UNIVERSITI PUTRA MALAYSIA

CHARACTERIZATION OF EARLY CELLULAR AND MOLECULAR MECHANISM OF NEWCASTLE DISEASE VIRUS AF2240-INDUCED APOPTOSIS IN MCF-7 CELLS

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By

GHRICI MOHAMMED

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

July 2011

Dedication

To my parents who instilled the values and confidence in me,
To my brothers, sisters, my wife and children for giving me the passion and encouragement to complete this study

Abstract of thesis presented to the Senate of Universiti Putra Malaysia
in fulfilment of the requirement for the degree of Doctor of Philosophy

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Chairman: Professor Datin Paduka AINI IDERIS, PhD

Faculty : Veterinary Medicine

Newcastle disease virus (NDV) is an oncolytic virus that has shown promising results in anti-tumor therapy. However, neither the mechanism of NDV oncolysis nor NDV induced apoptosis were fully understood.

The main objective of this study was to elucidate the mechanism by which NDV AF2240 induced apoptosis and to identify the signal that triggered apoptosis in MCF-7 cells. The cytotoxicity of NDV AF2240 against MCF-7 cells was assessed first. The NDV induced apoptosis was detected and analyzed by flow cytometry. Then the time kinetics of NDV replication via RT-PCR amplification of NP gene and the time kinetics of apoptosis detection based on caspase-8 activation were compared. Caspase-8 activation and flow cytometry were used to investigate ultra-violet (UV) inactivated NDV induced apoptosis and cycloheximide treated NDV infected MCF-7 cells. This latter was also treated with Z-

VAD-fmk and the titer of NDV progeny was determined by plaque forming unit assay. The co-localization of NDV antigens and apoptotic markers were analyzed by electron microscopic and dual staining assay. The involvement of mitochondrial mediated pathway was investigated via the detection of mitochondrial permeability transition pore assay opening activation. The involvement of NDV binding and sialic acid receptor in NDV AF2240 induced apoptosis was analyzed by antibody inhibition and neuraminidase assays. The whole NDV AF2240 HN gene was amplified and cloned into pDisplay expression vector. The HN expression was detected by indirect immunofluorescence and HN induced apoptosis was assessed by flow cytometry. There was a significant increase of cell death to 58.91% induced by increased NDV titer ($p < 0.0003$). In the same manner, flow cytometry analysis revealed a significant increase of apoptotic cells to 63.94% induced by all NDV AF2240 titers ($p < 0.0001$). The results suggested that oncolysis might probably be mediated by apoptosis and that both oncolysis and apoptosis were NDV AF2240 dose dependent. Apoptosis did not affect NDV infectivity but the treatment of NDV infected MCF-7 cells with the broad caspase inhibitor Z-VAD-fmk had an inhibitory effect on NDV progeny production. Caspase-8 activation was detected at 2 hr pi while expression of NDV genes started late at 6 hr pi. UV inactivated NDV AF2240 induced an increase of apoptosis of 20 % obtained at 72 hr pi. Treatment of NDV infected MCF-7 cells with cycloheximide did not inhibit apoptosis induction. This means that NDV induced apoptosis might be NDV replication and protein synthesis independent. NDV AF2240 induced apoptosis was mediated by both death receptor and mitochondrial mediated apoptosis. The antibody inhibition assay showed a significant decrease of apoptotic cells obtained after treatment with anti-NDV AF2240 antibody ($p < 0.0003$). Neuraminidase treatment showed a significant decrease of the percentage of apoptotic cells with the increased amount of

neuraminidase treatment ($p < 0.0003$). This inhibitory effect means that NDV induced apoptosis might be dependent on the binding of NDV to sialic acid receptor. The HN expression alone induced significant increase of apoptotic cells ($p < 0.0001$). HN induction of apoptosis was dose dependent.

As a conclusion, NDV AF2240 induced apoptosis at the attachment step of NDV life cycle and that NDV HN glycoprotein is most probably responsible for NDV AF2240 induction of apoptosis. Our findings suggest that the use of NDV AF2240 as an anti-cancer agent can be more beneficial for cancer patients since the virus induced a faster tumor cell death which can be translated into faster elimination of tumor cells and a reduction or elimination of chances of metastasis occurrence. The possibility of NDV AF2240 to selectively replicate and spread within tumor cells will eventually reach all the tumor cells and kill them by apoptosis is another benefit for cancer patients. Alternatively recombinant NDV HN glycoprotein which has shown an oncolytic effect on tumor cells can be used as an anti-tumor tool. Recombinant HN has more advantages than the whole virus such as its efficiency is stable over long storage, ease of mass production and absence of virus escapes mutants. However, recombinant pDisplay-HN need additional engineering before it can be used as an anti-cancer tool in cancer patients.

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

PENCIRIAN MEKANISMA MOLEKUL DAN AWAL SELULAR VIRUS PENYAKIT NEWCASTLE AF2240 DALAM INDUKSI APOTOSIS DALAM SEL MCF-7

Oleh

GHRICI MOHAMMED

Julai 2011

Pengerusi: Professor Datin Paduka AINI IDERIS, PhD

Fakulti: Perubatan Veterinar

Virus penyakit Newcastle (NDV) adalah salah satu virus onkolisis yang telah mencetuskan revolusi dalam kaedah terapi anti-tumor dan menunjukkan keputusan yang memberangsangkan. Walaubagaimanapun mekanisma onkolisis oleh NDV ini tidak diketahui, tetapi ia berkemungkinan dimangkinkan oleh NDV menggalakkan apotosis, sebagaimana yang telah ditunjukkan dalam perincian kajian terhadap pelbagai jenis sel tumor manusia. Namun mekanisma molekul bagi proses ini belum dapat difahami sepenuhnya.

Objektif utama kajian ini adalah untuk mengkaji mekanisma NDV AF2240 menggalakkan apotosis dan untuk mengenalpasti isyarat yang mencetuskan proses apotosis ini di dalam sel MCF-7. Penilaian dibuat terhadap proses kesitotoksikan yang dihasilkan oleh NDV strain AF2240 terhadap sel MCF-7, kemudian tindakbalas kesitotoksikan yang

dimangkinkan oleh NDV menggalakkan apotosis dikenalpasti dengan menggunakan analisis sitometri aliran. Perbandingan kinetik tempoh/masa replikasi NDV yang dianalisis menggunakan amplifikasi RT-PCR ke atas gene NP NDV, dan kinetik tempoh pengesanan tanda-tanda awal apotosis dikaji menggunakan caspase-8 pengaktifan dan liang peralihan ketelapan mitokondria pengaktifan perasmian. Seterusnya kajian bagi mengenalpasti samada NDV menggalakkan apotosis yang berlaku adalah replikasi NDV dan mandiri sintesis protein dijalankan. Penglibatan replikasi NDV terlebih dahulu dikaji dengan menyahaktifkan NDV AF2240 dengan sinaran ultra-ungu (UV), disusuli dengan UV NDV AF2240 menggalakkan apotosis, dikesan menggunakan caspase-8 pengaktifan dan analisis sitometri aliran. Penglibatan sintesis protein NDV dikaji menggunakan *cycloheximide*, sejenis perencat sintesis protein. Kesan antibodi anti-NDV AF2240 terhadap NDV menggalakkan apotosis dikaji menggunakan pengujian halangan antibodi. Seterusnya kajian dijalankan bagi mengenalpasti samada apotosis teraruh menghalang penghasilan progeni NDV, dianalisis menggunakan plak membentuk cerakinan unit dan rawatan Z-VAD-fmk. Kemudian kajian dibuat bagi mengenalpasti samada NDV menggalakkan apotosis berlaku dalam sel MCF-7 yang telah dijangkiti, menggunakan mikroskopi elektron dan dual berlumuran antigen NDV dan penanda apototik. Seterusnya pengenalpastian samada ekspresi HN menyebabkan apotosis, dikaji menggunakan RT-PCR untuk mengamplifikasikan keseluruhan gen HN, diikuti dengan pengklonan ke dalam vektor pCR 2.1 dan subpengklonan ke dalam vektor pernyataan pDisplay. Ekspresi gen HN dikenalpasti menggunakan imunopendarfluor tak langsung dan HN menggalakkan apotosis dikaji menggunakan analisis sitometri aliran. Hasil kajian menunjukkan terdapat peningkatan kematian sel yang signifikan dengan induksi sehingga 58.91% dengan kenaikan titer NDV ($p < 0.0003$). Juga didapati bahawa keputusan sitometri aliran

menunjukkan terdapat peningkatan kadar apotosis sel yang signifikan daripada kadar induksi sehingga 63.94% oleh semua titer NDV AF2240 ($p < 0.0001$). Keputusan kajian mencadangkan bahawa onkolisis yang berlaku kemungkinan dimangkinkan oleh apotosis dan kedua-dua proses onkolisis dan apotosis adalah bergantung kepada dos NDV AF2240. Apotosis tidak mengganggu infektiviti NDV tetapi rawatan terhadap sel MCF-7 yang dijangkiti oleh NDV dengan luas caspase perencat Z-VAD-fmk menunjukkan kesan inhibitori terhadap penghasilan progeni NDV. Pengaktifan caspase-8 dikesan pada 2 jam pi manakala ekspresi gen NDV bermula lewat pada 6 jam pi. NDV AF2240 ternyahaktif menggunakan UV menghasilkan peningkatan apotosis sebanyak 20% diperolehi pada 72 jam pi. Rawatan sel MCF-7 yang dijangkiti oleh NDV dengan *cycloheximide* tidak menghalang proses induksi apotosis. Ini bermaksud apotosis yang dihasilkan oleh NDV tidak bergantung kepada proses replikasi NDV dan sintesis protein. Proses apotosis oleh NDV AF2240 adalah dimangkinkan oleh reseptor kematian dan mitokondria mendamaikan apotosis. Pengujian halangan antibodi menunjukkan penurunan signifikan terhadap sel yang mengalami apotosis selepas rawatan menggunakan antibodi anti-NDV AF2240 ($p < 0.0003$). Rawatan menggunakan *neuraminidase* menunjukkan penurunan signifikan dalam peratusan sel-sel yang mengalami apotosis dengan peningkatan jumlah rawatan *neuraminidase* ($p < 0.0003$). Proses inhibitori ini menunjukkan bahawa NDV induced apotosis adalah bergantung kepada pengikatan NDV kepada reseptor asid sialik. Ekspresi HN sendiri menunjukkan peningkatan yang signifikan bagi sel-sel yang mengalami apotosis ($p < 0.0001$). Induksi HN terhadap apotosis adalah bergantung kepada dos.

Kesimpulannya, apotosis yang diinduksikan oleh NDV AF2240 berlaku pada langkah pengikatan dan pelekapan dalam kitar hidup NDV, dan glikoprotein NDV HN

berkemungkinan adalah faktor yang mencetuskan induksi apotosis oleh NDV AF2240. Hasil kajian ini menunjukkan bahawa penggunaan NDV AF2240 sebagai agen anti-kanser memberi lebih manfaat kepada pengidap kanser memandangkan virus mencetuskan kematian sel tumor dengan lebih pantas, seterusnya mempercepatkan proses penghapusan sel tumor dan merendah/menghapuskan risiko metastasis untuk berulang. Keupayaan NDV AF2240 untuk mereplikasi secara selektif dan tersebar ke dalam sel tumor akan sampai kepada semua sel tumor dan membunuh sel tersebut secara apotosis adalah satu lagi manfaat kepada pesakit kanser. Secara alternatif, glikoprotein NDV HN rekombinan yang mana telah menunjukkan kesan onkolitik ke atas sel tumor boleh digunakan sebagai bahan tumor anti. HN rekombinan mempunyai kelebihan berbanding sel virus sepenuhnya, seperti efisyensi yang stabil walaupun disimpan lama, mudah untuk dihasilkan dalam kuantiti yang banyak dan ketiadaan virus melepaskan diri mutan. Walaubagaimanapun, rekombinan pDisplay-HN memerlukan proses kejuruteraan molekular tambahan sebelum ia dapat digunakan sebagai bahan anti kanser kepada pesakit.

