UNIVERSITI PUTRA MALAYSIA

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

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MASTER OF SCIENCE
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By

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

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April 2010

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
berpotensi digunakan sebagai agen onkotik 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.

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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.

___________________________
ANUSHIA SWAMINATHAN

Date: 1 June 2010
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>ii</td>
</tr>
<tr>
<td>ABSTRAK</td>
<td>iv</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>vii</td>
</tr>
<tr>
<td>APPROVAL</td>
<td>viii</td>
</tr>
<tr>
<td>DECLARATION</td>
<td>x</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xiv</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xvi</td>
</tr>
<tr>
<td>LIST OF APPENDICES</td>
<td>xvii</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>xviii</td>
</tr>
<tr>
<td>CHAPTER</td>
<td></td>
</tr>
<tr>
<td>1  INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>2  LITERATURE REVIEW</td>
<td>6</td>
</tr>
<tr>
<td>2.1 Breast Cancer</td>
<td>7</td>
</tr>
<tr>
<td>2.1.1 Risk Factors of Breast Cancer</td>
<td>13</td>
</tr>
<tr>
<td>2.1.2 Classification and Stages of Breast Cancer</td>
<td>18</td>
</tr>
<tr>
<td>2.1.3 Treatment of Breast Cancer</td>
<td>23</td>
</tr>
<tr>
<td>2.2 Newcastle Disease Virus (NDV)</td>
<td>24</td>
</tr>
<tr>
<td>2.2.1 NDV Pathotypes and Classifications</td>
<td>25</td>
</tr>
<tr>
<td>2.2.2 NDV Characterization</td>
<td>26</td>
</tr>
<tr>
<td>2.2.3 NDV and Cancer Treatment</td>
<td>27</td>
</tr>
<tr>
<td>2.2.4 NDV Strain AF2240</td>
<td>28</td>
</tr>
<tr>
<td>2.3 Apoptosis</td>
<td>29</td>
</tr>
<tr>
<td>2.3.1 Morphology of Apoptosis</td>
<td>30</td>
</tr>
<tr>
<td>2.3.2 Molecular Process of Apoptosis</td>
<td>32</td>
</tr>
<tr>
<td>2.3.3 Apoptosis and Necrosis</td>
<td>33</td>
</tr>
<tr>
<td>2.3.4 Apoptosis and Tumour Suppressor Gene, p53</td>
<td>34</td>
</tr>
<tr>
<td>2.4 Tamoxifen</td>
<td>35</td>
</tr>
<tr>
<td>2.5 Animal Model in Cancer Research</td>
<td>36</td>
</tr>
<tr>
<td>2.5.1 Mouse as Model in Cancer Research</td>
<td>37</td>
</tr>
<tr>
<td>2.5.2 4T1 Mouse Mammary Cancer Cell Line</td>
<td>38</td>
</tr>
<tr>
<td>3  ONCOLYTIC EFFECT OF NDV AF2240 ON 4T1 MOUSE BREAST CANCER CELL LINE</td>
<td>39</td>
</tr>
<tr>
<td>3.1 Introduction</td>
<td>41</td>
</tr>
<tr>
<td>3.2 Materials</td>
<td>41</td>
</tr>
<tr>
<td>3.2.1 Virus</td>
<td>41</td>
</tr>
<tr>
<td>3.2.2 4T1 Mouse Breast Cancer Cell Line</td>
<td>41</td>
</tr>
<tr>
<td>3.3 Methods</td>
<td>41</td>
</tr>
<tr>
<td>3.3.1 Virus Propagation</td>
<td>41</td>
</tr>
<tr>
<td>3.3.2 Cell Culture</td>
<td>45</td>
</tr>
<tr>
<td>3.3.3 MTT Assay</td>
<td>47</td>
</tr>
<tr>
<td>3.4 Results</td>
<td>49</td>
</tr>
<tr>
<td>3.4.1 Virus Propagation</td>
<td>49</td>
</tr>
<tr>
<td>3.4.2 MTT Assay</td>
<td>49</td>
</tr>
</tbody>
</table>
3.5 Discussion

4 GROSS AND MORPHOLOGICAL STUDIES OF MICE INOCULATED WITH 4T1 BREAST CANCER CELLS TREATED WITH NDV AF2240

4.1 Introduction 53
4.2 Materials 56
  4.2.1 Experimental Animals 56
4.3 Methods 56
  4.3.1 BALB/c Mice Maintenance 56
  4.3.2 Breast Cancer Induction and Treatment Regimen 56
  4.3.3 Determination of Body Weight, Tumour Weight and Inhibition Rate
  4.3.4 Histology Processing 58
  4.3.5 Apoptotic and Mitotic Index 59
  4.3.6 Statistical Analysis 60
4.4 Results 61
  4.4.1 Gross Morphology of Tumour Growth 61
  4.4.2 Body Weight of Mice 63
  4.4.3 Tumour Weight 65
  4.4.4 Rate of Inhibition 67
  4.4.5 Mean Score of Apoptotic and Mitotic Index 68
4.5 Discussion 74

5 QUANTIFICATION OF APOPTOTIC CELLS IN BREAST CANCER TISSUE TREATED WITH NDV AF2240

5.1 Introduction 86
5.2 Materials and Methods 88
  5.2.1 TUNEL Assay 88
  5.2.2 Quantification of Apoptotic Cells 89
  5.2.3 Statistical Analysis 89
5.3 Results 90
  5.3.1 Confocal Micrographs of Breast Tumour 90
  5.3.2 Quantification of Apoptotic Cells 93
5.4 Discussion 95

6 EXPRESSION OF p53 TUMOUR SUPPRESSOR GENE IN BREAST CANCER TISSUE USING IMMUNOHISTOCHEMISTRY

6.1 Introduction 98
6.2 Materials and Methods 101
  6.2.1 IHC Staining for p53 101
  6.2.2 Scoring for IHC Staining 102
  6.2.3 Statistical Analysis 103
6.3 Results 104
  6.3.1 Light Micrographs of p53 Staining 104
  6.3.2 Mean Score of p53 Staining 105
6.4 Discussion 109