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RNAi has been rapidly adopted for the discovery and validation of gene function through the use of a sequence-specific short interfering RNA (siRNA), which was originally identified as 21–23-nucleotide double-stranded RNA fragments, generated by a cellular enzyme called Dicer [1]. These siRNA intermediates were found to form an RNA-induced silencing complex (RISC) with other cellular proteins, which then selectively degraded the complementary single-stranded target RNA in a sequence specific manner [1,2]. Not only is siRNA being used to characterize gene function, it is also being rapidly adopted in HTS for potential therapeutic targets in pharmaceutical research laboratories. A consequence of the growing success in using siRNA as a research tool in vitro and in vivo is an emerging interest in using siRNA as a therapeutic agent. However, the relatively small number of reports on in vivo siRNA studies indicates a lack of effective in vivo delivery methods, especially for achieving RNAi-mediated downregulation of specific gene targets in animal disease models. In this review, we will address the problems in in vivo siRNA delivery and discuss the approaches to overcome these problems.

Challenges of in vivo siRNA delivery
The function of a target gene can be validated by inhibiting the target protein and examining the induced phenotypic changes by various means, including biochemical, pharmacological and histological assays. Conventional target validation is a time-consuming process that can last for years, because of the time requested for screening small-molecule inhibitors of a target protein or generating gene knockout animals. As a potent and specific inhibitor of gene expression, siRNA is being rapidly adopted as the preferred tool for functional genomics research [3,4]. siRNA oligos are typically used to inhibit an individual gene, although targeting multiple genes or groups of genes is possible by using a combination of multiple siRNA sequences [5,6].

The success of siRNA in vitro has led to growing interest in in vivo applications of siRNA. These applications should eventually provide validated targets for conventional therapeutic modalities, such as small molecule and monoclonal antibody inhibitors, as well as validation of siRNA drug lead itself. In vivo siRNA target validation approach has greatly shorten the process of target validation because it takes only days to select a potent siRNA inhibitor to virtually any target gene, compared with months and years for screening small-molecule inhibitors. In cancer research, for example, the tumorigenesis process has been recognized as the result of abnormal overexpression of oncogenes, growth factors and mutant tumor suppressors, even if underexpression of other proteins can also play a crucial role. Efforts to identify and validate targets for developing cancer therapeutics have been mainly focused on those proteins overexpressed...
in the tumor tissue that might promote tumorigenesis. Among the well-known oncogenic growth factors, vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), fibroblast growth factor (FGF) and their receptors represent the most widely recognized targets, which originally took years to validate. In a recent study, siRNA-mediated downregulation of the pro-angiogenesis genes VEGF and VEGF receptor 2 (VEGFR2) in clinically relevant xenografts of tumor models resulted in a significant antitumor efficacy [7]. Using this technology, the functions of these two targets were rapidly revalidated in just a few weeks [7]. This example demonstrates the power of \textit{in vivo} target validation with siRNA inhibitors. Further, in this case, not only the roles of the proangiogenic factors were validated, but also the siRNA inhibitors themselves were validated as potential anticancer drugs.

Delivering siRNA \textit{in vivo} to animal tissues is complicated and involves using physical, chemical or biological approaches and in some cases a combination of them [7]. Because the main goal of \textit{in vivo} delivery is to have active siRNA oligos in the target cells, the stability of siRNA oligos in the extracellular and intracellular environments after systemic administration is the most challenging issue (Figure 1). The first hurdle is the size of the 21-nucleotide double-stranded siRNA oligos: these oligos are relatively small and thus are rapidly excreted through urine when administrated into the blood stream, even if siRNA molecules remain stable through chemical modifications. Second, the double-stranded siRNA oligos are relatively unstable in the serum environment and they can be degraded by RNase activity within a short period of time. Third, when siRNA is administered systemically, the nonspecific distribution of these oligos throughout the body will significantly decrease the local concentration where the disease occurs. In addition, the siRNA oligos need to overcome the blood vessel endothelial wall and multiple tissue barriers to reach the target cells. Finally, when siRNA reaches the target cells, cellular uptake of those oligos and intracellular RNAi activity require efficient endocytosis and intact double-stranded oligos, respectively.

