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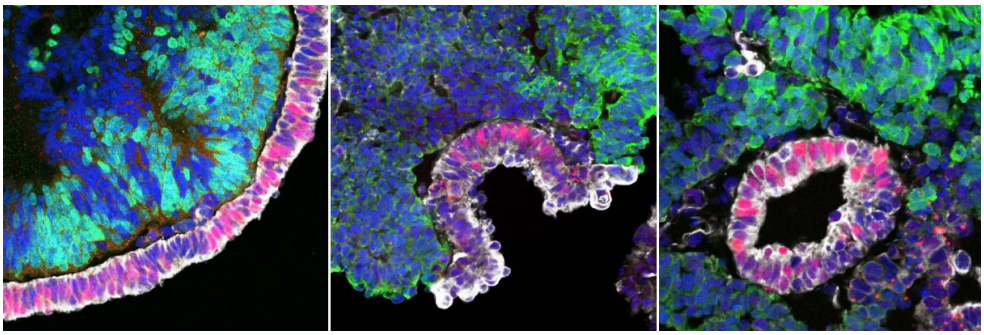
Functional pituitary tissue generated from human ESCs

February 26, 2016– The pituitary, located at the base of the brain, is the central regulator of the endocrine system, controlling hormone secretions within the body. It secretes different hormones in response to signals from the hypothalamus, and also controls hormone secretion levels in response to feedback signals from the body. Disruption of hormone production in the pituitary can lead to problems with blood pressure, electrolyte balance, growth, and fertility, highlighting the importance of this tissue in maintaining homeostasis. While hormone replacement therapies are currently used to treat some of these conditions, they need to be administered throughout the patient's life and cannot emulate the precise regulatory control of the natural endocrine system, which responds to the ever changing needs of the body. Thus, a method to produce pituitary tissue that is capable of responding to regulatory signals would be a step forward developing an effective therapy for pituitary diseases.

Now, a research collaboration between research associate Chifumi Ozone of the Laboratory for Organ Regeneration (Takashi Tsuji, Team Leader), associate professor Hidetaka Suga of Nagoya University and others has culminated in the successful generation of anterior pituitary tissue from human embryonic stem cells (ESCs). Furthermore, they were able to steer the induction of various functional hormone-producing cells of the anterior pituitary. Their achievements were published in the online journal, *Nature Communications*.



Co-induction of ventral neural epithelium (green) and non-neural ectoderm (white) from hESCs. A Rathke's pouch-like structure (LHX3+, red) began to form between culture days 26 (left) to 27 (middle and right).

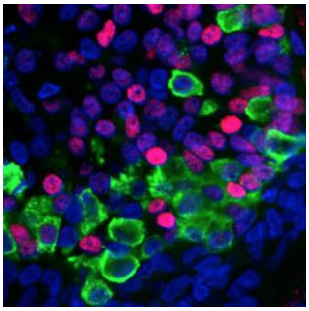
The current work builds on previous work by Suga and former group director Yoshiki Sasai (dec.) of the Laboratory for Organogenesis and Neurogenesis, in which the generated self-organizing pituitary tissue in vitro from mouse ESCs, using a 3D floating cell culture method called SFEBq which was developed by the same lab (*Science News: Nov. 14, 2011).

The pituitary primordium, also known as Rathke's pouch, emerges from the oral ectoderm upon receiving inductive signals from the neighboring hypothalamic neural epithelia (NE). The team began by determining the optimal conditions for inducing both hypothalamic NE and oral ectoderm in the same culture. After many attempts, they discovered that the addition of a hedgehog pathway agonist and BMP4 to cultured hESC-aggregates led to the efficient induction of oral ectoderm-like tissue around hypothalamic NE. After three weeks, they noticed a thickening of some sections of the oral ectoderm that expressed an early pituitary progenitor marker, LHX3, which eventually invaginated to form a Rathke's pouch-like structure, and the addition of FGF, important in early pituitary formation, produced Rathke's pouch-like structure at higher frequencies.

The group then examined whether their derived pituitary primordium could differentiate into mature pituitary hormone-producing cells. By culture day 67 to 70, they identified differentiation of adrenocorticotropic hormone (ACTH)-producing cells (corticotrophs) through immunostaining analyses and the observation of secretory granules in the cell cytoplasm. They also found that, consistent with reports from past studies, treating their derived pituitary tissue with glucocorticoids induced differentiation of somatotrophs, which produce growth hormone (GH), and at much lower frequencies, of cells producing prolactin (PRL) and thyroid stimulating hormone (TSH). When a Notch signaling inhibitor was added, they saw induction of gonadotrophs. Thus, their results show hESC-

derived pituitary primordium could differentiate into different hormone-producing cells of the anterior pituitary.

They also examined the regulatory responses of their derived corticotrophs and found that, similar to in vivo responses, ACTH release was stimulated with corticotropin-releasing hormones (CRH) whereas glucocorticoids inhibited ACTH release. Likewise, they found their hESC-derived somatotrophs responded similarly to natural ones in vivo.



Corticotrophs differentiated from hESCs (green, ACTH-positive)

Finally, they analyzed whether their hESC-derived pituitary could function in vivo by transplanting their pituitary tissue into the subrenal capsule of a hypopituitary mouse model. Extraction of the pituitary in mouse is lethal, causing death within a matter of weeks due to glucocorticoid deficiency arising from lack of ACTH. Ten days after transplantation, they observed engraftment of corticotrophs as well as a rise in ACTH levels stimulating glucocorticoid release. Comparisons between mice that received transplants and those that did not, revealed improvements to some hypopituitary symptoms, such as activity levels, which had dropped due to glucocorticoid deficiency, weight stability and length of survival. Three to four months after transplantation, the tissue graft showed signs of a vascular system and retained its hormone-secreting function.

“While there have been previous reports of pituitary induction from hESCs, our study differs in that we have recapitulated the in vivo pituitary development in vitro, and are the first to demonstrate that our derived pituitary tissue can respond to regulatory signals, and show some therapeutic effects when transplanted into hypopituitary mouse model,” says Suga. “Our work provides a platform for understanding pathogenesis of pituitary diseases and for developing future therapeutic applications.”

*Science news: Nov. 14, 2011
http://www.cdb.riken.jp/eng/04_news/articles/11/111114_pituitarytissue.html