Molecular Therapy
Letter to the Editor

In vivo confusion over *in vivo* conversion

In a recent report published in *Cell* entitled "Revisiting astrocyte to neuron conversion with lineage tracing *in vivo*,"[1] the group of Dr. Chun-Li Zhang raised questions on a series of studies published by my lab and others demonstrating that astrocytes in adult mouse brains can be converted into neurons.[2–6] While the article reports a lot of data from astrocytic reporter lines to test *in vivo* astrocyte-to-neuron conversion, the design of the study suffers from a fundamental error that culminates in confusing results and, even more importantly, unfounded conclusions. To avoid further confusion in the conversion field, the following analysis should help clarify some of the issues.

In 2010, AAV toxicity in brain tissue was first reported by Douglas Coulter's group.[7] In the experiments reported by Zhang's group,[1] a high dose of 1–2 × 10^{13} GC/mL, 1–2 μL AAV was used. Quantitatively, this amounts to 10–40 billion viral particles within 1 mm^3, which is a huge amount of virus for the brain to absorb. When neurons and glial cells encounter such a massive viral dose, they activate strong immune responses to defend themselves. If a viral dose is sufficiently strong, then both neurons and glial cells may die. The remaining surviving but injured neurons and glial cells will carry a high load of virus and will express the viral transgenes irrespective of any promoter restriction, leading to all sorts of unanticipated consequences, thus rendering data interpretation nearly impossible. Such a high dose of vector will also inevitably cause leakage of transgenes expressed by the AAV into neurons. Since the entire study employed high doses of AAV, the data essentially represent the effects of injured neurons and glial cells in response to a massive viral dose.

A further confounding feature of this study is the assumption that the astrocytic lineage-tracing mice used represent a viable standard for testing in vivo conversion. In fact, the astrocytic reporter lines were created for the purpose of lineage tracing, not for conversion. The Aldh1l1-CreERT2 mice are excellent for astrocytic lineage tracing in terms of specificity. However, one must keep in mind the origin of such specificity. The technology requires tamoxifen-induced Cre-*loxP* recombination in astrocytes, so that the transgenic mice can constitutively express a foreign reporter protein, such as GFP or tdTomato, in astrocytes. One must consider what happens to the astrocytes following Cre-*loxP* recombination with regard to their epigenetics. In fact, previous studies have reported Cre toxicity during the generation of such Cre lines.[8,9] Our group has tested such an astrocytic tracing line by crossing Aldh1l1-CreERT2 mice with Ai14 mice and obtained tdTomato-labeled astrocytes after administration of tamoxifen. We reported that while NeuroD1 could convert tdTomato-labeled astrocytes into tdTomato-labeled neurons, the timeline of conversion was delayed compared with that observed in wild-type mice.[10] The delayed conversion suggests that the Cre-*loxP* recombination in the lineage-traced astrocytes may have created a higher barrier for cell conversion. Currently, my lab is investigating whether transcription factors, such as NeuroD1, are expressed in the astrocytic reporter lines at a level similar to that in wild-type mice, and we are testing the capability of a variety of viruses to convert the Cre-*loxP*-recombinated astrocytes into neurons.

In summary, when conducting *in vivo* astrocyte-to-neuron conversion experiments, one must use a dose of AAV within the range of 10^{11} to 10^{12} GC/mL at 1–2 μL in mouse brains. Higher titers, such as 10^{13} GC/mL, can be detrimental to brain tissue and should be avoided in conversion experiments. Secondly, whereas one can use astrocytic lineage-tracing mice for conversion studies, a potentially higher barrier for astrocyte conversion must be considered in the experimental design. Strong promoter and enhancers should be used to maintain a high expression level of transcription factors so that they can convert the full astrocyte transcriptome into a neuronal transcriptome. Therefore, it is premature to assume that these reporter mice represent an adequate standard for measuring *in vivo* conversion until we have a better understanding of the effects of Cre-*loxP* recombination in these astrocytes. Until then, we may simply be creating *in vivo* confusion.

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