UNIVERSITI PUTRA MALAYSIA

LOCAL V4-UPM STRAIN NEWCASTLE VIRUS-INDUCED CELL DEATH OF BRAIN TUMOR CELL LINES

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By
ROHAYA BINTI IBRAHIM

Thesis Submitted to the School of Graduate Studies,
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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

LOCAL V4-UPM STRAIN NEWCASTLE VIRUS-INDUCED CELL DEATH OF BRAIN TUMOR CELL LINES

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Chair: Professor Abdul Manaf Ali, PhD

Faculty: Institute of Bioscience

V4-UPM strain is a local heat resistant variant of the avirulent Australian V4 (QUE) strain which has higher thermostabilities of infectivity and haemagglutination than the original. The aims of this study were to analyze the cytolytic effect and mode of cell death induced by V4-UPM strain of NDV on two human brain tumor cell lines, glioblastoma multiforme (DBTRG.05MG) and anaplastic astrocytoma cells (U87MG). In-vitro cytotoxic activity has been performed on both brain tumor cells via standard MTT assay. At 72 hours post-incubation, the IC50 values obtained were 30 haem agglutination (HA) unit/ml and 5 HA unit/ml for DBTRG.05MG and U87MG cells, respectively. In addition, V4-UPM strain inhibited the proliferation of both DBTRG.05MG and U87MG cells via the same method. Morphological changes of the treated cells also have been observed sequentially under the inverted light, fluorescence and electron microscopes. The virus-infected cells showed apoptotic features such as the cell shrinkage, cell blebbing and formation of apoptotic bodies under the inverted light

microscope. The differential uptake of acridine orange and propidium iodide dyes demonstrated the occurrence of apoptosis, mainly by chromatin condensation and DNA fragmentation that were observed under fluorescence microscope. The next morphological observation was based on color and nuclear morphology where the nuclei of treated-cells possessed DNA fragmentation were stained dark brown by 3',3'-diaminobenzidine. The distinct ultrastructural changes occurred in the nucleus, cellular organelles and plasma membrane structures were confirmed to be the apoptotic features via transmission electron microscopic (TEM) observation. The result of flow cytometric analyses based on annexin V-FITC that binds phosphatidylserine residue also suggested the onset of apoptosis for both groups. Meanwhile, the cell cycle was arrested at S phase followed by apoptosis. Based on these findings, it was confirmed that the V4-UPM strain induced apoptotic cell death on DBTRG.05MG and U87MG cells. Thus, this strain has the potential to be developed as an antitumor agent for future treatment particularly in grade-III brain tumor patients.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia

sebagai memenuhi keperluan untuk ijazah Master Sains

STRAIN TEMPATAN V4-UPM DARIPADA VIRUS PENYAKIT NEWCASTLE

MENYEBABKAN KEMATIAN KE ATAS SEL TUMOR OTAK

Oleh

ROHAYA BINTI IBRAHIM

Ogos 2011

Pengerusi: Profesor Abdul Manaf Ali, PhD

Fakulti: Institut Biosains

Strain V4-UPM adalah variasi resistan haba tempatan daripada strain V4 (QUE) Australia yang tidak berbisa. Ia mempunyai fungsi hemagglutinin and infektiviti yang lebih tinggi daripada induk asal. Tujuan utama kajian ini adalah untuk melihat kesan sitolitik dan cara kematian sel yang diakibatkan oleh strain V4-UPM ke atas kedua-dua sel tumor otak manusia iaitu 'glioblastoma multiforme' (DBTRG.05MG) dan 'anaplastic astrocytoma' (U87MG). Aktiviti sitotoksik dijalankan secara *in-vitro* ke atas kedua-dua sel tumor otak tersebut, melalui asai MTT piawai. Pada jam ke-72 masa inkubasi, sel DBTRG.05MG dan U87MG masing-masing memberikan nilai sebanyak 30 haem agglutination (HA) unit/ml dan 5 HA unit/ml. Melalui kaedah yang sama, proliferasi sel untuk kedua-dua sel DBTRG.05MG dan U87MG juga telah dikenalpasti. Seterusnya, perubahan morfologi sel yang dijangkiti diperhatikan menerusi rangkaian mikroskop cahaya inverted, fluoresen dan elektron. Menerusi mikroskop cahaya inverted, sel yang dijangkiti virus menunjukkan ciri-ciri apoptotik seperti pengecutan sel, 'blebbing' dan

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Wassalam…

I certify that a Thesis Examination Committee has met on 1 August 2011 to conduct the final examination of Rohaya Ibrahim on her Master of Science thesis entitled "Cytolytic effect of local V4-UPM strain of Newcastle disease virus of brain tumor cell lines" 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.
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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were 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 Universiti Putra Malaysia or at any other institution.

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ROHAYA BT IBRAHIM

Date: 1 August 2011

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