SID

فیلم های آموزشی

سامانه ویراستاری STES

بلاگ مرکز اطلاعات علمی

کارگاه های آموزشی

سرویس ترجمه تخصصی

سرویس های ویژه

کارگاه های آموزشی مرکز اطلاعات علمی جهاد دانشگاهی

کارگاه آنلاین آشنایی با پایگاه های اطلاعات علمی بین المللی و ترفند های جستجو

کارگاه آنلاین پروپوزال نویسی و پایان نامه نویسی

کارگاه آنلاین بررسی مقابله ای متون (مقدماتی)

Original Article

Oncolytic Effect of Newcastle Disease Virus AF2240 Strain on the MCF-7 Breast Cancer Cell Line

Fauziah Othman, Ph.D.[1]*, Aini Ideris, Ph.D.[2], Gholamreza Motalleb, Ph.D.[3], Zulkapli Bt. Eshak, M.Sc.[1], Asmah Rahmat, Ph.D.[4]

1. Human Anatomy Department, Faculty of Medicine and Health Sciences, University Putra Malaysia, Serdang, Selangor, Malaysia
2. Veterinary Clinical Studies Department, Faculty of Veterinary Medicine, University Putra Malaysia, Serdang, Selangor, Malaysia
3. Biology Department, Faculty of Science, University of Zabol, Zabol, Iran
4. Nutrition and Dietetic Department, Faculty of Medicine and Health Sciences, University Putra Malaysia, Serdang, Selangor, Malaysia

** Corresponding Address: Faculty of Medicine and Health Sciences, University Putra Malaysia, 43400, Serdang, Selangor, Malaysia*
Email: fauziah@medic.upm.edu.my

Received: 3/Jan/2009, Accepted: 28/Apr/2009

Abstract

Objective: This study was carried out to investigate the oncolytic effect of the Newcastle disease virus (NDV) strain AF2240 on the MCF-7 breast cancer cell line.
Materials and Methods: The NDV-AF2240 was propagated in 11 days old embryonated chicken eggs for 72 hours. The virus in the allantoic fluid was harvested and purified. The haemagglutination (HA) test was conducted on the purified virus to determine the virus titre which was 16384 haemagglutination units (HAUs). The microculture tetrazolium assay (MTA) was carried out via two methods-the monolayer and co-culture techniques- to determine the inhibitory concentration (IC_{50}) of NDV-AF2240 against the MCF-7 breast cancer cell line. Confocal laser scanning microscopy was carried out on polyclonal chicken antibody and fluorescein isothiocynate (FITC) conjugated goat anti-chicken antibody to observe virus localization in the cells. The terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) assay was conducted to quantify the percentage of apoptotic cells.
Results: IC_{50} value of NDV-AF2240 was two HAUs in both the monolayer and co-cultures. Virus particles were detected in the cytoplasm of MCF-7 breast cancer cell line after 24 and 48 hours post treatment. Virus budding was detected 72 hours post treatment. The number of apoptotic cells was significantly increased ($p<0.05$) 72 hours post NDV-AF2240 treatment.
Conclusion: The findings of this study show that NDV-AF2240 has an oncolytic effect against the MCF-7 breast cancer cell line. Further studies are needed to understand the anti cancer mechanism of this virus.

Keywords: Breast Cancer, Newcastle Disease Virus, Apoptosis, Confocal Laser Scanning Microscopy, In situ Nick End Labeling

Yakhteh Medical Journal, Vol 12, No 1, Spring 2010, Pages: 17-24

Archive of SID

Introduction

Despite recent advances in treatment, in 2005, an estimated 210,000 new cases and more than 40,000 deaths in the United States were attributed to breast cancer, making it the most common cancer and the third leading cause of cancer death among women in that country (1). In Malaysia, breast cancer is the commonest cancer regardless of ethnicity and age. This disease accounts for 30.4% of newly diagnosed cancer cases in Malaysian women (2). Existing breast cancer treatments such as chemotherapy and radiotherapy are not always effective and can cause significant side effects. New therapeutic modalities are needed to improve treatment of this disease (3). In recent years, there has been active interest in the potential use of replication-competent oncolytic viruses as therapeutic agents in the treatment of cancer (4). Newcastle disease virus (NDV) or paramyxovirus type 1 causes severe Newcastle disease, and in various avian species, it is characterized by inflammation of the respiratory and gastrointestinal tracts and the brain. Generally, this virus in humans is harmless, and only causes mild flu, conjunctivitis or laryngitis. NDV has been the subject of innumerable studies conducted on several different human tumor cell lines and tumor models world-

www.SID.ir

wide. The NDV strains widely used in these studies were the 73-T, MTH-68, Italian, Ulster and PV701 (5-12). NDV possesses several unique properties which make it an excellent anticancer agent; it has good cell binding properties, it binds specifically to tumor cells, it replicates selectively in tumor cell cytoplasm, it is relatively safe and it can act as an adjuvant (13). In this study, a Malaysian local strain of NDV (AF2240) was tested as an oncolytic agent on the MCF-7 breast cancer cell line. Although the concept of using viruses as anti-cancer agents is still new in Malaysia, recent advances in molecular biology and virology enable researchers to manipulate and enhance the possibility and the ability of NDV, or potentially another virus, as a future oncolytic agent in battling cancer in Malaysia.

