The findings of this study showed that NDV-AF2240 suppressed the growth of breast cancer and it is disseminated in blood vessels of the brain, lung and liver, however, found in the cells of the breast cancer.

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

PENGESANAN NEWCASTLE DISEASE VIRUS (NDV-AF2240) DI DALAM PAYUDARA BARAH 4T1 XENOTRANSPLANT MENCIT BALB/c MICE

Oleh

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September 2009

Pengerusi : Profesor Dr. Fauziah bt Othman, PhD

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Kaedah *in situ* reverse transcriptase polymerase chain reaction (*in situ* RT-PCR), antibodi poliklonal ayam dan antibodi kambing anti-ayam FITC menggunakan mikroskop pengimbasan laser konfokal dan pewarnaan negatif menggunakan mikroskop elektron pancaran telah dijalankan untuk mengesan virus NDV-AF2240 di dalam barah payudara, hati, otak dan paru-paru semasa suntikan intratumoral dalam xenotransplant ketumbuhan payudara dalam mencit betina BALB/c. Sejumlah 300 mencit betina BALB/c dibahagi secara rambang ke dalam 15 kumpulan (5 kumpulan tiada barah, 10 kumpulan berbarah) yang mempunyai 20 mencit sekumpulan. Kumpulan mencit normal (NC), normal dirawat dengan titer virus 8, 16, 32 dan 64 HA unit NDV-AF2240 masing-masing dinamakan N/NDV 8, N/NDV 16, N/NDV 32 dan N/NDV 64. Mencit dalam kumpulan berbarah disuntik subkutaneus

dengan sel kanser payudara mencit 4T1;ko-kultur dengan NDV-AF2240 atau bersama tamoxifen. Kumpulan diaruh barah dibahagi kepada barah kawalan (CC), barah dirawat dengan 5µg/ml tamoxifen citrate (CT), barah dirawat dengan 8, 16, 32, 64 dan 64 HA unit NDV-AF2240 masing-masing ditambah dengan tamoxifen; CT/NDV8, CT/NDV16, CT/NDV32 dan CT/NDV64 setiap hari selama 4 minggu. Mencit normal yang dirawat dengan 8, 16, 32, 64 HA unit NDV-AF2240 tidak menjejaskan jangka hayat. Semua mencit dalam kumpulan diaruh barah dan tidak diaruh barah hidup dan menghabiskan rawatan 4 minggu tersebut. Hanya 4 kumpulan mencit tersebut ada ketumbuhan barah payudara iaitu CC, CT, CT/NDV32 dan CT/NDV64, walaubagaimanapun mencit dalam kumpulan ini hidup hingga ke hujung 4 minggu rawatan. Terdapat perbezaan signifikan ($p<0.05$) dalam min berat badan antara N/NDV16, N/NDV64 dan NC. Antara kumpulan diaruh barah, min berat badan mencit kumpulan CC berbeza secara signifikan ($p<0.05$) dibandingkan kepada kumpulan C/NDV8, C/NDV32, CT/NDV 16, CT/NDV 32, dan CT/NDV64. Min isipadu dan berat barah CT/NDV32 dan CT/NDV64 tiada perbezaan signifikan ($p>0.05$) apabila dibandingkan sesama kumpulan ini dan juga kumpulan barah kawalan (CC), walaubagaimanapun, terdapat perbezaan signifikan ($p<0.05$) dalam perubahan isipadu dan berat barah dari awal hingga hujung eksperimen. Kumpulan CC dan CT mempunyai berat paru-paru yang lebih tinggi secara signifikan ($p<0.05$) dibandingkan kepada kumpulan lain. Kumpulan CC mempunyai berat hati yang tinggi secara signifikan ($p<0.05$) dibandingkan kepada kumpulan lain. Tiada perubahan signifikan berat otak ($p>0.05$) antara kumpulan CC dan kumpulan diaruh barah yang lain. *In situ* RT-PCR dijalankan untuk menentukan

pengekspresan gen HN NDV-AF2240 dalam seksyen tisu yang diawet formalin dan dibekukan dalam lilin. Gen HN diekspreskan dan ditemui dalam semua sel-sel barah payudara, walaubagaimanapun, ia ditemui khususnya dalam saluran darah otak, hati dan paru-paru. Keamatan pengekspresan gen HN dalam semua organ dalam kumpulan yang sama adalah serupa secara signifikan kecuali dalam tisu barah payudara. Tiada perbezaan signifikan ($p>0.05$) dalam keamatan gen HN antara CT/NDV 8 dan CT/NDV 16, walaubagaimanapun, terdapat perbezaan signifikan ($p<0.05$) apabila dibandingkan kepada CT/NDV 32 dan CT/NDV 64. Nampaknya penyebaran virus ditentukan oleh dos yang diberi semasa suntikan intratumoral. β actin sebagai kawalan dalam, diekspres dalam tisu barah payudara, otak, hati dan paru-paru. *In situ* RT-PCR menunjukkan keamatan tinggi ekspresi gen β actin yang serupa dalam semua tisu yang disebut sebelum ini. Immunofluoresence dan mikroskop pengimbasan laser konfokol telah berjaya mengesan partikel-partikel virus di dalam barah dan kesemua organ kumpulan diaruh barah semasa suntikan intratumoral. Dalam tisu barah, virus ditemui dalam sel-sel, tetapi dalam organ paru-paru, otak dan hati, virus banyak ditemui dalam saluran darah. Virus ini khususnya ditemui dalam vena sentral dan kapilari sinosodial hati. Fenomena ini adalah sama dengan keputusan *in situ* RT-PCR. Perwarnaan negatif menggunakan mikroskop elektron pancaran sebagai kaedah standard emas berjaya digunakan untuk mengesan NDV-AF2240 dalam sampel tisu barah, paru-paru, hati dan otak semasa suntikan intratumoral. Keputusan menunjukkan kehadiran NDV-AF2240 di dalam semua organ dalam kumpulan diaruh barah, tetapi bukan dalam kumpulan normal dirawat dengan virus. Morfologi virus newcastle

disease ditemui dalam bentuk pleomorf, sfera dan berukuran dari 60-320 nm. Virionnya ada sampul dan permukaan unjuran yang ketara. Adakalanya, virion nampak dalam bentuk rod. Selain daripada memerhatikan virus secara keseluruhan, nukleokapsid yang biasanya di dalam virion, kerap ditemui di luar virion dan ditemui berfilamentos. Keputusan projek ini menunjukkan NDV-AF2240 disebarkan ke otak, paru-paru dan hati semasa suntikan intratumoral barah payudara 4T1 dalam mencit betina BALB/c.

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I certify that an Examination Committee has met on 10 September 2009 to conduct the final examination of Gholamreza Motalleb on his Doctor of Philosophy thesis entitled “Localization of Newcastle Disease Virus (NDV-AF2240) in 4T1 Xenotransplant Breast Cancer BALB/c Mice” in accordance with 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 student be awarded the Doctor of Philosophy.

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Date : 16 November 2009

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.

_____

GHOLAMREZA MOTALLEB

Date : 7 September 2009

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