Pituitary disease and recovery: how are stem cells involved?

Charlotte Nys and Hugo Vankelecom

Laboratory of Tissue Plasticity in Health and Disease, Cluster of Stem Cell and Developmental Biology, Department of Development and Regeneration, KU Leuven (University of Leuven), 3000 Leuven, Belgium

Correspondence should be addressed to: Hugo Vankelecom, Laboratory of Tissue Plasticity in Health and Disease, Cluster of Stem Cell and Developmental Biology, Department of Development and Regeneration, KU Leuven (University of Leuven), Herestraat 49, Leuven, Belgium.

E-mail address: hugo.vankelecom@kuleuven.be

Abstract

The pituitary gland embodies our endocrine hub and rigorously regulates hormone balances in the body, thereby ruling over vital developmental and physiological processes. Pituitary dysfunction and disease strongly impact the organism's biology. Physical damage, tumour development and ageing all negatively affect pituitary state and functionality. On top of its hormone-producing cells, the pituitary contains a population of stem cells. Not only their physiological role is still largely unknown, also whether or how these stem cells are involved in pituitary disease and recovery from defective functionality remains enigmatic. Here, we summarize what is known on the phenotypical and functional behaviour of pituitary stem cells in diseased or dysfunctional gland, as particularly caused by injury, tumourigenesis and ageing.

Keywords: pituitary; stem cells; injury; regeneration; ageing; tumour

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Introduction

The pituitary gland acts, together with the hypothalamus, as the master endocrine regulator, coordinating essential physiological processes such as growth, puberty, metabolism, stress management and reproduction. In rodents, the gland consists of three lobes, i.e. the anterior lobe (AL), posterior (or neural) lobe (PL) and intermediate lobe (IL) (Cox et al., 2017) (Fig. 1). In humans, the IL structure regresses during embryonic development with its cells spreading over the AL (Cox et al., 2017). The PL contains axonal projections from the hypothalamus which store and secrete the hormones oxytocin and vasopressin, both produced in hypothalamic neurons. The adenohypophysis (i.e. AL and IL together) comprises hormone-producing cells, secreting prolactin (PRL), adrenocorticotropic hormone (ACTH), growth hormone (GH), luteinizing hormone (LH), follicle-stimulating hormone (FSH), thyroid-stimulating hormone (TSH) and melanocyte-stimulating hormone (MSH) (Andoniadou et al., 2013; Cox et al., 2017; Vankelecom, 2012). Function of the hormones is diverse, affecting multiple divisions of the endocrine system. PRL stimulates mammary gland development and regulates milk production during lactation. In addition, the hormone exerts a negative feedback on LH/FSH and sex hormone production. ACTH activates the adrenal cortex to produce stress hormones like cortisol. GH orchestrates body and organ growth through anabolic effects which are mainly mediated by insulin-like growth factor 1 (IGF1) from the liver. LH and FSH regulate ovarian follicle maturation and ovulation in females, and coordinate testicular spermatogenesis and testosterone production in males. TSH stimulates the thyroid gland to synthesize and secrete triiodothyronine (T3) and thyroxine (T4) which control processes such as metabolic rate, lipolysis and cardiac output. MSH activates skin melanocytes and hair follicles to produce melanin for pigmentation

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(Barkhoudarian and Kelly, 2017). Synthesis and secretion of hormones by the pituitary is intensely regulated by central and peripheral inputs, including stimulatory and inhibitory factors from the hypothalamus and feedback messengers from target glands (Barkhoudarian and Kelly, 2017; Vankelecom, 2012). Because of the hormones' widespread activities, pituitary disease and dysfunction with dysregulated production of one or more of the hormones have enormous impact on body development and physiology and can cause severe clinical symptoms and morbidity. Among others, diminished pituitary function (hypopituitarism) can result in dwarfism, delayed or absent puberty and infertility (Alexandraki, 2019).

In the past 15 years, evidence has been accumulating that the adenohypophysis, like many other adult tissues, contains a population of stem cells (Vankelecom, 2012; Vankelecom and Chen, 2014). The Vankelecom group first identified a so-called side population (SP) in the pituitary, based on the general property of stem cells to efflux potentially toxic compounds that, once in the cell, could threaten the stem cell's survival or integrity (Chen et al., 2009, 2006, 2005). This SP, visualized in flow cytometry through efflux of the dye Hoechst33342, was found to be enriched in cells expressing stemness markers, in particular sex determining region Y-box 2 (SOX2) and SOX9, and giving rise to clonal spheres (pituispheres), a further characteristic of stem cells. Moreover, the pituispheres were able to generate all pituitary hormonal cell types, thereby demonstrating the multipotent differentiation capacity of the sphere-forming (stem) cells (Chen et al., 2009). Other groups independently identified a $SOX2^+$ stem cell population in the pituitary (Fauquier et al., 2008; Garcia-Lavandeira et al., 2009) and more recent lineage tracing studies showed that $SOX2^+$ and $SOX9^+$ cells postnatally generate the variety of pituitary endocrine cell types, although at low level while most of the traced cells retain their

