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Study on the potential construction of giant virus gene transfer vectors

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Abstract. In 2003, a unique virus parasitic to amoebic protozoa was first isolated from water in a cooling tower in the United Kingdom . This virus possesses numerous characteristics that distinguish it from conventional viruses. Owing to its substantial genome capacity and physical volume, such viruses have been labeled as giant viruses. Due to their relatively recent discovery, limited existing research impedes the realization of their practical applications. Given their potential to carry extensive genetic information, this paper asserts the potential of these viruses to be fashioned into specialized virus transfer vectors and deliberates upon this topic. This study synthesizes domestic and international research on large viruses, analyzing their structural characteristics and host properties to explore the feasibility of constructing large virus transfer vectors. Large viruses possess traits not inherent in traditional viral vectors, such as the ability to survive in extremely short environments and accommodate vast genetic capacities. As a novel research domain in the past two decades, further exploration of the types, structural characteristics, and host properties of large viruses is necessary to ascertain their potential in constructing gene transfer vectors.

Keywords: Giant virus, gene transfer vectors, traditional viral vectors.

1. Introduction

Giant viruses are a recently discovered viral species characterized by their voluminous size, substantial genome capacity, and genetic stability. Considering these traits, large viruses hold the potential for development into gene transfer vectors. However, the current body of research on large viruses remains limited, necessitating intensified research efforts in the future to harness their inherent potential.

Genetic engineering technology involves transferring target genes into recipient cells to express specific traits. The key lies in constructing gene vectors by linking target genes with carriers through DNA ligases, forming an integrated entity that facilitates the subsequent transport of genes into recipient cells. Presently, using viruses as gene vectors to achieve transgenic purposes has matured, and various types of viruses can serve as mediators to construct vectors [1]. Existing viral gene vectors possess individual advantages and disadvantages, ultimately impacting gene expression, recipient cell health, and transfer efficiency [1]. For instance, viral vectors have limited gene capacities and cannot support the carriage and transcription of large-capacity genes. Current technology can support the construction of viral vectors carrying a 171-kbp gene [2]. However, considering the potential future demand for single-time transcription of larger capacity genes (nearing or exceeding 1 Mbp) to achieve

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the transfer of target genes, there's a need to identify a viral vector capable of supporting the single-time carriage and transcription of higher-capacity genes to meet the requirements of genetic engineering.

Presently, Giant viruses with substantial genetic capacities reaching 1 Mbp have been discovered, which is the viruses that can carry most amount of gene be found now[3]. Initially identified in amoebic protozoa in 2003, these viruses have genome sizes comparable to some smaller prokaryotes [4]. Despite the relatively recent discovery of giant viruses, they are widespread in nature, having ancient origins dating back around thirty thousand years, and they exhibit stability and infectivity in harsh environments [5-6]. There is ongoing controversy regarding the classification of giant viruses, but under current classification methods, known giant viruses belong to multiple virus families (common ones include Mimiviridae, Phycodnaviridae, Pandoraviridae, and Marseilleviridae), with substantial differences between these families [7]. As a recently discovered species, much information about giant viruses, including but not limited to their structure and function, hosts, infection mechanisms, and harmfulness, remains unclear. This knowledge gap poses a significant obstacle to further research and application of giant viruses.

