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RESEARCH LETTER

Gene Therapy With the DWORF Micropeptide Attenuates Cardiomyopathy in Mice

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Calcium dysregulation is a universal characteristic of heart failure (HF) and reduced SERCA (sarco/endoplasmic reticulum calcium-ATPase) activity plays a central role in disease progression.[1] Hence, increasing SERCA activity has been pursued as a clinical approach for treating HF and significant evidence supports its therapeutic potential.[1,2] Recently, we discovered the novel muscle-specific micropeptide dwarf open reading frame (DWORF), which enhances SERCA activity by displacing the SERCA-inhibitory peptide phospholamban.[3,4] In mice, cardiac-specific transgenic overexpression of DWORF enhanced SERCA activity, increased calcium cycling and contractility,[4] and rescued a genetic model of dilated cardiomyopathy,[3] suggesting that DWORF overexpression might be used to achieve SERCA activation in HF to normalize calcium homeostasis and prevent disease progression.

To explore the therapeutic potential of DWORF gene therapy in HF, we developed an adeno-associated virus (AAV) approach using cardiotropic serotype 9 (AAV9) and the cTnT (cardiac troponin-T) promoter (Addgene plasmid no. 69915).[5] All animal procedures were conducted in accordance with institutional guidelines and were approved by the Institutional Animal Care and Use Committee (IACUC). Both male and female mice were used. AAV9-cTnT-DWORF (AAV-DWORF) and control AAV9-cTnT-tdTomato (AAV-tdTomato) were validated in mice by delivery at postnatal day 5 by intraperitoneal injection at 5×10^{13} viral genomes/kg and Western blot analysis revealed cardiac-specific overexpression at postnatal day 28 (Figure [A]). The efficacy of AAV-DWORF gene therapy was assessed in a mouse model of dilated cardiomyopathy caused by gene deletion of muscle-specific LIM protein (MLP knockout [KO]). Consistent with the cardioprotection previously observed through transgenic overexpression of DWORF in MLP KO mice,[3] echocardiography of mice that received AAV-DWORF at postnatal day 5 showed significantly enhanced ventricular function compared with control MLP KO/AAV-tdTomato mice at 8 weeks (Figure [B]) and adverse cardiac remodeling was attenuated (Figure [B] and [C]). The degree of cardioprotection observed in MLP KO/AAV-DWORF mice was diminished compared to MLP KO/DWORF transgenic mice,[3] likely due to the reduced level of DWORF overexpression achieved by AAV-delivery (16.9±2.4-fold) compared with DWORF transgenic overexpression (58.5±14.7-fold; Figure [A]). Nevertheless, these results indicate that enhancing SERCA activity via DWORF gene therapy is a viable therapeutic strategy.

Next, we tested the potential of DWORF gene therapy in preventing adverse cardiac outcomes in a myocardial infarction (MI) model of HF. Mice received AAV-DWORF or AAV-tdTomato at postnatal day 5 and were subjected to sham surgery or MI by permanent ligation of the left coronary artery at 8 weeks of age and HF induction and progression were monitored for 12 weeks. Consistent with previous observations in other HF models,[3,4] endogenous DWORF protein expression was reduced in response to MI (3.4±1.0-fold; Figure [D]), likely contributing to the reduced SERCA activity underlying HF. Western blot analysis also indicated sustained AAV-mediated overexpression of DWORF in both sham (14.9±1.0-fold) and MI samples (17.0±4.8-fold) 12 weeks postsurgery (Figure [D]). Cardiac function was assessed in mice by echocardiography at baseline and post-MI and MI/AAV-DWORF mice showed significantly enhanced ventricular

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Nonstandard Abbreviation and Acronyms

AAV	adeno-associated virus
DWORF	dwarf open reading frame
HF	heart failure
MI	myocardial infarction
MLP KO	gene deletion of muscle-specific LIM protein
SERCA	sarco/endoplasmic reticulum calcium-ATPase

function (Figure [E]) and reduced cardiac dilation compared to MI/AAV-tdTomato mice (Figure [E] and [F]). Histological analysis of hearts with Masson's trichrome staining indicated no significant difference in infarct size between groups (Figure [F]).

Collectively, the data presented here indicate that DWORF gene therapy holds promise as a novel HF therapeutic. Future studies will continue to address the direct clinical relevance of DWORF gene therapy in established HF to build on the success of our prevention-based approach. The data supporting the findings of this study are available from the corresponding author upon reasonable request.

ARTICLE INFORMATION

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Disclosures

C.A. Makarewich, R. Bassel-Duby, and E.N. Olson are coinventors on a patent regarding the use of dwarf open reading frame (DWORF) for treatment of heart failure (US 10 570 183). The other authors report no conflicts.