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77 undifferentiated, stem cell phenotype (Andoniadou et al., 2013; Rizzoti et al., 2013).
78 The physiological function of the stem cells in the pituitary, apart from this limited
79 homeostatic contribution, remains largely unknown (Vankelecom, 2016). There are
80 some indications that they may contribute to hormonal cell adaptations in the gland
81 in response to endocrine demands of the body, such as in the increase in PRL-
82 producing cells during pregnancy and lactation, or the upsurge of specific hormonal
83 cells after deletion of their respective target organ (Levy, 2002; Rizzoti et al., 2013).
84 For instance, adrenalectomy is known to lead to a transient rise in ACTH-expressing
85 corticotropes (Nolan and Levy, 2006) and lineage tracing showed that a small part of
86 the new corticotropes originate from $SOX9^{+}$ stem cells (Rizzoti et al., 2013). From all
87 current knowledge, it appears that the stem cells in the adult pituitary are highly
88 dormant, and whether and how they are involved in remodeling processes in the
89 gland remains largely undiscovered. One glance behind the scenes may be their role
90 in tissue repair. Also in some other tissues (such as muscle and liver), stem cells are
91 deeply quiescent, and only obviously come into play under challenged conditions
92 such as injury (Hill et al., 2003; Huch et al., 2013b). Along this line, recent studies
93 provided strong hints toward the involvement of pituitary stem cells in the reaction to
94 damage in their tissue (Fu et al., 2012; Fu and Vankelecom, 2012; Willems et al.,
95 2016). In addition to this purported beneficial effect, stem cells may also be involved
96 in pathological processes in the pituitary including tumour growth (Andoniadou et al.,
97 2013; Mertens et al., 2015; Vankelecom and Roose, 2017). Moreover, genetic
98 defects in early-embryonic pituitary stem cells (generally referred to as progenitor
99 cells) also logically lead to subsided or absent pituitary function (Fang et al., 2016b;
100 Kelberman et al., 2009).

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In this review, we summarize and discuss the current findings on stem cell behaviour and/or function in diseased pituitary and in the gland's recovery from damage and hypofunction.

1. Pituitary progenitor cells in genetic hypopituitarism

Hypopituitarism can have a congenital origin, being sporadic or part of a hereditary syndrome. Some of the genetic mutations specifically affect the progenitor cells at the root of the embryogenic development of the pituitary (as in detail described in other reviews; (Fang et al., 2016b; Vankelecom, 2012; Vankelecom and Chen, 2014)).

The earliest transcriptional regulators expressed in Rathke's pouch (RP), the embryonic rudiment of the pituitary which initially is completely made up of embryogenesis-driving progenitor cells, are homeobox expressed in ES cells 1 (HESX1) and the bicoid homeodomain factors PITX1 and PITX2 (Vankelecom, 2012). Mutations in *HESX1* lead to septo-optic dysplasia in humans and a comparable phenotype in mice, including the occurrence of pituitary dysplasia which may at least partly be due to aberrant developmental behaviour (overproliferation) of the progenitor cells (Dattani et al., 1998; Fang et al., 2016a). Several different phenotypes are observed in *HESX1* mutants, ranging from lack of a pituitary to presence of multiple misplaced glands (Andoniadou et al., 2007; Kelberman et al., 2009). *PITX2* mutations are associated with Axenfeld-Rieger syndrome, also encompassing sporadic pituitary hypoplasia that can result in growth retardation (Tümer and Bach-Holm, 2009). PITX2 is involved in the early expansion of RP, and *Pitx2*- as well as *Pitx1*-deficient mice show severe pituitary hypoplasia missing most hormonal cells except for some sporadic ACTH^{+} cells (Suh et al., 2002; Charles et

