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Persistent Virus Presence during Experimental Oncolytic Virus Therapy in the Model of Subcutaneous Mouse Xenografts of Human Glyobolastoma Multiforme

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Abstract

The approach of using oncolytic viruses for the therapy of malignant diseases is considered as a promising alternative to cytotoxic chemotherapy. Especially relevant is the development of new therapies in the case of malignant glioblastomas, a disease against which effective treatments have not been developed to date. Viruses of many different families demonstrate selective activity against glioblastoma cells. In particular, a pronounced lytic activity against glioblastomas demonstrate non-pathogenic or vaccine strains of human enteroviruses. The main mechanism of oncolytic action of enteroviruses is associated with their ability to selectively replicate in tumor cells, although stimulation of the immune system also contributes to the oncolytic effect. The objective of the study was to determine the possibility of effective delivery of oncolytic enterovirus (vaccine strain type 1 poliovirus) to the subcutaneous xenografts of the human glioblastoma cell line (U87MG) and to determine the dynamics of viral oncolysis and the presence of the introduced virus in the body of tumor-bearing immunodeficient mice. It was found that in this experimental model, the delivery of the virus to the tumor is possible with the intravenous virus dose as low as 10^5 pfu. A single administration of the virus led to a gradual regression of tumors, while the virus was detectable in the blood of mice. However, the virus could no longer be detected two days after the tumor disappeared. The presence of the virus and the start of the virus regression process were accompanied by a significant increase in the survival rate of mice. According to the results of the study, it can be concluded that in the absence of a significant immune component, effective tumor oncolysis can occur through direct oncolytic action of enterovirus.

Keywords: multiform glioblastoma, poliovirus, viral oncolysis, athymic mice, xenografts of tumors, experimental therapy.

INTRODUCTION

Malignant transformation is accompanied by the elimination of intracellular control mechanisms that benefit the needs of the whole organism [1]. Mutations of the p53 gene or functional alterations of the p53-dependent mechanisms result in a breakdown of the safeguard system that ensures elimination of defective cells [2]. As the result, the cells enter the path of evolution within the organism, during which the most rapidly dividing and invasive cells are selected [2,3]. By acquiring the autonomy, a tumor cell gradually loses all the mechanisms that benefit the organism, including those preventing replication and spreading of viruses [4-11]. These mechanisms include the ability of the cell to recognize viruses and adaptively respond to the invasion by developing resistance to viruses [7, 12]. This is why cancer cells commonly are unable to induce the secretion of Type I interferons (IFNs) in response to viral infection, and to respond to IFN treatment by the induction of interferon response genes that act against replication of viruses and prevent their further spread [4, 7, 12-19]. These changes explain the increased sensitivity of tumor cells to viruses and provide the rationale for the development of oncolytic viruses for cancer therapy [12, 16, 19-21].

Meantime, oncolytic viruses act not only through the selective infection and destruction of cancer cells, but also through the induction of complex processes that involve an activation of natural mechanisms of anticancer immunity. These processes engage both innate and adaptive branches of anticancer immunity [22-26]. Studies indicate that viruses of many different families can act through these mechanisms at varying degrees. To understand the overall contribution to oncolysis of the direct killing of cancer cells by viruses, the authors decided to use *in vivo* model of immunodeficient athymic mice, in which many components of cellular immunity are compromised by a mutation in the FoxP3 gene [25] leading to a deficient adaptive T-cell mediated anticancer immunity. Another advantage of this model is because it has strongly compromised antiviral immunity that allows carrying out the treatment without rapid clearance of the virus from the organism.

In the present study the authors used mouse subcutaneous xenograft model of human U87MG glioblastoma cells [27] to test oncolytic activities of an enterovirus (Sabin's vaccine strain of Tyoe I Poliovirus) in order to measure the dynamics of direct oncolysis by the virus and to monitor the persistent persistence of the virus in the mice carrying a virus-infected tumor.

The cells of human glioblastoma cell line U87MG (ATCC HTB-14 [27]) were expanded as a monolayer culture, detached from plastic surface by treatment with trypsin solution, washed twice with phosphate buffered solution, and injected subcutaneously into rear hind region, both sides, in the amount of 1 million cells per injection, in 0.1 ml of the solution. The growth of tumors became noticeable starting from 12-16 days after the administration. When tumors reached size of a bean (about 10 mm), which corresponded to 24 days after the inoculation, Type 1 poliovirus was injected into tail vein in a volume of 0.2 ml of physiological saline at doses of 10^5, 10^6 and 10^7 plaque-forming units (pfu). There were two control groups of mice, one carrying a tumor, but not injected with the virus, the other not carrying tumors and injected with 10^7 pfu of the virus. Each group consisted of 15 mice, the total number of tumors was 30.

