UNIVERSITI PUTRA MALAYSIA

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MASTER OF SCIENCE
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2010

EFFECT OF NEWCASTLE DISEASE VIRUS AF2240 ON ALLOGRAFTED 4T1 BREAST CANCER CELLS IN BALB/*c* MICE

By

ANUSHIA SWAMINATHAN

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

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

EFFECT OF NEWCASTLE DISEASE VIRUS AF2240 ON ALLOGRAFTED 4T1 BREAST CANCER CELLS IN BALB/*c* MICE

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Chairman : Professor Dr. Fauziah Othman, PhD

Faculty : Medicine and Health Sciences

This study was carried out to observe the antitumor effect of NDV AF2240 *in vivo* using mouse 4T1 breast cancer cell line. One hundred and twenty female mice were assigned randomly into ten groups; negative control (CC), cancer treated with 0.5 μg/mL tamoxifen citrate (CT), cancer treated with NDV titre 8HA (CNDV8), NDV 16HA (CNDV16), NDV 32HA (CNDV32), NDV 64HA (CNDV64), combination of NDV 8HA+tamoxifen (CNDV8+T), NDV 16HA+tamoxifen (CNDV16+T), NDV 32HA+tamoxifen (CNDV32+T) and NDV 64HA+tamoxifen (CNDV64+T). These mice were induced with 4T1 cells and treatments were started concurrently and given daily for a month. Forty eight mice with tumour growth were euthanized weekly to remove tumour samples. At the end of the experiment, microscopic examinations were done on the cross-sections of tumour samples of these mice. Tumour growth was observed in groups; CC, CT, CNDV32+T and CNDV64+T, whereas, the rest of the groups had no tumour growth. CNDV32+T and CNDV64+T groups did not show any tumour

regression, having a very low apoptotic index (AI) and a high mitotic index (MI) throughout the one month treatment indicating that these treatments were not therapeutic. TUNEL assay was carried out to quantify apoptotic cells and the findings were concurrent with the AI results, where only CT group had an increase in apoptotic cells when compared at week 1 to week 4. Tamoxifen alone was able to regress the tumour but not with a significant difference. Tumours with an inactivated tumour suppressor gene, p53, produced p53 mutant proteins. Results showed that there was a strong direct correlation between the amount of mutant protein present in the nucleus of cancer cells in CC, CT, CNDV32+T and CNDV64+T groups with the MI score. Mutated p53 proteins were not able to inhibit growth of cancer cells, leading to high mitotic activity and increase in cell proliferation. In groups CNDV32+T and CNDV64+T, there was evidence that NDV caused cytoplasmic sequestration of p53 protein from the nucleus to the cytoplasm, indicating the enhancement by the virus to induce apoptosis on these cells. The breast tissues of CNDV8, CNDV16, CNDV32, CNDV64, CNDV8+T and CNDV16+T groups which had no tumour were also stained to detect localization of p53. It was found highly expressed in the cytoplasm of ductal epithelium similar to quiescent mammary glands, denoting these groups were tumour free. The findings of this study suggest NDV titres 8, 16, 32 and 64HA inhibit the growth of 4T1 cells, preventing tumour formation. Not all the combinations of NDV and tamoxifen were effective, the higher NDV titres combined with tamoxifen were neither able to inhibit nor regress tumour growth. In summary, NDV AF2240 alone can inhibit growth of 4T1 cancer cells and, thus, can be used as a potential oncolytic agent for breast cancer treatments. NDV is significantly more effective than tamoxifen and can be a very useful alternative anticancer agent for breast tumours.