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al., 2005; Lin et al., 1999). Mutations in LIM homeobox 3 (LHX3), which is also expressed at the RP phase, can lead to pituitary dysfunction with GH, TSH and LH/FSH deficiency and sometimes corticotrope deficits (Gangat and Radovick, 2017; Xatzipsalti et al., 2019). Another member of the LIM homeobox family, LHX4, is also present in RP and is involved in proliferative expansion of lineage precursors (Sheng et al., 1997). *LHX4* mutations give rise to a hypoplastic pituitary gland in which all hormonal cell types are present but in reduced numbers, in some cases leading to panhypopituitarism (Gangat and Radovick, 2017; Raetzman et al., 2002; Sheng et al., 1997). The stemness factor SOX2 is ubiquitously expressed in early RP progenitor cells and involved in progenitor proliferation and subsequent lineage specification toward hormonal cell types (Goldsmith et al., 2016; Jayakody et al., 2012). Transgenic *Sox2* deletion in RP results in severe hypoplasia due to decreased proliferation of the progenitor cells, which leads to underdevelopment of endocrine cell lineages, in particular the cell types that arise late during embryonic development (such as the GH-expressing somatotropes), and to deficient hypothalamic stimulation of the LH/FSH-producing gonadotropes (Goldsmith et al., 2016; Jayakody et al., 2012). Mutations in patients are associated with hypopituitarism (especially GH deficiency and low levels of FSH/LH) (Xatzipsalti et al., 2019; Jayakody et al., 2012). Another factor expressed in early progenitor cells is Prophet of Pit1 (PROP1). Although its main function has classically been situated in inducing the development of PIT1 lineages (i.e. GH, PRL and TSH) (Sornson et al., 1996), it is more and more evidenced that PROP1 plays a role in the migration of developing cells from the periluminal progenitor zone (i.e. zone around RP cleft) toward the developing AL (Raetzman et al., 2002; Ward et al., 2005). Recessive mutations of *PROP1* are the most frequent causes of combined pituitary hormone

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deficiency (Bertko et al., 2017; Xatzipsalti et al., 2019) with deficits in GH, PRL, TSH and LH/FSH. Patients with *PROP1* mutations often present with growth retardation and delayed or absent puberty, although time of onset and severity of phenotypes can differ (Xatzipsalti et al., 2019). Dwarfism and infertility are similarly observed in *Prop1*-deficient Ames dwarf mice (df/df) (Sornson et al., 1996).

Taken together, genetic mutations in factors meticulously regulating expansion and developmental progress in the progenitor cell pool during pituitary embryogenesis result in morpohological and functional deficits of the gland.

2. Pituitary stem cells in tissue recovery after damage

Pituitary hypofunction may result from damage inflicted in the gland by growing and expanding tumours which compress the neighbouring normal tissue; by surgical resection of those tumours, wounding the non-affected healthy tissue; and/or by traumatic brain injury as resulting from traffic accidents, falls, sport-related head injuries and acts of violence (Tanriverdi et al., 2015; Vennekens and Vankelecom, 2019; Willems and Vankelecom, 2014) (Fig. 1). To date, resultant pituitary deficiencies are covered with lifelong hormone replacement therapy, which however remains largely suboptimal due to adverse effects of supplemented hormones (e.g. development of diabetes) and the inability to reproduce the natural cyclic hormonal output of the pituitary (Willems and Vankelecom, 2014). Pursuing regeneration of destroyed tissue would therefore be an appealing alternative. Whether the gland has intrinsic capacity to repair damage remained unsettled for a long time. An older case report described possible regeneration of pituitary tissue in human patients after transsphenoidal electrocoagulation therapy of in-gland metastatized tumours (Landolt, 1973). In contrast, a study in rats questioned the ability of the pituitary to

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restore tissue, as was explored after fractional hypophysectomy (Saeger and Warnecke, 1980). Partial recovery of pituitary hormone levels was observed in some patients after traumatic brain injury-caused hypopituitarism (Tanriverdi et al., 2015; Vennekens and Vankelecom, 2019). However, it is not known whether pituitary cells were physically regenerated or merely regained functionality (e.g. responsiveness to hypothalamic hormones) (Vennekens and Vankelecom, 2019). Only in the last decade, convincing proof was provided that the adult (mouse) pituitary can repair damage (Fu et al., 2012; Fu and Vankelecom, 2012; Willems et al., 2016). In particular, a pituitary injury model was developed in which somatotropes were deliberately killed by treating transgenic GHCre/iDTR mice, expressing the diphtheria toxin (DT) receptor in the somatotropes (following GH promoter-driven Cre-mediated recombination), for a short 3-day period with DT. The large majority of somatotropes were obliterated, thereby inflicting pituitary tissue damage. Interestingly, the resident stem cell compartment showed an immediate reaction to the injury, displaying proliferative expansion and transcriptional activation (Fu et al., 2012). Moreover, the stem cells started to co-express GH, and somatotropes were replenished in the coming months (up to a level of 60%), resulting in considerable restoration of GH serum levels (Fu et al., 2012; Willems et al., 2016). Together, these observations uncovered that the adult (mouse) pituitary possesses the competence to repair damage and demonstrated that the resident stem cells acutely react to injury occurring in their 'home' tissue, associated with the regenerative response and process that is unfolding (Fu et al., 2012; Willems et al., 2016) (Fig. 1). Intriguingly, regeneration did no longer occur when the injury impact was prolonged (by treating the mice with DT for 10 instead of 3 days), although an initial stem cell response still developed (Willems et al., 2016). Pituitary stem cells may become exhausted after

