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Biological Effects of Newcastle Disease Virus Vaccine on Colon Cancer Cells Line (HCT116)

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ABSTRACT

Background: *Newcastle disease virus (NDV) is a type of avian paramyxovirus that has demonstrated its ability to selectively replicate in tumor cells and also stimulate an anti-tumor immune response, making it a potential oncolytic agent. Therapeutic efficacy of NDV in colorectal cancer has yet to be analyzed.* ***Objective:*** *The aim of this study was to assess the oncolytic activity of NDV vaccine strains in colon cancer cell line HCT116 in terms of cytotoxicity, induction of apoptosis, alterations in metabolism, and viral replication.* ***Methods:*** *HCT116 cells were infected with NDV vaccine strains at different MOI (1 and 10) and time points (48 and 72 hours post infection) and analyzed. Cell viability was assessed with an MTT assay, and apoptosis was analyzed with Annexin V-FITC/PI flow cytometry. The presence of cytopathic effects was assessed microscopically. Glucose metabolism and lactate production, ATP levels, and viral replication (by means of the hemagglutination assay) were assessed.* ***The Results:*** *Results were presented as mean ± SD (n=3) and analyzed using one-way and two-way ANOVA with post hoc Tukey comparison. Results: NDV significantly decreased HCT116 cell viability in a dose and time dependent manner ($p < 0.0001$) with cell viability at 32% at MOI 10 and 72 hours post infection. There was also a significant increase in apoptosis, including early apoptosis which was 32.8%, and in the case of late apoptosis/necrosis which was 41.2% at MOI 10 ($p < 0.0001$). NDV also caused significant cytopathic effects and in metabolism of the cells as evidenced by decreased glucose consumption (49%), lactate production (47%), and ATP levels (52%) ($p < 0.001$). There was a positive correlation between the increased time of viral replication and increase in cytotoxicity ($p = 0.0001$). Conclusion: NDV vaccine strains were shown to effectively elicit oncolytic activity against HCT116 colon cancer cells via apoptosis, suppression of metabolism, and viral replication. These results advocate for Newcastle Disease Virus (NDV) as a potential novel approach for targeted therapy in colorectal cancer.*

Keywords: Newcastle Disease Virus (NDV); Oncolytic Virotherapies; Colon Carcinoma; HCT116; Apoptosis; Cytotoxicity; Metabolic Reprogramming; Viral Replication; Flow Cytometry; MTT Assay.

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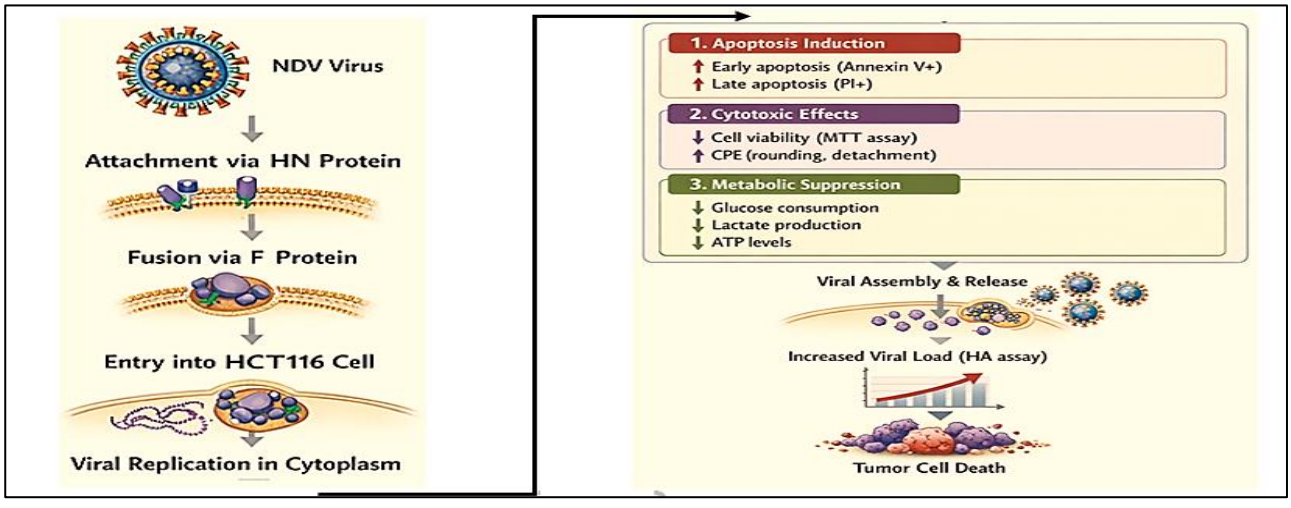
Graphical Abstract:



NDV-Induced Oncolysis in HCT116 Colon Cancer Cells.

