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COMMENTARY

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Reactive glia-to-GABAergic neuron reprogramming: a "golden touch" strategy to alleviate intractable seizures

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Abstract

This commentary highlights a research article published recently in *Cell Stem Cell* "Reprogramming reactive glia into interneurons reduces chronic seizure activity in a mouse model of mesial temporal lobe epilepsy". Generally, Lentini et al. reveal a strategy to fulfill in vivo glia-to-neuron reprogramming, which is a potential disease-modifying strategy for treatment of intractable seizures. Here, we describe exciting research advances in the treatment of intractable seizures based on this research article, summarizing its key findings, emphasizing its importance and providing further discussions. Further, issues worthy of further investigations are also postulated so that clinic translation can be better achieved.

Background

Mesial temporal lobe epilepsy (mTLE) is among the most common forms of treatment-refractory human epilepsy [1]. It is often characterized by recurrence of focal dyscognitive seizures and is associated with hippocampal sclerosis which is histopathologically characterized by neuronal loss and reactive gliosis [2]. Notably, a significant loss of hippocampal GABAergic interneurons [3] that leads to the imbalance between neuronal excitation and inhibition, has long been assumed to play a critical role in epileptic seizures in various phases, including seizure initiation, propagation and termination. Thus, augment of GABAergic transmission with various methods like drugs, optogenetics, and transplantation of GABAergic progenitors, plays important roles in controlling epileptic seizures. However, pro-GABAergic drugs often show side effects due to the unspecific targets distributed in the whole brain. Meanwhile, optogenetic activation of GABAergic neurons requires the co-administration of viral vectors, genetic manipulations, as well as specific light stimulation. Additionally, cell transplantation is a highly invasive approach with ethical issues and a potential risk of tumor formation; meanwhile, it also requires long-term immune suppression. Overall, all of them have limitations in the potential for clinical translation. Thus, alternative strategies are being explored to promote self-repair either by inducing the proliferation of residual cells or through fate conversion of other resident cells to the desired cell type.

Main text

In a recent paper published in *Cell Stem Cell* [4], Lentini et al. provided an elegant strategy to convert in vivo reactive glia, which has been reported to play a pro-seizure role in epilepsy, to GABAergic induced neurons (iNs). They also proved that these GABAergic iNs can functionally and stably integrate into the epileptic circuits and produce high therapeutic efficacies in a well-established animal model of mTLE.

First, the authors confirmed that the kainite (KA)-induced chronic mTLE model has not only a significant loss of GABAergic interneurons in the hippocampus

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across all subtypes, but also massive proliferation of reactive glial cells. Pioneering in vitro studies have made considerable progress on fate conversion of cultured glia into iNs by expression of the transcription factors that direct the generation of these different neuronal subtypes during embryonic forebrain development [5]. According to their previous studies, expression of the dorsal telencephalic fate-determinant neurogenin-2 instructs cortical astroglia to become glutamatergic neurons; meanwhile, the ventral telencephalic fate-determinant achaete-schute homolog 1 (*Ascl1*) or distal-less homeobox 2 (*Dlx2*) directs a GABAergic identity. Thus, the authors selected *Ascl1* and *Dlx2* to direct reprogramming in a mouse model of chronic mTLE and found that cortical glia with forced expression of *Ascl1* and *Dlx2* were reprogrammed into hippocampal GABAergic iNs. Further, immunohistochemistry showed that the iNs separately expressed vasoactive intestinal peptide (VIP; ~40%), somatostatin (SST; ~30%), or calretinin (~30%). Interestingly, no parvalbumin-positive iNs were found. This indicates that the current reprogramming strategy, although promising, may not be sufficient to completely restore the full spectrum of endogenous interneurons. Further studies should be conducted to provide fine tuning of the reprogramming strategy to accomplish complete restoration of endogenous interneurons, which is of great therapeutic significance.