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repetitive attempts to react to the continuous insult and to start the regenerative response (as also found in other tissues such as lung and hippocampus; (Ghosh et al., 2018; Sierra et al., 2015)). This exhaustion could fit within the postulated dormant nature of pituitary stem cells and the 'lazy', low-turnover character of the adult gland.

To enable in-detail study of the behaviour and phenotype of pituitary stem cells upon injury, as well as in other developmental, biological and pathological conditions, Cox et al. (2019) recently developed a new, innovative and powerful *in vitro* pituitary stem cell research organoid model. Organoids are structures that grow in 3D from tissue stem cells under specific culture conditions, and that reliably mimic multiple phenotypical and functional characteristics of the original tissue and/or its stem cells (Broutier et al., 2016; Huch et al., 2013a; Sato et al., 2009) (Fig. 1). Typically, organoids can be cultured and expanded for many months without losing their phenotype and while remaining genomically stable. Organoids can also be derived from diseased tissue, thereby reliably replicating pathobiological and genetic characteristics of the disorder, even in a patient-specific way. These organoids can be harnessed in drug screening platforms to evaluate the impact of existing and new drugs on the individual patient's tissue pathology.

Organoids were recently developed from adult mouse pituitary by culturing dissociated AL cells in an extracellular matrix scaffold (Matrigel) in the presence of a defined cocktail of growth factors, typically containing a generic organoid-culturing mixture with epidermal growth factor (EGF), noggin and WNT pathway activators (such as R-spondin 1 and/or WNT3A), and further enriched with factors that are crucial in embryonic development of the gland, in particular fibroblast growth factor 8 (FGF8), FGF10 and sonic hedgehog (SHH) (Cox et al., 2019, 2017). It was demonstrated that the pituitary organoids (clonally) originate from the resident

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SOX2$^+$ stem cells and largely retain stemness characteristics during culture. In addition, the organoids showed some expression of factors involved in pituitary embryonic development (e.g. *Eya1*, *Six1*, *Six6, Lhx3*) (Cox et al., 2019). The organoids could differentiate into specific cell types, both *in vitro* and *in vivo*, although at modest level. Importantly, (new) findings obtained in the organoids could be transposed to, and confirmed in the *in vivo* setting (such as expression of new stem cell markers), thereby indicating that this novel organoid model provides a relevant and reliable tool to study pituitary stem cell biology, and an interesting readout for pituitary stem cell behavior and activation (Cox et al., 2019) (Fig. 1). Indeed, the number of organoids augment upon stem cell activation following the injury inflicted in the transgenic (GHCre/iDTR) pituitary, showing increased expression of pituitary embryonic factors and upregulation of *Prrx1* and *Prrx2* as compared to control (undamaged) pituitary organoids. Expression of *Prrx1* and *Prrx2* was also found upregulated *in vivo* upon pituitary damage, further supporting organoids' translatability (Cox et al., 2019). Although expandable, the passaging capacity of the pituitary organoids remained limited which may have been due to the lack of essential factors in the culture medium to keep the stem cells activated, or which may be the result of an intrinsic feature of pituitary stem cells (e.g. just disposing of a restricted number of self-renewal rounds given the 'lethargic' turn-over character of the gland). Identification of pituitary stem cell-activating factors may thus relieve this roadblock, which would also be highly important to pursue regenerative routes for treating pituitary deficiency (Fig. 1). Completely understanding what is occurring upon injury, i.e. comprehending the role of the stem cells and the injury-induced microenvironment, will eventually be of major value.

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3. Pituitary stem cells in tissue ageing

At the organismal level, ageing is burdened with changes in metabolic, immunological and hormonal activities (Benayoun et al., 2015; Fontana et al., 2010; Oh et al., 2014; Straub et al., 2001). Menopause and somatopause (i.e. dropped GH levels and activity) are two important endocrine examples. At the organ level, ageing is associated with the accumulation of senescent cells (Fig. 1), which may arise because of several reasons such as DNA damage and telomere shortening (Hiraishi et al., 2018; Oh et al., 2014). Senescent cells are characterized by an irreversible cell cycle arrest and the secretion of 'senescence-associated secretory phenotype' (SASP) factors, including inflammatory cytokines like interleukin-6 (IL-6) and tumour necrosis factor-α (McHugh and Gil, 2018; Shufian et al., 2017). In addition, the ageing body and organs appear to be in a state of chronic, low-grade inflammation, generally referred to as 'inflammageing', further adding to increased levels of inflammatory signals, and contributing to the ageing process (Olivieri et al., 2018).

