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The Potential, Challenges and Solutions of Oncolytic Virotherapy

Xiyao Ling[1, *], **Xiaotong Sun**[2]

[1]Yali High School, Changsha, 410007, China
[2] First school of clinical medicine, Hebei North University, Zhangjiakou, 225800, China
*Corresponding author: lingxiyao1126@163.com

Abstract:

The traditional therapy of cancer has some limitations, for example the poor targeting, high toxicity, limited curative effect of the later period and so on. However, the oncolytic virus therapy can greatly kill the tumor cells. The OVs mainly use the direct dissolution of the tumor cells and the remodeling of TME to activate the anti-tumor activity to kill the tumor cells. The review generally finds out the oncolytic virus therapy still has three limitations. Firstly, The low targeting efficiency of OVs. Secondly, the inhibiting effect of TME on OVs. Thirdly, immune clearance and drug resistance. The review also finds three ways to help improve the curative effect of tumors such as Virus Modification, Regulating the tumor microenvironment and Immunological enhancement. The review thoroughly explores the killing mechanism of OVs, three present defects and three solutions. The exploration provides theoretical basis and thinkings for exploiting more curative oncolytic virus therapies in the future.

Keywords: Oncolytic therapy; Limitations; Improvement strategies.

1. Introduction

Tumors are still the main reason causing cancer-related death globally. The therapy of cancer has been facing multiple challenges. Traditional therapies with low targeting efficiency may lead to toxic reactions; the curative effect of radiation therapy is limited; the efficiency of immune checkpoint inhibitors are relatively low. All these challenges foster researchers explore new therapy, including the oncolytic virus (OV) therapy, which can selectively infect and lyse the tumor cell and activate the anti-tumor immune responses. The efficiency of the OV therapy depends on the replication capacity of OVs, tumor microenvironment (TME), and immune activation. The limitation of the single treatment strategy can be overcome through the multi-dimensional strategy of " virus modification + TME regulation + immune activation ".

The essay aims to systematically sort out the main challenges of oncolytic virotherapy, integrate the existing solutions and clinical evidence, and illustrate the necessity of the multi-dimensional strategy to provide a theoretical framework for the clinical translation of oncolytic virotherapy, promoting the advances in the cancer treatment.

2. The Killing Mechanism of Onco-

lytic Virus

OV is a promising method for curing the malignant tumors. OV can reach the targeted therapeutic effect by the direct dissolution of tumor and inducing specific anti-tumor immunity.

The main anti-tumor mechanism used by OVs are the direct dissolution of tumor and the remodelling of TME to activate the anti-tumor activity [1].

2.1 The Direct Dissolution of Tumor

OVs can selectively infect, replicate, split and kill cells by the direct cytolytic activity. After being infected with OVs, the virus can replicate in the tumor cells and cause dissolution of tumor [2]. When the tumor cells infected with virus are dead, the dead tumor cells will continue releasing new viruses to infect the nearby tumor cells. while the virus cannot replicate in the normal cells and even killing normal cells.

The mechanism of direct dissolution of tumor used by OVs is firstly much of them keeping replicating, then the injure of lysosome, autophagy, cell dissolution is immediately following. Secondly, OVs change their permeability and mitochondrial metabolism to conduct cell dissolution. Finally, OVs can induce the stress reaction of tumor cells, then followed with the cell dissolution and combining the superficial protein with TRAILR1 and TRAILR2 receptor, activating TRAIL directed cell apoptosis [1].

2.2 Reshaping TME to Activate the Anti-tumor Activity

The dissolution of tumor cells releases tumor antigen, the splitting tumor cells produce Damage-Associated Molecular Patterns (DAMP), Tumor-Associated Antigen (TAA), Pathogen-Associated Molecular Pattern and Immunostimulatory cytokine, triggering the anti-tumor immunoreaction [2]. These anti-tumor immunoreaction may turn Cold TME into Hot TME, then recruiting immune cells, for example DC cells, T cells, macrophage and NK cells [3].

2.2.1 DC Recruitment/T Cells Activation

OVs cause the Endoplasmic reticulum (ER) to stress, then followed with the cell apoptosis, the release of DAMP, TAA, DAMP and the activation of innate immunity. PAMP and virus combine with Toll Like Receptors (TLR) together become the reason of DC cells' maturity and cytokines' liberation [4].

