UNIVERSITI PUTRA MALAYSIA

IDENTIFICATION OF DIFFERENTIALLY REGULATED GENES IN BREAST (MCF-7) AND LUNG (H1299) CANCER CELL LINES DURING NEWCASTLE DISEASE VIRUS STRAIN AF2240 INFECTION

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By

NURHIDAYAH BINTI ROSLAN

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

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Chairman : Assoc. Prof. Norazizah Shafee, PhD

Faculty : Biotechnology and Biomolecular Sciences

Newcastle disease virus (NDV), a member of *Paramyxoviridae* family is known for its selective oncogenic effects in cancer cells. Even though NDV infections in cancer cells were shown to result in cell death through apoptosis, the actual mechanism of NDV-induced cell death process has not been thoroughly investigated. Therefore, in the present study, we investigated the effects of infection of a local velogenic NDV strain, designated as AF2240, on gene and cell cycle regulation of cancer cells. Two different cancer cell lines; MCF-7 breast cancer cell line with wild type p53 status, and H1299, a non-small cell lung cancer (NSCLC) cell line with p53-null were used. At 1 MOI, NDV AF2240 infection resulted in cellular morphological changes as early as 3 HPI in both MCF-7 as well as H1299 cells. At this time, viral proteins were also began to be detectable via immunoblotting. The infection caused massive cell death via apoptosis in MCF-7 cells by 12 HPI. The lack of p53 tumor suppressor protein in H1299 led to reduced apoptosis in H1299 cells. The cells however died

through necrosis after 12 hours post infection (HPI). These findings showed that NDV can induce apoptosis in both p53-dependent and p53-independent manner, although the former is more efficient.

Despite the p53-dependency, EIF4A1, a member of the RNA helicase family is crucial in NDV-induced cell death. Overexpression of EIF4A1 led to increase in translation of viral proteins. The increase in NDV viral proteins caused cells to trigger cell cycle arrest via upregulation of $p21^{Cip1}$ and $p27^{Kip1}$. The present of high levels (more than 0.5 fold) of these cyclin dependent kinase inhibitors at early stages of infection caused ploidy increase in MCF-7. In H1299, growth arrest at G_0/G_1 phase was observed.

Another important protein that was found to be involved in NDV-induced cancer cell killing was MBP-1. Due to the difference in the p53 status in MCF-7 versus H1299, the effects of MBP-1 regulation might be different. Since MBP-1 involves in both apoptotic as well as necrotic cell death pathways, the outcome of NDV infection in the cells were different. In MCF-7, NDV AF2240 infection caused massive apoptosis but not in H1299 cells.

This study started with preparation of pure virus and cell lines. Then the cells were either infected with NDV or mock-infected using saline and media. The cells were then harvested at different post-infection period (3, 6, 9, and 12 hours) where unlysed cells were used in apoptosis analysis using TUNEL assay and Typan blue staining method as well as in cell cycle analysis. Whereas, RNA and total lysate from lysed cells were subjected to GeneFishing, Real-time PCR and Western blotting.

Results obtained in this study showed that the outcome of NDV infection strongly depends on the genetic status of cell lines. This stresses the complexity of the pathways involve in NDV oncolytic properties. Nonetheless, our study has shed some light into further understanding of the effects of NDV on the gene and cell cycle regulation of MCF-7 and H1299 cells. Further studies however are needed in order to ensure the efficacy and efficiency of NDV as a potential anti-cancer agent for specific cancer types.

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

PENGENALPASTIAN GEN-GEN YANG DIKAWALATUR SECARA BERBEZA DI DALAM SEL KANSER SEMASA JANGKITAN VIRUS PENYAKIT NEWCASTLE STRAIN AF2240

Oleh

NURHIDAYAH BINTI ROSLAN

Disember 2012

Pengerusi : Prof. Madya Norazizah Shafee, PhD

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Virus penyakit Newcastle (NDV), yang merupakan ahli keluarga *Paramyxoviridae* telah dikenalpasti mempunyai kecenderungan menyebabkan kesan onkogenik terhadap sel-sel kanser. Walaupun jangkitan NDV di dalam sel kanser meyebabkan kematian sel secara apoptosis, mekanisma sebenar proses NDV merangsang kematian sel belum dikaji sepenuhnya. Oleh yang demikian, di dalam kajian ini, kami telah menyiasat kesan jangkitan NDV velogenik strain tempatan, dinamakan sebagai AF2240, ke atas pengawalaturan gen dan kitaran sel bagi sel-sel kanser. Dua jenis sel kanser berbeza; sel kanser payudara MCF-7 dengan status p53 biasa, dan H1299, sel kanser paru-paru jenis 'non-small cell lung cancer' (NSCLC) tanpa p53 ('p53-null') telah digunakan. Pada jangkitan secara berganda (MOI) 1, jangkitan NDV AF2240 menyebabkan perubahan morfologi sel seawal 3 jam selepas-jangkitan (HPI) kepada kedua-dua sel MCF-7 dan H1299. Pada ketika ini, protein-protein virus juga telah mula dikesan melalui kaedah 'immunoblotting'. Jangkitan tersebut telah