Recently, in vivo reprogramming of nerve/glial antigen 2 (NG2) glia, microglia as well as astroglia to generate GABAergic neurons have also been achieved, which makes the glia-to-neuron reprogramming a promising neuron-replacement strategy [6]. However, there still remains an important question on whether these iNs are competent enough to result in functional recovery under pathological circumstances, like epilepsy. Thus, in this study, the authors aimed to develop a strategy to convert endogenous hippocampal glia in epileptic mice in situ into functional GABAergic iNs. They found that injection of a retrovirus which encodes *Ascl1* and *Dlx2* into the hippocampus on the 5th day post KA injection efficiently induced reprogramming of hippocampal glia. About 75% of the $NEUN^+$ iNs differentiated into GABAergic neurons; some of them expressed VIP or neuropeptide Y and to a lesser extent SST. Although the fraction of GABAergic neurons generated in this way is different from that by grafted cortical glia into the hippocampus, these results strongly demonstrated that proliferating reactive glia of adult mTLE mice can be converted into GABAergic iNs in the hippocampus efficiently. According to the previous work by the authors, cortical astroglia can be reprogrammed and converted into glutamatergic and GABAergic neurons in vitro. In this study, they revealed that by forced expression of Ascl1 and Dlx2, both reactive glia in situ and cortical glia grafted into the epileptic hippocampus can be converted into GABAergic iNs. Moreover, Chen et al. reported that overexpressing NeuroD1 in cortical astrocytes after ischemic stroke mainly generated glutamatergic neurons [7]; however, in their more recent work, they found that forced overexpression of NeuroD1 in hippocampal astrocytes converted them into GABAergic neurons in an epileptic rat model [8]. These results indicate that regional differences (including extrinsic factors from the microenvironment) exert significant influence on the neuronal fate after conversion. Meanwhile, the influence of the starting population on the generation of different subtypes of neuron remains an important question to be addressed. Even if the same transcription factors were used, different neuronal identities were generated when different starting cells were targeted [6]. Thus, concerning the heterogeneity of the targeted reactive glia cells (namely NG2 glia, astroglia and microglia) in this study, it would be valuable to compare these subtypes on their converting efficiency to GABAergic iNs as well as the following in vivo functions and characteristics in epilepsy, which will facilitate more precise and more efficient conversion.

Next, the authors showed that iNs received innervations from many presynaptic granule cells (GCs) as well as projections from distant brain areas (such as the entorhinal cortex and the mammilary/supramillary bodies) by using retrograde monosynaptic tracing strategy. Meanwhile, these iNs also formed synaptic boutons on dendrites of successive GCs. Altogether, these findings indicate that iNs are both pre- and post-synaptic partners of GCs. Moreover, whole-cell patch-clamp recordings showed that iNs derived from in situ hippocampal glia or grafted astroglia were physiologically functional, and they formed GABAergic synapses with GCs, evidenced by the fact that blue light-evoked optogenetic activation of them reliably evoked inhibitory postsynaptic potentials (IPSPs) with a ~3.5 ms onset in GCs. Moreover, the recorded IPSPs were blocked by the application of gabazine, an antagonist of $GABA_A$ receptors. This provides direct evidence for the existence of monosynaptic connections between iNs and GCs, and glia-converted GABAergic iNs are functional in the hippocampus of mTLE mice. However, it remains to be determined whether the strength of synapses established by GABAergic iNs is comparable to or potentially different from endogenous synapses established under physiological conditions. Additionally, whether lineage reprogramming of glia cells would affect the local microenvironment is also worthy of studies in the future.

Finally, the authors evaluated the function of GABAergic iNs in the mTLE model and found that these GABAergic iNs significantly boosted a reduction (~50%) in

