Human cortical interneuron development unravelled

New understanding of principles of neurogenesis widens the use of preclinical models

by **Nicoletta Kessaris**

The cerebral cortex is the most enlarged component of the human brain. It is the seat for higher-order brain functions and the biggest information processing centre of the human brain. Interneurons, one of two major classes of neurons in the cortex, have expanded in number in proportion to cortical volume, but their diversity—the number of different subtypes—remains comparable to that of rodents. Shi *et al.* (*1*) reported conservation of the genetic networks that instruct interneuron identities between human and mouse, explaining how the same set of interneuron subtypes arise across species. On page 383 of this issue, Paredes *et al.* (*2*) find a distinctive cellular organization of the embryonic brain area where cortical interneurons are born and an extended period of neurogenesis in humans compared with rodents, explaining how interneuron numbers have increased. These studies aid our understanding of human cortical interneuron development and provide a framework for studying human disease.

Improvements in single-cell isolation techniques, large-scale gene expression technologies, and data analysis tools have enabled the characterization and comparison of individual neurons from different species. Between 40 and 60 different subtypes of interneurons have been identified in the adult mouse cortex (*3*), and homologs of these have been detected in humans, with variation in abundance, distribution, gene expression, and morphology (*4*, *5*). What is the importance of this diversity? Distinct functions have been identified for some of the most abundant populations, but the roles of all the different interneuron populations in cortical function and animal behaviour are far from understood. Progress has been hampered by our failure, until recently, to comprehend the full extent of interneuron diversity and our inability to identify different subtypes in live behaving animals. As genetic tools and technologies for detecting and imaging single neurons and their activities in intact brains improve, so will our insight into the function of interneuron diversity.

The origin of cortical interneurons in the embryonic brain has been extensively studied in mice. Two major sources have been identified in the subcortical brain: the medial and caudal ganglionic eminences (MGE and CGE) (*6*). Both are found in human embryonic brains, and immature cortical interneurons have been seen migrating out toward the developing cortex in slice cultures (*7*, *8*). How 40 to 60 different types of cortical interneurons arises from two pools of neural stem cells in the embryo remains unknown. Major regulators of gene expression have been identified in neural stem cells and immature cortical interneurons in rodents, but so far, these are insufficient to explain the complexity of the cortical interneuron population (*9*, *10*). The concept of "nature versus nurture" has been invoked: Could some diversity be imposed *after* cortical interneurons enter the cortex and settle at their final destinations?

To decipher the extent to which interneuron characteristics are determined at the source and to identify genetic pathways that generate diversity, transcriptomic analyses have been performed on single cells isolated from mouse (*11*, *12*) and human (*1*, *13*) embryonic ganglionic eminences. This involved the isolation of neuronal progenitors—neural stem cells—and young neurons from the embryonic brain and the characterization of gene expression in each cell. All four studies demonstrated that immature, but genetically discernible, cortical interneuron subtypes can be identified soon after they are generated in the ganglionic eminences and before they enter the cortex. This argues against major identity characteristics acquired de novo when these cells reach their destination. These findings also suggest that the transcriptomic signatures that interneurons exhibit upon maturation are largely defined at the source when these cells are born, through genetic programs that unfold gradually as the cells mature. Shi *et al.* also showed conservation between mouse and human gene networks that drive major developmental processes for cortical interneurons, including migration into the cortex, specialization, and acquisition of final characteristics. This provides a clear view of how the repertoire of cortical interneurons has been conserved between the two species. Major questions remain about how genetic networks and epigenetic modulators translate into distinct transcriptomic identities and how these developmental programs are influenced by external signals, either from neighbouring neurons and glia or from outside of the brain.

If genetic networks and diversity are conserved, how are interneuron numbers expanded in humans? Paredes *et al*. identified distinctive features in the cellular organization of the MGE in humans. Unlike the mouse MGE, where dividing interneuron progenitor cells reside mainly within a specific compartment of the MGE close to the central lumen, the progenitor zone in humans extends further into the parenchyma and becomes intermingled with newly born neurons (*7*). In addition, Paredes *et al*. discovered newly differentiated cells that are capable of continued cell division before generating postmitotic neurons. These findings show that not only the territory of the progenitor zone but also the

types of neurogenic cells within it and the duration of neurogenesis are expanded, thus allowing for amplification of the number of cortical interneurons that are generated in humans relative to rodents (see the figure). This increase in neuron numbers in humans is thought to underlie the highly evolved human brain (*14*); with greater numbers come greater brain size and capacity and a greater computational power that is not seen in other species. Embryonic development emerges from these and other studies as one of the most critical and, at the same time, vulnerable periods when neuronal diversity and cell numbers are set up. Genetic errors or external insults inflicted during embryogenesis are likely to cause permanent faults in brain development and later functions.

Cortical interneuron defects have been associated with childhood-onset disorders such as autism spectrum disorder and conditions of reduced interneuron function that result in epilepsy. MGE interneuron progenitors have been transplanted into the cerebral cortex of mice to replenish missing or faulty interneurons. Grafted cells were found to disperse long distances, integrate, and mature in the host brain. These experiments in mice have shown great promise for the treatment of interneuron-related deficiencies (*15*). Breakthrough studies have shown that cortical interneurons can be generated from mouse and human embryonic stem cells or induced pluripotent stem cells (iPSCs), opening up the possibility of developing personalized human therapies. Paredes *et al*. now show that human-derived embryonic MGE cells can fully mature and contribute to neural circuits when grafted into the mouse brain. Not only does this work reinforce the view that cortical interneurons are conserved between the two species but it also provides us with the exciting prospect of transplanting human progenitors or newly differentiated neurons into the rodent cortex to study human interneuron biology, interneuron disease states, and potential drug-based therapies in an *in vivo* setting.