Materials and Methods

Propagation of the NDV virus was carried out according to Blaskovic and Styk (14). Embryonated chicken eggs aged between ten and eleven days were obtained from the Linggi Poultry Farm, Negeri Sembilan, Malaysia. The eggs were sprayed with 70% ethanol to avoid contamination. They were candled and non-viable eggs were removed. Using a candling lamp, the margin of each egg's air sac was marked, and the eggs were incubated in humidified air at 37°C. All processes from candling of the eggs upon arrival to the harvesting of the virus were conducted in accordance with the Malaysian Biological Safety Cabinet's Guideline.

Preparation of seed virus dilution

Before propagation, an NDV-AF2240 virus seed was prepared. 9 ml of phosphate buffer saline (PBS), suck and filtered using 0.2μm filter (Millipore, USA) into 50 ml tubes and the fourth 50 ml tube, 27 ml of PBS was added since the total of 30 ml seed virus is was needed for inoculation of 100 eggs. Three 50 ml test tubes were filled with 9 ml phosphate buffer saline (PBS) which was poured-in through a 0.2 mm filter (Millipore, USA). A fourth test tube was filled in the same manner, but with 27 ml PBS; this tube was to hold the 30 ml final suspention needed for inoculation of 100 eggs. Using a 0.45 μm filter, approximately 1 ml of the virus was filtered, added to the first 50 ml test tube containing 9 ml PBS, and resuspended a few times. 1 ml of the resulting dilution was then added to the second test tube and again resuspended a few times before 1 ml of it was transferred and resuspended into the third test tube. A3 ml sample from the third test tube's dilution was then added to the fourth test tube containing 27 ml PBS in order to make a final dilution with 10^{-4} of virus to be used for the inoculation of the eggs.

Chicken egg inoculation

A hole approximately 1 mm in diameter was punched above the air sac margin of the eggs, and the virus seed suspension (0.1 ml) was injected into each one. The eggs were then incubated. After 24 hours, the eggs were candled to check for dead embryos. As embryo death may be caused by contamination, eggs containing dead embryos were removed and refrigerated at 4°C. They were candled and monitored daily for 120 hours or until 90% of the embryos were dead. The eggs were then refrigerated overnight to ensure blood vessel constriction before being harvested.

Harvesting

The egg membranes were punctured and their allantoic fluids separately collected and stored in sterile test tubes. All visibly contaminated eggs were rejected. Rapid-test using chicken red blood cells was conducted to confirm the presence of NDV in the allantoic fluids.

Virus clarification and purification

The allantoic fluids were clarified at 6000 g for 10 minutes at 4°C using a refrigerated centrifuge (Optima XL-100 K, Beckman Coulter). The resulting supernatants were centrifuged at 20000 rpm for 3 hours at 4°C using a T21 (Beckman, USA) rotor. They were then discarded and the pellet contents were resuspended and dissolved in 1 ml NTE buffer (NaCl, Tris-HCl, EDTA) using a 1 ml syringe. Sucrose gradients (filtered) at concentrations of 30%, 40%, 50% and 60% were prepared in Ultra-Clear tubes and kept in the refrigerator overnight. A few drops of the virus in NTE buffer were added into the sucrose gradient until all the Ultra-Clear tubes were balanced. The tubes were then centrifuged at 38000 rpm for 4 hours using a Beckman Coulter SW41 rotor. After centrifugation, a band of the purified virus was observed and marked under an inverted microscope. The band was collected using a 1 ml syringe and was transferred into polyallomer tubes. The polyallomer tubes were then topped-off with NTE buffer and centrifuged at 20000 rpm for 2 hours at 4°C. The pellet was dissolved in NTE buffer, filtered thru a 0.4 μm filter and kept at -80°C for further use.

Preparation of chicken red blood cell (RBC) for virus titration

Before blood was withdrawn from the chicken

Archive of SID

www.SID.ir

jugular vein, a 5 ml syringe was filled with a 1:1 mixure of PBS and EDTA to prevent the aspirated blood from clotting. The blood was then transferred using the syringe into a 15 ml test tube and centrifuged at 1000 rpm for 10 minutes at room temperature. The supernatant was discarded and the cells were resuspended in PBS and centrifuged again 3 times. For titration of the virus, 500 µl of RBC was diluted in 100 ml PBS to make a 0.5% suspension of RBC cell and PBS.