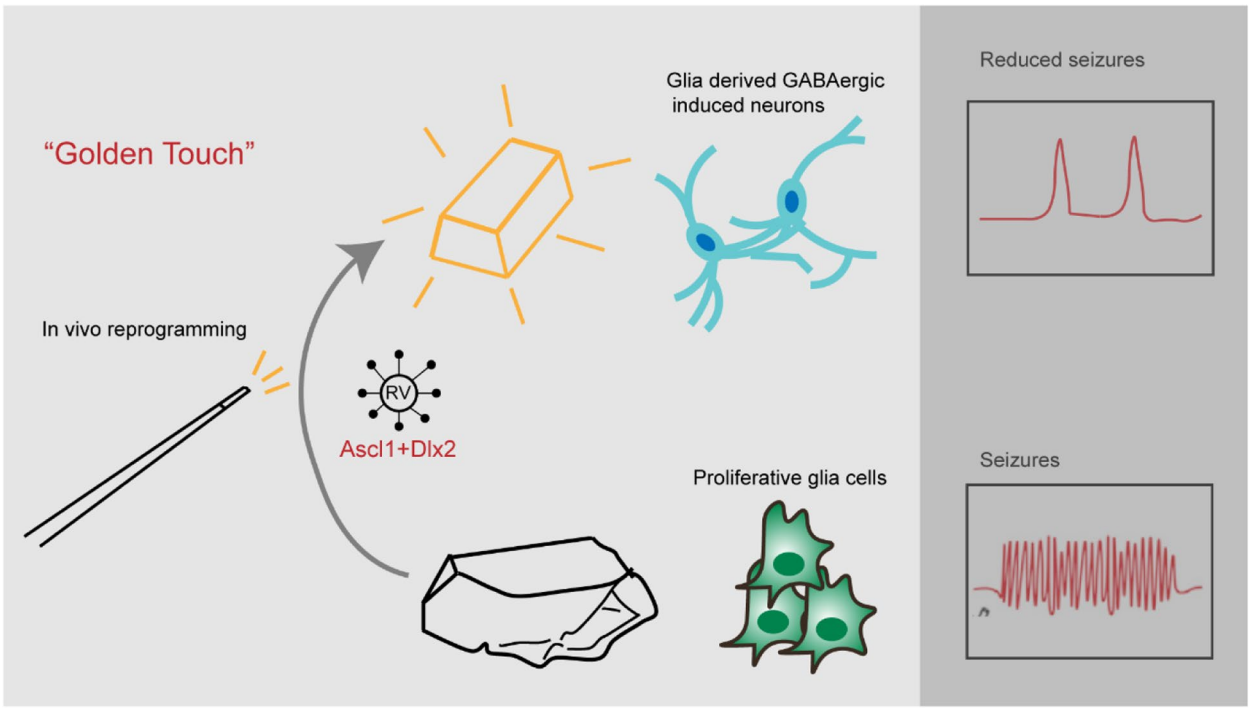

Fig. 1 Just like the story of "King Midas Touch" in ancient Greece, the in vivo reprogramming approach has an elegant power to turn "stone" ("bad" proliferative glial cells) into "gold" ("good" GABAergic induced neurons). By forced expression of *Ascl1* and *Dlx2*, reactive glia proliferating in the epileptic hippocampus can be successfully reprogrammed and converted into GABAergic iNs and exhibit anti-seizure effects in chronic epilepsy

spontaneous recurrent seizure (SRS) activity in chronic epilepsy, indicating promising anti-seizure effects. To better understand the functional characteristics of GABAergic iNs, the authors efficiently engineered astroglia in vitro alongside the reprogramming process so that their activity could be appropriately modulated with a chemogenetic approach. Clozapine-mediated excitation of the reprogrammed iNs led to a robust ~90% decrease in the frequency and cumulative duration of SRSs, demonstrating efficient anti-seizure functions of these GABAergic iNs in the mTLE model. As GABAergic neurons have divergent, age-specific, activity-dependent, and pathologically variable actions, they may bidirectionally exert both seizure-suppressing and seizure-promoting actions [9, 10]. Whether this strategy would overcome these two-sided actions in mTLE is worthy of further investigation. In addition, to facilitate clinical applications, other epilepsy models may be employed to test for a universal anti-seizure effect of the "glia-to-neuron reprogramming" strategy.

Conclusions

Taken together, the study by Lentini et al. reveals an elegant strategy to fulfill in vivo glia-to-neuron reprogramming through expression of *Ascl1* and *Dlx2* in a well-established mouse model of chronic mTLE. They show that these iNs are GABAergic and can functionally form GABAergic synapses with GCs. Further, these GABAergic iNs can significantly reduce SRS activity during the chronic phase of epilepsy, indicating a promising strategy for the treatment of intractable seizures (Fig. 1). Notwithstanding this promise, two issues may remain: (1) why the *Aslc1/Dlx2*-transduced cells differentiate into subtypes of GABAergic neurons; and whether the cellular origin (astroglia, NG2 glia, or microglia) would affect the reprogramming fate and thus anti-seizure efficacy; (2) whether lineage reprogramming of glial cells could affect the local microenvironment; and whether the strength of synapses established by iNs is comparable to or different from endogenous synapses established by GABAergic neurons under physiological conditions. Given that the dentate gyrus is closely associated with learning and memory, it would be informative to determine if the reprogramming would promote functional recovery and ameliorate cognitive impairments in mTLE. Last but not least, the clinical translation of gene therapies such as "glia-to-neuron reprogramming" is still facing several obstacles, including the invasive nature of the gene-encoding method and the irreversibility and toxicity of gene therapy vectors. Meanwhile, the long-term

therapeutic efficacy and safety in humans also need to be evaluated adequately. Nevertheless, the fast development of efficient in vivo delivery of reprogramming factors may facilitate the clinical translation of the glia-to-neuron reprogramming strategy.

Abbreviations
Ascl1: Achaete-schute homolog 1; *Dlx2*: Distal-less homeo box 2; GCs: Granule cells; iNs: Induced neurons; IPSPs: Inhibitory postsynaptic potentials; mTLE: Mesial temporal lobe epilepsy; NG2: Nerve/glial antigen 2; SST: Somatostatin; SRS: Spontaneous recurrent seizure; VIP: Vasoactive intestinal peptide.

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All authors collected references, wrote the manuscript and prepared the figure. Liying Chen substantively revised the manuscript. All authors read and approved the final manuscript.

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