Pituitary stem cells: what do we know?

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ABSTRACT

The pituitary gland is defined as a highly "plastic" gland, capable of adapting to the changing needs of the body over the course of a lifetime. In 1969, the first putative pituitary stem cells were isolated, defined as chromophobe cells because of their inability to secrete/incorporate hormones. Since then, studies have improved and have confirmed the presumed existence of a side population characterized by SOX2 expression in both the anterior and marginal pituitary lobes. From the numerous studies conducted to unravel the biological significance of these stem populations, it is currently believed that these cells, at least in the adult basal gland, are highly quiescent; however, their functions are still not well understood. This review reports the major advances achieved in recent years towards developing protocols for the isolation of pituitary stem cells from healthy tissue and pituitary adenomas, as well as the first studies on their use in regenerative medicine.

KEYWORDS

Stem cells, pituitary gland, stem cell therapy, regenerative medicine, regeneration, stemness potential, endocrine regeneration, endocrine transplantation, pituitary stem cells.

Introduction

The pituitary gland, or hypophysis, plays a central role in the endocrine system. It receives signals from the hypothalamus and, through hormones, regulates the other endocrine organs (Fig.1). In this way, hormone secretion is specifically regulated in each target gland based on very refined feedback mechanisms between the hypothalamus and the target glands themselves. Due to this activity, the pituitary gland is defined as a highly "plastic" gland, capable of adapting to the changing needs of the body throughout life.

Anatomically, the pituitary gland is divided into two parts: the anterior part, also known as the adenohypophysis, and the posterior part, also called the neurohypophysis. Both, due to the presence of different hormone-producing cell types, are responsible for the secretion of several types of hormones. The anterior part is composed of five hormone-producing cell types (somatotropes, thyrotropes, lactotropes, corticotropes, and gonadotropes), each responsible for the production of specific hormones. Somatotropes secrete growth hormone (GH), generally involved in the regeneration of bones and organs; lactotropes, which secrete prolactin (PRL), are essential during pregnancy and lactation; gonadotropes, which produce follicle stimulating hormone (FSH) and luteinizing hormone (LH), play a key role in fertility and reproduction; corticotropes, which secrete adrenocorticotropic hormone (ACTH), are essential for stress and immune responses. Finally, thyrotropes produce thyroid-stimulating hormone (TSH), which is necessary for correct metabolic control. Along with hormone cells, the anterior lobe is also composed of non-hormone-secreting cell types, such as endothelial cells, immune cells, and folliculostellate (FS) cells [1]. The posterior lobe contains axons of neurons, the cell bodies of which are located in the hypothalamus and are responsible for the secretion of arginine vasopressin (AVP) and oxytocin [2].

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Hypopituitarism is caused by the partial or complete loss of one or more pituitary hormones secreted from the anterior or the posterior pituitary gland [3]. It has an estimated incidence of 4.2 per 100000 per year and a prevalence of 45.5 per 100000 [4]. The etiology of hypopituitarism can be divided into congenital and acquired causes. Congenital hypopituitarism may be associated with structural pituitary abnormalities or other midline defects, as in septo-optic dysplasia (SOD), a disorder due to malformation of the anterior portion of the brain occurring towards the end of the first month of gestation and consisting of deficient development of the optic nerves, defects in midline brain structures, pituitary hormone deficiency [5], and craniofacial defects. Acquired hypopituitarism can result from damage to the pituitary gland associated with the presence of a tumor mass, infection, autoimmune disease, infiltrative disease, chemotherapy, radiation exposure, or some kind of trauma.

