



UNIVERSITI PUTRA MALAYSIA

***EVALUATION OF NEWCASTLE DISEASE VIRUS AS AN ONCOLYTIC
AGENT IN COLORECTAL CANCER CELL LINES***

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By

CHIA SUET LIN

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfilment of the Requirements for the Degree of Doctor of Philosophy**

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Chair: Professor Datin Paduka Khatijah Yusoff, PhD

Faculty: Biotechnology and Biomolecular Sciences

Reports showing tumour regression in cancer patients following viral infections appeared at the beginning of the last century. By the 1950s, several viruses including Newcastle disease virus (NDV) showed positive oncolytic activities. Since then, NDV with its natural ability to infect cancer cells has been widely studied as a candidate in viral oncolysis. In the present study, oncolytic effect of a local NDV isolate, strain AF2240, was analysed in a panel of colorectal cancer (CRC) cell lines. Prior to infection studies, a modified plaque assay using CRC cells as hosts was established. SW620 was found to be the best cell line to be used for NDV plaque assay because of the size, clarity, and also the number of plaques formed. The plaque forming unit (pfu)/ mL of NDV was determined and subsequently used for the infection studies.

All the CRC lines underwent apoptosis upon NDV infection. The presence of syncytia, a characteristic of paramyxoviral infection, proved that these cells were indeed infected by

the virus. In addition, sandwich ELISA and plaque assays performed on spent culture supernatants showed that infectious viral progenies were released into the medium.

The effects of NDV infection on the Ras/Akt/PKB pathway in these CRC cells with multiple gene mutations (in *K-Ras*, *p53*, and *APC*) were analysed. Comparison between these cell lines showed that all the cells experienced down-regulation of pAkt upon NDV infection. This down-regulation, however, did not affect its downstream effector, pGSK3 β . Thus the β -catenin protein level was also not down-regulated in all the cells. It is not surprising, however, that the pGSK3 β and β -catenin were down-regulated in the HCT116p53^{-/-} cells as these cells possess wild type APC and the *p53* gene is knocked out in both alleles. From these results, it is possible to suggest that it may not be effective to use therapeutic agents targeting the Ras/Akt/PKB pathway for CRC treatment. Regardless of the effect of NDV infection on the cell proliferation pathway, NDV did up-regulate BAD, down-regulate pBAD or reduce the Bax/Bcl-2 levels (depending on the cell lines) leading to apoptosis in these cells.

The SW480 cells experienced a down-regulation of the pAkt during a primary infection (at 25 hpi or earlier) but recovered gradually during a secondary infection (after 25 hpi) when the pAkt level increased. Repeated exposures of NDV to these cells did not induce any cellular apoptosis. These cells were then labelled as SW480P as they were found to be persistently infected with the virus. Microscopic analysis of the SW480P, with or without re-infection of NDV, showed an identical physical appearance to that of the parental SW480 cells. RT-PCR and immunofluorescent analyses of one of the most

abundant NDV protein, the nucleocapsid protein (NP) showed that the viral RNA and proteins were present in the uninfected SW480P. However, the newly synthesized viral progenies showed smaller plaques compared to wild type NDV suggesting that defective interfering particles (DIP) were produced. This abortive infection resulting in the production of DIPs may explain why some cancer cells resist the oncolytic effect by the virus during virotherapy.

This study demonstrated the potential of NDV as an oncolytic agent in various CRC cell lines. NDV mediated the oncolysis through suppression of the Ras/Akt/PKB pathway leading to apoptosis. Cellular apoptosis occur in CRC cells, regardless of the genetic mutations acquired, by the reduction of the Bax/Bcl-2 ratio, up-regulation of BAD and down-regulation of pBAD. Despite the robust pro-apoptotic ability of NDV, a small percentage (17%) of cells in the infected population, survived the infection even after 97 hpi. Following rescue and further propagation, these cells were found to be persistently infected with NDV. Viral progenies were actively secreted into the culture media. However, these secreted viruses were found to be less infectious and showing reduced oncolytic activities, characteristic of DIPs. Results obtained from this study will contribute towards further understanding of the mechanisms of NDV-induced oncolysis. This information will be useful for improving NDV as a candidate for cancer virotherapy.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**PENILAIAN VIRUS PENYAKIT SAMPAR AYAM SEBAGAI AGEN
ONKOLISIS DALAM SEL KANSER KOLOREKTAL**

