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Extrachromosomal plasmid vectors for gene therapy

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Extrachromosomal DNA is becoming widely utilized as a gene therapy vector. Plasmid DNA offers multiple advantages over viral gene therapy vectors, including large packaging capacity, stability without integration and reduced toxicity. Furthermore, plasmid DNA can be delivered to many different tissues, using a variety of delivery techniques currently being developed. This review will discuss the advantages of extrachromosomal DNA as a gene therapy vector, highlighting recent advances and successes in its use in vivo.

Keywords Extrachromosomal, gene delivery, gene therapy, genomic DNA, long-term expression, non-viral vector

Introduction

The ideal gene therapy vector would efficiently deliver the transgene of interest to the target tissue, without adverse immune responses and would provide sustained, therapeutic expression levels in vivo. Currently used gene therapy vectors can be placed into two broad categories, viral and non-viral, and each type has advantages, as well as disadvantages. Viral vectors are highly efficient at transfecting cells and delivering their transgene [1]. However, the disadvantages of viral gene therapy vectors are plentiful. Significant among these are viral toxicity and immunogenicity, limited packaging capacities, the complexity and expense of production and the potential for insertional mutagenesis in the case of integrating viral vectors. Due to these inherent limitations of viral vectors, a growth in non-viral gene therapy research, focusing on the use of extrachromosomal plasmid DNA, has been seen in recent years. These vectors have shown promise in a variety of gene therapy applications, including Factor IX therapy for hemophilia B, DNA vaccination and cancer therapy.

Extrachromosomal DNA has several advantages over traditional viral gene therapy vectors, including large transgene capacity, few insertional mutagenesis issues and reduced toxicity/immunogenicity. Extrachromosomal DNA can be used as a gene therapy vector in combination with a variety of delivery methods, making it applicable to a wide range of diseases and targetable to a variety of tissues in vivo. This review will focus on recent advances in the use of extrachromosomal plasmid DNA as a gene therapy vector, highlighting the advantages mentioned above.

Packaging capacity

A disadvantage of viral vectors is their limited packaging capacities, which could make them unsuitable for the delivery of large transgenes. For example, the dystrophin gene of Duchenne muscular dystrophy (DMD) is 2.5 Mb in size, with a 14 kb cDNA sequence. Adeno-associated virus (AAV) can only accommodate transgenes smaller than 4.5 kb, retroviruses have a limit of approximately 7.5 kb, and the recently developed ‘gutless’ adenoviral vectors have the ability to package up to 30 kb, but viral capsid proteins have the potential to induce immune responses in treated individuals, as can contaminating helper virus [2]. By contrast, extrachromosomal vectors have a tremendous carrying capacity, and are capable of incorporating transgenes of up to several hundred kb in size. This feature gives extrachromosomal vectors the ability to carry full genomic sequences, including endogenous promoters, enhancers, introns and matrix-attachment regions.

The inclusion of genomic sequences in a gene therapy vector may allow proper regulation of expression of the transgene. For example, genomic sequences often mediate tissue-specificity, as well as sustained levels of expression that are less subject to silencing, which commonly affects viral sequences/promoters. Stoll and colleagues [3-8] showed that an extrachromosomal vector carrying the 1.9 kb genomic sequence of human 6-antithymosin (hAA), along with Epstein-Barr virus (EBV) sequences, was capable of maintaining hAAAT serum levels of 300 µg/ml for at least 6 months in vivo. Expression from control extrachromosomal vectors carrying the hAAAT DNA under control of the Rous sarcoma virus (RSV) promoter fell to levels of ≈ 1 µg/ml within 3 weeks. Expression levels with the genomic hAAAT rivaled those obtained when the same 1.9 kb genomic sequence was included in a gutless adenoviral vector [4,5], but without the disadvantages of a viral system. Similarly, the 115 kb genomic locus encoding hypoxanthine phosphoribosyl transferase (HPRT), when included in a vector containing EBV sequences, has been shown to express stable, therapeutic levels of HPRT in human cells in vivo [8]. Episomally maintained EBV-YAC vectors have also been used to express the 200 kb human CETR gene in mouse cells in vitro [7]. This same genomic sequence was capable of rescuing the disease phenotype in transgenic mice null for the murine CETR gene [8].