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I certify that a Thesis Examination Committee has met on 27 july 2011 to conduct the final examination of Ghrici Mohammed on his thesis entitled "Characterization of early cellular and molecular mechanism of Newcastle disease virus AF2240-induced apoptosis in MCF-7 cells", in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U(A)] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

Members of the thesis Examination Committee were as follows:

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. 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 any other institution.

GHRICI MOHAMMED

Date: 27 July 2011

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Calcein AM: calcium acetoxymethyl ester

CHX: cycloheximide

CMC:carboxy methyl cellulose

CPE: cytopathogenic effect

dH_2O: distilled water

DIABLO: direct inhibitor of apoptosis binding protein with low pI

DISC: death inducing signalling complex

DMSO: dimethylsulfoxide

FAM: carboxyfluorescein group

FADD: Fas-associted death domain

FBS: fetal bovine serum

FITC: fluorescein isothiocyanate

FLICA: fluorescent inhibitor of caspases

HA assay: hemagglutination assay

HBSS: Hanks' balanced salt solution

HNSF: HN Sal I Forward primer

HNSR: HN Sac II Reverse primer

HN: hemagglutinin-neuraminidase

hr: hour

HAU: haemagglutinating units

INF-α: interferon-α

IRF-3: Interferon regulatory factor-3

kDa: kilodalton

LETD: (Leucine-Glutamic acid- Threonine-Aspartic acid) sequence

MCF-7: human breast carcinoma cell lines

min: minute

MTT: 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide

mU: milli-units

mw/cm^2: milli-watt/ cm^2

NA: neuraminidase

NPF: nucleoprotein forward primer

NPR: nucleoprotein reverse primer

PFU: plaque forming unit

pi: post-infection

PI: propidium iodide

PT: mitochondrial permeability transition pore complex

RE**:** Restriction enzyme

rpm: revolution per minute

Sec: second

SPF : specific pathogen free

TBE buffer: Tris base, boric acid, EDTA buffer

TNE buffer: Tris, NaCl, EDTA buffer

TNF-α: tumor necrosis factor-α

TNF-R: tumor necrosis factor-α receptor

TRAIL: tumor necrosis factor related apoptosis inducing ligand

μg/ml: microgram/millilitre

μl : microlitre

μM: micromole

UV-NDV: ultraviolet inactivated NDV

Vero cells: African green monkey kidney cells

Z-VAD-FMK: Benzoyloxycarbonyl-Val-Ala-Asp-fluoromethylketone

CHAPTER 1

INTRODUCTION

Cancer is widely spread and is one of the leading causes of mortality in the world and in Malaysia. Breast cancer, colorectal, cervix, ovary, thyroid gland, lung, uterus, stomach, brain and lymphoma are the most common in women in all the ethnic groups while colorectal, lung, nasophparynx, prostate and liver are the five most frequent in man. (Malaysian cancer statistics. 2006).

The current conventional cancer therapies are far from successful particularly for the advanced stages. The failure of these chemotherapy and radiotherapy was generally due in most cancers, to mutations in p53 induction of apoptosis (Levine, 1997) and / or the over-expression of anti-apoptotic genes such as Bcl-2 or BCR-ABL (Martin and Green, 1994; Rao and White, 1997). As a result, the anti-tumor therapies based on inducing apoptosis and in particular on inducing apoptosis that is independent of p53 function and / or the over-expression of Bcl-2 and its anti-apoptotic family members may hold a great chance of success. Hence, one of the strategies used in anti-tumor therapy was to re-activate the apoptotic pathway particularly at the decision stage of apoptosis which is blocked in great number of tumor cells, while the execution stage of apoptosis in these tumors is intact and functional (Thompson, 1995; Paulovich et al., 1997). In general there are three types of anti-cancer therapies. The first is the chemotherapy, either alone or in combination with surgery and radiotherapy. The second is the immunotherapy through the use of cytokines and interferon, known as immunomodulator factors. The third which is the most recent is virotherapy, based on the use of oncolytic viruses or targeting virus vectors specifically to tumor cells (Dachs et al., 1997; Roth and Christiano, 1997; Ring, 2002). The use of viruses as a potential

tumor killer is one of the promising strategy of anti-tumor therapy and have unlimited perspectives (Plaskin et al., 1994; Schirrmacher et al., 2001; Ring, 2002).

Newcastle disease virus (NDV) is one of the oncolytic viruses which has been used to treat human cancers and have shown promising oncolytic properties (Sinkovics and Horvath, 2000). The use of NDV as an anti-tumor agent have led to promising results such as partial to complete regression of various tumors, including advanced stages of tumors and those not responding to conventional therapies (Cassel et al., 1983; Reichard et al., 1992; Plaskin et al., 1994; Batliwalla et al., 1998; Csatary et al., 1998; Csatary and Bakacs, 1999; Schirrmacher et al., 2001). Significant regression of advanced tumors of the digestive tract was obtained following administration of autologous live cell NDV modified tumor vaccine (ATV) and NDV vaccine strain La Sota IV (Liang et al., 2003). Good responses were obtained from the use of NDV as an anti-tumor agent in wide variety of cancer including cervical carcinoma, neuroblastoma, osteosarcoma, bladder carcinoma and Wilm's tumor (Reichard et al., 1992). The local administration of live virus 73T strain led to regression of metastases melanoma, renal carcinoma, colorectal and lymphoma (Schirrmacher et al., 2001).

In Malaysia, most of the researches on NDV isolates have been done either for the purpose of poultry vaccine production (Ibrahim et al., 1983; Aini, 1987; 1989; Rabu et al., 2002) or for the diagnosis purposes (Kho et al., 2002). There was no reported NDV oncolytic clinical trials whether in animal or in human. As far as 2003, no studies have been reported on the use of NDV AF2240 strain or any other NDV strains in an anti-cancer research neither at pre-clinical nor at clinical stage, with the exception of one study on the killing of MCF-7 and MDA-231 cell lines by various NDV strains via induction of apoptosis (Fauziah et al., 2002). The studies have been limited to the

screening of the oncolytic activity of several NDV isolates on several commercially available tumor cell lines, CEM-SS, MCF-7, MDA (breast cancer), HT-29 (colorectal cancer) and HL-60 (acute promelocytic leukemia) by colorimetric microtiter (MTT) cytotoxicity assay. The results of these studies showed that cytotoxicity of NDV strains in various tumor cell lines was strain dependent and that AF2240 was more oncolytic on breast cancer cells but had no effect on normal cells (3T3). The oncolytic activity of NDV AF2240 was destroyed in inactivated virus (Omar et al., 2002). There were no reported studies on the molecular mechanism of NDV induced apoptosis until 2010 where NDV AF2240 infection was shown to induce conformational changes of Bax protein which in turn was translocated from the cytoplasm to mitochondria and this might have led to the release of cytochrome c which probably mediated AF2240 induced apoptosis (Molouki et al., 2010). However, the missing link between NDV AF2240 infection and the induced Bax conformational changes or the signalling mechanism by which NDV AF2240 induced Bax conformational changes was not identified.

NDV is intrinsically oncolytic but not all NDV strains have similar oncolytic effects, some induce direct oncolysis while other strains induce host immunity towards modified tumor expressing NDV antigen (Schirrmacher et al., 1997; Sinkovics and Horvath, 2000). Different NDV strains have different effects on the same cell line as shown with vaccine strain Roakin and B1 which destroy Daudi Burkitt's lymphoma cells (Tzadock-david et al., 1995) while 73T strain is harmless to these cells (Zorn et al., 1994). Most of the clinical trials used NDV vaccine 73T strain (Cassel and Garrett, 1965), MTH-68/H (Csatary and Bakacs, 1999) or the apathogenic NDV Ulster strain (Schirrmacher et al., 1998). All these strains were either attenuated or apathogenic.

Oncolytic efficiency can be further improved with the advent of reverse-genetic technology (Romer-oberdorfer et al., 1999) where it is possible to develop recombinant NDV with additional features such as the expression of cytokine genes, drug-sensitive genes, and cytotoxic genes while NDV non-essential genes can be removed and replaced. In addition, efficiency, simplicity and the cost of NDV administration can be improved much further. Obviously, these improvements depend on the research outcome and the level of advancement of technology. As a consequence, the main focus of the scientific community involved in anti-tumour therapies was on the molecular mechanisms of oncolysis *in-vitro* and *in-vivo*. The elucidation of this mechanism is essential for the understanding of viral oncolysis and for the development of novel anti-tumor therapies. The molecular mechanism of NDV oncolysis is still not fully elucidated. Earlier studies linked NDV oncolysis to the induction of TNF-α which in turn kill NDV-infected tumor cells (Lorence et al., 1988; Rood et al., 1990). NDV was also shown to induce NF-kB activation and nitric oxide (NO) production in murine macrophages (Umansky et al., 1996) and that murine macrophages stimulated by NDV can kill tumor cells *in vitro* (Lorence et al., 1988; Zorn et al., 1994) and *in vivo* (Schirrmacher et al., 2000).

NDV oncolysis might be mediated by NDV induced apoptosis. This might be the case after the first detection of NDV induced apoptosis in avian cells (Lam and Vasconcelos, 1994), followed by the detection of apoptosis in mammalian PC12 cells induced by NDV MTH-68/H (Fabian et al., 2001). Apoptosis was also induced in human tumor cell infected with NDV Ulster strain one to two days after production of cytokines, chemokines and up-regulation of antigen presenting HLA molecules (Washburn and Schirrmacher, 2002). NDV HN protein was able to induce TRAIL in tumor cells (Zeng et al., 2002). TRAIL induced by either NDV or HN was responsible

for the anti-tumor activity of NDV stimulated human monocytes (Washburn et al., 2003). TRAIL is a well known apoptosis inducer and exerts an anti-tumor activity (Nagata, 1997; Walczack et al., 1999; Green, 2000). Both apoptotic pathways, the death receptor and mitochondrial mediated pathways were induced by different recombinant NDV strains in various tumor cells (Elankumaran et al., 2006). Recent study has shown that NDV AF2240 induction of apoptosis in Hela cells might be mediated by induced a conformational change of Bax (Molouki et al., 2010). As a conclusion, these studies indicated that apoptosis may play a central role in NDV oncolysis activity.

