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Review

Applying horizontal gene transfer phenomena to enhance non-viral gene therapy

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ABSTRACT

Horizontal gene transfer (HGT) is widespread amongst prokaryotes, but eukaryotes tend to be far less promiscuous with their genetic information. However, several examples of HGT from pathogens into eukaryotic cells have been discovered and mimicked to improve non-viral gene delivery techniques. For example, several viral proteins and DNA sequences have been used to significantly increase cytoplasmic and nuclear gene delivery. Plant genetic engineering is routinely performed with the pathogenic bacterium *Agrobacterium tumefaciens* and similar pathogens (*e.g. Bartonella henselae*) may also be able to transform human cells. Intracellular parasites like *Trypanosoma cruzi* may also provide new insights into overcoming cellular barriers to gene delivery. Finally, intercellular nucleic acid transfer between host cells will also be briefly discussed. This article will review the unique characteristics of several different viruses and microbes and discuss how their traits have been successfully applied to improve non-viral gene delivery techniques. Consequently, pathogenic traits that originally caused diseases may eventually be used to treat many genetic diseases.

Contents

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1. Introduction

Horizontal gene transfer (HGT) is defined as the exchange of genetic material between different species. HGT occurs frequently between prokaryotes, allowing them to quickly adapt to environmental changes by sharing genes for antibiotic resistance [1] or metabolic enzymes [2,3]. This phenomenon revolutionized the field of biotechnology by allowing genetic engineers to transform bacteria with valuable eukaryotic genes for industrial production (*e.g.* insulin [4] and various antibodies [5]).

In contrast to the genetic promiscuity of prokaryotes, eukaryotes are much more resistant to HGT. Eukaryotic cells possess several barriers that repel foreign DNA, including a nuclear membrane and DNase enzymes in the cytosol [6]. However, several significant HGT events have been discovered in multicellular eukaryotes. For example, the red color of some aphids and spider mites has been attributed to the HGT of fungal genes for carotenoid biosynthesis [7,8]. The coffee berry borer beetle (*Hypothenemus hampei*) also expresses a mannanase gene of bacterial origin which allows the beetle to digest galactomannan, the major polysaccharide in coffee berries [9]. The most stunning example of eukaryotic HGT may be the photosynthetic sea slug, *Elysia chlorotica*, which is able to harvest and support algal plastids for several months by expressing plastid maintenance genes of algal origin [10].

While the previous examples are highly random and isolated events, there are other examples of eukaryotic HGT which are more frequent. For example, viruses are highly efficient HGT vectors that transfer viral genes and even some host genes between cells [11,12]. The bacterium *Agrobacterium tumefaciens* infects plant tissues by transferring oncogenes to plant cells to induce tumor formation [13]. Finally, *Trypanosoma cruzi* is an intracellular eukaryotic parasite which infects human cells and is responsible for adverse HGT events which may cause chronic Chagas Disease [14]. The purpose of this review is to highlight the mechanisms that these pathogens use to transfer genetic material and show how those mechanisms have been applied to improve modern gene delivery techniques. In addition, the natural transfer of nucleic acids between host cells *via* plasmodesmata, nanotubes, vesicles, and carrier proteins will also be discussed.

2. Highly evolved HGT: Viruses

Viruses have evolved over millennia into highly efficient gene delivery vehicles. Their efficiency is highlighted by the success of many clinical trials with viral gene therapy [15]. For example, recombinant viruses have been used to successfully treat Leber's congenital amaurosis (LCA, a type of blindness) [16] and Severe Combined Immunodeficiency (SCID) [17]. Unfortunately, the clinical progress of viral gene therapy has been hindered by severe side effects, including immune responses [18,19], inflammation [20], and even oncogenesis [21]. Additional concerns associated with viral gene therapy include restrictions on gene size (<5–40 kb, depending on the virus) [15] and the relative difficulty of manufacturing viruses. Therefore, interest in non-viral gene delivery has grown significantly over the past few decades. Many non-viral gene delivery techniques have been developed (cationic polymers, lipids, dendrimers, peptides, *etc.*), but these techniques are typically much less efficient than viral gene delivery. This section will focus on the unique characteristics of viruses that have been used to increase the efficiency of non-viral gene delivery techniques, including methods of DNA protection/transport, cell invasion, endosomal escape, nuclear transport, and transgene expression/maintenance (see Fig. 1 for overview).

