Use of Adeno-Associated Virus Vector for Cardiac Gene Delivery in Large-Animal Surgical Models of Heart Failure

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The advancement of gene therapy-based approaches to treat heart disease represents a need for clinically relevant animal models with characteristics equivalent to human pathologies. Rodent models of cardiac disease do not precisely reproduce heart failure phenotype and molecular defects. This has motivated researchers to use large animals whose heart size and physiological processes more similar and comparable to those of humans. Today, adeno-associated viruses (AAV)-based vectors are undoubtedly among the most promising DNA delivery vehicles. Here, AAV biology and technology are reviewed and discussed in the context of their use and efficacy for cardiac gene delivery in large-animal models of heart failure, using different surgical approaches. The remaining challenges and opportunities for the use of AAV-based vector delivery for gene therapy applications in the clinic are also highlighted.

Keywords: AAV virus, cardiac gene delivery, animal models of heart failure, heart disease

INTRODUCTION

THE PATHOGENESIS OF MANY congenital and acquired cardiac diseases is closely associated with changes to the genetic and molecular programs in the heart. The increased understanding of molecular mechanisms that drive and support cardiovascular disorders have made gene therapy approaches an attractive tool to target key molecules and pathways, and serve as a viable alternative to current pharmacological treatments. For the management of inherited cardiomyopathies, gene therapy is arguably the single most promising therapeutic platform.

Successful use of cardiac gene therapy requires that the DNA encoding for the gene of interest is efficiently delivered into cardiomyocytes, and in most cases, long-lasting gene expression is desired. Today, adeno-associated viruses (AAV)-based vectors are undoubtedly among the most promising DNA delivery vehicles. Advantages of AAV as a cardiac gene delivery vector include: (1) efficient transduction into cardiomyocytes, (2) eliciting modest cellular immune response, and (3) triggering persistent expression of therapeutic

gene of interest, even in the absence of genome integration.

AAV BIOLOGY

AAV is a small non-enveloped virus that can only replicate in the presence of a helper virus such as adenovirus or herpesvirus.¹ The AAV genome consists of a single-stranded DNA that encodes only two genes, the *Rep* gene, which encodes proteins essential for DNA replication and packaging, and the *Cap* gene, which encodes the capsid proteins and, in an alternative reading frame, the assembly activating protein. The approximately 4.7 kb-long AAV genome is flanked by two inverted terminal repeats that are the only cis-elements required for the production of recombinant AAVs (rAAVs). As a result, rAAVs do not encode any viral proteins, an attribute that likely contributes significantly to their limited immunogenicity.

The biology of rAAV transduction into the cell is still incompletely understood, but available data suggest that the AAV receptor consists of a glycan component.² Several secondary receptors such as

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fibroblast growth factor receptor, hepatocyte growth factor receptor, and α V β 5 integrin have been described in the literature, but their importance for AAV transduction remains controversial.² Recently, a putative universal AAV receptor has been described, but its function in AAV transduction remains to be elucidated.³ After receptor binding, the virions are efficiently endocytosed via the CLIC/GEEC pathway,⁴ although a less efficient, dynamin-dependent pathway may also play a role in AAV infection. In fact, it has been reported that AAV can also be endocytosed by clathrin-mediated endocytosis.⁵ Following endocytosis, most AAV serotypes traffic to the Golgi in a syntaxin 5-dependent process.⁶ Interestingly, transduction of adult rat cardiomyocytes with the cardiotropic AAV serotype 9 (AAV9) appears to be less affected by inhibition of syntaxin 5 function than other AAV serotypes or other cell types.⁶ The point of escape of AAV from the endocytic system remains to be determined, but escape is dependent on the N-terminal domain of the large AAV capsid protein, VP1. This domain has been shown to have phospholipase A2 activity that is essential for transduction and escape into the cytosol.⁷ Once in the cytosol, AAV virions are imported as intact particles into the nucleus, where capsid disassembly and release of the viral DNA occurs. Next, synthesis of the second strand occurs, which is necessary for transcription.⁸

It can be assumed that the tropism of AAV serotypes is largely determined by differential receptor expression, but studies show that the tropism is also influenced by trafficking steps downstream of receptor binding.^{1,2,5}

Comments

In recent years, significant progress has been made in understanding AAV biology and the

development of new serotypes with cardiac tropism. However, knowledge is still incomplete, and future research needs to be done to find out AAV capsid processing, intracellular and extracellular uptake with trafficking mechanisms.