That pituitary and hormonal aspects affect ageing is also clear from genetic mouse models altering longevity. $Prop1^{df/df}$ (Ames dwarf) and $Pit1^{dw/dw}$ (Snell dwarf) mice live 40% longer than control animals, with the Ames model reported as the first mammalian organism with increased lifespan (Brown-borg and Borg, 1996; Flurkey et al., 2001). Decline in GH levels in the models is thought to be the main cause underlying life extension since homozygous GH receptor knockout mice also live longer (Conschigano et al., 2000) and, by contrast, overexpression of GH significantly reduces lifespan (Bartke et al., 2002). Reduced GH leads to lower liver-produced IGF1 levels. Of note, the GH/IGF1 axis is also downgraded by caloric restriction which is more and more associated with extended lifespan (Fontana et al., 2010; Ren et al., 2017; Vitale et al., 2019). Caloric restriction is also linked to

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augmented number and function of tissue stem cells, as observed in skeletal muscle and lung (Cerletti et al., 2012; Hegab et al., 2019; Oh et al., 2014). On the other hand, reduction of GH levels, as occurring during the physiological age-related somatopause, has multiple adverse effects such as bone frailty and metabolic defects (Lin et al., 2012; Locatelli and Bianchi, 2014; Vijayakumar et al., 2011). There are indications that diminished hypothalamic stimulation of pituitary GH production is involved in the GH decline observed at somatopause, but whether (also) pituitary ageing with decreased functionality plays a role is not known yet (Ajdžanovi et al., 2018; Veldhuis, 2013).

Homeostatic and regenerative capacity of tissues also progressively fades with ageing, and more and more evidence is provided that tissue stem cells undergo an ageing process which may contribute to the organ's physical and functional decline. Stem cell ageing may be caused by degenerative changes in stem cell function and regulation, both at the local (niche) and systemic level (Oh et al., 2014; Ren et al., 2017). Whether stem cell ageing is also occurring in the pituitary is at present not clear. A recent study described telomere shortening in the glandular cells of the AL of elderly people as analysed after autopsy (Hiraishi et al., 2018). However, stem cells were not looked at. In this context, one other study revealed that the stem cells present in the marginal zone (i.e. the cell layer bordering the cleft between AL and IL; MZ; Fig. 1) contain the longest telomeres in the gland, and that cellular telomere length gradually decreases toward the AL parenchyma with the shortest chromosome ends present in hormonal cells (Garcia-Lavandeira et al., 2009). A recent study hinted to pituitary stem cell ageing by discovering that regenerative capacity of the pituitary disappears in older mice, already surfacing from 8-10 months of age (Willems et al., 2016). Regeneration of somatotropes was no longer

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observed after their DT-induced ablation in the older GHCre/iDTR mice. In parallel, the number of pituitary stem cells and their functionality (as probed by pituisphere formation efficiency) were decreased in old mice, strongly suggesting that pituitary stem cells decline in number as well as in activation responsiveness at ageing. Also in other tissues (such as skeletal muscle and liver), it has been found that stem cell number declines and functional ageing occurs which underlies the waning regenerative capacity following injury in these tissues at ageing (Sousa-victor et al., 2018; Timchenko, 2009). Whether pituitary stem cells become senescent, less responsive to regulatory (activating) factors or deteriorate because of being embedded in a chronic inflammatory (micro)environment is at present not clear. A recent study touched upon the upregulation of senescent markers (such as p16 and p53) in the pituitary SOX2^{+} stem cell population of 6-month-old mice (Gonzalez-Meljem et al., 2017). In other tissues such as muscle, it has been found that stem cells retain intrinsic functionality at ageing but are oppressed by the microenvironment, thus showing 'rejuvenation' when changed to a young environment (Conboy et al., 2005).

Taken together, although not much is known yet on the mechanisms underlying pituitary ageing, declining pituitary functionality may, at least partly, be explained by deteriorating pituitary stem cell phenotype and activity. However, how pituitary stem cells precisely age and to what extent this process is involved in waning pituitary endocrine function, is not known yet. The inflammatory environment and associated dysfunction and senescence may provide one explanation which would be interesting to investigate.