Since apoptosis is involved in both, the development and elimination of tumors, it can be exploited for tumor treatments (Evan et al., 1992; Revillard et al., 1998). Any increase or decrease in apoptosis rate can cause diseases (Evan et al., 1992). Tumour development and spread is the result of this apoptosis unbalance (Evan et al., 1992; Revillard et al., 1998). Apoptosis is a very well controlled mechanism for cell turnover and for the specific killing of cell during development and differentiation. When this control disappears, diseases occur (Evan et al., 1992). A complete understanding of apoptosis induction and regulation mechanisms will have a great impact on cancer therapy as well as in other diseases treatments (Revillard et al., 1998). The increase of the scientific literature about the identification and characterization of viral genes and proteins involved in the modulation of apoptosis and their cellular counterparts will result in the development of new and advanced anti-tumor therapies. Since apoptosis plays a crucial role in cell growth and death, scientists have turned their efforts into studying apoptosis in the hope to find a breakthrough treatments or new ways of treatments for diseases such as degenerative diseases, AIDS, diseases of the immune system and cancer (Vaux, 1993; Revillard et al., 1998). Eliminating tumor cells by the

induction of apoptosis has become an important approach in cancer therapy (Sach and Lotem, 1993). A defect in apoptosis machinery was the source of the resistance of many tumors to therapies and the disruption of the genetic control or effectors mechanism has profound implication for the cells, tissues and the organism (Malcomson et al., 1996). The findings that apoptosis can be activated by extrinsic signals that require no new gene expression can be exploited by using apoptosis as a powerful tool to eliminate cancerous cells. There are two check points in apoptosis, the decision step and the execution step. In the majority of tumors, the decision step is disabled but the execution step is still functional, this means that tumor cell will die if we provide the decision in the form of apoptotic signal (McDonnell et al., 1995). It is very critical to identify the initiator factors that trigger apoptosis (Cohen, 1997). The identification of these initiators is necessary for the design of therapeutic tool or for the manipulation of this highly genetically regulated mechanism for therapeutic purposes.

There are two types of viruses with antagonist functions regarding apoptosis. Some inhibit the apoptotic functions and other type induces apoptosis in order to replicate and spread in the host (O'Brien, 1998). Newcastle disease virus belongs to the latter group. NDV induces apoptosis in wide variety of tumour cells *in-vivo* and *in-vitro* (Schirrmacher et al., 1997; Washburn and Schirrmacher, 2002; Zeng et al., 2002; Washburn et al., 2003; Elankumaran et al., 2006). NDV is a naturally occurring anti-tumour virus. This property is well established since the sixties (Cassel and Garrett, 1965). However, the mechanism of its tumoricidal activity is not fully known.

NDV AF2240 is a velogenic, viscerotropic strain isolated from a Malaysian field outbreak in the 1960s and is responsible for high morbidity and mortality of chicken (Lai, 1985). NDV AF2240 has been used as a challenge virus in many vaccination

trials and it has been shown to cause 100% mortality in susceptible flocks. The common lesions caused by NDV AF2240 strain is necrotic haemorrhages in the proventriculus, small intestines, caecal tonsils and trachea (Ibrahim et al., 1980).

The mechanism of NDV AF2240 oncolytic activity at the cellular and molecular level is not fully understood. The molecular mechanism of NDV AF2240 induction of apoptosis and the identification of the initiator or a causative factor (s) that triggers apoptosis are not known. This study was undertaken to redress these shortcomings. The choice of NDV AF2240 was based on the reported anti-tumor advantages. MCF 7 cell line was chosen as a model that can be replicated in any other type of tumor cell.

The hypothesis is that the oncolytic activity of NDV AF2240 might be due to apoptosis induced at early stage of NDV AF2240 life cycle during the binding of HN glycoprotein to sialic acid containing receptor of MCF-7 cells or just after the entry of the virus into the cells and no virus gene expression was necessary for this induction. Therefore the main objective of this study was to identify the signal that triggered apoptosis induction in MCF-7 cell line. This identification can be achieved by characterizing the early cellular and molecular mechanisms of NDV AF2240 induced apoptosis in MCF-7 cells.

The specific objectives of the study were as follows:

1. To determine the cytotoxicity of NDV AF2240 strain to MCF-7 cells
2. To demonstrate that NDV AF2240 cytotoxicity was mediated by NDV AF2240 induced apoptosis
3. To identify whether apoptosis was induced by NDV gene (s) or by cellular factors. This objective was achieved via a set of sub- objectives including:

a. To compare the time kinetic of NDV replication and the time kinetic of detection of early sign of apoptosis
b. To demonstrate whether NDV AF2240 induced apoptosis was NDV replication and protein synthesis independent
c. To demonstrate that NDV AF2240 induced apoptosis was dependent on NDV binding to cell surface and to sialic acid containing receptor

4. To demonstrate whether NDV AF2240 induced apoptosis inhibited NDV progeny production.
5. To confirm that NDV AF2240 induced apoptosis in NDV infected MCF-7 cells.
6. To demonstrate that NDV HN protein expression alone induced apoptosis in MCF-7 cells

REFERENCES

Ahlert, T. and Schirrmacher, V. 1990. Isolation of a human melanoma adapted Newcastle disease virus mutant with highly selective replication patterns. *Cancer Research* 50: 5962-5968.

Aini, I., Ibrahim, A.L., Spradbow, P.B. and Ch'ng, H.S. 1987. Development of food pellet Newcastle disease virus vaccine. In Copland, J.W. (ed). *Newcastle disease in poultry. A new food pellet vaccine*. ACIAR Monograph Nbre. 5, pp 20-23. Canberra.

Aini, I. 1989. *Vaccination of village chickens against Newcastle disease*, PhD Thesis, Universiti Pertanian Malaysia.

Allan, W.H. and Gough, R.E. 1974. A standard haemagglutination inhibition test for Newcastle disease: 1. A comparison of macro and micro method. *Veterinary Record* 95: 120-123.

Amendola, A., Lombardi, G., Oliverio, S., Colizzi, V. and Piacenti, M. 1994. HIV-1 gp120-dependent induction of apoptosis in antigen-specific human T cell clones is characterized by tissue transglutaminase expression and prevented by cyclosporine A. *FEBS Letters* 339: 258-264.

Ameisen, J.C. and Capron, A. 1991. Cell dysfunction and depletionin AIDS: the programmed cell death hypothesis. *Immunology Today* 12: 102-105.

Anouya, F., Watiez, R., Mousset, S. and Caillet-Fauquet, P. 1997. The cytotoxicity of the parvovirus minute virus of mice non-structural protein NS1 is related to changes in the synthesis and phosphorylation of cell proteins. *Journal of Virology* 71: 4671-4678.

Arends, M.J., Morris, R.G. and Wiley, A.H. 1990. Apoptosis: the role of the endonuclease. *American Journal of Pathology* 136: 593-608.

Arends, M.J. and Wyllie, A.H. 1991. Apoptosis: mechanisms and roles in pathology. *International Review of Experimental Pathology* 32: 223-254.

Banda, N.K., Bernier, J., Kurahana, D.K., Kurrle, R., Haigwood, N., Sekaly, R.P. and Finkel, T.H. 1992. Cross-linking CD4 by human immunodeficiency virus gp120 primes T cells for activation-induced apoptosis. *Journal of Experimental Medicine* 176: 1099-1106.

Barber, G.N. 2001. Host defence, viruses and apoptosis. *Cell Death and Differentiation* 8: 113-126.

Barton, E.S., Connolly, J.L., Forrest, J.C., Chappell, J.D. and Dermody, T.S. 2001a. Utilization of sialic acid as a coreceptor enhances reovirus attachment by multi-step adhesion-strengthening. *Journal of Biological Chemistry* 276: 2200-2211.

Barton, E.S., Chappell, J.D., Connolly, J.L., Forrest, J.C. and Dermody, T.S. 2001b. Reovirus receptor and apoptosis. *Virology* 290: 173-180.

Batliwalla, F.M., Baterman, B.A. and Serano, D. 1998. A 15 year follow up of AJCC stage III malignant melanoma patients treated postsurgically with Newcastle disease virus (NDV) oncolysate and determination of alterations in the CD8T cell repertoire. *Molecular Medicine* 4: 783-794.

Bellamy, C.O.C., Malcomson, R.D.G., Harrison, D.J. and Wyllie, A.H. 1995. Cell death in health and disease: the biology and regulation of apoptosis. *Semin Cancer Biology* 6: 3-12.

Benson, A.A. 1966. On the orientation of lipids in chloroplast and cell membranes. *Journal of American Oil Com Society* 43: 265-270.

Bian, H., Fournier, P., Moormann, R., Peeters, B. and Schirrmacher, V. 2005. Selective gene transfer to tumor cells by recombinant Newcastle disease virus via a bispecific fusion protein. *International Journal of Oncology* 26: 431-439.

Bian, H., Fournier, P., Peeters, B. and Schirrmacher, V. 2005. Tumor-targeted gene transfer in vivo via recombinant Newcastle disease virus modified by a bispecific fusion protein. *International Journal of Oncology* 27: 377-384.

Bian, H., Fournier, P., Moormann, R., Peeters, B. and Schirrmacher, V. 2005. Selective gene transfer *in vitro* to tumor cells via recombinant Newcastle disease virus. Cancer *Gene Therapy* 12: 295-303.

Bitzer, M., Prinz, F., Bauer, M., Spiegel, M., Neubert, W.J., Gregor, M., Schulze-Osthoff, K. and Lauer, U. 1999. Sendai virus infection induces apoptosis through activation of caspase-8 (FLICE) and caspase-3 (CPP32*). Journal of Virology* 73: 702-708.

Blumberg, K.E., Giorgi, C., Roux, L.; Raju, R., Dowling, P., Chollet, T. and Kolakofsky, D. 1983. Sequence determination of the Sendai virus HN gene and its comparison to the influenza virus glycoproteins. *Cell* 41: 269-278.

Bonifacino, J.S., Dasso, M., Harford, J.B., Lippincott-schwartz, J. and Yamada, K.M. 2004. *Short Protocols in Cell Biology*. *A compendium of methods from current protocols in cell biology.* pp 11.21- 11.22. John Wiley & Sons.

Bossy-Wetzel, E., Newmeyer, D.D. and Green, D.R. 1998. Mitochondrial cytochrome c release in apoptosis occurs upstream of DEVD-specific caspase activation and independently of mitochondrial transmembrane depolarization. *European Molecular Biology Organisation Journal* 17: 37-49.

Bowen, H.A. and Lyles, D.C. 1981. Structure of Sendai viral proteins in plasma membranes of virus infected cells. *Journal of Virology* 37: 1079-1082.

Brand, K., Arnold, W., Bartels, T., Lieber, A., Kay, M.A., Strauss, M. and Dorkin, B. 1997. Liver-associated toxicity of the HSV-tk/ GCV approach and adenoviral therapy *Cancer Gene Therapy* 4: 9-16.

Bratt, M.A. and Gallaher, W.R. 1970. Comparison of fusion from within and from without by Newcastle disease virus. *In Vitro* 6: 3-14

Brojatsch, J., Naughton, J., Rolls, M.M., Zingler, K. and Young, J.A.T. 1996. CAR1, a TNFR-related protein, is a cellular receptor for cytopathic avian leucosis-sarcoma virus and mediates apoptosis. *Cell* 87: 845-855.

Burge, B.W.and Huang, A.S. 1970. Comparison of membrane protein glycopeptides of Sindbis virus and VSV. *Journal of Virology* 6: 176-182.

Cain, K., Bratton, S.B., Longlain, C., Walker, G., Brown, D.G., Sun, X.M. and Cohen, G.M. 2000. Apaf-1 oligomerizes into biologically active ~700-kDa and inactive ~1.4 MDa apoptosome complex. *Journal of Biological Chemistry* 275: 6067-6070.

Carrascosa, A,L., Bustos, M.J., Nogal, M.L., Gonzalez de Buitrago, G. and Revilla, Y. 2002. Apoptosis induced in an early step of African swine fever virus entry into Vero cells does not require virus replication. *Virology* 294: 372-382.

Cassel, W.A. and Garrett, R.E. 1965. Newcastle disease virus as an antineoplastic agent. *Cancer* 18: 863-868.

Cassel, W.A., Murray, D.R. and Phillips, H.S. 1983. A phase II study on the postsurgical management of stage II malignant melanoma with a Newcastle disease virus oncolysate. *Cancer* 52: 856-860.