TRANSITIONING FROM CARDIAC MODELS OF RODENTS TO CLINICALLY RELEVANT LARGE-ANIMAL MODELS

Animal models are fundamental for human heart research. With the development and advancement of molecular biology, animal models have become instrumental to preclinical studies. In cardiac research, animal studies are used to explore neurohumoral, hemodynamic, and cellular processes of cardiac diseases, and are extensively used to develop new experimental and clinical approaches for the treatment of a variety of cardiac diseases.⁹ However, many considerations should be undertaken in order to model human cardiac disease closely in animals. Such considerations include appropriate choice of animal model, experimental design, and proper analysis of data.¹⁰ Small-animal models are still widely used for initial proof-of-concept cardiac studies.

Within the rodent cardiac system, different AAV serotypes have demonstrated distinct patterns of transduction. Of the 13 known serotypes, AAV 1, 6, 8, and 9 have demonstrated cardiac tropism.¹¹ The current view is that the route of AAV delivery is as important as the choice of serotype. The two basic, primarily used methods of AAV delivery into the heart are intramyocardial injection and intravascular infusion (Table 1). In rodents, AAV1 and AAV6 appear more suitable for cardiac gene transfer through intramyocardial, intrapericardial, or intravascular (intracoronary) routes, while AAV8 and

Table 1. AAV trafficking per routes of administration in vivo

Intramuscular injection		Intravascular administration	
Biological barriers	Host response	Biological barriers	Host response
1. Interstitium <ul style="list-style-type: none"> • Fibrillar connective tissue • Extracellular fluid • Supporting protein structures 	1. Acute inflammation from needle stick	1. Blood	1. AAV degradation from DNAsases; DNA fragmentation, endonucleosis, mononuclear phagocyte system, DNA liposome complexes, neutralizing antibodies
2. Capillary network	2. Local muscle injury with fibrosis	2. Transport to interstitium from blood compartment's endothelial barriers	2. Permeability properties of vascular endothelium
3. Cellular membrane	3. Heterogenous vector distribution between sites	3. Navigation through extracellular matrix	
4. Nucleus	4. Extracellular matrix component interference to capillary network	4. Cellular membrane	
		5. Nucleus	

AAV, adeno-associated virus.

AAV9 can achieve more efficient cardiac transduction only via the transvascular route.¹² Indeed, in neonatal mice, intravenous delivery of 1×10^{11} gc rAAV2/8 and rAAV2/9 yielded around 20- and 200-fold higher transduction levels than rAAV2/1 delivery, respectively. The authors suggested that the high transduction efficiency of rAAV2/9 is most likely attributable to the presence of receptors or ligands located on the outside of the viral capsid, which display a strong affinity for cardiac tissue.¹³ Interestingly, vector dose significantly affects transduction levels. At a high vector dose (2.5×10^{11} gc), all serotypes (1, 6, 7, 8, and 9) were efficiently transduced into the hearts of mice, as measured by the LacZ reporter gene. In contrast, at an intermediate dose (2.5×10^{10} gc), AAV9 was the only serotype that continued to show high transduction levels in the heart.¹⁴ Intracoronary delivery appears more efficient than intravenous delivery in mice, and its use yields a 2.6- to 28-fold higher transgene expression in the heart 3 weeks after AAV injection, depending on AAV serotype. The highest level of cardiac gene expression in rodents was achieved with intracoronary AAV9 delivery (5×10^{11} gc). Intracoronary delivery of AAV6 and AAV5 was less efficient.¹⁵

Results in rodents are informative and lay the ground for additional, in-depth investigations in more clinically relevant experimental models. There are significant differences in cardiac physiology and molecular biology between small and large animals that need to be taken into consideration when moving from a rodent model to a large-animal model. The main differences are: (1) the heart rate of rodents is fivefold higher than that of large mammals and humans, and the force–frequency relation is inverse; (2) myocardium exhibits a very short action potential, which normally lacks a plateau phase; (3) removal from the cytosol is predominated by the activity of the sarcoplasmic reticulum calcium pump, whereas $\text{Na}^+/\text{Ca}^{2+}$ -exchanger activity is less relevant; and (4) in rodent myocardium, α -myosin heavy-chain isoform predominates, and a shift toward the β -myosin isoform occurs only with hemodynamic load or hormonal changes.¹⁶ Additionally, there is a significant difference in cardiac gene epigenetic regulation between rodents and large mammals. Comparison of 4,000 genes in humans/large animals and mice revealed differences in transcription factor binding sites in 41–89% of cases.¹⁷ Difficulty in extrapolating results from rodents to humans stems not only from differences in anatomy and physiology, but also because rodent models are limited in their ability to mimic cardiac disease etiology. In contrast, large animals such as pigs and sheep have consistent body

