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New Horizons in Oncolytic Virotherapy for Osteosarcoma

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Abstract: Osteosarcoma is a highly aggressive malignant bone tumor characterized by an immunosuppressive microenvironment, with the five-year survival rate of metastatic patients remains dismally low. Oncolytic virotherapy has emerged as a promising strategy, utilizing both direct tumor lysis and immunity activation. This review systematically examines the latest advancements in oncolytic virus monotherapy Oncolytic monotherapy and combination therapy for osteosarcoma, drawing from preclinical models (e.g., mouse and canine studies) and clinical trials. In monotherapy, genetically engineered viruses such as VCN-01 (targeting integrin αvβ3/5 via an RGD sequence and secreting hyaluronidase for stromal degradation) and Delta-24-ACT (expressing 4-1BBL to enhance T-cell activation) demonstrated significant tumor reduction and prolonged survival. VSV-IFNβ-NIS was well tolerated in canine models, improving long-term survival and upregulating T-cell immune responses. In combination therapy, oncolytic viruses enhanced the efficacy of chemotherapy (e.g., OBP-702 with doxorubicin), radiotherapy (e.g., E4orf6 virus), targeted drugs (e.g., VSVΔ51 with PI3K inhibitor ZSTK474), and immune checkpoint inhibitors (e.g., CAV2-AU-M2 delivering anti-PD-1 antibodies). Despite these promising outcomes, challenges such as viral toxicity, limited delivery modes, and species-specific variability remain. Future strategies may include gene editing for improved tumor targeting, oncolytic virus-T-cell chimeras, and novel systemic delivery approaches to enhance therapeutic efficacy. Oncolytic virotherapy, in combination with multidisciplinary treatments, holds great potential to overcome current limitations amd improve survival outcomes for vosteosarcoma patients.

Keywords: Osteosarcoma, Oncolytic virotherapy, Combination therapy

1. Introduction

Osteosarcoma is the most common primary malignant bone tumor, characterized by a bimodal age distribution, with peak incidence occurring in adolescents (15-25 years) and older adults (>60 year). The incidence in children and adolescents is approximately 3-4.5 cases per million per year, accounting for 5% of pediatric malignancies [1, 2]. While the five-year event-free survival rate for non-metastatic osteosarcoma is approximately 60%, the prognosis for metastatic cases remains poor. At initial diagnosis, 10% to 20% of patients present clinically detectable metastases, most commonly in the lungs, and the five-year survival rate for metastatic osteosarcoma is less than 30% [1, 2].

The current standard treatment involves extensive surgical resection combined with neoadjuvant/adjuvant chemotherapy, primarily using the MAP regimen (methotrexate, dobifloxacin, and cisplatinis). Radiotherapy is generally reserved for unresectable tumors in the pelvis or

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craniofacial regions [1]. Despite some advances, targeted therapy and immunotherapy for osteosarcoma remain challenging due to the tumor's genetic heterogeneity, immunosuppressive microenvironment, and intrinsic drug resistance. Consequently, overall survival rates for osteosarcoma have not significantly improved over the past four decades, underscoring the urgent need for novel therapeutic strategies [2].

This article focuses on the potential of oncolytic virus therapy in osteosarcoma treatment, evaluating its clinical prospects and discussing research directions in this emerging field.

2. Characterization and mechanism of action of oncolytic viruses

Oncolytic viruses are classified into naturally occurring and genetically engineered types. The latter are modified to enhance tumor-selective cytotoxicity and immune activation, reducing damage to normal cells simultaneously [3, 4]. Moreover, these modifications enable the viruses to evade host immune recognition, prolonging their persistence in the body and enhancing therapeutic efficacy. [4].

Oncolytic viruses exert anti-tumor effects through four primary mechanisms.

