UJEMI PEARLS

Herpes simplex virus 1 in oncolytic virotherapy for treatment of triple negative breast cancer

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SUMMARY Breast cancer is the leading cause of death in women under 40, with triple negative breast cancer (TNBC) being the most aggressive form of the disease with the poorest overall survival rates of all breast cancer types. TNBC represents 15-20% of all breast cancers, and the treatment options for advanced stage breast cancers including TNBC are limited, with survival rates hovering around 25% over the past two decades. Hence, there is an urgent need for development of therapeutic strategies to combat this deadly disease. Here, we have proposed the use of oncolytic herpes simplex virus 1 (oHSV-1) for treatment of advanced stage TNBC. The large genome of oHSV-1 allows for flexibility in engineering transgenes in the oncolytic virus (OV), such as the 15-hydroxyprostaglandin dehydrogenase, which degrades tumor-promoting prostaglandin E2. The oHSV-1 can be further modified to specifically target TNBC cells by engineering of the gD glycoprotein to target the androgen receptor, which is overexpressed in TNBC cells. oHSV-1 facilitates killing of TNBC cells both by oncolysis and by inducing an antitumor immune response that increases infiltration of CD8+ T-lymphocytes for cytotoxic killing of tumor cells. While TNBC cells may develop immune resistance by expressing the immune checkpoint molecule programmed cell death ligand 1 (PD-L1), this resistance can be mitigated by using oHSV-1 in combination with an immune checkpoint blockade such as anti-PD-1 antibodies. Finally, to improve the effectiveness of oHSV-1 as a therapeutic agent post intratumoral administration, we propose using mesenchymal stem cells (MSCs) as cell carriers of the OV to improve its systemic delivery, in addition to modifying the oHSV-1 surface proteins to express polyethylene glycol to reduce sequestration by the mononuclear phagocytic system in the liver and spleen. Therapeutics for TNBC are most effective early in the disease, highlighting the importance of future work to explore strategies for early diagnosis of TNBC, which will allow for increased effectiveness of our proposed oHSV-1 treatment, ultimately leading to improved overall prognosis and outcomes of individuals with TNBC.

INTRODUCTION

Breast cancer is the most common malignancy in women around the world (1), with the disease being the leading cause of death in women under 40 (2). Triple negative breast cancer (TNBC) is androgen receptor-positive (3) but lacks expression of the estrogen receptor, progesterone receptor, and epidermal growth factor receptor 2 (4). TNBC is the most aggressive form of breast cancer and is often observed in individuals carrying a mutation in the BRCA1 gene (4). Current treatments for breast cancer include cytotoxic, immunotherapeutic, and hormonal approaches, which have limited efficacies in treating TNBC and advanced stage metastatic disease, for which patients have less than 25% survival rates (2). Further, current therapies are limited in their effectiveness due to the heterogeneity of TNBC as well as permanent side effects such as potential cognitive impairment (5) and therapeutic resistance. Therefore, there is an urgent need to address the treatment of TNBC using additional therapeutic strategies. Oncolytic viruses (OV) are antitumor viruses that selectively infect and kill tumor cells while sparing normal host cells (6). During cellular transformation, tumor cells acquire changes that distinguish them from normal cells, including overexpression of certain cell surface antigens and downregulation of the interferon response (5), both of which are seen in TNBC. These changes allow for

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specific recognition and targeting by OVs. Herpes simplex virus 1 (HSV-1) is an enveloped double-stranded DNA virus with over 80 genes (7), allowing flexibility in target genes for engineering an oncolytic herpes simplex virus 1 (oHSV-1). oHSV-1 kills tumor cells both by oncolysis and by increasing the CD4+ and CD8+ T-lymphocyte density in addition to inducing CD8+ T-lymphocyte infiltration to the tumor site, generating an antitumor immune microenvironment (8). Previous work has demonstrated the efficacy of using oHSV-1 to target tumor cells, for example by engineering oHSV-1 to target cells that are deficient in the interferon pathway (9). This has led to the development of a clinical trial evaluating the oHSV-1 talimogene laherparepvec (T-VEC) which demonstrated complete regression in 8 of 50 cases of melanoma (10). One of the most promising aspects of using oHSV-1 for therapeutics is the presence of acyclovir, an FDA-approved anti-HSV drug (11), which is a nucleoside analogue that allows for the blockage of HSV replication. This provides the ability to terminate oHSV-1 replication in patients, if necessary, at any time point during the treatment regimen.