vessel anatomy, coronary arterial distribution, and lack of collateral circulation. Importantly for cardiac studies, induction of infarctions in large animals is predictable in size and location. Taken together, while rodent cardiac studies provide basic insight into cardiac biology and disease, large-animal models more closely resemble human cardiac physiology and pathology and thus are more suitable and relevant to study cardiac disease such as myocardial ischemia and post-infarction left ventricle (LV) remodeling.¹⁸

Comments

Studies carried out on rodent models have led to important advances in identifying AAV serotypes with cardiac tropism, selecting the mode and dose of viral delivery. They have laid the foundation for future research on large animals.

USE OF AAV VECTOR IN LARGE ANIMALS

About two decades ago, it was shown that AAV vectors can efficiently transfer marker genes into myocardial cells in a large-animal model.¹⁹ Viral particles (10^7 – 10^8) of AAV plasmid/ β -gal were injected antegrade into the coronary arteries of adult pigs. Three days after injection, a large number of LV cells within the area perfused by the circumflex artery were positive by staining. Interestingly, the distribution of positive cells at 6 months had a similar pattern to that observed at 3 days after injection.¹⁹ Later, it was demonstrated that the level of gene expression was dependent on AAV dose: no fluorescent signal was detected after intracoronary delivery of low dose of AAV2/EGFP (2.64×10^{13} DNase resistant particles [drp/mL]) to the pigs. However, at least 12% of the cardiomyocytes in the LV were GFP positive in animals that had received the highest dose of AAV2/EGFP (5.28×10^{13} drp).²⁰

The differences between AAV serotypes 1 and 2 in the mediation of onset of transgene expression were examined in a porcine model, in which the vectors, expressing LacZ and VEGF, were delivered into the myocardium by intramuscular injection.²¹ Both LacZ and VEGF expression was detected 3 days after AAV1-mediated gene transfer. In contrast, no gene expression was detected at 3 days after AAV2-mediated gene transfer. Based on those findings, the authors concluded that AAV1 mediates earlier onset of transgene expression.²¹ In another study, the transduction efficiency of AAV2 vectors versus AAV6 vectors was compared. In both groups, AAV vectors (3.5×10^{10} vg) were applied to the left anterior descending artery

(LAD) area by selective pressure-regulated retro infusion in the pig's anterior interventricular vein. After 28 days, AAV6 vectors demonstrated 48-fold more reporter activity in the target territory compared to AAV2 vectors.²² AAV9 serotype was investigated in a newborn dog model. The study showed that intravenous injection of $2\text{--}2.5 \times 10^{14}$ vg/kg of AAV9 resulted in widespread gene transfer in skeletal muscle but not in the heart.²³ In contrast, $7.14\text{--}9.06 \times 10^{14}$ vg/kg of AAV8 gene transfer resulted in robust expression in both skeletal muscle and the heart.²³ Interestingly, increasing AAV8 dose only moderately improved heart transduction. The authors proposed that the observed changes between cardiac transduction by AAV8 and AAV9 are likely related to the intrinsic property of the viral capsid rather than the vector dose.^{23,24}

Comments

The data demonstrate discrepancies between rodents and large animals, underlining the limitations of the use of small rodent models to investigate biology and feasibility of gene therapy. Taken together, it appears that cardiac muscle in large animals is well transduced by several rAAV serotypes, with the greatest cardiac tropism in most large-animal models demonstrated with AAV6 and AAV9.