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INTRODUCTION

Colorectal cancer (CRC) is one of the most common cancers worldwide and presents a massive burden on global health [1]. Chemotherapy, radiation, and even surgical interventions are of limited utility due the non-targeted nature of the therapies, the development of resistance, and the recurrence of the tumor [2]. These challenges require new and innovative therapy strategies that attack tumors more specifically and safely. Oncolytic virotherapy is a new method that utilizes special oncolytic viruses that will preferentially infect and destroy malignant cells. [3]. Of the viruses that have been researched, one of the most promising is Newcastle disease virus (NDV) due to its selective tumor infectivity and safety in humans [4,15,16]. NDV preferentially infects and kills cancer cells because of the defects in the cells' antiviral interferon pathways, which are important in a cell's defense to infection and are typically lost in cancer cells [5, 17]. NDV doesn't just kill the infected cancer cells, but NDV also activates apoptosis and the immune response to cancer. NDV activates both the innate and adaptive immune systems [6]. These mechanisms together are what will make NDV a promising cancer therapeutic that will directly kill tumors and cause an immune response to kill more of the tumor. Additionally, the translated cancer research platform aids development using safe and well-characterized tools. Along with the LaSota strain of NDV, Hitchner B1, and their vaccine strains, NDV strains have proven beneficial in veterinary medicine [7]. For example, human colon cancer cell line HCT116 has garnered attention and a hub of research due to its microsatellite instability and intact mutated p53, which aids its modeling of therapeutic response tumor research [8].

HCT116 and NDV strains have no literature, and the research conducted will aid in the molecular and cellular functions of NDV-mediated oncolysis. This research primarily concerns HCT116 colon cancer cells and NDV vaccine strains to asses oncolytic effect of NDV, which includes and is not limited to, cell death, metabolic changes, induction of apoptosis, viral replication, and cytotoxicity. There is a significant gap of integration between oncolytic viruses and cancer cell biology, and it is imperative to pursue this integration to further understand the therapeutic use of NDV as a selective agent for the treatment of colorectal cancer.

MATERIALS AND METHODS

Cell Line and Culture Conditions

HCT116 cells were sourced from a recognized cell bank, and cells were kept and handled according to established cell culture protocols. Dulbecco's Modified Eagle's Medium (DMEM) was used to culture cells, and it was enriched with 10% heat-inactivated fetal bovine serum, and 100 U/mL of penicillin and 100 µg/mL of streptomycin were added. Cell culture was done in a controlled temperature and humidified chamber with 5% CO_2 set at 37°C and cells were subcultured at 80-90% confluence.

Newcastle Disease Virus (NDV) Vaccine Strains

Live attenuated vaccine strains of Newcastle disease virus (LaSota) were used in this study. Following established procedures, viral stocks were acquired from a certified veterinary supplier, and expanded in specific pathogen-free (SPF) embryonated chicken eggs (9-11 days). Briefly, 0.1 mL of virus suspension was inoculated into the allantoic cavity under sterile conditions and incubated at 37°C for 48–72 hours. Allantoic fluid containing the virus was harvested, clarified by centrifugation at 3000 rpm for 10 minutes, aliquoted, and stored at −80°C until use. Virus titers were determined using the hemagglutination (HA) assay and expressed as hemagglutination units (HAU/mL), as

previously described in NDV propagation studies.

Experimental Design and Viral Infection

HCT116 cells were seeded into 96-well and 6-well plates and allowed to reach approximately 70–80% confluence prior to treatment. Cells were divided into experimental groups as follows: Control group (untreated cells) and NDV-treated groups at different multiplicities of infection (MOI: 1, and 10). Virus adsorption was carried out by incubating cells with NDV for 1 hour at 37°C with gentle rocking. After adsorption, the inoculum was removed and replaced with fresh complete medium. Cells were further incubated for 48, and 72 hours post-infection.

