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Expert Opinion

Leptin gene therapy in the fight against diabetes

Yuqing Wang, Akihiro Asakawa, Akio Inui & Ken-ichiro Kosai†
†*Kagoshima University Graduate School of Medical and Dental Sciences, Department of Gene Therapy and Regenerative Medicine, Kagoshima, Japan*

Importance of the field: The incidence of diabetes is increasing worldwide, yet current treatments are not always effective for all patient or disease types.
Areas covered in this review: Here, we summarize the biologic and clinical roles of leptin in diabetes, and discuss candidate viral vectors that may be employed in the clinical use of central leptin gene therapy for diabetes.
What the reader will gain: We discuss how studies on leptin, a regulator of the insulin–glucose axis, have significantly advanced our understanding of the roles of energy homeostasis and insulin resistance in the pathogeneses of metabolic syndrome and diabetes. Recent studies have demonstrated the long-term therapeutic effects of central leptin gene therapy in obesity and diabetes via decreased insulin resistance and increased glucose metabolism. Many of these studies have employed viral vectors, which afford high *in vivo* gene transduction efficiencies compared with non-viral vectors.
Take home message: Adeno-associated viral vectors are particularly well suited for central leptin gene therapy owing to their low toxicity and ability to drive transgene expression for extended periods.

Keywords: adeno-associated viral vector, diabetes, gene therapy, leptin, viral vector

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1. Introduction

Diabetes is a genetic disorder in which environmental factors precipitate phenotypic expression of the disease. The young and adult patient populations with obesity-related diabetes have recently been increasing at an alarming pace worldwide [1]. Current treatments for diabetes aim to establish glycemic control, and several medications that lower blood glucose levels, including insulin, have been developed. Although some of these medications are useful for certain patient types with diabetes, there is significant room for improvement. For instance, repeated daily administration of medications can be troublesome for patients, and some glucose-lowering medications are ineffective for certain diabetes types. Thus, novel therapeutic strategies, including those that provide prolonged effects after each application, are necessary to treat patients with diabetes more effectively.

Adipocyte-derived leptin is a pleiotropic hormone that peripherally and centrally regulates food intake and energy homeostasis [2-4]. Central administration of leptin inhibits appetitive drive, body weight gain and adiposity, and stimulates nonthermogenic energy expenditure via brown adipose tissue [3,5-7]. Several studies have suggested that leptin also contributes to hypothalamic control of the insulin–glucose axis. For example adeno-associated viral vector (AAV)-mediated central leptin gene therapy via an intracerebroventricular (i.c.v.) route was recently demonstrated to increase hypothalamic leptin levels to ameliorate hyperglycemia and hyperphagia in diabetic, insulin-deficient nonobese Akita mice and leptin-deficient obese *ob/ob* mice and also to promote survival in insulin-deficient mice studied by Kojima *et al.* [8,9]. The technique aided by AAV produces a robust and durable central leptin supply, which reinstates euglycemia and energy homeostasis for

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Article highlights.

- The biologic and clinical roles of leptin in diabetes.
- Central leptin gene therapy provides long-term therapeutic effects.
- Characteristic features of the representative viral vectors.
- Adeno-associated viral vectors may be suitable for central leptin gene therapy.
- Possible adverse effects of future leptin gene therapy are discussed.

This box summarizes key points contained in the article.

an extended period in the absence of insulin and is expected to be an effective therapeutic strategy for type 1 and type 2 diabetes. Here, we discuss diabetes, leptin and gene therapy vectors, including the potential of central leptin gene therapy as a substitute for conventional insulin therapy.

2. Diabetes

Diabetes is a chronic illness that requires continuous medical care and patient education on self-management to prevent acute complications and reduce the risk of long-term morbidity and mortality [10]. Diabetes afflicts an estimated 6% of the adult population in the Western hemisphere, and is increasingly problematic as the Eastern hemisphere adopts Western life-style approaches [11]. The WHO estimates that 170 million people worldwide have diabetes, and that the population will increase to 366 million by 2030 [12]. The rapid increasing of obesity is the most important pathogenic factor in diabetes [11].

