UNIVERSITI PUTRA MALAYSIA

EFFECTS OF NEWCASTLE DISEASE VIRUS ON GENE EXPRESSION PROFILING, AND NITRIC OXIDE AND GLUTATHIONE PRODUCTION IN MCF-7 BREAST CANCER CELL LINE

MOHAMED KALID ALI

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By

MOHAMED KALID ALI

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

APRIL 2011

Specially dedicated to,

My beloved Ikram, Abshiro, Khalid, Uncle Abdulahi and Aunty Fadumo, Brother In-law Said Hussen Id.

AND

The rest of my Family and Friends

For their invaluable love, understanding, patience, support and care

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

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Chairman: **Profesor Datin Paduka Khatijah Mohd Yusoff, PhD**

Faculty: **Biotechnology and Biomolecular Sciences**

Breast cancer is a major cause of death for many women around the world. Progress has been made in the survival of breast cancer patients due to improved understanding of the molecular processes, diagnostic techniques and knowledge of the treatment using chemotherapy, radiotherapy, and virotherapy. The field of virotherapy has emerged from the past decade by using oncogenic viruses to selectively kill cancerous cells without harming normal cells. Newcastle disease virus (NDV), an avian paramyxovirus, induces apoptosis in a variety of human malignant cells such as breast cancer cells. In this study, the cytolytic properties of NDV strain AF2240 on gene expression profiling, measurement of Nitric oxide (NO) free radical and Glutathione antioxidant (GSH) were investigated by using RT-PCR, capillary electrophoresis, flow cytometry both under normoxic and hypoxic condition.

Multiplex Gene Expression Kit was used whereby the relative expressions of 25 different genes were measured at the mRNA level. The results showed that treatment of MCF7 cells with NDV caused apoptosis, NO production and GSH depletion and changed the expression level of most of the genes involved in tumour progression, cell cycle regulation, cell growth and differentiation, apoptosis, cancer suppression, and DNA damage, as measured by RT-PCR and capillary electrophoresis. Since the mode of apoptosis by NDV is NO and GSH dependent, comparisons of NDV with NO-Donors such as DETA-NONOate and NO-Scavengers like cPTIO were also studied. Increased production of Nitric oxide (NO) and depleted levels of Glutathione (GSH) after treatment of NDV at normoxic condition compared NDV at hypoxic condition were observed. However, addition of DETA-NONOate to MCF-7 cells has induced NO production, GSH depletion. Moreover, NO production and apoptosis were attenuated, and GSH increased after addition of cPTIO either alone or with combination of either NDV or DETA-NONOate to the MCF-7 cells. In addition to the above, co-treatment of NDV+DETA-NONOate depleted NO production and cell death compared to the treatment of NDV alone to cells. From this study, it was concluded that NDV induces apoptosis, Gene expression, NO production and GSH depletion in MCF-7 cells which makes it an effective anticancer agent due to its ability to kill breast cancer cell lines in normoxic and hypoxic conditions.

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

KEBERKESANAN NEWCASTLE DISEASE VIRUS DALAM PROFIL EKSPRESI GEN DAN PENGELUARAN NITRIK OKSIDA SEL KANSER PAYU DARA MCF-7

Oleh

MOHAMED KALID ALI

APRIL 2011

Pengerusi : Profesor Datin Paduka Khatijah Mohd Yusoff, PhD

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Kanser payu dara merupakan pembunuh terbesar bagi wanita di seluruh dunia. Peningkatan kadar kelansungan hidup bagi penghidap kanser payu dara mula ditunjukkan apabila kefahaman mengenai proses molekul, teknik diagnostik dan pengetahuan mengenai rawatan seperti kemoterapi, radioterapi dan viroterapi semakin berkembang. Kaedah viroterapi mula dikenali sejak sedekad yang lepas apabila virus-virus onkogenik mula digunakan sebagai sasaran untuk membunuh sel kanser tanpa menggangu sel-sel yang lain. Newcastle Disease Virus (NDV) yang tergolong dalam kumpulan Avian Paramyxovirus, menyebabkan kematian sel dalam pelbagai jenis kanser yang merbahaya seperti kanser payu dara. Dalam kajian ini, kebolehan membunuh sel kanser oleh NDV AF2240 melalui “gene expression profiling”, penyukatan radikal bebas Nitrik Oksida (NO), dan “Glutathione” anti oksida telah dikaji dengan menggunakan

teknik RT-PCR, “ capillary electrophoresis”, “flow cytometry” di bawah kondisi “hypoxic” dan “normoxic”. “Multiplex Gene Expression Kit” telah digunakan dan sementara itu, ekspresi 21 gen yang berhubung-kait boleh diukur pada peringkat mRNA. Keputusan kajian ini menunjukkan bahawa sel MCF-7 yang dirawat dengan menggunakan NDV telah menyebabkan kematian sel secara berprogram, tiada pengurangan atau pengeluaran GSH dan perubahan ekspresi sebahagian besar gen yang terlibat dalam pembesaran tumor, kawalan kitaran hidup sel, pertumbuhan dan diferensiasi sel, kematian sel secara berprogram, pembantutan pertumbuhan kanser dan kerosakan DNA seperti yang diukur melalui RT-PCR and “capillary electrophoresis”. Memandangkan cara NDV membunuh sel bergantung kepada NO and GSH, perbandingan NDV dengan NO-Donors seperti DETA-NONOate dan NO-Scavengers seperti cPTIO turut dijalankan. Peningkatan pengeluaran Nitrik Oksida (NO) and penurunan kadar Glutathione (GSH) selepas dirawat dengan NDV di bawah kondisi normoxic berbanding hypoxic turut diamati. Walau bagaimanapun, penambahan. DETA-NONOate terhadap MCF-7 telah menyebabkan pengeluaran NO dan pengurangan GSH. Tambahan lagi, pengeluaran NO dan kematian sel secara berprogram berkurangan dan GSH meningkat apabila ditambah cPTIO sama ada secara sendiri atau digabungkan dengan NDV atau DETA-NONOate terhadap MCF-7. Malah, gabungan rawatan NDV+DETA-NONOate mengurangkan NO dan kematian sel berbanding dengan rawatan NDV sendirian.

Daripada kajian ini dapatlah dirumuskan bahawa NDV menyebabkan kematian sel secara berprogram, ekspresi gen, pengeluaran NO dan pengurangan GSH di dalam sel MCF-7 yang mana membuatkan ia sebagai rawatan anti kanser yang efektif kerana mampu membunuh sel kanser payu dara di bawah kondisi normoxic dan hypoxic.

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I certify that a Thesis Examination Committee has met on 4 April 2011 to conduct the final examination of Mohamed Kalid Ali on his thesis entitled “Effects of Newcastle disease virus on Gene Expression Profiling, and Nitric Oxide and Glutathione Production in MCF-7 Breast Cancer Cell Line” 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 Master of Science degree.

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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 Master of Science. The members of the

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Date: 4 April 2011

DECLARATION

I declare that the thesis is my original work except the 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 UPM or at any other Institution.

Mohamed Kalid Ali

Date: 4 April 2011

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