Cassel, W.A. and Murray, D.R. 1986. Viral oncolysate in the treatment of regional metastases of melanoma. In *Cancer in the neck: evaluation and treatment*, ed. D.L. Larson,O.M. Guillamondegui, and A.J. Ballantyne, pp. 235-242. NewYork: McMillan

Cassel, W.A. and Murray, D.R. 1992. A ten year follow up on stage II malignant melanoma patients treated postsurgicaly with Newcastle disease virus oncolysate. *Med. Oncol Tumor Pharmacther* 9: 169-171.

Chaudhary, P.M., Eby, M.T., Jasmin, A., Kumar, A., Liu, L. and Hood, L. 2000. Activation of the NF-kappaB pathway by caspase-8 and its homologs. *Oncogene* 19: 4451-4460.

Cheville, N.F. and Beard, C.W. 1972. Cytopathology of Newcastle disease and the influence of bursal and thymic lymphoid systems in the chicken. *Laboratory Investigation* 27: 129-143.

Cheville, N.F., Stone, H., Riley, J. and Ritchie, A.E. 1972. Pathogenesis of virulent Newcastle disease virus in chickens. *Journal of American Veterinary Medical Association* 161: 169-179.

Chinaiyan, A.M. and Dixit, V.M. 1996. The cell death machine. *Current Biology* 6: 555-562.

Choppin, P.W. and Compans, R.W. 1975. Reproduction of paramyxoviruses. In *Comprehensive virology: large RNA viruses*, ed. H.Fraenkel-Conrat, and R.R.Wagner, pp 95-178. New York: Plenum press.

Choppin, P.W. and Scheid, A. 1980. The role of viral glycoproteins in adsorption, penetration, and pathogenecity of viruses. *Review of Infectious Diseases* 2: 40-61.

Clarke, P., Meintzer, S.M., Gibson, S., Widmann, C., Garrington, T.P., Johnson, G.L. and Tyler, K.L. 2000. Reovirus-induced apoptosis is mediated by TRAIL. *Journal of Virology* 74: 8135

Cleverley, D.Z. and Lenard, J. 1998. The transmembrane domain in viral fusion: essential role for a conserved glycine residue in vesicular stomatitis virus G protein. *Proceeding of the National Academy of Science USA* 95: 3425-3430.

Clinkscales, C.W., Bratt, M.A. and Morrison, T.G. 1977. Synthesis of Newcastle disease virus polypeptides in a wheat germ cell free system. *Journal of Virology* 22: 97-101.

Coffey, M.C., Strong, J.E., Forsyth, P.A. and Lee, P.W.K. 1998. Reovirus therapy of tumors with activated Ras pathway. *Science* 282: 1332-1334.

Cohen, J.J. 1991. Programmed cell death in the immune system. *Advances in Immunology* 50: 55-85.

Cohen, G.M. 1997. Caspases: the executioners of apoptosis. *Biochemistry Journal* 326: 1-16.

Collins, P.L. and Hightower, L.E. 1982. Newcastle disease virus stimulates the cellular accumulation of stress (heat shock) mRNAs and proteins. *Journal of Virology* 44: 703-707.

Collins, P.L., Hightower, L.E. and Ball, L.A. 1978. Transcription and translation of Newcastle disease virus mRNA in vitro. *Journal of Virology* 28: 324-336.

Compton, M.M. 1992. A biochemical hallmark of apoptosis: Internucleosomal degradation of the genome. *Cancer Metastasis Research* 11: 105-119.

Connolly, J.L., Barton, E.S. and Dermody, T.S. 2001. Reovirus binding to cell surface sialic acid potentiates virus-induced apoptosis. *Journal of Virology* 75: 4029-4039.

Coukos, G., Makrigiannakis, A., Kang, E.H. 1999. Use of carrier cells to deliver a replication-selective herpes simplex virus-1 mutant for the intraperitoneal therapy of epithelial ovarian cancer. *Clinical Cancer Research* 5: 1523-1537.

Creasey, A.A., Reynolds, M.T. and Laird, W. 1986. Cures and partial regression of murine and human tumors by recombinant human tumor necrosis factor. *Cancer Research* 46: 5687-5690.

Csatary, L.K. 1971. Viruses in the treatment of cancer. *Lancet* 2: 825

Csatary, L.K, Eckhardt, S., Czegledi, F., Bokusza, I., Fenyvesi, C., Gergely, P., Bodey, P. and Csatary, C.M. 1993. Attenuated veterinary virus vaccine for the treatment of cancer. *Cancer Detection and Prevention* 17: 619-627.

Csatary, L.K., Moss, R.W., Beuth, J., Töröcsik, B., Szebereuyi, J. and Bakacs, T. 1998. Beneficial treatment of patients with advanced cancer using a Newcastle disease virus vaccine (MTH 68/H). *Anticancer Research* 19: 635-638.

Csatary, L.K. and Bakàcs, T. 1999. Use of Newcastle disease virus vaccine (MTH-68/H) in a patient with high-grade glioblastoma. Research letters. *Journal of the American Medical Association.* 281: 1588-1589.

Dachs, G.U., Dougherty, G.J., Stratford, I.J. and Chaplin, D.J. 1997. Targeting gene therapy to cancer: a review. *Oncological Research* 9: 313-325.

Davis, M.A. 2002. *Apoptosis methods in pharmacology and toxicology*. *Approaches to measurement and quantitation*. Human Press.

De Rossi, A., Ometto, L., Roncella, S., D'andrea, E., Meuin, C., Calderazzo, F., Rowe, M., Ferrarini, M. and Chieco-Bianchi, L. 1994. HIV-1 induces down-regulation of bcl-2 expression and death by apoptosis of EBV-immortalized B cells: a model for a persistent "self-limiting" HIV infection. *Virology* 198: 234-244.

Deveraux, Q.L., Roy, N., Stennicke, H.R., Van Arsdale, T., Zhou, Q., Srinivasula, S.M., Alnemri, E.S., Salversen, G.S. and Reed, J.C. 1998. IAP's block apoptosis events induced by caspase-8 and cytochrome c by direct inhibition of distinct caspases. *European Molecular Biology Organization Journal* 17: 2215-2223.

Du, C., Fang, M., Li, Y., Li, L.and Wang, X. 2000. Smac, a mitochondrial protein that promotes cytochrome c dependent caspase activation by eliminating IAP inhibition. *Cell* 102: 33-42.

Duvall, E. and Wyllie, A.H. 1986. Death and the cell. *Immunology Today* 7: 115-119.

Eick, D. and Hermeking, H. 1996. Viruses as pacemakers in the evolution of defence mechanisms against cancer. *Trends in Genetics* 12: 4-6.

Elankumaran, S., Rockemann, D. and Samal, S.K. 2006. Newcastle disease virus exerts oncolysis by both intrinsic and extrinsic caspase-dependent pathways of cell death. *Journal of Virology* 80: 7522-7534.

Eskes, R., Desagher, S., Antonsson, B. and Martinou, J.C. 2000. BID induces the oligomerization and insertion of Bax into the outer mitochondrial membrane. *Molecular Cell Biology* 20: 929-935.

Esolen, L.M., Park, S.W., Hardwick, J.M. and Griffin, D.E. 1995. Apoptosis as a cause of death in measles virus-infected cells. *Journal of Virology* 69: 3955-3958.

Evan, G.I., Wyllie, A.H., Gilbert, C.S., Littlewood, T.D., L., H., Brooks, M., Walters, C.M., Pen, L.Z. and Hancock, D.C. 1992. Induction of apoptosis in fibroblasts by c-myc protein. *Cell* 69: 119-128.

Everett, H. and McFadden, G. 1999. Apoptosis: an innate immune response to virus infection. *Trends in Microbiology* 7: 160-165.

Fábián, Z., Töröcsik, B., Kiss, K., Csatary, L.K., Bodey, B., Tigyi, J., Csatary, C. and Szeberenyi, J. 2001. Induction of apoptosis by a Newcastle disease virus vaccine (MTH-68/H) in PC12 rat phaeochromocytoma cells. *Anticancer Research* 21: 125-136.

Fauziah, O., Omar, A.R., Patimah, I. and Aini, I. 2002. Microscopic evaluation of Newcastle disease virus (NDV) a killer in chicken but a possible life saver in human. *Journal of Electronic Microscopy Society of Thailand* 16: 272.

Fawthrop, D.J., Boobis, A.R. and Davis, D.S. 1991. Mechanisms of cell death. *Archives of Toxicology* 65: 437-444.

Finkel, T.W., Tudor-williams, G., Banda, H.K., Cotton, M.F., Curiel, T., Monks, C., Baba, T.W., Ruprecht, R.M. and Kupfer, A. 1995. Apoptosis occurs predominantly in bystander cells and not in productively infected cells of HIV-infected and SIV-infected lymph nodes. *Nature Medicine* 1: 129-134.

Flanagan, A.D., Love, R. and Tesar, W. 1955. Propagation of Newcastle disease virus in Ehrlich ascites cells in vitro and in vivo. *Proceedings of the Society for Experimental Biology and Medicines* 90: 82-86.

Fournier, P., Zeng, J. and Schirrmacher, V. 2003. Two ways to induce innate immune responses in human PBMCs: paracrine stimulation of IFN-α responses by viral protein dsRNA. *International Journal of Oncology* 23: 673-680.

Fournier, P., Zeng, J., Van der lieth, C.W., Washburn, B., Ahlert, T. and Schirrmacher, V. 2004. Importance of serine 200 for functional activities of the hemagglutinin-neuraminidase protein of Newcastle disease virus. *International Journal of Oncology* 24: 623-634.

Fransen, L., Van der Heyden, J., Ruyssehaert, R. and Fiers, W. 1986. Recombinant tumor necrosis factor: its effect and its synergism with interferon-gamma on variety of normal and transformed human cell lines. *European Journal of Cancer and Clinical Oncology* 22: 419-426.

Freytag, S.O., Rogulski, K.R., Paielli, D.L., Gilbert, J.D. and Kim, J.H. 1998. A novel three-pronged approach to kill cancer cells selectively: concomitant viral, double suicide gene, and radiotherapy. *Human Gene Therapy* 9: 1323-1333.

Garten, W., Koharma, T. and Klenk, H.D. 1980. Proteolytic activation of the hemagglutinin-neuraminidase of Newcastle disease virus involves loss of a glycopeptide. *Journal of General Virology* 51: 207-211.

Gotoh, B., Sakaguchi., T., Nishikawa, K., Inocencio, N.M., Hamaguchi, M., Toyoda,T. and Nagai, Y. 1988. Structural features unique to each of the three antigenic sites on the hemagglutinin-neuraminidase protein of Newcastle disease virus. *Virology* 163: 174-182.

Gougeon, M.L. and Montagnier, L. 1993. Apoptosis in AIDS. *Science* 260: 1269-1270.

Green, D.G. and Scott, D.W. 1994. Activation-induced apoptosis in lymphocytes. *Current Opinion Immunology* 6: 476-487.

Green, D.R. and Reed, J.C. 1998. Mitochondria and apoptosis. *Science* 281: 1309-1312.

Green, D.R. 1998. Apoptotic pathways: the roads to ruin. *Cell* 94: 695-698

Green, D.R. 2000. Apoptotic pathways: paper wraps stone blunts scissors. *Cell* 102: 1-4.

Haines, D.S., Strauss, K.I. and Gillespie, D.H. 1991. Cellular response to double-stranded RNA. *Journal of Cell Biochemistry* 46: 9-20.

Hammon, W., Yohn, M.D.S., Casto, B.C. and Atchison, R.W. 1963. Oncolytic potentials of nonhuman viruses for human cancers. I.Effects of twenty-four viruses on human cancer cell lines. *Journal of National Cancer Institute* 31: 329-345.

