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Review article / Обзорная статья

Measles Virus as a Vector Platform for Glioblastoma Immunotherapy (Review)

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Abstract

Introduction. Oncolytic virotherapy is one of the approaches in immunotherapy of solid brain tumors. Measles virus vaccine strains are prospective agents for the therapy of cancers such as neuroblastoma, mesothelioma, and glioblastoma multiforme. The hyperexpression of the CD46 and other receptors on the surface of malignant cells allows the measles virus to infect and lyse the tumor, thus inducing an immune response. However, widespread immunization of the population and the resistance of neoplasms to oncolysis present difficulties in clinical practice.

Text. This review covers approaches to modifying the measles virus genome in order to increase specificity of virotherapy, overcome existing immunity, and enhance the oncolytic effect. It was shown that expression of proinflammatory cytokines on viral particles leads to tumor regression in mice and triggers a T-cell response. Several approaches have been used to overcome virus-neutralizing antibodies: shielding viral particles, using host cells, and altering the epitope of the protein that enables entry of the virus into the cell. Furthermore, the insertion of reporter genes allows the infection of target cells to be monitored in vivo. A combination with the latest immunotherapies, such as immune checkpoint inhibitors, demonstrates synergistic effects, which suggests the successful use of combined approaches in the therapy of refractory tumors.

Conclusion. Measles virus attenuated strains appear to be an easy-to-modify and reliable platform for the therapy of solid brain tumors.

Keywords: oncolytic viruses, measles virus, immunotherapy, CD46 receptor, virotherapy

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Вирус кори как векторная платформа для иммунотерапии опухолей головного мозга (обзор)

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Резюме

Введение. Одним из подходов в иммунотерапии солидных опухолей головного мозга является применение онколитических вирусов. Вакцинные штаммы вируса кори рассматривают в качестве перспективных кандидатов для терапии мезотелиомы, нейробластомы и мультиформной глиобластомы. Гиперэкспрессия рецептора CD46 и других белков на поверхности злокачественных клеток позволяет вирусу кори таргетно инфицировать и лизировать опухоль, индуцируя иммунный ответ. Однако широкая иммунизация населения и устойчивость новообразований к онколизу представляют трудности в клинической практике.

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Текст. В настоящем обзоре обсуждаются подходы к модификации генома вируса кори с целью повысить таргетность виротерапии, преодолеть существующий иммунитет и усилить онколитический эффект. Показано, что экспрессия провоспалительных цитокинов на вирусных частицах приводит к регрессии опухоли у мышей и запускает Т-клеточный ответ. Для преодоления вирус-нейтрализующих антител применяются подходы по экранированию вирусных частиц, использованию клеток-носителей и изменению эпитопа белка, обеспечивающего проникновение вируса в клетку. Кроме того, вставка репортерных генов позволяет отслеживать инфицирование таргетных клеток *in vivo*. Комбинация с новейшими методами иммунотерапии, такими как ингибиторы иммунных контрольных точек, демонстрирует синергизм эффектов, что позволяет рассчитывать на успешное применение сочетанных подходов в терапии рефрактерных опухолей.

Заключение. Атенуированные штаммы вируса кори представляют собой удобную и безопасную платформу для иммунотерапии опухолей головного мозга.

Ключевые слова: онколитические вирусы, вирус кори, иммунотерапия, рецептор CD46, виротерапия

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INTRODUCTION

In recent years, approaches aimed at enhancing the antitumor immune response have been actively introduced into oncological practice. The use of oncolytic viruses targeting various tumors is one of the promising directions in immunotherapy [1]. The mechanism of oncolytic action is based not only on the direct lysis of tumor cells, but also on the activation of the antitumor response due to the release of hazard signals (PAMPs, DAMPs) and tumor-specific proteins, which makes it possible to overcome the immunosuppressive tumor microenvironment [2].

Glioblastoma multiforme (GBM), or glioblastoma, is the most common and most aggressive tumor of the central nervous system in adults. Overall survival for GBM is 15 months, and 5-year survival is 5–7 % after the initial diagnosis [3]. There are numerous problems associated with the treatment of glioblastoma, including ineffective delivery of drugs or agents across the blood-brain barrier, extensive intratumoral and intertumoral heterogeneity, redundant signaling pathways, and an immunosuppressive microenvironment. Oncolytic viruses represent a promising therapeutic approach as, in addition to their lytic activity, they can overcome immunosuppression by stimulating innate immunity. As of 2021, there are 20 clinical trials in the world using oncolytic viruses for the treatment of GBM; 4 clinical trials for neuroblastoma [4].

