Abstract: 25O

AAV-directed expression of Neuregulin 1-β1 drives cardiac enlargement in neonatal mice – a critical role for Neuregulin 1-β1 and ErbB4 in postnatal cardiomyocyte survival

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Heart failure (HF) is a leading cause of death worldwide. With a lack of effective treatments, there is rising interest in stimulation of myocardial regeneration through cardiomyocyte proliferation, with the goal of recapitulating healthy cardiac tissue. ErbB4, an epidermal growth factor receptor, and its ligand neuregulin-1-β1 (NRG-1-β1), represent a dynamic signalling cascade important in myocardial development, including cardiomyocyte proliferation and survival (Odiete, Hill and Sawyer, 2012). In this study, adeno-associated viruses (AAV, detailed below) were utilised to determine the importance of ErbB4/NRG axis in the heart. P1 neonates were anaesthetised by placing them on ice for 60 seconds and the relevant AAV administered via a temporal vein injection ($2x10^{11}$vgc/neonate, 30-gauge needle). Neonates were then returned to their mother after recovering under a warming lamp with close supervision. Mice were then be culled at P8 by decapitation. Firstly, we developed an adeno-associated virus (AAV) directing expression of NRG-1-β1 (AAV-NRG1β1) in cardiomyocytes. Neonates infected with AAV-NRG1β1 at P1 developed a significant increase in cardiac mass within 8 days (~200% of control hearts). Cardiac enlargement was coincident with increased proliferation (BrdU, injected P5 and P7, culled at P8; ~10% increase). We next evaluated the role of ErbB4 in a model of Cre-Lox recombination model, facilitated by adeno-associated virus (AAV)-mediated iCre delivery, to delete ErbB4 from cardiomyocytes in floxed neonatal mice. ErbB4 floxed ($ErbB4^{ff}$) neonates that received AAV-iCre on P1 died on P9 from a rapid development of dilated cardiomyopathy, with a significant decrease in ejection fraction evident from P6. ErbB4 cardiac knockdown (cKD) mice in failure (P8) did not exhibit changes in cardiomyocyte size, proliferation (BrdU or PH3) or endothelial cell populations. Then, we assessed the impact of upregulation of exogenous NRG-1-β1 expression in ErbB4 cKD mice (AAV-iCre-T2A-NRG1β1 in $ErbB4^{ff}$ mice). Excess NRG-1-β1 rescued cardiac survival in failing ErbB4 cKD hearts. Interestingly, rescue was associated with significant upregulation of ErbB3 expression, suggesting an alternative pathway for NRG-1-β1 signalling to facilitate rescue. Thus, this study identifies a critical role for the ErbB4/NRG axis in the immediate postnatal period, amenable to augmentation with AAV-NRG-1-β1.

Reference:

Odiete O, Hill MF, Sawyer DB. 2012. *Neuregulin in Cardiovascular Development and Disease. Circulation Research*.**111**(10):1376-85.