Expression of human Factor IX cDNA is greatly enhanced by inclusion of the ApoB gene hepatic lectin control region (FLCR), the hepatic-specific hAAAT promoter, and the Factor IX intron A and 3'-untranslated region (UTR) [9]. When this 6.1 kb Factor IX minigene, in a Bluescript plasmid backbone, was injected into the tail vein of mice, therapeutic serum levels of Factor IX (0.5 to 2 ng/ml) were observed that persisted for at least 225 days. In comparison, a plasmid containing the Factor IX cDNA under control of the same hAAAT promoter, but lacking the hAAAT-FLCR control region, intron A and 3'-UTR, provided only transient high expression, falling to low/undetectable levels (<10 ng/ml) within 2 weeks after injection. Both vectors were retained extrachromosomally in mouse liver cells, but only the Factor IX minigene continued to express the therapeutic protein, up to 240 days post-injection [10]. These results are consistent with the observations made by Stoll and colleagues [3-8].
that showed significantly increased and sustained expression levels from hAAT genomic sequence, compared with cDNA sequence. These studies highlight the utility of genomic sequences in gene therapy vectors to enhance and maintain transgene expression levels in vivo. The exact mechanism of the genomic sequence-enhanced expression is unclear, and may include resistance of these sequences to silencing, overenhanced stabilization of mRNA transcripts and/or other influences.

In addition to mammalian genomic sequences, recent findings have revealed that alternative DNA forms are also capable of high-level, persistent expression in vivo. It has been observed that linearized DNA, when injected into the mouse tail-vein by hydrodynamic methods, is capable of providing stable supraphysiological levels of serum Factor IX that are approximately 200-fold higher than those observed for identical closed circular DNA [11]. Analysis of the DNA state revealed that the linear DNAs formed concatemers that were maintained extrachromosomally in the cells. It is hypothesized that the large concatemers cause a DNA conformation that is more amenable to transcriptional activity than closed circular DNA.

Stability of extrachromosomal DNA
A theoretical advantage of some viral gene therapy vectors is their ability to integrate into the host/patient genome, thereby ensuring the permanent presence of the therapeutic transgene. A drawback to this approach, however, is that the integration event is often random, leading to the possibility of inserional mutagenesis, or, alternatively, integration into transcriptionally silent regions [2]. Extrachromosomally maintained plasmids are advantageous in that there is little risk of these integrative problems. Additionally, in dividing tissue, such as liver, less extrachromosomal vectors is minor. Zhang and colleagues [12] used hydrodynamic tail-vein injection to transfuse mouse hepatocytes with naked plasmid DNA encoding the hAAT cDNA under control of the cytomegalovirus (CMV) promoter, with no other viral sequences for mammalian replication or retention. By injecting 10 μg of naked plasmid DNA, serum AAT levels of 2 to 5 μg/mL were observed, which persisted for at least 6 months. While there was an initial decrease in total vector copy number per day 1, hAAT DNA was maintained extrachromosomally in the liver cells for at least 180 days. Therefore, at least in the case of non-dividing liver cells, plasmid DNA can be maintained and expressed over the long-term without integration or the use of viral replication/retention sequences. These findings are similar to those observed by Spell and colleagues [3**, in which both high-expressing genomic-EBV plasmids and low-expressing cDNA plasmids (both with and without EBV retention sequences) were maintained extrachromosomally in liver cells. A significant finding of this study was the importance of genomic sequences for stable, high-level transgene expression in vivo. Only plasmids containing the genomic hAAT sequence continued to express high levels of serum AAT, over 100-fold greater than that obtained from a cDNA transfected. Gill and colleagues [13••] recently demonstrated that the use of the cellular elongation factor 1α (EF1α) and ubiquitin C (UbC) promoters gave increased persistence of transgene expression in the lungs. Whereas luciferase reporter gene expression fell drastically within 7 days after delivery via intranasal instillation when expressed from the CMV promoter, expression from the EF1α and UbC promoters gave approximately 10-fold higher expression levels for 4 and 24 weeks, respectively. Quantitative PCR analysis of plasmid vector in lung tissue revealed that there were no significant differences in plasmid copy number in the CMV versus EF1α or UbC promoter vectors. Together, these results suggest that transgene/promoter silencing, rather than vector loss, may be the most significant cause of reduced expression levels in vivo. These findings again highlight the importance of using mammalian genomic sequences (promoters, enhancers and intron sequences), as opposed to viral expression sequences, in order to achieve maximal therapeutic levels of a transgene in vivo.