Haemagglutination(HA) test
The 2nd thru 24th wells of a 96-well plate were filled with 50 µl PBS. 100 µl of the purified virus was transferred into the first well. About 50 µl of the purified virus was transferred from the 1st well into the 2nd well and was resuspended a few times to make a two-fold dilution of the virus; this process was continued consecutively with a 50 µl dilution from each well being transferred to the next until the 23rd well contained a 50 µl dilution from the 22nd well. 50 µl of the 0.5% chicken RBC suspension was added into all the wells which were then left at room temperature for 30 minutes. The first well containing the purified virus and chicken RBCs acted as a positive control, whereas the 24th well containing PBS and chicken RBCs served as a negative control. red button appeared in all wells except the 24th well which represented the virus HA titer.

Cell-culture maintenance
The MCF-7 breast cancer cell line (ATCC # HTB-22) and the 3T3 mouse fibroblast (ATCC # CRL-6361) were used in this study. The MCF-7 and 3T3 cell lines were maintained in RPMI-1640 cell culture media (cat. no. R1383, Sigma) supplemented with 10% fetal calf serum (FCS) (cat. no. N4637, Sigma) and 1% penicillin/streptomycin (cat. no. P0781, Sigma) in a humidified incubator supplied with 5% CO_2 at 37°C. The incubator was humidified with copper sulfate ($CuSO_4$) diluted in distilled water. The media was changed every 2 days as it turned from red to light yellow.

Cell sub-culturing method
Depending on cell growth rate, the cells (MCF-7 and 3T3 cells) were sub-cultured every 3 days, or when the cultures reached 70% confluency. Old media was poured out of each flask and the cells were washed twice with PBS or with cell culture media without supplements. After washing, 1 ml of trypsin was added into the flask and tilted a few times to ensure equal distribution of the trypsin. The flask was then incubated for 3 to 5 minutes, tapped sharply and observed under an inverted microscope for the presence of cell detachment. Approximately 20 ml of supplemented cell culture media was added into the flask. The flask was shaken a few times to disperse the cells and 10 ml of the suspension was transferred to another tissue culture flask.

Microculture tetrazolium assay (MTA)
MTA was conducted in order to evaluate viability of the cells against the NDV-AF2240 virus. Monolayer and co-culture methods were used in virus inoculation for the MTA.

Monolayer method
The MCF-7 and 3T3 cells were grown in 25 cm^2 flasks (Nunc, Denmark) until they reached 100% confluency; they were then trypsinized and counted using a haemocytometer. The specific cell culture medium for each cell line was added to the flask to get the reach a concentration of 1×10^5 cells/ml. 100 µl of each cell suspension was pipeted into a 96-well flat bottom plate (Nunc, Denmark). The cells were then incubated for 24 hours in a humidified incubator at 37°C supplied with 5% CO_2 for attachment of the cells. The old media in each well was removed and replaced with 50 µl of new cell culture medium without supplement. 50 µl purified NDV-AF2240 virus was put into the first column consisting of 8 wells. The virus was then resuspended and transferred to the second column to make a 2-fold dilution of the virus and this process continued from the highest titre available to the lowest titre. For control, instead of virus inoculation, the wells were incubated 45 minutes in 50 µl PBS in a humidified incubator. Each well was filled with 150 µl of completely supplemented cell culture medium and incubated for 72 hours in a humidified incubator at 37°C in an atmosphere of 5% CO_2.

Co-culture method
Approximately 150 µl of MCF-7 and 3T3 cellsuspension was dropped into each well on 96-well plate and incubated 4 hours to allow cell attachment. The medium was removed and replaced with 50 µl PBS. Then, 50 µl of the purified (specify the virus) virus was added into the first column. The dilution was resuspended and transferred to the next column to make a 2-fold dilution of the virus (from the highest to the lowest titre). The plates were then incubated 45 minutes

www.SID.ir
Archive of SID

in a humidified incubator at 37°C. The wells were filled again with 150 µl of completly supplemented cell culture medium (5% FCS, 1% penicillin/streptomycin). The cells were then incubated for 72 hours.

Microculture tetrazolium assay (MTA) (Phytotech Labs, USA)

MTA was conducted using an ELISA microplate reader (Anthos LX-800, Denmark) (model number seems incorrect). The absorbance was read at 550 and 590nm wavelengths as reference. To determine the best condition for NDV to induce 50% killing of the cells, two methods were used,; the monolayer and co-culture methods. After 72 hours of incubation, 20 µl (5 mg/ml PBS) of MTT solution were added into each well and incubated for 4 hours in a humidified incubator. The media from each well was removed and 100 µl of 100% DMSO was added to each well. The well contents were then mixed thoroughly and left at room temperature for 30 minutes until the dark blue crystal dissolved.

Percentage of cell viability was determined as follows:

$$\% \text{ viability} = \frac{\text{OD sample}}{\text{OD control}} \times 100\%$$

Percent cell viability versus virus titer (HA unit) graphs were obtained and the IC_{50} values were determined.