To increase their stability in the extracellular and intracellular environments, siRNA oligos can be chemically modified by a variety of methods, including changes of the oligo backbone, replacement of individual nucleotides with nucleotide analogues and addition of conjugates to the oligo [8]. The chemically modified siRNA demonstrated a significant serum resistant and higher stability [8], but this did not solve the problems of urine excretion and targeted delivery. Therefore, a delivery system capable of protecting siRNA oligos from the urine excretion and RNase degradation, transporting siRNA oligos through the physical barriers to the target tissue and enhancing cellular uptake of the siRNAs is the key to the success of \textit{in vivo} siRNA application.

The accessibility of different tissue types, various delivery routes and a variety of pharmacological requirements makes it impossible to have a universal \textit{in vivo} delivery system suitable to every scenario of siRNA delivery. In terms of \textit{in vivo} delivery vehicles for siRNA, the ‘nonviral’ carriers are the major type being investigated so far, although some physical and viral delivery approaches are also very effective. The routes of \textit{in vivo} deliveries are commonly categorized as local or systemical. Some of the delivery vehicles and delivery routes are very efficient for target validation in animals but might not be useful for delivery of siRNA therapeutics in humans (Figure 2). Therefore, \textit{in vivo} siRNA delivery carriers and methods can also be classified as clinically viable or nonclinically viable, according to their suitability in humans.
Delivering siRNA in vivo using nonviral carriers

Many nonviral carriers used in the delivery of DNA for gene therapy have been adopted for siRNA delivery. Because RNAi is active in the cytoplasm, delivery of siRNA is relatively easier than delivery of plasmid or viral DNA, which has to be in the nucleus for expression. Nonviral siRNA delivery to the disease tissue does not elicit an immune response, which has a great advantage for its application in drug target validation and also allows multiple administrations of siRNA, which are crucial for siRNA therapeutic applications.

Cationic lipids and polymers are two major classes of nonviral siRNA delivery carriers and both are positively charged and can form complexes with negatively charged siRNA. The siRNA–carrier complex can be condensed into a tiny nanoparticle with size ~100 nm, allowing very efficient cellular uptake of the siRNA agent through the endocytosis process. In a mouse model, the reporter gene silencing and downregulation of the tumor necrosis factor α (TNF-α) expression were achieved after intraperitoneal (i.p.) administration of siRNA–lipoplexes [9]. A recent study reported that the use of a cationic derivative of cardiolipin to form lipoplexes with siRNA targeting the c-raf oncogene led to inhibition of tumor growth in a sequence specific manner [10]. In another study, a family of highly branched histidine-lysine (HK) peptides was found to be an effective carrier of siRNA [11]. The Raf-1 expression in MDA-MB-435 xenografts was significantly inhibited by intratumoral injection of Raf-1 siRNA complexed with the HK polymer [12]. Another recent study demonstrated a significant inhibition of HER-2 expression and tumor growth through i.p. injection of HER-2 siRNA formulated with polyethylenimine (PEI) [13]. These studies demonstrated that cationic lipids and polymers can enhance siRNA delivery in vivo through systemic routes either intravenously (i.v.) or i.p. Those carrier-administered siRNA agents were efficiently knocking down the target genes and achieved antitumor efficacies. By contrast, direct intratumoral injection of VEGF siRNA without carrier did not generate any significant antitumor efficacy [14]. In an antiviral study, i.v. administration of siRNA specific targeting influenza virus RNA genome complexed with PEI was able to inhibit influenza virus production in mice [15].

Some ligand-targeted siRNA delivery systems have been developed using the cationic liposome complex and polymer complex systems. Recent successes of using ligand-targeted complexes to deliver therapeutic siRNA into tumor tissues and liver tissues suggest that targeted systemic delivery for siRNA therapeutics is very promising [16–18]. We demonstrated an efficient systemic delivery of the siRNA reaching the tumor neovascular tissue in the tumor model [16] and the ocular neovascular tissue in a herpes simplex virus (HSV) eye infection model [5] through a ligand-targeted nanoparticle [19]. The Arg-Gly-Asp (RGD)-motif peptide ligand is specific to the activated integrin receptor, a marker of the endothelium and tumor cells. The ligand-targeted nanoparticle maintains the stability of the siRNA payload, targets the tumor neovascularization and enhances the cellular uptake of the siRNA targeting VEGFR2 mRNA. As a result, antiangiogenesis effects were observed in xenografts of the tumor model and in ocular neovascularization in the HSV disease model [5]. More importantly, this siRNA nanoparticle delivery system is clinically viable and can be used for various applications of siRNA therapeutics.