Sheehan's syndrome, for example, develops as a result of ischemic pituitary necrosis due to severe postpartum hemorrhage

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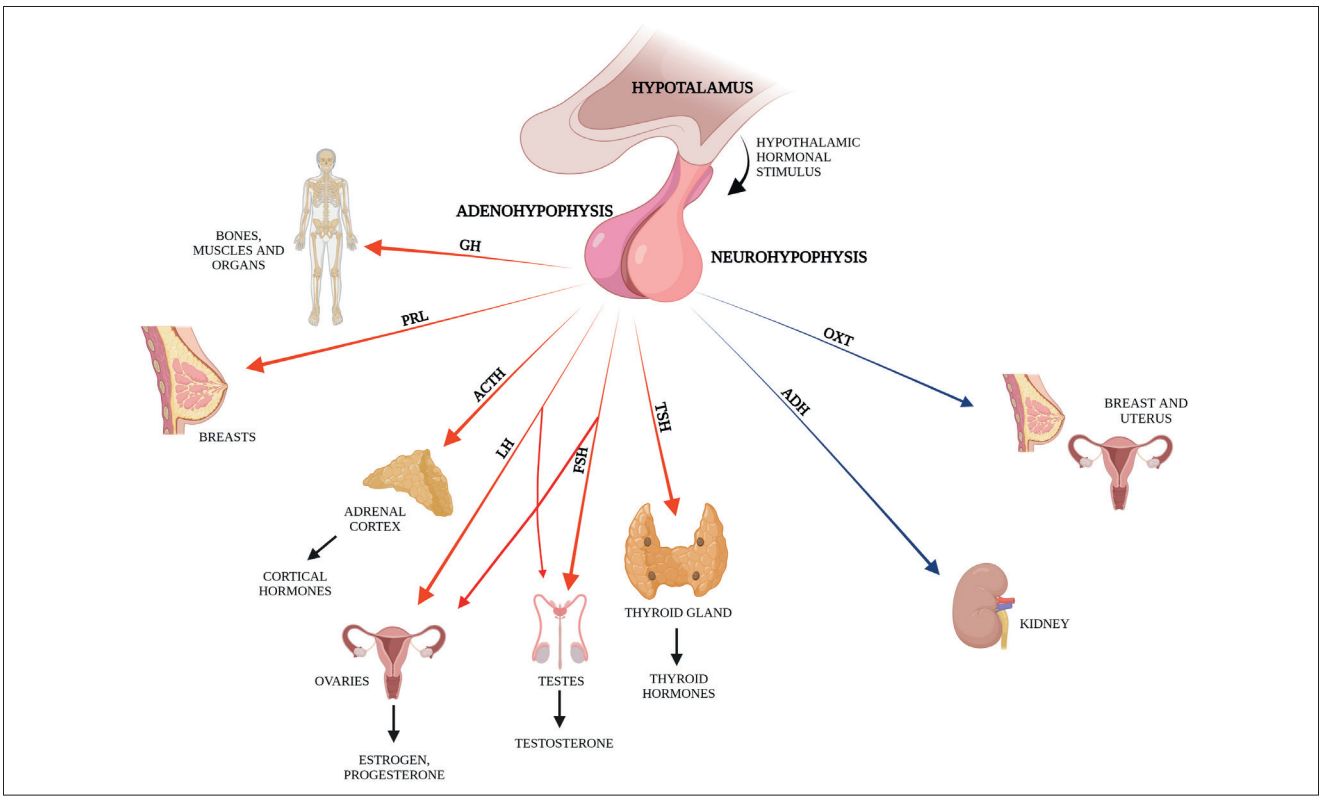
Figure 1 Hypothalamic-pituitary axis. Figure created in BioRender.com.



and is characterized by various degrees of hypopituitarism [6]. The clinical presentation of hypopituitarism varies considerably depending on the number and severity of hormone deficiencies. The existence of pituitary stem cells was only theorized for many years, until SOX2+ side population (SP) cells were isolated [7,8]. The isolation of this cellular subpopulation strengthened the hypothesis of the existence of a stem cell population within the adult gland, which is an important first step to understanding not only the biology of the hypophysis, but also whether these cells might provide a future therapeutic strategy against pituitary disorders.

In this review, we briefly analyze studies carried out to assess the potential role of pituitary stem cells in regenerative medicine, and look at how researchers have tried to isolate them over the years.