RESEARCH QUESTIONS

There is a clear deficit in therapeutics for treatment of TNBC, with minimal progress in this field for the past two decades (5). New strategies, such as the use of oHSV-1, a promising OV for the treatment of melanoma, must be explored to determine their applicability and effectiveness in treating TNBC. However, there are various considerations that must be accounted for during the development of oHSV-1 for oncolytic virotherapy, including the specific engineering of oHSV-1, the potential for combination therapy, and strategies to increase the effectiveness of oHSV-1 once administered. To address these topics, this article will focus on the following three research questions:

1. How can oHSV-1 be engineered to target TNBC cells?
2. How can oHSV-1 be used in combination therapy for treatment of TNBC?
3. How can the therapeutic potential of oHSV-1 be maximized?

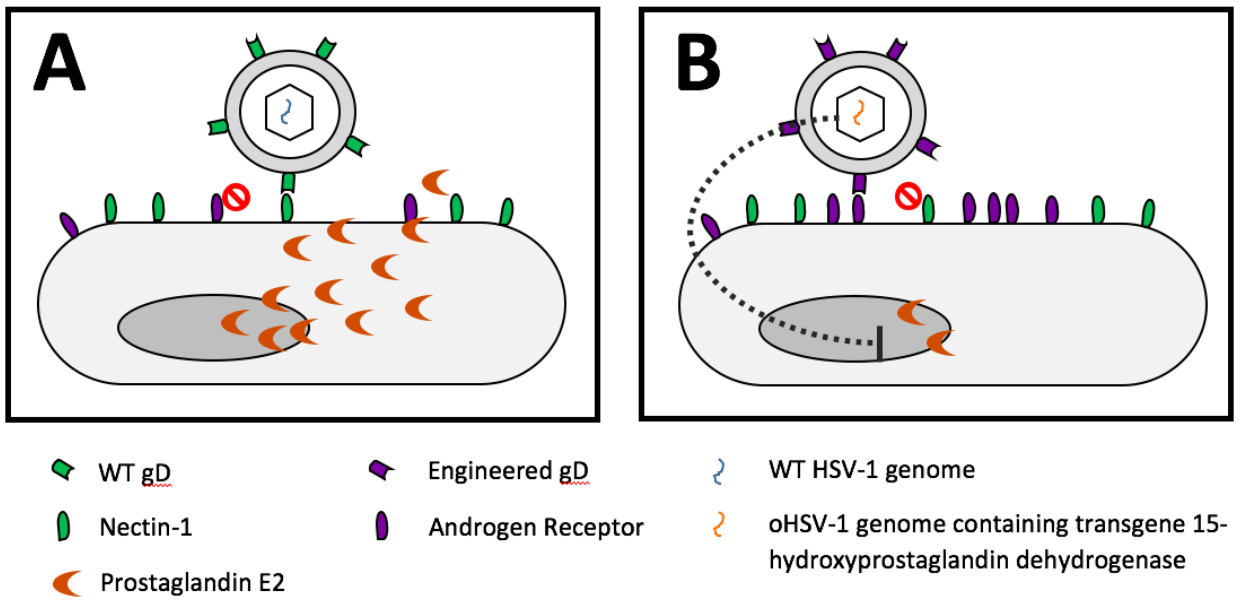
PROJECT NARRATIVE

How can oHSV-1 be engineered to target TNBC cells? A promising aspect of OVs is its ability to target, replicate in and kill tumor cells while sparing normal cells (6). While this is an essential property of OVs (12, 13), their effectiveness can be enhanced by engineering. Although OVs are specific in their targeting and will spare normal noncancerous cells, there may be increased off-target replication of oHSV-1 in patients who are immunocompromised, especially if oHSV-1 is administered systemically (10). One strategy to mitigate this issue is to modify the normal tropism of oHSV-1. By performing a knockout of the γ134.5 gene, the replication of oHSV-1 is no longer able to occur in neurons (2). To further restrict oHSV-1 replication to TNBC cells, the OV can be engineered to specifically target TNBC cells (10, 14-16), such as by modifying and retargeting the entry mediator glycoprotein gD to bind to the androgen receptor, which is specifically overexpressed in TNBC (3). Engineering of OVs is not limited to modification of the viral entry receptors: OVs can be "armed" with antitumor transgenes that contain additional therapeutic power. Prostaglandins are lipid autacoids synthesized in the human body which modulate the inflammatory response (17). Prostaglandin E2 plays a large role in cancer, promoting tumor formation, progression, and metastasis by acting directly on cancer cells (18). Thus, one strategy to reduce tumorigenesis and tumor progression is to reduce the levels of prostaglandin E2 in the body. To achieve this, oHSV-1 can be armed with the 15-hydroxyprostaglandin dehydrogenase transgene, which encodes an enzyme that degrades prostaglandin E2 (19). By engineering oHSV-1 to restrict its replication to TNBC cells and to express an enzyme that decreases the levels of tumor-promoting prostaglandin E2, we are able to specifically target TNBC cells and decrease the progression of cancer. Due to the heterogeneity of breast cancer, it is important to note that the engineered oHSV-1 must be tailored to the type of breast cancer. For example, modifying glycoprotein gD to target the androgen receptor is only effective for TNBC; other types of breast cancers express different surface receptors such as human epidermal growth factor receptor 2 (HER2) and would require engineering the OV to specifically target HER2 (16).