At various intervals after the virus administration, the condition of mice was monitored by regular measurement of tumor sizes and collecting blood for detecting virus titers (Table 1).

The table shows the results of tumor measurements with volume calculation according to the formula $V = L \times W^2 \times 0.5$, where L and W are linear sizes of tumors (length and height), as well as virus titers in the blood taken at different time intervals after the administration into the tail vein. Virus titers were measured by infection of the sensitive human rhabdomyosarcoma RD cells with serially diluted blood samples obtained from the tail vein.

It should be noted that within the range of applied virus doses (10^5, 10^6 and 10^7, PFU), an apparent oncolytic effect was observed, which consisted of tumor growth abrogation, and subsequent gradual reduction of tumor volumes, with final complete disappearance of tumors. On day 20 after the injection, there was only a small palpable grain of compaction in place of the tumors, which presumably corresponded to a scar tissue. Further observation for two months did not reveal a secondary growth, and the virus in the blood was no longer detectable. Of the group infected with the virus, 100% of the mice survived, while in the control group of the tumor-bearing mice, only 6 mice remained at day 15, by day 20 all mice were euthanized as the tumor sizes increased allowable limits.

Dynamics of virus detection in the blood of mice was also monitored. In the group of control mice not carrying tumors, the virus was detected during first 2 hours after injection. Further measurements did not reveal virus in the blood of the control mice.

In the group of tumor-bearing mice, with the introduction of three doses of the virus, two hours after injection the virus in the blood was detected only in mice injected with 10^6 pfu (10 μl of undiluted blood was minimal for the detection) and with 10^7 pfu (at the blood dilution of 1:100). However, at later time points the virus was readily isolated from all the infected mice, approximately at the same level (at 1:100 dilution), up to day 20 when the virus was no longer detected. At this time point, the mice did not already have visible tumors.

Table 1. Dynamics of growth and degradation of tumors of U87MG cells with the introduction of different doses of vaccine poliovirus type 1, as well as titers of the virus in the blood.

Group of mice	Day 0		Day 5		Day 10		Day 15		Day 20	
	Tumor volume	Virus in the blood	Tumor volume	Virus in the blood	Tumor volume	Virus in the blood	Tumor volume	Virus in the blood	Tumor volume	Virus in the blood
No tumors	X	10^2	X	nd	X	nd	X	nd	X	nd
No virus	920±22	nd	1243±54	nd	1576±48	nd	6/15 1987±98	nd	0/15 (all perished)	nd
Virus, 10^5 pfu	952±38	nd	948±56	10^3	634±59	10^2	108±32	10^2	scar	nd
Virus, 10^6 pfu	1012±32	10	821±42	10^3	433±46	10^2	104±45	10^2	scar	nd
Virus, 10^7 pfu	987±46	10^2	876±32	10^3	388±52	10^2	98±12	nd	scar	nd

Thus, during this study the authorsu found that subcutaneous xenografts can be efficiently infected with the oncolytic virus with the intravenous doses of the virus starting at 10^5 pfu. Therefore, it can be concluded that in the mouse model the intravenous administration can deliver infectious viral particles to tumor sites to initiate the process of infection of susceptible tumor cells. The destruction of the tumor under the influence of the virus is accompanied by the release of the infectious virus into the blood, which ensures a permanent presence of viral particles at a level that is sufficient for the reinfection of the remaining tumor cells. Certainly, the persistence of the virus in the mouse depends on the presence of tumor-susceptible tumor cells, since even a high virus dose introduced to non-tumor-bearing mice does not ensure the virus presence in the blood 5 days after the injection, while it was found at high titers in the blood of the tumor bearing mice. Only after the tumors completely disappear, the virus can no longer be detected in the blood.

The authors found that xenografts of human glioma cells in athymic mice are an effective and sensitive system for studying viral delivery to tumor sites. This model could be found suitable for testing other modes of administration, such as the use of cell-based carriers infected with the virus *in vitro* and then introduced into the blood stream. Such mode of virus administration would reduce the initial load of the virus and might increase the penetration efficiency into the tumor.

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