2.1. Nucleic acid protection: Capsids & envelopes

One of the simplest ways viruses enhance gene delivery is by storing their nucleic acids within protein capsules (capsids), which may also be surrounded by a lipid membrane or "envelope" from the previous host cell [22,23]. Capsids protect their nucleic acid cargo during intercellular transport from degradation by plasma nucleases [24] and scavenging by albumin [25]. In addition, the size and shape of viral particles directly influence their circulation half life, since filamentous capsids have been shown to persist 10 times longer (~1 week) in the circulation than spherical capsids [26]. Specialized capsid proteins also play key roles in cell binding and invasion. However, capsid proteins have also been shown to initiate immune responses, thereby significantly reducing the effectiveness of some viral gene therapies after the initial treatment [27]. Some viral capsids may also cause inflammation and even apoptosis in certain cells [28].

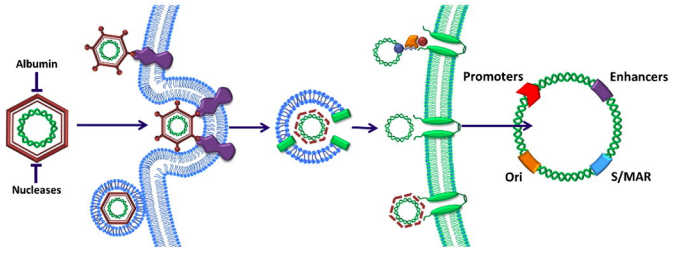

Fig. 1. Useful traits of viral gene delivery and expression that have been used to enhance non-viral gene delivery. From left to right: in the extracellular space, viruses protect their nucleic acid cargo from plasma scavengers and nucleases with protein capsids and/or lipid membranes (envelopes). Antigens on the capsid or envelope surface allow viruses to bind one or more receptors on specific cell types and directly fuse with the cell membrane (enveloped viruses) or induce endocytosis. As pH decreases within the maturing endosome, capsid proteins change conformation and destabilize the endosomal membrane to release viral nucleic acids, with or without the capsid. Nuclear import of viral nucleic acids is then facilitated either by binding to host transcription factors or viral proteins with nuclear localization signals that interact with nuclear pore complexes. Finally, viral gene expression within the nucleus is enhanced by highly efficient promoters and enhancers while origins of replication (Ori) and/or scaffold/matrix attachment regions (S/MAR) ensure plasmid replication and sustained gene expression.

Many non-viral gene delivery vehicles have aimed to mimic the beneficial/protective properties of capsids while avoiding the immune and inflammatory effects of capsids [29–31]. For example, cationic polymers and peptides readily bind to anionic plasmid DNA to form polyplexes, thereby condensing the DNA and protecting it from serum nucleases [32,33]. Cationic polymers (PEI and poly-lysine) have also been used to coat non-infectious viruses to create polymer–virus hybrids that are able to transduce a wide variety of cells and sustain gene expression for a considerable period (up to 40 days) [34] with much lower doses of hybrid than native virus [35].

Development of artificial capsids for gene delivery has also been the focus of much research, but controlling the crucial factors of size and shape while packaging bulky plasmid DNA has proven to be a considerable challenge. Nonetheless, Lim et al. were able to synthesize a self-assembling filamentous capsid containing siRNA by using self-assembling β-sheet peptides with poly-lysine sequences for DNA binding and covalently attached glucose ligands for cell-specific receptor binding. This synthetic capsid was able to deliver siRNA and silence GFP expression in HeLa cells just as well as lipofectamine (~70% reduction in GFP expression) [36]. Malay et al. also showed that gold nanoparticles could be used to catalyze formation of capsids with cysteine rich *trp* RNA-binding attenuation protein (TRAP) monomers. However, the diameter of these synthetic capsids was quite small (15–22 nm) and they did not contain any nucleic acids [37]. It is also worth mentioning that polyplexes of plasmid DNA and a cationic peptide from the HIV protein Vpr (aa 52–93) were shown to have transfection efficiencies 100–1000 fold higher than poly-lysine (but roughly equivalent to PEI) [38].

There have also been significant efforts to mimic enveloped viruses. For example, Muller et al. synthesized a PEI–lipid–RGD peptide conjugate that formed artificial virus-like envelopes (AVEs) loaded with plasmid DNA and presenting RGD peptides for cell-specific binding to HUVEC cells [39]. These micelles were able to transfect nearly 100% of HUVEC cells *in vitro*, while non-RGD micelles and PEI polyplexes transfected only 50% and 5% of cells, respectively [39]. Similar AVEs