How can oHSV-1 be used in combination therapy for treatment of TNBC? While using oHSV-1 to target oncolysis in breast cancer cells to decrease cancer progression is a promising strategy, it is not without limitations. Immune checkpoints, which are inhibitory signals for T-cell receptor (TCR) antigen recognition and activation, are tightly regulated for maintenance of self-tolerance (20). Cancer cells, including TNBC, have exploited this mechanism as a strategy to confer immune resistance, and this is particularly prominent in highly immunogenic cancers (20). For example, the programmed cell death 1 (PD-1) and programmed cell death ligand 1 (PD-L1) molecules, the latter being overexpressed in the tumor microenvironment especially after administration of an OV, have been proposed to be a mechanism for resistance to oncolysis and for preventing TCR activation (21). Accordingly, the effectiveness of using oHSV-1 in isolation to treat TNBC may be compromised by the ability of the cancer cells to upregulate PD-L1, leading to decreases in oncolysis and immune activation. One potential strategy to address this challenge is the use of oHSV-1 in combination with an immune checkpoint blockade such as anti-PD-1 antibodies. Recent work has demonstrated the effectiveness of intratumoral injection of oHSV-1 in combination with an immune checkpoint blockade in promoting an antitumor response (22, 23). It was shown that T-VEC (the first FDA-approved oHSV-1) altered the tumor microenvironment of patients with advanced melanoma by increasing the levels of circulating CD4+ and CD8+ T-lymphocytes as well as increasing tumor infiltration of CD8+ T-lymphocytes which express PD-1 (21). While this increased concentration of T-lymphocytes in the tumor microenvironment should enhance the antitumor immune response, tumor cells expressing PD-L1 can escape TCR activation. Introducing a PD-1 blockade such as pembrolizumab into the treatment regimen in combination with oHSV-1 alleviates this concern and allows for improved clinical outcomes compared to the use of either treatment in isolation, including increased tumor regression and sensitivity to PD-1 blockades (21).

Similarly, combination therapy using Newcastle disease virus as the OV and anti-cytotoxic T-lymphocyte associated protein 4 (CTLA-4) antibodies as the immune checkpoint blockade has also been shown to be effective at achieving tumor rejection (24). The suggested mechanism of action highlights the synergistic effects of using these two

FIG. 1 Engineering of oHSV-1 to target TNBC cells. Panel **A**: WT HSV-1. Glycoprotein gD binds to nectin-1 but not androgen receptor. The tumor cell produces tumor-promoting prostaglandin E2. Panel **B**: oHSV-1. Engineered gD binds to overexpressed androgen receptor. Armed oHSV-1 contains transgene 15-hydroxyprostaglandin dehydrogenase which reduces expression of tumor-promoting prostaglandin E2.



therapeutics in combination. While the OV induces inflammatory immune infiltration in distant tumors, creating an inflammatory phenotype at distant and metastatic lesions that increase their susceptibility and sensitivity to CTLA-4 blockades (24), the immune checkpoint blockade reduces immune escape by the tumor cells (20, 21). It has been demonstrated that this combinatorial approach does not increase toxicity compared to single-agent therapy (21) and thus the proposed use of oHSV-1 and PD-1 blockade is a very promising combination therapy to be further explored in treatment of TNBC.

How can the therapeutic potential of oHSV-1 be maximized? Development of therapeutic strategies to target TNBC is at the forefront. While T-VEC, the oHSV-1 that has been in clinical trials, demonstrated effectiveness in cancer regression (10), its intratumoral administration restricts the widespread delivery of the OV, and thus limits its potential for treating advanced stage TNBC. Further, it is known that systemic delivery of therapeutic agents such as oHSV-1 has limited efficacy because it is rapidly cleared by the body as a result of sequestration by the mononuclear phagocytic system in the liver and spleen (25). Two challenges that must be overcome are identified here. First, a strategy for effective systemic delivery of oHSV-1 must be developed. Second, once the oHSV-1 is delivered systemically, sequestration of the OV by splenic and hepatic macrophages must be reduced.