Measles virus: advantages and limitations

Attenuated strains of measles virus (MV) are considered as promising candidates for oncolytic therapy. A long history of the use of vaccines containing attenuated strains of measles viruses indicates their safety for humans due to their genetic stability. Despite the wide coverage of vaccination, MV as an oncolytic agent has shown sufficient efficacy and safety in preclinical and clinical trials against many types of cancer, including mesothelioma, glioma and GBM [5, 6]. At the same time, MV vaccine strains are convenient for cloning, which makes it possible to modify the virus, increasing its oncolytic efficacy.

MV is a single-stranded (–)RNA virus belonging to the *Paramyxoviridae* family. MV entry into the cell is mediated by attachment of the viral hemagglutinin (H) protein to at least one of the three known cell surface receptors: a membrane cofactor protein, a member of the complement regulatory protein family (CD46), a signaling molecule that activates lymphocytes (SLAM), or nectin-4 [7]. Wild-type MV strains mainly bind to SLAM receptor, attenuated vaccine strains MV Edmonston B (MV-Edm) and Leningrad-16 (L-16) [8] penetrate the CD46 receptor, while nectin-4 can be used by both wild-type and MV-Edm strains [7].

Neoplastic cells overexpress CD46 receptors on the cell surface, mediating the tumor-specific lytic effect of attenuated virus strains [9]. However, although CD46

molecules are abundantly expressed on the surface of the of cholic cells, which contributes to their effective infection, some cells show resistance to oncolysis after virus entry, indicating that other processes may affect its oncolytic efficiency [9].

Indeed, recent studies have identified the expression of the interferon-induced transmembrane protein 1 (IFITM1) gene as the interferon-stimulated gene (ISG) responsible for limiting MV replication in human tumor cells [10].

MV sensitivity to antiviral reactions was also found in a study by Kurokawa et al. [11]. In particular, mice bearing human GBM xenografts with a defective interferon pathway were shown to be more responsive to MV treatment (interferon-stimulated gene, ISG). The production of infectious virions of the offspring increased by 387 times compared with mice carrying GBM with an intact interferon pathway. Moreover, the analysis of gene expression in tumor samples from GBM patients treated with MV (NCT00390299) showed an inverse correlation between ISG expression and viral replication [11]. Thus, IFITM1 expression may serve as a biomarker for MV virotherapy resistance in GBM patients.

Approaches to modification of the MV genome

With the development of genetic technologies, it became possible to enhance the oncolytic properties of MV by arming the virus with genes encoding proteins that enhance the antitumor effect, or by increasing the selectivity of the modified virus for tumor cells.

The first MV genome that became available for genetic modifications was obtained by cloning the MV-Edm vaccine strain after passage on Vero cells [12]. Additional transcription units can be inserted fairly easily into MV antigenome sequences. Thus, it is possible to create various modifications of MV differing in replicative capacity, cytotoxicity [13], tropism for receptors, or effects on the induction of cellular interferon response and apoptosis of target cells [14], which together reflect the antitumor activity of viruses.

At the same time, the MV genome has two distinctive features that significantly complicate the task of obtaining recombinant strains based on them [15]:

1. As with all (-)RNA viruses, the MV genome is a template necessary for the formation of mRNA, with which antigenomic RNA replicates, which in turn acts as a template for the synthesis of genomic RNA. The transcription step is characterized by the production of short transcripts, which are usually capped and polyadenylated, while the replication steps produce unmodified full-length transcripts.
2. Genomic RNA is biologically active only when it is present as a ribonucleoprotein (RNP), that is, it is

associated with the nucleocapsid protein and viral RNA-dependent RNA polymerase. RNP has a very rigid structure, which makes the virus completely resistant to cleavage by cellular RNases. Thus, genomes and antigenomes should be encapsulated inside cultured cells, i.e., as long as they are artificially synthesized from a cloned cDNA template by foreign DNA-dependent RNA polymerases co-expressed in host cells.

There are several approaches to modifying the MV genome. In a simple approach, transcripts are introduced into cells parallel infected with the appropriate parent virus. A more complex option assumes transfection together with genome-wide sequence of minireplicons encoding encapsulating proteins – nucleocapsid (N), phosphoprotein (P) and RNA polymerase (L) for the formation of biologically active RNPs in cells also infected with vaccinia virus (vTF7-3) encoding T7 RNA polymerase at high levels. All sequences should contain T7 promoters. Such a strategy ensures efficient transcription of all virus-specific cloned segments in transfected cells.