Oleh

CHIA SUET LIN

Mei 2012

Pengerusi: Profesor Datin Paduka Khatijah Yusoff, PhD

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Terdapat beberapa laporan yang menunjukkan regresi tumor pada pesakit kanser berikutan jangkitan virus muncul pada awal abad yang lalu. Menjelang 1950-an, beberapa virus termasuk virus penyakit sampar ayam (NDV) menunjukkan aktiviti onkolitik positif. Sejak itu, NDV dengan kebolehan semulajadi untuk menjangkiti sel-sel kanser telah dikaji secara meluas sebagai calon dalam virus onkolitik. Dalam kajian ini, kesan onkolitik NDV strain tempatan, AF2240, telah dianalisa dalam sekumpulan sel kanser kolorektal (CRC). Sebelum kajian jangkitan NDV, asai plak dengan menggunakan sel-sel CRC sebagai perumah telah dihasilkan. SW620 merupakan sel CRC yang terbaik untuk digunakan bagi asai plak NDV kerana saiz, kejelasan, dan juga bilangan plak yang terbentuk. Unit pembentukan plak (pfu)/ mL NDV telah ditentukan dan kemudiannya digunakan untuk kajian jangkitan.

Kesemua sel CRC mengalami apoptosis apabila dijangkiti NDV. Kehadiran sinsitia, ciri jangkitan paramyxovirus, membuktikan bahawa sel ini sememangnya telah dijangkiti oleh virus tersebut. Di samping itu, sandwic ELISA dan asai plak menggunakan supernatan kultur menunjukkan bahawa progeni virus berjangkit hadir di dalam medium.

Kesan jangkitan NDV pada laluan Ras/Akt/PKB dalam sel CRC yang mempunyai mutasi gen berganda (dalam K-Ras, p53, dan APC) dianalisa. Perbandingan antara sel kanser yang berlainan menunjukkan bahawa kesemua mengalami penurunan-kawalaturan pAkt apabila berlakunya jangkitan NDV. Penurunan-kawalaturan ini, bagaimanapun tidak memberi kesan terhadap pGSK3 β . Oleh itu, tahap β -catenin protein juga tidak diturun-kawalaturan dalam kesemua sel. Walau bagaimanapun, pGSK3 β dan β -catenin telah diturun-kawalaturan dalam sel HCT116p53^{-/-} yang mempunyai APC tanpa mutasi dan gen p53 yang telah disingkirkan pada kedua-dua alel. Keputusan yang didapati mencadangkan bahawa agen terapeutik yang menasaskan laluan Ras/Akt/PKB untuk rawatan CRC adalah tidak berkesan. Tanpa mengira kesan jangkitan NDV pada laluan proliferasi sel, NDV menaik-kawalaturan BAD, menurunkan-kawalaturan pBAD atau mengurangkan nisbah Bax/Bcl-2 (bergantung kepada jenis sel) yang menyebabkan apoptosis kepada sel ini.

Sel SW480 mengalami penurunan-kawalaturan pAkt semasa jangkitan pertama (pada 25 hpi atau lebih awal) tetapi pulih secara beransur-ansur semasa jangkitan sekunder (selepas 25 hpi) di mana tahap pAkt semakin meningkat. Pendedahan ulangan NDV terhadap sel ini tidak menyebabkan apoptosis. Sel ini kemudiannya dilabelkan sebagai

SW480P kerana ianya didapati dijangkiti oleh virus secara berterusan. SW480P, sama ada dijangkiti semula atau tidak oleh NDV, menunjukkan penampilan fizikal yang sama dengan sel induk SW480 apabila dianalisa secara mikroskopik. RT-PCR dan analisa imunopendaflouran salah satu protin yang paling banyak dalam NDV, protin nukleokapsid (NP), menunjukkan bahawa RNA dan protin virus hadir dalam SW480P yang tidak dijangkiti. Walau bagaimanapun, progeni virus yang baru disintesis menghasilkan plak yang lebih kecil berbanding dengan NDV mencadangkan bahawa zarah gangguan cacat (defective interfering particle; DIP) telah dihasilkan. Jangkitan yang menghasilkan DIP ini menjelaskan mengapa sesetengah sel kanser dapat mengelak kesan onkolitik virus semasa viroterapi.