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4. Pituitary stem cells in tissue tumourigenesis

Pituitary tumours are in general non-malignant and slow-growing lesions (adenomas), but can cause significant morbidity and increased mortality risk due to overproduction of one or several hormones, compression of the healthy neighbouring pituitary tissue (leading to hypopituitarism) and of neural structures (such as the optic chiasm, resulting in visual defects), and/or infiltration into adjacent head structures (causing, among others, severe headache) (Melmed, 2020, 2011). Patients often need lifelong hormonal supplementation since healthy pituitary tissue may be compressed and damaged by the expanding tumour tissue, or may be harmed during transsphenoidal resection of the lesion.

Not much is known on the pathogenesis of pituitary tumours (now referred to as pituitary neuroendocrine tumours or PitNETs; (Mete and Lopes, 2017; Trouillas et al., 2020)) and the molecular pathways involved. Only 5% of the PitNETs can be traced back to a germline mutation, often part of a hereditary syndrome (such as mutations in the aryl hydrocarbon receptor-interacting protein (AIP) in familial isolated pituitary adenoma (FIPA); (Caimari and Korbonits, 2016; Melmed, 2011)), whereas alterations in classical oncogenes or tumour suppressor genes are only rarely present (Melmed, 2020).

The link between pituitary tumourigenesis and stem cells is at present unclear. Actually, a distinction should be made between ‘in-tumour’ stem cells (so-called tumour stem cells or TSC) and the resident stem cells (outside of the tumour) in the tissue where tumourigenesis is occurring. TSC (in malignant cancers referred to as ‘cancer stem cells’) are hypothesized to represent the driving force of tumour development and growth. In *stricto sensu*, these cells generate the tumour with its heterogeneous cell types, are (more) resistant to therapy and thus regrow the

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tumour after treatment toward the original (or even more severe) form. Experimentally, TSC must be capable of regrowing the tumour when transplanted into immunodeficient mice, most strictly in a clonal manner. Ontologically, TSC may derive from the tissue stem cells that become mutated, or from differentiated tissue cells that de-differentiate and regain 'stem cell' features such as defense against toxic (e.g. chemotherapeutic) compounds, self-renewal and proliferative capacity, and 'multipotency' thus generating the different cell types comprising the tumour. A number of studies have provided indications that pituitary tumours may contain a TSC population by using different experimental approaches, including (tumour-)sphere formation, stemness marker expression (e.g. OCT4, nestin, SOX2, CD133, CD15) and therapy resistance (Chang et al., 2016; Manoranjan et al., 2016; Peverelli et al., 2017; Würth et al., 2017; Xu et al., 2009) (reviewed in detail elsewhere; (Vankelecom, 2012; Vankelecom and Roose, 2017)). However, experimental support remained limited and was not always convincing or concordant with the TSC definition as described above (e.g. no therapy resistance, no convincing proof for 'multipotency' and no clonal *in vivo* outgrowth) (Vankelecom, 2012; Vankelecom and Roose, 2017). In another study, TSC were searched for using the SP approach (Mertens et al., 2015). A SP was detected in all human pituitary tumours analyzed, comprising 1.5-2% of the tumour cells, irrespective of the hormonal phenotype. The SP was found enriched in cells expressing TSC markers (such as chemokine (C-X-C motif) receptor-4 (CXCR4) and CD44) and could generate spheres, whereas the remaining bulk of the tumour cells did not display sphere-forming capacity (Mertens et al., 2015). The spheres expressed the stem cell markers SOX2 and nestin, showed some, albeit limited, self-renewal (passaging) capacity and displayed low-level spontaneous hormone differentiation, thus deviating the cells from the

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stemness path which may be one of the reasons of the limited passageability, as also observed in another study (Peverelli et al., 2017). Mertens et al. (2015) also found out that human pituitary tumour fragments or (SP) cells did not develop and grow into tumours upon subcutaneous or subrenal xenotransplantation in immunodeficient mice. To circumvent this hurdle, the SP of a pituitary tumour cell line (i.e. from the mouse corticotrope AtT20 cell line) was tested *in vivo*. The AtT20 SP, also displaying upregulated expression of CXCR4, CD44 and SOX2, showed higher tumour-forming and -growing capacity after subcutaneous transplantation than the remaining bulk of the AtT20 cells. Tumour growth could be slowed down and reduced in size by treatment with the CXCR4 antagonist AMD3100 (Mertens et al., 2015). Moreover, the same study found that the pituitary of Drd2 knockout ($Drd2^{-/-}$) mice, in which prolactinomas develop because of absence of tonic dopamine inhibition (Kelly et al., 1997), harboured a larger SP than the pituitary of control mice (Mertens et al., 2015). In addition, a rise in colony-forming and $SOX2^{+}$ cells was detected, at least partially assigned to higher proliferative activity, with $SOX2^{+}$ cell clusters found nearby as well as within the PRL^{+} tumour region (Mertens et al., 2015). However, lineage tracing showed that the $Drd2^{-/-}$ tumours were not derived from the $SOX2^{+}$ stem cells (Vankelecom and Roose, 2017), thus excluding these cells as true TSC. Taken together, resident pituitary ($SOX2^{+}$) stem cells appear activated during tumourigenesis in their gland, but do not directly give rise to the tumours. This stem cell activation may be a passive result of the tumourigenic insult, or may be an active reaction of the stem cells to the menacing tumour development, with either beneficial or in contrast detrimental impact. For instance, the tumour may hijack the stem cells to its own benefit, to activate its development and growth by claiming growth and survival factors from the stem cells (Fig. 1). This hypothesis is

