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Hippo Pathway Knockdown Gene Therapy in the Heart

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Background

After a myocardial infarction (MI), scar tissue formation and loss of cardiac muscle negatively affect the heart's ability to contract, resulting in pathologic remodeling and eventual heart failure (HF). Current paradigms hold that cardiomyocytes (CMs) cannot proliferate and repair the heart after MI. The Hippo signaling pathway is an inhibitory kinase cascade that represses adult CM proliferation and renewal after MI.[1] Interestingly, though, neonatal mouse and pig hearts can regenerate within a limited time frame after birth,[2-4] and anecdotal reports suggest that the neonatal human heart also has a regenerative capacity.[5] Neonatal mouse heart studies indicate that newly formed heart muscle cells or CMs originate from preexisting CMs, implying that it is possible to manipulate adult CMs to promote heart regeneration in humans directly.[6]

Current Limitations

A long-standing and unmet clinical goal in cardiovascular medicine is to uncover and effectively manipulate endogenous genetic mechanisms to induce post-MI cardiac repair. Many specialized cells in the adult mammalian body, including CMs, enter a postnatal state of cell cycle quiescence through poorly understood mechanisms. Although research conducted in mice has enhanced the field's understanding of CM and tissue regeneration in a broader context, it is still uncertain how these findings will translate into treatments for chronic, incurable human conditions such as HF. Recent studies reveal that excessively stimulating the proliferation of CMs in mice and pig models can result in the animal's death.[7-9]

Recent Developments

An adeno-associated virus 9 (AAV9)–based gene therapy has been developed to locally knock down the Hippo signaling pathway gene *Sav* in CMs of the border zone microenvironment in a pig model of ischemia/reperfusion–induced MI.[10] A catheter-based technology using NOGA electromechanical mapping (Biologics Delivery Systems/Johnson & Johnson) was used to deliver *Sav,* packaged in a gene therapy viral vector, specifically to border zone CMs in the post-MI heart. Advantages to this approach include improved delivery of gene therapy products to cells of interest and a reduced amount of viral material administered per kilogram of body weight. This translational work

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showed that the knockdown of *Sav* in the border zone of the pig heart 2 weeks after MI led to the induction of regenerative repair with improved cardiac function (Fig. 1).[10] In these hearts, AAV9-*Sav*-short hairpin RNA delivery promotes CM cell cycle reentry and division concomitant with transient sarcomere breakdown and capillary formation.[10] In addition, fibrosis is markedly reduced in hearts treated with gene therapy (Fig. 1). This work was a direct follow-up to mouse studies in which CM deletion of *Sav* 3 weeks after MI in a mouse model of HF resulted in Hippo signaling pathway reduction and regenerative repair.[1]

Future Directions

These functional data reveal that the Hippo signaling pathway inhibits CM growth and regeneration in large animals, suggesting that this regulation is conserved in humans. AAV9-*Sav*-short hairpin RNA treatment induces a steady improvement in cardiac function in mice and pigs in a safe and effective manner, providing exciting, new avenues to improve HF therapies and to potentially broaden clinical indications. In a logical next step to bring *Sav* knockdown with short hairpin RNA gene therapy to the clinic, it is imperative to determine how human CMs respond to this therapy and to perform dose-ranging studies to determine functional outcomes.

Abbreviations and Acronyms

AAV9	adeno-associated virus 9
CM	cardiomyocyte
MI	myocardial infarction

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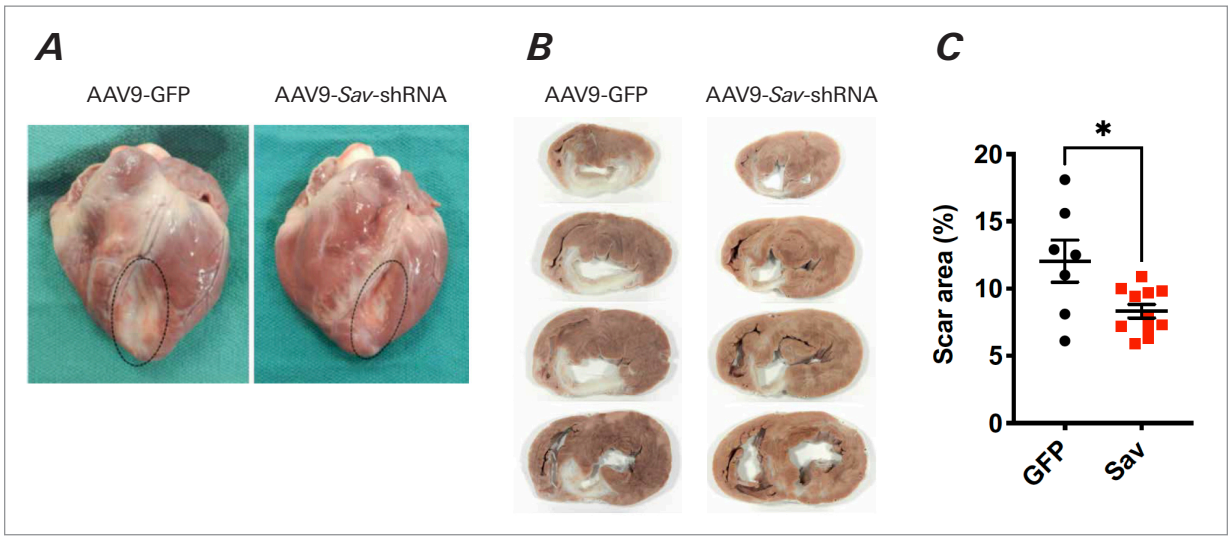