Hanon, E., Meyer, G., Vanderplasschen, A., Dessy-Doize, C., Thiry, E. and Pastoret, P.P. 1998. Attachment but not penetration of Bovine Herpesvirus 1 is necessary to induce apoptosis in target cells. *Journal of Virology* 72: 7638-7641.

Heicappell, R., Schirrmacher, V., Von Hoegen, P., Ahlert, T. and Appelhans, B. 1986. Prevention of metastatic spread by postoperative immunotherapy with virally modified autologous tumor cells. I. Parrameters for optimal therapeutic effects. International *Journal of Cancer* 37: 569-577.

Heise, C.C., Williams, A-M., Xue, S., Propst, M. and Kirn, D.H. 1999. Intravenous administration of ONYX-015 a selectively replicating adenovirus induces antitumor efficacy. *Cancer Research* 59: 2623-2628.

Heylbroeck, C., Balachandran, S., Servant, M.J., Duluca, C., Barber, G.N., Lin, R. and Hiscott, J. 2000. The IRF-3 transcription factor mediates Sendai virus induced apoptosis. *Journal of Virology* 74: 3781-3792.

Hiebert, S.W., Paterson, R.G. and Lamb, R.A. 1985. Hemagglutinin-neuraminidase protein of the paramyxovirus simian virus 5: nucleotide sequence of the mRNA predicts an N-terminal membrane anchor. *Journal of Virology* 54: 1-6.

Hightower, L.E. and Bratt, M.A. 1974. Protein synthesis in NDV infected chicken embryo cells. *Journal of Virology* 13: 788-800.

Hinshaw, V.S., Olsen, C.W., Dybdahl-Sissoko, N. and Evans, D. 1994. Apoptosis: a mechanism of cell killing by influenza A and B viruses. *Journal of Virology* 68: 3667-3673.

Hiscott, J., Grandvaux, N., Sharma, S., Tenoever, B.R., Servant, M.J. and Liu, R. 2003. Convergence of the NF-Kappa B and interferon signalling pathways in the regulation of antiviral defense and apoptosis. *Annals of New York Academy of Science* 1010: 237-248.

Hockenberry, D.M. 1994. Bcl-2 in cancer, development and apoptosis. *Journal of Cell Science* S18: 51-55.

Huang, Z., Krishnamurthy, S., Panda, A. and Samal, S.K. 2003. Newcastle disease virus V protein is associated with viral pathogenesis and functions as an alpha interferon antagonist. *Journal of Virology* 77: 8676-8685.

Hugin, A.W., Vacchio, M.S. and Morse, H.C. 1991. Virus-encoded "superantigen" in a retrovirus-induced immunodeficiency syndrome of mice. *Science* 252: 424-427.

Ibrahim, A.L., Chulan, U. and Babjee, A.M. 1980. The immune response of chickens vaccinated against Newcastle disease with Newcastle disease V4 vaccine. *Australian Veterinary Journal* 56: 29-33.

Ibrahim, A.L., Lai, C.M. and Aini, I. 1983. Spray vaccination with an improved F Newcastle disease virus vaccine. *British Veterinary Journal* 139: 213-219.

Igney, F.H. and Krammer, P.H. 2002. Death and anti-death. Tumor resistance to apoptosis. *Nature Research Cancer* 2: 277-288.

Iorio, R.M. and Bratt, M.A. 1984. Monoclonal antibodies as functional probes of the HN glycoprotein of Newcastle disease virus: antigenic separation of the hemagglutinating and neuraminidase sites. *Journal of Immunology* 133: 2215-2219.

Jacobson, M.D., Weil, M. and Raff, M. 1997. Programmed cell death in animal development. *Cell* 88: 347-354.

Jan, J.T. and Griffin, D.E. 1999. Induction of apoptosis by Sindbis virus occurs at cell entry and does not require virus replication. *Journal of Virology* 73: 10296-10302.

Janicke, R.U., Sprengart, M.L., Wati, M.R. and Porter, A.G. 1998. Caspase-3 is required for DNA fragmentation and morphological changes associated with apoptosis. *Journal of Biological Chemistry* 273: 9357-9360.

Jeurissen, S.H., Wagenaar, F., Pol, J.M., Van der eb, A.J. and Noteborn, M.H. 1992. Chicken anemia virus causes apoptosis of thymocytes after in vivo infection and of cell lines after in vitro infection. *Journal of Virology* 66: 7383-7388.

Joe, A.K., Foo, H.H., Kleeman, L. and Levine. B. 1998. The transmembrane domains of Sindbis virus envelope glycoproteins induce cell death. *Journal of Virology* 72: 3935-3943.

Jorgensen, E.D., Collins, P.L. and Lomedico, P.T. 1987. Cloning and nucleotide sequence of Newcastle disease virus hemagglutinin-neuraminidase mRNA: identification of a putative sialic acid binding site. *Virology* 156: 12-24.

Jurianz, K., Haas, C., Hubbe, M., Ertel, C., Brunner, G., Altevogt, P., Schirrmacher, V. and Hoegen, P. 1995. Adhesive function of Newcastle disease virus hemmagglutinin in tumor-host interaction. *International Journal of Oncology* 7: 539-545.

Kerr, J.F.R., Wyllie, A.H. and Currie, A.R. 1972. Apoptosis: a basic biological phenomenon with wide ranging implications in tissue kinetics. *British Journal of Cancer* 26:239-257.

Kerr, J.F., Winterford, C.M. and Harmon, B.V. 1994 Apoptosis, its significance in cancer and cancer therapy. *Cancer* 73: 2013-2026.

Kho, C.L., Mohd-Azmi, M.L., Arshad, S.S. and Yousoff, K. 2000. Performance of an RT-nested PCR ELISA for detection of Newcastle disease virus. *Journal of Virological Methods* 86: 71-83.

Kingsbury. D.W. 1985. Orthomyxo- and paramyxoviruses and their replication. In *Virology,* ed B.N. Fields et al, pp. 1157-1178. New York: Raven Press.

Kirchner, H.H., Anton, P. and Atzpodieu, J. 1995. Adjuvant treatment of locally advanced renal cancer with autologous virus-modified tumor vaccines. World Journal of Urology. 13: 171-173.

Kirshner, J.R., Karpova, A.Y., Kops, M. and Howley, P.M. 2005. Identification of TRAIL as an interferon regulatory factor 3 transcriptional target. *Journal of Virology* 79: 9320-9324.

Klenk, H.D. and Choppin, P.W. 1969. Lipid of plasma membranes of monkey and hamster kidney cells and of parainfluenza virus grown in these cells. *Virology* 38: 253-268.

Klenk, H.D., Caliguili, L.A. and Choppin, P.W. 1970. The proteins of parainfluenza virus SV5. II. The carbohydrate content and glycoproteins of the virion. *Virology* 42: 473-481.

Kohn, A. and Fuchs, P. 1969. Cell fusion by various strains of Newcastle disease virus and their virulence. *Journal of Virology* 3: 539-540.

Kooby, D.A., Carew, J.F., Halterman, M.W., Mack, J.E., Bertino, J.R., Blumgart, L.H., Federoff, H.J. and Fong, Y. 1999. Oncolytic viral therapy for human colorectal cancer and liver metastases using a multi-nutated herpes simplex virus type-1 (G207). *FASEB J* 13: 1325-1334.

Kotelkin, A., Prikhod'ko, E.A., Cohen, J.I., Collins, P.L. and Burkreyer, A. 2003. Respiratory syncytial virus infection sensitizes cells to apoptosis mediated by tumor necrosis factor-related apoptosis induced ligand. *Journal of Virology* 77: 9156-9172.

Koyama, A.H. and Adachi, A. 1997. Induction of apoptosis by herpes simplex virus type 1. *Journal of General Virology* 78: 2909-2912.

Labrada, L., Bodelón, G., Viñuela, J. and Benavente, J. 2002. Avian reoviruses cause apoptosis in cultured cells: viral uncoating, but not viral gene expression, is required for apoptosis induction. *Journal of Virology* 76: 7932-7941.

Lai,C.M. 1985. *A study on a velogenic viscerotropic Newcastle disease virus in-vitro and in-vivo.* PhD thesis, Universiti Pertanian Malaysia.

Lai, M.C. and Ibrahim, A.L. 1987. Velogenic viscerotropic Newcastle disease virus. P33-34. In *Newcastle disease in poultry. A new food pellet vaccine,* Ed. J.W. Copland,

pp 33-34. Camberra: Australian Centre for International Agriculture Research (ACIAR)

Lam, K.M. and Hao, Q. 1987. Induction of lymphocyte agglutination and lysis by Newcastle disease virus. *Veterinary Microbiology* 15: 49-56.

Lam, K.M. and Vasconcelos, A.C. 1994. Newcastle disease virus-induced apoptosis in chicken peripheral blood lymphocytes. *Veterinary Immunology and Immunopathology* 44: 45-56.

Lam, K.M. 1995. Apoptosis in chicken embryo fibroblasts caused by Newcastle disease virus. *Veterinary Microbiology* 47: 357-363.

Lamb, R.A. and Kolakofsky, D. 1996. Paramyxoviridae: the viruses and their replication. In *Virology*, ed. B.N. Fields, D.M.Knipe., P.M. Howley, et al, pp 1177-1204. Philadelphia: Lippincott-Raven Publishers.

Landsberger, F.R., Compans, R.W., Choppin, P.W. and Lenard, J. 1973. Organization of the lipid phase in viral membranes. Effects of independent variation of the lipid and the protein composition. *Biochemistry* 12: 4498-4502.

Laurent-Crawford, A.G., Krust, G.B., Muller, S., Riviere, Y., Rey-Cuille, M.A., Bechet, J.M., Montagnier, L. and Hovanessian, A.G. 1991. The cytopathic effect of HIV is associated with apoptosis. *Virology* 185: 829-839.

Laurent-Crawford, A.G., Krust, B., Riviere, Y., Desgranges, C., Muller, S., Kieny, M.P., Dauguet, C. and Hovanessian. A.G. 1993. Membranes expression of HIV envelope glycoproteins triggers apoptosis in CD4 cells. *AIDS Research Human Retrovirus* 9: 761-773.

Laurent-Crawford, A.G., Coccia, E., Krust, B. and Hovanessian, A.G. 1995. Membrane-expressed HIV envelope glycoprotein heterodimer is a powerful inducer of cell death in uninfected CD4+ target cells. *Research Virology* 146: 5-17.

Levine, B., Huang, Q., Isaacs, J.T., Reed, J.C., Grifrdfin, D.E. and Hardwick, J.M. 1993. Conversion of lytic to persistent alphavirus infection by the Bcl-2 cellular oncogene. *Nature* 361: 739-742.

Levine, A.J 1997. p53, the cellular gatekeeper for growth and decision. *Cell* 88: 323-331.

Li, C.J., Friedman, D.J., Wang, C., Metler, V. and Pardee, A. 1995. Induction of apoptosis in uninfected lymphocytes by HIV-1 Tat protein. *Science* 268:429-437.

Liang, W., Wang, H., Sun, T.M., Yao, W.Q., Jiu, Y., Li, C.L. and Meng, F.J. 2003. Application of autologous tumor cell vaccine and NDV vaccine in treatment of tumors of digestive tract. *World Journal of Gastroenterology* 9: 495-498.

Liu, C.K., Wei, G. and Atwood, W.J. 1998. Infection of glial cells by the human polyomavirus JC is mediated by an N-linked glycoprotein containing terminal α (2-6)-linked sialic acids. *Journal of Virology* 72: 4643-4649.