ADVANTAGES OF USING SELF-COMPLEMENTARY AAV VECTORS IN LARGE ANIMALS

Self-complementary (sc) AAV vector has a higher transduction efficiency and faster kinetics of transduction than conventional single-stranded (ss) genome AAV vector.²⁵ Conventional AAV vector is converted into the active double-stranded DNA form by host-mediated DNA synthesis. Thus, the generation of duplex DNA is a limiting step in AAV vector transduction. scAAV is suitable as a vehicle for small genes, such as short hairpin RNAs (shRNAs) and microRNAs, due to its compact size.²⁵ The gene transfer efficiency of ssAAV9 versus scAAV9 was compared after percutaneous transendocardial delivery in dogs. A lower dose of scAAV9 (5×10^{11} gc/kg) mediated 50-fold greater cardiac gene transfer expression than a higher dose of ssAAV9 (2×10^{13} gc/kg). It was also shown that scAAV8 gene transfer efficiency was similar to that of scAAV9. Furthermore, scAAV6/GFP transduction resulted in reporter gene expression in approximately 60% of cardiomyocyte area, the most efficient gene transfer of all vectors.²⁶ Similar data were demonstrated in a sheep model in which

the delivery of scAAV6/EGFP resulted in robust gene expression, especially when compared to ssAAV6 or ssAAV9 delivery.²⁷ An additional study assessed the feasibility of transferring scAAV6 expressing shRNA for phospholamban (PLB). Vector was delivered via a percutaneous inserted cardiac catheter to the LV, and resulted in a 16-fold reduction of PLB mRNA expression. However, the efficient scAAV6-mediated cardiac gene transfer of shRNA was associated with severe toxicity.²⁸

Comments

Data accumulated from numerous studies now indicate that scAAV vectors have a superior transduction efficiency compared to the traditional ssAAV vectors, even with serotypes that have shown reduced cardiac tropism. Moreover, the transgene expression of scAAV vector improved with time in large-animal models, highlighting its tremendous potential in clinical applications.

THERAPEUTIC EVALUATION OF AAV VECTORS USING DIFFERENT SURGICAL APPROACHES IN LARGE-ANIMAL MODELS OF HEART FAILURE

Myocardial ischemia and infarction model

Historically, induction of myocardial ischemia and/or myocardial infarction (MI) have been the most common models to evaluate the pathophysiological mechanisms of post-infarction remodeling and heart failure (HF) development and progression. Existing models vary, and include permanent surgical coronary ligation (Fig. 1A), endovascular coronary artery occlusion with balloon and implantation of the embolic coil (Fig. 1B), ischemia/reperfusion, intracoronary micro embolization, and creation of chronic ischemia with ameroid constrictors. One of the first studies with AAV vector was performed in dogs. Acute infarction of the anterior LV wall was induced by permanent occlusion of the LAD artery. Four hours later, 5×10^{12} viral particles of rAAV2/VEGF165 were directly injected into the LV wall. Gene delivery enhanced both angiogenesis and cardiomyocyte viability in the infarcted myocardium.²⁹ In another study, MI was induced in pigs by endovascular balloon occlusion of the left circumflex coronary artery for 2 h. AAV9-S100A1 (1.5×10^{13} viral particles) was transferred 2 weeks later in a retrograde fashion via the cardiac anterior interventricular vein during blocking the venous outflow and LAD arterial inflow. At 14 weeks, AAV9-S100A1 delivery prevented cardiac deterioration and reversed ventricular remodeling by re-establishing

Figure 1. (A) Animal model of myocardial ischemia after permanent coronary ligation. (B) Animal model of myocardial ischemia after endovascular coronary artery occlusion with balloon. (C) Animal model of ischemia/reperfusion.

S100A1 expression level. Furthermore, S100A1 expression reconstituted cytosolic and SR Ca^{2+} cycling as well as energy homeostasis.³⁰ A similar delivery technique was described using AAV6 vector (1×10^{13} vg) encoding βARKct gene 2 weeks post MI. Robust and long-term βARKct expression was found after delivery, leading to significant amelioration of LV hemodynamics and contractile function in pigs with HF compared to control animals.³¹ In a different study, the researchers chose to use a new chimeric vector generated from AAV2 and AAV8 (BNP116), encoding inhibitor-1c, a SERCA2a/phospholamban-complex upstream regulator. One month after an anterior MI, pigs received intracoronary injection of either a high (1.0×10^{13} vg) or low dose (3.0×10^{12} vg) of BNP116/I-1c. Results showed improvement of cardiac function and promising efficacy of this novel vector for cardiac gene transfer.³² Similar results were demonstrated after intracoronary delivery of rAAV9 carrying I-1c gene post MI in pigs. Injection of AAV9/I-1c prevented further deterioration of cardiac function and led to a decrease in scar size compared to control.³³ In a sheep model of acute MI, investigators coupled the molecular cardiac surgery with recirculating delivery (MCARD) technique with a promising potential therapeutic transgene βARKct , delivered by a scAAV6. Quantitative poly-

