News Release

## Title

**Functional anterior pituitary generated in self-organizing culture of human embryonic stem cells**

## Key Points

- **Establishment of differentiation method into human anterior pituitary tissues from human ESCs using 3D floating culture**
- **Formation of pituitary placode by recapitulating embryogenesis**
- **Generation of functional pituitary hormone-producing cells**
- **Therapeutic effects of transplantation to hypopituitary mice**

## Summary

Assistant Professor Hidetaka Suga (corresponding author) in the Department of Endocrinology and Diabetes (Professor: Hiroshi Arima), Nagoya University Graduate School of Medicine (Dean: Masahide Takahashi), Team Leader Takashi Tsuji, Research Associate Chikafumi Ozone (1st author), Team Leader Mototsugu Eiraku in RIKEN Center for Developmental Biology (Dean: Hiroshi Hamada) and their collaborators established the differentiation method of functional anterior pituitary generated in self-organizing culture of human embryonic stem cells (hESCs).

During early embryogenesis, the systemic hormonal center adenohypophysis, or anterior pituitary, is derived from oral ectoderm under the inductive influence from overlying ventral hypothalamic neuroepithelium. In this study, they demonstrate efficient generation of pituitary tissues from hESCs by recapitulating the *in vivo* interactions. In aggregation culture of hESCs suitable for forebrain differentiation, ventral hypothalamic differentiation and oral ectroderm differentiation are simultaneously induced by optimized treatments with hedgehog and BMP signals. In the co-presence of these two tissues, pituitary placodal tissues self-form and, after long-term culture, differentiate into pituitary hormone-producing cells such as corticotrophs and somatotrophs. The hESC-derived corticotrophs secrete ACTH in response to CRH *in vitro*. When grafted to pituitary-resected mice, these cells can rescue activity and survival as well as hormone levels in the hosts. Thus, recapitulation of tissue interactions in self-organizing culture provides a useful methodology for functional pituitary generation towards future replacement therapy.

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## Research Background

The adenohypophysis, or anterior pituitary, is a key endocrine center of systemic hormones. In response to releasing hormones from the hypothalamus, the anterior pituitary secretes important hormones for basal life support, growth, homeostasis and reproduction,

such as adenocorticotrophin (ACTH), growth hormone (GH), prolactin (PRL), thyroid stimulating hormone (TSH) and gonadotrophins (LH/FSH). Malfunction of pituitary causes various serious systemic problems. For instance, ACTH insufficiency causes severe adrenal hypofunction, which could be life-threatening. In addition to insufficiency of individual hormones, there are cases for pan-hypopituitarism, in which the production of all pituitary hormones is impaired.

During early embryogenesis, the pituitary primordium, or pituitary placode, arises as a thickened epithelium of the dorsal oral ectoderm. Previous studies have indicated that the pituitary placode specification is promoted by inductive signals from the overlying ventral hypothalamic neuroepithelium (NE), while the molecular nature of these signals remains elusive. In their previous study, they have shown that pituitary placodal tissues are generated from mouse ES cells (ESCs) in three-dimensional (3D) culture called the SFEBq (serum-free floating culture of embryoid body-like aggregates with quick reaggregation) method. In this culture, when hypothalamic NE is co-induced with non-neural ectoderm within the same ESC aggregates in SFEBq culture by high density cell plating and with optimized medium, pituitary placodal tissues self-form from non-neural ectoderm via local interactions with hypothalamic NE. They can produce functional corticotrophs that secrete ACTH in response to corticotropin-releasing hormone (CRH) both *in vitro* and *in vivo*. Furthermore, transplantation of mouse ESC-derived pituitary tissues can rescue the survival of hypophysectomized mice, which otherwise would die in a few weeks.

These results raise the possibility that ES/induced pluripotent stem (iPS) cell-derived pituitary tissues may be applicable to therapies of hypopituitarism. As a step towards this goal, it is necessary to generate pituitary tissues from human pluripotent stem cells.