After mature DC cells have taken in and processed the antigen, DC cells express antigen on the cell membrane in the form of antigen peptide-MHC class 2 molecular compound, present molecular compound to CD4 T cells and provide the starting signal of naive T cells activation. The mature DC cells also highly express CD80, CD86 and so on costimulatory molecules, providing the second signal for T cells activation. The cytokines produced by the DC cells furtherly induce the activated T cells to proliferation and differentiation, then integrally starting immune response. T cells mainly kill the tumor cells with the help of Cytotoxic T-lymphocyte (CTL) cells. CD8 T cells through further differentiation become the CTL. T cells can also fight tumor through the direct anti-tumor effects of the cytokines they secrete (for instance tumor necrosis factor TNF-α, interferon IFN-γ and interleukins IL-2) [5].

2.2.2 Macrophages Activation

In the TME, OVs act on M2 macrophage, these M2 macrophages are repolarized into M1 macrophage. These M1 macrophages express different kinds of proinflammatory cytokine and chemokines and so on to enhance the anti-tumor environment.

M2 Macrophage can promote the growth of tumors versatilely. The action of repolarizing M2 macrophage into M1macrophage is good for killing tumors. M1 macrophage can synthesize IL-12, Inducing the CD4 naive T cells proliferate and differentiate into CD4 Th1 cells, taking part in the immune response mediated by T cells. Moreover, M1 macrophage can kill the tumors by the IgGFc receptor on the M1 macrophages surface or the cytotoxic effect mediated by antibody dependent cells. M2 macrophage can make the expression of MHC molecule and CD80/86 costimulatory molecules become low through compounding, secreting IL-10. These MHC molecules and CD80/86 costimulatory molecules are presented on the surface by antigen. M2 macrophage restrains the adaptive immune response [5].

2.2.3 NK Cells Activation

The infection and replication of OVs firstly happen in cancer cells. After experiencing fast virus replication, infected tumor cells break down and yield new virus to make other uninfected tumor cells become infected. The broken tumor cells become necrotic cancer cells and activate endogenous NK cells as anti-tumor reaction. The cytokines released by necrotic tumor cells take responsibility for activating NK cells [6]. At the same time, OVs are able to split tumor cells, activate death of immunogenic cells, then release DAMP. These DAMP can be detected by DC cells, then inflammation happens. Furthermore, OVs infect DC cells, as the reaction to being infected, tumor and myeloid cells yield chemokines to collect NK cells in cancer [1]. But NK cells do not express specific or pan-specific antigen recognition receptor. NK cells express regulatory receptor related to their activation and restraint. Through these regulatory receptors, NK cells can

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recognize the body's self and non-self components and kill tumor and so on target cells selectively. NK cells also have IgGFc receptor (FcyRIIIA/CD16) on the surface and antibody-dependent cell-mediated cytotoxicity (ADCC) effect to kill tumor cells [5].

3. The Low Targeting Efficiency of OV

3.1 Targeting Efficiency

Systematic drug administration may lead to the non-specific distribution of virus, infecting the normal tissues accidentally and causing potential toxicity [7]. The defect of targeting efficiency is due to the dis-match between the receptors on the surface of virus and tumor cell ligands, as well as the lack of tumor selective tropism. For cerebral glioma, the Blood Brain Barrier (BBB) pricks up difficulties of targeting. It's hard for Mainlined OVs to penetrate the BBB and accumulate in cerebral tumor, which causes limitations of therapeutic efficacy of OVs in the malignant tumors of the central nervous system [8,9]. Moreover, in systematic circulation, OV may be captured by non-tumor cells, such as liver cells or immune cells, lowering its ability to reach tumors [10].

Genetic engineering modification and surface modification can help the OV to increase its targeting efficiency and avoid the clearance. For example, modify the viral capsid protein to express tumor-specific ligands (like Angiopep-2 for glioma), enabling the virus to bind to the receptors overexpressed by tumor cells, thereby enhancing the blood-brain barrier penetration ability and tumor accumulation [8]. Inserting tumor-specific promoters (such as telomerase reverse transcriptase promoters) can confine viral replication within tumor cells and reduce off-target toxicity [7]. Coating the surface of the virus with polyethylene glycol (PEG) or nanoparticles can shield the virus from recognition by neutralizing antibodies and phagocytosis by macrophages, thereby prolonging its systemic circulation time [9].

3.2 Efficiency of Delivering

The delivering efficiency of OV is limited by multiple reasons. First, systematic administered viruses are eliminated by immune system rapidly and easily: antibodies in the blood and phagocytosis by macrophages (such as through pattern recognition receptors) will reduce their load obviously before the viruses reach the tumors [7]. Next, the physical barriers of solid tumors (like the compact extracellular matrix (ECM) and high interstitial pressure) limit the Infiltration and diffusion of the OV in the tumors [9]. For brain glioma, although avoids the systematic elimination, the physiologic barrier, BBB, will prevent it from entering the brain parenchyma [8]. Moreover, the low replication efficiency of the virus within tumor cells caused by host-limiting factors further weakens the delivery effect [10].

