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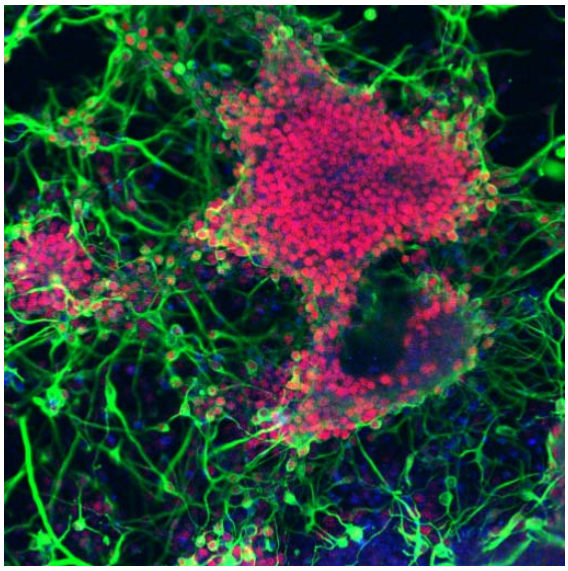
Head first: Novel method for the induction and regional specification of forebrain precursors from ES cells

February 7, 2005 – The mammalian central nervous system is notoriously non-regenerative, and the treatment of disorders in which neural function has been compromised represents one of the greatest challenges remaining to modern medicine. Much attention has been paid in recent years to the promise of cell replacement therapy as a potential means to restore damaged nervous systems to health. This method, which involves the selective culture of cells for transplantation into patients deficient in a specific cell type, represents one of the best hopes on the horizon for patients suffering from a host of currently incurable afflictions.

Embryonic stem (ES) cells may make an especial contribution to the realization of the promise of regenerative medicine, as these cells have the potential to give rise to any of the body's myriad cell types. However, while researchers in recent years have developed methods allowing them to steer the differentiation of ES cells into a variety of neuronal types, efforts to induce the selective differentiation of precursors to the embryonic forebrain (called the telencephalon) have been frustrated by the strong tendency of neurons differentiated from ES cells using extant culture protocols to assume more posterior (caudal) neuronal fates. In a breakthrough achievement published in the advanced online edition of *Nature Neuroscience*, Yoshiki Sasai (Group Director; Laboratory for Organogenesis and Neurogenesis) and colleagues at the RIKEN Center for Developmental Biology (Kobe, Japan) and three Japanese universities announce their development of a technique enabling the highly selective differentiation of telencephalic precursors from mouse ES cells.



Telencephalic precursor cells induced by SFEB culture (Bf1 shown in red)

This is the latest in a series of methodological advances for the high-efficiency differentiation of neurons from ES cells by the Sasai laboratory. In previous reports, Sasai described the application of a method based on the ability of feeder cells (called stromal cell derived inducing activity, or SDIA) used in combination with various growth factors to steer mouse and primate ES cells to differentiate into dopaminergic, sensory and enteric neurons at efficiencies of up to 90%. But these

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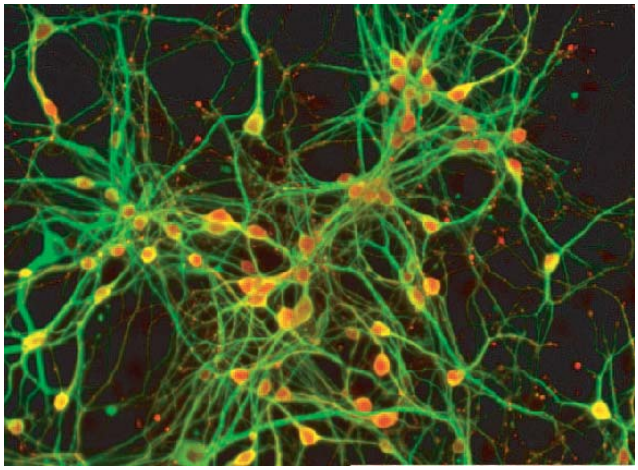
SDIA-based approaches failed to generate forebrain precursors (as distinguished by the expression of certain genes) at high frequency.

The group switched to a different tack to test the feasibility of inducing telencephalic precursor differentiation in vitro, using a culture technique that required neither feeder cells nor culture serum, a method they named SFEB (for Serum-free Floating culture of Embryoid Body-like aggregates). Embryoid bodies are agglomerates of embryonic stem cells that have been observed in traditional ES cell culture experiments. When Sasai and colleagues treated the SFEB colonies with antagonists of Wnt and Nodal, both of which are neural suppressing factors, they found that the cells showed neural differentiation at a near-perfect selectivity of about 90%. By adding an additional factor, Dkk1, to the cocktail, they were further able to steer nearly 40% of the cells down the path to a telencephalic fate (as evidenced by the expression of the Bf1 marker) – a first in the guided differentiation of ES cells.



GABAergic basal telencephalic neurons (red) induced using the SFEB method

They next sought to take the SFEB-cultured precursors one step further down the forebrain pathway by treating them with Wnt3a – a signaling factor that blocks neural differentiation early in embryogenesis, but interestingly promotes the adoption of a pallial (dorsal) fate in cells already committed to the telencephalic lineage. Their experiments bore out the hypothesis that the same would occur under SFEB culture, with significant dose-dependent increases in the population of cells expressing pallial markers following late-stage treatment with Wnt3a. Further experiments in which the SFEB aggregates were treated with Sonic hedgehog (Shh) after between 4-10 days of culture sent the cells in the opposite direction, triggering an increase in number of basal telencephalic neurons arising from the precursors.

The ability to generate the cellular forebears of the telencephalon from mouse ES cells represents a landmark in stem cell biology research. From a purely developmental perspective, the close mirroring of in vivo gene expression patterns by the externally induced effects seen in SFEB culture experiments provides new food for thought for scientists studying the genetic regulation of neurogenesis, particularly in its implications for the neural default model, which states that ectodermal cells tend to assume a neural fate in the absence of molecular messages to the contrary. And the proof-of-principle demonstration of the amenability of ES cells to forebrain differentiation and regional specification provides new avenues for biomedical researchers and clinicians to explore in the struggle to find cures for a

range of human neurological disorders, including Huntington's and Alzheimer's disease, that affect the mind's highest functions.

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