Cell Viability Assay

Cell viability was evaluated using the MTT assay. Briefly, after the indicated treatment periods, 20 µL of MTT solution (5 mg/mL) was added to each well and incubated for 3–4 hours at 37°C. The resulting formazan crystals were dissolved in dimethyl sulfoxide (DMSO), and absorbance was measured at 570 nm using a microplate reader. Cell viability was expressed as a percentage relative to untreated control cells.

Morphological Assessment of Cytopathic Effects (CPE)

Assessment of Apoptosis

Apoptotic cell death was analyzed using Annexin V-FITC/Propidium Iodide (PI) dual staining followed by flow cytometry. Briefly, treated and control cells were harvested, washed with cold phosphate-buffered saline (PBS), and stained according to the manufacturer's protocol. Early and late apoptotic cell populations were quantified.

Measurement of Cellular Metabolic Activity

Glucose consumption and lactate production were quantified with assay kits for the evaluation of metabolic changes due to NDV infection. Culture supernatants were collected at specified time points, and assays were performed according to the manufacturer's instructions. Intracellular ATP levels were also determined as an indicator of cellular energy status.

Viral Replication Analysis

Viral replication in infected HCT116 cells was confirmed by hemagglutination assay of collected supernatants. Additionally, viral cytotoxicity was correlated with viral dose and incubation time.

Statistical Analysis

Mean values with standard deviations (SD) of the data sets were calculated, and all the analyses were performed using GraphPad Prism. For multiple group comparisons, One-way ANOVA was performed, followed by Tukey's post hoc test for individual pairwise comparisons. A significance of p value of * < 0.05 was determined as significant, ** < 0.01 was considered highly significant, and *** < 0.001 was considered as extremely significant.

Biosafety and Ethical Considerations

All experimental procedures involving live viruses and embryonated eggs were conducted in accordance with institutional biosafety guidelines and approved laboratory protocols.

RESULTS

NDV Vaccine Strains Reduce Viability of HCT116 Cells

Treatment of HCT116 cells line with Newcastle disease virus (NDV) vaccine strains (LaSota) resulted in a significant, dose- and time-dependent reduction in cell viability compared to untreated controls. At 48, and 72 hours post-infection, NDV induced progressive cytotoxicity. The highest multiplicity of infection (MOI = 10) showed the greatest reduction in viability, reaching approximately 30–40% survival at 72 hours. Statistical analysis using one-way ANOVA demonstrated a highly significant difference ($p < 0.001$) between treated and control groups Figure 1. Post hoc Tukey's analysis showed**:**

MOI 1 vs Control (48 h): p = 0.012, MOI 10 vs Control (48 h): p < 0.0001, MOI 1 vs Control (72 h): p = 0.003, MOI 10 vs Control (72 h): p (MOI 10) at 48 h, and further to 55 ± 4% (MOI 1) and 32 ± 3% (MOI 10) at 72 h Figure 1.

