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Research Report

Hes1 and *Hes5* are required for differentiation of pituicytes and formation of the neurohypophysis in pituitary development

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ABSTRACT

The pituitary gland is a critical endocrine organ regulating diverse physiological functions, including homeostasis, metabolism, reproduction, and growth. It is composed of two distinct entities: the adenohypophysis, including the anterior and intermediate lobes, and the neurohypophysis known as the posterior lobe. The neurohypophysis is composed of pituicytes (glial cells) and axons projected from hypothalamic neurons. The adenohypophysis derives from Rathke's pouch, whereas the neurohypophysis derives from the infundibulum, an evagination of the ventral diencephalon. Molecular mechanisms of adenohypophysis development are much better understood, but little is known about mechanisms that regulate neurohypophysis development. *Hes* genes, known as Notch effectors, play a crucial role in specifying cellular fates during the development of various tissues and organs. Here, we report that the ventral diencephalon fails to evaginate resulting in complete loss of the posterior pituitary lobe in *Hes1*$^{-/-}$; *Hes5*$^{+/-}$ mutant embryos. In these mutant mice, progenitor cells are differentiated into neurons at the expense of pituicytes in the ventral diencephalon. In the developing neurohypophysis, the proliferative zone is located at the base of the infundibulum. Thus, *Hes1* and *Hes5* modulate not only maintenance of progenitor cells but also pituicyte *versus* neuron fate specification during neurohypophysis development.

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1. Introduction

The pituitary gland is a critical endocrine organ, which is essential for homeostasis, metabolism, reproduction, and growth (Davis et al., 2013; Hojo et al., 2008; Kita et al., 2007; Zhu et al., 2005; Zhu and Rosenfeld, 2004). It is composed of two distinct entities: the adenohypophysis and the neurohypophysis. The adenohypophysis includes the anterior and intermediate lobes, and the neurohypophysis constitutes the posterior lobe. The anterior lobe contains five hormone-producing cell types: corticotropes secreting adrenocorticotrophic hormone (ACTH), thyrotropes secreting thyroid-stimulating hormone (TSH), gonadotropes secreting luteinizing hormone (LH) and follicle-stimulating hormone (FSH), somatotropes secreting growth hormone (GH) and lactotropes secreting prolactin (PRL), and the intermediate lobe contains one hormone-producing cell type: melanotropes secreting alpha melanocyte-stimulating hormone (α-MSH). By contrast, the neurohypophysis is composed of pituicytes and axons projected from hypothalamic neurons that release arginine vasopressin and oxytocin. Unlike other pituitary hormonal cells, pituicytes are defined as glial cells.

During embryonic development, the pituitary gland originates from two separate germinal tissues. The adenohypophysis derives from Rathke's pouch, whereas the neurohypophysis derives from the infundibulum, an evagination of the ventral diencephalon (Hojo et al., 2008; Kaufmann, 1992; Kita et al., 2007). The oral ectoderm thickens and invaginates to form Rathke's pouch at embryonic day 8.5 (E8.5) in mice. The dorsal portion of Rathke's pouch directly contacts the ventral diencephalon, which evaginates at E10 to form the infundibulum. Rathke's pouch separates from the oral ectoderm and further develops and differentiates. It had been believed that the stratified appearance of endocrine cells in the anterior lobe results from an ordered specification of cell types by interacting gradients of bone morphogenetic protein (BMP) and fibroblast growth factor (FGF) signaling. Recently, Davis et al. (2010) have proposed a new model for pituitary cell specification that focuses on intrinsic factors including the BMP, WNT, and Notch signaling pathways. These factors are necessary for cell specification between E11.5 and E13.5, and cell–cell communication likely plays an important role in regulating this process (Davis et al., 2010, 2013).