Proliferating tissues, such as rapidly dividing tumor cells, are likely to experience a high rate of loss of extrachromosomal plasmid DNA. In order to overcome this problem, some extrachromosomal gene therapy vectors incorporate viral or mammalian genomic sequences that can provide replication and/or retention ability to the vector. EBV sequences have been used in this respect. Presence of the EBV nuclear antigen 1 (EBNA-1) protein and the oriP family of repeat sequences on an extrachromosomal vector provides nuclear retention in dividing mammalian cells [14]. EBNA-1 binds to the family of repeats and to the chromosomal scaffolding in the mammalian nucleus [15]. Thus, as the cells divide, the extrachromosomal vector remains associated with the chromosomes.

The EBNA-1 protein is also able to interact with the EBV oriP dyad symmetry element to replicate the extrachromosomal vector in primate cells [16-18]. However, EBV has limited replication ability in rodent cells [19,20]. Some mammalian genomic sequences have been shown to function in rodent and primate cells as extrachromosomal vector mammalian origins of replication [21]. The 19 kb genomic locus of hAAT, in addition to its aforementioned high expression levels, is able to act as an origin of replication in rapidly dividing human 293 and mouse HepaLA cell lines [3•]. When included on a plasmid with EBNA-1 and the EBV family of repeats, this vector was able to replicate and be sustained, under selection, for at least 25 cell divisions. EBV-based plasmids have also been used to deliver and maintain a dystrophic minigene in mouse skeletal muscle, which resulted in increased and sustained dystrophin expression for at least 10 weeks, compared with a non-EBV extrachromosomal vector [22]. When the HSV thymidine kinase (tk) suicide gene was delivered to melanoma tumor cells in vivo, followed by ganciclovir treatment, tumor growth was significantly slowed when the tk gene was introduced on a plasmid containing EBV sequences as compared with mice receiving non-EBV plasmid DNA [23].

In addition to the replication function of EBNA-1, this protein is also able to enhance transgene expression from vectors containing the oriP sequence in cfs [24]. Addition of EBV sequences to a Factor IX minigene plasmid resulted in a 10- to 100-fold increase in expression levels compared to a non-EBV minigene plasmid, when injected into mouse tail-vein hydrodynamically [GR Salmen et al, unpublished data]. In this case, expression occurred in the
non-dividing liver, so the increase in expression from these EBV plasmids was due primarily to the enhancer activity of EBNA-1, rather than its replication or retention activity. It has also been demonstrated that reporter gene expression can be reactivated in vivo following transfection, with an EBNA-1 expression plasmid [25]. This effect was only observed in mice originally transfected with an episomal plasmid, and not a plasmid lacking an EBV oriP, indicating that the reactivation and enhancement of expression was due to a specific interaction between EBNA-1 and the EBV oriP sequence on the extrachromosomal transgene vector.