Lorence, R.M., Rood, P.A. and Kelley, K.W. 1988. Newcastle disease virus as an antineoplastic agent: induction of tumor necrosis factor-α and augmentation of its cytotoxicity. *Journal of National Cancer Institute* 80: 1305-1312.

Lorence, R.M., Reichard, K.W., Katubig, B.B., Reyes, H.M., Phuangsab, A., Sassetti, M.D., Walter, R.J. and Peeples, M.E. 1994a. Complete regression of human fibrosarcoma xenografts after local Newcastle disease virus therapy. *Cancer Research* 54: 6017-6021.

Lorence, R.M., Reichard, K.W., Katubig, B.B., Reyes, H.M., Phuangsab, A., Mitchell, B.R., Cascino, C.J., Walter, R.J. and Peeples, M.E. 1994b. Complete regression of human neuroblastoma xenografts in athymic mice after local Newcastle disease virus therapy. *Journal of National Cancer Institute* 80: 1305-1312.

Malcomson, R.D.G., Oram, S.H. and Harrison, D.J. 1996. The importance of apoptosis: is it real or imaginary?. *Biological*s 24: 295-299.

Malaysia Cancer Statistics. 2006, Ministry of Health, Malaysia

Marcellus, R.C., Teodoro, J.G., Wu, T., Brough, D.E., Ketner, G., Shore, G.C. and Branton, P.E. 1996. Adenovirus type 5 early region 4 is responsible for E1A-induced p53-independent apoptosis. *Journal of Virology* 70: 6207-6215.

Martin, S.J. and Green, D.R. 1994. Apoptosis as a goal of cancer therapy. *Current Opinion of Oncology* 6: 616-621.

Martin, S.J., Green, D.R. and Cotter, T.G. 1994. Dicing with death: dissecting the components of the apoptosis machinery. *Trends in Biochemical Science* 19: 26-30.

Mattson, M.P. and Furukawa, K. 1997. Anti-apoptotic actions of cycloheximide: blockade of programmed cell death or induction of programmed cell life. *Apoptosis* 2: 257-264.

McCabe, M.J. and Orrenius, S. 1992. Deletion and depletion: the involvement of viruses and environmental factors in T-lymphocyte apoptosis. *Laboratory Investigations* 66: 403-406.

McDonnell, T.J., Meyn, R.E. and Robertson, L.E. 1995. Implications of apoptotic cell death regulation in cancer therapy. *Seminars Cancer Biology* 6: 53-55.

McDanial, H.A. and Osborne, J.S. 1973. Diagnosis of velogenic viscerotropic Newcastle disease virus. *Journal of American Veterinary Medicine Association* 163: 1075-1079.

McGinnes, L.W. and Morrison, T.G. 1986. Nucleotide sequence of the gene encoding the Newcastle disease virus fusion protein and comparisons of paramyxovirus fusion protein sequences. *Virus Research* 5: 343-356.

McGinnes, L.W., Wilde, A. and Morrison, T. G. 1987. Nucleotide sequence of the gene encoding the Newcastle disease virus hemagglutinin-neuraminidase protein and

comparison of paramyxovirus hemagglutinin-neuraminidase protein sequences. *Virus Research* 7: 187-202.

McGinnes, L., Sergel, T. and Morrison, T. 1993. Mutations in the trans-membrane domain of the HN protein of Newcastle disease virus affect the structure and activity of the protein. *Virology* 196: 101-110.

Meyaard, L., Otto, S.A., Jonker, R.R., Mijnster, M.J., Keet, R.P. and Miedema, F. 1992. Programmed death of T cells in HIV-1 infection. *Science* 257: 217-219.

Millar, N.S., Chambers, P. and Emmerson, P.T. 1986. Nucleotide sequence analysis of the hemagglutinin-neuraminidase gene of Newcastle disease virus. *Journal of General Virology* 67: 1917-1927.

Molouki, A., Hsu, Y.T., Jahanshiri, F., Rosli, R. and Yusoff, K. 2010. Newcastle disease virus infection promotes Bax redistribution to mitochondria and cell death in Hela cells. *Intervirology* 53: 87-94.

Moolten, F. 1986. Tumor chemosensitivity conferred by inserted herpes thymydine kinase gene: paradigm for a prospective cancer control strategy. *Cancer Research* 46: 5276-5281.

Morrison, T., Ward, L.J. and Semerjian, A. 1985. Intracellular processing of the Newcastle disease virus fusion protein. *Journal of Virology* 53: 851-857.

Morrison, T.G. and Portner, A. 1991. Structure, function, and intracellular processing of the glycoproteins of Paramyxoviridae. In *the paramyxoviruses,* ed D.W. Kingsbury, pp 347-382. New York : Plenum Press

Muzio, M., Salvesen, G.S. and Dixit, V.M. 1997. FLICE induced apoptosis in a cell-free system. Cleavage of caspase zymogenes. *Journal of Biological Chemistry* 272: 2952-2956.

Mymryk, J.S., Shire, K. and Bayley, S.T. 1994. Induction of apoptosis by adenovirus type 5 E1A in rat cells requires a proliferation block. *Oncogene* 9: 1187-1193.

Nagai, Y., Klenk, H.D. and Rott, R. 1976. Proteolytic cleavage of the viral glycoproteins and its significance for the virulence of Newcastle disease virus. *Virology* 72: 494-508.

Nagai, Y., Hamaguchi, M. and Toyoda, T. 1989. Molecular biology of Newcastle disease virus. *Progress in Veterinary Microbiology and Immunology* 5: 16-64.

Nagata, S. 1997. Apoptosis by death factor. *Cell* 88: 355-365.

Nemunaitis, J., Khuri, F., Ganly, I., Arseneau, J., Posner, M., Vokes, E., Kuhn, J., McCarty, T., Landers, S., Blackburn, A., Romel, L., Randlev, B., Kayes, S. and Kirn, D. 2001. Phase II trial of international administration of ONYX-015, a replication-selective adenovirus, in patients with refractory head and neck cancer. *Journal of Clinical Oncology* 19: 289-298.

Nicholson, D.W. and Thornberry, N.A. 1997. Caspases: killer proteases. *Trends biochemical science* 22: 299-306.

Nicoletti, I., Migliorati, G., Pagliacci, M.C., Grignani, F. and Riccardi, C. 1991. A rapid and simple method for measuring thymocyte apoptosis by propidium iodide staining and flow cytometry. *Journal of Immunological Methods* 139: 271-279.

Noteborn, M.H., Todd, D., Verschueren, C.A., de Gauw, H.W., Curran, W.L., Veldkamp, S., Douglas, A.J., McNulty, M.S., Van der eb, A.J. and Koch, G. 1994. A single chicken anemia virus proteininduces apoptosis. *Journal of Virology* 68: 346-351.

Oberhammer, F., Wilson, J.W., Dive, C., Morris, I.D., Hickman, J.A., Wakeling, P.R. and Sikorska, M. 1993. Apoptotic death in epithelial cells: cleavage of DNA to 300 and/or 50 kb fragments prior to or in the absence of internucleosomal fragementation. *European Molecular Biology Organisation Journal* 12: 3679-3684.

O'Brien, V. 1998. Viruses and apoptosis. *Journal of General Virology* 79: 1833-1845.

Ockert, D., Schirrmacher, V., Beck, N., Stoelber, E., Ahlert, T., Flechtenmacher, J., Hagmuller, E., Nagel, M. and Saeger, H.D. 1996. Newcastle disease virus infected intact autologous tumor cell vaccine for adjuvant active specific immunotherapy of resected colorectal carcinoma. *Clinical Cancer Research* 2: 21-28.

Ogasawara, T., Gotoh, B., Suzuki, H., Asaka, J., Shimokata, K., Rott, R. and Nagai, Y. 1992. Expression of factor X and its significance for the determination of paramyxovirus tropism in the chick embryo. *European Molecular Biology Organisation Journal* 11: 467-472.

Old, L.J. 1986. Tumor necrosis factor (TNF). *Science* 230: 630-632.

Oldstone, M.B. 1997. How viruses escape from cytotoxic T lymphocytes: molecular parameters and players. *Virology* 234: 179-185.

Ohshima, T., Iwama, M., Ueno, Y., Sugiyama, F., Nakajima, T., Fukamizu, A. and Yagami, K. 1998. Induction of apoptosis in vitro and in vivo by H-1 parvovirus infection. *Journal of General Virology* 79: 3067-3071.

Omar, A.R., Aini, I., Ali, A.M., Othman, F., Yusoff, K., Abdullah, J.M., Wali, H.S.M., Zawawi, M. and Meyyappan, N. 2002. An overview on the development of Newcastle disease virus as an anti-cancer therapy. *Malyasian Journal of Medical Sciences* 9: 4-12.

Ozawa, K., Ayub, J., Kajigaya, S., Shimada, T. and Young, N. 1988. The gene encoding the non-structural protein of B19 (Human) parvovirus may be lethal in transfected cells. *Journal of Virology* 62: 2884-2889.

Pahl, P.M., Horwitz, M.A., Horwitz, K.B. and Horwitz, L.D. 2001. Desferri-exochelin induces death by apoptosis in human breast cancer cells but does not kill normal breast cells. *Breast Cancer Research* 69: 69-79.

Palladino, M.A., Shalaby, R.R. and Kramer, S.M. 1987. Characterization of the antitumor activities of human tumor necrosis factor-α and the comparison with other

cytokines: induction of tumor-specific immunity. *Journal of Immunology* 138: 4023-4032.

Park, M.S., Shaw, M.L., Manoz-Jordan, J., Cros, J.F., Nakaya, T., Bouvier, N., Palese, P., Garcia-Sastre. and Basler, C.F. 2003. Newcastle disease virus (NDV)-based assay demonstrates interferon antagonist activity for the NDV V protein and the Nipah virus V, W and C proteins. *Journal of Virology* 77: 1501-1511.

Pasternak, C.A., Alder, G.M., Bashford, C.L., Buckley, C.D., Micklen, K.J. and Patel, K. 1985. Cell damage by viruses, toxins and complement: common features of pore-formation and its inhibition by Ca2+. *Biochemistry Society Symposium* 50: 247-264.

Paulovich, A.G., Toczysuk, D.P. and Hartwell, L.H. 1997. When check points fail. *Cell* 88: 315-321.

Peeples, M.E. 1988. Newcastle disease virus replication. In *Newcastle disease*, ed. D.J. Alexander, pp 45-78. Kluwer Academic Press Publishers.

Pieterson, A.M., Van der eb, M.M., Rademaker, H.J., Van den wollenberg, D.J.M., Rabelink, M.J.W.E., Kuppen, P.J.K., Diesendock, J.H., Van Ormondt, R.C.,Masman, D., Van de velde, C.J.H., Van der eb, A.J., Hoeben, RC. and Noteborn, M.H.M. 2000. Specific tumor cell killing with adenovirus vectors containing the apoptin gene. *Gene Therapy* 6: 882-892.

Pieterson, A.M. and Noteborn, M.H.M. 1999. Apoptin. In *cancer therapy: past achievement and future challenges*. Ed. Habib pp 153-161. New York: Kluwer Academic/ Plenum Publishers.

Pitti, R.M., Marsters, S.A., Ruppert, S., Douahme, C.J., Moore, A. and Ashkenazi, A. 1996. Induction of apoptosis by Apo-2 ligand, a new member of the tumor necrosis factor cytokine family. *Journal of Biochemistry* 271: 12687-12690.