2. Problem to use giant viruses as gene transfer vectors

As of the present stage, giant viruses, with the largest known gene-carrying capacity among viruses, harbor immense potential as gene engineering vectors. However, numerous potential issues exist that could affect the feasibility of their development as viral transfer vectors. Due to the insufficient known information regarding giant viruses mentioned above, various factors might hinder the creation of giant viruses as transfer vectors, such as high research costs or other unfavorable factors. One primary issue is that the hosts of most giant viruses are currently unknown [7]. The majority of large viruses use amoebic protozoa as hosts, differing from common targets of genetic engineering like bacteria, plants, and animals. Although reports suggest signs of large virus infections in the human body [8-10], attempts have been made to culture-related viruses in human T-cells [11], and there are reports indicating potential hosts like certain algae and vertebrates, it cannot be ruled out that large viruses infect hosts indirectly through amoebas [6-7]. If the traditional biological targets of genetic engineering cannot serve as potential hosts for giant viruses, considering the mechanism of viral transfer vectors involves enabling the virus transformed into a transfer vector to directly infect hosts and perform transcription, translation, and expression in host cells, giant viruses might not effectively serve as gene carriers. Another potential issue is that due to substantial differences in composition and structure between giant viruses and traditional viruses, it remains unclear whether the conventional methods of creating viral gene vectors can be applied to construct giant virus gene vectors. Failure to do so would undoubtedly escalate the difficulty and cost of research and might even lead to unsuccessful outcomes. A final potential issue is the uncertainty regarding the harmfulness of giant viruses [5-6]. As it is currently unknown whether large viruses can infect humans or other organisms and what symptoms such infections might cause, estimating their risks and potential harms poses challenges, potentially involving risks in research (Figure 1). The aforementioned issues represent challenges in researching the preparation of giant viruses as gene engineering vectors.

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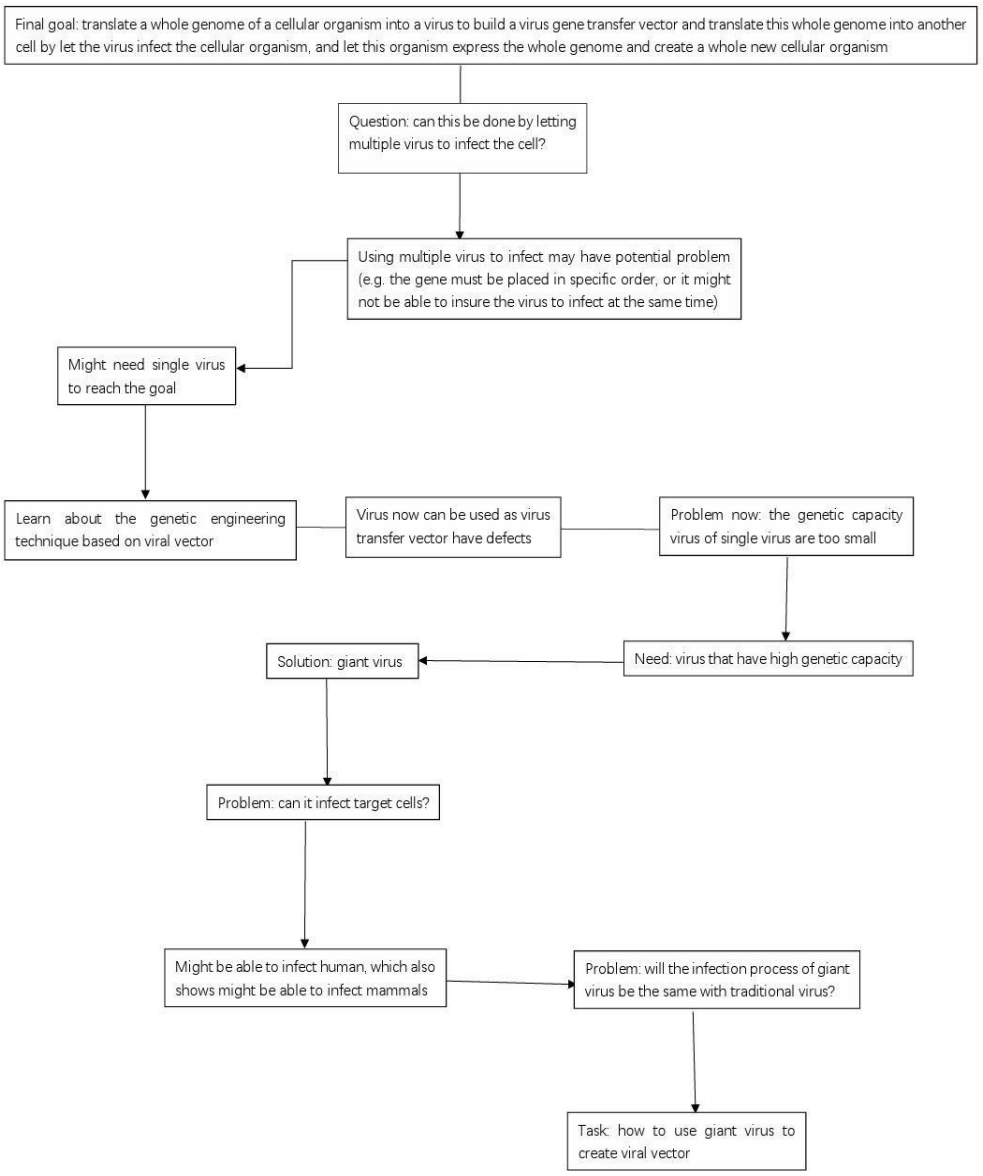

