STEM CELLS

Making human microglia in a dish

Researchers have developed a protocol for differentiation of microglia-like cells from human pluripotent stem cells.

Microglia are resident macrophages of the central nervous system that carry out multiple functions, from guiding brain development to serving as sentinels that detect and engulf damaged or diseased cells. Recently, microglia have also emerged as important regulators of the pathogenesis of several neurological disorders, such as Parkinson's and Alzheimer's disease. In spite of the increasing interest in the biology of these cells, they remain enigmatic.

Growing human microglia in culture is challenging, as these cells are not easily accessible and tend to alter their defining features when removed from their natural milieu. In addition, they differ considerably from their mouse counterparts, which limits the utility of information gathered from mouse studies and highlights the need for new approaches for cultivating human microglia in a dish that would faithfully reflect their *in situ* characteristics.

To address this issue, a research group from the Whitehead Institute for Biomedical Research led by Rudolf Jaenisch has established a protocol that allows for differentiation of human induced pluripotent and embryonic stem cells into microglia-like cells. First, the team developed a cell growth medium that mimics the natural environment of human microglia—cerebrospinal fluid—and that lacks serum, which until now was considered necessary for microglia growth but also was suspected to unpredictably change their features. Then they developed a step-wise procedure based on differential cell adhesiveness to the culture dish and on responsiveness to CSF1-receptor agonists to enrich for embryoid bodies containing the microglia-specific progenitor cells. The scientists noticed that cells delaminating from small cystic embryoid bodies expressed the master myeloid transcription factor PU.1 that is necessary for microglial differentiation and maintenance. When these cells were harvested and plated on a highly adhesive surface, they switched on the expression of microglia-specific markers and were thus dubbed pluripotent-stem-cell-derived microglia-like cells, or

BIOPHYSICS

UNRAVELING MAGNETOGENETICS

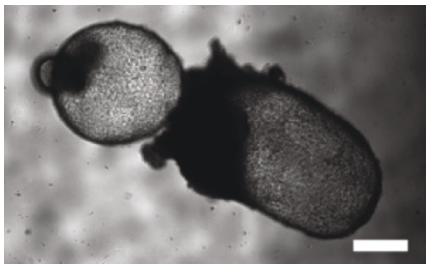
Recent reports on the magnetogenetic manipulation of neurons are being called into question.

Magnetogenetics has just emerged as a way to noninvasively activate neurons. The technology involves fusing or targeting the iron-binding protein ferritin to TRP channels in the neuronal membrane. A magnetic field is then thought either to exert a magnetic force on ferritin complexes or to heat the iron-containing complexes, both of which would open up the associated TRP channels and result in neuronal activation.

After reading a report on a molecular magnetic compass and later the reports on magnetogenetics, Markus Meister from the California Institute of Technology in Pasadena questioned the findings "because of the incredibly minuscule amount of iron involved and the relatively tiny magnetic fields they were using," he says. His intuition arose from his early work on bacterial taxis, where he also encountered magnetotactic bacteria. "That's why I had kind of an intuition for how big a compass needle needs to be in order to orient itself in the earth field," explains Meister.

According to him, the physical limitations of magnetogenetics stem from the paramagnetic nature of ferritin complexes as well as the small size of their iron-containing core. The smallest known iron particles that are permanently magnetic (i.e., ferromagnetic) contain about a million iron atoms within a diameter of 30 nanometers. In contrast, the iron in ferritin complexes is paramagnetic, because the iron core is too small to be ferromagnetic. Paramagnetic materials are only weakly magnetic and require the application of an external magnetic field to exhibit magnetism.

On the basis of physics principles, Meister assessed the plausibility of different scenarios that could potentially explain the mechanisms underlying magnetogenetics. He put forward several hypotheses for how magnetic force could gate an ion channel. For one, a magnetic field applied to paramagnetic particles such as ferritin complexes



Cystic embryoid bodies in culture. Reproduced from Muffat *et al.* (2016) with permission.

pMGLs. The pMGLs were endowed with key characteristics of resident microglia such as high motility, phagocytic ability and secretion of specific cytokines and chemokines. In addition, the gene expression profile of pMGLs closely resembled that of human fetal microglia kept in the same conditions, and it was different from the gene expression signature of distantly related cells such as peripheral macrophages. The pMGL differentiation protocol was efficient, robust and reproducible in 20 different human pluripotent stem cell lines derived from both healthy and diseased individuals.

The team then genetically tweaked pMGLs to express green fluorescent protein to facilitate their visualization, and they cocultured the fluorescent cells with differentiating neural progenitors in a 3D culture system. The cohabitation with neural cells stimulated pMGLs to differentiate into resting and yet dynamically motile microglia characterized by fast-moving tentacles used for immune surveillance. A focal laser injury of mixed pMGLs and neural cell aggregates caused a prompt reaction by the microglia, which extended their cellular processes to make contact with the damaged zone and then rapidly migrated to the injured area—behavior typical for microglia of the central nervous system.

This protocol for generating microglial cells from human embryonic stem cells and induced pluripotent stem cells derived from healthy and diseased individuals offers countless possibilities for future research into diseases, neural differentiation and immune responses in which microglia have a key role.

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Muffat, J. *et al.* Efficient derivation of microglia-like cells from human pluripotent stem cells. *Nat. Med.* http://dx.doi.org/10.1038/nm.4189 (2016).

could be pulled by a force that is proportional to the magnetic field gradient. Alternatively, multiple ferritin complexes targeted to the same ion channel could tug in different directions. Another mechanism could be a rotational force on the channel that arises from a possible asymmetric magnetizability of ferritin. Finally, the effect on the ion channel could be more indirect through stress on the membrane that could be generated cumulatively by many ferritins. According to Meister's calculations, the forces that could be achieved by these mechanisms are five to ten orders of magnitude too small to open ion channels.

Similarly, the proposed alternative mechanism of magnetically induced heat, which acts on temperature-sensitive TRP channels, is physically implausible according to Meister. Ferromagnetic nanoparticles have been shown to heat up in a size-dependent manner. However, these nanoparticles are more susceptible to heating than the paramagnetic ferritin complexes are. In addition, the size of the iron core in ferritin complexes is smaller than the particle size that is considered useful for heating applications in the nanoparticle field. Even under the most benevolent assumptions, Meister found that the achievable temperature change would be in the range of nano-Kelvins, which is not large enough to open temperature-sensitive TRP channels.

While Meister questions the current magnetogenetics approaches, further modifications or alternative approaches may emerge. But some basic experimental standards should be applied in such studies. Meister finds that the magnetosensation literature is full of reports of single, isolated responses without indication of repeated stimulation. "The literature is just filled with accidental coincidences like that," Meister cautions. Considering the stakes for people trying to repeat experiments, Meister thinks that "there is a moral obligation to go beyond chasing the spectacular story and spending more time on checking things out."

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Meister, M. Physical limits to magnetogenetics. *eLife* **5**, e17210 (2016).