



UNIVERSITI PUTRA MALAYSIA

**CYTOLYTIC EFFECTS OF NEWCASTLE DISEASE VIRUS STRAIN
AF2240 ON DBTRG.05MG AND U-87MG BRAIN TUMOR CELL LINES**

ROLA ALI SAEED

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By

ROLA ALI SAEED

**Theses Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfilment of the Requirements for the Degree of Master of Science
February 2008**



Especially to.....

*To my parent
To my husband...Aied
To my daughters...Raghd and Rand
To my sisters and brother*

*To my country, Republic of Yemen
To all cancer patients all over the world*



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

CYTOLYTIC EFFECTS OF NEWCASTLE DISEASE VIRUS STRAIN AF2240 ON DBTRG.05MG AND U-87MG BRAIN TUMOR CELL LINES

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February 2008

Chairman: Professor Abdul Manaf Ali, PhD

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Newcastle disease virus (NDV) is a potential oncolytic agent as it can replicate up to 10,000 times better in human cancer cells than in most normal human cells. Several strains of NDV were reported to induce cytolysis to various cancerous cell lines.

In this study, the cytolytic effects of local strain NDV AF2240 toward DBTRG.05MG (glioblastoma multiform) and U-87MG (anaplastic astrocytoma) cell lines were determined using microtetrazolium assay (MTT) for both monolayer and co-culture methods. The value of (IC₅₀) inhibition concentration, fifty percent at which the titer of NDV as hemagglutination units (HAU) that reduce 50% of cell population as compared to the untreated control was determined after 72 hours. The IC₅₀ values for cytolytic effects of NDV strain AF2240 on DBTRG.05MG cell line were 955 HAU/ml and 460 HAU/ml for the monolayer and co-culture methods, respectively. For U-87MG cell line, the IC₅₀ values were 380 HAU/ml and 52 HAU/ml for monolayer and



co-culture methods, respectively. No significant cytolytic effect was observed on normal HCN-2 and 3T3 cell lines at the same titre used in the brain tumor cell lines. The cell proliferation rate of treated brain tumor cell lines was reduced significantly with time and titration of the virus as compared to the untreated control.

It was confirmed that the mode of cell death in response to infection by NDV strain AF2240 on brain tumor cell lines was by apoptosis. Morphological features of apoptosis were observed by Phase Contrast Microscopy, Fluorescence Microscopy (Acridine Orange (AO) and Propidium Iodide (PI) staining) and Transmission Electron Microscopy. Features observed included chromatin condensation and margination along the inner nuclear membrane, cytoplasmic condensation, and membrane blebbing without disintegration of the cellular membrane. These were further confirmed with DNA laddering in agarose gel electrophoresis assay and terminal deoxyribonucleotide transferase-mediated dUTP-X nick end-labeling staining (TUNEL) assay. However, analysis of the cellular DNA content using PI showed that the virus caused an increase in sub-G1 region. The apoptosis peaks (sub-G1) found in DBTRG.05MG cells treated with NDV strain AF2240 were 18.40 and 37.40% for 24 and 48 hours, respectively whereas in U-87MG cells treated with NDV strain AF2240 the peaks were 10.29 and 19.45% for 24 and 48 hours, respectively. Early apoptosis was also observed by annexin V flow cytometry method. The amounts of apoptotic cells were 3.7 and 4.26% for DBTRG.05MG cells and U-87MG cells 6 hours post-inoculation, respectively. It was concluded that NDV strain AF2240 is a potent antitumor agent and the mode of cell death induced by this virus is apoptosis.



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

**KESAN SITOTOSIK DARI VIRUS PENYAKIT NEWCASTLE JENIS AF 2240
TERHADAP DBTRG.05MG DAN U-87MG TUMOR SEL OTAK**

Oleh

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Virus penyakit Newcastle (NDV) merupakan ejen pembunuh barah (or “oncolytic”) yang berupaya disebabkan virus ini dapat mereplikasi 10,000 kali lebih baik di sel barah manusia berbanding dengan sel normal manusia. Beberapa jenis NDV telah dilapor dapat mencetuskan “cytolysis” terhadap beberapa jenis sel barah. Di dalam ujikaji ini kesan sitotosik dari jenis NDV AF2240 tempatan terhadap sel DBTRG.05MG (glioblastoma multiform) dan U-87MG (anaplastic astrocytoma) telah ditentu dengan menggunakan analisa methyl thiazolyl tetrazolium (MTT) untuk kedua-dua cara kultur satu lapis dan kultur campuran. Nilai bagi kepekatan perencatan lima puluh peratus (IC_{50}) di mana titer bagi NDV sebagai unit “hemagglutination” (HAU) yang mengurangkan 50% sel populasi apabila dibandingkan dengan kawalan yang tidak dirawat selepas 72 jam. Nilai IC_{50} bagi kesan sitotosik NDV jenis AF2240 atas DBTRG.05MG cell line ialah 995 HAU/mL dan 460 HAU/mL bagi cara kultur satu lapis dan kultur campuran masing-masing. Bagi sel U-87MG, nilai IC_{50} adalah



