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Research review paper

Design, preparation and application of nucleic acid delivery carriers

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ABSTRACT

Gene delivery vectors must deliver their cargoes into the cytosol or the nucleus, where DNA or siRNA functions *in vivo*. Therefore it is crucial for the rational design of the nucleic acid delivery carriers. Compared with viral vectors, non-viral vectors have overcome some fatal defections in gene therapy. Whereas the most important issue for the non-viral vectors is the low transfection efficiency, which hinders the progress of non-viral carriers. Sparked by the structures of the virus and understanding of the process of virus infection, various biomimic structures of non-viral carriers were designed and prepared to improve the transfection issues *in vitro* and *in vivo*. However, less impressive results are achieved. In this review, we will investigate the evolution of the virus-mimicking carriers of nucleic acids for gene therapy, especially in cancer therapy; explore and discuss the relationship between the structures, materials and functions of the carriers, to provide guidance for establishing safe and highly efficient non-viral carriers for gene therapy.

Contents

1. Introduction

Nucleic acids, which include DNA (deoxyribonucleic acid) and RNA (ribonucleic acid), have many therapeutic applications. They could be used as pharmaceutical agents to treat diseases, involving correction of genetic defects and gene augmentation for chronic diseases including cancers. Herein, gene therapy with nucleic acids has attracted more and more attentions. However, it is generally difficult for the naked nucleic acid to deliver into cells of the body mainly due to enzymatic degradation by the nucleases (Takakura et al., 2001), which limit serum half-life of DNA to 10 min (Kawabata et al., 1995) and unmodified small interfering RNA (siRNA) to 5–60 min (Soutschek et al., 2004). Moreover, nucleic acids are negatively charged biomacromolecules, which hinder them across the cellular membranes without the aid of external force.

How to introduce foreign nucleic acid into cells? Researchers get the answers from the process of virus infection. Generally, viruses could enter the cells via endocytosis and release viral genome, which could replicate and transcribe in host cells. Then viral mRNAs are translated and proteins are processed to make multiple copies of viruses. Finally, viruses assemble inside the host cells and escape to the exterior (Fig. 1). Through understanding the virus infection process, researchers exploit the delivery carriers for nucleic acids. The viral carriers, like adenoviral vectors (Hartman et al., 2008) and retroviral/lentiviral vectors (Cooray et al., 2012; Ellis, 2005) were first used as they could bind to

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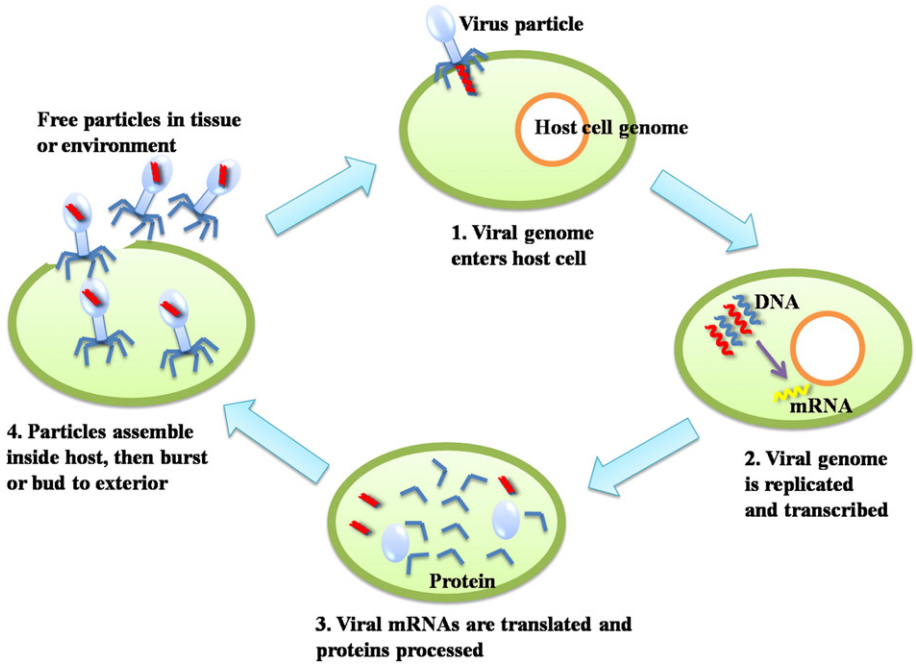

Fig. 1. The process of virus infection.