2.1 Classification and clinical manifestations

Diabetes can be categorized into four clinical classes. Type 1 diabetes is a chronic autoimmune disorder characterized by destruction of insulin-producing pancreatic β cells [13]. Type 2 diabetes results from a progressive defect in insulin secretion on a background of insulin resistance; symptoms can manifest at any age. The disorder is influenced by a persistent metabolic imbalance that is engendered by a range of internal and external environmental factors, including diet and other lifestyle choices [1,14-17]. Type 2 diabetes is characterized by a range of metabolic disturbances – for example chronic hyperglycemia, insulin insensitivity in fat and muscle cells, hepatic glucose production in the prandial state, and decreased β cell effectiveness, which disrupts the first-phase insulin response to nutrient ingestion [12]. Most diabetes patients suffer from either type 1 or type 2 diabetes, with type 2 insulin-resistant diabetes accounting for 90 – 95% of all diabetes cases [11]. Another type of diabetes is caused by specific disorders, such as diseases of the exocrine pancreas (e.g., cystic fibrosis), or genetic defects in β cell function or insulin signaling. Finally, gestational diabetes mellitus (diabetes diagnosed during pregnancy) is generally classified as an independent diabetes type [10].

2.2 Current treatments

In addition to symptomatic treatment of disease complications, current diabetes therapy attempts to supplement insulin, increase insulin sensitivity, reduce excessive hepatic glucose production. and/or enhance glucose-stimulated insulin secretion [11]. Medications include recombinant insulin, oral anti-diabetes drugs, anti-hypertensive medications, and anti-dyslipidemic agents [12]. Diabetes is accompanied by hypertension, cardiovascular disease and microvascular disorders, which complicate treatment and can result in blindness, non-traumatic limb amputation, and renal failure [10]. Clinical studies have demonstrated that glycemic control is crucial for avoiding and/or delaying the onset of diabetes complications. The American Diabetes Association (ADA) and European Association for the Study of Diabetes (EASD) have established < 7.0% glycosylated hemoglobin (HbA_{1c}) as a treatment goal for adults with diabetes [18]. Although conventional treatments, such as diet, exercise and insulin replacement regimens, can effectively lower HbA_{1c} levels in diabetes patients, none address obesity or insulin resistance, or maintain normoglycemia permanently. Therefore, novel therapeutic strategies that persistently normalize the abnormal phenotypes of diabetes are eagerly awaited.

3. Leptin

3.1 Biology of leptin

Leptin is a 167 amino-acid protein product of the obese (*ob*) gene, which was identified in 1994 based on the morbid obesity phenotype that results from its absence [19-21]. Leptin is secreted from adipocytes in white adipose tissue, stomach, placenta, and mammary glands [19,22-25]. It acts primarily on the hypothalamus to modulate food intake and energy expenditure. The leptin receptor (OB-R) is alternatively spliced into six isoforms (OB-Ra, OB-Rb, OB-Rc, OB-Rd, OB-Re and OB-Rf) [3,26,27]. The short isoforms contain a C-terminally truncated intracellular domain (OB-Ra, OB-Rc, OB-Rd, OB-Rf) and may transport leptin through physiological barriers. OB-Ra is expressed in the endothelium of the vasculature and epithelium of the choroid plexus of the circumventricular organs, where it transports leptin across the blood-brain barrier. The long isoform (OB-Rb), which contains an extensive C-terminal intracellular domain, is abundantly expressed in hypothalamic neurons, including the hypothalamic arcuate nucleus (ARC), dorsomedial hypothalamic nucleus, paraventricular nucleus (PVN), ventromedial hypothalamic nucleus (VMH), and lateral hypothalamic nucleus of the CNS as described by Friedman and Halaas in 1998 [3]. OB-Rb activates JAK/signal transducer and activator of transcription signaling, affecting expression of hypothalamic neuropeptides. The soluble isoform (OB-Re), which contains only the extracellular domain, is a serum

leptin-binding protein. Leptin circulates as a free form or OB-Re–bound forum, and the sum of the two is generally measured as the total leptin level.