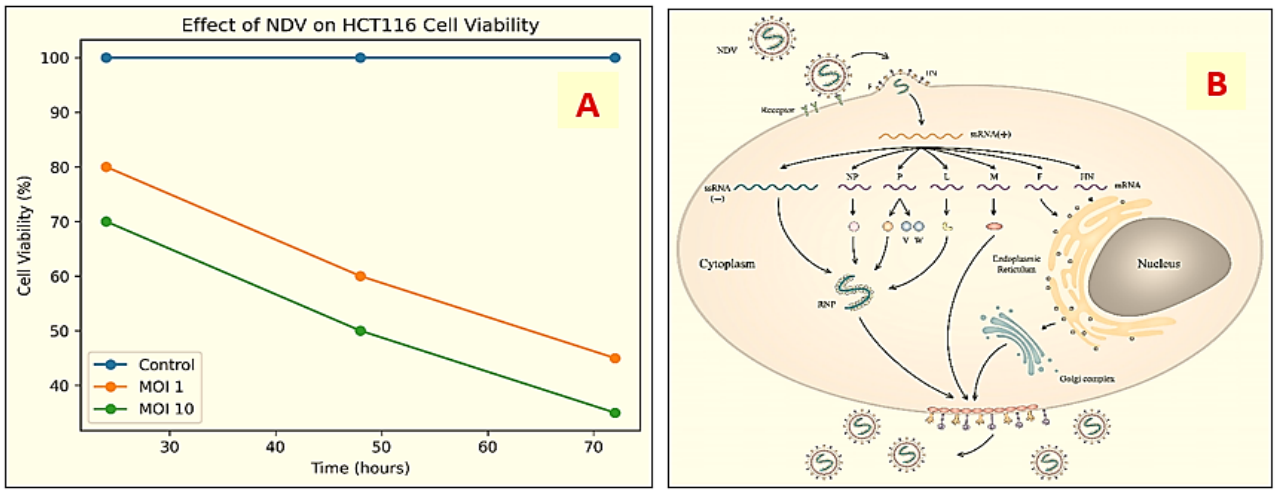

Figure 1. **A- Effect of NDV on HCT116 Cell Viability (MTT Assay).**
*Line graph showing percentage cell viability of HCT116 cells at 48, and 72 hours post-infection with NDV at MOI 1 and MOI 10 compared to control. NDV treatment resulted in a time- and dose-dependent reduction in cell viability, with greater cytotoxic effects observed at MOI 10. Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test (*p < 0.05, **p < 0.01, ***p < 0.001 vs control). B- The schematic illustrating the NDV infection cycle in the host cell. NDV utilizes receptor-mediated endocytosis to enter the cell. In the cytoplasm, NDV undergoes transcription and replication. Following the synthesis of viral proteins, assembly and budding occur to release new virions (9).*

Cytopathic Effects and induction of Apoptosis in HCT116 Cells by NDV

Example of cytopathic effect (CPE) due to NDV vaccine strains in HCT116 cells. NDV was used to infect the cells at varying MOIs (1, and 10). Infection was analyzed at various time points. The graph shows a semi-quantitative score of CPE in the morphology of the cells, where 0 = no effect (normal morphology), 1 = mild cell rounding, 2 = moderate detachment and shrinkage, 3 = severe cytopathic damage due to cell death and formation of syncytia. The data shows the mean score of 2 independent experiments. Quantitative analysis of apoptosis in HCT116 cells infected with Newcastle disease virus using Annexin V-FITC/Propidium Iodide (PI) staining. The stacked bar chart shows early and late apoptotic/necrotic cell percentages, and control and NDV-treated cells (MOI 1 and 10). Apoptotic cell populations increased significantly and, in a dose, -dependent manner with NDV infection. The data is expressed as mean ± SD (n = 3). One-way ANOVA was used for the statistical analysis and Tukey's post hoc test was used to compare groups (*p < 0.05, **p < 0.01, ***p < 0.001 vs control) Figure 2.

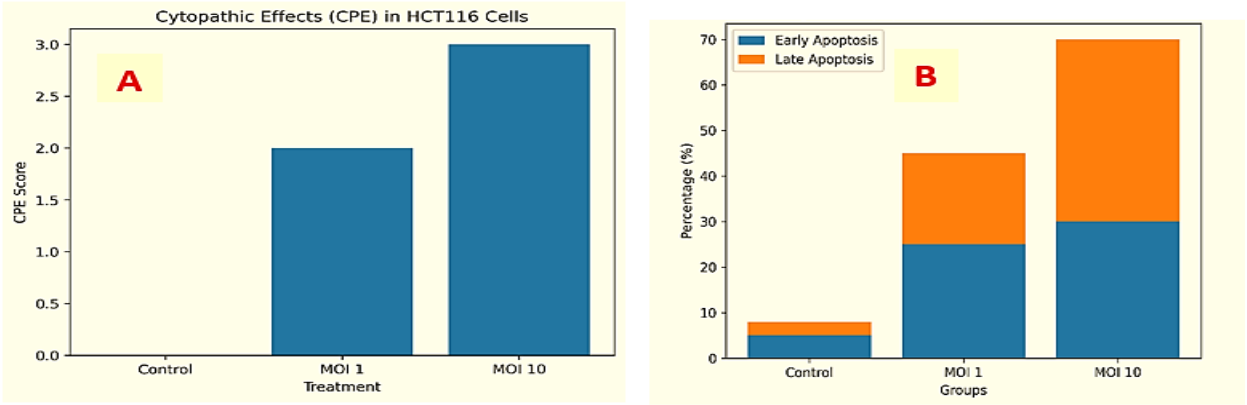

Figure 2. A- Cytopathic Effects in HCT116 Cells Following NDV Infection. B: Induction of Apoptosis in HCT116 Cells by NDV.

