Short Communication

Non-Specific Immunostimulatory Capacity of Newcastle Disease Virus (NDV) and Suppression of Breast Cancer Cells

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\textbf{ABSTRACT}

Newcastle disease virus (NDV) is an enveloped single stranded RNA virus that causes deadly infection to over 250 species of birds, comprising domestic and wild-type, thus resulting in substantial economic loss to poultry industry across the globe. NDV possesses several distinctive properties that make it an outstanding anti-cancer agent. In humans it is reported to have oncolytic and immune-stimulatory effects, precisely replicates in tumour cells while sparing normal cells and causes oncolysis. Although NDV has been extensively studied by researchers there is still need for a vigorous research on its potential use as a new treatment modality to cancer patients through a known process termed viroimmunotherapy. This paper deals with an overview of the research which has been carried out worldwide in the use of immune-stimulatory properties of NDV as an anti-cancer agent.

\textbf{Keywords:} Newcastle disease virus, immune-stimulation, anti-cancer, cytokines.

\textbf{INTRODUCTION}

Breast cancer remains a major cause of deaths in humans. Regardless of the amazing scientific advancement in the prognosis and treatment of tumour, large numbers of people are still coming down with the disease, especially due to resistance to treatment and relapse\textsuperscript{33}. Surgery, chemotherapy, hormone therapy and radiotherapy are the current available treatment for breast cancer\textsuperscript{34}. However one of the several new approaches to treatment of cancer is oncolytic virotherapy\textsuperscript{8} which exploits the potential of naturally occurring viruses to selectively replicate in and causes cytotoxicity to tumor cells\textsuperscript{4} thus the use of NDV to treat cancer patients is an attractive adjunct to conventional therapy\textsuperscript{19}. Newcastle disease virus is one of the numerous naturally occurring oncolytic viruses, which selectively infect, replicate in, and kill tumor cells\textsuperscript{13}. For a long time, the therapeutic efficacy was thought to depend on the direct viral oncolysis, however direct NDV induced cytolysis may not be the only factor that plays a role in anti-tumour efficacy\textsuperscript{32}. The host immune system was considered as a brake that decreases virus delivery and spread, thus researchers paid much of their attention to enhancing virus tumour selectivity and cytotoxicity, but with the discovery of indirect Oncolytic mechanism induced by virus such as anti-tumour immunity following viral infection, many research turned their direction toward the arena\textsuperscript{33}. Indeed, tumor-specific immune cells persist post-therapy and can search and destroy any tumour cells that escape the oncolytic virus, and thus immune memory may prevent relapse of the disease.

Understanding how the host immune system act together with NDV to accomplish antitumor immunity is essential for effective tumour suppression. NDV has a long history as an immune-stimulant, inducing a rapid type I interferon (IFN) response in infected cells\textsuperscript{12}. Studying the immune effector molecules produced by NDV infected cancer cells, and the signalling pathways involved in stimulating cytokines and chemokine production in tumour cells, is essential in designing recombinant NDV with enrich immunogenicity that could help recruit the body’s own proinflammatory mechanism for immune mediated clearance of transformed cells\textsuperscript{1}. Therefore, this mini review will summarize researches in the field of NDV immunotherapy and/or immune-stimulatory properties of NDV in cancer therapy specially breast cancer.

Newcastle disease virus and the innate immunity: The pleiotropic immunostimulatory properties of NDV in addition to its noble cell binding and selective proliferation in replicating cells have since been documented\textsuperscript{37,33}. Study conducted in vitro, indicated that infection of human immune cells with NDV, stimulate production and released of cytokines, interferon-alpha (INF-α) and tumour necrosis factor alpha (TNF-α)\textsuperscript{40}. Also, infection of human cancer cells with NDV makes the cells more sensitive to the cytotoxic effects of TNF-α\textsuperscript{35}, although the exact mechanism leading to the stimulation of the human

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The immune system is still under exploration. One allied important feature is its capacity to induce large amounts of type 1 IFN response during interaction with human peripheral blood cells. This is associated to the nature of the dsRNA structures, which are produced within the cytoplasm through the viral replication, thereby stimulating a robust cellular IFN response.

Interferon-induction by NDV for tumor selectivity: NDV express a dissimilar pattern of replication in normal cells upon comparison with tumour cells. Its weak replication in normal cells can be associated with a well-organised antiviral response within the infected cells. In contrast, its efficient replication in tumour cells has some connection with a weak antiviral response of the cancer cells. Many NDV strains have better replication ability in transformed cells than in non-transformed cells. This may be the reasons for classifying the virus as essentially harmless in humans and for the importance of its use as tumour therapeutics, surprisingly NDV has strong ability to persuade type I interferon response. Replication of viral RNA in infected cells instigates the initiation of an innate antiviral response that recruit the transcription of RNA responsive genes, this response encompasses gene regulation by the interferon regulatory factor (IRF) family of transcription factor. NDV motivate interferon induced genes like antiviral enzyme protein kinase R (PKR), RNaseL, MxA, dsRNA-responsive protein kinase and a dynamin-like GTPase with antiviral activity. RNaseL was lately reported to show generation of a small self-RNA, thus increasing the augmentation of innate antiviral immunity.