Immunolabelling of the apoptotic cells

The cover slips were pre-treated with 100% ethanol for 45 minutes. They were placed on a sterile Petri dish. Approximately 1×10^6 MCF-7 cells were seeded on the cover slips. They were maintained in a humidified 37°C incubator supplied with 5% CO_2 until 50% confluency was reached before being treated with NDV-AF2240. The cells were incubated in the serum-free media virus dilution for 45 minutes. Complete media was added after the treatment. All the cell lines were grown in RPMI-1640 media supplemented with 5% FCS and 1% penicillin/streptomycin. They were then treated with 2 HA units of NDV-AF2240. The cells were observed for 24, 48 and 72 hours post treatment.

Immunolabeling of the virus

The MCF-7 cells were washed with 80 µl of PBS 3 times within 15 minutes before being fixed with 50 µl of 4% paraformaldehyde (158127, Sigma) in PBS for 15 minutes. The cover slips were washed 3 times within 15 minutes with PBS. To block unspecific antibodies, the cells were incubated in 50 µl of 0.1% Triton X-100 (Sigma), 10% BSA (cat. no. A1933, Sigma) in PBS for 15 minutes followed by being washed with PBS three times within 15 minutes. They were permeabilized with 0.5% Triton X-100 in PBS for 10 minutes and were washed again with PBS several times. They were incubated with 50 µl of primary antibody (37°C, 45 minutes) in a humidified incubator. Polyclonal anti-chicken antibody (Biology Labs, UPM) was diluted 1:100; the secondary antibody, FITC conjugated goat anti-chicken (cat. no.C7182, Sigma) was diluted 1:100. Again, the cells were washed with PBS three times within 15 minutes before being incubated in 50 µl of secondary antibodies for 45 minutes under the same conditions as the primary antibodies. They were then washed three times with PBS before being drained and mounted on cover slips with anti-fading agent. The cover slips were sealed using either nail polish or Depex mounting medium and stored at 0°C prior to viewing. Viewing was done using confocal laser scanning microscopy (Biorad, MRC-1024).

TUNEL assay

Detection of apoptotic cells was done using Apoptosis Detection System, Fluorescein (Promega, USA). The MCF-7 cells were grown on chambered slides (Lab-Tek, USA), and treated with 4 HA units of NDV strain AF2240. After 24, 48 and 72 hours post inoculation, the slides were washed with PBS and processed for detection of apoptotic cells. They were fixed by immersion in freshly prepared 4% paraformaldehyde and PBS solution (pH 7.4) in a Coplin jar for 25 minutes at 4°C. The cells were then washed twice with fresh PBS for 5 minutes before being immersed in a 0.2% Triton X-100 solution in PBS for 5 minutes. Then, rinsing of the cells with PBS was conducted twice for 5 minutes at room temperature. The excess liquid was removed by tapping the slides and the MCF-7 cells were covered with 100 µl of equilibration buffer and equilibrated at room temperature for 5-10 minutes. While the cells were equilibrating, the nucleotide mixture was thawed on ice and sufficient TdT incubation buffer for all experimental reactions was prepared. Next, the equilibrated areas were blotted with tissue paper and 50 µl of TdT incubation buffer was added to the cells on a 5 cm2 area. The saline sodium citrate (SSC) ×20 was diluted 1:10 with deionized water and was complemented with 2×SSC to fill a standard Coplin jar (40 ml). The reactions were terminated by immersing the slides in 2×SSC in a Coplin jar for 15 minutes at room temperature. The slides were washed 3 times in fresh PBS for 5 minutes at room temperature to remove unincorporated fluorescein-12-dUTP. Finally, they were stained in a Coplin jar by immersing the slides in 40 ml of

Archive of SID

www.SID.ir

propidium iodide (PI) solution freshly diluted to 1 µg/ml in PBS for 15 minutes at room temperature in the dark. The slides were then washed in deionized water 3 times for 5 minutes at room temperature. One drop of anti-fade agent (Fluoroguard, Bio-Rad) was dropped to the area containing the cells and the slides were then mounted using glass cover slips. The cell samples were immediately viewed under a confocal microscope (Bio-Rad, MRC-1024).

Statistical analysis

The numbers of apoptotic cells were compared using the independent t-test (SPSS version 15). $P<0.05$ was considered statistically significant.

Results

MTA assay

The highest titer obtained for NDV-AF2240 was 214 or 16384 HA units. For monolayer and co-culture methods, the virus titer needed to induce 50% killing of MCF-7 cells was 2 HA units (Fig 1).

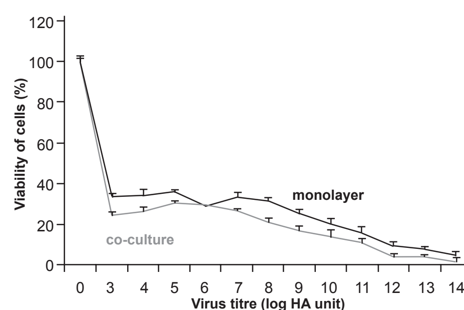


Fig 1: Inhibition concentration percentage of NDV-AF2240 inoculated MCF-7 cells in monolayer and co-culture methods 72 hours post-inoculation.