their hosts and introduce their genetic materials into the host cells. The viral vectors were prepared by removing the virus' own cargo and subsequently packaging the genes of interest into the viral shells (Selkirk, 2004). These viral delivery systems showed relatively high transfection efficiency both *in vitro* and *in vivo*. However, the death of an 18-year old participant in virus treatment using adenovirus vector to deliver therapeutic DNA to the liver in the University of Pennsylvania study resulted in a significant setback to gene therapy (Lehrman, 1999; Marshall, 1999). It revealed the risk of overwhelming inflammation from the virus treatment. Apart from the clinical safety issue (Check, 2005), the viral delivery systems exhibited some other critical problems, such as small cargo capacity, lack of long-term transgene expression, resistance to repeated administration, difficulty in large-scale pharmaceutical grade production and quality control and so on (Chowdhury, 2009; Collins et al., 2008), which led to a reconsideration for the use of viral vectors and accelerated the research on non-viral carriers since they could overcome toxicity issues for viral delivery.

In order to deliver therapeutic genes into the target cells safely and efficiently, it is especially crucial for the rational design of the non-viral delivery carriers. As we known, virus consists three parts, including the genetic materials like DNA or RNA, a protein coat that covers and protects these genes, and an envelope of lipids surrounding the protein coat. Sparked by the structures of the virus, many virus-mimicking non-viral carriers are developed for therapeutic applications. They exhibit low immunogenicity, low toxicity, ease of production, and the potential of transferring large pieces of nucleic acids into cells (Chen and Huang, 2008; Gary et al., 2007; Guo and Huang, 2012).

Besides the safety issues, the viral vectors have low specific targeting, like tumor-targeting in cancer therapy. Non-viral vectors have presented some crucial advantages over viral vectors to improve the toxicity and targeting problems by using nanotechnology in tumor tissues, naming the enhanced permeability and retention (EPR) effect with the characteristics of tumor vasculature (Fig. 2) (Iyer et al., 2006; Maeda et al., 2003; Torchilin, 2011). Non-viral vectors within nanoscale could pass through tumor blood vessels and get more accumulation in tumor tissues than in normal tissues. More importantly, to overcome the extracellular and intracellular barriers during the delivery of nucleic acids, various non-viral carriers have been designed and prepared. These carriers include lipids (Ewert et al., 2010; Gomes-da-Silva et al., 2012; Yang et al., 2011), biomaterials (e.g. chitosan, cyclodextran) (Li and Loh, 2008; Saranya et al., 2011), synthesized polymers (Martello et al., 2012; Patnaik and Gupta, 2013; Son et al., 2012) and dendrimers (Percec et al., 2010). But until now, only few of these vectors step into clinical trials or commercial availability. The most important issue for the non-viral vectors is the low transfection efficiency which hinders the progress of non-viral carriers.

For systemic administration of these non-viral vectors into target cells, there are many extracellular and intracellular barriers that must be overcome to get efficient transfection, including effective circulation in the blood, extravasation across the vascular endothelial membranes, diffusion through the extracellular matrix, cellular association and uptake, endosomal escape, unpacking of the complexes of nucleic acids and release of the intact therapeutic nucleic acids in the cytoplasm

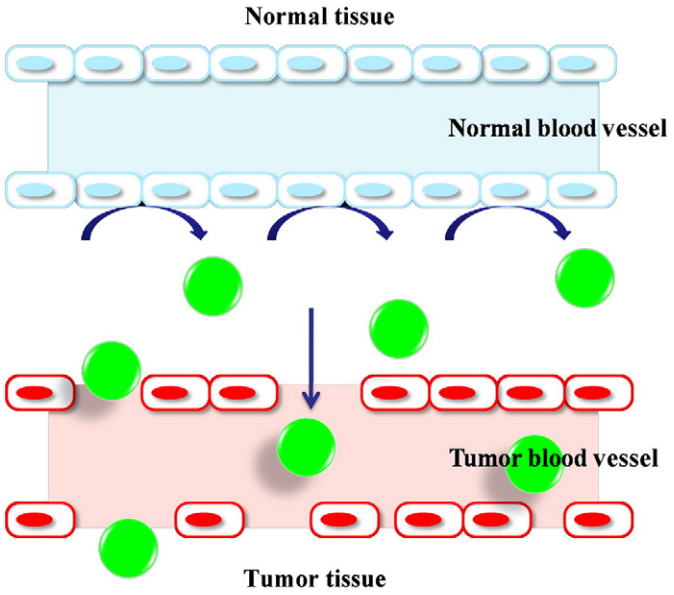

Fig. 2. Schematic representation of the passive tumor targeting of non-viral vectors and enhanced permeability and retention (EPR) effect.