merase chain reaction data showed robust βARKct expression in the LV. MCARD-mediated AAV6/ βARKct gene transfer resulted in preservation of regional and global systolic function after acute MI, though without arresting ventricular remodeling.³⁴ The same group demonstrated that MCARD-mediated delivery of scAAV9/SERCA2a following ovine ischemic cardiomyopathy resulted in a significant increase of SERCA2a expression in the left ventricular walls compared to other organs. Twelve weeks post MI, scAAV9/SERCA2a animals had superior LV function and attenuated myocyte hypertrophy compared to control.³⁵ The study also found that HF was significantly less pronounced in sheep transduced with recombinant AAV1/SERCA2a post major MI compared to control animals. Moreover, AAV1/SERCA2a gene delivery restricted the progression of fibrosis and prevented cardiac remodeling.³⁶

Ischemia/reperfusion model

This model is very attractive for researches because it resembles the clinical state following percutaneous coronary intervention (PCI) in the setting of acute MI (Fig. 1C). Reperfusion injury after restoring coronary blood flow in acute MI is assumed to cause many molecular changes due to

oxidative stress, accumulation of metabolic intermediates, endothelial damage with inflammatory response, and cellular dysfunction. Despite the attractiveness of this model and the relative ease of its creation, few studies have been dedicated to its investigation. This is due to the difficulties associated with obtaining consistent results, complicated pathophysiology of myocardial reperfusion injury, large mortality related to reperfusion-induced arrhythmias, myocardial stunning, and progressive death of cardiomyocytes. Ischemia/reperfusion was used to study the effect of rAAV9/Heme Oxygenase-1 (1×10^{13} viral particles) delivery in a pig model, in which hearts were subjected to 60 minutes of ischemia followed by 24 h of reperfusion. The study demonstrated that Heme Oxygenase-1 expression in the heart attenuates inflammation and improves post-ischemic function, and appears to be a suitable approach to treat reperfusion injury.³⁷ An additional study evaluated the effect of SUMO-1 gene transfer in a pig model. One month after balloon occlusion of the proximal LAD artery followed by reperfusion, the animals were randomized to receive AAV1/SUMO-1 or AAV1/null gene transfer via antegrade coronary infusion (5×10^{12} or 1×10^{13} viral particles). The study has demonstrated that SUMO-1 gene transfer using AAV1 improved cardiac function and stabilized LV volumes compared to control.³⁸

Volume overload models

Techniques to induce volume overload conditions include the creation of mitral, aortic or tricuspid valve regurgitation, or the creation of an arterio-venous fistula (most often aorto-caval shunt). Altered loading conditions and decreased

contractility in cases of valvular regurgitation result in progressive heart-chamber dilatation. Increased cardiac filling pressures, reduced cardiac output, and excessive peripheral vasoconstriction are the established hallmarks in volume overload models.¹⁶ The effectiveness of the volume overload model was confirmed by creating mitral regurgitation (MR) through the rupture of chordae tendinae of the mitral valve apparatus in a pig model (Fig. 2A). After 2 months, pig underwent intracoronary injection of rAAV1/SERCA2a (1×10^{12} viral particles). Four months post injection, SERCA2a expression resulted in positive inotropic effect and improvement of LV remodeling. In addition, SERCA2a expression levels were restored to normal in the treatment group.³⁹ An additional study that used a similar volume overload model found that AAV1/SERCA2a increased eNOS activity and calcium-storage capacity, and improved coronary vascular reactivity in the setting of MR.⁴⁰ In another study, MR was created with implanted LV to left atrium shunt with a teflon graft (Fig. 2B). Two weeks prior to graft implantation, AAV6/SERCA2a (5×10^{12} vg) was injected into LAD and circumflex arteries. After 3 months, SERCA2a-expressing animals had significantly better global LV function and lower end-systolic and end-diastolic dimensions.⁴¹

Pressure overload models

Pressure overload models are very popular in rodent studies. Pressure overload is accompanied by reductions in ventricular chamber compliance and diastolic dysfunction. Reduced compliance requires higher pressure to fill the ventricles. LV

Figure 2. (A) Volume overload animal model by creation of mitral regurgitation through the rupture of chordae tendinae of the mitral valve apparatus. (B) Volume overload animal model by implantation left ventricle to left atrium shunt with teflon graft.

pressure overload can be created with devices narrowing the ascending or descending thoracic aorta with a variety of surgical approaches. For right ventricle pressure overload, pulmonary artery banding is employed.¹⁶ No data were found on the use AAV vector in large animals using pressure overload models.