Tunnel assay

The untreated MCF-7 cells were labeled red (Fig 2A) indicating viable cells. There were no cells with fragmented nuclei among the uninfected cells. The treated MCF-7 cells were labeled orange to red indicating viable cells, and yellow to green indicating apoptotic cells (Fig 2B-D).

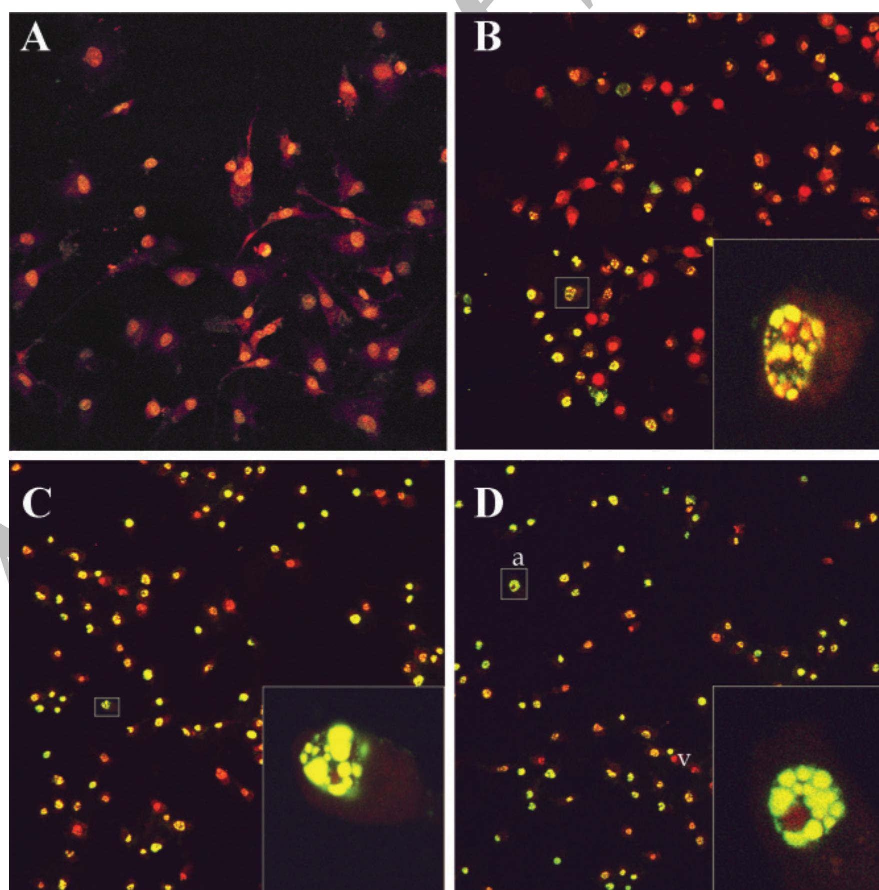


Fig 2: Confocal micrographs of untreated and NDV-AF2240 treated MCF-7 cells stained using the TUNEL technique: (A) untreated (B, C and D) treated for 24, 48 and 72 hours respectively. Cells were double stained with fluorescein-12-dUTP and propidium iodide. Viable (V) cells showed orange to red nuclei, whereas apoptotic (a) cells showed yellow to green nuclei. Note the fragmented nuclei (B, C and D). Magnification: A-D: ×40, inserted pictures (B-D): ×120.

www.SID.ir

The fragmented MCF-7 cell nuclei were clearly seen after 24 hours of treatment (Fig 2B). Prolonged exposure of both infected and uninfected MCF-7 cells to the NDV-AF2240 virus increased the number of cells with fragmented nucle (Fig 2C-D).

Apoptotic cell quantification

The number of apoptotic cells among the MCF-7 cells treated with NDV-AF2240 for 24, 48 and 72 hours significantly increased ($p<0.05$). 24 hours post-treatment, 24% of the MCF-7 cells became apoptotic, and the number of apoptotic cells increased 3-folds 72 hours post treatment.

Table 1: Percentage of MCF-7 apoptotic cells treated with AF2240 strain of NDV.

NDV strain /	AF2240		
Cell	24 hours	48 hours	72 hours
MCF-7	145/600 (24%± 4.08)	302/600 (50%)	486/600 (81.00 ± 3.89)

Data are expressed as Mean ± Standard deviation. The experiment was replicated six times to achieve average results.

The treated MCF-7 cells showed NDV-AF2240 particles in their cytoplasm within 24, 48 and 72 hours post treatment (Fig 3). Virus budding was clearly noted after 72 hours post treatment (Fig 3D).