## Research Results

In this study, they have shown that pituitary precursors can be generated from hESCs using 3D culture. They co-induced hypothalamic and non-neural ectodermal tissues in one hESC aggregate. For non-neural ectodermal induction, timed treatment with BMP4 was effective, and pituitary placode tissues are generated from the non-neural ectoderm presumably under the influence of adjacent hypothalamic tissues, as seen *in vivo*.

One intriguing difference between mESC and hESC cultures for pituitary differentiation is the formation of Rathke's pouch-like vesicles. In their previous study using mESC culture, pituitary placode tissues efficiently invaginated to form pouches (a few to several vesicles per aggregate) and generated hormone-producing cells, while the rest of the non-neural ectoderm stayed on the surface and formed thin epithelium. In hESC culture, although some portion of non-neural ectoderm formed $Lhx3^+$ pouches, especially in the presence of Fgf2, a major portion did not invaginate and remained on the surface. Nevertheless, in long-term culture, these tissues became $Lhx3^+$ pituitary placode tissues on the surface and also generated hormone-producing cells in hESC culture. Thus, the morphological formation of pouch structures does not matter in the terminal specification into hormonal cells. They infer

that early differentiation into pituitary placodal cells may not occur all at once in hESC culture but rather as a gradual process, unlike mESC culture.

In long-term culture, $ACTH^+$ and $GH^+$ cells were detected by immunostaining. By day 90, functional corticotrophs that released ACTH in response to CRH were generated. It is important to see that this release was suppressed by a negative feedback mechanism by the downstream hormone glucocorticoid. These findings show that their culture produced human corticotrophs with proper responses to upstream and downstream signals. In contrast, GH release by GHRH was not efficiently seen in this culture. GH release was substantially increased with hESC-derived pituitary tissues were cultured in the presence of glucocorticoid, which has been shown to promote somatotroph differentiation. Since GH-like immunoreactivity was seen even in culture without glucocorticoid, it is likely that glucocorticoid has a positive effect on maturation of somatotrophs such that they can respond to GRF and release GH.

Finally, they examined the in vivo effects of hESC-derived pituitary tissue by transplantation into hypophysectomized mice. Their present study clearly demonstrated that hESC-derived pituitary tissues in our culture had biological (or therapeutic) impacts on the hypopituitarism phenotypes in addition to the elevation of hormones. The daily activities of the host mice were recovered and their survival was clearly elongated. In this study, hESC-derived pituitary tissues were ectopically grafted into the subcapsular spaces of the mouse kidneys. Although CRH, secreted from the hypothalamus, should be dilute in the peripheral site, hESC-derived pituitary tissues rescued survival and activities, suggesting that the basal secretion from these tissues was sufficient to have those effects. In the future, for understanding the detailed functionality controlled by endogenous hypothalamic releasing hormones, it is necessary to examine the orthotopic graft of the hESC-derived pituitary tissues into the sella turcica using transplantation into larger animals such as primates. Although this should be technically demanding, it is important to develop these techniques, including the reconstitution of local blood supply, for stepping forward to clinical applications of the stem cell-derived pituitary tissues.

## Research Summary and Future Perspective

In this study, they optimize the co-induction conditions for non-neural ectoderm and hypothalamic NE in human ESC (hESC) aggregates, and the formation of pituitary placodal tissues that have the ability to differentiate into hormone-producing cells. Another important step is to show the functionality of hESC-derived pituitary tissues. So far, no report has been made for therapeutic ability of human ES/iPS cell-derived pituitary tissues in animal transplantation. They therefore test the in vivo functionality of hESC-derived pituitary tissues by focusing on their effects on animal survival. Thus, their present study investigates the feasibility of the human pluripotent stem cells-based approach at these two critical technical milestones towards replacement therapy for impaired endocrine tissues *in vivo*.

**Publication**

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