UNIVERSITI PUTRA MALAYSIA

MECHANISMS OF ONCOLOYTIC ACTIVITY OF NEWCASTLE DISEASE VIRUS STRAIN AF2240 IN HUMAN RENAL CARCINOMA CELL LINE

CH’NG WEI CHOONG

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MECHANISMS OF ONCOLYTIC ACTIVITY OF NEWCASTLE DISEASE
VIRUS STRAIN AF2240 IN HUMAN RENAL CARCINOMA CELL LINE

By

CH’NG WEI CHOONG

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

May 2014
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Newcastle disease virus (NDV) is an oncolytic virus that is known to selectively replicate in cancer cells compared to normal cells. It has been proposed that this preference is due to a defect in the cancer cells' interferon (IFN) responses. The exact mechanism underlying this process, however, remains unknown. In the present study, the antiviral response towards NDV infection by clear cell renal cell carcinoma (RCC) cells was examined. The most common first line treatment of RCC is using IFN. Unfortunately, most RCC cases are diagnosed at a late stage and often are resistant to IFN therapies. Alternative treatment approaches, including virotherapy, using oncolytic viruses, are currently being investigated. The present study used proteomic, molecular, immunological and biochemical techniques to investigate the mechanistic pathways that are involved in the response of RCC cells with defective or reconstituted wild type (wt) von Hippel-Lindau (VHL) gene activity to an oncolytic NDV infection.

It was observed that NDV induced activation of NF-κB in RCC cells by inducing phosphorylation of IκBα and its subsequent degradation. IκBα was phosphorylated as early as 1 hour post-infection and resulted in rapid NF-κB nuclear translocation and activation. Importantly, p38 MAPK phosphorylation occurred upstream of the NF-κB activation. These data provide evidence for the involvement of the p38 MAPK/NF-κB/IκBα pathway in NDV infection and eventual apoptosis of RCC cells. Since the results indicated that there was a possible correlation between the pathway and IFN-β signaling, additional experiments were performed to further understand the IFN-β signalling, specifically STAT pathway, in NDV-infected RCC cells under various microenvironmental factors.

The complexity of solid tumor microenvironments includes regions of hypoxia. In these regions, the transcription factor, hypoxia inducible factor (HIF), is active and
regulates expression of many genes that contribute to aggressive malignancy, radio-
and chemo-resistance. To investigate the oncolytic efficacy of a highly virulent
(velogenic) Newcastle disease virus (NDV) in the presence or absence of HIF-2α,
renal cell carcinoma (RCC) cell lines with defective or reconstituted wild type (wt)
von Hippel-Lindau (VHL) gene activity were used. The data showed that these RCC
cells responded to NDV by producing only IFN-β, but not IFN-α and are associated
with increased STAT1 phosphorylation. Restoration of wt VHL expression enhanced
NDV-induced IFN-β production, leading to prolonged STAT1 phosphorylation and
increased cell death. Hypoxia augmented NDV oncolytic activity regardless of the
cells' HIF-2α levels.

In summary, this study demonstrates IFN-β may play important role in NDV
oncolysis through activation of p38 MAPK/NF-κB/IκBα and STAT pathways in
renal cell carcinoma. Altogether, these findings provide a better mechanistic
understanding of NDV-mediated cell death and also highlight the potential of
oncolytic local strain of NDV AF2240 as a potent therapeutic agent against
normoxic and hypoxic cancer cells, especially renal cell carcinoma.
MEKANISMA AKTIVITI ONKOLITIK VIRUS PENYAKIT SAMPAR
AYAM STRAIN AF2240 DALAM JUJUKAN SEL KARSINOMA GINJAL
MANUSIA

Oleh

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Mei 2014

Pengerusi: Assoc. Prof. Norazizah Shafee, PhD

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Kawasan hipoksia selalunya dijumpai dalam tumor pepejal. Faktor induksi hipoksia (HIF) adalah sejenis faktor transkripsi yang aktif di kawasan tersebut. Ia mengawal ekspresi gen-gen yang menyumbang kepada keagresifan maglinan yang rintang terhadap rawatan. Kajian selanjutnya dilakukan untuk mengkaji pula keberkesanan onkilotik virulen NDV di dalam jujukan sel RCC yang mempunyai tahap ekspresi HIF-2α yang berbeza, iaitu jujukan sel RCC yang memiliki jenis liar von Hippel-Lindau (VHL) dan satu lagi tidak memilikinya. Keputusan daripada kajian ini menunjukkan bahawa penghasilan IFN-β dan peningkatan pemfosforilan STAT1 berlaku apabila sel-sel tersebut bertindak balas dengan NDV. Walau bagaimanapun, penghasilan IFN-α tidak dapat dikesan selepas infeksi NDV. Pemulihan jenis liar von Hippel-Lindau (VHL) meningkatkan penghasilan IFN-β, sekali gus menyebabkan pemfosforilan STAT1 yang berpanjangan dan peningkatan kematian sel. Hipoksia juga meningkatkan aktiviti onkilotik tanpa mengira tahap HIF-2α dalam sel-sel tersebut.