Kajian ini menunjukkan potensi NDV sebagai agen oncolytic dalam sel CRC. NDV menyebabkan onkolisis melalui penindasan laluan Ras/Akt/PKB yang membawa kepada apoptosis. Apoptosis berlaku di dalam sel CRC, tanpa mengira mutasi genetik yang diperolehi, melalui pengurangan nisbah Bax/Bcl-2, penaik-kawalaturan BAD dan penurun-kawalaturan pBAD. Terdapat peratusan yang kecil (17%) sel yang dijangkiti NDV, selamat daripada jangkitan walaupun selepas 97 hpi. Sel-sel tersebut dibiakkan dan didapati berjangkit dengan NDV secara berterusan. Progeni virus dirembeskan ke dalam media tetapi ianya didapati kurang berjangkit dan menunjukkan aktiviti onkolitik yang rendah - ciri-ciri zarah gangguan cacat. Keputusan yang diperolehi daripada kajian ini akan menyumbang ke arah kefahaman yang lebih lanjut tentang mekanisme onkolisis oleh NDV. Maklumat ini juga berguna untuk menambahbaikkan NDV sebagai calon viroterapi bagi kanser.

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I certify that a Thesis Examination Committee has met on **10th of May, 2012** to conduct the final examination of **Chia Suet Lin** on his thesis entitled “Evaluation of Newcastle disease virus as an oncolytic agent in colorectal cancer cell lines” in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1988. The Committee recommends that the student be awarded the Doctor of Philosophy.

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DECLARATION

I declare that the thesis is my original work except for equations 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 other institution.

CHIA SUET LIN

Date: 10 May 2012

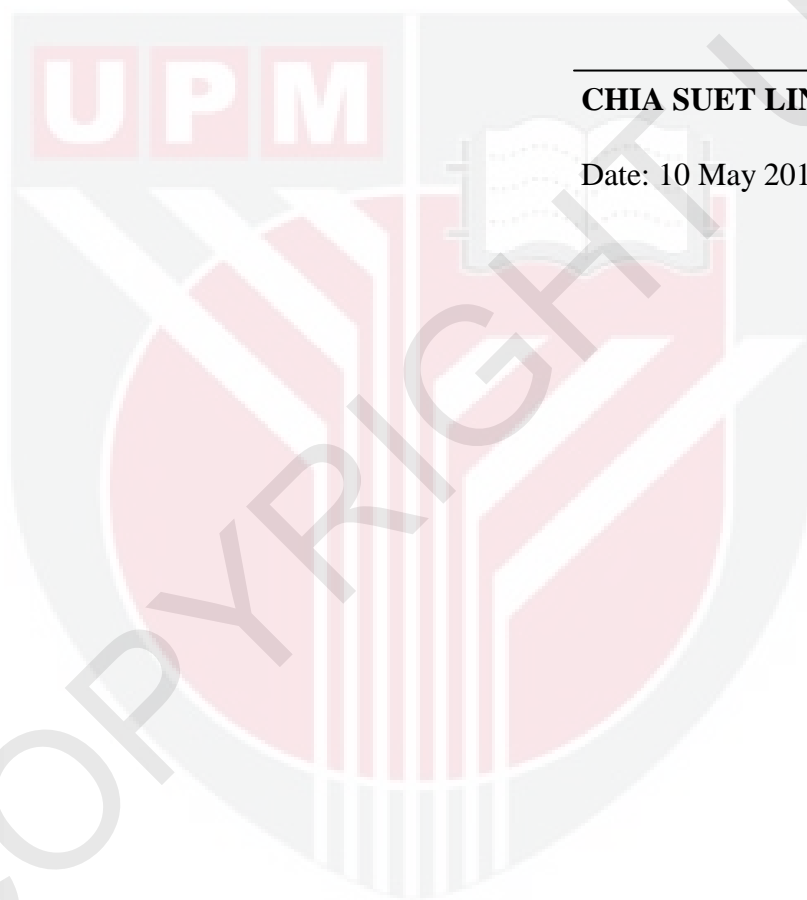


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