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Review article

In vivo methods for acute modulation of gene expression in the central nervous system

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ABSTRACT

Accurate and timely expression of specific genes guarantees the healthy development and function of the brain. Indeed, variations in the correct amount or timing of gene expression lead to improper development and/or pathological conditions. Almost forty years after the first successful gene transfection in *in vitro* cell cultures, it is currently possible to regulate gene expression in an area-specific manner at any step of central nervous system development and in adulthood in experimental animals *in vivo,* even overcoming the very poor accessibility of the brain. Here, we will review the diverse approaches for acute gene transfer *in vivo*, highlighting their advantages and disadvantages with respect to the efficiency and specificity of transfection as well as to brain accessibility. In particular, we will present well-established chemical, physical and virus-based approaches suitable for different animal models, pointing out their current and future possible applications in basic and translational research as well as in gene therapy.

1. Introduction

Proper development of the central nervous system (CNS) determines its function and consequent behaviors. Accordingly, numerous gene alterations during development lead to brain disorders characterized by a variety of abnormal behaviors, often depending on which brain area is mostly affected. On the other hand, gene alterations during adulthood may also lead to a variety of brain-related diseases and neurodegenerative disorders that vary in their symptoms, depending on the affected brain areas. This complexity highlights the need for temporal and spatial regulation of specific genes for proper brain function. Accordingly, the development of reliable techniques for gene transfection *in vivo* has recently attracted the attention of an increasing number of researchers as a means to study and understand the roles of the diverse genes underlying the basic mechanisms of CNS development and function (basic research) and to study genes involved in CNS disorders to find new possible treatments (translational research). In particular, in recent years, basic research has benefited from new tools for gene editing (*e.g.*, CRISPR-Cas9 technology; Ahmad et al., 2018) and neuronal-activity modulation (optogenetics and chemogenetics; Dobrzanski and Kossut, 2017; Towne and Thompson, 2016), which both need to be coupled to a nucleic acid delivery system. For translational research, a fast-growing field of study focuses on the possibility of treating CNS disorders by manipulating gene expression (gene therapy) rather than by classical pharmacology, which has proven highly ineffective in the last 10 years (Gribkoff and Kaczmarek, 2017). Thus, regardless of the final application, the development of novel methods for modulating gene expression *in vivo* has acquired increasing

Abbreviations: AAV, adeno-associated viruses; ADV, adenoviruses; ApoE, apolypoprotein E; BBB, blood brain barrier; cap, encapsulation; Cas9, CRISPR associated protein 9; cDNA, coding DNA; CMV, cytomegalovirus; CNS, central nervous system; CNT, carbon nanotubes; CRISPR, clustered regularly interspaced short palindromic repeats; DCX, doublecortin; DNA, deoxyribonucleic acid; DOPE, 1,2-dioleoyl-phosphatidyl-ethanolamine; EGFP, enhanced green fluorescent protein; EUE, exo utero electroporation; EYFP, enhanced yellow fluorescent protein; gRNA, guide RNA; HBD, heparin-binding domain; HD-ADV, helper-dependent ADVs; HIV, human immunodeficiency virus; HSV, herpes simplex viruses; HV, helper virus; ICV, intracerebroventricular; iPS, induced pluripotent stem-cells; IT, intrathecal; IUE, *in utero* electroporation; LNE, lipid nanoemulsion; LPS, lipopolysaccharide; LRP, lipoprotein receptor-related protein; mHTT, mutant huntingtin; miRNA, micro-RNA; MNP, magnetic nanoparticles; mRNA, messenger RNA; PAMAM, polyamidoamine; PEG, polyethylene Glycol; PEI, polyethylenimine; rep, replication; RNA, ribonucleic acid; RVG, rabies virus glycoprotein; SF, spherical fullerenes; shRNA, short hairpin RNA; siRNA, small-interfering RNA; SLN, solid lipid nanoparticles; ssDNA, single-stranded DNA; SV40, simian virus 40; SVZ, subventricular zone; TMC, trimethylated chitosan; TNF-α, tumor necrosis factor alpha; US, ultrasound; VSV-G, vesicular stomatitis virus glycoprotein

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Table 1
Chemical methods for transfection.