To further explain the mechanism of the differences in vulnerability of normal and tumour cells to viral infection, the pathways for the interferon-induced antiviral enzymes was examined. Of which, the result obtained showed many defect in the antiviral interferon defence response of the tumor cells, there was no response to UV-inactivated NDV, however normal cells responded significantly with high degree of antiviral enzymes. This indicate that, early and strong induction of an antiviral response in normal cells may explain the reasons for the break of the NDV replication cycle after making of the positive stranded RNA, perhaps this result could be the reason for the progressive replication cycle of the virus leading to high expression of the viral protein.

NDV activation of macrophages: Non-specific immune stimulating potential of Newcastle disease virus (NDV) and its various anti-tumour activity received much attention recently. Activation of macrophages to tumoricidal state is a multistep process resulting in production of cytotoxic factors, which eventually destroy neoplastic cells. A research carryout to examine the capability of NDV to trigger anti-tumor activity in murine macrophages discovered that, macrophages were activated

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**Table 1:** Indicates of some related researches on effect of NDV induced immune cells derived products and their tumoricidal roles

<table>
<thead>
<tr>
<th>Immune cells</th>
<th>Cytokines released</th>
<th>Tumoricidal role</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macrophages</td>
<td>IL, NO, TNF-α, TRAIL, IFN-α</td>
<td>Cytostatic</td>
<td>(40)</td>
</tr>
<tr>
<td>Monocytes</td>
<td></td>
<td>Cytotoxic/Apoptosis</td>
<td>(38)</td>
</tr>
<tr>
<td>Natural killer cells (NK-Cells)</td>
<td>TRAIL, IFN-α</td>
<td>Cytotoxic/Apoptosis</td>
<td>(Sedener et al., 1999)</td>
</tr>
<tr>
<td>Peripheral blood mononuclear cells</td>
<td>TRAIL, TNF-α</td>
<td>Cytotoxic</td>
<td>(Takeda et al., 2001)</td>
</tr>
<tr>
<td>Dendritic cells (DC)</td>
<td>TRAIL, CD40, CD36</td>
<td>Cytotoxic</td>
<td>(Takeda et al., 2001)</td>
</tr>
</tbody>
</table>