Figure 1. Construction of gene transfer vectors

3. The structure, characteristics, and differences between giant viruses and traditionalviruses

The most conspicuous feature of giant viruses is their significantly larger size and genome capacity compared to traditional viruses. Their genome capacity even surpasses that of certain cellular organisms (with the genome sizes of pneumoniae, Chlamydia trachomatis, and Treponema pallidum being 816,394bp, 1,042,519bp, and 1,138,011bp, respectively, whereas the genome capacity of the Mimivirus, belonging to the Megavirales order, can reach 1,181,404bp). Additionally, the number of genes contained within their genomes is also greater than that of traditional viruses [12]. However, despite large numbers of protein-encoding genes within giant viruses, a substantial portion remains uncharacterized, possibly serving to expand their physical size [3]. While this conclusion may seemingly conflict with the highly compressed internal genes of such viruses, resulting in relatively empty interiors, if true, it signifies immense potential for becoming gene carriers. These uncharacterized genes could be replaced by target genes and equipped with promoters for stable expression. The enlargement of the genome in giant viruses, aiming to mimic bacteria, is further

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evidenced by some giant viruses enclosing themselves in polysaccharide membranes, thus facilitating their entry into hosts through phagocytosis, a method utilized by their host amoebas [5, 12].

Another distinction between giant viruses and traditional viruses lies in the former harboring a greater number of genes. Several genes previously unknown in traditional viruses have been discovered in giant viruses, including but not limited to protein translation-related genes, DNA repair enzyme-related genes, and topoisomerase-related genes [13]. Prior to this discovery, the capability to translate proteins was considered a significant distinction between viruses and traditional cellular life. However, the existence of giant viruses renders this distinction ineffective (though giant viruses still require the assistance of hosts to complete their entire expression cycle and cannot independently replicate, translate, or express DNA). Currently, it is believed that the purpose of carrying such genes in giant viruses lies in their ability to shut down the translation process in some hosts. In such instances, these genes enable giant viruses to complete their replication, translation, and expression lifecycle [14]. This ability offers a new perspective. If giant viruses are engineered into gene transfer vectors, there might be opportunities to edit genes in cells that no longer undergo translation, enabling these specific cells to achieve additional functionalities. This approach could be aimed at hosts that can shut down their translation abilities, closing specific functions (such as toxin production) by creating gene transfer vectors and infecting hosts. Simultaneously, it enables the expression of genes carried by the viral vector to achieve specific objectives.