Regulate the TME can clear the physical and biological barriers. Co-deliver matrix-degrading enzymes (like hyaluronidase) can degenerate the compact extracellular matrix, promoting the spread of OVs in solid tumors [7]. Using both OVs and anti-VEGF antibody can normalize the tumor vessels, improve blood perfusion and enhance the main permeation of OVs for tumors [9]. For brain glioma, using instantaneous permeant (like focused ultrasound) to break the tight connection of BBB can increase the cross-barrier penetrating efficiency of Ovs [8].Moreover, clearing the immunosuppressive cells in TME (like M2 type macrophages) can reduce the consumption of viruses and maintain their activity [7].

4. The Inhibiting Effect of TME on Oncolytic Virus

TME is made up of many cells, for instance macrophages T-cells and so on. This section lay emphasis on the influence of macrophages on oncolytic viruses. Tumor cells and immunosuppressive cells jointly secrete growth factors, cytokines, thereby suppressing common anti-cancer immune responses, cancer development and angiogenesis get support from factors and cytokines, the growth of tumor get progressed and limiting therapeutic responses [11]. When monocytes enter surrounding tissues, the monocytes' activation mode depends on the signals received from local microenvironment, which explains their functional plasticity and heterogeneity. Primitive monocytes can differentiate into M1 and M2 macrophages under the action of different cytokines.

4.1 Macrophages Restrain OV's Anti-tumor Effect

Macrophages can devour and kill OVs. Macrophages are extremely effective at discerning and combining with the antigenicity of pathogens and other foreign substances through surface pattern recognition receptors and regulatory receptors and take these pathogens and other antigenic foreign substances into the cell through receptor-mediated endocytosis. Macrophages can also take antigenic foreign substances such as pathogens into macrophages through non-receptor -mediated macrocytosis [5]. Since both subtypes of macrophages can swallow oncolytic viruses, it will be more beneficial for exerting anti-tumor effects if macrophages are induced to polarize more into M1 type.

The reasons are M1 macrophages produce NO and ROS related to bactericidal and tumor-killing activities at first. Moreover, M1-polarized macrophages use chemokines CXCL9 and CXCL10 to recruit new Th1 cells, and produce pro-inflammatory cytokines, such as IL-6, TNF-α, IL-1β, IL-23 and IL-12 [12]. So M1 macrophages are considered as a type of anti-tumor macrophages phenotype. Next, M2 macrophages make antigen-presentation ability get lost. M2 macrophages are important parts of tissue remodeling, removing debris and immune adjustment. M2 macrophages sustain angiogenesis through secretion of adrenomedullin, vascular endothelial growth factor (VEGF) and high expression of immunosuppressive molecule, such as programmed death ligand 1 (PD-L1), transforming growth factor β (TGF-β) and IL-10. M2 macrophages are deemed to a kind of tumor-helping macrophage phenotype [11]. But activated M1 macrophages yield a mixture of tumor necrosis factors (IL-6, IL-12 and TNF-α), these tumor necrosis factors will induce proliferate iNOS. INOS can eliminate pathogens [13]. Electively activated M2 macrophages yield anti-inflammatory cytokines (TGF-β and IL-10), restraining inflammatory response, expressing arginase1(arg1) and easy to lead to immune tolerance [13]. So M1 macrophages have stronger virus-killing ability compared with M2 macrophages.

4.2 Strategies to Promote OV Treatment by Optimizing Macrophages

If M2 macrophages are reprogrammed into M1 macrophages, it will not only give full play to the anti-tumor effect rather than promoting tumor growth but also improve the therapeutic effect of oncolytic viruses. But the lethal effect of M1 macrophages on oncolytic viruses should be considered in the meantime. So, people can consider adding cytokines to oncolytic viruses that can convert M2 macrophages into M1 macrophages. Since monocytes can polarize into M1 macrophages with the help of IFN-γ and GM-CSF, M2 macrophages may also be able to be reprogrammed into M1 macrophages in this method. T-VEC is a kind of HSV-1 that expresses GM-CSF, which is approved Food and Drug Administration (FDA) of the United States for the first batch of OVA for treating patients with advanced melanoma [11]. Its mechanism of action is that OVs expressing GM-CSF can attract monocytes to polarize into macrophages and DCs, converting TAM (tumor-associated macrophage) from the M2-like phenotype to the M1-like phenotype. The converting progress makes expression of pro-inflammatory cytokines IL-6, TNF-α become higher [14]. Although the inflammatory factors produced by macrophages and the phagocytic action of macrophages themselves are powerful weapons for killing tumor cells, macrophages will swallow OVs and make the transport of OVs to tumors become less efficient, hence the requirement of OVs' direct delivery needs a larger viral load to neutralize strong OV-clearing of M1 macrophages and increase the virus transported to the tumor site [11].