The synthesis and secretion of leptin are markedly increased in obese subjects [28]. Notably, leptin replacement either peripherally or centrally in leptin-deficient subjects [3,29-33], or selective introduction of the leptin receptor into the hypothalamus of OB-Rb mutant rats has been shown to normalize food intake and body weight [34]. Taken together, these results highlight the important metabolic roles of adipose tissue.

3.2 Leptin and diabetes

A number of studies in mice, rats, and humans have demonstrated that leptin is a key hormone in the regulation of not only food intake and energy expenditure but also insulin secretion and glucose metabolism via hypothalamic control of pancreatic insulin–glucose homeostasis [2-6,28,35-43]. Insulin is secreted from pancreatic β cells to stimulate adipogenesis and increase fat deposition in adipocytes [2,44-49]. Recent studies have shown that leptin inhibits episodic and postprandial insulin hypersecretion from the pancreas and modulates insulin activity in adipocytes, myocytes, and hepatocytes. C57BL/6H *ob/ob* mice, which carry a mutation in the obese gene, are obese and diabetic, and show reduced activity, metabolism, and body temperature [42]. Injections of recombinant leptin in C57BL/6H *ob/ob* and wide-type mice reduced body weight, the percentage of body fat, food intake and serum concentrations of glucose and insulin [20,21,42]. Furthermore, white and brown adipose tissues completely disappeared for an extended period in transgenic skinny mice that overexpressed leptin in the liver [28]. Glucose metabolism in the skinny mice increased in concert with an activation of insulin signaling in skeletal muscle and liver. Moreover, the livers of these mice were small and showed decreased stores of glycogen and lipid. Similar effects were found after acute intracerebroventricular infusion of leptin, which led to a significant increase in glucose turnover without altering the plasma insulin concentration [41]. Central administration of leptin has been shown to increase glucose turnover and uptake, and to decrease hepatic glycogen storage, suggesting that the effects of leptin on glucose metabolism are mediated largely via central mechanisms [8,40]. Leptin increases glucose uptake in peripheral tissues in humans independently of weight loss, which may be beneficial to some patients with type 1 or type 2 diabetes [2,3,49]. Together, these studies suggest a number of potential pathophysiologic and therapeutic roles for leptin in diabetes [13,34].

4. Gene therapy

Gene therapy is a novel therapeutic strategy that introduces a specific gene into patients to treat their disease. To date, more than 1,500 clinical trial protocols have been approved around the world [50]. A key issue for successful gene therapy is the development of an appropriate vector for the target disease. General requirements for vectors that can be used to treat congenital or acquired diseases (outside of cancer, which is a somewhat special case) include high *in vivo* gene transduction efficiency in target tissues and/or cell types, a clinically acceptable safety profile, long-term expression of the transgene, and the ability to carry and transduce a full-length gene, including transcriptional control elements. Although ideal vectors have not yet been developed, recombinant viral vectors have been widely used for clinical trials owing to their high *in vivo* gene transduction efficiency.

4.1 Features of representative vectors and their potential for central leptin gene therapy

Gene therapy with leptin is a promising treatment strategy for diabetes. Here, we summarize characteristic features of representative vectors and discuss their potential utility for central leptin gene therapy (**Table 1**).

4.1.1 Adenoviral vector (ADV)

Adenovirus is double-stranded DNA virus with a 36 – 40 kb genome, which encodes more than 70 gene products. The viral genome contains five early transcription units (E1A, E1B, E2, E3 and E4), two early delayed (intermediate) units (pIX and IVa2), and five late units (L1 – L5), which encode structural proteins for the capsid and internal core. Inverted terminal repeats at the end of the viral chromosome function as replication origins. ADVs can be easily prepared to high titer and can transduce genetic material into a variety of tissues *in vivo*, such as heart, liver, kidney, CNS, and cancers, irrespective of whether the cells are dividing [45,51-59]. A particularly important clinical advantage of ADVs is the relatively low potential for carcinogenesis, which is largely due to the episomal nature of the transgene (i.e., the transgene is not integrated into the chromosome).

