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# Preservation of cardiac function in left ventricle cardiac hypertrophy using an AAV vector which provides VEGF-A expression in response to p53

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ABSTRACT

Here we present the application of our adeno-associated virus (AAV2) vector where transgene expression is driven by a synthetic, p53-responsive promoter, termed PG, used to supply human vascular endothelial growth factor-$A_{165}$ (VEGF-A). Thus, p53 is harnessed to promote the beneficial expression of VEGF-A encoded by the AAVPG vector, bypassing the negative effect of p53 on HIF-1α which occurs during cardiac hypertrophy. Wistar rats were submitted to pressure overload induced by thoracic aorta coarctation (TAC) with or without concomitant gene therapy (intramuscular delivery in the left ventricle). After 12 weeks, rats receiving AAVPG-VEGF gene therapy were compared to those that did not, revealing significantly improved cardiac function under hemodynamic stress, lack of fibrosis and reversal of capillary rarefaction. With these functional assays, we have demonstrated that application of the AAVPG-VEGF vector under physiologic conditions known to stimulate p53 resulted in the preservation of cardiac performance.



## Introduction

Pathologic cardiac hypertrophy is a chronic condition characterized by increased ventricular mass in response to an increased load in the presence of hypertension, aortic stenosis, heart valve defects, or myocardial infarction (Mill et al., 2011). Cardiac muscle hypertrophy can provide a short term beneficial compensatory response by reducing diastolic wall stress and increasing developed force, but, at subsequent stages of cardiac remodeling, hypertrophy culminates in contractile failure and ventricular dilatation (Frey and Olson, 2003; Gardin and Lauer, 2004) and is associated with increased morbidity and mortality (Levy et al., 1990).

The hypertrophic muscle mass develops disproportionally to neovascularization, a mismatch that results in tissue hypoxia and contributes to the transition to heart failure (Izumiya et al., 2006; Shiojima et al., 2005). Hypoxia signals mediators that can satisfy the demand for increased capillary density. For example, activation of the transcription factor HIF-1α (hypoxia-inducible factor-1α) is known to induce expression of vascular endothelial growth factor (VEGF) and thus promote neovascularization (Carmeliet, 2000; Forsythe et al., 1996; Tirziu and Simons, 2005). However, muscle mass can accumulate faster than HIF-1α and VEGF can stimulate angiogenesis, resulting in continued hypoxia, a cellular stress that induces the accumulation of p53 (Das et al., 2010; Ravi et al., 2000; Sano et al., 2007).

The p53 protein is a potent transcription factor (Beckerman and Prives, 2010) and has been the subject of several studies related to biomechanical stress and cardiac hypertrophy (Leri et al., 1998, 2000; Tsukamoto et al., 2006). Under hypoxic conditions, increased p53 protein levels and activity were noted in heart cells (Das et al., 2010; Long et al., 1997). The increase in p53 was associated with inactivation of HIF-1α and reduction in VEGF expression (Ravi et al., 2000; Sano et al., 2007). PUMA, a transcriptional target of p53 and

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activator of apoptosis, is also an important mediator of cell death in the transition to heart failure (Mandl et al., 2011). Thus the p53 pathway plays a central role in down-regulating VEGF expression and promoting cardiac cell death.

The mammalian VEGF family consists of the variants VEGF-A, VEGF-B, VEGF-C, VEGF-D and placenta growth factor. All forms of VEGF are secreted and may differ in binding affinity with one, two or all three VEGF receptors. Splice variants of VEGF-A yield a variety of isoforms, though VEGF-$A_{165}$ has been studied extensively (Giacca and Zacchigna, 2012). Experimental studies have shown that VEGF-$A_{165}$ (referred to here as VEGF-A) is an essential endothelial mitogenic agent in angiogenesis and vasculogenesis (Pettersson et al., 2000), induces mitosis of adult cardiomyocytes (Laguens et al., 2002) and is also associated with the recruitment and activation of resident cardiac stem cells to repair sites of ischemic tissue (Ferrarini et al., 2006; Vera Janavel et al., 2006). Administration of recombinant VEGF-A has shown therapeutic benefit in animal models of cardiac hypertrophy induced by transaortic coarctation (Friehs et al., 2006), though the administration of recombinant protein may prove to be inefficient for prolonged treatments.

Gene therapy is a promising alternative to the application of recombinant VEGF. The use of viral vectors that efficiently transduce cardiac cells *in vivo* can provide localized concentrations of VEGF, thus increasing the effect of the transgene (Giacca and Zacchigna, 2012). In addition, gene therapy may provide an opportunity for preemptive treatment, especially in the case that the gene transfer vector provides on-demand expression of VEGF. Controlled expression of VEGF is critical to avoid undesired effects such as hemangioma formation (Lee et al., 2000; Schwarz et al., 2000). To this end, our laboratory has developed several gene transfer vectors where expression of the transgene is controlled by p53 acting upon a synthetic p53-responsive promoter, termed PG. We have shown that these retroviral, adenoviral and adeno-associated virus vectors offer high-level expression only in the presence of active p53 (Bajgelman et al., 2013; Bajgelman and Strauss, 2008; Merkel et al., 2010; Strauss and Costanzi-Strauss, 2004). However, the use of adeno-associated virus (AAV) may be especially well suited for cardiac gene transfer since these vectors combine the advantages of low immune response against transduced cells and the possibility of long term expressions of the therapeutic gene (Bennett, 2003; Raake et al., 2008).