However, due to the large size of the MV genome, it rarely replicates at high titers and produces large polyploid virions that are easily inactivated by chemical and physical factors. Thus, it is preferable to use the virus assembly method without the purification step from helper viruses. This is achieved by transfection with plasmids encoding N and P proteins and T7 RNA polymerase under the control of CMV promoters.

Obviously, much weaker expression of T7 RNA polymerase is possible from the plasmid than is achievable with the helper virus. However, in the selected strategy, the plasmids encoding the N and P viral proteins, which are required in large quantities, do not depend on T7 promoters. At the same time, a relatively low level of T7 RNA polymerase is considered sufficient for the synthesis of antigenomic RNA and mRNA encoding a large viral polymerase, which is required in small amounts [15].

It should be noted that helper cells stably transfected with plasmids encoding T7, N, and P RNA polymerase were initially used to increase efficiency. They were then transiently transfected with plasmids encoding the full-length antigenome sequence and large viral RNA polymerase. Thus, using this approach, MV Ed-tag was obtained from a single syncytium [12].

Further modifications to the MV assembly include a gradual transition from using stably transfected cells to transiently transfected cell lines. With this approach, standard cell lines should be simultaneously transfected with all plasmids containing CMV promoters recognized by cellular RNA polymerase II located in the nuclei, and not by T7 promoters [16].

Measles virus retargeting

As noted earlier, the CD46 receptor is overexpressed on the surface of many tumor cells [17]. However, CD46 molecules are also present in healthy tissues, which can lead to infection of non-tumor cells. Therefore, approaches have been proposed to increase the selectivity of oncolytic MVs for tumor cells.

To enhance the selectivity of an oncolytic virus, it is necessary that life cycle of the virus depends on the properties of specific tumor cells. The most attractive strategy for retargeting the virus is to target it to a receptor specific for a particular type of a tumor cell. This can be achieved by introducing specific mutations into the viral protein hemagglutinin H, which is responsible for binding to MV receptors, for its additional binding to a specific receptor recognition site on the surface of a selected tumor cell [18]. In addition to natural receptors such as CEA, CD20, CD30, CD38, CD133, CD138, IL-13R, uPAR, engineered ankyrin repeat proteins (DARPin)s have been tested against EpCAM, IGFR [19], single chain antibody fragments (scFv's) [20] to CEA, EGFR, folate receptor, HER2/neu; T cell receptors (TCR) [21], and cysteine nodes [22]. Thus, simultaneous specific targeting to two different structures of the surface of tumor cells increased the effectiveness of therapy [23]. On the other hand, the penetration of MV into the cell critically depends on the proteolytic activation of the viral fusion protein F [24]. The F protein is normally activated in the Golgi trans network of virus-producing cells via cleavage into two subunits F1 and F2 by ubiquitous furin-like proteases [13]. Replacing the polybasic splitting motif in the F protein to a site for tumor-associated proteases, such as matrix metalloproteases, hyperactivated in tumors and promoting their invasive growth due to the destruction of the extracellular matrix, MV replication may be limited to tumor tissue rich in proteases. Such targeting of the virus to proteases can be combined with highly effective retargeting to specific receptors on the surface of tumor cells, which allows increasing the effectiveness of virotherapy.

In addition, targeting after viral penetration is possible by integrating miRNA target sites into viral RNA [25]. To enhance the oncolytic effect, a combination of miRNA target sites may be optimal.

Thus, retargeting the virus can increase its specificity for tumor cells, which increases the safety and efficacy of virotherapy.

Enhancing the oncolytic effect

Although the principle of action of oncolytic viruses initially assumes direct lysis of tumor cells, it is now generally accepted that the main mechanism of action of

virotherapy in general is the immune response caused by the ongoing viral infection of the tumor tissue. Thus, immunologically "cold" tumors become "hot" and available for immune-mediated destruction. Indeed, the main mechanism of action of the modified oncolytic MV encoding GM-CSF has been shown to induce an antitumor response in an immunocompetent mouse model [26]. But even without additional enhancement of efficacy, MV infection of neoplastic cells itself elicits a strong immune response. MV infection stimulates the activation of many immune cells, such as myeloid and plasmacytoid dendritic cells, as well as macrophage cells [27]. Natural killers (NK), neutrophils, and cytokine-induced killers intensively infiltrate the tumor [28]; the adaptive immune system is also activated, which manifests itself in increased infiltration and induction of tumor-specific T cells [29]. Such mediated induction of antitumor immunity is non-targeted and therefore cannot guarantee targeted targeting of critical tumor antigens.