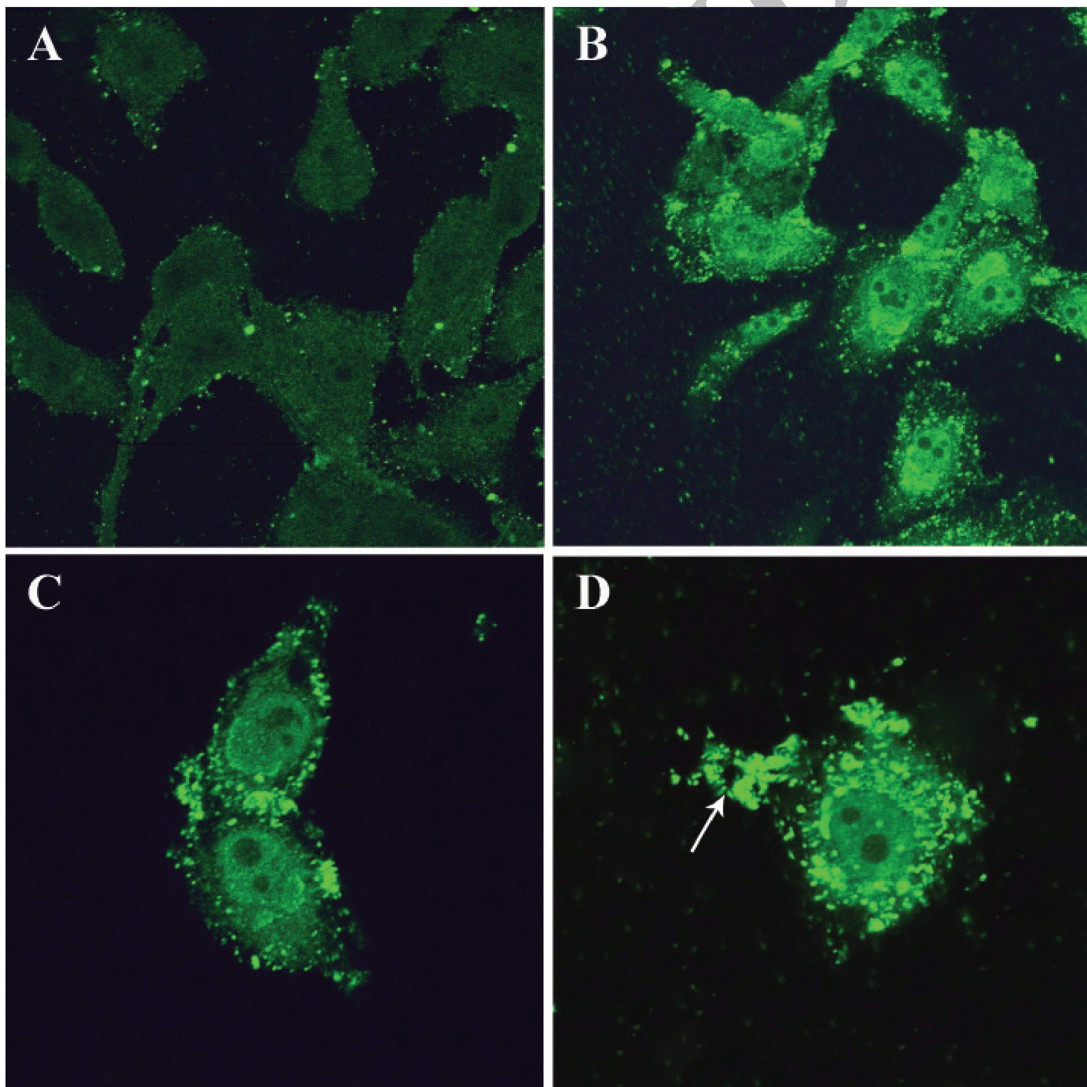


Fig 3: Confocal laser scanning micrographs of untreated and NDV-AF2240 treated MCF-7 cells labeled with polyclonal antibody and anti-chicken FITC: untreated (A) and treated for 24, 48 and 72 hours (B, C and D respectively). Note the fluorescent staining of the cytoplasm at 24 and 48 hours post treatment (B, C) and budding of the virus (arrow) at 72 hours post-treatment (D). Magnifications: A & B ×60, C & D ×120.

