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The use of oncolytic virus to combat neuroblastoma

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Abstract. Neuroblastoma is a type of cancer that arises from immature nerve cells in the body and often appears in children. The current treatment of chemotherapy and radiotherapy is not a successful method of treatment due to its harm to the child's body and its inability to effectively pass the blood-brain barrier (BBB) in the brain to effectively target the tumor. Recent studies into the field of oncolytic viruses have shown the possibility to target neuroblastoma cancer cells in the brain by engineering specific viruses to express NY-ESO-1, an antigen that is tumor-specific and commonly expressed in neuroblastoma. Research also showed a method to integrate protein A into the envelope protein of retroviruses which allows monoclonal antibodies' Fc region to bind with the virus, allowing specificity to an antigen. This paper combines the ideas of previous studies to design a novel model of oncolytic virus treatment that specifically targets neuroblastoma. Since the oncolytic virus can be injected directly at the site and the virus is small enough to penetrate the BBB, the paper hypothesize that the model is a valid treatment for neuroblastoma in children.

Keywords: NY-ESO-1, HSV-1, Protein-A Integration, Monoclonal Antibodies, GM-CSF.

1. Introduction

Neuroblastoma is a childhood tumor that involves neuroblasts and is the most common external tumor in children which occurs in the sympathetic nervous system [1]. Current clinical treatments can successfully deal with low and middle-risk neuroblastoma, but high-risk patients have event-free survival (EFS) of only about 45%. The rationality of toxicity for current chemotherapies contributes to the low EFS, and it is less efficient for high-risk patients [2]. For this reason, we want to focus on a novel immunotherapy based on oncolytic modification for high-risk patients in this article to reduce neuroblastoma with more efficiency and less harm [3].

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For more efficient immunotherapy, we aim to discover a more efficient antigen from target cancer cells that T cells can better capture. The outstanding result acquired by herpes simplex virus (HSV) in treating melanoma inspired us to adopt a similar treatment with higher affinity [4]. Thus, we managed to modify HSV used previously with neuroblast-specific antibodies. This new technique reduces toxicity and improves current immunotherapy's poor efficiency. Effective HSV with target antibody is the final result of our experiment, and further experiments on mice are designed to prove the high affinity and curative effects.

2. Summaries of Previous Research

BL Liu et al. introduced Herpes simplex virus type-1 (HSV1) can direct tumor-specific cell lysis in 2003. This enhanced oncolytic potential is through the inactivation of ICP34.5, which encodes neurovirulence factor. The main findings of the article suggest that recent clinical isolates of HSV1 are more effective in the lytic ability in human tumor cells compared to the passaged laboratory isolation. Firstly, the removal of ICP 34.5 in HSV resulted in improved human tumor cell killing. Furthermore, the deletion of ICP34.5, and ICP 47 by the up-regulation of US11, and insertion of GM-CSF, further improves the oncolytic and immune-stimulating properties of the virus. Moreover, the testing of the constructed viruses in vitro with human tumor cells and in vivo with mice both display significant anti-tumor effects. In conclusion, the evidence suggests that JS1/ICP34.5-/ICP47-/GM-CSF is a powerful oncolytic agent that could be a proper treatment of solid tumor types in humans [5].

Michael T. Bethune et al. shed light on a novel carceral antibody. The article aims to pilot an approach to extend T cell receptor (TCR) gene therapies to patients beyond those expressing HLA-A2. HLA-A2-restricted TCRs are common among TCR gene therapy, but patients expressing HLA-A2 allele cannot acquire the treatment. The authors isolate TCRs that collectively recognize multiple NY-ESO-1-derived epitopes presented by multiple MHC alleles and compare their dextramer binding with TCRs to reveal their strength and affinity differences. NY-ESO-1 is a tumor-specific, immunogenic public antigen expressed across various tumor types and is safe to target in a clinic. The finding offers a potential alternative treatment for patients expressing HLA-A2 allele [4].