To enhance the systemic delivery of oHSV-1, the OV can be packaged with carrier cells, which are engineered cells that transport the OV to the tumor site, thus improving the delivery of OVs to target cells (8, 26-27). One strategy to achieve systemic delivery is to take advantage of the tumor microenvironment which can be used to attract carrier cells (26). In this approach, mesenchymal stem cells (MSCs) can be used as cell carriers of oHSV-1. MSCs accumulate in the tumor stroma due to the hypoxic and pro-inflammatory characteristics of the tumor microenvironment (26). In this way, in addition to engineering the oHSV-1 to target TNBC cells specifically, the use of MSCs as cell carriers for oHSV-1 can improve its infiltration and delivery to the tumor location, reducing immune-mediated inactivation of the OV (8).

Following successful delivery of oHSV-1 to the tumor site, there must be minimal sequestration of the OV by the mononuclear phagocytic system in the liver and spleen in order to maximize the effectiveness of oHSV-1 as a therapeutic agent. Sequestration is normally mediated by opsonization with antibodies, complement, coagulation factors, and other serum proteins which facilitate recognition be splenic and hepatic macrophages (25). One strategy to alleviate this challenge is by modifying the viral coat proteins on oHSV-1 with biocompatible polymers, such as polyethylene glycol (PEG) (28). This reduces the ability of the splenic and hepatic macrophages to opsonize and sequester the OV. The PEG-modified oHSV-1 viral coat proteins should also be engineered to express the target cell receptor binding ligands (28) in order for the PEG modification to not alter the tropism, targeting and infectivity of the OV.

Although oHSV-1 is very promising in the treatment of TNBC, its effectiveness is greatly decreased if there is no method of facilitating its systemic delivery and reducing its sequestration. By PEGylation of the oHSV-1 viral coat proteins while restoring its target cell receptor binding ligand onto the surface of the PEG molecules, and using MSCs as cell carriers of oHSV-1 to improve delivery and infiltration to the tumor location, we have highlighted here a promising strategy to address these two challenges.

CONCLUSIONS

The current treatments for TNBC only allow for survival rates of 25%, and this number has not changed over the past two decades (2), deeming TNBC the most fatal form of breast cancer (29). Therefore, timey advancement in therapeutic strategies for this deadly disease is critical. We have proposed the use of oHSV-1 for treatment of advanced stage TNBC. The advantage to using oHSV-1 is that its large genome facilitates flexibility in engineering transgenes to allow for lysis of TNBC cells and to enable degradation of tumor-promoting factors. In addition to oncolysis, oHSV-1 facilitates killing of TNBC cells by inducing an antitumor immune response by increasing CD8+ T-lymphocyte infiltration for cytotoxic killing of tumor cells. The challenge of TNBC cells evading immune recognition by hijacking the immune checkpoint mechanism can be addressed by using oHSV-1 in

combination with an immune checkpoint blockade such as anti-PD-1 antibodies. Finally, enhanced systemic delivery of oHSV-1 and reduced sequestration by the mononuclear phagocytic system were identified as novel approaches to improve the effectiveness of oHSV-1 as a therapeutic agent.

There are multiple approaches to further modifying the therapeutic potential of oHSV-1. For example, previous work has shown that oHSV-1 may be used in combination therapy with doxycycline, an antibiotic that was shown to increase cellular caspase 8 expression, leading to increased apoptosis which facilitates enhanced oHSV-1 infection and spread (30). While there may not be one perfect combination or treatment, there is great potential for oHSV-1, and immunotherapy in general, in targeting breast cancer, as demonstrated by the recent FDA approval of the Tecentriq-Abraxane combination immunotherapy (31). Despite these promising therapeutic strategies, it is important to realize that therapeutics for TNBC are most effective early in the disease (22). Recently, the use of noninvasive strategies such as liquid biopsies for detection of biomarkers in disease progression has been gaining momentum in the field. Work in early diagnostics for TNBC should be rapidly explored in this area, such as using liquid biopsies to allow for early detection of disease biomarkers for early diagnosis of the disease, which will facilitate earlier administration and increased effectiveness of the proposed oHSV-1 treatment regimen, translating into improved prognosis and clinical outcomes of individuals with TNBC.

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