Group	Type/Helper	Toxicity	*In vivo* delivery method	Efficiency /Stability	BBB accessibility	Most Prominent Applications (references)
Lipids	Liposomes	↑	N/A	↓	N/A	Nayerossadat et al., 2012
	Cationic lipid mix/PEG	↓	Intraventricular injection	→	N/A	Hassani et al., 2005; Nayerossadat et al., 2012; Yin et al., 2014;Zhi et al., 2018
	Cationic lipid mix/DOPE	↓	Intraventricular injection, Intratumoral (glioblastoma) injection	↑	N/A	Roessler and Davidson et al., 1994; Hassani et al., 2005; Pulkkanen and Ylä-Herttuala, 2005; Lagarce and Passirani, 2016;Cikankowitz et al., 2017
	Lipid Nanoemulsions	↓	Intranasal	↑	N/A	Ramamoorth and Narvekar, 2015; Yadav et al., 2016
	Solid lipid nanoparticles	↓	Intravenous injection	↑	Possibly	Jin et al., 2011; Pathak et al., 2017
Nanoparticles	Silica	↓	Subventricular injection, Intracortical injection	↑	N/A	Bharali et al., 2005; Luo and Saltzman, 2006
	Gold nanoparticles	↓	Systemic injection	↑	+	Jensen et al., 2013; Escudero-Francos et al., 2017; Takeuchi et al., 2018; Hu et al., 2018
	Carbon Nanotubes	↓	Intraventricular injection	↑	N/A	Al-Jamal et al., 2011; Costa et al., 2016
Polymers	Polyethylenimine	↑	Intracortical injection	↓	(+ with PEG)	Abdallah et al., 1996; Lungwitz et al., 2005; Nouri et al., 2017
	Polyethylenimine/RVG	↑	Tail injection	↓	+	Hwang et al., 2011
	Chitosans/PEG	↓	Intratumoral (glioblastoma) injection	↑	–	Duceppe and Tabrizian, 2010; Danhier et al., 2015; Ramamoorth and Narvekar, 2015
	ChitosansTrimethylated/PEG + RVG	↓	Intravenous injection	↑	+	Gao et al., 2014
	Polyamidoamine (with various functionalizations)	↓	Tail injection, Systemic injection, Intravenous administration	↑	+	Huang et al., 2007;Huang et al., 2008;Ke et al., 2009;Zarebkohan et al., 2015

↑high / →medium / ↓low / +yes / -no / N/A-information not available.

importance in recent years. This modulation can be achieved either by the generation of genetically modified animals or by acute procedures for gene expression modulation. Here, we will only review the latter.

Generally, an acute modulation of gene expression requires a transfection or transduction process (*i.e.*, a procedure that introduces foreign nucleic acids, such as DNA/RNA, into a cell) to produce genetically modified cells or organisms by nonviral or viral methods, respectively. Indeed, many molecules, such as nucleic acids and certain drugs, are not able to diffuse through the lipophilic cell membrane due to their physicochemical properties (*e.g.*, hydrophilicity, charge) and/or size. Thus, the support of specific carriers or chemical/physical stimulation is often necessary to increase the efficiency of the transfection process.

Although highly efficient, acute introduction of DNA into mammalian cells *in vitro* was achieved a long time ago (Graham and van der Eb, 1973), for the last four decades, scientists have struggled to increase the efficiency of this process *in vivo* (Crystal, 2014). For example, circulating nucleic acids for transfection have a very short half-life *in vivo* because they are degraded by circulating nucleases in the blood. Moreover, targeting specific organs and cell types at discrete times is generally challenging *in vivo* and is particularly difficult in the case of the brain for a number of reasons. First, the brain is an isolated, inaccessible environment due to the presence of the skull and the blood-brain barrier (BBB), which separates circulating blood from the brain's extracellular fluid. Second, the brain contains several different areas that are each characterized by specific functions, rendering area-specific transfection crucial in this organ. Third, the CNS contains hundreds of billions of neuronal and glial cells characterized by high diversity (*e.g.*, even among neurons, there is a wide variety of diverse types with diverse functions). Finally, neurons are postmitotic cells that do not divide, requiring a cell cycle-independent introduction of genetic material.

Since almost ninety-five percent of the animals used in research are mice and rats (Badyal and Desai, 2014), we will focus this review on rodents. For a long time, the dominant approach for acute gene transfer *in vivo* in rodents was the design of different viral vectors with increasingly higher efficiency of transfection and tissue specificity (see viral methods below). Nevertheless, due to limitations related to the safety of viral gene transfer, many physical strategies have also been adopted, such as electroporation and sonoporation (see physical methods below). However, physical methods require strong conditions (*e.g.*, strong electric field or ultrasound) for efficient transfection, and thus a range of synthetic carriers for nucleic acids suited for chemical transfection have also been created (see chemical methods below; Yin et al., 2014). In recent years, different methods have also been combined (*e.g.*, physical and chemical methods or viruses and physical methods) to try to overcome the shortcomings of one method *vs* the other while taking advantage of the positive features of both.

In this review, we describe the currently available methods for the delivery of nucleic acids to the CNS *in vivo*. First, we focus on chemical methods, which include a wide selection of nucleic acid carriers that allow crossing of the cell membrane. Second, we describe physical methods, which take advantage of physical forces to increase membrane permeability and possibly direct nucleic acids to the desired location. Third, we address viral-based techniques, which explore the intrinsic transfection ability of viruses. Interestingly, all of the described techniques are very different, but they each present some level of overlap, creating a great portfolio to choose from when designing diverse experiments with gene transfer *in vivo*. Here, we will note the advantages and disadvantages of each described method and will indicate their best-suited applications.

2. Chemical methods for transfection

Chemical methods of transfection are a set of techniques that rely on external carriers characterized by specific chemical properties that are