Fig. 1 AAV9-*Sav*-shRNA gene therapy improves cardiac function in pigs after MI. **A**) Representative pig hearts harvested 90 days after viral vector injection (AAV9-GFP control vs AAV9-*Sav*-shRNA). Pigs experienced MI at 92 days of age, received viral vector at 106 days, and were euthanized at the age of 196 days. Scale bar, 2 cm. **B**) Representative heart slices for AAV9-GFP control and AAV9-*Sav*-shRNA pigs. Scale bar, 2 cm. **C**) Scar size quantification (AAV9-GFP, n = 7; AAV9-*Sav*-shRNA, n = 11). Mann-Whitney test was used for the comparison. Data presented as mean (SEM). **P* < .05.

AAV9, adeno-associated virus 9; GFP, green fluorescent protein; MI, myocardial infarction; shRNA, short hairpin RNA.

From Liu S, Li K, Wagner Florencio L, Tang L, Heallen TR, Leach JP, Wang Y, Grisanti F, Willerson JT, Perin EC, Zhang S, Martin JF. Gene therapy knockdown of Hippo signaling induces cardiomyocyte renewal in pigs after myocardial infarction. *Sci Transl Med.* 2021;13(600):eabd6892. doi:10.1126/scitranslmed.abd6892. Reprinted with permission from AAAS.[10]

Conflict of Interest Disclosure: J.F.M. is a co-founder of and owns shares in YAP Therapeutics. J.F.M. and T.R.H. are co-inventors on the following patents associated with this study: patent no. US20200206327A1 entitled "Hippo pathway deficiency reverses systolic heart failure post-infarction"; patent No. 15/642200.PCT/US2014/069349 101191411 entitled "Hippo and dystrophin complex signaling in cardiomyocyte renewal"; and patent No. 15/102593.PCT/US2014/069349 9732345 entitled "Hippo and dystrophin complex signaling in cardiomyocyte renewal."

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References

1. Leach JP, Heallen T, Zhang M, et al. Hippo pathway deficiency reverses systolic heart failure after infarction. *Nature.* 2017;550(7675):260-264. doi:10.1038/nature24045
2. Zhu W, Zhang E, Zhao M, et al. Regenerative potential of neonatal porcine hearts. *Circulation.* 2018;138(24):2809-2816. doi:10.1161/CIRCULATIONAHA.118.034886
3. Ye L, D'Agostino G, Loo SJ, et al. Early regenerative capacity in the porcine heart. *Circulation.* 2018;138(24):2798-2808. doi:10.1161/CIRCULATIONAHA.117.031542
4. Porrello ER, Mahmoud AI, Simpson E, et al. Transient regenerative potential of the neonatal mouse heart. *Science.* 2011;331(6020):1078-1080. doi:10.1126/science.1200708
5. Haubner BJ, Schneider J, Schweigmann U, et al. Functional recovery of a human neonatal heart after severe myocardial infarction. *Circ Res.* 2016;118(2):216-221. doi:10.1161/CIRCRESAHA.115.307017
6. Martin JF, Perin EC, Willerson JT. Direct stimulation of cardiogenesis: a new paradigm for treating heart disease. *Circ Res.* 2017;121(1):13-15. doi:10.1161/CIRCRESAHA.117.311062
7. Gabisonia K, Prosdocimo G, Aquaro GD, et al. MicroRNA therapy stimulates uncontrolled cardiac repair after myocardial infarction in pigs. *Nature.* 2019;569(7756):418-422. doi:10.1038/s41586-019-1191-6
8. Tian Y, Liu Y, Wang T, et al. A microRNA-Hippo pathway that promotes cardiomyocyte proliferation and cardiac regeneration in mice. *Sci Transl Med.* 2015;7(279):279ra38. doi:10.1126/scitranslmed.3010841
9. Monroe TO, Hill MC, Morikawa Y, et al. YAP partially reprograms chromatin accessibility to directly induce adult cardiogenesis in vivo. *Dev Cell.* 2019;48(6):765-779.e7. doi:10.1016/j.devcel.2019.01.017
10. Liu S, Li K, Wagner Florencio L, et al. Gene therapy knockdown of Hippo signaling induces cardiomyocyte renewal in pigs after myocardial infarction. *Sci Transl Med.* 2021;13(600):eabd6892. doi:10.1126/scitranslmed.abd6892