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**Fig. 1:** NDV activation of innate immunity for cytotoxic activity on tumor cells. NDV interact with cells of the innate immunity on the surface of infected tumour cells where it causes the expression of TRAIL on monocytes and NK-cells, it causes the synthesis of nitric oxide (NO) and tumor necrosis factor (TNF-α) on macrophages.
following infection with different NDV strains. Several macrophage enzymes become up regulated and anti-tumour effector molecules such as nitric oxide (NO) and tumor necrosis factor (TNF-α) were also established in the supernatants.\textsuperscript{24} See Table 1 below. The NDV activated macrophages displayed cytotoxic anti-tumour activity in vitro and were active against tumor cell lines such as mammary carcinoma, lungs and mastocytoma.\textsuperscript{29} Antitumor activity by NDV activated macrophages could be transferred in vivo. This result demonstrated that NDV can strongly and effectively activate macrophages to perform anti-tumor activity in vitro and in vivo.\textsuperscript{13} Study by\textsuperscript{30} indicated that tumoricidal activity of NDV stimulated macrophages is mediated by TRAIL with high expression of mRNA for TRAIL, 14 hours after NDV macrophage activation anti-tumour cytotoxic activity that kills the TRAIL-R2 receptor expressing tumour line was observed. This cytotoxic activity may be stop by soluble TRAIL-Fc but not by recombinant TNF-α Fc-binding protein.\textsuperscript{37} Induction of NO production in NDV activated macrophages is associated with activation of nuclear factor-kB (NF-kB). These reactions are part of an activation mechanism comprising of stimulation of ADA and inhibition of 5'-nucleotidase, suggesting that signalling requirements of NF-kB activation and NO generation are similar in NDV-activated macrophages.\textsuperscript{36} NDV activation of natural killer cells (NK-cells): Infection with NDV have been described previously to cause increase cytotoxic activity of NK-cell fraction in peripheral blood lymphocyte, correlating with virus induced INF-α production.\textsuperscript{38,39} The mechanisms of NK activation by NDV are largely unresolved,\textsuperscript{40} in a study to investigate whether NDV infection of tumour target results in the direct activation of NK cells through the induction of NK- activating ligands. The established human carcinoma cell lines Panc-1, HeLa, and A549 and the recently isolated melanoma cell line Ma-Mel-8a were infected with lytic and nonlytic NDV strain, where it was demonstrated that NK cells exercise significantly improved cytolitic activity in vitro in contrast to many tumour cell lines infected with NDV.\textsuperscript{40} Both the nonlytic NDV strain and the lytic strain instigate direct NK-triggering effect. Moreover, it has shown that the incubation of NK cells with inactivated NDV particles was able to enhance the cytotoxic activity of the NK cells against uninfected targets. In addition to the previously reported IFN-α/β mediated induction of the death receptor ligand TRAIL on NK cells (Sato et al., 2001), the direct activation of NK cells by NDV may thus contribute to the known oncolytic properties of certain NDV strains in vivo.\textsuperscript{39} It has also been shown that NDV infected tumor cells induced NK cells to secrete increased amount of IFN-α and TNF-α can contribute to the antitumor cytotoxicity of NDV activated macrophages.\textsuperscript{29} These results suggest that direct activation of NK cells contributes to the antitumor effects of NDV. Dendritic cells (DCs) pulsed with NDV oncolysates: Research has shown that dendritic cell is associated with innate recognition of danger signals and induction of immune response, which is a critical link between innate and adaptive immune responses.\textsuperscript{21} Their ability of picking up, processing and presentation of antigen to naive memory T-cells could be through in vitro or in vivo, all the characteristics exhibited by DCs make them unique candidates for immunotherapy aiming at inducing effective T cell-mediated anti-tumor immunity,\textsuperscript{2} large amount of DCs can be generated from PBMC derived monocytes, the activation, maturation and protection of DCs is said to be driven by dsRNA.\textsuperscript{3} The systematic data presented in this review provide new understanding into the strategy and mechanism of function of NDV-induced DC, the virus serves as a potent mediator to Th1 response, favouring the induction of DC maturation, the release of pro-inflammatory cytokines and the enhancement of antigen cross-presentation,\textsuperscript{12} all these stages are crucial for the priming and activation of a CD8+ T cell-mediated tumor-protective immune response.\textsuperscript{11} Dendritic cells activated with NDV oncolysate were reported to be effective in stimulating autologous T-cells from cancer patients.\textsuperscript{13} This research shows that DCs from breast cancer patients were pulsed with lysate from MCF-7 cancer cell line or from NDV treated MCF-7 cells and compared for stimulatory capacity in an ELISPOT technique response of the autologous bone marrow-derived memory T-cells.\textsuperscript{2} DC pulsed with viral oncolysates showed increased expression of co-stimulatory molecules in comparison with culture of T-cells and DCs pulsed with noninfected tumor lysates and induced significantly higher ELISPOT memory T-cell responses.\textsuperscript{2} Supernatants from co-cultures of MTC and TuN-L pulsed DC contained increased titers of IFN-alpha and IL-15. NDV infection of tumour cells resulted in a number of differences in protein expression including a heat-shock protein which became phosphorylated.\textsuperscript{11} The results suggest that a DC preparation pulsed with viral oncolysate includes danger signals (e.g. dsRNA, cytokines, HSP molecules) and is superior for MTC stimulation to a DC preparation pulsed with lysate from non-infected tumor cells.\textsuperscript{2} These studies highlighted the importance of the immunostimulatory component of NDV therapy and demonstrated the potential of both naturally and recombinant NDV expressing a tumor-associated antigen to be used as a therapeutic cancer vaccine vector.

CONCLUSION

It is generally believed that oncolytic NDV therapy follows in two stages, an initial stage in which the virus mediates direct oncolysis of tumor cells, leading to the second stage in which it induced immune response carrying on to facilitate tumour damage after the viral vector has been cleared. NDV is a promising clinical candidate as it shows sign of inducing anti-tumoural immunity, and this is surely a step forward to success for this agent. Different forms of NDV such as live once, heat attenuated and UV inactivated has been tested and proved to be effective in killing tumour cells,\textsuperscript{20} in all the cases NDV provoked the production of weak tumour antigens, destruction of tumour immune tolerance and production of immune response against the tumour antigen.\textsuperscript{2} The increase in TRAIL expression in NDV activated peripheral blood mononuclear cells
(PBMC), DCs, and NK-cells indicated that TNF induced apoptosis could be the central mechanism in oncolytic NDV induced apoptosis, however the exact mechanism by which NDV induced cytokines suppresses the tumor cells has not been completely elucidated, thus finding the sequence of immunological/ cytokines reactions that mediate the NDV induced oncolysis will significantly help in constructing genetically engineered NDV strain with enhance oncolytic activity which could be more safer to the patients.

REFERENCE


