

IRSF Informal Research Update

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A gene therapy for Rett syndrome should be focused on 1) developing a reagent to achieve global CNS gene delivery and 2) to test whether delivery of MeCP2 (or other transgenes) can be efficacious for Rett Syndrome. We've been attacking both avenues simultaneously.

Where we are now (delivery)

On the problem of efficient and global CNS delivery, we have identified AAV vectors that can globally deliver a transgene to the CNS in adult mice after intravenous injection in a dose-dependent manner. In collaboration with the Oregon National Primate Research Center, we replicated this experiment at a single dose in 4 non-human primates. The delivery to neurons was not as robust as in mice, and pre-existing neutralizing antibodies to AAV were identified as a barrier to efficient gene delivery. We view an intravascular approach as a valuable research tool and proof-of-concept, but the high vector delivery to peripheral organs and reduces efficacy in monkeys raises serious concerns about human translation. As an alternative strategy, we have identified intra-CSF injections as a way to achieve broad CNS delivery with minimal exposure of peripheral organs. The delivery efficiency to spinal cord motor neurons can mimic the efficiency of intravascular delivery, but brain delivery is broad albeit relatively inefficient in mice. Through a collaboration with Nick Boulis at Emory University, we injected pigs by the intrathecal route and saw robust cerebellum delivery at or above the levels we get with intravenous injection in mice, with global delivery to the rest of the brain at a lower efficiency.

Where we're going (delivery)

Using the existing AAV serotypes, our future directions are to optimize injection parameters into the cisterna magna, which can tolerate higher volumes, allowing higher doses in closer proximity to the brain than an intrathecal injection. We have initiated studies comparing 2 serotypes (AAV9 and AAV2.5) in non-human primates, based on our preliminary mouse data. As an alternative strategy to enhance delivery, our next future goal is to build upon our experience with AAV capsid directed evolution and shuffling to develop a modified AAV capsid tailored to achieve broad and efficient CNS transduction with an intracisternal or intravenous route.

Where we are now (MeCP2 gene replacement)

We've produced AAV vectors packaging the MeCP2 gene under a strong ubiquitous promoter (CMV/CBA), a mostly neuron-specific promoter (synapsin), an 800 bp mouse MeCP2 promoter fragment, and a truncated 229 bp MeCP2 promoter fragment. These vectors utilize a myc-epitope tag on MeCP2 to distinguish it from the endogenous protein. We've characterized the expression of these in HEK293 cells and observed the following strength of expression: CBA >> SYN > 800MeCP2 > 229MeCP2. We predict that the 229 bp MeCP2 promoter fragment will be the most suitable for a therapeutic application, and we've also produced an AAV vector using this promoter to drive GFP. The GFP vector has been injected intravenously in mice to assess expression strength and cell/tissue specificity in vivo, and we hope to have these results by the end of July.

Where we are going (MeCP2 gene replacement)

At this year's IRSF meeting, I made an offer to provide the AAV/MeCP2 reagents available to anyone interested in using them as research tools or to investigate efficacy. Several investigators indicated some interest in this regard. I am also preparing for my own experiments to deliver the CBA-MeCP2 and 229-MeCP2 vectors into a RTT model by intracranial and intravenous injection. The endpoints of these studies will be limited, but hopefully they will address the feasibility of a MeCP2 gene replacement strategy. Another potential approach is to delivery BDNF or another factor downregulated by MeCP2 (such as Serpine2 or STMN2) to improve the CNS environment, but this approach is only in a conceptual stage of development.

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