Apart from their genetic content, the shell proteins of giant viruses also exhibit certain differences compared to traditional viruses. These differences not only involve variations in protein types, shell sizes, or functions but also encompass distinct morphological structures. In contrast to the diverse structural shapes found in traditional virus capsid proteins (such as the icosahedral viruses like adenoviruses, enveloped viruses like influenza viruses and coronaviruses, helical viruses like tobacco mosaic viruses, bullet-shaped viruses like rabies viruses, complex viruses like T4 bacteriophages, filamentous viruses like Ebola viruses and Marburg viruses), giant viruses are generally categorized into icosahedral and non-icosahedral shapes, mostly spherical, elliptical, or oval [6-7]. Some structures can be observed under an optical microscope due to their size [15]. Considering the significantly lower cost of optical microscopy compared to electron microscopy, this could potentially reduce the cost of studying giant viruses in certain aspects compared to the requirements for traditional virus observation, mostly reliant on electron microscopy. However, besides their shapes, giant viruses have a specific genetic outlet in their structure compared to most traditional viruses. This outlet is used to release genes when infecting hosts and generally divides into “stargate” and “corks” types [6,15]. The stargate appears in icosahedral configurations of large viruses, exhibiting a star-shaped, operable opening. The existence of the stargate means that icosahedral giant viruses are not perfectly symmetrical, unlike traditional viruses with icosahedral structures. The corks typically appear in non-icosahedral large viruses, and one or more corks can exist. These openings allow giant viruses to maintain extremely high stability and survive in extreme environments where conventional viruses cannot persist (active giant viruses have been found in extreme environments like alkaline lakes, permafrost, and depths of up to 3 kilometers in the sea). However, these openings also cause thermodynamic barriers, making giant viruses less sensitive to changes in the external environment and rendering the process of infecting hosts more passive compared to traditional viruses. Based on current information, the process by which giant viruses infect hosts differs from traditional viruses, which can actively inject genetic material into host cells. Instead, the infection of giant viruses relies on host cells’ phagocytic behavior toward viral particles [6, 16]. If the phagocytic behavior of the host is a necessary condition for the infection of giant viruses, it might present a certain obstacle to creating giant viruses as gene engineering vectors, making the selection of target infections more stringent. Specifically, under the premise of not further modifying giant viruses themselves, it might not infect cells that do not engage in phagocytic behavior, such as bacteria, some mature cells (like sperm cells), or inactive cells (Figure 2). As bacteria are one of the primary targets of current genetic engineering, and infecting germ cells is a primary method for heritable genetic engineering, this peculiarity potentially negatively affecting numerous similar aspects.

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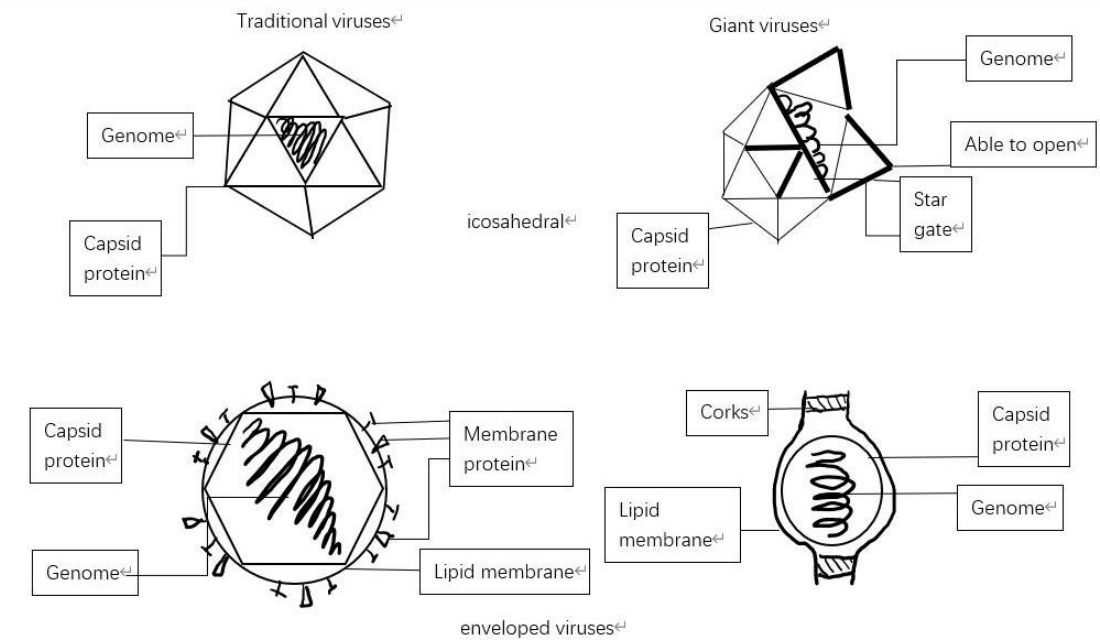