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

KESAN NEWCASTLE DISEASE VIRUS AF2240 KE ATAS ALLOGRAF SEL BARAH PAYUDARA 4T1 PADA MENCIT BALB/*c*

Oleh

ANUSHIA SWAMINATHAN

April 2010

Pengerusi : Professor Dr. Fauziah Othman, PhD

Fakulti : Perubatan dan Sains Kesihatan

Kajian ini telah dijalankan untuk menyelidik kesan antikanser virus penyakit Newcastle (NDV) AF2240 secara *in vivo* menggunakan sel kanser payudara mencit 4T1. Sebanyak 120 mencit betina dibahagikan secara rambang kepada sepuluh kumpulan; kawalan negatif (CC), kanser yang dirawat dengan 0.5 μg/mL tamoxifen (CT), kanser yang dirawat dengan NDV titer 8HA (CNDV8), NDV 16HA (CNDV16), NDV 32HA (CNDV32), NDV 64HA (CNDV64), gabungan NDV 8HA+tamoxifen (CNDV8+T), NDV 16HA+tamoxifen (CNDV16+T), NDV 32HA+tamoxifen (CNDV32+T) dan NDV 64HA+tamoxifen (CNDV64+T). Mencit ini disuntik dengan sel 4T1 dan rawatan dimulakan serentak, setiap hari selama sebulan. Sebanyak 48 ekor mencit mempunyai ketumbuhan tumor payudara dan dibunuh setiap minggu dan sampel tumor dikumpul. Ketumbuhan tumor hanya dilihat pada mencit di dalam kumpulan CC, CT, CNDV32+T dan CNDV64+T, manakala tiada ketumbuhan pada mencit di dalam kumpulan rawatan yang lain. Tiada regresi pada pertumbuhan tumor untuk kumpulan CNDV32+T dan

CNDV64+T, malah menunjukkan indeks apoptotik (AI) yang sangat rendah dan indeks mitotik (MI) yang tinggi sepanjang rawatan dijalankan. Ini menandakan rawatan tersebut bukan terapeutik. Ujian TUNEL dijalankan untuk menentukan kuantiti sel apoptotik dan keputusannya adalah selari dengan keputusan AI, iaitu hanya kumpulan CT menunjukkan peningkatan dalam sel apoptotik apabila dibandingkan pada minggu pertama sehingga minggu keempat. Kumpulan yang dirawat dengan tamoxifen sahaja dapat merencatkan tumor tetapi tidak signifikan. Tumor dengan gen perencat tumor, p53, menghasilkan protin p53 yang mutan. Terdapat juga korelasi yang kuat antara protin mutan di dalam nukleus sel tumor dalam kumpulan CC, CT, CNDV32+T dan CNDV64+T dengan skor MI. Protin p53 yang mutan tidak dapat menginhibitasi pertumbuhan sel kanser, menyebabkan aktiviti mitotik tinggi dan peningkatan dalam proliferasi sel. Dalam kumpulan CNDV32+T dan CNDV64+T, terdapat bukti bahawa NDV menyebabkan pemencilan sitoplasmik p53 protin dari nukleus ke sitoplasma, dan ini menunjukkan bahawa virus tersebut memainkan peranan di dalam merangsangkan apoptosis pada sel. Tisu payudara mencit kumpulan CNDV8, CNDV16, CNDV32, CNDV64, CNDV8+T dan CNDV16+T yang tidak mempunyai ketumbuhan tumor juga diwarnakan untuk mengesan lokasi p53. Ia banyak terekspres di dalam sitoplasma duktus epitelium yang serupa dengan kelenjar payudara yang tidak aktif, menandakan kumpulan-kumpulan ini bebas daripada tumor. Keputusan kajian ini mencadangkan NDV titer 8, 16, 32 dan 64HA menginhibitasi pertumbuhan sel 4T1, dengan tidak membenarkan formasi tumor. Tidak semua kombinasi NDV dan tamoxifen berkesan, titer NDV yang lebih tinggi apabila digabungkan dengan tamoxifen tidak dapat menghalang ataupun merencatkan pertumbuhan tumor. Kesimpulannya, rawatan dengan NDV AF2240 sahaja boleh menginhibitasi pertumbuhan sel kanser 4T1. Oleh itu, ia

berpotensi digunakan sebagai agen onkolitik untuk rawatan kanser payudara. NDV lebih efektif secara signifikan berbanding dengan tamoxifen dan boleh dijadikan agen antikanser alternatif yang berfaedah untuk kanser payudara.

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I certify that a Thesis Examination Committee has met on 9 April 2010 to conduct the final examination of Anushia d/o Swaminathan on her thesis entitled "Effect of Newcastle Disease Virus AF2240 on Allografted 4T1 Breast Cancer Cells 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 student be awarded the Master of Science.

Members of the Thesis Examination Committee were as follows:

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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 were as follows:

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Date: 15 July 2010

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 at any other institution.

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ANUSHIA SWAMINATHAN

Date: 1 June 2010

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