Amputated amphibians advance regenerative medicine

Amphibians are an interesting clade for the study of regenerative medicine. Whereas most other tetrapods are unable to regenerate lost limbs, some amphibians can. Throughout their lives, urodeles (newts and salamanders) are able to regenerate the complete structure of a lost limb in a smaller but fully functioning replacement. Juvenile anurans (frogs and toads) have a similar ability during the larval stage, but adults only regenerate 'spikes' or cartilaginous rods that lack the joints and structure of the lost limb. This is the case with adult *Xenopus laevis*, the African clawed frog that has become a popular model for biomedical research.

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Because anurans show this incomplete regeneration in adulthood, they are considered an intermediate model between the complete regenerative ability of urodeles and the absence of regenerative ability in mammals. Researchers, including Rio Tsutsumi and colleagues at Kyoto University (Japan), hope that by achieving functional limb regeneration in frogs they can help bridge the gap between regenerative amphibians and non-regenerative tetrapods, such as humans. Building upon previous work that described the regeneration of amputated joints in a species of newt, Tsutsumi's team hypothesized that an adult frog might be able to regenerate a complete elbow joint under specific circumstances. The researchers amputated limbs at the elbow and studied the cartilaginous spike that grew in its place. Although they had removed the distal side of the elbow joint, frogs were able to bend and stretch the regenerated forelimb at the elbow (*Regeneration* doi:10.1002/reg2.49; published online 6 January 2016).

Using epifluorescence image capture, Tsutsumi *et al.* were able to reconstruct three-dimensional images that demonstrated the structure of this regenerated limb and the concave, socket-like morphology of the regenerated joint. They also observed in these images that the major flexor and extensor muscles (the biceps and triceps, respectively) inserted into the regenerated spike in a pattern than resembled that of the intact forelimb. Together these findings suggest that a functional elbow structure was regenerated at the elbow-end of the spike cartilage.

To explain these findings, the researchers propose that regeneration depends upon interactions between remaining tissues and the regenerating tissues. Such interactions guide the growth and integration of regenerated tissues into remaining tissues in a process that Tsutsumi *et al.* call 'reintegration'. By further studying these interactions and the process of reintegration, the scientists hope to unravel the mechanisms that allow functional regeneration in vertebrate species.

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CRISPR/CAS9 CORRECTS RETINAL DYSTROPHY IN RATS

Gene therapy is a therapeutic strategy where, as opposed to using traditional drugs, doctors manipulate specific genes in patients suffering from inherited genetic diseases, such as cystic fibrosis and severe combined immunodeficiency (SCID). Although conceptualized in the 1970s, gene therapy has had little clinical success and has suffered several setbacks as an approach to treating disease. Much of the difficulty in applying gene therapy lies in the technical difficulty of targeting and disrupting faulty genes. However, recent advances in gene editing technology, including the development of the clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 system, are providing new options and excitement for realizing the potential of gene therapy.

As a proof-of-principle study, Benjamin Bakondi and colleagues at Cedars-Sinai Medical Center (Los Angeles, CA) have now successfully applied CRISPR/Cas9 to a rat model of retinitis pigmentosa, an inherited degenerative eye disease that causes severe loss of photoreceptors leading to visual impairment and blindness (*Mol. Ther.* doi: 10.1038/mt.2015.220; published online 15 December 2015). Transgenic S334ter rats suffer from an autosomal-dominant mutation in an allele for the rhodopsin gene (*Rho*), and display similar visual phenotypes to humans suffering from retinitis pigmentosa caused by *Rho* mutations. Using CRISPR/Cas9, Bakondi *et al.* were able to disrupt the allele-specific Rho^{S334} and rescue the effects of retinitis pigmentosa.

Bakondi *et al.* injected plasmids containing targeting-guide RNA and the Cas9 enzyme directly into the eyes of S334ter rat pups at age P0, along with a fluorescent dye for histological verification of the spread of the injection. Using fluorescent confocal microscopy, they confirmed that the plasmids were successfully taken up by photoreceptors in the injected retinas. To test how well the CRISPR/Cas9 strategy functionally rescued retinal degeneration, the researchers performed histology on injected retinal tissue at different periods throughout development of the S334ter rats and into adulthood. Bakondi *et al.* found that injected retinas had significantly higher levels of photoreceptors compared with retinas in non-injected eyes. Further, they showed immunohistochemical evidence that synaptic connectivity was maintained between photoreceptors and downstream neurons. Most importantly, they found that intact eyes with the injections had enhanced visual acuity (as tested by the optokinetic reflex response) compared with eyes that were not injected, demonstrating that this CRISPR/Cas9 strategy functionally rescued the deleterious effects of the Rho^{S334} mutation. Although only a proof-of-principle study, this work provides an important advancement for developing gene therapy as a treatment option for patients suffering from genetic disorders.

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