Plaskin,D., Porgador, A., Vadai, E., Feldman, M., Schirrmacher, V. and Eisenbach, L. 1994. Effective anti-matastatic melanoma vaccination with tumor cells transfected with MHC genes and /or infected with Newcastle disease virus (NDV). *International Journal of Cancer* 59: 796-801.

Polyak, K., Waldman, T., He, T.C., Kinzler, K.W. and Vogelstein, B. 1996. Genetic determinants of p53-induced apoptosis and growth arrest. *Genes and Development* 10: 1945-1952.

Portner, A. 1981. The HN glycoprotein of Sendai virus: analysis of site(s) involved in hemagglutinating and neuraminidase activities. *Virology* 115: 375-384.

Rabu, A., Tan, W.S., Kho, C.L., Omar, A.R. and Yusoff, K. 2002. Chimeric Newcastle disease virus nucleocapsid with parts of viral hemagglutinin-neuraminidase and fusion proteins. *Acta Virologica* 46: 211-217.

Ramsey-Ewing, A. and Moss, B. 1998. Apoptosis induced by a post-binding step of Vaccinia virus entry into Chinese hamster ovary cells. *Virology* 242: 138-149.

Rao, L and White, E. 1997. Bcl-2 and the ICE-family of apoptotic regulators: making a connection. *Current Opinion in Genetic Development* 7: 52-58.

Ravindra, P.V., Tiwari, A.K., Sharma, B., Rajawat, Y.S., Ratta, B., Palia, S., Sundaresan, N.R., Chaturvedi, U., Aruna Kumar, G.B., Kantaraja, C., Meeta, S., Subudhi, P.K., Rai, A. and Chauhan, R.S. 2008. HN protein of Newcastle disease virus causes apoptosis in chicken embryo fibroblasts cells. *Archives of Virology* 153: 749-754.

Reeve, P. and Poste, G. 1971. Studies on the cytopathogenicity of Newcastle disease virus: relation between virulence, polykaryocytosis and plaque size. *Journal of General Virology* 11: 17-24.

Reichard, K.W., Lorence, R.M., Cascino, C.J., Peeples, M.E., Walter, R.J., Fernando, M.B., Reyes, H.M. and Greager, J.A. 1992. Newcastle disease virus selectivity kills human tumor cells. *Journal of Surgical Research* 52: 448-453.

Revillard, J-P., Adorini, L., Goldman, M., Kabelitz, D. and Waldmann, H. 1998. Apoptosis: potential for disease therapies. *Trends in Immunology Today* 19: 291-293.

Ring, C.J.A. 2002. Cytolytic viruses as potential anti-cancer agents. *Journal General of Virology* 83: 491-502.

Roizman, B. and Palese, P. 1996. Multiplication of viruses: an overview. In *virology*, ed. B.N.Fields., D.M. Knipe., P.M. Howley et al., pp 101-111. Philadelphia. Lippincott, Raven publishers.

Rojko, J.L., Fulton, R.M., Renanza, L.J., Williams, L.L., Copelan, E., Cheney, C.M., Reichel, G.S., Neil, J.C., Mathes, L.E., Fisher, T.G. and Cloyd, M.W. 1992. Lymphocytotoxic strains of feline leukaemia virus induce apoptosis in feline T4-thymic lymphoma cells. *Laboratory Investigation* 66: 418-426.

Romer-Oberdrfer, A., Mundt, E., Mebatsien, T., Buchlz, U and Mettinleiter, T. 1999. Generation of recombinant Newcastle disease virus from cDNA. *Journal of General Virology* 80: 2987-2995.

Rommelaere, J. and Tattersall, P. 1990. Oncosuppression by parvoviruses. In *Handbook of parvoviruses*, Ed. P.Tijssen vol. 2, pp. 41-57. Boca Raton, FL: CRC Press.

Rood, P.A., Lorence, R.M. and Kelly, K.W. 1990. Serum protease inhibitor abrogation of Newcastle disease virus enhancement of cytolysis by recombinant tumor necrosis factors alpha and beta. *Journal of National Cancer Institute* 82: 213-217.

Roth, J.A. and Christiano, R.J. 1997. Gene therapy for cancer: what have we done and where are we going?. *Journal of National Cancer Institute* 89: 21-39.

Rutter, G. and Manweiler, K. 1977. Alterations of actin-containing structures in BHK 21 cells infected with Newcastle disease virus and Vesicular Stomatis virus. *Journal General of Virology* 37: 233-242.

Sachs, L. and Lotem, J. 1993. Control of programmed cell death in normal and leukaemia cells: new implications for therapy. *Blood* 82: 15-21.

Sastry, K.J., Marin, M.C., Nehete, P.N., McConnell, K., El-Naggar, A.K. and McDonnell, T.J. 1996. Expression of human immunodeficiency virus type 1 tat results in down regulation of bcl-2 and induction of apoptosis in hematopoitic cells. *Oncogen* 13: 487-493.

Schaper, U.M., Fuller, F.J., Ward, M.D., Mehrotra, Y., Stone, H.O., Stripp, B.R. and De Buyssher, E.V. 1988. Nucleotide sequence of the envelope protein genes of a highly virulent, neurotropic strain of Newcastle disease virus. *Virology* 165: 291-295.

Scheid, A., Caliguiri, L.A., Compans, R.W. and Choppin, P.W. 1972. Isolation of paramyxovirus glycoproteins: Association of both hemagglutinating and neuraminidase activities with the larger SV5 glycoprotein. *Virology* 50: 640-652.

Scheid, A. and Choppin, P.W. 1974. Identification of biological activities of paramyxovirus glycoproteins. Activation of cell fusion, hemolysis and infectivity by proteolytic cleavage of an inactive precursor protein of Sendai virus. *Virology* 57: 475-490.

Schirrmacher, V., Ahlert, T., Heicappell, R., Appelhans, B. and Von Hoegen, P. 1986. Successful application of non-oncogenic viruses for anti-metastatic cancer immunotherapy. *Cancer Review* 5: 19-49.

Schirrmacher, V., Von Hoegen, P. and Heicappell, R. 1988. Postoperative activation of tumor specific T cells by immunization with virus-modified tumor cells and effects on metastasis. *Advances Experimental Medicine Biology* 233: 91-96.

Schirrmacher, V., Haas, C., Bonifer, R. and Ertel, C. 1994. Virus potentiation of tumor vaccine T cell stimulatory capacity requires cell surface binding but not infection. *Clinical Cancer Research* 3: 1135-1148.

Schirrmacher, V., Haas, C., Bonifer, R. and Ertel C. 1997. Virus potentiation of tumor vaccine T-cell stimulatory capacity requires cell surface binding but not infection. *Clinical Cancer Research* 3: 1135-1148.

Schirrmacher, V., Ahlert, T., Probstle, T., Steiner, H.H., Herld-Meude, C., Gerhards, R., Hagmuller, E. and Steiner, H.A. 1998. Immunization with virus-modified tumor cells. *Seminars in Oncology* 25: 677-696.

Schirrmacher, V., Haas, C., Bonifer, R., Ahlert, T., Gerhards, R. and Ertel, C. 1999a. Human tumor cell modification by virus infection: an efficient and safe way to produce cancer vaccine with pleiotropic immune properties when using Newcastle disease virus. *Gene Therapy* 6: 63-73.

Schirrmacher, V., Jurianz, K., Roth, C., Griesbach, A., Bonifer, R. and Zawatzky, R. 1999b. Tumor stimulator cell modification by infection with Newcastle disease virus: analysis of effects and mechanism in MCTC-CML cultures. *International Journal of Oncology* 14: 205-215.

Schirrmacher, V., Bai, L., Umansky, V., Yu, L., Xing, Y. and Qian, Z. 2000. Newcastle disease virus activates macrophages for anti-tumor activity. *International Journal of Oncology* 16: 363-373.

Schirrmacher, V., Griesbach, A. and Ahlert, T. 2001. Anti-tumor effects of Newcastle disease virus in vivo: local versus systemic effects. *International Journal of Oncology* 18: 942-952.

Schirrmacher, V., Beckhove, P., Choi, C., Griesbach, A. and Mahnke, Y. 2005. Tumor-immune memory T cells from the bone marrow exert GvL without GvH reactivity in advanced metastatisized cancer. *International Journal of Oncology* 27: 1141-1149.

Schlegel. R.A. and Williamson, P. 2001. Phosphatidylserine, a death knell. *Cell Death and Differentiation* 8: 551-563.

Schulze-Osthoff, K., Ferrari, D., Los, M., Wesselborg, S. and Peter, M.E. 1998. Apoptosis signalling by death receptors. *European Journal of Biochemistry* 254: 439-459.

Schuy, W., Garten, W., Linder, D. and Klenk, H.D. 1984. The carboxyterminus of the hemagglutinin-neuraminidase of Newcastle disease virus is exposed at the surface of the viral envelope. *Virus Research* 1: 415-426.

Sedger, L.M., Shows, D.M., Blanton, R.A., Peschon, J.J., Goodwin, R.G., Cosman, D. and Wiley, S.R. 1999. IFN-γ mediates a novel antiviral activity through dynamic modulation of TRAIL and TRAIL receptor expression. *Journal of Immunology* 163: 920-928.

Servant, M.J., Ten Oever, B., LePage, C., Couti, L., Gessani, S., Julkunen, I., Lin, R. and Hiscott, J. 2001. Identification of distinct signalling pathways leading to the phosphorylation of interferon regulatory factor 3. *Journal of Biological Chemistry* 276: 355-363.

Shen, Y. and Shenk, T.E. 1995. Viruses and apoptosis. *Current Opinion in Genetics and Development* 5: 105-111.

Shetty, S., Gladden, J.B., Henson, E.S., Hu, X., Villanueva, J., Haney, N. and Gibson, S.B. 2002. Tumor necrosis factor related apoptosis inducing ligand (TRAIL) up-regulates death receptor 5 (DR5) mediated by NF-kappaB activator in epithelial cell lines. *Apoptosis* 7: 413-420.

Sinkovics, J. and Horvath, J. 1993. New developments in the virus therapy of cancer: a historical review. *Intervirology* 36: 193-214.

Sinkovics, J. and Horvath, J. 2000. Newcastle disease virus (NDV): a brief history of its oncolytic strains. *Journal of Clinical Virology* 16: 1-15.

Srinivasula, S.M., Ahmad, M., Fernandes-Alnemri, T. and Alnemri, E.S. 1998. Autoactivation of procaspase-9 by Apaf-1-mediated oligomerization. *Molecular Cell* 1: 949-957.

Steiner, H.H., Bonsanto, M.M., Beckhove, P., Brysch, M., Geletneky, K., Ahmadi, R., Schuele-Freyer, R., Kremer, P., Ranaie, G., Matejic, D., Bauer, H., Kiessling, M., Kunze, S., Schirrmcher, V. and Herold-mende, C. 2004. Antitumor vaccination of patients with glioblastoma multiforme: a pilot study to assess feasibility, safety and clinical benefit. *Journal of Clinical Oncology* 22: 4272-4281.

Stojdl, D.F., Lichty, B., Knowles, S., Marius, R., Atkins, H., Sonenberg, N. and Bell, J.C. 2000. Exploiting tumor-specific defects in the interferon pathway with a previously unknown oncolytic virus. *Nature Medicine* 6: 821-825.

Strack, P.R., Frey, M.W., Rizzo, C.J., Cordova, B., George, H.J., Meade, R., Ho, S.P., Corman, J., Tritch, R. and Koran, B.D. 1996. Apoptosis mediated by HIV protease is preceeded by cleavage of Bcl-2. *Proceeding of the National Academy of Science USA* 93: 9571-9576.