www.SID.ir

Discussion

The double immunolabeling method was applied for detection of the NDV-AF2240 virus. Labeling of the virus was carried out by using a polyclonal antibody and FITC conjugated goat anti-chicken antibody which emits a bright green fluorescent color. The NDV particles were observed in the cytoplasm of the treated MCF-7 cells 24, 48 and 72 hours post treatment. This finding corroborates the fact that replication of a *paramyxovirus* or any RNA virus normally takes place in the cytoplasm (14, 15). Matured progeny of NDV were released from the host cells into the extracellular matrix via a process called ‘budding’ (16-18). The budding virus particles of the MCF-7 cells were observed 72 hours post treatment. This study proves the vital role of NDV and its progeny in their oncolytic effect on malignant cells. AF2240 is not the only virulent strain of NDV showing an antineoplastic property. Other virulent strains of NDV such as Cassel’s 73-T, MTH-68, Italian and Hickman have been proven to be potent against various types of cancer cells (19, 5, 20, 8). A major pathway of NDV induced cell death is apoptosis. Apoptosis plays a central part in normal tissue homeostasis and has a role in a variety of clinical diseases characterized by either increased or decreased cell survival (21). Apoptosis is often characterized by cellular shrinking, chromatin condensation and margination, and ruffling of the plasma membrane with eventual breaking up breakage of the cell into apoptotic bodies (22, 23). Morphological and chemical changes of apoptotic cells can be differentiated from any another cell death. Cells having undergone apoptosis can be detected and differentiated by using the TUNEL and COMET assays and can later be visualized either using confocal laser scanning or fluorescence microscopy (24). In this study, confirmation of MCF-7 cell apoptosis initiated by NDV-AF2240 was done using the TUNEL assay. The assay is non-radioactive and provides a simple, accurate and rapid detection of apoptotic cells. With the help of the TdT enzyme, assessment of damaged DNA of apoptotic cells was done by catalytically incorporating flourescein-12-dUTP at the 3’-OH DNA ends (25). The cells were then counter-stained with propidium iodide (PI) which stains the nucleus of both apoptotic and non apoptotic cells with a fluorescent bright red color (26). Cells with fragmented fluorescein-12-dUTP incorporated DNA exhibit yellow to green fluorescence whereas viable cell DNAs not incorporateed with fluorescein-12-dUTP only emit a red fluorescent color visualized by using a confocal microscope (27). The TUNEL assay confirmed that NDV-AF2240 in cell cultures induces cell death via the apoptotic pathway. The number of apoptotic cells revealed that in treated MCF-7 cells the percentage of apoptotic cells proportionally increased with post-inoculation time. These findings confirm that long-term exposure and continuous usage of NDV bincreases virus efficacy (27, 28) and coincide with the works carried out by Narayani (27). The number of apoptotic MCF-7 cells towards compared to MDA-MB-231 cells was significantly fewer. These results prove that NDV-AF2240 selectively induces cytotoxic activity against estrogen dependent cells. Further studies are required to understand the mechanism underlying the action of the NDV-AF2240 virus toward different types of cells. Confirmation of apoptosis using the TUNEL assay has been used in various studies and has confirmed that NDV induces death via apoptotic pathway in both tissue and cell cultures (27, 29). Kommers et al. (29) used six types of isolated pigeon-origin NDV on chickens and used the TUNEL assay to detect apoptotic cells five days post infection. An in vitro study by Narayani et al. (27) revealed that NDV-AF2240 was more responsive toward MDA-MB-231 cells compared to MCF-7 cells, and that the apoptotic cells were prominently detected at 3 days post infection. The results of this study were in agreement with previous studies indicating that the TUNEL assay is the best IHC method in detecting apoptotic cells (30).

Conclusion

Our results indicate that NDV-AF2240 inhibits MCF-7 cancer cell proliferation and eventually leads to apoptosis. This study also provides a basis for exploration of NDV-AF2240 as an anti-cancer agent.

Acknowledgments

The authors would like to extend their thanks to Putra University, Malaysia and the Malaysian National Cancer Council for the use of their laboratory facilities. There is no conflict of interest in this article.

References

1. Jemal A, Murray T, Ward E, Samuels A, Tiwari RC, Ghafoor A, et al. Cancer Statistics, 2005. CA Cancer J Clin. 2005; 55(1): 10-30.
2. Lim GCC, Yahaya H, Lim TO. The first report of the national cancer registry cancer incident in Malaysia 2002. National Cancer Registry. 2003; 138-176.
3. Stoff-Khalili MA, Dall P, Curiel DT. Gene therapy for carcinoma of the breast. Cancer Gene Ther. 2006; 13(7): 3: 633-647.
4. Schirrmacher V, Haas C, Bonifer R, Ahlert T, Ger-