Studies in preclinical models are essential before cortical interneuron transplantation therapies can comfortably move to the clinic. Many questions can be addressed in these models, including identifying the disorders that can be treated with transplantation and which interneuron subtypes are essential in each case. Such models can also be used to study the optimal therapeutic window for each disorder and how cortical interneurons develop, differentiate, integrate, and survive in transplantation settings. They can also help to ascertain how effective these therapies might be. Although such refinements are taking place in preclinical settings, the first-in-human clinical trial for interneuron cell therapy to treat patients with unilateral mesial temporal lobe epilepsy is already being prepared (NCT05135091), spearheading human cortical interneuron transplantation therapy.

REFERENCES AND NOTES

1. Y. Shi *et al*., *Science* 374, eab6641 (2021).
2. M. F. Paredes *et al*., *Science* 375, eabk2346 (2022).
3. B. Tasic *et al*., *Nature* 563, 72 (2018).
4. R. D. Hodge *et al*., *Nature* 573, 61 (2019).
5. F. M. Krienen *et al*., *Nature* 586, 262 (2020).
6. C. P. Wonders, S. A. Anderson, *Nat. Rev. Neurosci.* 7, 687 (2006).
7. D. V. Hansen *et al*., *Nat. Neurosci*. 16, 1576 (2013).
8. T. Ma *et al*., *Nat. Neurosci*. 16, 1588 (2013).
9. J. S. Hu, D. Vogt, M. Sandberg, J. L. Rubenstein, *Development* 144, 3867 (2017).
10. N. Kessaris, L. Magno, A. N. Rubin, M. G. Oliveira, *Curr. Opin. Neurobiol*. 26, 79 (2014).
11. C. Mayer *et al*., *Nature* 555, 457 (2018).
12. D. Mi *et al*., *Science* 360, 81 (2018).
13. Y. Yu *et al*., *Nat. Neurosci*. 24, 1745 (2021).
14. S. Herculano-Houzel, *Proc. Natl. Acad. Sci. U.S.A*. 109 (Suppl 1), 10661 (2012).
15. R. F. Hunt, S. C. Baraban, *Cold Spring Harb. Perspect. Med*. 5, a022376 (2015).

ACKNOWLEDGMENTS

I thank W. Richardson, D. Kullmann, and H. Kessaris for comments and discussion. Funding is provided by the UK Biotechnology and Biological Sciences Research Council (BB/N009061/1) and the UK Wellcome Trust (108726/Z/15/Z).

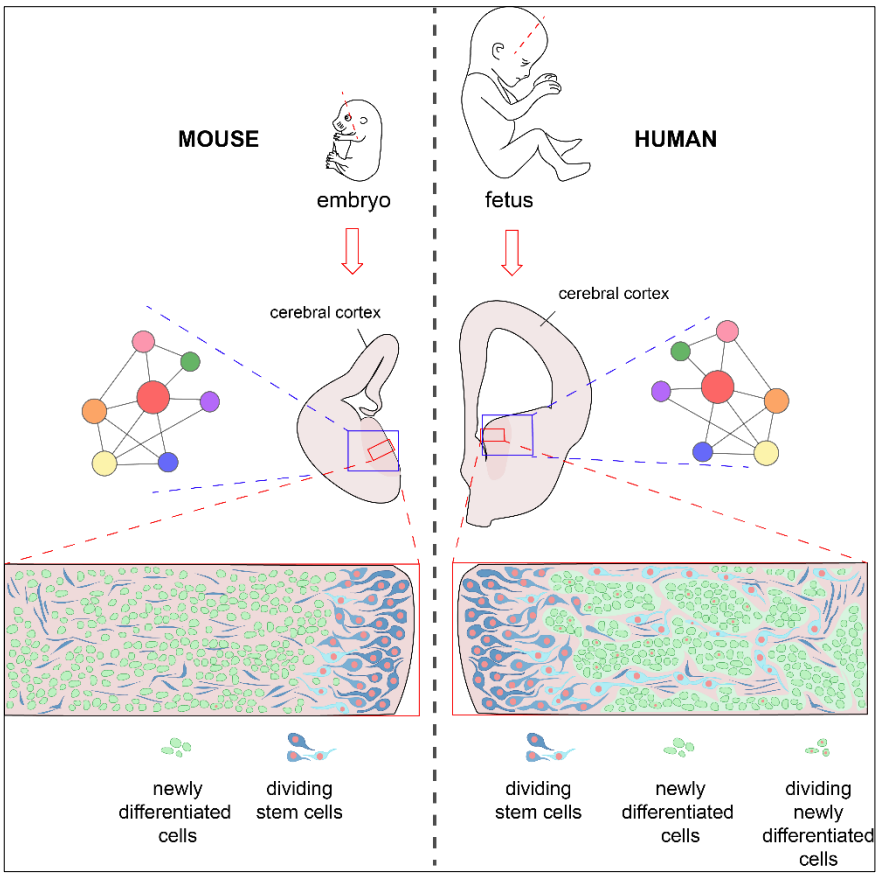

Figure: Building the human cerebral cortical interneuron repertoire

Basic developmental processes and genetic networks underlying the generation of cortical interneurons are similar between mice and humans, allowing the same subtypes of interneurons to arise but with different population sizes. Additions to the stem cell pool, with differentiated cells being able to divide, allowed the expansion of interneurons in humans.