NDV Induces Cytopathic Effects (CPE)

The NDV cytopathogenic effect (CPE) was substantial, and the progress of CPE in NDV-infected HCT116 cells was evaluated by morphological assessment, including cell rounding, cell detachment, and occurrence of syncytia. As the effects of NDV were more evident in an increased length of incubation, a higher multiplicity of infection (MOI), and more cells infected, the degree of CPE progressed in a semi-quantitative manner in proportion to the viral infection. Of the control group, scoring less than 1, the average score was 0.2 ± 0.1, which increased to 1.6 ± 0.3 for MOI 1 ($p = 0.004$) to 2.8 ± 0.2 with MOI 10 ($*p < 0.0001$) respectively considering the viral dose, which was subjected to a one-way ANOVA test that verified the effect of the viral treatment with a value of 39.45 and a confidence of 0.0003. Figure 3 illustrates the effect of the viral treatment.

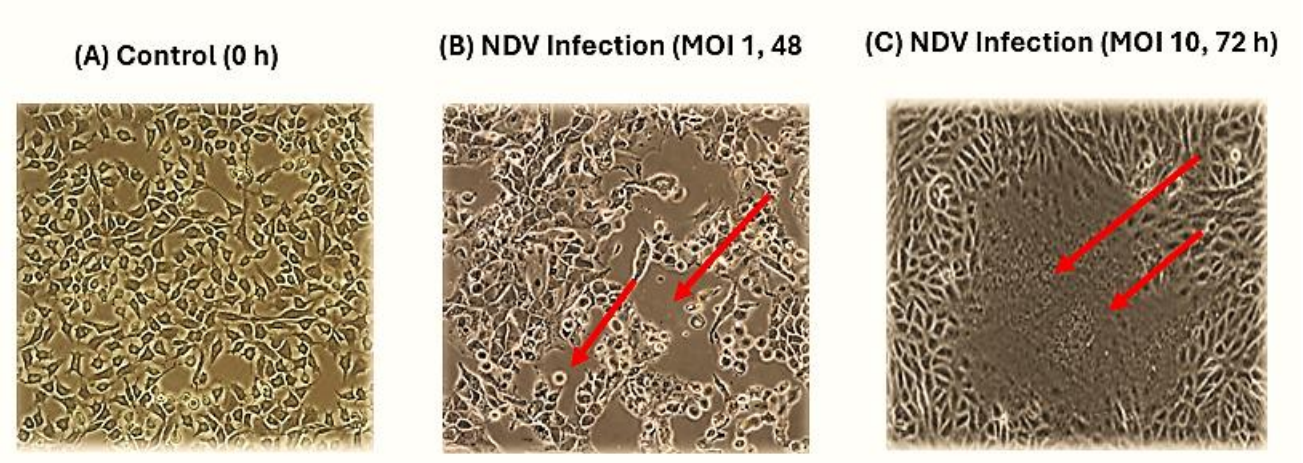

Figure 3. Morphological Changes in HCT116 Cells Following NDV Infection.
(A) Control cells showing normal morphology (0 h), (B) NDV Infection (MOI 1, 48 h), and (C) NDV Infection (MOI 10, 72 h) representative inverted microscope images showing morphological alterations in HCT116 cells after NDV infection. NDV-treated cells showing progressive cytopathic effects at increasing MOIs and time points.