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supported by another paradigm of stem cell-tumour interaction occurring in the pituitary (sellar) region. Adamantinomatous craniopharyngioma (ACP), a mostly benign but burdening tumour often occurring in children, is hypothesized to develop from RP remnants (Andoniadou et al., 2012; Gaston-Massuet et al., 2011; Nielsen et al., 2011). Two-third of ACP show activating mutations in the β-catenin (*CTNNB1*) gene, leading to constitutive activation of the WNT pathway (Hölsken et al., 2014; Prieto and Pascual, 2018). Juan-Pedro Martinez-Barbera and colleagues developed a mouse model of ACP by genetically expressing a mutant, degradation-resistant (thus constitutively active) form of β-catenin in early-embryonic pituitary progenitor cells (using $Hesx1^{Cre/+}$;$Ctnnb1^{+/lox(ex3)}$ mice; (Gaston-Massuet et al., 2011)) or in $SOX2^+$ stem cells (using tamoxifen-inducible $Sox2^{CreERT2/+}$;$Ctnnb1^{lox(ex3)/+}$ mice; (Andoniadou et al., 2013)). The animals developed pituitary tumours that closely resembled human ACP, in particular showing the presence of typical nucleocytoplasmic β-catenin$^+$ cell foci. These clusters were found to express the stemness markers SOX2 and nestin (Andoniadou et al., 2013; Gaston-Massuet et al., 2011). As observed for $Drd2^{-/-}$ prolactinomas (Vankelecom and Roose, 2017), the ACP-mimicking tumours did not derive from the $SOX2^+$ stem cells as investigated by lineage tracing (Andoniadou et al., 2013; Gonzalez-Meljem et al., 2017). Nonetheless, the tumours did not develop when the mutant β-catenin was expressed in differentiated pituitary cells such as $Pit1^+$, GH^+ or PRL^+ cells, indicating that stem cells formed an essential part of the tumourigenic machinery (Gaston-Massuet et al., 2011). Further studies showed that the nucleocytoplasmic β-catenin$^+$ stem cell clusters displayed senescent features (such as expression of p16, p21 and p53; presence of DNA damage and activation of a DNA damage response; and expression of lysosomal β-D-galactosidase) and showed a SASP, producing (at least

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426 at the mRNA level), among others, IL-6, basic fibroblast growth factor (bFGF), IL-1β,
427 vascular endothelial growth factor receptor 1 (VEGFR1), chemokine (C-X-C motif)
428 ligand 1 (CXCL1) and CXCL11 (Gonzalez-Meljem et al., 2017). Thus, the clustered,
429 mutated stem cells activate a senescence programme and secrete SASP factors,
430 which appear to lead to changes in the microenvironment and induce proliferation
431 and transformation of nearby non-stem cells (Fig. 1). Intriguingly, also here, stem
432 cells in older pituitary appeared less active since almost no tumourigenesis was
433 observed when expression of the mutant β-catenin was induced in the $SOX2^{+}$ stem
434 cells in 6-9 months old mice as compared to earlier induction during embryonic
435 development or in 4-6 weeks young mice (Andoniadou et al., 2013; Gonzalez-
436 Meljem et al., 2017). Alternatively, senescence and SASP responses may be less
437 pronounced in the aged mice because of cellular senescence already occurring
438 (Gonzalez-Meljem et al., 2017). Taken together, the described mouse model also
439 supports a key role for stem cells in pituitary tumourigenesis, most plausibly through
440 paracrine communication that leads to transformation of neighbouring cells and
441 feeding of the tumour, thus acting as a signaling center that sends out cytokines,
442 chemokines and other molecules like EGF, SHH and FGF (Andoniadou et al., 2012)
443 (Fig. 1). Hence, interference with this SASP-associated signaling (e.g. by using anti-
444 IL-6 antibodies or small-molecule inhibitors of EGFR; (Andoniadou et al., 2013; Chen
445 et al., 2018) may obstruct ACP growth and progression. Intriguingly, Cox et al.
446 (2019) described the development of a specific type of organoids (so-called ‘dense’
447 organoids) from the adult mouse pituitary which may be derived from residual oral
448 ectoderm or RP cells still present in the postnatal gland and expresses squamous
449 epithelium markers. Because ACP is also squamous in nature and also postulated to
450 originate from RP or remnants, it is tempting to speculate that the dense organoid