Secara keseluruhannya, kajian ini menunjukkan bahawa IFN-β memainkan peranan yang penting dalam onkolis NDV melalui pengaktifan laluan p38 MAPK/NF-κB/IκBα dan laluan STAT bagi sel karsinoma ginjal. Hasil daripada kajian ini memberi pemahaman yang mendalam tentang mekanisma yang terlibat dalam aktiviti onkilotik dan ia juga menunjukkan bahawa NDV AF2240 onkilotik strain tempatan mempunyai potensi yang tinggi sebagai agen terapeutik untuk membunuh sel-sel kanser terutamanya sel karsinoma ginjal dalam keadaan normoksia dan hipoksia.
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Last but not least, I would like to extend my special gratitude to my family members: my parents Ch’ng Ah Leik and Chu Sai Kim, sisters and brothers, for their love, blessings, understanding, warm encouragement and inspiration. Thank you.
I certify that a Thesis Examination Committee has met on 20 May 2014 to conduct the final examination of Ch’ng Wei Choong on his thesis entitled “Mechanisms of Oncolytic Activity of Newcastle Disease Virus Strain AF2240 in Human Renal Carcinoma Cell Line” 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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LIST OF ABBREVIATIONS

CPE  Cytopathic effect
DAPI  4’,6-Diamidino-2-Phenylindole, Dihydrochloride
FACS  Fluorescence-activated cell sorting
HAU  Hemagglutination unit
HIF  Hypoxia inducible factor
HIF-1α  Hypoxia inducible factor-1 alpha
HIF-2α  Hypoxia inducible factor-2 alpha
hpi  Hour(s) post-infection
IFN  Interferon
IFN-α  Interferon-alpha
IFN-β  Interferon-beta
JAK/STAT  Janus kinase / signal transducer and activator of transcription
MAPK  Mitogen-activated protein kinase
MOI  Multiplicity of infection
MTT  Methylthiazolyldiphenyl-tetrazolium bromide
NDV  Newcastle disease virus
NP  Nucleocapsid protein
PARP1  Poly (ADP-ribose) polymerase 1
PHD  Prolyl hydroxylase domain
PKR  Protein kinase R
RCC  Renal cell carcinoma
RIPA  Radio-immunoprecipitation assay
SOCS  Suppressor of cytokine signaling

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<td>Signal transducer and activator of transcription 1</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumor necrosis factor-alpha</td>
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<td>TUNEL</td>
<td>Terminal deoxynucleotidyl transferase dUTP nick end labelling</td>
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<td>VHL</td>
<td>Von Hippel-Lindau</td>
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<td>VSV</td>
<td>Vesicular stomatitis virus</td>
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CHAPTER 1

INTRODUCTION

Newcastle disease virus (NDV) is a type of avian virus belonging to the Paramyxoviridae family (Yusoff and Tan, 2001). It is of interest to cancer researchers due to its oncolytic properties. In cancer cells with naturally occurring defective antiviral defense systems, the virus can replicate up to 10,000 times better compared to normal cells (Reichard et al., 1992). In recent years, many scientific reports and phase I/II/III clinical trials revealed that NDV can act as a potent and promising therapeutic agent against cancers (Lam et al., 2011; N.C.I., 2013). Despite various studies, NDV has not been approved by the U.S. Food and Drug Administration for cancer treatment. This is because, in some clinical trials, positive outcomes were not significantly observed (N.C.I., 2013). NDV-modified tumor cells vaccine has been shown to improve both recurrence-free and overall survival of patients with colon carcinoma in a phase II trial (Schlag et al., 1992). Some advanced renal cell carcinoma patients displayed partial responses including partial remission (15%) and stable disease (30%) after the treatment (Pomer et al., 1995). Such vaccine, however, did not show remarkable clinical efficacy in melanoma patients (Voit et al., 2003). The main obstacle in reducing the unfavourable outcome is the lack of sufficient understanding of the mechanisms of NDV infection in cancer cells. The complexity and heterogeneity of the various types of cancers also are major factors.

Renal cancer is a common adult malignancy worldwide (Globocan, 2012). Majority of patients are asymptomatic over a long period of time until the disease become locally advanced. Clear cell renal cell carcinoma (RCC) is the most lethal and dominant subtype of adult renal cancer (Eble et al., 2004; Thomas and Tawfik, 2008; Zhou and He, 2013). This subtype is less susceptible to conventional oncologic treatments including radiotherapy and chemotherapy. To date, several molecular-targeted agents are approved by the U.S. Food and Drug Administration for RCC treatment (Fisher et al., 2013). Unfortunately, complete responses are very rare, with undesirable side effects.

Currently, the first line treatment option available for RCC is using interferon (IFN) therapy. Even though it is the first line option, therapeutic response of patients with metastasis is low, around 15-20% (Unnithan and Rini, 2007). IFN secreted by cells in response to virus infections has been shown to be beneficial, with oncolytic viruses. The specificity of NDV-mediated killing of cancer cells has been proposed to be due to defects in the type I interferon (IFN-α/β) response of the cells (Stojdl et al., 2000; Fiola et al., 2006). Cancer cells responded to NDV infection by producing only IFN-β production (Elankumaran et al., 2010). The efficacy and safety of vesicular stomatitis virus (VSV) as an oncolytic agent has been shown to be enhanced by IFN-β, through immune-mediated mechanisms, in mesothelioma (Willmon et al., 2009). This observation leads to the possibility of manipulating the exclusive IFN-β induction by NDV as a potential strategy to boost the efficacy and
safety of NDV as an oncolytic agent in clinical settings. This option could be closely examined if the detailed mechanism of cellular responses to NDV infection is known.

In the present study, the oncolytic activities of a local isolate of NDV (designated as AF2240) in RCC cell lines was investigated. It is hypothesized that NDV oncolytic properties can be enhanced in renal carcinoma cells through the manipulation of interferon-related pathways. To test this hypothesis, the study was designed with the main objective to investigate the molecular mechanisms underlying NDV oncolysis in human clear cell renal cell carcinoma (RCC) cell lines. The specific aims of the study were:

1. To examine the oncolytic activity of NDV in renal carcinoma cells.
2. To study the response of the p38MAPK/NF-κB/1xBα pathway in NDV-infected renal carcinoma cells.
3. To investigate the involvement of interferons in the oncolytic activity of NDV in renal carcinoma cells.
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