AAV9 and Cre: a one-two punch for a quick cardiac knockout

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Online publish-ahead-of-print 3 September 2014

This editorial refers to ‘Rapid and highly efficient inducible cardiac gene knockout in adult mice using AAV-mediated expression of Cre recombinase’ by S. Werfel et al., pp. 15–23, this issue.

Knockout mice have revolutionized our ability to conduct biomedical research and no area has benefited more than cardiovascular disease. The knockout (or genetic deletion) of (a) specific gene(s) has provided a clearer understanding of the roles a gene plays in development, normal physiology, and in responses to injury, vasomotor regulation, and excitation/contraction coupling to name a few. These advances notwithstanding, the utility of traditional knockout mice can be limited in cases where the gene deletion causes embryonic lethality, or when a tissue-specific knockout is desired to avoid having off-target effects limit the study. The application of Cre-lox technology to transgenesis has provided a powerful tool in helping investigators create tissue-specific knockouts.¹

The Cre-lox strategy requires the breeding of two separate lines of genetically manipulated mice: ‘floxed’ mice in which the gene of interest has been flanked by two loxP sites using ‘knock-in’ techniques, and another line of transgenic mice in which a tissue-specific promoter is used to drive the expression of Cre-recombinase. By crossing these two strains, mice are obtained in which tissue-specific expression of Cre results in deletion of the gene of interest due to the action of the Cre-recombinase enzyme that excises the genomic region flanked by the loxP sites. The deletion is tissue-specific because it can only occur in tissues where Cre is expressed. In a further refinement of this system, the Cre-recombinase has been made tamoxifen-inducible by adding two modified oestrogen receptor (MER) ligand-binding domains on either end of the fusion protein.² The temporal control over gene deletion provided by this MER-Cre-MER system enables investigators to overcome embryonic lethality, to study gene function during different phases of a disease process, and to conduct lineage-tracing studies.

In the current article, Werfel et al.³ employed a one-two punch with AAV9 and Cre to expedite cardiac gene inactivation in adult mice by using adenov-associated viral vectors of serotype 9 (AAV9) to deliver Cre-recombinase under the control of a cardiac-specific promoter (cTnT). Although this approach still requires the generation of ‘floxed’ mice, these mice can then be treated with a simple intravenous injection of the AAV9 vector expressing Cre-recombinase, as opposed to the typical strategy of crossing with a transgenic line expressing Cre. Using the AAV9 approach, gene knockout is virtually complete and the timing of recombination can be chosen by the investigator to meet the needs of the experimental design. One can appreciate how such temporal control could be superimposed upon animal models of diseases such as diabetes or hypertension without the need for additional (i.e. tamoxifen) treatment since even moderate doses of tamoxifen in αMHC-MerCreMer mice induce a DNA damage response, leading to cardiac dysfunction, fibrosis, and even failure at higher doses.⁴

The authors also nicely show that the human cTnT promoter provides better cardiac specificity than the alpha-myosin heavy chain (Myh6) promoter and they demonstrate proof of principle by using AAV9/MER-Cre-MER to knockout the gene encoding the serum response factor (SRF) in adult mice.

As noted by the authors, advantages of the AAV-cTnT-Cre approach when compared with conventional transgenesis include the reduction in breeding needed for conditional knockout experiments, given that at least two rounds of mating are required to cross a Cre-expressing line with homozygous floxed mice. A potential disadvantage of this approach is the low level of recombination detected in off-target tissues including the liver, lung, and spleen. As noted by the authors, off-target Cre expression in the liver can be suppressed by incorporating miR-122 sites behind the Cre cDNA to selectively destabilize Cre mRNA in the liver.⁵ Other limitations might include the moderate dose of AAV9 vector required to achieve Cre-mediated excision in 80–90% of cardiomyocytes, but this is balanced against the negative effects of tamoxifen in the hearts.

One should note that the generalizability of the AAV/Cre approach involving IV injection to other tissues depends greatly on the tissue tropism of the AAV serotype combined with the specificity of the tissue-specific promoter. The combination of the liver-tropic AAV8 capsid and the liver-specific thyroxine-binding globulin (Tbg) promoter has previously been used to specifically delete floxed Hdac3 in the liver.⁶ Similarly, one can predict that the combination of muscle-tropic AAV9 and the muscle-specific CK6 promoter⁷ would provide for the specific targeting of Cre to skeletal myofibers, particularly in ischaemic hindlimbs.⁸

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As noted by Werfel et al., the combination of AAV and Cre has already proven very powerful in the field of neuroscience, where the local delivery of AAV by stereotactic injection has been used for over a decade for optogenetic studies and for tracking neural connectivity. Thus, in the brain, local injection long ago provided the tissue specificity that in the heart can now be achieved by intravenous injection of AAV9 carrying cardiac-specific promoters. Because of this, the utility of combining AAV with Cre recombination has been more thoroughly explored in the brain than in the heart, resulting in such refinements as the reciprocal arrangement in which transgenic mice expressing cell-specific Cre mediate the recombination of floxed alleles delivered via AAV. One visually obvious example of this can be found in the Brainbow system. Another innovation in AAV-mediated recombination involves using the Cre-LoxP and Flp-FRPs systems in combination, which could facilitate the tracking of fibroblasts that have been transdifferentiated into cardiomyocytes, epicardial progenitor cells programmed to contribute to cardiac regeneration, or cardiomyocytes that have been reprogrammed to replicate. With the right combination of targeted AAV vectors expressing the appropriate regulatory factors in tandem with Cre, Flp, and other recombinases, it may one day be possible to determine the relative contributions of each strategy to promoting the regeneration of regions of myocardium that had previously been infarcted, as illustrated in Figure 1. Given that research efforts are already underway to downsize the CRISPR/Cas9 gene editing system so that it can be packaged into AAV capsids, the future indeed looks quite bright for AAV-mediated gene editing and recombination!

Acknowledgements

B.A.F. acknowledges support from NIH (R01 HL115225 and R01 HL116455) and B.H.A acknowledges support from NIH (R01 HL116455).

References


Figure 1 Stylized depiction of what fully regenerated myocardium might resemble after successful cardiac regeneration of a myocardial infarct using AAV-mediated gene therapy coupled with the expression of multiple recombinases. Each colour indicates a lineage of cells resulting from a particular regenerative strategy (fibroblast transdifferentiation, cardiomyocyte replication, etc.). The transgenic mice used for such a demonstration would need to carry multiple genes encoding fluorescent proteins flanked by the target recognition sites for each recombinase. Modified from Prasad et al.