It has been reported that siRNA can trigger ‘off-target effects’ [20,21] and activate the cellular interferon pathway, especially when delivered with cationic liposome or polymer transfection reagents [22,23]. These issues raise concerns for the integrity of target validation studies and the safety and selectivity of the potential siRNA therapeutics. However, the majority of these alarming results were obtained from in vitro studies and many more studies have shown that siRNA inhibitors are highly specific in vitro and in vivo [24,25]. A recent study that used systemic delivery of unmodified and unformulated siRNA oligos into mice revealed a lack of interferon response [26]. Two recent reports have revealed that siRNA oligos, containing the 5′-UGUGU-3′ motif, were able to induce a Toll-like receptor-mediated interferon response only when they were delivered in vivo with cationic lipid or polymer carriers, through either i.v. or i.p. administration [27,28]. In contrast, neither the unformulated siRNA oligos containing the 5′-UGUGU-3′ motif, nor siRNA containing no 5′-UGUGU-3′ motif but with cationic carriers, were able to induce the interferon response. For this reason, any 5′-UGUGU-3′ motif and other potential immunostimulatory motifs should be eliminated from the siRNA oligos if the cationic lipid or polymer carriers are going to be used.

Delivering siRNA in vivo using local administrations

The choice between local and systemic delivery depends on what tissues and cell types are targeted. For example, skin and muscle can be better accessed using local siRNA delivery, whereas lung and tumor can be reached efficiently by local and systemic siRNA deliveries. There is increasing evidence that siRNA can be efficiently delivered to various tissue types using different approaches (Figure 2).

Intranasal

Airway delivery of siRNA is a very useful method for target validation and therapeutic development because of the relevance of the respiratory system in various diseases. In a recent study, intranasal delivery of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) specific siRNA mixed with pulmonary surface active material (Infasurf™) and elastase resulted in a lowered GAPDH protein levels in the lung, heart, and kidney by ~50–70% on day 1 and day 7 after administration, when compared with scrambled siRNA control [29]. Direct delivery of unformulated siRNA into mouse airway led to knockdown of heme oxygenase-1 (HO-1) expression in the lung [30]. Intranasal administration of cationic liposome-formulated siRNA specifically targeting the influenza virus RNA genome into mouse lung infected with the influenza virus resulted in a significantly reduced lung virus titer in infected mice and protected animals from lethal challenge [31]. However, in vivo delivery of siRNA with cationic polymer carriers, such as PEI, is often associated with severe toxicity in the host and might induce nonspecific interferon response through the Toll-like receptor pathway, as discussed above. Therefore, pulmonary siRNA delivery might require formulations without cationic carriers. Recently, we have successfully delivered siRNA with D5W (5% d-glucose in water) solution into mouse and monkey lungs, achieving effective knockdown of severe acute respiratory syndrome (SARS) coronavirus RNA [32].

Intraocular

An increasing number of clinical protocols have been approved for treating eye diseases with nucleic acid drugs, such as antisense oligonucleotides or RNA aptamers. Delivery of nonformulated siRNA specific to VEGF to the subretinal space in a mouse model
of retinal neovascularization resulted in a significant reduction of angiogenesis in the eye. Importantly, this study indicated that chemical protection of the siRNA was not essential, at least in the intravitreous compartment of the eye, in contrast to antisense oligonucleotides or RNA aptamers, which need chemical modification for applications in the eye [33]. Using a murine model of herpetic stromal keratitis, which develops from the herpes simplex virus corneal infection, we found that subconjunctival administration of siRNA targeting several genes in the VEGF pathway significantly inhibited the corneal angiogenesis and disease symptoms [5]. Subconjunctival delivery of siRNA specific to the transforming growth factor β (TGF-β) significantly reduced the inflammatory response and matrix deposition in a wound-induced mouse model of ocular inflammation [34]. The evidence also provided clinically viable means for the local delivery of siRNA for gene function validation in various eye disease models. Local delivery of siRNA to the front of the eye subconjunctivally or to the back of the eye intravitreously is highly efficient in silencing target gene expression and therefore these are effective administration routes for target validation of eye diseases. However, the frequency and time intervals between repeated deliveries might be the limiting factors of these delivery routes, especially for clinical application of siRNA therapeutics.