Kouki Morizono et al. examine the use of retrovirus as a vector to reach target stable transduction to deliver gene therapy to patients. Researchers have found that the envelope of alphavirus Sinubis contains two transmembrane envelope proteins: E1 and E2. E1 is responsible for pH-dependent fusion while E2 is responsible for binding with receptors. This allows the possibility of Sinubis vectors where E2 protein is modified to contain the Fc-binding domain of protein A and brings significant increases in binding specificity for MHC molecules and T helper cells when monoclonal antibodies are present. This finding is crucial as it has overcome previous limitations such as decreasing the infectivity after modification and the method theoretically is applicable to bind viral vectors to any cell surface with a specific reagent to bind to [6].

3. Methods

3.1. Introduction, isolation, and expansion of NY-ESO-1 antibody

To acquire adequate wanted antibodies, we adapt existing methods from previous findings. The previous study used the dextramer binding ability to represent TCR's 'strength,' a term that describes the affinity between TCRs and NY-ESO-1. Researchers adopt specific methods and discover that 3A1 TCR, compared with most clinically used 1G4 TCR, has a higher affinity towards NY-ESO-1. To isolate and expand target 3A1 TCR, researchers first prepare NY-ESO-1 9-10-mer peptide sequences and then use them to stimulate reactive T cells, sorted via FACS using cognate peptide–MHC tetramers [4]. Target T cells are extracted and cloned separately; thus, the NY-ESO-1 antibody can be successfully extracted.

3.2. HSV modification

HSV is an ideal treatment that has proven efficient in treating melanoma [5]. We are interested in this phenomenon and planning to discover the efficiency of similar treatment to neuroblastoma. The keys of

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HSV modification contain three parts with three aims: reduce toxicity, target neuroblasts and eliminate them. Gene modification and editing are introduced to reduce toxicity and eliminate target cells. We add the NY-ESO-1 antibody introduced previously onto the envelop protein via gene edit for better targeting.

To reduce toxicity, ICP 34.5 and ICP 47 are knocked out from their genome in HSV. These two genes, ICP 34.5 and ICP 47, are essential in the HSV escape mechanism and toxicity. Knocking them out prevents the virus from producing harmful compounds that could damage healthy tissue [7]. GM-CSF is an important cytokine that promotes the differentiation and proliferation of macrophages and granulocytes. We insert the gene of GM-CSF into HSV, an existing technique adapted to treat melanoma [5]. This insertion helps with the elimination of target neuroblasts. Direct insertion of NY-ESO-1 antibody onto the virus' envelope is too complicated and unpredictable. Thus, we introduce protein A, an envelope protein allowing the binding of antibodies. Studies show that protein A can bind to antibodies, including NY-ESO-1 antibody; thus, adding the gene of protein A into the virus can lead to better recognition [5].

3.3. Mechanism of this treatment

The mechanism of this treatment is that when the engineered virus targets NY-ESO-1 on the neuroblast membrane, the virus undergoes an endocytosis process. This either results in cell lysis by replicating virus DNA in the cancer cells or releasing GM-CSF, the important cytokine introduced. GM-CSF released by the neuroblasts will recruit phagocytes which initiate the adaptive immune system.

NY-ESO-1 antibodies need to bind with the virus with protein A, and we reach this outcome by mixing them. Protein A needs to be expressed on the envelope protein of the virus to bind to antibodies. Without protein A, the virus will lack the ability to specifically bind to the antigen we have found on the tumor. Antibodies, mainly IgG, sometimes IgA, and IgM, can bind with protein A with sufficient affinity. Therefore, these antibodies are engineered to be on the protein A to initiate binding between the vector and the tumor. Protein A itself can be integrated into the envelope protein by injecting the gene of protein A into the genetic material of HSV, allowing HSV to express protein A on their surface as they proliferate. Protein A, in this way, can be integrated into the membrane protein, which allows the virus to bind to antibodies that bind to NY-ESO-1 in neuroblastoma [5-6].

After the virus has gained its specificity, the mixture will be injected into the tumor site. Once injected, the virus would bind to NY-ESO-1 antigen on the surface of the tumor. Virus replication kills the neuroblast by breaking its membrane and, disseminating the therapeutic agent, further through the tumor tissue than non-replicating therapies. This results in a decrease in the size of the tumor as well as an increase in T-cell activity.