First-generation ADVs, which lack E1 and E3 regions are viral-replication defective, have been used clinically for *in vivo* gene therapy directed at a variety of diseases [60,61]. Although first-generation ADVs can package and transduce up to 7 kb of sequence, they produce only transient transgene expression over the course of weeks when they are administered to immunocompetent hosts, because even low levels of certain viral proteins transcribed from the vector induce cytotoxic immune responses. To address this issue, helper-dependent ADVs (HD-ADVs), were developed by deleting all viral coding sequences from the vector backbone. *In vivo* administration of HD-ADVs in rodents resulted in persistent transgene expression. An additional advantage of HD-ADVs is their ability to package up to 37 kb of transgene sequence. A comparative study of leptin gene therapy using HD-ADVs and first-generation ADVs demonstrated that HD-ADVs efficiently delivered the transgene, causing prolonged elevations of serum leptin levels and weight loss. Furthermore, the liver toxicity, inflammation and cellular infiltration observed with first-generation ADVs were

Table 1. Characteristic features of the representative viral vectors.

	ADV			AAV	RV	LV
	1G-ADV	HD-ADV	CRA			
Capacity for transgene	~ 7 kb	~ 37 kb	*	~ 4.5 kb	~ 7 kb	~ 7 kb
In vivo gene transduction efficiency	High	Medium	Very high	High	Very low	Medium
Integration into host genome (frequency)	Low	Low	Low	Low	Very high	Very high
Duration of transgene expression	Transient	Persistent	N.D.	Persistent	Persistent	Persistent
Cytotoxic immune response (severity)	Relatively high	Minimal	*	Medium	Minimal	Minimal
Titer	Very high	Medium	Very high	High	Low	Medium
Clinical protocols[‡] (total: 815)	387[§]			71	336	21
Possible usefulness for central leptin gene therapy	Medium	High	N.D.	Very high	Very low	N.D.

*Depends on CRA construction and/or a protocol for *in vivo* use.

[‡]Gene Therapy Clinical Trial Worldwide, 2010 (http://wiley.co.uk/genmed/clinical/).

[§]Total number of ADV clinical protocols.

1G-ADV: First generation ADV; AAV: Adeno-associated virus; ADV: Adenovirus; CRA: Conditionally replicating ADV; HD-ADV: Helper-dependent ADV; LV: Lentivirus, N.D.: Not determined; RV: Retrovirus.

significantly better with HD-ADVs [62]. Potential problems with the clinical use of HD-ADVs include a relatively low titer and possible contamination from the helper virus.

On the other hand, most clinical trials of ADV-based gene therapy have been conducted in the field of oncology, which can be used to formulate plans for ADV-based leptin gene therapy [63]. A major obstacle to cancer gene therapy is inefficient and nonspecific gene delivery to cancer cells, leading to unsatisfactory outcomes in clinical trials due to tumor recurrence from nontransduced cancer cells [64]. Conditionally replicating ADVs (CRAs), which selectively replicate in tumor cells but not in normal cells, may circumvent this problem to achieve efficient, tumor-specific gene delivery. Moreover, we have recently developed a novel method to construct m-CRAs, highly engineered CRAs that are regulated by multiple factors [63,64]. Although the utility of current CRAs for central leptin gene therapy is unknown, the lessons from ADVs may be useful in the development of ideal ADVs for central leptin gene therapy.

4.1.2 *Adeno-associated viral vector (AAV)*

AAVs are single-stranded DNA parvoviruses. They are not pathogenic and can efficiently infect both dividing and nondividing cells. Serotype 2 AAV is often used for gene therapy because wild-type serotype 2 AAV is not associated with any human diseases. AAV does not contain any genes that encode viral proteins, eliminating the risk of potentially undesirable immune responses. High titers of AAV ($> 10^{12} - 10^{13}$ particles/ml) can be stably prepared. Since the first AAV-based gene therapy trial in human patients was performed in 1995 [65], several additional clinical trials of AAV-mediated gene therapy have been initiated for cancer and other diseases. Long-term transgene expression, which is required for many gene therapy applications, has been observed after AAV-mediated *in vivo* gene transduction into lung, liver, muscle, heart, and brain [34,66-74]. New AAV serotypes have advanced our ability to target specific cell types [75], facilitating studies in a diverse range of disease models, including Parkinson's disease, Alzheimer's disease, hemophilia, diabetes, obesity, α1-antitrypsin deficiency, Canavan disease, cystic fibrosis, lysosomal storage diseases and Duchenne muscular dystrophy [76]. Some treatments have progressed to clinical use, and AAV is thought to be a promising viral vector for clinical gene therapy. A disadvantage of AAV is that the size of the packaged transgene is limited to less than 5 kb.