First, they directly induce tumor cell lysis. Many tumor cells harbor specific mutations that impair antiviral signaling pathways, rendering them more susceptible to viral replication and selective oncolysis [4, 5]. Second, oncolytic virotherapy activates anti-tumor immunity and modulates the tumor microenvironment, particularly by converting "cold tumors" into "hot tumors", thereby enhancing immune responses. Specifically, oncolytic viral infection leads to the release of tumor-associated antigens and cellular debris, which are processed by dendritic cells and presented to T cells, activating tumor-specific immune responses.Additionally, during virus infection, the release of damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs) induces cytokine production, and immune cell infiltration, and activation, ultimately promoting immunogenic cell death (ICD) of tumor cells. This process further enhances the anti-tumor immune response [2, 4, 5]. Lastly, oncolytic viruses can serve as gene therapy vectors to deliver transgenes encoding therapeutic proteins, further enhancing their anti-tumor effects.

These multifaceted mechanisms have made oncolytic virotherapy a promising strategy for osteosarcoma treatment.

3. Advances in oncolytic virus therapy for osteosarcoma

To date, a number of preclinical and clinical studies on oncolytic virus therapy for osteosarcoma have been conducted, demonstrating promising results. This section focuses on the research progress of several genetically engineered oncolytic viruses.

3.1. Monotherapy

VCN-01 is a genetically modified adenovirus derived from human adenovirus type 5, engineered to enhance tumor cell infection via integrin αvβ3/5, bypassing the CAR receptor. Additionally, its fiber protein's heparan sulfate glycosaminoglycan (HSG)-binding domain has been replaced with an RGD sequence, further enhancing its tumor cell infectivity. The E1A gene of VCN-01 contains a 24-bp deletion in the pRb-binding site, ensuring that viral replication and oncolysis occur only in pRb-deficient cells. In addition, VCN-01 encodes a secreted human PH20 hyaluronidase, which degrades hyaluronic acid (HA) in the tumor extracellular matrix, facilitating viral dissemination within tumor tissues [6]. In preclinical studies of osteosarcoma, a mouse model transplanted with human primary osteosarcoma cells received intravenous VCN-01 injections at doses of 10^7 or 10^8 PFU (Plaque Forming Units) over four administrations, administered on days 7 and 14 post-tumor inoculation. In preclinical studies, VCN-01 significantly reduced tumor size in mouse models [7]. At Day 60, the tumor surface area in mice injected with 10^7 PFU VCN-01 was 0.14%, compared to

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2.06% in the control group. Similarly, in the 10^8 PFU VCN-01 group, the tumor surface area was 0.4%, further highlighting its therapeutic efficacy [7].

Delta-24-ACT, a genetically engineered variant of the oncolytic adenovirus Delta-24-RGD, is modified by inserting the 4-1BB ligand (4-1 BBL) gene into the E3 locus via homologous recombination to enhance immunostimulatory effects. As a co-stimulatory molecule, 4-1BBL expression promotes T-cell activation, proliferation, and anti-tumor immune responses. [8]. In in vitro experiments, Delta-24-ACT effectively infected and lysed both human and murine osteosarcoma cells, inducing immunogenic cell death (ICD), as evidenced by ATP release, HSP-90α release, and calreticulin (CRT) translocation to the cell membrane. In in vivo studies, Delta-24-ACT treatment significantly increased median survival time, reduced lung metastases, and induced long-term anti-tumor immune memory [8].

A study evaluating the safety and long-term efficacy of systemic oncolytic virotherapy in canines with naturally occurring osteosarcoma demonstrated its potential to improve clinical outcomes, particularly in targeting primary and metastatic tumors while eliciting durable anti-tumor immune responses [9]. In this study, a recombinant vesicular stomatitis (VSV) expressing interferon-β (IFN-β) and the sodium-iodine symporter (NIS) gene (VSV-IFNβ-NIS) was developed to enhance both direct tumor lysis and immune activation [9]. VSV-IFNβ-NIS selectively replicates in tumor cells, promoting oncolysis while IFN-β expression enhances anti-tumor immune responses. The NIS gene further enables radioiodine-based imaging and potential radioisotope therapy. The study found that VSV-IFNβ-NIS treatment was well tolerated, with no serious adverse events observed. Moreover, the proportion of long-term survivors was higher in the treatment group than in the control group, demonstrating potential efficacy. Meanwhile, RNA sequencing (RNA-seq) analysis showed increased expression of T-cell-anchored immune gene clusters in all long-term responders, suggesting that VSV-IFNβ-NIS may enhance anti-tumor immunity, offering a new direction for future therapy [9].