menyebabkan kematian sel-sel MCF-7 yang sangat banyak melalui apoptosis pada 12 HPI. Ketiadaan protein penindas tumor p53 dalam H1299 telah menyebabkan kurangnya sel-sel H1299 yang mengalami apoptosis. Sel-sel tersebut bagaimanapun telah mengalami necrosis selepas 12 jam jangkitan dilakukan. Penemuan ini menunjukkan bahawa NDV mampu merangsang apoptosis samada secara bergantung kepada p53 ataupun tidak, walaupun kaedah yang pertama didapati lebih banyak berlaku.

Di sebalik kebergantungan terhadap p53, EIF4A1 yang merupakan salah satu ahli keluarga 'RNA helicase', juga didapati terlibat dan penting dalam kematian sel yang disebabkan oleh jangkitan NDV. Pengekspressian secara berlebihan EIF4A1 telah membawa kepada peningkatan dalam penghasilan protein-protein virus. Peningkatan protein-protein virus NDV telah menyebabkan sel-sel mencetuskan penahanan kitaran sel ('cell cycle arrest') melalui pengawalaturan positif $p21^{Cip1}$ dan $p27^{Kip1}$. Kehadiran perencat 'cyclin dependent kinase' ini dengan kadar yang tinggi pada peringkat awal jangkitan telah menyebabkan peningkatan ploidi dalam MCF-7. Manakala dalam H1299 pula, ia telah menyebabkan suatu penahanan kitaran sel.

Protein lain yang didapati terlibat dalam kematian sel kanser yang disebabkan oleh NDV adalah MBP-1. Disebabkan perbezaan status p53 dalam MCF-7 dan H1299, kesan terhadap pengawalaturan MBP-1 juga berbeza. Oleh kerana MBP-1 terlibat di dalam kedua-dua laluan apoptosis dan juga necrosis, hasil jangkitan NDV dalam sel-sel tersebut juga mungkin berbeza. Dalam MCF-7, jangkitan NDV AF2240 menyebabkan kematian sel secara apoptosis yang banyak tetapi tidak bagi sel H1299.

Kajian ini dimulakan dengan penyediaan virus tulen dan sel-sel. Kemudian sel-sel tersebut telah samada dijangkitkan dengan NDV atau menggunakan air garam dan media. Sel-sel tersebut kemudiannya dituai pada masa-masa yang berbeza (3, 6, 9, dan 12 jam selepas jangkitan) dimana sel yang tidak diletuskan digunakan dalam analisis apoptosis menggunakan kaedah esei TUNEL dan pewarnaan 'Trypan blue' juga analisis kitaran sel. Sementara RNA dan protein daripada cell yang telah diletuskan digunakan untuk experimen 'GeneFishing', 'Real-time PCR' dan 'Western blotting'.

Hasil yang diperolehi dalam kajian ini menunjukkan bahawa jangkitan NDV sangat bergantung kepada status genetik sesuatu sel. Ini menunjukkan kekompleksan laluan-laluan yang terlibat dalam tindakbalas onkolitik NDV. Walaubagaimanapun, kajian kami sedikit sebanyak telah menyumbang ke arah pemahaman yang lebih baik tentang kesan NDV ke atas pengawalaturan gen dan kitaran sel dalam sel-sel MCF-7 dan H1299. Kajian lanjut bagaimanapun perlu dibuat untuk memastikan keberkesanan dan tahap kecekapan NDV sebagai agen anti-kanser yang berpotensi bergantung kepada jenis-jenis sel tertentu.

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I certify that an Examination Committee has met on 2nd of December 2011 to conduct the final examination of **Nurhidayah binti Roslan** on her thesis entitled **Identification of Differentially Regulated Genes in Breast (MCF-7) and Lung (H1299) Cancer Cell Lines during Newcastle disease virus Strain AF2240 Infection"** 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 **Doctor of Philosophy**.

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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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NURHIDAYAH BINTI ROSLAN

Date: 2 December 2012

TABLE OF CONTENTS