Embryogenesis, plasticity, and stemness potential

The pituitary gland has two different embryological origins: thickening of the rostral ectoderm, called the pituitary placode, initiates the formation of Rathke's pouch, followed by the formation of the anterior and the intermediate pituitary lobes; the posterior lobe, on the other hand, arises from the neuroectoderm. Transcription factors, such as Hesx1, Pitx1/Pitx2, and others [9,10], allow modulation of the differentiation potential of the various pituitary hormone-producing cells within the anterior lobe [9,10]. Nowadays, what we know about pituitary embryogenesis is thanks to studies performed mainly in mouse models [11-14]. Cell differentiation in the murine pituitary takes place in different stages of embryogenesis: a) Tbx19+ corticotropes are the first cell lineage to achieve differentiation in week E12.5, followed by b) differentiation of thyrotropes in week E14.5, and c) differentiation of lactotrope and gonotrope Pit1 lineages in weeks E15.5 and E16.5, respectively [9,11,12]. Notably, downregulation of the Notch signaling pathway induces expression of Tbx19, which promotes expression of the ACTH precursor proopiomelanocortin (POMC) and, consequently, differentiation of the last hormone-producing cells, the corticotropes [15]. On the other hand, activating the Notch signaling pathway results in upregulation of Prop1 through interaction with β-catenin [15,16]. Differentiation of the somatrotope cells is regulated by retinoic acid, thyroid hormones, and growth hormone releasing hormone (GHRH). Lactotrope cell differentiation is dependent on estrogen stimulation, while gonotropic development of Pit1 is dependent on Gata2 due to the upregulation of Sf1, which promotes the expression of FSH-β and LH-β Gata2 [17-19]. Gata2 also activates TSH-β gene transcription in synergy with Pit1 [17]. The pituitary gland undergoes remodeling not only in the postnatal phase, but also throughout adult life. The adult pituitary gland can adapt its cellular compartments in a manner strictly dependent on changes in physiological conditions, potentially thanks to a contribution from a hypothalamic stimulus [20]. For example, the pituitary gland increases the number of GH-secreting cells in puberty, and increases the number of PRL-secreting cells in pregnancy and during lactation. Another important feature of the pituitary gland is its ability to regenerate itself. Several studies [21-23] have demonstrated that the pituitary gland can restore lost hormone-producing cell types after tissue damage thanks to stem cell differentiation and transdif-

ferentiation between various pituitary cell phenotypes. However, these studies have not yet provided a clear demonstration of the presence of these regenerative processes [24-26], making further studies necessary. Over the years, several studies have demonstrated the presence of stem cells in human organs (heart [27], brain [28], muscles [29], etc.). Stem cells are a particular type of cell, pluripotent and undifferentiated, from which all other differentiated and specialized cells are generated. They are characterized by the expression of specific markers (i.e., CD44 and CD105 for mesenchymal stem cells [30]; CD133 and CD90 for embryonic stem cells [31]; CD34 and CD117 for hematopoietic stem cells [32]; CD45 and VEGFR1/2 for endothelial stem cells [33], etc.). Over the years, it has been demonstrated that the different plasticity of organs and tissues is closely related to the presence of a stem cell population [34].

The pituitary gland is also subject to several changes throughout life, and its ability to adapt to these changes seems to be supported by the presence of adult stem cells inside the gland [7,22,35,36]. The presence of stem cells within the pituitary gland was discovered through *in vitro* studies in mouse models. In these studies, researchers described the isolation of spherical colonies from pituitary gland tissue; termed "pituispheres" [22,25,37], these were positive for the presence of stemness markers, such as Sox-2 [7], NESTIN [36], Sca-1 [38], and CD133 [38]. However, studies on the presence of stem cells in human pituitary tissue are still limited by several issues, in particular, the difficulty accessing this gland due to its anatomical position, and the low cell yield from pituitary gland tissue.

Pituitary stem cells

In addition to hormone-producing cells, the pituitary gland is characterized by the presence of a group of cells, called chromophobe cells, that do not express hormone markers [39,40], The absence of hormone production/secretion by these cells has been demonstrated using the periodic acid of Schiff method.

