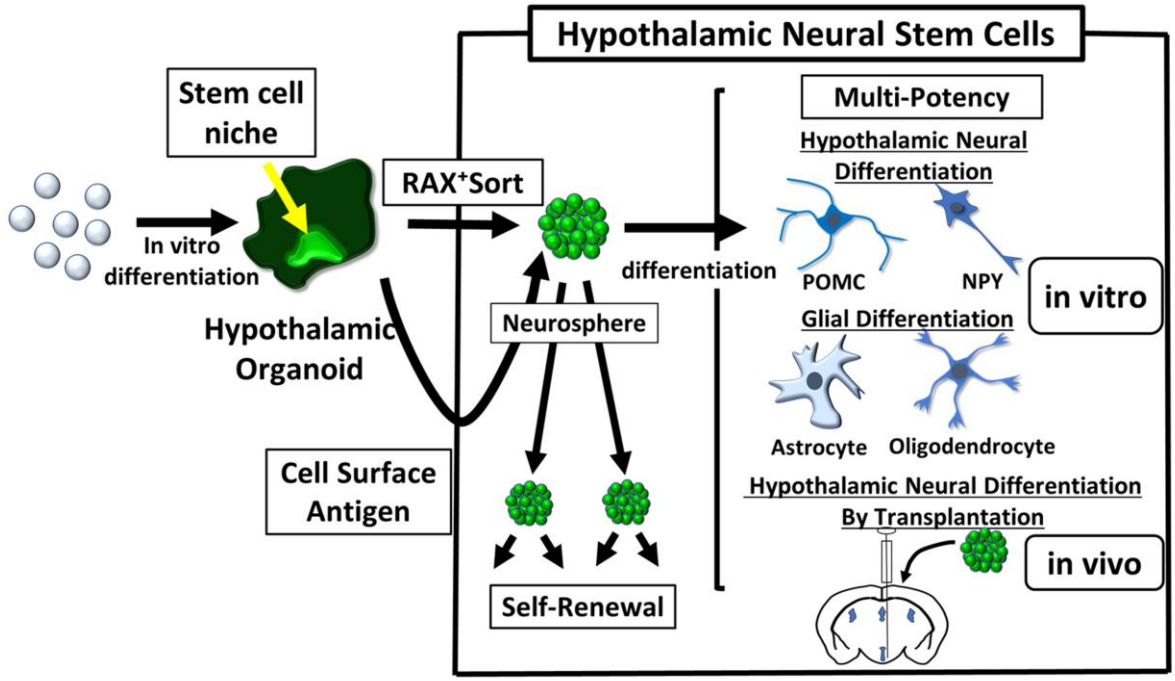

News Release

Title

Generation of hypothalamic neural stem cell-like cells in vitro from human pluripotent stem cells

Key Points

- Successful generation from human ES cells to hypothalamic neural stem cell-like cells.
- Transplantation of hypothalamic neural stem cell-like cells into immunodeficient model mice confirmed the viability and ability to differentiate into hypothalamic neurons.
- Successful generation of hypothalamic neural stem cell-like cells from wild-type human ES cells using cell surface antigens.
- Expected to contribute to regenerative medicine for patients with hypothalamic disorders and to aging research.

Summary

The hypothalamus is an essential region of the brain that maintains physiological homeostasis. Once damaged, it is currently impossible to recover its function. However, there is currently no curative treatment.

Tanycytes are radial glial cell-like ependymal cells found in the ventral hypothalamus of rodents. Recent studies have reported that tanycytes in rodents possess both self-renewal and multilineage properties, indicating that

they are hypothalamic neural stem cell-like cells. Recently, we succeeded in generating tanycyte-like cells from mouse embryonic stem cells (mESCs) and showed that these cells have hypothalamic neural stem cell-like cell properties. In this study, we induced human hypothalamic neural tissue organoids using human ESCs (hESCs), attempted to fractionate hypothalamic neural stem cell-like cells from the organoids, and examined whether these fractionated cells have tissue stem cell properties.

We focused on RAX because its expression is gradually restricted to tanycytes during the late embryonic stage. We differentiated RAX::VENUS knock-in hESCs into hypothalamic organoids and sorted RAX-positive cells from mature organoids. The isolated RAX-positive cells formed neurospheres and exhibited self-renewal and multipotency. Neurogenesis was observed when neurospheres were transplanted into the mouse hypothalamus. We isolated RAX-positive hypothalamic neural stem cell-like cells from wild-type human ES organoids. This is the first study to differentiate human hypothalamic neural stem cell-like cells from pluripotent stem cells.

Research Background

The hypothalamus is an essential region of the brain that maintains physiological homeostasis. It can be damaged by a number of factors such as brain tumors, hereditary diseases, and inflammatory diseases. Patients with hypothalamic disorders suffer lifelong symptoms of endocrine disorders, obesity, and associated lifestyle-related diseases such as diabetes. Once damaged, it is currently impossible to recover its function. Therefore, treatment for hypothalamic disorders involves only symptomatic therapy such as hormone replacement therapy, and there is currently no curative treatment.