Strauss, J.H., Burge, B.W. and Darnell, J.E. 1970. Carbohydrate content of the membrane protein of Sindbis virus. *Journal of Molecular Biology* 47: 437-448.

Strong, J.E., Coffey, M.C., Tang, D., Sabinin, P. and Lee, P.W.K. 1988. The molecular basis of viral oncolysis: usurpation of the Ras signalling pathway by reovirus. *European Molecular Biology Organization Journal* 12: 3351-3362.

Suarez, P., Diazguerra, N., Prieto, C., Esteban, J., Castro, J.M., Nieto, A. and Ortin, J. 1996. Open reading frame-5 of porcine reproductive and respiratory virus as a cause of virus-induced apoptosis. *Journal of Virology* 70: 2876-2882.

Sugawara, K.E., Tashiro, M. and Homma, M. 1982. Intermolecular association of HANA glycoprotein of Sendai virus in relation to the expression of biological activities. *Virology* 117: 444-455.

Sugarman, B.J., Aggarwal, B.B., Hass, P.E., Figari, I.S., Palladino, M.A. and Shepard, H.M. 1985. Recombinant human tumor necrosis factor-alpha: effects on proliferation of normal and transformed cells in vitro. *Science* 230: 943-945.

Suliman, A., Lam, A., Datta, R. and Srivastava, R.K. 2001. Intracellular mechanisms of TRAIL: apoptosis through mitochondrial-dependent and independent pathways. *Oncogene* 20: 2122-2133.

Takizawa, T., Matsukawa, S., Higuchi, Y., Nakamura, S., Nakanishi,Y. and Fukuda, R. 1993. Induction of programmed cell death (apoptosis) by influenza virus infection in tissue culture cells. *Journal of General Virology* 74: 2347-2355.

Teodoro, J.G., Shore, G.C. and Branton, P.E. 1995. Adenovirus E1A protein induces apoptosis by both p53-dependent and p53- independent mechanisms. *Oncogene* 11: 467-474.

Teodoro, J.G. and Branton, P.E. 1997. Regulation of apoptosis by viral gene products. *Journal of Virology* 71: 1739-1746.

Ten, R.M., Blank, V., Le Bail, O., Kourilsky, P. and Israel, A. 1993. Two factors, IRF1 and KBF-1/ NF-kappa B, cooperate during induction of MHC class I gene expression

by interferon alpha beta or Newcastle disease virus. *C.R Academy of Science* III 16: 496-501.

Thompson, S.D., Lauer, W.G., Murti, K.G. and Portner, A. 1988. Isolation of a biologically active soluble form of the hemagglutinin-neuraminidase protein of Sendai virus. *Journal of Virology* 62: 4653-4660.

Thompson, C.D. 1995. Apoptosis in the pathogenesis and treatment of disease. *Science* 267: 1456-1462.

Tollefson, A.E., Toth, K., Doronim, K., Kuppuswamy, M., Doronina, O.A., Lichtenstein, D.L., Hermiston, T.W., Smith, C.A. and Wold, W.S. 2001. Inhibition of TRAIL-induced apoptosis and forced internalization of TRAIL receptor 1 by adenovirus proteins. *Journal of Virology* 75: 8875-8887.

Toyama, S. 1977. Altered cell fusion capacity of lines of KB cells resistant to Sendai virus induced cytolysis. *Virology* 76: 503-515.

Tozawa, H., Watanabe, M. and Ishida, N. 1973. Structural components of Sendai virus: serological and physiological characterization of hemagglutinin subunit associated with neuraminidase activity. *Virology* 55: 242-253.

Tuosto, L., Montani, M.S.G., Lorenzetti, S., Cundari, E., Moretti, S., Lombardi, G. and Piccolella, E. 1995. Differential susceptibility to monomeric HIV gp120-mediated apoptosis in antigen-activated CD4+T cell populations. *European Journal of Immunology* 25: 2907-2916.

Tyler, K.L., Squier, M.K.T., Rodgers, S.E., Schneider, B.E., Oberhaus, S.M., Grdina, T.A., Cohen, J.J. and Dermody, T.S. 1995. Differences in the capacity of reovirus strains to induce apoptosis are determined by the viral attachment protein σ1. *Journal of Virology* 69: 6972-6979.

Tzadok-David, Y., Metzkin-Eizenberg, M. and Zakay-Rowes, Z. 1995. The effect of a mesogenic and a lentogenic Newcastle disease virus strain on Burkit lymphoma Daudi cells. *Journal of Cancer Research and Clinical Oncology* 121: 169-174.

Umansky, V., Shatrov, V.A., Lehmann, V. and Schirrmacher, V. 1996. Induction of NO synthesis in macrophages by Newcastle disease virus is associated with activation of nuclear factor-kB. *International Immunology* 8: 491-498.

Van der eb, M.M. 1998. Severe hepatic dysfunction after adenovirus-mediated transfer of the herpes simplex virus thymidine kinase gene and ganciclovir administration. *Gene Therapy* 5: 451-458.

Vasconcelos, A.C. and Lam, K.M. 1994. Apoptosis induced by the infectious bursal disease virus. *Journal of General Virology* 75: 1803-1806.

Vasconcelos, A.C and Lam, K.M. 1995. Apoptosis in chicken embryos induced by the infectious bursal disease virus. *Journal of Comparative Pathology* 112: 327-338.

Vaux, D.L. 1993. Toward an understanding of the molecular mechanisms of physiological cell death. *Proceedings of the National Academy of Science USA* 90: 786-789.

Vaux, D.L. and Strasser, A. 1996. The molecular biology of apoptosis. *Proceedings of the National Academy of Science USA* 93: 2239-2244.

Verhagen, A.M., Ekert, P.G., Pakush, M., Sike, J., Connolly, L.M., Reid, G.E., Moritz, R.L., Simpson, R.J. and Vaux, D.L. 2000. Identification of DIABLO, a mammalian protein that promotes apoptosis by binding to and antagonising IAP proteins. *Cell* 102: 43-53.

Vidalain, P.O., Azocar, O., Lamonille, B., Astier, A., Rabourdin-Combe, C. and Servet-Delpart, C. 2000. Measles virus induces functional TRAIL production by human dendritic cells. *Journal of Virology* 74: 556-559.

Walczack, H., Miller, R.E., Arial, K., Gliniak, B., Griffith, T.S., Kubin, M., Chin, W., Jones, J., Woodward, A. and Le, T. 1999. Tumoricidal activity of tumor necrosis factor-related apoptosis-inducing ligand in vivo. *Nature Medicine* 5: 157-159.

Walkers, P.R., Leblanc, J., Smith, B., Pandey, S. and Sikorska, M. 1999. Detection of DNA fragmentation and endonucleases in apoptosis. *Methods* 17: 329-338.

Wallach, D., Varfolomer, E.E., Malinin, N.L., Golstev, Y.V., Kovalenko, A.V. and Boldin, M.P. 1999. Tumor necrosis factor receptor and Fas signalling mechanisms. *Annuar Review of Immunology* 17: 331-367.

Washburn, B. and Schirrmacher, V. 2002. Human tumor cell infection by Newcastle disease virus leads to upregulation of HLA and cell adhesion molecules and to induction of interferons, chemokines and finally apoptosis. *International Journal of Oncology* 21: 85-93.

Washburn, B., Weigand, M.A., Grosse-Wilde, A., Stahl, H., Rieser, E., Sprick, M.R., Schirrmacher, V. and Walczak, H. 2003. TNF-related apoptosis-inducing ligand mediates tumoricidal activity of human monocytes stimulated by Newcastle disease virus. *Journal of Immunology* 170: 1814-1821.

Wathelet, M.G., Lin, C.H., Parekh, B.S., Ronco, L.V., Howley, P.M. and Maniatis, T. 1998. Virus infection induces the assembly of co-ordinately activated transcription factors on the IFN-β enhancer in vivo. *Molecular Cell* 1: 507-518.

Webb, H.E. and Smith, C.E. 1970. Viruses in the treatment of cancer. *Lancet* 1: 1206-1208.

Weldon, C.B., Parker, A.P., Patten, D., Elliott,S., Tang, Y., Figo, D.E., Dugan, C.M., Coakley, E.L., Buttler, N.N., Clayton, J.L., Alam, J., Curiel, T.J., Beckman, B.S., Jaffe, B.M. and Burrow, M.E. 2004. Sensitization of apoptocally resistant breast carcinoma cells to TNF and TRAIL by inhibition of p38 mitogen-activated protein kinase signalling. *International Journal of Oncology* 24: 1473-1480.

Westendorp, M.O., Frank, R., Ochsenbauer, C., Stricker, K., Dhein, J., Walczak, H., Debatin, K.M. and Krammer, P.H. 1995. Sensitization of T cells to CD95-mediated apoptosis by HIV-1 Tat and gp120. Nature 375: 497-500.

White, E. 1994. Tumor biology, p53, guardian of Rb. *Nature* 371: 21-22.

White, E. 1996. Life, death, and the pursuit of apoptosis. *Genes and Development* 10: 1-15.

Wilcox, M.E., Yang, W., Senger, D., Rewcastle, N.B., Morris, D.G., Brasher, P.M., Shi, Z.Q., Johriston, R.N., Nishikawa, S., Lee, P.W. and Forsyth, P.A. 2001. Reovirus as an oncolytic agent against experimental human malignant gliomas. *Journal of National Cancer Institute* 93: 903-912.

Wilde, A. and Morrison, T.G. 1984. Structural and functional characterization of Newcastle disease virus polycistronic RNA species. *Journal of Virology* 51: 71-76.

Wilkinson, M. 1988. A rapid and convenient method for isolation of nuclear, cytoplasmic and total cellular RNA. *Nucleic Acids Research* 16: 10934.

Willingham, M.C. 1999. Cytochemical methods for detection of apoptosis. *Journal of Histochemistry and Cytochemistry* 47: 1101-1110.

Wilson, C., Gilmore, R. and Morrison, T.G. 1987. Translation and membrane insertion of the hemagglutinin-neuraminidase glycoprotein of Newcastle disease virus. *Molecular Cell Biology* 7:1386-1392.

Wyllie, A.H., Kerr, J.F.R. and Currie, A.R. 1980. Cell death: the significance of apoptosis. *International Review of Cytology* 68: 251-306.

Wyllie, A.H. 1987. Apoptosis: cell death in tissue regulation. *Journal of Pathology* 153: 313-316.

Wyllie, A.H. 1992. Apoptosis and the regulation of cell numbers in normal and neoplastic tissues: an overview. *Cancer Metastasis Research* 11: 95-103.

Yamada, T., Yamaoka, S., Goto, T., Tsujimoto, Y. and Hatanaka, M. 1994. The human T- cell leukaemia virus type 1 Tax protein induces apoptosis which is blocked by the Bcl-2 protein. *Journal of Virology* 68: 3374-3379.

Ying, H., Zaks, T.Z., Wang, R.F., Irvine, K.R., Kammula, U.S., Marincola, F.M., Leither, W.W. and Restifo, N.P. 1999. Cancer therapy using a self-replicating RNA vaccine. *Nature Medicine* 5: 823-827.

Zeng, J., Fournier, P. and Schirrmacher, V. 2002. Induction of interferon-9 and tumor necrosis factor-related apoptosis-inducing ligand in human blood mononuclear cells by hemagglutinin-neuraminidase but not F protein of Newcastle disease virus. *Virology* 297: 19-30.

Zorn, U., Dallmann, I., Grosse, J., Kirchner, H., Poliwoda, H. and Atzpodien, J. 1994. Induction of cytokines and cytotoxicity against tumor cells by Newcastle disease virus. *Cancer Biotherapy* 9: 225-234.