Pacing induced models

This model allows the effect of rapid pacing on ventricular function in dilated cardiomyopathy to be evaluated. The advantages of the rapid pacing model are its relative simplicity, and the ability to sustain atrial or ventricular pacing to produce biventricular systolic and/or diastolic dysfunction without concomitant changes in the coronary vessels.¹⁶ A study in dogs with tachypacing-induced dilated cardiomyopathy showed that direct myocardial injection of AAV9/VEGF-B₁₆₇ (1×10^{12} gc) delays functional myocytes derangement without affecting myocardial blood perfusion, inducing angiogenesis.⁴² In a similar study, AAV9/VEGF-B₁₆₇ was infused intracoronary. Compared to control subjects, VEGF-B₁₆₇ expression markedly preserved diastolic and contractile function and attenuated ventricular chamber remodeling, halting the progression of HF.⁴³ To test the efficiency of AAV2/SERCA2a the authors explored the effects of different doses of this gene construct on cardiac function after creating pacing-induced HF. Cardiac recirculation delivery of AAV1/SERCA2a elicited a dose-dependent improvement in cardiac performance. In contrast, simple antegrade intracoronary infusion did not cause any effect on ventricular function.⁴⁴

Comments

HF is a molecular-clinical complex associated with functional, neurohumoral, and genetic abnormalities. Progression of HF depends on basic pathologic processes such as ischemia, pressure overload, volume overload, and more. In many cases, surgical models in large animals can closely mimic the human conditions, making them instrumental for the investigation of potential gene treatments with distinct phenotypic and pathogenetic features of HF.

CONCLUSIONS

In recent years, AAV vectors have gained popularity in the setting of cardiac gene transfer due to a lack of pathogenicity and low immunogenicity in humans, lack of integration into the human host genome, ability to infect post-mitotic cells such as

myocytes, and ability to provide expression of the transgene for prolonged periods of time. However, studies have also highlighted critical challenges of AAV as a vector, including: (1) variability among different species, (2) transduction to off-target organs, and (3) neutralization caused by preexisting humoral immunity. Evaluation of AAV-mediated gene transfer in the hearts of large animals is highly relevant to clinical application. This review shows the significant differences between transduction efficiency as well as the kinetics of transgene expression of AAV serotypes in the different large-animal models of HF. Those differences are attributed to inequality in cellular uptake, intracellular trafficking mechanisms of AAV vectors in various species, and structural differences between AAV serotypes at the capsid level. Moreover, AAV transduction greatly depends on the dose and route of delivery. An optimal, clinically translatable technique for cardiac AAV transfer should incorporate a sophisticated, minimally invasive system with multiple recirculations with a long adhesion time of the vector with the coronary endothelium, the ability to apply pharmacological mediators, and the use of physical and mechanical methods of improving cellular permeability.

In summary, recombinant AAV serotypes 1, 6, 8, and 9 are currently the most often used vectors for cardiac gene delivery in large-animal models of HF. This review summarizes current knowledge on the use of AAV as a delivery vehicle of different genes to treat a failing heart by enhancing contractility, attenuated cardiac remodeling, and preventing the progression of HF. It also highlights the importance of using the most relevant animal models to study heart disease and reviews the experience of AAV delivery using different surgical approaches. Taken together, accumulating data validate the efficacy and safety of AAV gene therapy approach for successful clinical application to treat HF in the clinic.