Figure 2. The Structure, characteristics, and differences between giant viruses and traditional viruses

Despite the vast differences between giant viruses and traditional viruses, several crucial similarities persist between the two. These similarities primarily revolve around overlaps in genetic content and certain protein characteristics [17]. Additionally, the prevailing viewpoint suggests that the origin of giant viruses resulted from the fusion of numerous smaller traditional viruses within host organisms. There is also a hypothesis that their origin aligns with viruses escaping from host cell genomes [3]. Based on these hypotheses, it can be speculated whether there exists an intermediate species between traditional viruses and giant viruses that could harness advantages from both, or whether there's a possibility of artificially creating such intermediate states.

4. Confirmation of hosts and infectivity of giant viruses

The current understanding of giant viruses remains relatively insufficient, especially concerning the hosts of these viruses, which remains a topic of debate. Current studies suggest that known hosts of giant viruses include one or multiple amoeba types, with some known hosts being exclusively amoebas [7]. The current data do not exclude the possibility of giant viruses exhibiting specificity for infecting amoebas. As numerous amoebas themselves are parasitic, the potential for giant viruses to indirectly infect hosts through amoeba parasitism adds complexity to confirming the actual hosts of giant viruses. There's speculation regarding sponges being potential hosts for giant viruses, supported by some evidence but yet insufficient for conclusive proof [18]. Given that sponge organisms possess amoeba-like cells internally, it's reasonable to consider sponges as potential direct hosts for large viruses. Previous studies have indicated the ability of researchers to culture giant viruses using human T-cells [11]. While this demonstrates the capacity of giant viruses to infect T-cells, considering the substantial similarities between T-cells and amoebas and the lack of further information, it's uncertain whether giant viruses possess the ability to infect most cells similar to amoebas or other cells within the same host that are significantly different from amoebas.

Existing data indicate potential hosts for giant viruses, including corals, leeches, dipteran insects (flies), mammals (cattle and monkeys, marine protists, mollusks (oysters), and fish [18-24]. However, except for marine protists, there isn't sufficient evidence to directly prove other hosts being infected by giant viruses rather than indirect infection through amoeba-parasite interactions. One possible method to research and verify the direct hosts of giant viruses involves purifying virus particles and co-culturing them with cells under specific conditions (simulating a normal biological environment).

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Based on the characteristic that giant viruses can be observed under an optical microscope, observations can be directly made post-culturing. If the results show the absence of amoebas but the presence of active and replicating giant virus particles within cells, it can be considered that the organism is a direct host for giant viruses.