The above results demonstrate that persistence of extrachromosomal DNA in vivo can be obtained and that instability need not pose significant limitations on the use of extrachromosomal DNA as a gene therapy vector.

Toxicity and immunogenicity
A primary concern with viral vectors is their potential immunogenicity, typically elicited by viral proteins, either in the viral capsid or expressed intracellularly in infected cells. Extrachromosomal plasmid DNA is typically free of these immunomodulatory properties. With respect to naked plasmid DNA, the transgene itself represents the most likely source of elicited immune responses and only becomes a problem if the therapeutic protein has not been seen by the host's immune system, a case which is typically reserved for full mutations and some protein truncations. In the absence of an immune response, there is no barrier to repeated administration of the gene therapy vector, an additional advantage of extrachromosomal vectors. In the case of cystic fibrosis (CF), for example, where the target lung epithelial cells turn over frequently, non-viral delivery of CFTR plasmid DNA has proven successful for the administration of multiple doses of a DNA-liposome complex into CF patients [26].

It has been suggested that unmethylated CpG motifs contained in gene therapy vectors may elicit an immune response, which would affect both extrachromosomal and viral vectors alike. As a defense against viral and bacterial infection, immune systems have evolved the ability to mount an immune response against these CpG motifs, the unfortunate outcome of which is an immune response mounted against gene therapy vectors [27]. Yew and colleagues [28,29] have recently shown that deletion of CpG motifs in plasmid DNA resulted in a decreased immune response, and increased and sustained expression of transgenes in vivo. Serum levels of Factor IX were sustained at approximately 1 μg/ml for at least 42 days after transfection when delivered by hydrodynamic tail-vein injection, a level that is considered therapeutic. In comparison, a similar control plasmid bearing a full set of CpG motifs dropped to approximately 1 ng/ml after 14 days. These results indicate that reducing CpG motifs in plasmid DNA could be a simple way to boost further expression and persistence and reduce immunoreactivity of transgenes in vivo from extrachromosomal gene therapy vectors.

Delivery
Several recent advances have been made in the delivery of plasmid DNA to various tissues, including the liver, lung, muscle and skin, and are applicable to both naked and complexed plasmid DNA vectors.

Systemic injection
The recently developed method of hydrodynamic tail-vein injection in mice, developed by Wolf and Liu [30,31], involves the rapid injection of a large volume of saline, including naked plasmid DNA, into the tail vein of mice. The result is a highly efficient (approximately 40%) transfection of liver cells in vivo. This hydrodynamic injection method has been used to study the in vivo expression of many extrachromosomal DNA constructs, AAT [32*,33], Factor IX [10,11], hepatocyte growth factor (HGF) [34*] and mannose-binding lectin (MBL) [35]. This high-pressure injection technique is being evaluated for its utility in larger animals. Recent work has demonstrated the applicability of this technique in obtaining efficient transgene expression in the limbs of rhesus monkeys [36].

As an alternative to hydrodynamic tail-vein injection for targeting the liver, Liu and Huang [37] have demonstrated the feasibility of mechanical massage of the liver (MMIL) in enhancing transfection following intravenous injection of naked plasmid DNA. While this technique is only approximately 10% as efficient at transfecting hepatocytes as the hydrodynamic method, it is significantly simpler and less toxic than hydrodynamic tail-vein injection.

Though hydrodynamic injection is not always a feasible approach, systemic delivery is desirable for many genetic disease treatments. Duchenne muscular dystrophy (DMD) is a degenerative disease of the musculature of the body that typically results in death from respiratory failure, partly due to the progressive degeneration of the diaphragm muscle. Liu and colleagues [38] attempted to systematically deliver plasmid DNA encoding the luciferase reporter protein to the diaphragm. The plasmid DNA solution was injected into the tail-vein, followed by clamping of the vena cava immediately below the diaphragm. This method resulted in the diaphragm being the major target of vector delivery and expression. Using a plasmid DNA construct encoding the 14 kb full-length dystrophin cDNA, Liu et al observed that approximately 40% of diaphragm muscle fibers were transfected and properly expressed dystrophin. Furthermore, dystrophin expression resulted in functional rescue of the degenerative phenotype at 7 days after transfection, with dystrophin gene expression still detectable after 6 months.