4.1.3 *Retroviral and lentiviral vectors (RV and LV)*

RV was used for the first clinical gene therapy trials in human patients. RV can accommodate up to 7 kb of transgene sequence and can integrate into the host genome, resulting in persistent transgene expression [52,77,78]. A disadvantage of this vector is the requirement for active host cell replication at the time of gene transduction, which is particularly problematic for *in vivo* neuronal gene therapy. To address this issue, recently developed LVs can transduce genes into nondividing cells, including neurons, after *in vivo* administration. Both RV and LV integrate the transgene into the host genome, a critical safety concern in clinical trials.

4.2 AAV-mediated central leptin gene therapy for diabetes

In summary, ADV and AAV have the advantage of high *in vivo* gene transduction efficiency and safety compared with RV and LV. Thus, we will focus on ADV- and AAV-mediated leptin gene therapy. In 1995, injections of recombinant leptin were shown to reduce body weight and fat deposition [21,42], through the direct activities of leptin on neuronal networks that control feeding and energy balance [20]. At that time, first-generation ADV were being developed and

used for gene therapy studies of many disease models. In 1996, obesity and diabetes in leptin-deficient *ob/ob* adult mice were treated effectively with tail-vein injections of ADV expressing mouse leptin [79]. ADV-mediated leptin gene therapy for *ob/ob* mice led to serum leptin levels that were 70 times higher than those observed in control C57BL/6J mice; the treated *ob/ob* mice demonstrated a rapid reduction in food consumption and marked weight loss. Notably, the elevated serum leptin levels in the treated animals did not persist beyond 2 – 3 weeks due to a cytotoxic immune response against proteins encoded by the first-generation ADV [80,81]. Interestingly, complete amelioration of the obese phenotype was achieved after leptin gene transfer in *ob/ob* mice despite the lack of persistent leptin gene expression. A rapid resumption of food intake to pretreatment values and gradual body weight gain were observed after the serum leptin concentrations fell to undetectable levels, indicating that treatment did not result in long-lasting adverse effects. The data also showed that hyperinsulinemia and insulin resistance were improved and fasting blood glucose was reduced after leptin gene transduction, which together eliminated the non-insulin-dependent diabetic phenotype in the *ob/ob* mice.

Then, in 1997, a single intramuscular injection of AAV encoding mouse leptin (AAV-lep) to *ob/ob* mice was shown to prevent obesity and diabetes for more than six months by maintaining normal circulating levels of leptin (2 – 5 ng/ml) [31]. Although systemic injection of AAV-lep is effective for obesity and diabetes in *ob/ob* mice, enhancing ectopic leptin production in peripheral tissues (skeletal muscle or liver) is not an appropriate weight control strategy because of the pleiotropic effects of leptin [65,76,82,83]. Chronic increases in the peripheral leptin concentration after intraperitoneal or subcutaneous injection led to reduced food intake in lean and, to a lesser extent, in diet-induced-obese mice. Both groups, however, developed peripheral leptin resistance, which could be overcome by intracerebroventricular injection of a leptin dose that was 4,000-fold lower than the peripherally applied dose [32,84]. Peripheral but not central, leptin resistance in this murine model of obesity suggests that leptin resistance can be overcome by a central leptin supply. In addition, high levels of peripheral leptin in overweight individuals do not appear to cross the blood–brain barrier [85,86]. In accordance with the observation that the weight-reducing effects of leptin are predominantly mediated through the hypothalamus, AAV-mediated gene transduction and leptin overexpression in the hypothalamuses of normal rats suppressed age-related body weight gain and increases in fat mass, adiposity and serum insulin for more than six months without affecting food consumption was reported by Dhillon *et al.* in 2001 [87]. In normal rats, at six weeks after i.c.v. injection of AAV-leptin, which mediated leptin overexpression, the hypothalamus leptin mRNA levels were three times higher than those in control rats. As reported at two weeks post-injection, reduced serum leptin correlated with significant reductions in body weight due to a loss of fat depots with no change in lean mass. The expression of uncoupling protein-1 (UCP1) mRNA, a measure of thermogenic capacity, in brown adipose tissue doubled in the treatment group, indicating that thermogenic energy expenditure was augmented in the treated rats.