**Intracerebral**

The brain tissue is the foundation of the central nervous system (CNS), obviously a very important biological system that draws interest for functional genomics research and therapeutic development [35]. A recent study showed that infusion of an aqueous solution of chemically protected siRNA oligonucleotides directly into the brain was able to selectively inhibit gene expression [36]. Treatment of rats with aqueous siRNA against α-2A adrenergic receptors [α(2A)-ARs] on day 2–4 after birth resulted in an acute decrease in the levels of α(2A)-AR mRNA in the brainstem into which siRNA were injected [37]. Nonviral infusion of siRNA in the brain provided a unique approach to accelerate target validation for neuropsychiatric disorders that involve a complex interplay of gene(s) from various brain regions. For example, infusion of siRNA specific to an endogenous dopamine transporter (DAT) gene in regions (ventral midbrain) far distal to the infusion site resulted in a significant downregulation of DAT mRNA and protein in the brain and elicited a temporal hyperlocomotor response similar to that obtained upon infusion of GBR-12909, a pharmacologically selective DAT inhibitor [38]. However, the difficulty of performing surgical implantation of an infusion pump delivering high dose of siRNA limits its usefulness as a tool for functional genomics. Another recent study on the use of cationic formulations for siRNA delivery to the brain revealed that delivery was more efficient using lipid carriers than polymer carriers [39]. Electroporation is a physical approach that has been used for introduce DNA into the cells. During the process of electroporation, an electric field pulse induces pores (electropores) in cell membranes to allow DNA molecules to enter the cell. Recently, electroporation procedures have been adopted for local delivery of siRNA. In one study, siRNA introduced into the hippocampus region by local electroporation led to a marked reduction in the levels of DAT mRNA and protein in the brain and elicited a temporal hyperlocomotor response similar to that obtained upon infusion of GBR-12909, a pharmacologically selective DAT inhibitor [38]. However, the difficulty of performing surgical implantation of an infusion pump delivering high dose of siRNA limits its usefulness as a tool for functional genomics. Another recent study on the use of cationic formulations for siRNA delivery to the brain revealed that delivery was more efficient using lipid carriers than polymer carriers [39]. Electroporation is a physical approach that has been used for introduce DNA into the cells. During the process of electroporation, an electric field pulse induces pores (electropores) in cell membranes to allow DNA molecules to enter the cell. Recently, electroporation procedures have been adopted for local delivery of siRNA. In one study, siRNA introduced into the hippocampus region by local electroporation led to a marked reduction in the expression levels of the mRNA and protein of the target genes, such as glutamate receptor 2 (GluR2) and cyclooxygenase 1 (Cox-1), without affecting the expression levels of other proteins [40].

**Intramuscular**

The skeletal-muscle tissue is accessible for local siRNA administration. Direct injection of siRNA formulated with cationic lipids or polymers can be considered for local delivery, although
inflammation caused by the injection is a common problem. A recent study with nonformulated siRNA delivered by direct injection into mouse muscle followed by electroporation demonstrated a significant gene silencing that lasted for 11 days [41]. The electroporation method was also applied in a different study targeting several reporter genes in the murine skeletal muscle [42]. A local hydrodynamic approach, in which a sufficient volume of siRNA was rapidly injected into a distal vein of a limb, which was transiently isolated by a tourniquet or blood pressure cuff, was tested for siRNA delivery in muscles of animal models and demonstrated a knockdown of both reporter and endogenous gene [43].

**Intratumoral**

Intratumoral delivery of siRNA is a very attractive approach for functional validation of tumorigenic genes. We observed inhibition of tumor growth in two human breast cancer xenografts using intratumoral delivery of VEGF specific siRNA [44]. It was reported that atelocollagen, a collagen solubilized by protease, can protect siRNA from being digested by RNase when forming a complex with siRNA. In addition, the siRNA can be slowly released from atelocollagen to efficiently transduce into cells, allowing a long-term target gene silencing [45,46]. In a mouse xenograft tumor study, after administration of atelocollagen–luc-siRNA complex intratumorally, a reduced luciferase expression was observed. Furthermore, intratumoral injection of atelocollagen–VEGF-siRNA showed an efficient inhibition of tumor growth in an orthotopic xenograft model of a human nonsemionomatous germ cell tumor [45]. A similar result was observed in a PtdCho-3 human prostate tumor xenograft using the same siRNA delivery approach [46]. Therefore, the atelocollagen-based siRNA delivery method could be a reliable approach to achieve maximal inhibition of gene function in vivo. On the basis of the successful validation of a group of novel genes for their roles in tumorigenesis by using intratumoral delivery of formulated siRNA [2,4], we believe that intratumorally delivering siRNA into tumor xenografts is a very useful platform for in vivo target validation.