On the other hand, understanding the effects of giant viruses on hosts (whether directly or indirectly) is also crucial. As previously mentioned, giant viruses harbor numerous unexpressed genes, rendering predictions about their impact on hosts via genetic technologies potentially inaccurate. Due to numerous cases isolating giant virus samples from pneumonia patients, it's suggested that giant viruses might be associated with some human pneumonias, although some data partially disagree with this view [25-29]. Moreover, the role and impact of amoebas in this process cannot be ignored. Firstly, in natural environments, as most giant viruses reside within amoebas, it's assumed that documented cases of giant virus infections also simultaneously involve amoeba parasitism. Current data indicate the presence of numerous potential pathogens causing pneumonia within amoebas in nature [30-32]. Thus, differentiating between the impact caused by these pathogens and that caused by giant viruses is challenging. Secondly, it's unclear whether amoebas themselves, without pathogens, possess the capability to induce pneumonia. Simultaneously, the combined effects generated by amoebas and large viruses could potentially alter infection symptoms. Given that certain nematodes display varying harm to hosts based on the presence or absence of pathogens, this possibility should be considered [33]. Therefore, if assuming giant viruses can directly infect specific hosts, the role of amoebas as a necessary infection route should also be considered. Studies have demonstrated the pathogenicity of specific giant virus types in mice after inoculation, but these studies did not account for these aforementioned factors [34]. Researching these questions could involve cultivating pure amoebas (ensuring they are pathogen-free) and pure giant viruses (not influenced by amoebas' presence) and observing their effects on specific hosts after inoculation.

5. The future prospects of genetic engineering based on giant viruses

Despite the considerable lack of clarity surrounding giant viruses at present, their diverse characteristics endow them with the potential to become viral transfer vectors. Their unique features, distinct from traditional viruses, also suggest the possibility of achieving other specialized functions. As previously mentioned, giant viruses possess an immense genomic information capacity distinct from traditional viruses, allowing them to carry substantial amounts of DNA and enabling the transportation of larger individual genes. Additionally, due to the inherent protein translation genes mentioned earlier, giant virus transfer vectors can target transgenes for inactive cells or deactivate specific cellular functions, while retaining the ability to express the carried genes. Their inherent stability allows storage under conditions where traditional virus carriers cannot be stored.

However, utilizing giant viruses as viral transfer vectors still presents numerous potential issues, and conducting research to acquire sufficient information about these concerns is imperative. Currently, the primary obstacle to the development of large viruses lies in insufficient information. The foremost issue is the host. Considering that the direct hosts of known giant viruses include only algae and amoebas, while algae may be an ideal target for genetic engineering, other multicellular plants, animals, bacteria, and traditional transgenic targets are not included. Simultaneously, due to the distinct infection mode of giant viruses, their demonstrated infection capability is currently limited to amoeba-like cells (such as macrophages and other immune cells). Does this mean that giant viruses are only capable of infecting these specific cell types? If so, the practical value of virus carriers made using giant viruses will be significantly reduced. Lastly, it remains unclear whether amoebas serve as intermediate hosts in the infection process of giant viruses or if their presence is necessary during infection. Additionally, existing information cannot confirm whether giant viruses, their amoeba hosts, or the amoebas themselves would cause substantial adverse effects on the host. If amoebas are proven to play a necessary role in infection and cause unacceptable harm to target cells, successfully constructing giant viruses as virus carriers for widespread use remains unfeasible.

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The value of giant viruses is not limited to constructing virus carriers. Firstly, their complex structure presents high value for the development of virology through actual attempts at synthesizing viruses artificially. If artificial synthesis is not feasible, there is a particular parasitic species that are able to effect giant viruses [35]. These parasitic viruses, named virophage, resembling bacteriophages, might have developed from them due to extreme similarities between giant viruses and bacteria in some aspects. The existence of this virus could potentially serve as a means for large virus gene editing, while research into it alongside giant viruses might offer substantial information for virology, biological evolution, and the study of gene and protein transcription and translation processes. Due to the significant similarities between giant viruses and bacteria, it might even be plausible to explore the possibility of directly using whole bacteria as gene transfer vectors implanted into cells based on studies on giant viruses. Lastly, research has suggested that giant viruses have the ability to provide certain advantages to their eukaryotic hosts, suggesting considerable research value in this area [36].

6. Conclusion

Despite the current lack of information about giant viruses, their enormous potential in genetic engineering and other fields is evident. Research directed towards them can provide advantageous information across multiple disciplines. There is a pressing need to intensify research efforts focused on giant viruses to fully unlock their potential.

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