On the other hand, gene transduction therapy with AAV bearing leptin receptor (AAV-OB-Rb) was shown to improve energy balance and reproductive status in obese female Koletsky rats [34]. When AAV-OB-Rb was microinjected into the media preoptic area, PVN, VMH, ARC or dorsal vagal complex in the brainstem, all groups showed marked expression of human leptin receptor mRNA. Evaluation based on body weight, food intake and UCP-1 mRNA, leptin, insulin and glucose levels revealed that injections in the PVN were generally ineffective, whereas injections in the ARC were most effective. These results suggest that AAV can be used to achieve long-term expression of functional leptin receptors in the CNS, and that leptin acts at specific brain locations to affect food intake, energy expenditure, and reproduction. Intracerebroventricular AAV-mediated leptin gene therapy can produce a stable leptin supply in the hypothalamus, and the exogenous leptin is not transported to extra-hypothalamic sites [9,29,38,39,87] or the periphery [33,88]. Therefore, increasing the local supply of leptin via ADV- or AAV-mediated gene therapy may represent a novel therapeutic approach for diabetes patients [2,14,45,89-92].

Hyperinsulinemic, obese *ob/ob* mice (leptin mutants) and severely insulin-deficient, nonobese Akita mice (insulin 2 mutants) were used to examine the effects of intracerebroventricularly administered AAV-lep by Kalra and colleagues [88]. A key to the prevention of type 2 diabetes is suppression of hyperglycemia through decreased insulin resistance and increased glucose disposal [3,47]. AAV-leptin-treated *ob/ob* mice show elevated mRNA levels of GLUT1 and GLUT4, glucose transporters that play important roles in regulating glucose uptake in brown adipose tissue. This result may reflect increased glucose uptake in brown adipose tissue if glucose transporter expression is regulated solely through the hypothalamus.

One possible mechanism to improve glucose metabolism is related to central leptin-mediated increases in UCP1 expression and enhanced glucose uptake in brown adipose tissue. Increased UCP1 mRNA expression has been detected in wild-type, *ob/ob*, and Akita mice after treatment with AAV-lep. Studies of viral-vector-based central leptin gene therapy in insulin-deficient or leptin-deficient animal models have provided profound insights into the regulatory effects of leptin on glucose homeostasis, insulin resistance, food intake, and body weight. Data from a number of sources support central leptin gene therapy as a potential alternative to current insulin therapy for diabetic patients to ameliorate hyperglycemia and hyperphagia. Of particular interest is the observation that a single course of central leptin gene therapy effectively

and durably reinstates euglycemia and energy homeostasis in the absence of insulin. Thus, a local enhancement of the leptin supply in the hypothalamus offers an alternative anti-diabetic treatment paradigm for both type 1 and type 2 diabetes. Results that support treatment of type 1 diabetes include the observed amelioration of hyperglycemia and diabetes in Akita mice after a single intracerebroventricular injection of AAV-leptin, which enhanced the rate of glucose disposal and insulin sensitivity [88]. The key to preventing type 2 diabetes with leptin gene therapy is to suppress hyperglycemia, decrease insulin resistance, and increase glucose disposal [24,46,47,88]. Furthermore, central leptin gene therapy may also be associated with anti-obesity and anti-aging effects, as described by Kalra *et al.* [2,89,91].