Recently, tissue generation from pluripotent stem cells has attracted attention as a treatment for such refractory diseases. However, the transplantation of mature neuronal cells alone does not result in successful neuronal engraftment. Therefore, trial transplantation of neural progenitor cells or neural stem cells has been applied in clinical practice. In contrast, the presence of neural stem cells in the human hypothalamus has been unknown.

Tanycytes are radial glial cell-like ependymal cells found in the ventral hypothalamus of rodents. Recent studies have reported that tanycytes in rodents possess both self-renewal and multilineage properties, indicating that they are hypothalamic neural stem cell-like cells. Recently, we succeeded in generating tanycyte-like cells from mouse embryonic stem cells (mESCs) and showed that these cells have properties of hypothalamic neural stem cell-like cells.

However, there are limited reports on whether tanycytes have the same properties in humans due to the difficulty in obtaining human third ventricular periventricular cells. In this study, we induced human hypothalamic neural tissue organoids using human ESCs (hESCs), attempted to fractionate hypothalamic neural stem cell-like cells from the organoids, and examined whether these fractionated cells have tissue stem cell properties.

Research Results

We focused on the transcription factor retina and the anterior neural fold homeobox (RAX) as markers of hypothalamic neural stem cell-like cells. We used a RAX::VENUS knock-in hESC line and differentiated into hypothalamic-pituitary organoids rich in RAX-positive cells. (Figure1)

Fig1.

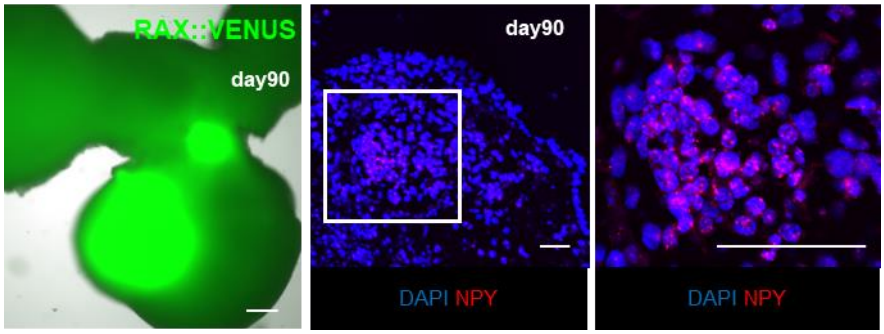

Fig1. RAX-positive cells expressed in hypothalamic tissue generated from human ES cells

Tissues that were positive in mature hypothalamic neuron marker (red) expressed RAX (green), suggesting that these cells may be the equivalent of tanycytes.

We isolated RAX::VENUS-positive cells from the hESC-derived hypothalamic tissues, and they formed cell aggregates called 'neurospheres'. The neurospheres also show self-renewal and multipotency. All these properties are observed in neural stem cells. (Figure2)

Fig2.

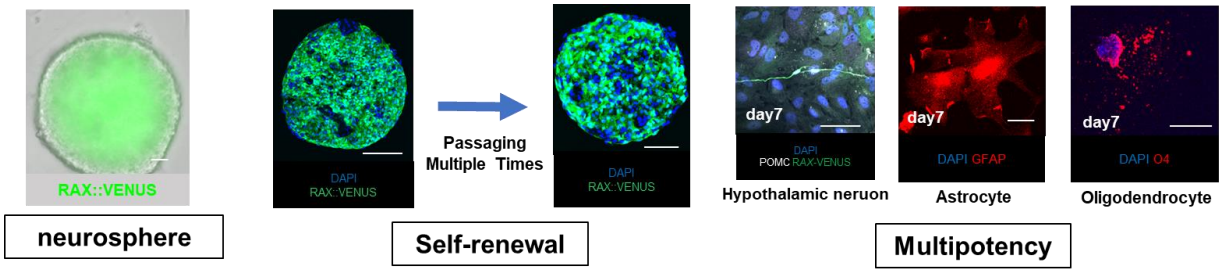

Fig2. Neurospheres have the properties of neural stem cells
The left figure shows cell aggregates called neurospheres composed of RAX-positive cells. As shown in the center figure, neurospheres can re-aggregate even after multiple passages (Self-renewal), and they can differentiate into cells of the three lineages that compose the nervous system (neurons, astrocytes, and oligodendrocytes) as shown in the right figure (multipotency).

When the NEUROSPHERES were transplanted into the ventral hypothalamus of immunodeficient mice, the neurospheres were engrafted in the mouse brain, and some cells were found to have differentiated into hypothalamic neurons (Neurogenesis). (Figure 3)

Fig3.