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AUTHOR DISCLOSURE

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REFERENCES

- Samulski RJ, Muzycka N. AAV-mediated gene therapy for research and therapeutic purposes. *Ann Rev Virol* 2014;1:427–451.
- Nonnenmacher M, Weber T. Intracellular transport of recombinant adeno-associated virus vectors. *Gene Ther* 2012;19:649–658.
- Pillay S, Meyer NL, Puschnik AS, et al. An essential receptor for adeno-associated virus infection. *Nature* 2016;530:108–112.
- Nonnenmacher M, Weber T. Adeno-associated virus 2 infection requires endocytosis through the CLIC/GEEC pathway. *Cell Host Microbe* 2011;10:563–576.
- Bartlett JS, Wilcher R, Samulski RJ. Infectious entry pathway of adeno-associated virus and adeno-associated virus vectors. *J Virol* 2000;74:2777–2785.
- Nonnenmacher ME, Cintrat JC, Gillet D, et al. Syntaxin 5-dependent retrograde transport to the trans-Golgi network is required for adeno-associated virus transduction. *J Virol* 2015;89:1673–1687.
- Farr GA, Zhang LG, Tattersall P. Parvoviral virions deploy a capsid-tethered lipolytic enzyme to breach the endosomal membrane during cell entry. *Proc Natl Acad Sci U S A* 2005;102:17148–17153.
- Sonntag F, Bleker S, Leuchs B, et al. Adeno-associated virus type 2 capsids with externalized VP1/VP2 trafficking domains are generated prior to passage through the cytoplasm and are maintained until uncoating occurs in the nucleus. *J Virol* 2006;80:11040–11054.
- Katz MG, Swain JD, White JD, et al. Cardiac gene therapy: optimization of gene delivery techniques *in vivo*. *Hum Gene Ther* 2010;21:371–380.
- Ishikawa K, Tilemann L, Hajjar RJ. Gene delivery methods in cardiac gene therapy. *J Gene Med* 2011;13:566–572.
- Zincarelli C, Soltys S, Rengo G, et al. Analysis of AAV serotypes 1–9 mediated gene expression and tropism in mice after systemic injection. *Mol Ther* 2008;16:1073–1080.
- Asokan A, Samulski RJ. An emerging adeno-associated viral vector pipeline for cardiac gene therapy. *Hum Gene Ther* 2013;24:906–913.
- Pacak CA, Mah CS, Thattaiyath BD, et al. Recombinant adeno-associated virus serotype 9 leads to preferential cardiac transduction *in vivo*. *Circ Res* 2006;99:e3–e9.
- Bish LT, Morine K, Sleeper MM, et al. Adeno-associated virus (AAV) serotype 9 provides global cardiac gene transfer superior to AAV1, AAV6, AAV7, and AAV8 in the mouse and rat. *Hum Gene Ther* 2008;19:1359–1368.
- Fang H, Lai NC, Gao MH, et al. Comparison of adeno-associated virus serotypes and delivery methods for cardiac gene transfer. *Hum Gene Ther Methods* 2012;23:234–241.
- Katz MG, Fargnoli AS, Tomasulo CE, et al. Model-specific selection of molecular targets for heart failure gene therapy. *J Gene Med* 2011;13:573–586.
- Odom DT, Dowell RD, Jacobsen ES, et al. Tissue-specific transcriptional regulation has diverged significantly between human and mouse. *Nat Genet* 2007;39:730–732.
- Dixon JA, Spinale FG. Large animal models of heart failure: a critical link in the translation of basic science to clinical practice. *Circ Heart Fail* 2009;2:262–271.
- Kapliitt MG, Xiao X, Samulski RJ, et al. Long-term gene transfer in porcine myocardium after coronary infusion of an adeno-associated virus vector. *Ann Thorac Surg* 1996;62:1669–1676.
- Kaspar BK, Roth DM, Lai NC, et al. Myocardial gene transfer and long-term expression following intracoronary delivery of adeno-associated virus. *J Gene Med* 2005;7:316–324.
- Su H, Yeghiazarians Y, Lee A, et al. AAV serotype 1 mediates more efficient gene transfer to pig myocardium than AAV serotype 2 and plasmid. *J Gene Med* 2008;10:33–41.
- Raake PW, Hinkel R, Muller S, et al. Cardiospecific long-term gene expression in a porcine model after selective pressure-regulated retroinfusion of adeno-associated viral (AAV) vectors. *Gene Ther* 2008;15:12–17.
- Pan X, Yue Y, Zhang K, et al. Long-term robust myocardial transduction of the dog heart from a peripheral vein by adeno-associated virus serotype-8. *Hum Gene Ther* 2013;24:584–594.
- Pan X, Yue Y, Zhang K, et al. AAV-8 is more efficient than AAV-9 in transducing neonatal dog heart. *Hum Gene Ther Methods* 2015;26:54–61.