In order to launch a targeted immune response through critical driver genes encoding key factors on which the development of tumor cells depends, strategies are used to combine oncolytic viruses with factors modulating the tumor microenvironment. To date, strains of MV Edmonston B, Schwarz, and Moraten have been modified and tested *in vitro* and *in vivo* against GBM, multiple myeloma, as well as melanoma, lymphoma, pancreatic cancer, lung cancer, etc. To increase effectiveness, recombinant MVs with the following built-in genes are described:

- ✓ NIS (sodium iodine symporter), which allows to enhance the therapeutic effect due to local 133I radiotherapy;
- ✓ PNP (purine nucleoside phosphorylase), which converts fludarabine to the more toxic form 2-fluoroadenine;
- ✓ SCD (super cytosine desaminase) converting 5-FC to 5-FU for local chemotherapy;
- ✓ GM-CSF (granulocyte-macrophage colony stimulation factor), which attracts T-cells, neutrophils and induces antitumor immunity;
- ✓ IL-12, IL-15 activating NK and T cells;
- ✓ IFN β , which stimulates the immune response;
- ✓ α PDL1, α CTLA4 blocking immune checkpoints;
- ✓ muCLDN-6, hTERT presenting TAA (tumor associated antigen);
- ✓ NAP [neutrophil activating protein (*H.pylori*)], which activates neutrophils;
- ✓ α CD3/ α CD20, α CD3/ α CEA, activating T cells;
- ✓ wild MV genes that suppress the innate immune response;

- ✓ BNIP3, reactivating apoptosis;
- ✓ endostatin-angiostatin inhibiting neoangiogenesis, etc. [30].

Thus, the immunostimulatory effect of MV encoding hTERT as TAA was shown in studies on IFNAR^{-/-}CD46Ge mice (transgenic expressing human CD46 against the background of deficiency of type I interferon receptors), which are currently the most preferred preclinical model [31].

Preclinical and clinical studies

The determination which cells or tissues are infected with an oncolytic virus is greatly facilitated by the use of reporter genes, which allow non-invasive detection of the virus in living cells and the body. Marker proteins that do not interfere with the life cycle of the virus, such as fluorescent proteins, are widely used to track the replication and spread of oncolytic MV. In particular, the human gene encoding the iodide symporter protein (NIS) was cloned into MV-Edm [32]. The NIS protein enables visualization of *in situ* replication of an oncolytic virus in the parenchyma of gliomas using a gamma camera after systemic administration of radioactive isotopes ¹²³I, ¹²⁴I, ¹²⁵I or ¹³¹I, which accumulate in infected tumor cells. NIS-modified MV has been tested in preclinical studies [32]. It has been shown that the therapeutic effect of MV is increased by radiation therapy through local accumulation of ¹³¹I. In mouse models, the NIS-modified virus increased their survival and, at the same time, the cytopathic effect compared to MV-CEA. MV-CEA is another modified MV-Edm encoding for carcinoembryonic antigen (CEA) to monitor viral replication in glioma cells *in vivo* using a blood test since this factor is released and detectable in the blood. Animal models have shown significant tumor regression after intratumoral administration of MV-CEA.

Phase I clinical trials using MV-CEA for the treatment of GBM were carried out in 23 relapsed patients. One group received the virus directly into the resection cavity, and the other group received the virus directly before the operation through a catheter. No significant differences were observed between groups in progression-free survival at 6 months (NCT00390299).

Thus, the introduction of marker genes into the composition of the virus genome also makes it possible to evaluate the effectiveness of the selected therapeutic approach.

MV-GFP-H_{AA}-scEGFR is a retargeted virus that expresses a single chain antibody at the C-terminus of the H protein that binds epidermal growth factor receptor (EGFR). EGFR overexpression is characteristic of GBM cells, which determines the specificity of MV-GFP-H_{AA}-scEGFR.

Significant regression and induction of apoptosis in cells infected with MV-GFP-H_{AA}-scEGFR was shown *in vivo*. At the same time, the introduction of MV-GFP-H_{AA}-scEGFR into the central nervous system of mice expressing CD46 did not lead to neurotoxicity [33].

MV-141.7 and MV-AC133 are other recombinant viruses retargeted at the CD133 receptor. CD133 is often expressed by GBM cells. The introduction of the MV-141.7 virus into mice with orthotopic glioma resulted in a higher survival compared to the unmodified virus [34]. In addition, the combination of CD133 retargeting and SCD virus armament approaches shows greater safety and high specificity for the lysis of CD133-positive cells compared to modified vesicular stomatitis virus in an orthotopic glioma model [35].