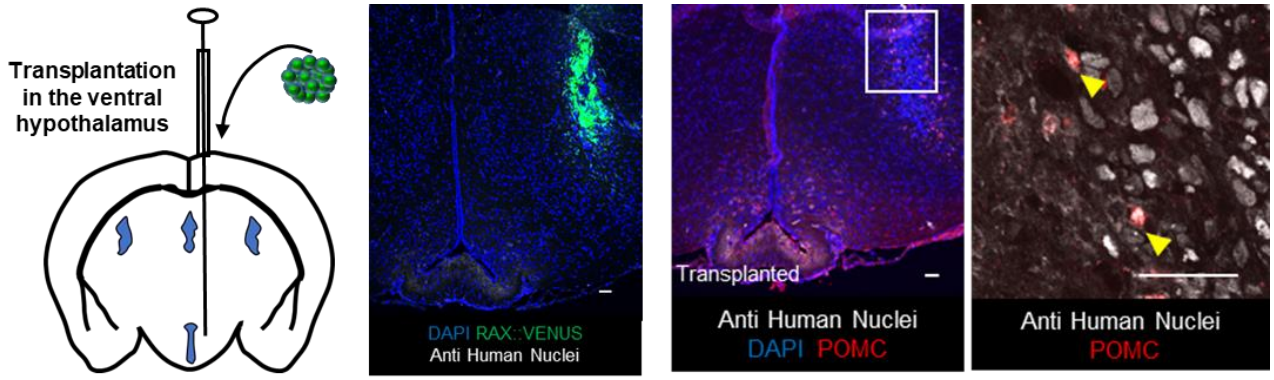

Fig3. Neurospheres are engrafted and differentiate into hypothalamic neurons in the mouse brain
The left figure shows a schematic diagram of the transplantation of neurospheres into the ventral hypothalamus of an immunodeficient mouse. As shown in the middle figure, neurospheres are still expressed one week after transplantation (green), confirming that they are engrafted. As shown in the right figure, some cells of the neurospheres express a hypothalamic neuron marker (red).

We attempted to generate hypothalamic neural stem cells from non-transgenic wild-type human ES cells. As a result, we were able to generate cells with the properties of hypothalamic neural stem cells by using cell surface antigens. (Figure 4)

Fig4.

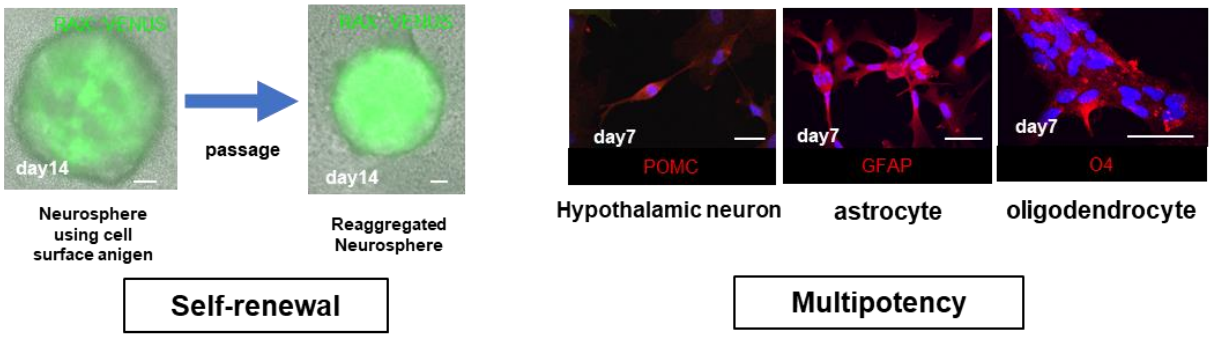

Fig4. Neurospheres sorted using cell surface antigens have the properties of neural stem cells

The left figure shows neurospheres composed of cell surface antigen-positive cells. These neurospheres can passage (Self-renewal), and they can differentiate into cells of the three lineages that compose the nervous system (neurons, astrocytes, and oligodendrocytes) as shown in the right figure (multipotency).

Research Summary and Future Perspective

We have generated hypothalamic neural stem-like cells using human ES cells.

Therefore, we expect that this technology can be applied to regenerative medicine for patients suffering from hypothalamic disorders.It has also been reported that exosomes secreted from mouse hypothalamic neural stem cells have anti-aging properties. Therefore, hypothalamic neural stem cells generated from human ES cells may also be useful for research on aging.

Publication

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Authors:

Tsutomu Miwata[1], Hidetaka Suga[1], Yohei Kawaguchi[1], Mayu Sakakibara[1], Mayuko Kano[2], Shiori Taga[1,3], Mika Soen[1], Hajime Ozaki[1], Tomoyoshi Asano[1], Hiroo Sasaki[4], Takashi Miyata[1], Yoshinori Yasuda[1], Tomoko Kobayashi[1], Mariko Sugiyama[1], Takeshi Onoue[1], Hiroshi Takagi[5], Daisuke Hagiwara[1], Shintaro Iwama[1], Hiroshi Arima[1]

Affiliations:

[1]Department of Endocrinology and Diabetes, Nagoya University Graduate School of Medicine, Nagoya, Japan.

[2]Division of Metabolism and Endocrinology, Department of Internal Medicine, St. Marianna University School of Medicine, Kanagawa, Japan.

[3]Regenerative & Cellular Medicine Kobe Center, Sumitomo Pharma Co., Ltd., Kobe, Japan.
[4]Department of Neurosurgery, Graduate School of Medicine, Nagoya University, Nagoya, Japan.
[5] Department of Gastroenterology and Metabolism, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan.

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