380 HAU/mL dan 52 HAU/mL bagi kultur satu lapsi dan kultur campuran masing-masing. Tidak ada kesan sitotoksik yang ketara diperhatikan terhadap sel normal HCN-2 dan 3T3 pada titre yang sama seperti digunakan di atas sel barah otak. Kadar sel proliferasi sel otak telah dikurangkan secara ketara mengikut masa dan nilai IC_{50} oleh virus jika dibandingkan dengan kawalan yang tidak dirawat. Ini telah dipastikan bahawa cara sel mati yang disebabkan oleh jangkitan NDV jenis AF2240 terhadap sel barah otak berlaku secara apoptosis. Keistimewaan morfologi bagi apoptosis diperhatikan melalui fasa kontras mikroskopik, Fluorescence mikroskopik (Acridine orange (AO) dan Propidium Iodide (PI) pewarnaan) dan pancaran elektron mikroskopik termasuk kondensasi kromatin dan biraian sepanjang membran nuklear, citoplasmik kondensasi, dan membrane blebbing tanpa penguraian membran sel. Semua ini telah dikesan selanjutnya dengan DNA laddering dalam analisa agarose gel electrophoresis dan analisa terminal deoxyribonucleotide transferase-mediated dUTP-X nick end-labeling staining (TUNEL). Walau bagaimanapun, analisa atas kandungan sel DNA melalui PI menunjukkan bahawa virus telah menyebabkan peningkatan pada lingkungan subG1. Puncak apoptosis (sub-G1) yang didapati di DBTRG.05MG sel yang dirawat dengan NDV strain AF2240 adalah 18.40% dan 37.40% untuk 24 dan 48 jam masing-masing sedangkan pada sel U-87MG yang dirawat dengan NDV strain AF2240 adalah 10.29% dan 19.45% untuk 24 dan 48 jam masing-masing. Awal apoptosis juga diperhatikan melalui cara Annexin V aliran sitometrik, jumlah apoptosis sel adalah 3.7% dan 4.2% untuk sel DBTRG.05MG dan U-87MG selepas 6 jam inokulasi. Kesimpulannya NDV jenis AF2240 merupakan ejen antitumor yang berpotensi dan cara cell mati yang dicetuskan oleh virus ini adalah melalui apoptosis.



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I certify that an Examination Committee has met on 29th February 2008 to conduct the final examination of Rola Ali Saeed on her Master of Science thesis entitled “Cytolytic Effects of Newcastle Disease Virus Strain AF2240 on DBTRG.05MG and U-87MG Brain Tumor Cell Lines” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are 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 UPM or at any other institution.

ROLA ALI SAEED

Date: 26 February 2009



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LIST OF ABBREVIATIONS

| | |
|-----------------|--------------------------------------|
| µl | microlitre |
| °C | degree Celcius |
| aa | Amino acid |
| AA | Anaplastic astrocytoma |
| AO | Acridine Orange |
| ATCC | American Type Culture Collection |
| ATV | Antibiotic- trypsin-versine |
| BBB | Blood–Brain Barrier |
| bp | Base pair |
| CAS | Chorioallantoic sac |
| cm | Centimeter |
| cm ² | Centimeter square |
| cm ³ | Centimeter cube |
| CO ₂ | Carbon dioxide |
| DMSO | Dimethylsulphoxide |
| DNA | Deoxyribonucleic acid |
| dUTP | Deoxyuridine Triphosphate |
| ECACC | European Collection of Cell Cultures |
| EDTA | Ethylenediaminetetraacetic acid |



| | |
|------------------|--|
| ELISA | Enzyme-Linked Immunosorbent Assay |
| F | Fusion |
| FCM | flow cytometry |
| Fig. | Figure |
| g | Gram |
| GBM | Glioblastoma multiform |
| HA | Hemagglutination |
| HAU | Hemagglutination unit |
| HN | Hemagglutinin- neuraminidase |
| HSV | Herpes simplex virus |
| IC ₅₀ | Inhibition concentration |
| IFN | Interferon |
| IL | Interleukin |
| Kbp | kilo base pair |
| M | Molar |
| mg | Milligram |
| ml | Milliliter |
| MTT | 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide |
| MV | Measles virus |
| NA | Neuraminidase |
| NDV | Newcastle disease virus |