Molecular mechanisms of adenohypophysis development are much better understood, but little is known about mechanisms that regulate neurohypophysis development (Davis et al., 2013; Hojo et al., 2008; Zhu et al., 2005; Zhu and Rosenfeld, 2004). Previous studies have reported that deletion of the homeobox gene *Nkx2.1* leads to loss of the infundibulum during pituitary development (Kimura et al., 1996; Takuma et al., 1998). Deletion of the homeobox gene *Lhx2* also results in loss of the infundibulum (Zhao et al., 2010). In *SOX3*-deficient embryos, the evagination of the infundibulum is less pronounced (Rizzoti et al., 2004). However, mechanisms of differentiation of pituicytes have not been examined at all in these studies.

In many organs, cell proliferation and differentiation are antagonistically regulated by multiple basic helix–loop–helix (bHLH) genes (Kageyama et al., 2005; Ohsawa and Kageyama, 2008). The repressor-type bHLH genes include *Hes* genes, homologs of *Drosophila hairy and Enhancer of split* [*E(spl)*]. Notch is a transmembrane protein and activated by its ligands such as Delta. Although Notch signaling is not the sole regulator of Hes factors, Hes1 and Hes5 are essential effectors for Notch signaling. *Hes* genes regulate the maintenance of stem cells and progenitors, and control the normal timing of cell differentiation. In addition, *Hes* genes regulate binary cell fate decision in many tissues and organs: *Hes* genes promote astrocyte *versus* neuron, enterocyte *versus* non-enterocyte, billiary cell *versus* hepatocytic cell, exocrine cell *versus* endocrine cell, and T cell *versus* B cell fate decisions (Kageyama et al., 2007, 2008a, 2008b, 2009).

The analysis of targeted mouse mutants has demonstrated roles of the Notch signaling pathway in adenohypophysis development (Davis et al., 2010; Himes and Raetzman, 2009; Hojo et al., 2008; Monahan et al., 2009; Nantie et al., 2014; Raetzman et al., 2004, 2007; Zhu and Rosenfeld, 2004; Zhu et al., 2007). We have reported that in conditional *Hes1*; *Hes5* double-mutant mice, the pituitary gland is severely hypoplastic and dysmorphic (Kita et al., 2007). In the absence of *Hes1* and *Hes5*, cell differentiation is accelerated and progenitors are prematurely differentiated in the developing pituitary gland. Although we have also reported that the neurohypophysis is lost in conditional *Hes1*; *Hes5* double-mutant mice, the molecular mechanism of this phenotype was not investigated at all. *Hes1* also regulates migration of hypothalamic neurons and axonal projection to the pituitary gland (Aujla et al., 2011). Here, we report that *Hes1* and *Hes5* modulate the differentiation of pituicytes, and are essential for formation of the neurohypophysis during mouse pituitary development.

2. Results

2.1. *Hes1 is compensated by Hes5 in the developing ventral diencephalon*

We have previously reported the expression pattern of *Hes1* in mice during pituitary development (Kita et al., 2007). Here, we examined the expression pattern of *Hes1* in the developing ventral diencephalon and neurohypophysis. At E10.5, *Hes1* is strongly expressed in the ventral diencephalon (Kita et al., 2007). At E12.5, *Hes1* expression was diminished in the evagination of the ventral diencephalon (the infundibulum) (Fig. 1A). In this region, *Hes1* expression was restricted in the periluminal side of the base of the infundibulum (Fig. 1A, arrowhead). At E14.5, *Hes1* expression is further downregulated in the ventral diencephalon (Kita et al., 2007). These findings suggest that *Hes1* may control the evagination of the ventral diencephalon and the development of the neurohypophysis.

Since we have reported that *Hes5* compensates *Hes1* during the development of various organs (Hatakeyama et al., 2004; Kita et al., 2007; Kitagawa et al., 2013), we next examined *Hes5* expression in the developing ventral diencephalon. In wild-type mice, *Hes5* was not detected in the ventral diencephalon (Fig. 1B). However, *Hes5* was upregulated in the ventral diencephalon of *Hes1*-null mice (Fig. 1C),