Electroporation
Recent years have seen the development of electroporation-enhanced in vivo delivery of naked plasmid DNA to a variety of tissues, including liver, muscle and tumors [39]. Lucas and Haller [38] used electroporation to deliver naked plasmid DNA encoding interleukin-12 (IL-12) to mouse skeletal muscle in vivo. Not only was a significant increase in serum IL-12 observed in electroporation-enhanced plasmid-injected mice, but also increased and sustained expression levels following multiple treatments was demonstrated, indicating that electroporation is a feasible treatment protocol that allows adjustment of expression levels over time through simple re-administrations of extrachromosomal DNA. Most recently, Lucas and Haller have demonstrated that electroporation of IL-12 plasmid DNA into B16.F10 melanoma cells in vivo results in complete regression of tumors in 47% of treated mice, resistance to
additional B16F10 challenge in five out of seven 'cured' mice, and reduced tumor angiogenesis [39*]. In addition to muscle and tumor cell delivery, electroporation has also been used to enhance delivery of intravenously injected plasmid DNA in mice [40]. The abundance of vasculature in the skin makes it an attractive target for gene therapy when protein secretion is the desired outcome. Using this method [40], enhanced IL-12 expression levels were observed, and these results were similar to those obtained in mice treated intramuscularly [38].

Skeletal muscle is an easy target for electroporation-enhanced gene transfer, and its vascular accessibility makes it an ideal target for gene therapy for secreted proteins. Using a syringe-electrode device, Liu and Huang [41] observed transfection of 20% of muscle fibers. Yin and Tang [42] observed a 50% increase in blood glucose levels in streptozotocin-induced diabetic mice following electroporation-mediated plasmid DNA transfection of the heart pre-pro-insulin gene into skeletal muscle, accompanied by approximately normal serum levels of insulin and significantly decreased mortality rates. Additionally, Vilquin and colleagues [43] have demonstrated that electroporation of naked plasmid DNA into dystrophic mice is not significantly affected by the fibrotic state of the muscle tissue. Given the large size of the dystrophin gene, extrachromosomal plasmid DNA is probably the most applicable gene therapy vector for treatment of muscular dystrophy. The applicability of electroporation-enhanced gene delivery to dystrophic tissue is therefore a very attractive delivery mechanism for the treatment of this disease.

Ultrasound
Recent research has demonstrated the utility of ultrasound-mediated transfection of tissue in vivo. In principle, ultrasound can be used to non-invasively target almost any location in the human body, given that the depth and size of the tissue does not hinder penetration. Huber and Pfeifer [44] showed that ultrasound sonication enhanced intramuscular plasmid DNA injection by 10-fold compared with tumors injected with DNA in the absence of ultrasound treatment. Intramuscular injection of a hepatocyte growth factor (HGF) plasmid into the hindlimb of a rabbit ischemia model resulted in significant improvement in angiographic score, number of limb blood vessels, and blood flow and pressure, as compared with animals treated with naked plasmid DNA in the absence of ultrasound treatment [45].

Complexed DNA
While recent developments in physical methods of transfecting DNA into living tissue have made significant advances in efficiently delivering plasmid DNA, these methods are not effective for all the tissues in the body. Therefore, plasmid DNA is often complexed with other biological molecules in order to obtain better delivery and transfection frequencies. Liposomes are the best known of these complexing agents, but several other methods have been used recently, including TerplexDNA, polyethyleneimine and lipopolymers.