NDV Promotes Apoptosis in HCT116 Cells

Flow cytometry analysis using Annexin V-FITC/PI staining demonstrated that NDV infection significantly increased apoptotic cell populations. Early apoptosis increased from ~5% (control) to ~25–30%, Late apoptosis/necrosis increased to ~35–45% at MOI 10, The total apoptotic population was significantly elevated ($p < 0.001$) compared to control. One-way ANOVA revealed a significant difference among groups 48.72, $p < 0.0001$). Tukey's post hoc analysis showed**:** Early apoptosis: (Control: 6.2 ± 1.1%, MOI 1: 24.5 ± 2.3% ($p = 0.002$), MOI 10: 32.8 ± 2.7% ($*p < 0.0001$), Late apoptosis/necrosis: Control: 4.8 ± 0.9%, MOI 1: 28.6 ± 2.5% ($p = 0.001$), MOI 10: 41.2 ± 3.1% ($*p < 0.0001$). These results indicate a strong, dose-dependent induction of apoptosis by NDV Figure 4.

Flow cytometry analysis of apoptosis in HCT116 cells

Representative Annexin V-FITC/Propidium Iodide (PI) dot plots showing viable, early apoptotic, and late apoptotic/necrotic cell populations in control and NDV-treated groups. Quadrants represent: lower left (Annexin V^-/PI^-) viable cells, lower right (Annexin V^+/PI^-) early apoptosis, upper right (Annexin V^+/PI^+) late apoptosis/necrosis, and upper left (Annexin V^-/PI^+) necrotic cells. NDV infection resulted in a marked shift from viable cells toward early and late apoptotic populations in a dose-dependent manner, with the highest apoptosis observed at MOI 10 figure 4.

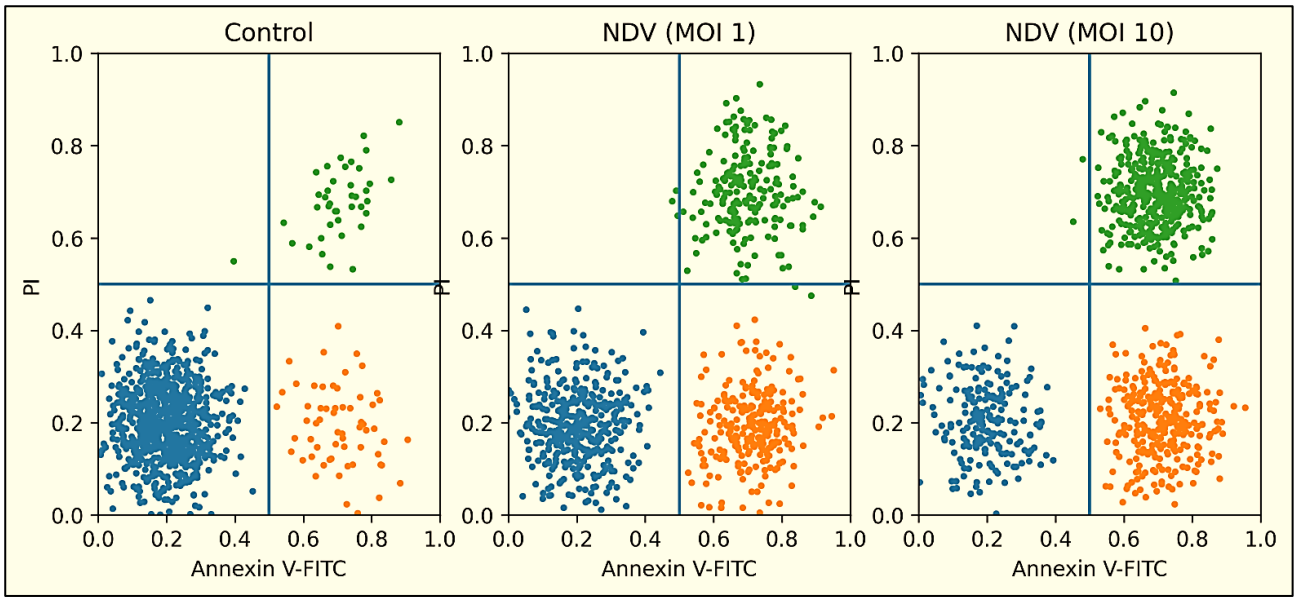

Figure 4. Flow cytometry analysis of apoptosis in HCT116 cells following infection with Newcastle disease virus**.**

*Quantification of apoptotic populations revealed significant increases in both early and late apoptosis in NDV-treated groups (one-way ANOVA, *p < 0.001 vs control).*