3.2. Combination therapy

Due to the highly heterogeneous, drug-resistant, and immune-evasive nature of osteosarcoma, the anti-tumor effects of single-agent oncolytic virus therapy are limited [10]. Therefore, combination therapy has emerged as a novel approach, integrating chemotherapy, radiotherapy, targeted drugs, and immune checkpoint inhibitors to significantly improve the therapeutic efficacy of osteosarcoma.

Combining oncolytic viruses with chemotherapy can reduce the potential complications of oncolytic viruses and the toxic side effects of chemotherapeutic agents. Moreover, it maintains overall treatment efficacy, decreases the likelihood of chemoresistance, and may even reverse it in tumors. [4, 11]. For instance, OBP-70, a oncolytic adenovirus expressing the tumor suppressor p53, has been shown to reduce the number and viability of both DOX-sensitive and DOX-resistant osteosarcoma cells in a dose-dependent manner when administered alongside a fixed dose of DOX in human osteosarcoma cell lines. Additionally, OBP-702, an oncolytic adenovirus, significantly reduced the number and viability of DOX-resistant osteosarcoma cells. DOX-resistant osteosarcoma cells exhibited high levels of multidrug resistance protein 1 (MDR1), whereas OBP-702 enhanced DOX-induced apoptosis. The combination of OBP-702 and DOX significantly inhibited tumor growth compared to monotherapy, making it a promising strategy to reverse chemoresistance in osteosarcoma patients by inhibiting MDR1 expression in an MNNG/HOS xenograft tumor model [11]. Thus, chemotherapy plays an important role in the treatment of osteosarcoma and has a promising prospect for development [12].

The combination of mycoviruses with radiotherapy can enhance cell lysis through radiation synergy or increase virus-mediated cellular sensitivity to radiotherapy [4, 5]. A preclinical study demonstrated that a novel oncolytic adenovirus, E4orf6, combined with radiotherapy significantly

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increased apoptosis rates and inhibited the proliferation of osteosarcoma cells in vitro, broadening the scope idea of combining oncolytic viruses with other therapies [4, 13].

Targeted drugs can enhance the efficacy of oncolytic viruses through synergistic mechanisms. For example, inhibition of the PI3K-Akt pathway not only induces endoplasmic reticulum stress but also significantly enhances virus-induced apoptosis, thereby enhancing the cytotoxic effects of oncolytic viruses. Studies have shown that the combination of the oncolytic VSVΔ51 with the PI3K inhibitor ZSTK474 exhibited significant cytotoxicity against osteosarcoma cells in vitro. In addition, in vivo experiments demonstrated increased immune effector cell infiltration in tumor tissues and greater tumor suppression in the combination therapy group [14]. Another example involves the use of the Semliki Forest Virus (SFV) expressing an inhibitor of galactose lectin-3 (Gal3), a protein upregulated in osteosarcoma and other tumors which promotes cell adhesion, migration, invasion and chemoresistance. Vivo studies showed that SFV expressing Gal3-N-C12 (a peptide inhibitor of Gal-3) induced strong antitumor responses in situ in K7M2 and MOS-J osteosarcoma models, leading to complete tumor regression in 47% and 30% of mice, respectively [15]. This therapy combination significantly enhances the anti-tumor effects of the oncolytic virus therapy and provides a novel strategy for osteosarcoma treatment.

Well-studied tumor immunotherapy strategies include immune checkpoint inhibitors, adoptive immune cell therapy, cytokine therapy, oncolytic virus therapy, and tumor vaccines [5]. However, due to the complexity and regulation of the immune system, single-agent immunotherapies often suffer from low response rates and significant individual variability, limiting their clinical applicability [5,16]. Since oncolytic viruses can stimulate anti-tumor immunity and enhance the response to tumor immunotherapy, combining oncolytic viruses with immunotherapy can significantly improve therapeutic efficacy.