**Delivery siRNA in vivo using systemic administration**

Systemic administration of siRNA agent is a very attractive approach for drug target validation and therapeutic application, if the hurdles described in the Figure 1 can be addressed. Usually, the purpose of systemic administration is not to deliver siRNA throughout the entire body, but to reach specific disease tissues. Two tissue types have been of particular interest for many siRNA delivery efforts: liver and tumor.

**Liver delivery**

Some of the first published results showing the activity of siRNA in mammals involved delivery of siRNA into mouse liver by using the hydrodynamic method; this consists of a rapid injection of a large volume of aqueous solution into the mouse tail vein, which creates a high pressure in the vascular circulation, leading to an extensive delivery of siRNA into hepatocytes [47–51]. This procedure allows high efficiency of siRNA uptake and potent siRNA activity in hepatocytes and, thus, is a useful tool for functional genomic studies in liver. However, this procedure is not clinically viable because of the potential damage of the liver and other organs and, therefore, is limited only to research on liver function and metabolism or liver infectious diseases such as hepatitis [52,53]. Hepatocyte specific targeting carriers for siRNA delivery into the liver are very attractive approaches for development of siRNA therapeutics for hepatic diseases and are currently under investigation. As one step towards the liver-targeting delivery, liver delivery of chemically modified oligonucleotide with cholesterol conjugates was tested, as described in recent publications [8,54]. The data suggested that at least three challenges must be addressed: adequate protection from serum degradation of the siRNA oligonucleotide on the way to the liver; protection of the siRNA oligonucleotide from rapid glomerulofiltration by the kidney into the urine; selective uptake by the target hepatocytes. In addition, the high dose used for cholesterol-conjugated siRNA i.v. delivery indicated a widespread distribution rather than liver targeted. A recent report [55] indicated that the lipid-encapsulated siRNAs targeting hepatitis B virus (HBV) RNA were able to exert a potent and persistent antiviral activity in the HBV mouse model, through i.v. injection. Although the conclusion of the report was that the chemical modification of siRNA is very important for the potency, the report did not clarify whether this modification is crucial or not when the lipid-encapsulation is applied.

**Targeting of the tumor and neovasculature**

Malignant tumors grow fast and spread throughout the body via blood or the lymphatic system. Metastatic tumors established at distant locations are usually not encapsulated and, thus, more amenable for systemic delivery. Local siRNA administration methods discussed above can meet the requirements for most functional genomics studies by acting on primary tumors or xenograft models, which form the basis of most cancer biology research. However, systemic delivery of siRNA is needed not only for studies related to gene functions, but also for siRNA-based cancer therapeutics.

Systemic siRNA delivery imposes several requirements and greater hurdles than local siRNA delivery. It requires stable oligonucleotides in the blood and in the local environment to enter the target cells. In addition, the siRNA needs to pass through multiple tissue barriers to reach the target cell. A recent study in pancreas xenografts used the systemic administration of CEACAM6-specific siRNA without protection and formulation. The study demonstrated a significant suppression of primary tumor growth by 68% versus control siRNA, associated with a decreased proliferation index of the tumor cells, impaired angiogenesis and increased apoptosis. Treatment of CEACAM6-specific siRNA completely inhibited metastasis and significantly improved survival, without apparent toxicity [56].

Recent studies showed that tumor-targeting siRNA delivery was achieved by using an RGD peptide ligand-directed nanoparticle and that its application in antiangiogenic treatment for cancer [57] was obtained by systemic siRNA delivery [19], as reviewed elsewhere recently [58]. We studied the RGD ligand-targeted nanoparticle for targeting the neovasculature in ocular neovascularization models [5]. The anti-angiogenesis efficacy observed in ocular neovascularization models further demonstrated this approach as a clinically viable method for siRNA therapeutics. In addition, we demonstrated that siRNA oligos specific for several genes can be combined in the same nanoparticle to give a better inhibition of the disease pathology [5,58]. This targeted siRNA delivery provides a unique therapeutic approach as the dual-targeted therapeutics.
Recently, antibody-mediated delivery of siRNA was reported for tumor targeting via cell-surface receptors [59]. When the siRNA cocktail targeting c-Myc, MDM2 and VEGF was complexed with an antibody-protamine, it was able to be efficiently delivered into B16 cell formed tumors with overexpression of gp160 surface protein by either local or systemic deliveries, achieving anti-tumor activity. This antibody-mediated binding to the artificial tumor cells is interesting for proof of concept, but might have limitations for clinically viable delivery.