5. Immune Clearance and Drug Resistance

The main challenges the OV therapy faces in the clinical application lies in the host immune clearance and tumor cell drug resistance. As an exogenous pathogen, the viruses are easily recognized and eliminated by the host immune system. For example, the Herpes simplex virus type 1 (HSV-1) will experience targeted binding of neutralizing antibodies, activation of the complement system, and the phagocytic function of phagocytes during the delivery process [15]. This immune clearance mechanism is considered as the main barrier that limits the curative efficiency of OV therapeutics; even the viruses that undergo the genetic engineering modification are difficult to avoid the problem completely [16]. The tumor cells can escape the virus infection through adaptive mutation, forming drug resistance. According to the research of Chouljenko et al., tumor cells may change the expression of viral receptors, restrain the viral replication-related signaling pathways, or activate the anti-apoptotic mechanism, making the viruses can't disassociate tumors effectively [17]. For example, specific oncolytic viruses designed for carcinoembryonic antigen (CEA) -positive tumors may lose their targeting due to mutations that result in the absence of CEA expression in tumor cells [18]. These mechanisms jointly lead to phenomenon of the low response rate or treatment failure of the OV therapeutics in some patients, highlighting the urgency of developing the combined strategies to overcome the immune clearance and drug resistance.

Using both OVs and immune regulator can improve the curative effect of the virus. Enabling virus to express immune-stimulating cytokines (such as GM-CSF or IFN-γ) through genetic engineering can recruit and activate dendritic cells, Cytotoxic T cells, and natural killer cells, changing the "cold" TME into "hot" TME [9]. Using the OV combined with immune checkpoint inhibitors (such as PD-1/PD-L1) can prevent the T cells from exhausting, stopping the immune escape of tumor cells [7]. These strategies can improve the anti-tumor immunity and reduce immune-mediated virus clearance, creating a cycle that is beneficial for maintaining the activity of the virus [10].

The drug resistance of the OV therapy can be improved

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by the virus modification, pedigreline enhancement, and genetic regulation. Modifying the virus envelop or capsid protein through the genetic engineering can improve the targeting and binding capacity of tumor cell specific receptors, thus increase the efficiency of the virus to entry tumor cells [9]. Using the OV combined with nano-carriers, as well as the EPR effect, to increase the enrichment of virus in tumor areas can prevent virus from being degraded by the tumor interstitial protease and break through the extracellular matrix barrier of tumor cells [9]. Through the genetic modification to regulate the antiviral pathways of tumor cells can inhibit the IFN-γ/STAT1 signal-mediated viral clearance mechanism and keep OVs to continuously replicate in tumor cells [10].

6. Conclusion

OV therapy shows a remarkable potential of oncotherapy through the combination of direct tumor lysis and TME remodeling. However, clinical applications are still limited by three main challenges. First, low targeting and delivering efficiency, such as immune clearance during systemic administration and physical barrier limitations like the blood-brain barrier; second, the inhibitory effect of TME, such as the phagocytosis and immunosuppression of M2-type macrophage; third, the immune clearance and drug-resistance of tumor cells, including neutralizing antibodies clear the virus and tumor cells escape through mutations.

Current studies have shown that by employing multi-dimensional strategies such as virus modification (e.g., genetic engineering to target ligands, PEG encapsulation), TME regulation (e.g., degradation of extracellular matrix, re-polarization of macrophages), and immune enhancement (e.g., combination with immune checkpoint inhibitors, armed cytokines), the therapeutic efficacy can be significantly improved. In the future, it is necessary to further optimize the combination regimens and promote the clinical translation of personalized virus construction to accelerate the development of this therapy as a widely applicable treatment for tumors. In-depth research on the dynamic interaction mechanisms between oncolytic viruses and immune cells in the TME, and the development of „oncolytic virus + dual immune checkpoint inhibitors + metabolic regulators“ combined therapies, can provide more precise treatment options for various tumors and ultimately achieve the transition from „effective killing“ to „long-term cure“.

Authors Contribution

All the authors contributed equally and their names were listed in alphabetical order.

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