In combination with immune checkpoint inhibitors, Programmed death-ligand 1 (PD-L1) is positively associated with tumor metastasis and recurrence in osteosarcoma, highlighting the importance of immune checkpoint inhibitors in osteosarcoma treatment [17]. Conditionally replicating adenoviruses (CRAds) have emerged as promising cancer therapeutic tools due to their selective replication in tumor cells, efficient gene delivery, robust transgene expression, broad tropism, and ability to induce effective anti-tumor immune responses [18]. Notably, when combined with immune checkpoint inhibitors, CRADs exhibit enhanced therapeutic efficacy [18]. For example, CAV2-AU-M2, a modified canine adenovirus with an E3 deletion and insertion of anti-PD-1 single domain antibody (sdAb) and DsRed coding sequences, demonstrated selective replication in canine osteosarcoma cell lines and induced effective tumor cell lysis in in vitro [18]. This virus also produces anti-PD-1sdAb locally within the tumor microenvironment upon infection, minimizing the systemic side effects associated with immune checkpoint inhibitors [18]. In addition, telomerase-specific oncolytic adenovirus OBP-502 has been shown to enhance the effect of. PD-1 blockade in murine osteosarcoma models by inducing immunogeic cell death (ICD). When combined with an anti-PD-1 antibody, OBP-502 significantly increased tumor-infiltrating CD8+ T cells, thereby significantly inhibiting K7M2 and NHOS tumor growth [19].

Oncolytic-based therapies have also been explored in combination with adoptive immune cell therapy for osteosarcoma treatment. Studies have shown that oncolytic-iduced adoptive cell therapy prolonged survival in rat models, while IL2-armed oncolytic promoted T-cell infiltration and the generation of tumor-reactive tumor-inflitrating lymphocytes (TILS) in low-immunogenic tumors, offering new insight for the treatment of osteosarcoma [20]. Furthermore, a strategy involving oncolytic expressing bispecific T-cell engagers (BiTEs) has been investigated. By inserting the full-length BiTE sequence between the viral glycoprotein and polymerase genes, researchers constructed VSVΔ51/BiTE [21]. The virus significantly inhibited osteosarcoma growth in mice while activating anti-tumor immune responses, making it a promising therapeutic strategy [21].

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Cytokine-based combination therapy has also been explored, with granulocyte colony-stimulating factor (G-CSF) serving as an example. Since osteosarcoma cells typically exhibit defects in the retinoblastoma (Rb) pathway, oncolytic adenoviruses (OAds) can be engineered to selectively target virally-infected human mesenchymal stem cells (MSCs) and administered in combination with G-CSF [22]. G-CSF is commonly used to reduce neutropenic complications in high-risk cancer patients and has shown efficacy in treating osteosarcoma [23]. Both in vitro and in vivo studies utilizing the OAd dlE102-MSC model for osteosarcoma treatment have shown favorable results. When combined with G-CSF, tumor size was reduced, and survival rates improved in OS mouse models models. Moreover, OAd-MSCs and combination therapy induced lymphocyte infiltration, especially TILs, into tumor tissues, thereby improving the immunosuppressive tumor microenvironment of osteosarcoma [22].

4. Conclusion

Oncolytic virotherapy, a treatment with a 100-year history of development, has emerged as one of the most promising anti-osteosarcoma immunotherapies. Despite the exciting results in terms of preclinical and clinical data, several challenges remain. In terms of safety, the virus exhibits certain toxic side effects on the organism due to off-target effects, mutations, proliferation, and other uncontrollable factors in terms of delivery method, the most commonly used method is intratumoral injec. Regarding the delivery method, intratumoral injection is most commonly used; however, this approach limits application in some deep metastases and other sites. Intravenous injection, on the other hand, results in rapid virus clearance due to the virus's strong immunogenicity, thereby limiting efficacy. The tumor microenvironment of osteosarcoma itself is highly immunosuppressive, further restricting the use of oncolytic virus therapies. Addtionally, the animal models used in preclinical studies differ from human in species, which can affect drug development and application.

Currently, several promising oncolytic therapies for the treatment of osteosarcoma are being explored, including the design of synovial adenoviral platforms, the use of viruses as vectors for the precise delivery of antisense peptide nucleic acids to regulate the overexpression of PD-L1 in cancer cells, and the utilization of oncolytic virus-T-cell chimerism to enhance the immune response to the target site. Moreover, the rapid development of gene editing technology and the in-depth understanding of viral gene structure and expression have enabled the regulation of lysovirus gene expression, providing a relatively reliable guarantee for the effectiveness and safety of oncolytic therapy.

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