4. Results

4.1. Mouse Model

In order to test our potential treatments, we used the LSL-MYCN; Dbh-iCre mice model. Initially, we isolated tumor cells from these mice, which were then cultured in vitro to create mBN-A1 cells. These cultured cells, or mBN-A1 cells, possess neuronal structures similar to human neuroblastoma cells. Furthermore, mBN-A1 cells are engineered to express luciferase, a bioluminescent protein, which is a valuable marker for testing [8]. Through bioluminescence imaging, we would confirm positive luciferase expression in mNB-A1 cells on Dbh-iCre tumors and expressed MYCN mRNA and protein levels similar to the LSL-MYCN; Dbh-iCre tumors, further confirming that their MYCN mRNA and protein levels comparable to LSL-MYSN; Dbh-iCre tumors [9]. To test tumorigenic potential, mNB-A1 cells were injected into NSG mice (immunodeficient mice) Due to mNB-A1 cells, the mice started developing tumors, and the growth was observed with bioluminescence imaging in real-time [10].

4.2. Experiment conduct

The first treatment involves using only phosphate-buffered saline (PBS). This is often used as a control in scientific experiments. In the second treatment, we used a combination of HSV-1's oncolytic

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properties and GM-CSF's immune-stimulating properties. The third treatment consists of HSV-1's oncolytic properties, GM-CSF's immune-stimulating properties, and NY-ESO-1's immune response-provoking properties. For the fourth treatment, we continue to utilize HSV-1's oncolytic properties, GM-CSF's immune-stimulating properties, and added protein A. While for the fifth treatment, we contained everything in the fourth treatment and added A6, a monoclonal antibody. The last one, the sixth treatment, comprises all the contents stated above, including HSV-1, GM-CSF, protein A, A6, and NY-ESO-1, which is thought to be the most effective one among all the treatments. (Fig. 1)

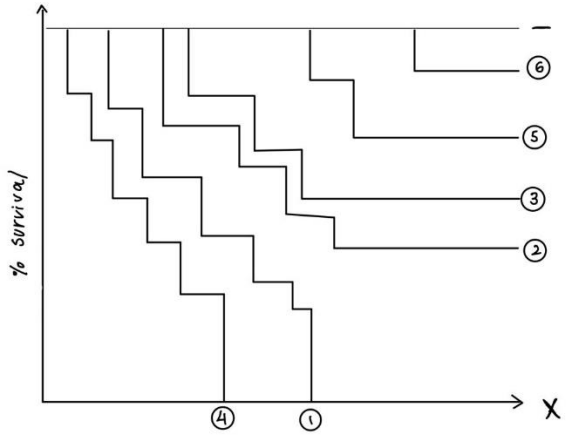

Figure 1. Hypothetical mice survival percentage of each treatment FACS and MHC tetramer detects tumor-specific T-cell.

To better understand the efficiency of T-cells in these treatments, we will use two specific technologies. The first one is fluorescence-activated cell sorting (FACS). FACs provide a method for sorting a heterogeneous mixture of biological cells into two or more containers, one cell at a time, based on each cell's specific light scattering and fluorescent characteristics [5]. This is used to target and isolate T-cells. The second one is the MHC tetramer, which contains four MHC molecules of HLAA2 to help analyze T-cell immunity. We categorized the six treatments into two significant groups of modified HSV-1: one containing NY-ESO-1 and the other without. Through the results (Fig. 2 A and B), we can see there are more tumor-specific T-cells in MHC containing NY-ESO-1.