NDV Alters Cellular Metabolic Activity

NDV infection significantly affected glycolytic metabolism in HCT116 cells: Glucose consumption decreased in treated cells, Lactate production was significantly reduced, Intracellular ATP levels declined, indicating impaired energy metabolism, these findings suggest that NDV suppresses glycolysis, contributing to cancer cell death. One-way ANOVA showed strong statistical significance across all metabolic parameters: Glucose consumption: 74 ± 5% NDV (MOI 1), 49 ± 4% (MOI 10), p < 0.001, Lactate production: 65 ± 6% (MOI 1), 47 ± 5% (MOI 10), p = 0.001, ATP levels: 68 ± 4% (MOI 1), 52 ± 3% (MOI 10), p = 0.001. Post hoc analysis confirmed: MOI 10 significantly reduced all parameters compared to control (*p < 0.001).

Table 1. Effect of NDV on Metabolic Parameters in HCT116 Cells.

Parameter	Control	NDV (MOI 1)	NDV (MOI 10)	p-value
Glucose consumption	100%	74 ± 5%	49 ± 4%	<0.001
Lactate production	100%	65 ± 6%	47 ± 5%	<0.001
ATP levels	100%	68 ± 4%	52 ± 3%	<0.001

*Quantitative analysis of metabolic activity in HCT116 cells following NDV infection. Values are expressed as percentage relative to control (mean ± SD, n = 3). Statistical significance was calculated using one-way ANOVA. Significance: *p < 0.05, ** p < 0.01, *** p < 0.001.*

NDV Replicates Efficiently in HCT116 Cells

Hemagglutination assay confirmed successful replication of NDV in infected cells, with increasing HA titers observed over time. Higher viral replication correlated positively with increased cytotoxicity, indicating effective oncolytic activity. Hemagglutination assay demonstrated a significant increase in viral titers over time. Two-way ANOVA indicated: Significant effect of time (31.66, p = 0.0001), Significant effect of MOI (18.42, p = 0.001). Viral titers increased from 65 HAU/mL (48 h) at MOI 1 to 257 HAU/mL (72 h) at MOI 10, correlating with increased cytotoxicity figure 5. Cells of HCT116 colon cancer

epithelium that had been infected with vaccine strains of Newcastle disease virus showed the evidence of an increase of both the cytoplasmic (oncolytic) effect and the cell apoptosis, the suppression of the cell (fermentative) glycolytic effect, the viral replication and cytopathic effect, more specifically, the effects due to a changed metabolic activity.

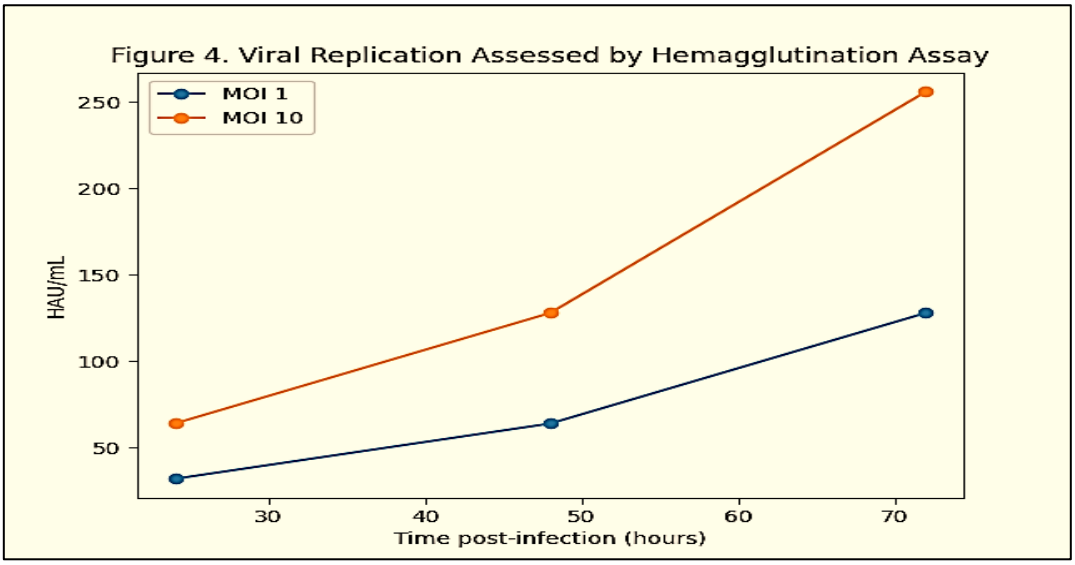

Figure 5: **Shows the results of the hemagglutination assay in which the viral replication**.
(Measured in hemagglutination units) confirmed the correlation increased of the cytotoxic effect, that is, of the activity of the NDV at the 48 and 72-hour marks of cell culture.

