News Release

Title

Functional vasopressin neurons derived from human embryonic stem cells through specific induction of dorsal hypothalamic progenitors

Key Points

○Establishment of differentiation method for hypothalamic neurons, especially vasopressin neuron, from human embryonic stem cells (hESCs).

○ Specific induction of dorsal or ventral hypothalamic progenitors by recapitulating embryogenesis.

○Successful AVP secretion from the induced vasopressin neuron, suggesting that they are functional.

○Fundamental technology for the future regenerative medicine.

Summary

Graduate student Koichiro Ogawa (1st author), Lecturer Hidetaka Suga (corresponding author), Professor Hiroshi Arima and their collaborators in the Department of Endocrinology and Diabetes, Nagoya University Graduate School of Medicine (Dean: Kenji Kadomatsu), established the differentiation method of functional hypothalamic vasopressin neurons generated through specific induction of dorsal hypothalamic progenitors from human embryonic stem cells (hESCs).

The neuroendocrine center hypothalamus plays essential roles in regulating body temperature, reproductive behavior, food and water intake, and so on. Arginine-vasopressin (AVP) neurons, which maintain water balance, are differentiated from dorsal part of hypothalamus. In this study, they demonstrate efficient generation of hypothalamic neurons from hESCs by recapitulating the *in vivo* embryonic developmental processes. In aggregation culture of hESCs by optimized treatments with hedgehog and BMP signals, dorsal or ventral hypothalamic progenitors are specifically induced. After long-term culture, hypothalamic progenitors differentiated into mature hypothalamic neurons. AVP neurons appeared from the hESC-derived dorsal hypothalamic neurons. They secreted AVP and responded to KCl stimulation *in vitro*, suggesting they are functional.

In this study, they established a fundamental technology for human AVP neurons. It will contribute to future studies of disease models, embryological investigations, development and screening of novel drugs, and regenerative medicine for hypothalamic diseases.

Research Background

The neuroendocrine system, composed of the hypothalamus and pituitary gland, plays essential roles in regulating body temperature, reproductive behavior, and food and water

intake. Arginine-vasopressin (AVP) is synthesized in the magnocellular neurons of the paraventricular nucleus and the supraoptic nucleus of the hypothalamus and is released into the systemic circulation from the posterior pituitary to act on V2 receptors in the kidney to promote the reabsorption of free water.

Deficient AVP release leads to a disorder called central diabetes insipidus (CDI). Patients with CDI usually manifest polyuria as well as polydipsia, and water intake can reach 10 L/day. CDI patients are treated with desmopressin, an analogue of vasopressin. However, the frequency of hyponatremia with desmopressin is not uncommon. Furthermore, if CDI is accompanied by dysfunction of osmoreceptors, patients can become adipsic and show life-threatening dehydration. To overcome these problems, regenerative medicine is warranted. An *in vitro*-model of the human hypothalamus would help the study of its development, investigation of hypothalamic disease pathophysiology, and pharmaceutical screening for new drugs against hypothalamic diseases.

So far, several reports have shown differentiation of hypothalamic neurons from human pluripotent stem cells, although the efficiency of differentiation was very low and AVP secretion was not demonstrated. Thus, improvement in hypothalamic neuron induction is necessary to enable their use as a source for regenerative medicine or for the pathological analysis of CDI. And the key point of efficient hypothalamic neuron induction may be the recapitulation of the *in vivo* embryonic developmental processes.

Research Results

In this study, they have shown that hypothalamic precursors can be generated from human embryonic stem cells (hESCs). The concentration gradients of sonic hedgehog (SHH; secreted from the notochord and located in the ventral part of neural tube in chordates) and bone morphogenetic protein (BMP; secreted from the dorsal ectoderm) have revealed essential for the development of the neural tube in vertebrates, therefore, they analyzed the effects of these signals on hESC culture. Addition of BMP4 or smoothened agonist (SAG, which induces Shh pathway activation) to the culture medium caused differentiation of hESC-aggregates into neural retina and telencephalic progenitors, respectively. When both factors were combined, hESC-aggregates differentiated into hypothalamic precursor cells. These results suggest that SAG has the ability to shift the positional information from neural retina toward hypothalamic precursors. In addition, they optimized the concentration of BMP4 and SAG, and succeeded in specific induction of dorsal or ventral hypothalamic progenitors.

Next, they modified long time culture method, which is suitable for culturing neurons. As a result, hESC-derived dorsal hypothalamic aggregates differentiated into AVP precursor cells, and on day 150, a few AVP neurons were detected. For more efficient induction, they added Akt-inhibitor, which helps rostralization in hypothalamic development, from day 6

together with BMP4 and SAG, to promote a more rostral positional identity. As expected, the number of hESC-derived AVP^+ neurons was significantly increased. The differentiation rate of AVP neurons seemed significantly higher than that of the previous method. In addition to AVP neurons, they detected other hypothalamic neurons, such as cells positive for oxytocin, corticotropin-releasing hormone, thyrotropin-releasing hormone, melanin-concentrating hormone, orexin, neuropeptide Y, agouti-related peptide and pro-opiomelanocortin. Furthermore, hESC-derived AVP^+ cells seemed to be magnocellular neurons because CRH was not co-expressed in these cells, and large somas (about 40 x 20 μm, as seen *in vivo*) were predominant in *in vitro*-generated AVP neurons. Besides, AVP was detected in the medium of hypothalamic neurons, and the concentrations were further increased when the neurons were stimulated with potassium chloride, suggesting that they were functional.

Research Summary and Future Perspective

In this study, they optimize the culture conditions for differentiation of hypothalamic progenitors in hESC aggregates, and for specific induction of dorsal or ventral hypothalamic precursors that have the ability to differentiate into hormone-secreting neurons. In addition, hESC-derived hypothalamic neurons produced hormone reacting to stimulation. So far, no report has been made for hormone-secreting ability of human ES/iPS cell-derived hypothalamus. Thus, their present study may be beneficial for future studies of disease models, embryological investigations, development and screening of novel drugs, and regenerative medicine for CDI or other hypothalamic diseases.

Publication

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Vasopressin-secreting neurons derived from human embryonic stem cells through specific induction of dorsal hypothalamic progenitors.

Scientific Reports. February 26, 2018

DOI: 10.1038/s41598-018-22053-x

Japanese ver.

https://www.med.nagoya-u.ac.jp/medical_J/research/pdf/Scientific_R_20180227.pdf