4.3 Possible adverse effects

In most fields of biomedical research, including gene therapy, experimentally promising results have not always resulted in successful outcomes in actual clinical trials. Adverse effects, which are sometimes unpredictable or undetected in preclinical studies, are the most critical types of failure in clinical trials. One possible side effect may be caused by long-term leptin production, because the previously reported systems for administering central leptin gene therapy, which were described in the earlier subsections, involve the constitutive overexpression of leptin without native transcriptional regulation. Although the possibility of production of an antibody against leptin may be low or none, such aberrantly overexpressed leptin may have the possibility of side effects related to an imbalance of the leptin feedback regulatory loop. To avoid or reduce the possible side effects, the optimal dose of leptin-expressing vector should be carefully determined in preclinical studies. Other side effects may also be caused by viral vectors as follows.

Adverse events after RV-mediated gene therapy are mainly caused by insertional mutagenesis. The vector sequence of the mouse leukemia long-terminal repeat, when integrated into chromosome transactivated neighboring promoters, leading to aberrant gene expression and a growth advantage for the transduced cells in which the oncogene is abnormally activated, thereby resulting in the later onset of leukemia in patients [93]. Although, in theory, any integration vectors such as LV may carry a similar risk of insertional mutagenesis, it is quite uncertain that this side effect occurring after retroviral *ex vivo* gene therapy to hematopoietic cells would be similarly reproduced after *in vivo* local administration of LV, for example after LV-mediated leptin gene transduction into the CNS.

In contrast, recombinant ADV has a safety advantage due to the episomal nature of the transgene, leading to much lower risk of insertional mutagenesis, and the safety in the case of local injection of ADV has been well established by a number of clinical trials in human cancer patients. On the other hand, only a single case of the death of a patient after ADV-mediated gene therapy has been reported; however, this clinical trial was quite special, and several questions as to its suitability were raised from clinical and scientific viewpoints [94,95].

Infusion of a large amount of ADV into the right hepatic artery – that is a delivery system similar to systemic ADV administration – of a patient with congenital metabolic disease has been shown to result in lethal systemic inflammatory response syndrome [95]. It is uncertain the degree to which side effects related to the immune reaction are induced by local injection of a small amount of ADV, including HD-ADV, into the CNS of diabetic patients in clinical trials.

Compared with the first-generation ADV, recombinant AAV has safety advantages not only in terms of the episomal nature of the transgene, but also due to the reduced induction of cellular immunity. However, it must also be considered that a patient with rheumatoid arthritis died during an AAV-mediated gene therapy trial, which employed the intra-articular delivery of a TNF-α antagonist through the AAV delivery system [96]. Although a later investigation demonstrated that the patient's death was primarily the result of disseminated histoplasmosis as an opportunistic infection with subsequent bleeding complications and multiorgan failure, the contribution of an immune response to the AAV could not be evaluated [96]. In this regard, not only should the safety concerns associated with ADV be extensively examined in preclinical studies, but these concerns should also be carefully monitored in actual human clinical trials of AAV-mediated central leptin gene therapy.

5. Expert opinion

Ideal anti-diabetes therapies would allow safe and long-lasting glycemic control without perturbing physiological homeostasis. In addition to conventional insulin therapy, central leptin gene therapy may develop into an effective therapeutic strategy for diabetes patients. Particularly positive findings with leptin therapy include decreased body weight and food intake via activity in brown adipose tissue, whereas these parameters increase with insulin therapy. Moreover, the effects of leptin therapy result in improvements in insulin resistance. Therefore, central leptin gene therapy that results in local hypothalamic supplies of leptin is a promising approach for diabetes patients, and may even substitute for insulin therapy in the future, as a previous review also suggested [90].

A key issue in central leptin gene therapy is the development of appropriate vectors for human clinical trials. Vectors should result in high *in vivo* gene transduction efficiency, long-term transgene expression and minimal immunogenicity and toxicity. Although AAV is a promising vector for gene therapy, clinical evidence supporting its use is still embryonic. In practice, an unexpected death occurred in a human clinical trial that used AAV, although the possible contribution of an immune response to the AAV could not be evaluated [96,97]. This event, together with lessons from

clinical gene therapy trials for other diseases, suggests that extensive preclinical studies focusing on safety should be performed before testing central leptin gene therapy in human patients with diabetes.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

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