The recently developed novel gene delivery system TerplexDNA consists of plasmid DNA complexed with spermyl-poly-L-lysine and low-density lipoprotein [46]. This system was used to increase transfection and expression of a therapeutic plasmid DNA into myocardial cells in rabbits and in rats. The results show that TerplexDNA provides more uniform and uniform transfection of myocardial cells near the injection site, as well as 10- to 100-fold increased expression of luciferase reporter gene for at least 30 days after treatment.

Polyethyleneimine-DNA complexes have been shown to be effective for transfection of lung tissue following aerosol delivery [47]. Aerosol and intranasal delivery of these polyethyleneimine-DNA complexes resulted in high-level, persistent antibody response to human growth hormone that was comparable to immune responses achieved through intramuscular injection, suggesting that aerosol and intranasal delivery could prove to be an effective and less invasive method of delivering DNA vaccines for respiratory pathogens. This method may also prove effective in the treatment of CF, for which lung epithelial cells are the target for gene therapy.

Malato and colleagues [48] used water-soluble lipopolymers to deliver plasmid DNA encoding IL-12 by intranasal injection into mice with subcutaneous tumors. Higher expression levels and slowed tumor growth in mice treated with WS3P/ plasmid DNA were observed compared with mice treated with polyethyleneimine/plasmid DNA or naked plasmid DNA. Alternative plasmid DNA delivery systems 'stabilized plasmid-lipid particles' (SLPs) [49], which were shown to be more stable (increased circulation half-life), less toxic (non-oxidative serum amniontransferases) and exhibited moderately increased reporter gene expression in tumor cells following systematic intranasal injection in vivo compared with traditional plasmid DNA-lipid complexes.

Targeting of specific tissues is an obstacle for most gene therapy vectors, both viral and non-viral. The inclusion of ligands or antibodies, either on the viral capsid or conjugated to plasmid DNA, is a promising method for targeting to specific cell types. Recently, an extrachromosomal DNA system has been constructed that non-covalently complexes plasmid DNA with histone H1, influenza virus hemagglutinin (H1A) peptide and a retinal cell carcinoma (RCC)-specific monoclonal antibody (mAb) [50]. The result is a vector that is able to specifically target RCC cells (via mAb), is capable of translocating out of the endosome and into the cytosol (via H1A peptide), and once there, is protected from degradation and efficiently transported into the nucleus (via histone H1). Using this system in vivo, Dias and colleagues [50] observed transfection specific to the Renal G259 tumors in three out of four treated mice following intravenous injection of the plasmid DNA and other components. This conjugated plasmid DNA methodology shows great potential for targeting specific cell types of the body and could prove useful in gene therapy of a variety of diseases. For instance, a major drawback of suicide gene therapy in tumor cells is the requirement of specific targeting to the tumor cell, to avoid damage to surrounding normal tissue. Additionally, this method may be useful in specifically targeting other tissues of the body, such as lung epithelial cells for CF.

DNA vaccines
Extrachromosomal plasmid DNA has also found utility in DNA vaccines. As opposed to protein vaccination, plasmid
DNA vaccines express the recombinant allergen intracellularly, allowing them to be presented onto MHC class I and II molecules, which in turn allows for the development of both humoral and cellular immune responses [51-52]. Additionally, plasmid DNA vaccines have the benefit that they can be easily and quickly produced, as opposed to protein vaccines, which may contain contaminants that could induce an immune response. These features give plasmid DNA great potential as a vaccination method for a variety of diseases.