In 1969, chromophobe cells were identified as the pituitary stem cell population [40]. Yoshimura *et al*. evaluated their proliferation and differentiation capacity. They purified chromophobe cells from murine pituitary glands and transplanted them into the hypothalamic pituitary area following surgical removal of the pituitary gland. New acidophilic and basophilic cell structures were seen to form following this transplantation. This research was followed by *in vitro* studies of the capacity of chromophobe cells to differentiate into acidophilic and basophilic cells in a hypothalamic hormone-enriched culture medium [41], but pluripotency of these cells was not demonstrated. Chromophobe cells are a highly heterogeneous group that includes agranular (FS), follicular, marginal zone, degranulated, mesenchymal, and immune cells. This cellular heterogenicity could explain, in part, the failure to detect pluripotency. With this in mind, pituitary stem cell research began to focus on the stemness of FS cells and pituitary marginal cells. In recent years, studies performed to evaluate the presence of stem markers in the mouse pituitary gland have demonstrated the simultaneous expression of these markers on FS cells [7,8,42,43].

Lepore *et al*. explored adult pituitary stem cell isolation using a method based on the study of murine cell cultures derived from the low cell density areas of the intermediate and anterior pituitary lobes. They observed very low cell adhesion capacity and a concomitant tendency to form small colonies, whose cells morphologically resembled FS cells and were positive for S100 expression [44]. The ability to form colonies was assessed by sorter analysis, based on the cells' ability to take up b-Ala-Lys AMCA (the fluorescent dipeptide derivative that FS cells incorporate). The results of the analysis showed that the AMCA+ cells were derived from both the anterior part of the pituitary gland (zone of residence of FS cells), and from the marginal layer, where the FS population is absent; these results did not exclude the possibility that another cell line could be the putative pituitary stem line [45]. Later, scientists demonstrated the ability of the isolated cells to differentiate into GH-secreting cells, such as AMCA+ [45]. Garcia-Lavandeira *et al*. also defined a cell group (GPS cells) with the ability to form colonies; these cells express GRFa2/Prop1/Stem markers [42]. Unlike the cells isolated by Lepore *et al*. [44], they had a round morphology and formed scattered colonies, but were more compact when grown in feeder layers [42]. Interestingly, amplification of these undifferentiated cells was obtained for several generations, providing evidence of a good self-renewal capacity. An important feature for the characterization of pituitary stem cells is the ability to migrate, and thus to perform the epithelium-mesenchyme transition (EMT). Chemokines also play a key role in migration. It has been observed that CXCL12 and its receptor CXCR4 are critical for pituitary gland development [46]; it has also been found that FS cells produce CXCL12 and that the binding of this ligand to its receptor CXCR4 induces FS cell network formation within the anterior pituitary lobe. This interaction has been described in a study conducted in an *in vitro* human cellular model [43,46]. Two niches of different types have been observed within the pituitary gland, one being the region lining the anterior and posterior portions of the pituitary gland, and the other lying in the marginal cell layer [7,47]. SOX2-positive cells capable of self-renewal and of giving rise to all types of hormone-secreting cells are distributed in these niches [48,49]. The CAR (coxsackievirus and adenovirus receptor) marker has also been found in stem cells residing in these niches [50] with a presence of SOX2+ cells during both the developmental and the postnatal periods. CAR-positive cells have been shown to have a greater proliferative capacity than SOX2-positive cells, although the latter also express epithelial mesenchymal markers (i.e., E-cadherin and vimentin). Hormone-secreting cells do not express the CAR marker in either embryonic or adult stages, but express SF S100 [50]. Fauquier *et al*. demonstrated that a group of SOX2-positive cells had the ability to form "pituispheres" [7]. Initially, there was co-expression of only SOX2 and E-cadherin markers; however, after one week of culture, the spherical colonies were also positive for S100 and SOX9 markers [7], leading the authors to hypothesize that the SF cells were probably progenitor cells and not stem cells. Cox *et al*. published a protocol for the creation of organoids from pituitary tissue, allowing a more accurate study of pituitary stem cells and their role in gland organogenesis [51]. However, due to the heterogeneity of primary cultured pituitary stem cells, many questions remain to be answered.