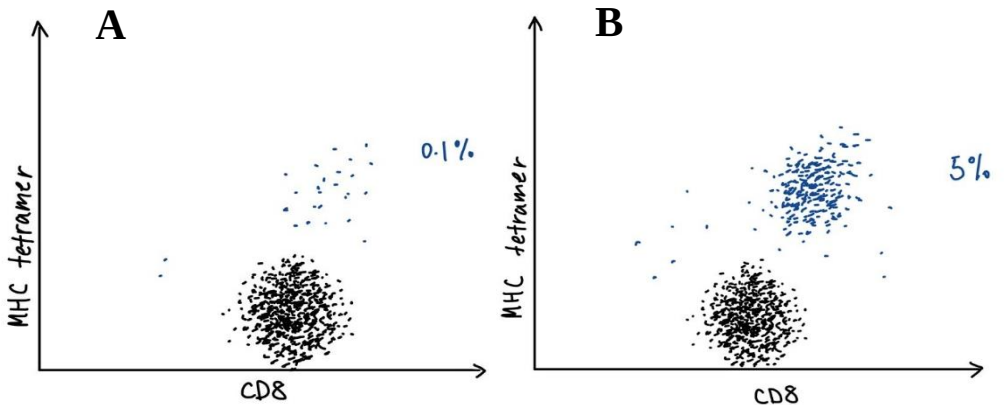

Figure 2. Detection of T-cells using FACS. FACS provides a method to sort a heterogeneous mixture of biological cells into two or more containers, one cell at a time, based on each cell's specific light scattering and fluorescent characteristics. (*A*) Modified HSV-1 with protein A. Little luminance is detected. Around 0.1% T-cells are detected. (*B*) Modified HSV-1 with protein A and NY-ESO-1 antibody. Significant rise in luminance. Around 5% of T-cells are detected.

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4.3. Experiment results

An approach called immunofluorescence (IF) staining is introduced to examine further the nature of the treatment shown in Figures 2 below [9]. Immunofluorescence staining is an excellent immunochemical technique that allows visualization of many different components (a wide range of antigens) in a tissue or a cell type. We find out that the best result since modified HSV-1 attacks tumor cells are those with protein A and NY-ESO-1 antibodies. They function as attacking neuroblasts but have minimal impact on normal brain tissues. Modified HSV-1 with protein A and without protein A groups are brought about mainly to illustrate the function of protein A and NY-ESO-1 antibody. (Fig. 3)