Here we propose a new therapeutic strategy to prevent the deterioration of cardiac function associated with hypertrophy. For this, we employed our adeno-associated vector which expresses the human VEGF-A cDNA driven by a chimeric promoter responsive to p53 (Bajgelman et al., 2013). In theory, as p53 levels increase during the transition to decompensated cardiac hypertrophy, the p53-responsive AAV vector should provide expression of VEGF-A. In this way, the negative role of p53 in response to physiologic stress is counterbalanced by using p53 to drive on-demand VEGF-A transgene expression which, in turn, should contribute to neovascularization and preservation of cardiac function.

## Results

### *AAVPG-VIG provides p53-responsive expression of VEGF in transduced primary cells*

The AAVPG-VIG vector encodes the human VEGF-$A_{165}$ (VEGF-A) cDNA driven by the chimeric p53 responsive promoter, PGTxβ (Bajgelman et al., 2013; Bajgelman and Strauss, 2008). In order to verify the functionality of the vector in primary cardiac cells, cardiomyocytes isolated from neonatal rat heart were transduced with the AAVPG-VIG vector and subjected to increasing concentrations of doxorubicin, an anthracycline known to induce transactivation by p53, yielding significant amounts of secreted human VEGF-A, as measured in the cell culture medium by ELISA (Fig. 1A). In Fig. 1B, we demonstrate that a similar response also occurs using AAVPG-luc virus, encoding the luciferase reporter gene. The fall in transgene expression at the highest drug dose may have been due to toxicity, but this appears not to have altered vector expression since the result is consistent even in the absence of normalization (Fig. S1). These assays show that a treatment known to activate p53 results in the induction of expression from AAVPG vectors in a comparable manner.

We have directly tested whether the co-transduction of the AAVPG and AAVCMV vectors is associated with any cross talk between the vectors. As seen in Fig. S2, the performance of AAVPG-luc was the same whether or not AAVCMV-LacZ was present. Therefore, the co-transduction approach yields a reliable internal control.

### *In vivo cardiac transduction using viral vectors expressing the LacZ reporter gene*

We performed *in vivo* cardiac transduction followed by observation of β-galactosidase (LacZ) expression driven by the constitutive CMV promoter. Wistar rats were injected in the left ventricle using either $10^8$ genome copies (GC) AAVCMV-LacZ (serotype 2) or $10^8$

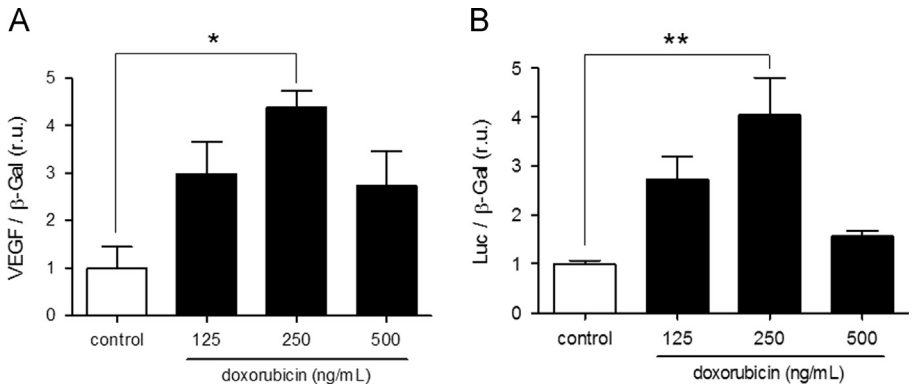


**Fig. 1.** Expression of AAVPG viruses can be induced in cardiomyocytes isolated from neonatal rat heart. (A) Cells were co-transduced with an equimolar mixture of the adeno-associated vectors AAVPG-VIG and AAVCMV-LacZ. After 24 h, medium was changed and cells were subjected to the indicated concentrations of doxorubicin. Human VEGF-A expression was quantified in the supernatant by ELISA, and normalized by β-gal activity presented by the cellular lysates. The condition without doxorubicin was called 1. (B) Cells were co-transduced with an equimolar mix of the adeno-associated vectors AAVPG-luc and AAVCMV-LacZ. After 24 h, medium was changed and cells were subjected to the indicated concentrations of doxorubicin. The cells were harvested and luciferase (Luc) activity was quantified. β-gal was used to normalize Luc activity. The condition without doxorubicin was called 1. $^*P < 0.05$ and $^{**}P < 0.01$ *vs.* control. One-way ANOVA followed by Bonferroni *posthoc* test. Mean+SEM, $n=3$.