Pituitary adenoma stem cells (PASCs)

In recent years, several studies have focused on the isolation of stem cells derived from pituitary adenomas (PASCs). In almost all the studies published so far [22,25,37], it was found to be possible to isolate PASCs able to grow in suspension as spheroids, "pituispheres", a characteristic considered (as previously mentioned) an index of self-renewal. Generally, the isolation of such cells was performed using serum-free culture media enriched with growth factors, such as EGF and bFGF [52]. Unfortunately, the rate of stem cell isolation from pituitary adenomas is still well below 100%. Würth *et al*. [53] developed a protocol to isolate PASCs from GH-secreting adenomas *in vitro* and found that only 68% of adenomas were able to proliferate, giving rise to spheroids when selected in culture medium permissive only for stem cells. In a study by Peverelli *et al*. [54], the stem cell isolation rate was only 69.6%. Zhao *et al*. [55] obtained a different result. They selected CD133- and NESTIN-positive co-expressing cells directly from dispersed pituitary adenoma cells. These cells accounted for about 3% of the total adenoma cells but were able to promote sphere formation for multiple passages. In another study [56], stem cell selection was done differently, based on the stem characteristic in which these cells show overexpression of ATP-binding cassette; this characteristic confers cellular resistance against toxic stimuli, including pharmacological ones. In this study, the authors analyzed cultures derived from pituitary adenomas and selected the cells by cytofluorimetry, based on their ability to extrude the Hoescht 33342 dye. They were able to isolate a SP in which PASCs represented about 1.9% of the population.

In recent years, characterization studies of PASCs have also been done [53,56,57]. Chen *et al*. [57] demonstrated that adenoma-derived sphere colonies express CD133, NESTIN, and other typical neural stem cell markers, such as NCAM and B-tubulin II (neuron-specific). Other studies [56] have shown that, in addition to the NESTIN marker, cells isolated as SPs were CD44+, CXCR4+, KIT+, KLF4+, and SOX2+, and EMT-related gene expression was up-regulated in cells that are candidates to be PASCs compared with non-SPs, whereas epithelial markers were down-regulated [56]. Orciani *et al*., in a subset of GHomas and Non-functioning pituitary adenomas (NFPAs), observed that there was also expression of typical mesenchymal stem cell (MSC) surface markers; in fact, the medium used for the isolation of PASCs was an MSC-permissive medium, and it was reported that, under appropriate differentiation conditions, these cells had the ability to differentiate in an osteogenic, adipogenic, and chondrogenic sense [58]. The same result was reported in another study [59], in which cells isolated from GHomas, prolactinomas, and NFPAs showed morphological characteristics of MSCs, expression of surface markers such as CD73, CD90, CD105, CD44, and vimentin, and the ability to differentiate into osteocytes and adipocytes. Peverelli *et al*. [54] reported that PASCs isolated from 46 NFPAs were SOX2+, OCT4+, and KLF4 mRNA+. They also confirmed the expression of nuclear PROP1 (2-10% of cells) in SOX2+ cells, whereas in SOX– cells, PROP1 was cytoplasmic. The authors were therefore led to think that there were heterogeneous populations of stem cells (PROP1+/SOX2+) and precursors (PROP1+/SOX2–) within the pituitary gland primary cell cultures.

Pituitary stem cells in regenerative medicine

Since 2007, when Takahashi published a protocol to reprogram human somatic cells into induced pluripotent stem cells (iPSCs) [60], science has made great strides in generating specific stem cells for research on pathophysiology and personalized treatments. Episomal vectors were later developed to obtain iPSCs without the use of retroviral/lentiviral vectors [61,62], and this allowed scientists to generate specific iPSCs without the occurrence of mutations caused by the integration of genetic material, thus creating increasingly clinical grade iPSCs.