Several key advances have been made recently in the development of vaccines against HIV. Berzofsky and colleagues [53] administered plasmid DNA encoding the simian immunodeficiency virus (SIV) strain SFVmac239 gag protein and the HIV-1 Env gp160 protein, intramuscularly to rhesus monkeys, either alone or in conjunction with IL-2/IL-10 protein or plasmid. This treatment resulted in the monkeys developing significant circulating CD4+ cytophilic T-lymphocytes (CTLs) specific to epitopes of the SIV gag and HIV-1 env proteins. Each monkey was then challenged intravenously with an infectious dose of chimeric virus SHIV89.6P. Monkeys receiving DNA vaccination, augmented with IL-2/IL-10, showed significant reduction in viral loads, no clinical manifestation of the disease and no morbidity, as compared with control monkeys that developed high viral loads, significant progression of disease and 50% morbidity within 140 days after challenge. Similar results were observed when a DNA vaccine followed by a booster of recombinant modified vaccinia Ankara (MVA) was used in rhesus monkeys [54], even when a challenge was administered 7 months after the booster treatment. These results indicate that plasmid DNA vaccines can efficiently induce immune responses that are capable of the long-term control of pathogenic challenge. DNA vaccines have also been applied to the prevention of allergic responses. Perg and colleagues [55] successfully used plasmid DNA encoding the mite allergen Der11 to intramuscularly vaccinate mice.

The skin is an attractive tissue for vaccination, due to its easy accessibility and its immunoreactivity mediated by an abundance of antigen-presenting cells. ID Technologies has developed microneedle arrays (MEAs) for the efficient delivery of plasmid DNA into the epidermis of mice [54+]. This technique is relatively non-invasive and non-traumatizing, and results in reporter gene expression levels similar to, or greater than, those achieved by intramuscular or intradermal injection. Expression levels with MEAs were >10-fold higher than those achieved by topical application of the DNA solution. Additionally, 100% seroconversion in mice transfected by MEA with plasmid DNA encoding the hepatitis B surface antigen was observed, compared with 40 to 50% seroconversion achieved by injection, indicating usefulness of this technique in DNA vaccination protocols.

Conclusion

Extrachromosomal DNA is a viable gene therapy vector. Extrachromosomal vectors have large DNA carrying capacities, enabling them to deliver transgenes of several hundred kilobases in size. This feature allows them to deliver full genomic sequences, which are typically too large to be packaged by viruses. Genomic transgene sequences provide many advantages, including increased expression levels and specific transgene regulation through the use of endogenous promoters. In relatively quiescent cells, plasmid DNA has been shown to be maintained extrachromosomally, with expression seemingly dependent on the transgene sequences. In rapidly dividing cells, when extrachromosomal vectors may be lost, EBV sequences can be included in cis to provide stable maintenance of the plasmid in vivo, without relying on integration into the genome. These sequences can also act as enhancers to boost expression levels. Extrachromosomal vectors typically do not elicit potentially adverse immune responses, which is a tremendous advantage over viral gene therapy vectors. Furthermore, it is easy and inexpensive to produce large amounts of high-quality plasmid DNA, a significant advantage compared to the complex methods required for viral vector production. Additionally, plasmid DNA can be used in conjunction with a variety of delivery techniques, and can thus be used to target many different cell types in vivo. Gene therapy is no longer restricted to the treatment of inherited diseases. Current research is making use of gene therapy to treat cancer, cardiovascular disease, and to vaccinate against a variety of acquired diseases. The versatility of extrachromosomal DNA makes it an attractive gene therapy vector for these and multiple other human diseases.

References

** of outstanding interest
* of special interest


** These results indicate that genomic sequences provide prolonged and high expression levels in vivo, suggesting that gene therapy vectors would benefit greatly from using full genomic sequences whenever possible. This paper also demonstrates that a mammalian gene can function as a mammalian open reading frame in an extrachromosomal vector in vivo, which may provide stability to the vector in proliferating tissues.


* These results demonstrate that naked plasmid DNA can be efficiently delivered intravenously to primate limbs. This is a significant result because it demonstrates that common methods for delivering plasmid DNA in mice can be adapted for primate delivery.


* This paper reports the development of a simple, non-invasive, non-traumatic skin-dissection device for efficient delivery of plasmid DNA to the epidermis. This device holds promise as a delivery tool for DNA vaccines and other epitope-delivered gene therapies.