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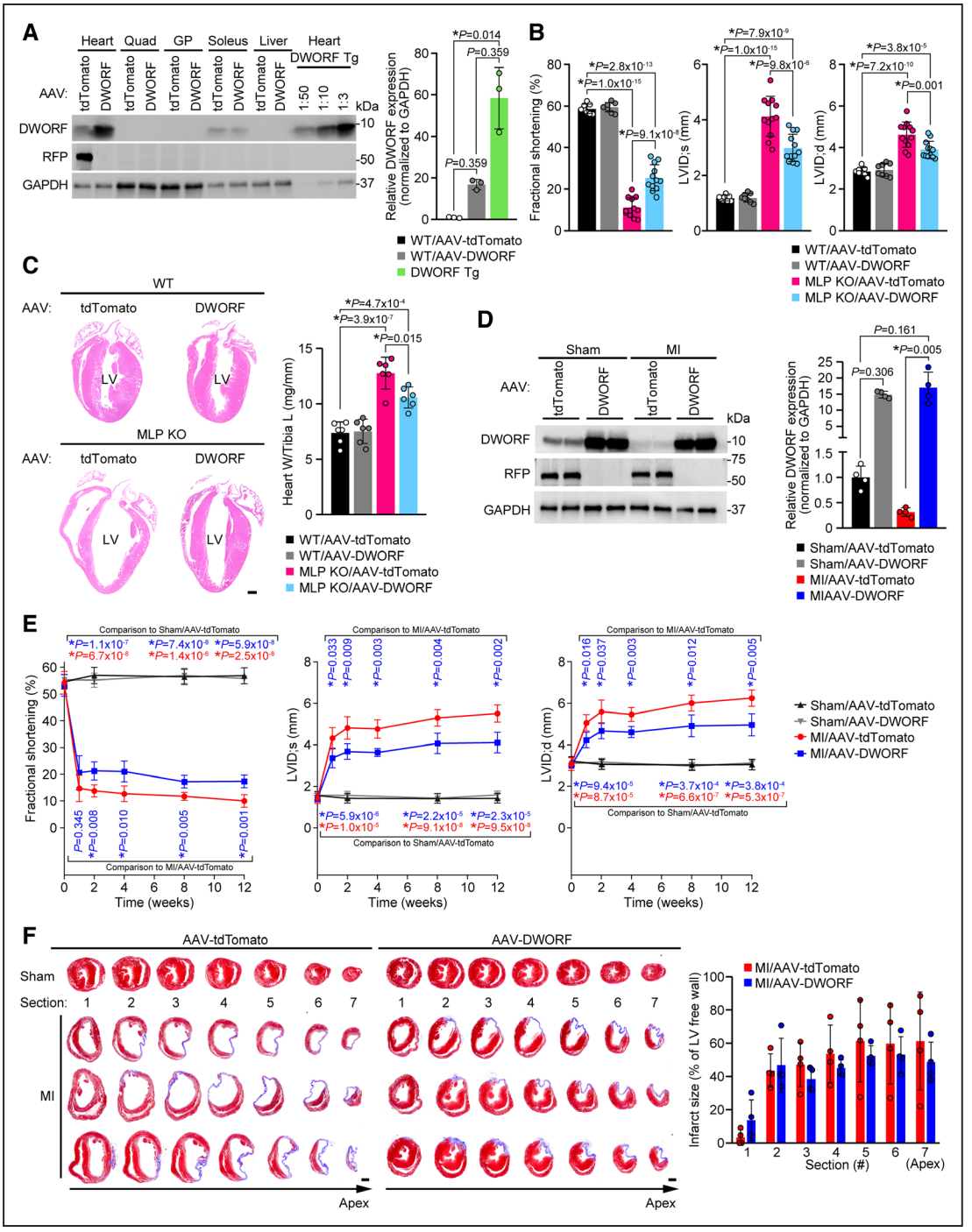

Figure. Development of dwarf open reading frame (DWORF) gene therapy as a heart failure (HF) therapeutic.
A, Western blot analysis and DWORF quantification of lysates from mice 4-weeks post-adeno-associated virus (AAV)-delivery (n=3/group). DWORF Tg (DWORF transgenic) heart lysates were diluted to compare the level of AAV-DWORF-mediated overexpression with DWORF Tg mice. **B**, Echocardiography analysis of left ventricular (LV) fractional shortening and internal diameter (LVID) during systole (s) and diastole (d). n=8 (WT/AAV-tdTomato, WT/AAV-DWORF) or n=12 (MLP KO [muscle-specific LIM protein knockout]/AAV-tdTomato, MLP KO/AAV-DWORF). **C**, **left**, Representative H&E staining of heart sections (n=4/group). Scale=1 mm. **Right**, Heart weight (W) to tibia length (L) measurements for n=6/group. **D**, Western blot analysis and quantification of heart lysates 12 weeks after sham/myocardial infarction (MI) surgery (n=4/group). **E**, Echocardiography analysis of fractional shortening and LVID at baseline (0 wk) and post-sham/MI surgery for n=5 sham mice (sham/AAV-tdTomato, sham/AAV-DWORF) or n=6 MI mice (MI/AAV-tdTomato, MI/AAV-DWORF). **F**, **left**, Masson's trichrome staining on serial heart sections taken at 0.5 mm increments starting at the suture (section 1) and numbered (1–7) for quantification. Scale=1 mm. **Right**, Quantification of infarct size of each section calculated as the length of the scar/length of total LV free wall for n=4 mice/group. Statistics: Data shown as mean±SD. The Shapiro-Wilk normality test was used for distribution. Statistical analysis included nonparametric Kruskal-Wallis test with Dunn-multiple comparison (**A** and **D**) or Mann-Whitney test (**F**) and 2-way ANOVA with Tukey post hoc test (**B** and **C**) or mixed-effects analysis with Geisser-Greenhouse correction and Tukey post hoc test (**E**). *P* values are corrected for multiple testing with $P<0.05$ considered statistically significant. GP indicates gastrocnemius plantaris; RFP, red fluorescent protein; and WT, wild type.