In 2011, a study reported the possibility of differentiating ACTH-secreting cells into murine stem cells [63]. This was achieved by placing hypothalamic tissue cells in a 3D sphere surrounded by Pitx1/2+ oral ectoderm cells and using a BMP4 stimulus in the culture medium. The authors used the Sonic Hedgehog (Shh) pathway agonist, Smoothened agonist (SAG), to promote Lhx3 expression by oral ectoderm cells, thus inducing cell invagination that could develop into a kind of Rathke's pocket. At that point, Lhx3+ cells began to differentiate toward all hormone-secreting cell lineages. To produce ACTH-secreting cells, they added DAPT (Notch inhibitor) to the culture medium. To see the functionality of these cells, the scientists transplanted them into the renal sub-capsule of hypophysectomized mice and found that blood products of ACTH were increased, and, in addition, that there was spontaneous locomotor activity and overall higher survival than in the murine control (non-transplanted mice). In 2020, Kasai *et al*. [64], using the protocol described by Suga *et al*. [63]no efficient stem-cell culture for its generation is available, partly because of insufficient knowledge about how the pituitary primordium (Rathke's pouch, demonstrated that the combined differentiation of pituitary cells and hypothalamic cells compensates for the imbalance in gland function levels and these levels are comparable to those of healthy tissue.

Currently, iPSCs are used to study the mechanisms that lead to diseases such as hypopituitarism; in fact, it has been seen that patients with pituitary hormone deficiency can have mutations in the *PROP1*, *LHX3*, *LHX4*, *HESX1*, *POU1F1*, *GLI2* and *OTX2* genes [65].

Another recent study [66] showed that iPSCs derived from patients with a heterozygous variant in OTX2 lacked the ability to secrete hormones, unlike iPSCs used as controls. The authors reported that, by correcting the mutation using the CRISPR/Cas-9 technique, iPSCs from patients regained the ability to differentiate into hormone-secreting cells. This demonstrated that the variant was the cause of the patients' hypopituitarism.

The question of whether or not *in vitro* addition of various concentrations of BMP2 and FGF8 to the culture medium can modulate and promote the proportion of pituitary cells has been investigated. High concentrations of BMP2 and low concentrations of FGF8 were found to induce the production of LH and FSH cells; with equal concentrations of both factors, there was increased stimulation of GH- and TSH-secreting cells, whereas low concentrations of BMP2 and high concentrations of FGF8 promoted POMC+ cells [67]. In two studies [67,68], a combination of BMP4, SHH, FGF8, and FGF10 in culture medium was used to isolate hormone-secreting monolayer stem cells *in vitro*; however, this method relies on sorter selection of GFP+/SIX+ cells.

Nowadays, recombinant hormone therapy is the standard approach to hormone deficiencies caused by glandular hypofunction or total glandular dysfunction. This type of therapy has certainly improved the lives of patients by significantly reducing morbidity and mortality, but, unfortunately, drug therapy does not allow total restoration of the hormone levels to those of a healthy individual, nor does it vary hormone levels following stress or circadian stimuli [69]. In fact, the healthy pituitary gland, being an extremely plastic and responsive gland, can modulate the production and quantity of each hormone group according to the type of feedback, positive or negative, it receives. For this reason, exogenous hormonal pharmacological administration cannot completely replace the patient's hormonal homeostasis. Regenerative medicine, understood as the transplantation of differentiated stem cells, could be a viable future approach for the correction of genetic and/or cellular defects in patients. At the same time, the use of patient-specific iPSCs could be useful for therapeutic decisions in the context of personalized medicine [69]. However, both types of techniques, whether 2D or 3D, have yet to be perfected for use in clinical therapeutic settings.

Conclusions

In conclusion, several studies have reported the hypothesis of the existence of multipotent stem cells in the adult pituitary, both murine and human; however, not all reach the same conclusions. We can confirm that in most publications the existence of an SP characterized by the expression of SOX2 in both the anterior pituitary lobe and the marginal zone is presumed. From the numerous studies conducted to unravel the biological significance of these stem cell populations, it is currently believed that these cells, at least in the basal adult gland, are highly quiescent; however, their functions are not yet well understood [70]. What is certain is that a more in-depth characterization of stem cells is needed to clearly understand the biological basis of hypopituitarism and adenoma development. In addition, greater knowledge in this area could facilitate the study of new ways of treating endocrine disorders of the pituitary gland, thus opening the way to the world of regenerative medicine and, therefore, stem cell therapy.

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