Archive of SID
www.SID.ir

hards R, Ertel C. Human tumor cell modification by virus infection: an efficient and safe way to produce cancer vaccine with pleiotropic immune stimulatory properties when using Newcastle disease virus. Gene Ther. 1999; 6: 63-73.
5. Cassel WA, Garret RE. Newcastle disease virus as an ntineoplastic agent. Cancer. 1965; 18: 863-868.
6. Liebrich W, Schlag P, Manasterski M. In vitro and clinical characterisation of a Newcastle disease virus-modified autologous tumour cell vaccine for treatment of colorectal cancer patients. Eur J Cancer. 1991; 27(6): 703-710.
7. Csatary LK, Eckhardt S, Bukosza I, Czagledi F, Fenywesi C, Geregely P, et al. Attenuated veterinary virus vaccine for the treatment of cancer. Cancer Detect Prev. 1993; 17(6): 619-627.
8. Mallmann P. Autologous tumor-cell vaccination and ymphokine-activated tumor-infiltrating lymphocytes (LAK-TIL). Hybridoma. 1993; 12(5): 559-566.
9. Schild H, Von Hoegen P, Schirrmacher V. Modification of tumour cells by a low dose of Newcastle disease virus. Lancet 1998; 19: 23-28.
10. Bohle W, Schlag P, Liebrich W, Hohenberger P, Manasterski M, Moller P, et al. Postoperative active specific immunization in colorectal cancer patients with virus-modified autologous tumor-cell vaccine. First clinical results with tumor-cell vaccines modified with live but avirulent Newcastle disease virus. Cancer. 1990; 66(7): 1517-1523.
11. Phuangsab A, Lorence RM, Reichard KW, Peeples ME, Walter RJ. Newcastle disease virus therapy of human tumor xenografts: antitumor effects of local and systemic administration. Cancer Lett. 2001; 172; 27-36.
12. Washburn B, Schirrmacher V. 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. Int J Oncol. 2002; 21: 85-93.
13. Schirrmacher V, Ahlert T, Probstle T, Steiner HH, Herold-Mende C, Gerhand R, et al. Immunization with virus-modified tumor cells. Semin Oncol. 1998; 25(6): 677-696.
14. Blaskovic D, Styk B. Laboratory methods of virus transmission in multicellular organisms. Methods in Virology. 1967; 1: 194-197.
15. Garoof H, Hewson R, Opstelten DJE. Virus maturation by budding. Microbiol Mol Biol Rev. 1998; 62(4): 1171-1190.
16. Washburn B, Schirrmacher V. 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. Int J Oncol. 2002; 21: 85-93.
17. Fauziah O. Pathogenicity of Velogenic Viscerotropic Newcastle Disease in the bursa fabricious of chicken. Presented for the M.Sc. Putra. Universiti Putra Malaysia. 1993.
18. Harvey W. Newcastle disease virus: an evolving pathogen virus? Avian pathol. 2001; 30: 5-11.
19. Wheelock EF, Dingle JH. Observations on the repeated administration of viruses to a patient with acute leukemia. A preliminary report. N Engl J Med. 1964; 271(13): 645-651.
20. Csatary LK, Moss RW, Beuth J, Torocsik B, Szeberenyi J, Bakacs T. Beneficial treatment of patients with advanced cancer using a Newcastle disease virus vaccine (MTH-68/H). Anticancer Res. 1999; 19(1b): 635-638.
21. Fleisher TA. Apoptosis. Ann Allergy Asthma and Immunol. 1997; 78: 245-249.
22. Cruchten SV, Broeck WV. Morphological and biochemical aspects of apoptosis, oncosis and necrosis. Anat Histol Embryol. 2002; 31: 214-223.
23. Fabian Z, Torocsik B, Kiss K, Csatary LK, Bodey B, Tigyi J, et al. Induction of apoptosis by a Newcastle disease virus vaccine (MTH-68/H) in PC12 rat phaeochromocytoma cells. Anticancer Res. 2001; 21: 125-135.
24. Yasuhara S, Zhu Y, Matsui T, Tipirneni N, Yasuhara Y, Kaneki M, et al. Comparison of comet assay, electron microscopy, flow cytometry for detection of apoptosis. J Histochem Cytochem. 2003; 51: 873-885.
25. Rohwer F, Azam F. Detection of DNA damage in prokaryotes by terminal deoxyribonucleotide transferase-mediated dUTP nick end labeling. Appl Environ Micribiol. 2000; 1001-1006.
26. Timm EA, Stewart CC. Discrimination between dead and viable apoptotic cells using two-color TdT assay and surface labeling as detected by flow cytometry. Biochemica. 1996; 1: 1-4.
27. Narayani M. Oncolytic effect of Newcastle disease virus on the MCF-7 and MDA-MB-231 breast cancer cell lines. Presented for the M.Sc. Putra. University Putra Malaysia.1993.
28. Pecora AL, Rizvi N, Cohen GI, Meropol NJ, Sterman D, Marshall JL, et al. Phase I Trial of Intravenous administration of PV701, an oncolytic virus, in patients with advanced solid cancers. J Clin Oncol. 2002; 20(9): 2251-2266.
29. Kommers GD, King DJ, Seal BS, Carmichael KP, Brown CC. Pathogenesis of six pigeon-origin isolates of Newcastle disease virus for domestic chickens. Vet Pathol. 2002; 39: 353-362.
30. Molino A, Colombatti M, Bonetti F, Zardini M, Pasini F, Perini A, et al. A comparative analysis of three different techniques for the detection of breast cancer cells in bone marrow. Cancer. 1991; 67: 1033-1036.

Archive of SID

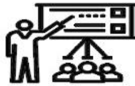
www.SID.ir

SID

فیلم های
آموزشی

سامانه ویراستاری
STES

بلاگ
مرکز اطلاعات علمی


کارگاه های
آموزشی

سرویس ترجمه
تخصصی

سرویس های
ویژه

کارگاه های آموزشی مرکز اطلاعات علمی جهاد دانشگاهی

کارگاه آنلاین آشنایی با پایگاه های اطلاعات علمی
بین المللی و
ترفند های جستجو

کارگاه آنلاین
پرپوزال نویسی و پایان نامه نویسی

کارگاه آنلاین
بررسی مقابله ای متون (مقدماتی)