| | |
|-------|---|
| NK | Natural killer |
| nm | Nanometer |
| NTE | NaCl-Tris-HCl-EDTA buffer |
| OD | Optical density |
| PBMCs | Peripheral Blood Mononuclear Cells |
| PBS | Phosphate buffered saline |
| PCD | programmed cell death |
| PI | Propidium iodide |
| PS | phosphatidylserine |
| RBC | Red blood cells |
| RNA | Ribonucleic acid |
| rpm | Revolution per minute |
| rTdT | Recombinant Terminal Deoxynucleotidyl |
| SSC | Saline Sodium Citrate |
| ssRNA | Singe stranded RNA |
| TEM | Transmission electron microscopy |
| TNF | Tumor necrosis factor |
| TUNEL | Terminal deoxyribonucleotide transferase-mediated dUTP-X nick end- labeling |
| UPM | Universiti Putra Malaysia |



| | |
|-----|----------------------------|
| UV | Ultra violet |
| v/v | Volume/ Volume |
| VSV | Vesicular stomatitis virus |
| w/v | Wight/Volume |
| WHO | World Health Organization |



CHAPTER I

GENERAL INTRODUCTION

Brain tumors are considered benign as they do not spread outside the brain. However, they are like cancerous tumors and grow in the brain. They can be dangerous because of the importance of the brain and the limited amount of space inside the skull. The brain contains cells called neurons which are specialized for the processing and transmission of cellular signals. Glial cells are non-neuronal cells, they are supportive cells of the central nervous system and most of the brain is made up of these cells. These cells provide support and nutrition, maintain homeostasis, form myelin, and they help neurons do their work. The majority of these cells are called astrocytes. There are many different kinds of brain tumor based on whether they are primary or secondary. Primary tumors are tumors that originated in the brain. They can be further divided into astrocytomas, glioblastoma, oligodendrogliomas, meningiomas, medulloblastomas, neuronomas and schwannomas based on the cell type involved. Secondary tumors are tumors which result from metastasis. Gliomas such as anaplastic astrocytoma and glioblastoma multiforme are the most common type of primary brain tumors (Mangiardi and Kane, 2003).

The treatment of primary brain tumors is difficult because of polyclonicity, the blood brain barrier, the diffuse infiltrative nature of these tumors, and the perilous location



of some tumors. So, to cure brain tumors some consideration must be taken to kill all cells within the tumor and spare the remaining normal brain cells. There are three standard types of treatment for patients with primary brain tumors: surgery, radiation therapy, and chemotherapy (Mangiardi and Kane, 2003). New cancer treatments with novel mechanisms of action are needed. Viral therapy for cancer (virotherapy) has significantly been identified to show some promise in cancer therapy. Virotherapy involves the treatment of cancer by using a virus specifically to infect cancer cells while leaving normal cells unharmed (You *et al.*, 2004). At least 10 different viral species have been shown to have this potential and some are already being used in clinical trials (Kirn *et al.*, 2001). These viruses infect, replicate in and kill human cells through diverse mechanisms (Evarts and ven der Poel, 2005).

The Newcastle disease virus (NDV) is a member of the new genus *Avulavirus* within the family *Paramyxoviridae*. The virus causes a highly contagious disease in poultry and wild birds infecting 27 to 50 orders. Exposure to humans however, results in mild conjunctivitis, laryngitis and influenza-like symptoms (Fenner *et al.*, 1987). A very virulent strain of the virus known as strain AF2240 has been shown to be responsible for a very high mortality and morbidity among poultry flocks in Malaysia (Lai and Ibrahim, 1987).

Interest in the use of NDV as an anticancer agent has arisen from the ability of the virus to selectively kill human tumor cells with limited toxicity to normal cells. It has oncolytic activity that can destroy tumor cells and stimulate the immune system.



Strains 73-T, MH68, Italian, Ulester, Rokin, PV701 (MK107) and HUI strains of NDV have been shown to exhibit an oncolytic activity. In addition, the oncolytic effects of six Malaysian strains of NDV, AF2240, 01/C, Ijuk, S, F, and V4, have also been studied on several tumor cell lines (Omar *et al.*, 2003; Freeman *et al.*, 2006; Niederhuber, 2006). However, no studies have yet been made on NDV strain AF2240 oncolytic activity on brain tumor cells. Therefore, in this study the oncolytic effects of NDV strain AF2240 was tested *in vitro* against two types of brain tumor cell lines DBTRG.05MG and U-87MG.

The objectives of this study are:

- i. To determine the cytolytic effects of NDV strain AF2240 on brain tumor cell lines DBTRG.05MG (glioblastoma multiforme) and U-87MG (anaplastic astrocytoma).
- ii. To determine the cytolytic effects of NDV strain AF2240 on normal cell lines HCN-2 (Human brain cells, cerebral cortex neurons) and 3T3 (normal mouse fibroblast cells).
- iii. To study the effects of NDV strain AF2240 on the proliferation of brain tumor cell lines DBTRG.05MG and U-87MG.
- iv. To determine the morphological changes, biochemical changes and the mode of cell death induced by the virus.
- v. To identify the phase of the cell cycle affected by the virus.