Gene targeting technology and gene therapy of the brain

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Many serious disorders of the CNS are resistant to conventional small-molecule therapy but could be treated, even cured, with gene therapy of the brain. In current practice, delivery of the therapeutic gene to the brain requires drilling a hole in the head followed by insertion of the gene incorporated in a viral vector. The advantage of craniotomy-based gene delivery is that the gene can be expressed in a highly circumscribed area of the brain with an effective treatment volume of 1–10 μl. However, craniotomy-based delivery does not enable the expression of the therapeutic gene widely throughout the brain or even to a relatively localized area such as a brain tumor, which could have a volume greater than several milliliters. However, craniotomy has to be used currently because the virus does not cross the blood–brain barrier (BBB) *in vivo*.

Viruses have been the vector of choice because the virus-coat proteins trigger endocytosis of the virus into the target brain cell. The two most commonly used viral vectors are adenovirus or herpes simplex virus (HSV). The problem with both these viruses is that, because they are common, humans have a pre-existing immunity. This immunity generates an inflammatory response with even a single intracerebral injection of the virus: increased expression of antigen presenting cells, leukocyte infiltration, gliosis and demyelination all occur in the brain of rodents, primates and humans following a single intracerebral injection of either adenovirus or HSV[1,2]. The inflammatory response is even caused by replication-deficient virus, because the inflammation follows from an immune reaction to the viral coat proteins.

Figure 1. β-Galactosidase histochemistry of a rat brain removed 48 h after a single intravenous injection of a β-galactosidase gene carried by a plasmid that is packaged in the interior of 85 mm liposomes[3]. The surface of the liposome is covered by thousands of strands of 2000 Da polyethylene glycol (PEG), and this stabilizes the liposome in the blood and prolongs the circulation time in the plasma. Approximately 2% of the PEG strands that project from the liposome surface are tethered to a monoclonal antibody that targets the transferrin receptor. This receptor is expressed both on the brain capillary endothelium, which forms the blood–brain barrier *in vivo*, and on the neuronal plasma membrane. Targeting the immunoliposomes to the transferrin receptor enables transport across both the blood–brain barrier and the neuronal plasma membrane *in vivo*. The use of gene targeting technology enables widespread expression in the brain of an exogenous gene following a single intravenous administration of a non-viral gene formulation.

Gene targeting technology

Craniotomy and viruses are first-generation brain gene delivery systems. Future gene therapy of the brain could use delivery systems that are both non-invasive and non-viral. A brain gene delivery system should enable widespread expression of a therapeutic gene throughout the brain following a simple intravenous injection. Such goals can only be achieved with gene targeting technology that engineers a formulation with the following functions.

First, the exogenous gene packaged in a non-viral plasmid vector is interiorized within a nanocontainer, much like exogenous genes are packaged in the interior of viruses. This protects the therapeutic gene from the ubiquitous endonucleases in the body. Second, the nanocontainer is non-immunogenic and formed by either natural lipids or other non-immunogenic polymeric substances. Third, the nanocontainer carrying the exogenous gene is stable in the bloodstream with optimal plasma pharmacokinetics following an intravenous injection. Fourth, the surface of the nanocontainer is modified in a way that triggers transcytosis across microvascular endothelial barriers such as the BBB and then endocytosis into target neurons or glial cells in brain. The BBB is 400 miles long in the humans, so a brain gene targeting technology that enables the nanocontainer to traverse the BBB will distribute the therapeutic gene throughout the entire brain volume.

Development of gene therapy

The development of non-invasive, non-viral gene therapy of the brain requires a molecular formulation that can only emerge with advanced drug targeting technology that brings together several

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disciplines including liposome technology, polymer technology, monoclonal antibody targeting technology, genetic engineering, and the molecular biology of therapeutic gene discovery. These technologies have been recently used to engineer a non-viral formulation that enables widespread expression of an exogenous gene throughout the brain following a single intravenous injection (Fig. 1)[3]. The non-viral plasmid containing an exogenous gene, β-galactosidase, is packaged in the interior of 85 nm liposomes.

These neutral liposomes contain a small quantity of cationic lipid to stabilize the DNA. Any DNA on the exterior of the liposome is removed by nuclease digestion. An injection of a liposome into the bloodstream, however, is followed by rapid uptake by cells lining the reticuloendothelial system (RES). The rapid RES uptake can be blocked by coating the surface of the liposome with several thousand strands of 2000 Da polyethyleneglycol (PEG), a process called pegylation. The pegylated liposomes are stable in the bloodstream and have long blood circulation times. However, pegylated liposomes are relatively inert and must be further modified to enable targeting through both the BBB and the neuronal plasma membrane. This is accomplished by tethering the tips of 1–2% of the PEG strands with a targeting monoclonal antibody (MAb) to form an immunoliposome.

Owing to expression of the transferrin receptor (TfR) on both the BBB and the neuronal plasma membrane, the use of an anti-TfR MAb causes the pegylated immunoliposome to undergo transport through both the BBB and the neuronal plasma membrane *in vivo*. The liposomal lipids fuse with the endosomal membrane inside neurons, which releases the plasmid into the cytosolic space of target neurons, where it can then diffuse to the nuclear compartment. The only immunogenic component of the formulation is the MAb and the immunogenecity of murine MAbs in humans can be eliminated with genetic engineering and 'humanization' of the MAb.

There is diffuse, widespread expression of the β-galactosidase gene throughout the brain at 2 days after a single intravenous injection of the pegylated immunoliposomes (Fig. 1). When the anti-TfR MAb is replaced with a mouse immunoglobulin IgG_{2a} isotype control, there is no expression of the exogenous gene in the brain[3]. The exogenous gene is expressed in neurons, which are visible at low magnification in Ammon's horn of the hippocampus, thalamic nuclei and the supraoptic nuclei. There is also gene expression in the epithelium of the choroid plexus lining the ventricles.

Use of gene therapy to the brain

There are many diseases where it would be advantageous to have widespread expression of an exogenous gene throughout the brain including the inborn errors of metabolism such as the lysosomal storage disorders, fragile X syndrome, Rett's syndrome, Canavan's disease and the inherited epilepsies. Other conditions that would be amenable to non-invasive, non-viral gene therapy of the brain include cerebral AIDS, brain tumors and neurodegenerative diseases such as Parkinson's disease. In the case of Parkinson's disease, it could be argued that it is desirable to augment dopamine production only in the basal ganglia and not in the cortex. However, it is possible that the insertion of tissue and cell specific promoters at the 5′ end of the gene, and tissue-specific mRNA stabilizers at the 3′ end of the gene, can enable tissue-specific gene expression in particular regions of the brain. The persistence of plasmid-based gene formulations in brain cells *in vivo* can also be optimized and this will determine the periodicity of repeated administration of the gene medicine.

Animals to humans

Gene therapy of the brain originated from gene discovery that took place in the absence of a parallel program in gene targeting technology. Following gene discovery, the intracerebral injection of viral vectors provided a quick solution to the 'delivery problem', and enabled gene expression in the brain of experimental animals. However, the transfer of this technology to humans has not been successful. There is little interest within large pharmaceutical companies of administering viruses to the brain in humans via craniotomy. The application of genomics and the availability of the human genome sequence will only accentuate the need for practical approaches to brain gene therapy in the future. What is required is a technology that enables the widespread expression of a therapeutic gene throughout the brain following the intravenous injection of a non-immunogenic formulation. This goal is possible if advanced gene targeting technology is developed in parallel with gene discovery.

References

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