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model may in the future be instrumental to help deciphering ACP pathogenesis and pathology. And more in general, developing organoids from human pituitary tumours (including ACP) may pave the way to a better understanding of pituitary tumourigenesis and in particular of the involvement of stem cells in this pathogenic process (Fig. 1). Development of proper organoid models may further also solve the problem of growing primary human pituitary tumour cells *in vitro* for research and drug screening aims, at present not possible or only with very low efficiency (using spheres) and reliability (Fig. 1). Transplanting these cells orthotopically in the pituitary region of immunodeficient mice will in the end be needed to unravel their tumour-initiating capacity.

Summary and conclusion

Disease and dysfunction of the pituitary gland, as key endocrine regulator, strongly impact organism physiology. Whether pituitary anomalies have a ground in, or link to the tissue's stem cells, is at present far from clear. Here, we summarized the still limited number of studies touching upon this possible connection.

Aberrant regulation and behaviour, most often genetically based, of the progenitor cells initiating and driving pituitary embryogenesis logically leads to a hypofunctional and often dysmorphic gland.

Tumourigenesis in the gland is found to go hand in hand with stem cell activation, which appears to boost development and growth of the tumour. At present, no convincing proof has been found that pituitary stem cells act as true TSC, in a straight way giving rise to the tumour itself.

Tissue damage in the gland also leads to stem cell activation which this time seems beneficial and results in, or contributes to tissue repair and hormone restoration.

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Finally, declined stem cell number and functionality at ageing may be a causative factor of age-related fading of pituitary function including receding regeneration, but underlying mechanisms are still unclear.

Taken together, although data have been provided that may support a role for pituitary stem cells in gland dysfunction and disease (and its recovery), much work has to be done to compile a more clear, detailed and convincing picture. The newly developed pituitary organoid model represents an innovative tool that can powerfully help to reach this goal. Moreover, organoids from diseased pituitary, being reliable models that are at the same time expandable (thereby meeting an important need in pituitary research), may be translatable into drug-screening platforms. In the end, new insights in 'pituitary disease-stem cell' links may lead to novel treatment options including activation or dampening of stem cell activity *in situ*, or restoring (stem) cell populations by transplantation.

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Declaration of interest

The authors declare no potential conflict of interest.

Author contributions

C.N. and H.V. wrote the manuscript.

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Figure legend

Fig. 1. Stem cells in pituitary disease

A. Pituitary disorders in which the tissue stem cells may be involved. Schematic representation of the human pituitary (upper left) and cross-section of the mouse pituitary, showing the marginal-zone (MZ) stem cell niche and the stem cell clusters scattered in the AL parenchyma, surrounded by hormonal cells. Pituitary disease/dysfunction can result from congenital defects (e.g. mutations in developmental genes as listed) or can be contracted during life through physically damaging impacts (potential causes as listed). Decreased pituitary function also occurs naturally upon ageing (potential causes as listed). Finally, tumour development and growth in the gland can cause pituitary dysfunction (potential causes as listed). Whether stem cells are involved in these pathological conditions, is not clear yet. Possible contributions, as discussed in the text, are indicated. (1) Impact of mutations on embryonic progenitor cells may lead to aberrant pituitary development and function. (2) Pituitary stem cells may be activated upon tissue injury to play a role in regenerative response and repair. (3) Decrease in stem cell number and functionality (e.g. faded reaction to injury) may contribute to pituitary ageing. (4) Stem cells may act as a factory of paracrine signals and inflame neighbouring cells to become tumourigenic, resulting in tumour formation and expansion. AL: anterior lobe, IL: intermediate lobe, PL: posterior lobe, MZ: marginal zone.

B. Organoid technology to study and harness pituitary stem cells.

Organoids, developing from pituitary stem cells of normal gland (Cox et al. 2019) or of diseased (e.g. tumour) tissue, have broad application potential. Organoids can be used as powerful research models to decipher pituitary stem cell biology and

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disease. They can be applied to amplify pituitary stem cells for regenerative purposes. Last but not least, pituitary disease organoids can be cryopreserved and biobanked, and used for drug screening, even in a patient-personalized manner.

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