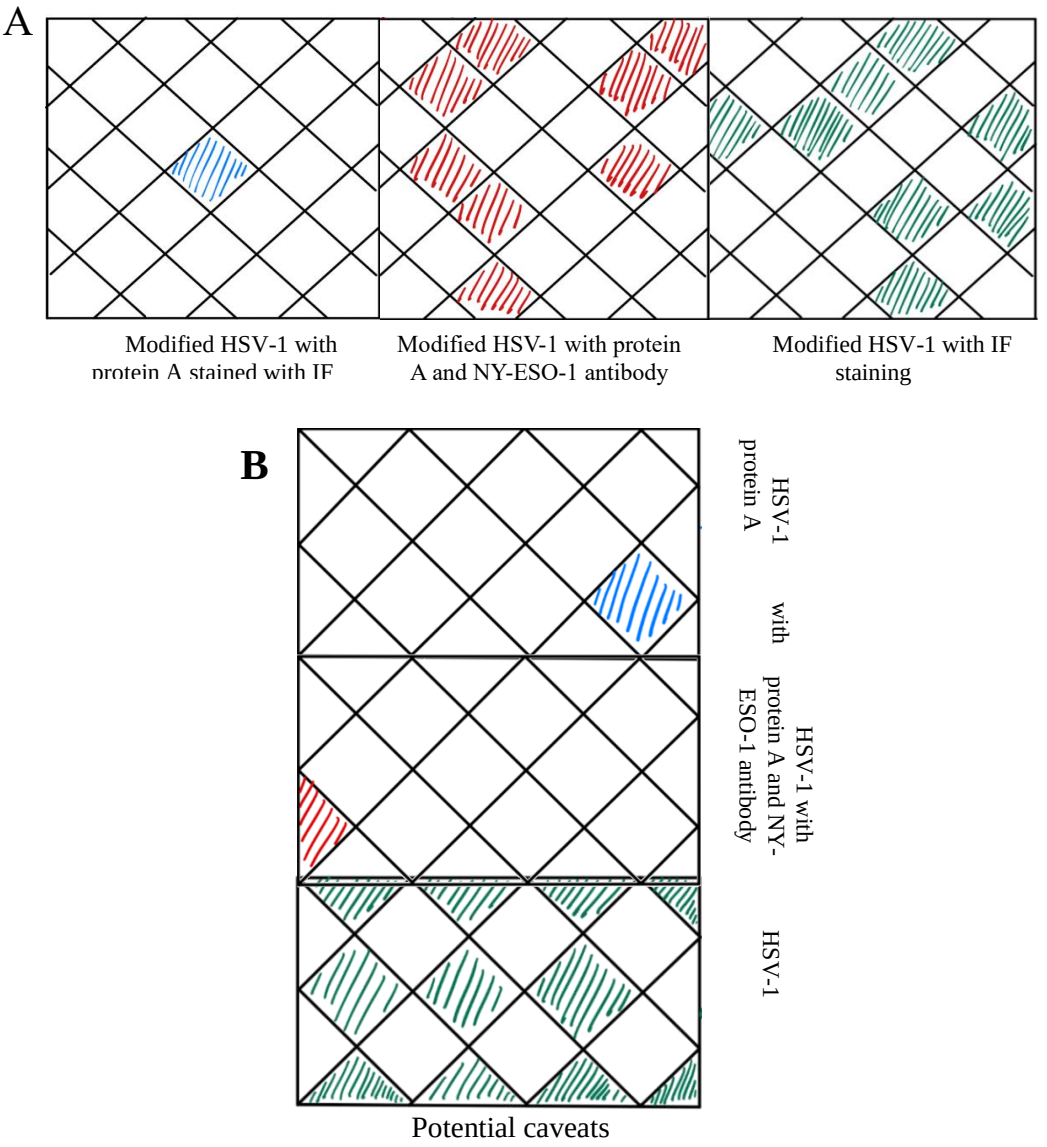

Figure 3. Immunofluorescence (IF) staining is introduced to examine further the nature of the treatment. IF staining is an excellent immunochemical technique that allows visualization of many different components. In this case we exam the staining property from both neuroblasts and normal neural cells with three different types of materials. (A) In neuroblasts, modified HSV-1 with protein A and NY-ESO-1 has a significant higher affinity due to the higher luminance. This indicate that NY-ESO-1 is a proper antigen found on neuroblasts, and the binding is efficient. (B) In normal neuro cells, only modified HSV-1 without protein A has high luminance. This indicate that NY-ESO-1 does not bind to normal neuro cells without specific antigen.

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4.4. No T cell response

In some circumstances, T cells do not come to the tumor site. This condition happens because GM-CSF, crucial in regulating the host's defense against pathogens, was not released from infected cells. Therefore, differentiation of macrophages and granulocytes won't happen.

4.5. No viral replication

There is an absence of important information (inactivation/loss of viral component) required for viral replication or the patient's immune system may neutralize HSV before its replication starts and apply its anti-tumor effects – the host immune response. Furthermore, for patients with pre-existing immunity to HSV, the patient's immune system will suppress the replication. Specific tumor microenvironments may also affect the virus' ability to proliferate and infect within the tumor.

4.6. Alternative method

There are two other potential curations of neuroblastoma tumors. The first hypothetical alternative is using adoptive T-cell therapy. In this therapy, we extract T- cells from the patient and modify or train their genes so they can recognize neuroblastoma tumor cells. After growing the T-cells in the lab, we insert them back into the patient to attack the tumor. However, when growing those T-cells, the patient would likely be required to take chemotherapy or radiation therapy. The other possible treatment involves modifying the HSV-1 virus to express peptides or ligands that target blood vessels associated explicitly with neuroblastoma. This aims to disrupt the blood supply to the tumor, impeding its growth and progression.

5. Conclusion

In conclusion, this article examines the possibility of using a new virus vector system to target neuroblastoma in patients who are considered high-risk specifically. Since one of the significant drawbacks of conventional treatment is how healthy brain tissue is also affected, we propose that this modified HSV can bind specifically to the NY-ESO-1 antigen in neuroblastoma and stimulate an adaptive immune response by releasing GM-CSF inside the cancer cell. This can be a breakthrough in treating neuroblastoma, as neuroblastoma is hugely prevalent in children, which means that their bodies will not be able to handle the dosage of chemotherapy and radiotherapy necessary to control the tumor. This treatment will be able to prevent children from unnecessary damage from chemotherapy while also working effectively enough to limit the growth of tumors. The anticipated results of the study suggest that there can be significant implications in using an oncolytic virus as a prominent way of cancer treatment as it possesses several advantages over chemotherapy and radiotherapy. The specificity will allow minimal damage to the patient's body, which also results in fewer side effects on the patient. The immunological memory also results in less likelihood of cancer returning to affect the patient again.

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Victor Zhao, Oscar Hu, Laura Li, and Elisa Pan contributed equally to this work and should be considered co-first authors.

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