- McCarty DM. Self-complementary AAV vectors: advantages and applications. *Mol Ther* 2008;16:1648–1656.
- Bish LT, Sleeper MM, Brainard B, et al. Percutaneous Transendocardial delivery of self-complementary adeno-associated virus 6 achieves global cardiac gene transfer in canines. *Mol Ther* 2008;16:1953–1959.
- White J, Thesier DM, Swain JB, et al. Myocardial gene delivery using molecular cardiac surgery with recombinant adeno-associated virus vectors *in vivo*. *Gene Ther* 2011;18:546–552.
- Bish LT, Sleeper MM, Reynolds C, et al. Cardiac gene transfer of short hairpin RNA directed against phospholamban effectively knocks down gene expression but causes cellular toxicity in canines. *Hum Gene Ther* 2011;22:969–977.
- Ferrarini M, Arsic N, Recchia FA, et al. Adeno-associated virus-mediated transduction of VEGF165 improves cardiac tissue viability and functional recovery after permanent coronary occlusion in conscious dogs. *Circ Res* 2006;98:954–961.
- Pleger ST, Shan C, Ksienzyk J, et al. Cardiac AAV9-S100A1 gene therapy rescues post-ischemic heart failure in a preclinical large animal model. *Sci Transl Med* 2011;3:92ra64.
- Raake PW, Schlegel P, Ksienzyk L, et al. AAV6- β ARKct cardiac gene therapy ameliorates cardiac function and normalizes the catecholaminergic axis in a clinically relevant large animal heart failure model. *Eur Heart J* 2013;34:1437–1447.
- Ishikawa K, Fish KM, Tilemann L, et al. Cardiac I-1c overexpression with reengineered AAV improves cardiac function in swine ischemic heart failure. *Mol Ther* 2014;22:2038–2045.
- Fish KM, Ladage D, Kawase Y, et al. AAV9.I-1c delivered via direct coronary infusion in a porcine model of heart failure improves contractility and mitigates adverse remodeling. *Circ Heart Fail* 2013;6:310–317.
- Swain JD, Fargnoli AS, Katz MG, et al. MCARD-mediated gene transfer of GRK2 inhibitor in ovine model of acute myocardial infarction. *J Cardiovasc Transl Res* 2013;6:253–262.
- Katz MG, Fargnoli AS, Williams RD, et al. Safety and efficacy of high-dose adeno-associated virus 9 encoding sarcoplasmic reticulum Ca(2+) adenosine triphosphatase delivered by molecular cardiac surgery with recirculating delivery in ovine ischemic cardiomyopathy. *J Thorac Cardiovasc Surg* 2014;148:1065–1072.
- Katz MG, Brandon-Warner E, Fargnoli AS, et al. Mitigation of myocardial fibrosis by molecular cardiac surgery-mediated gene overexpression. *J Thorac Cardiovasc Surg* 2016;151:1191–1200.
- Hinkel R, Lange R, Petersen B, et al. Heme oxygenase-1 gene therapy provides cardioprotection via control of post-ischemic inflammation: an experimental study in a pre-clinical pig model. *J Am Coll Cardiol* 2015;66:154–165.
- Tilemann L, Lee A, Ishikawa K, et al. SUMO-1 gene transfer improves cardiac function in a large-animal model of heart failure. *Sci Transl Med* 2013;5:211ra159.
- Kawase Y, Ly HQ, Prunier F, et al. Reversal of cardiac dysfunction after long-term expression of SERCA2a by gene transfer in a pre-clinical model of heart failure. *J Am Coll Cardiol* 2008;51:1112–1119.
- Hadri L, Bobe R, Kawase Y, et al. SERCA2a gene transfer enhances eNOS expression and activity in endothelial cells. *Mol Ther* 2010;18:1284–1292.
- Beeri R, Chaput M, Guerrero JL, et al. Gene delivery of sarcoplasmic reticulum calcium ATPase inhibits ventricular remodeling in ischemic mitral regurgitation. *Circ Heart Fail* 2010;3:627–634.
- Pepe M, Mandani M, Zentilin L, et al. Intramyocardial VEGF-B167 gene delivery delays the progression towards congestive failure in dogs with pacing-induced dilated cardiomyopathy. *Circ Res* 2010;106:1893–1903.
- Witek F, Zentilin L, Hoffman NE, et al. Intracoronary cytoprotective gene therapy: a study of VEGF-B167 in a pre-clinical animal model of dilated cardiomyopathy. *J Am Coll Cardiol* 2015;66:139–153.
- Byrne MJ, Power JM, Prevolos A, et al. Recirculating cardiac delivery of AAV2/1SERCA2a improves myocardial function in an experimental model of heart failure in large animals. *Gene Ther* 2008;15:1550–1557.

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