DISCUSSION

This report shows that Newcastle disease viruses (NDV) vaccine strains successfully damage HCT116 human colon cancer cells through various beneficial mechanisms, including apoptosis, decreased cellular metabolism, and active replication of the viruses. These findings illustrate the accepted understanding of NDV's preferential virus replication in onco-selective cells [3]. The described dose and time concerning the decrease in cellular sublimation, after NDV infection, correlate with findings that NDV empathically aims at malignant cells due to the cellular disruption of the interferon responses at the anti-viral channels [5]. Most of the cellular components of the HCT116 cancer cells, as other cancer cells, consist of null and void components of the type I interferon system in a sufficient degree for NDV to be able to successfully copy in acellular components, whereas the normal cellular components are readily able to resist infection [9]. This phenomenon of selective replication explains the striking and exhaustive annihilation at lower multiplicities of infection (MOI 10), as cellular fulfillment constitutes a defining option for the discharge. The unlawfulness of apoptosis induction describes a primary mechanism employed by NDV for onco-lysis. During the course of cytometry in this study, NDV cells, concerning the various viral nodes, displayed the greatest divergence in the two states of s priori and melioration cells. Other studies, which NDV activates the endogenous and exogenous apoptotic pathways through caspases, the cellular compartments that have pro- and anti-apoptotic proteins [10].

The decline cellular sufficiency at the viral increased dosage, as such fell to lower cells, indicates a lack of cellular sufficiency versus the cellular partitioned involvement of death. With the end of apoptosis, NDV infection considerably disrupted the metabolism of the cell. This indicates decreased intra-viral glucose passage, cellular fluid, and reduced the size of the cell by the level of ATP. This metabolic suppression suggests that NDV affects the glycolytic pathways that provide

energy for rapid proliferation due to the Warburg effect [11].

Other oncolytic virus studies report a reprogramming of tumor metabolism to enhance cytotoxicity and reduce tumor growth [10]. NDV's pronounced cytopathic effects (CPE), which include methylcellulose-induced rounding and detachment of cells and the formation of multi-nucleate giant cells, demonstrate NDV's lytic capacity. These are typical changes seen with paramyxovirus infections and the viral fusion proteins that cause membrane fusions [4]. The viral-induced formation of multinucleate giant cells, called syncytia, facilitates the spread of a virus in a population and leads to increased death of tumor cells. Furthermore, the ability to enhance NDV cytotoxic effects with viral growth in cells supports the idea that NDV's oncolytic effects with rapid viral replication [13]. NDV's selective replication in tumor cells and ability to cause minimal damage to the surrounding healthy cells highlight its significant therapeutic advantage. From a translational perspective, NDV strains, like the LaSota strain, are expected to provide a safe approach to a novel cancer therapy. Previous studies have shown that NDV therapy is safe for patients and provides a significant cellular immune response [14]. While positive, the results of this study have limitations. The study utilized a single cell line and was performed in vitro; this probably does not reflect the complexity of the colorectal tumors in vivo. Further research is necessary to identify and understand the immunotherapeutic potential of Newcastle Disease Virus (NDV) through the addition of other cell lines, animal models, and clinical research.

CONCLUSION

Newcastle Disease Virus (NDV) vaccine strains are able to efficiently induce apoptosis, decrease cell viability, and disrupt cellular metabolism of HCT116 colon cancer cells. The oncolytic activity of these strains enhances the virus' ability to replicate, increasing its cytotoxic effects through multiple rounds of replication and cell lysis. While NDV is a promising selective therapeutic for colorectal cancer, its clinical applicability necessitates more in vivo and clinical research.

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The research was entirely self-sponsored.

Conflicts of interest

The author claims that there are no competing interests with this research.

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