Overcoming antiviral immunity

Although oncolytic MVs have many advantages as a therapeutic agent, the presence of neutralizing antibodies prevents their widespread use. Owing to the availability of safe and effective measles vaccines in the vaccination schedule, many cancer patients have significant levels of neutralizing antibodies (nAbs) against MV in plasma. This poses a problem for systemic administration of an oncolytic virus because neutralizing antibodies can bind MV before it reaches the tumor.

However, there are several strategies to overcome this potential hurdle. For example, modification of HMV protein epitopes demonstrates antitumor efficacy even in the presence of antibodies [36]. Another approach is shielding, using polyethyleneimine polymers [37], as well as its combination with graphene oxide [38], to hide the surface of the virion from neutralization with nAbs.

Another option for the systemic delivery of oncolytic MVs to the tumor and its metastases is the use of carrier cells. Various types of cells have been proposed as potential viral carriers, including directly malignant cells, stem cells, immature and mature dendritic cells, activated T-lymphocytes, and peripheral blood mononuclear cells [39–41].

The adaptability of the use of MV allows for synergy with other immunotherapies. Thus, the combination of tumor cells pre-infected with MV with autologous vaccines based on dendritic cells (DC) can overcome the problem of the tumor barrier and activate antitumor immunity [42].

Combination with other treatments

The next logical step would be to combine the oncolytic virus with checkpoint inhibitors to overcome the immunosuppression of the tumor and its microenvironment. Oncolytic viruses attract cytotoxic T-lymphocy-

tes to the tumor and regulate PD-L1 expression on both GBM cells and immune cells, making them an ideal candidate for combinations [43]. Phase III clinical trials of antibodies against PD-L1 in the treatment of GBM (NCT02017717) showed promising efficacy and safety in patients resistant to conservative therapy. According to a study, PD-L1 expression was observed in 88 % of patients with primary GBM and 72.2 % of recurrent GBM [44]. However, although most GBMs express PD-L1, their levels remain low (approximately 2.7 %). In addition, PD-1 expression is also observed in other tumor-infiltrating cells. An inverse correlation is known between PD-L1 expression and prognosis, suggesting an immunosuppressive effect of PD-1+ T cells in the GBM microenvironment [45]. STAT3 activation induced by IL-10 in tumor-associated macrophages can maintain high levels of PD-L1, resulting in an anti-inflammatory M2 macrophage state that directly suppresses T-cell activation by mediating an immunosuppressive microenvironment [46]. Thus, the combined use of viruses and antibodies against PD-L1 may have synergistic effects in the treatment of GBM.

Studies show that MV enhanced by insertions of PD-L1 and CTLA-4 sequences, firstly, is able to induce antibody synthesis, and secondly, significantly increases the response to virotherapy in an immunocompetent mouse melanoma model [47]. This proves that in the future it is possible to use such a combination as a monotherapy.

It should also be noted that antibodies (α EGFR, α IFN, α PD1, α CTLA4), immune cells (CD8+NKG2D+, NK), inhibitors of various cell signaling pathways, and other therapeutic strategies such as radiation, chemotherapy, or combination with other therapeutic viruses have been tested in a number of studies, revealing varying degrees of synergy [48]. Thus, the combination of oncolytic MV with activated NK cells led to an increase in the release of cytolytic enzymes by NK cells and, as a result, accelerated death of tumor cells compared with in vitro monotherapy [49]. On the other hand, simultaneous administration of activated NK cells and replicating oncolytic viruses may reduce the therapeutic potential of the latter due to their elimination by NK cells [50]. However, the strategy of using MV encoding interleukin-12 (MeVac FmIL-12) and interleukin-15 (MeVac FmIL-15) avoids virus clearance while mediating the increased T- and NK-cell response and thus increases therapeutic efficacy, especially in tumors controlled by NK cells. MeVac FmIL-15 virus infection increased tumor infiltration with T and NK cells. However, the MeVac FmIL-12 virus was characterized by a more stable expression of viral genes and activation of the immune system, and, accordingly, a greater antitumor efficacy [51].

CONCLUSION

Oncolytic viral immunotherapy is a promising approach in the treatment of solid brain tumors, primarily glioblastoma multiforme. Attenuated measles virus appears to be a convenient and safe immunotherapy platform for targeting the virus to tumor neoantigens. In addition, the ability to amplify the virus by introducing sequences encoding cytokines, immune checkpoint inhibitors, and other molecules into the genome provides the flexibility and efficiency of the proposed therapeutic approach based on the measles virus as a vector platform.

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