**Conclusion**

Currently, *in vivo* siRNA delivery, through either the local or systemic route, is mainly serving as a research tool in functional genomics and the proof of principle for potential RNAi therapeutics (Table 1). Therefore, examining the utility of each siRNA delivery method *in vivo* requires confirmation of its robustness during the target validation process, with repeated testing in the preclinical models. A significant advantage of siRNA is the rapidity with which different siRNA sequences and the matching genes can be studied, which is particularly useful for drug target validation. Moreover, developing and optimizing siRNA delivery in various types of animal disease models is a challenging but worthy effort to accelerate the novel drug discovery process. With growing knowledge of *in vivo* siRNA delivery, the potential therapeutic applications of siRNA agents are emerging. To date, two siRNA therapeutics protocols were tested in Phase I clinical trials for the treatment of ocular neovascularization diseases and many more are in their late stage of preclinical studies for various indications [32,55,58]. Ultimately, the effort for *in vivo* siRNA delivery will be translated into many clinically viable administration methods for siRNA-based therapeutics to treat various cancer, viral infection, autoimmune and CNS diseases.

**Table 1**

| Applications  | Targets                  | Carrier          | Route               | Refs       |
|--------------|--------------------------|------------------|---------------------|------------|
| Oncology     | VEGF and VEGFR2          | Polymer          | Intratumoral        | [2,4,44]   |
|              | VEGFR2                   | Polymer          | Intratumoral        | [2,4]      |
|              | VEGF                     | N2A, syngenic tumor | Ligand-targeted nanoparticle | i.v. injection | [16,19,58] |
|              | VEGF                     | PtdCho-3, xenograft tumor | Atelocollagen | Intratumoral injection | [45]     |
|              | c-Raf                    | J78, xenograft tumor | Saline             | i.p. injection | [9]      |
|              | CEACAM6                  | MDA-MB-231, SCID | Cardiolipin analogue | i.v. injection | [10]    |
|              | EGFR                     | Orthotopic pancreatic, nude | Naked siRNA | i.p.  | [56] |
| Ophthalmology| VEGF Mouse (laser)       | Saline           | Local injection     | [33]      |
|              | VEGF, VEGFR1             | Photocoagulation | Ligand-targeted    | i.v., subconjunctival | [5]   |
|              | VEGF2                    | Mice (HSV induction) | Ligand-targeted     | i.v., subconjunctival | [5] |
|              | TGF-β II                  | Mice (wound induction) | Polymer nanoparticle | Subconjunctival | [34] |
| Rheumatology  | TNF-α Mouse              | Saline, electroporation | Intra-articular injection | [41]   |
| Infectious Diseases | Influenza A virus | C57BL/6 mouse | PEI | i.v. administration | [15] |
|              | Influenza A virus BALB/c mouse | PBS, oligofectamine | Intranasal | [31] |
|              | SARS Rhesus monkey        | DSW              | Intranasal         | [32]      |
|              | HBV BALB/c mouse         | PBS              | I.v. hydrodynamic injection | [51] |
|              | HBV BALB/c mouse         | Liposome         | I.v. injection     | [55]      |
| CNS          | P2X3 Rat                 | Saline           | Intrathecal injection | [36]   |
|              | DAT Mouse                | Saline           | Intraventricular infusion | [38,39] |
|              | α(2A)-ARs Rat            | Saline           | Intra-hippocampus   | [37]      |
|              | GluR2, Cox-1 Mouse       | Saline, electroporation | Intra-hippocampus | [40] |
| Others       | TNF-α Mouse              | DOTAP            | I.p. injection       | [9]       |
|              | GAPDH Mouse              | InfaSurf™       | Intra-nasal administration | [29] |
|              | HO-1 Mouse               | Naked            | Intra-nasal administration | [30] |
|              | Apo8 C57BL/6 mouse       | Stabilized Chol-siRNA | I.v. injection | [8] |
|              | Caspase-8, caspase-3 C57BL/6 mouse | 10% lipiodol | High-volume portal vein injection | [50] |

Abbreviations: P2X3, P2X purinoceptor 3; Apo8, apolipoprotein B; PBS, phosphate buffered saline; DOTAP, dioleoyl trimethylammonium.

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