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Stem cells and their role in pituitary tumorigenesis

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ABSTRACT

The presence of adult pituitary stem cells (PSCs) has been described in murine systems by comprehensive cellular profiling and genetic lineage tracing experiments. PSCs are thought to maintain multipotent capacity throughout life and give rise to all hormone-producing cell lineages, playing a role in pituitary gland homeostasis. Additionally, PSCs have been proposed to play a role in pituitary tumorigenesis, in both adenomas and adamantinomatous craniopharyngiomas. In this manuscript, we discuss the different approaches used to demonstrate the presence of PSCs in the murine adult pituitary, from marker analyses to genetic tracing. In addition, we review the published literature suggesting the existence of tumor stem cells in mouse and human pituitary tumors. Finally, we discuss the potential role of PSCs in pituitary tumorigenesis in the context of current models of carcinogenesis and present evidence showing that in contrast to pituitary adenoma, which follows a classical cancer stem cell paradigm, a novel mechanism has been revealed for paracrine, non-cell autonomous tumor initiation in adamantinomatous craniopharyngioma, a benign but clinically aggressive pediatric tumor.

1. Introduction

The pituitary gland is a master endocrine organ that orchestrates the release of several hormones as a consequence of hypothalamic and peripheral organ signals (Denef, 2008). The finely tuned release of these hormones is essential for many physiological processes such as growth, metabolism and reproduction (Drouin, 2011). During recent years a substantial amount of work has revealed the existence of pituitary stem cells (PSCs), which reside in the embryonic and postnatal pituitary (Castinetti et al., 2011; Vankelecom and Chen, 2013). These undifferentiated progenitor/stem cells have the capability to commit and give rise to all three main pituitary hormone-producing cell lineages (through the activation of essential transcription factors: PIT1, TPit and SF1) and ultimately differentiate into the mature hormone-producing populations (Dasen and Rosenfeld, 2001; Zhu et al., 2007). These PSCs have been shown to contribute to organ homeostasis and tumorigenesis (Andoniadou et al., 2013; Rizzoti et al., 2013).

In this concise review, we aim to address the growing body of evidence that suggests the importance of PSC in the formation and progression of pituitary tumors. Specifically, we will focus on Adenomas and Adamantinomatous Craniopharyngioma. The regulation of the pituitary stem cells and its signaling pathways is the subject of a separate review in this issue (S. Camper and MI. Perez-Millan, this Issue).

1.1. The properties and identity of pituitary stem cells

Stem cells (SCs) are characterized by the capacity to self-renew and to divide asymmetrically giving rise to progenitors with multipotent differentiation capability. These progenitors can either terminally differentiate or become transit-amplifying cells which further proliferate in order to sustain a pool of undifferentiated tissue-specific progenitors (Hsu et al., 2014). SCs are therefore able to maintain and replenish the pool of tissue-specific progenitor cells which are essential for embryonic development and plastic tissue adaptation, such as that occurring during homeostatic turnover or in regeneration after injury (Patel et al., 2013; van Es et al., 2012).

SCs can be experimentally characterized by the expression of defined stem cell-associated factors (e.g. SOX2, NANOG, OCT4, SCA1 and CD44), their in-vitro clonogenic potential or the identification

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of a side population by flow cytometry (Goodell et al., 1996; Lepore et al., 2005; Puck and Marcus, 1956). A decade of research has provided convincing evidence that adult PSCs exist. Firstly, it was shown that pituitary cells grown in stem cell-promoting media form adherent colonies, which actively uptake the fluorescent dipeptide β-Ala-Lys-Nε-AMCA and express S100β, both markers of pituitary folliculostellate cells (FS) (Lepore et al., 2005). These adherent colonies were also able to differentiate into hormone producing cells, indicating their multipotent capacity. Another study reported that non-adherent sphere colonies, referred to as "pituispheres", could be grown in clonal conditions and were shown to express stem cell-associated markers such as OCT4 and NANOG. This pituitary cell population had the capacity to efflux verapamil-sensitive Hoechst dye, which allowed the identification of a side population with SC properties by flow cytometry (Chen et al., 2005). Cells within the side population expressed Stem Cell Antigen 1 (SCA1), showed increased expression of SOX2, SOX9, CD44 and CD133, as well as the activation of developmental pathways essential for stem cell homeostasis and embryogenesis (i.e. Notch, Wnt and Shh) (Chen et al., 2009).

Since the first characterization of PSC populations, several other cell populations have been identified with *in-vitro* clonogenic potential such as those expressing NESTIN, PROP1, PRX1/2, GFRα2 or CXCR4 (Garcia-Lavandeira et al., 2009; Gleiberman et al., 2008; Higuchi et al., 2014; Horiguchi et al., 2012; Nomura et al., 2009; Rizzoti et al., 2013). NESTIN has been shown to have overlapping expression with SOX2+ cells in the marginal zone (MZ) of the anterior pituitary (AP) and in pituispheres. However, expression of NESTIN is not restricted to PSCs and is heterogeneous, including non-hormonal pituitary cell types (Krylyshkina et al., 2005; Vankelecom, 2007). Expression of PROP1, an essential embryonic pituitary transcription factor, has been confirmed in adult PSCs (Garcia-Lavandeira et al., 2009; Yoshida et al., 2009). Additionally, it has been shown that expression of SOX2+/PROP1+ must be downregulated postnatally for hormonal cell differentiation (Chen et al., 2009; Fauquier et al., 2008; Gremeaux et al., 2012; Yoshida et al., 2009). Recently, it was also shown that PROP1 expression is required to maintain a normal pool of PSCs both embryonically and postnatally by orchestrating an EMT-like process (Pérez Millán et al., 2016). PRX1 and 2 are paired related homeodomain proteins that have been identified in the PSC side populations, including a population of SOX2+/PROP1+ periluminal embryonic pituitary cells (Susa et al., 2012), and it has been suggested that expression of PRX1/2 is essential for the maintenance and proliferation Rathke's pouch progenitor cells up until differentiation (Vankelecom, 2010). The chemokine receptor CXCR4 has been found to be expressed in FS cells and in other cells of the AP (Barbieri et al., 2007; Horiguchi et al., 2012). In addition, CXCR4 and its ligand CXCL12 were identified in the stem cell-enriched side population of the mouse pituitary gland (Vankelecom, 2010). The well-known chemotactic and trophic properties of CXCR4 and its ligand suggest a possible role for migration and maintenance of PSCs (Barbieri et al., 2007). Finally, a population expressing GFRα2, RET and PROP1 (referred to as "GPS" cells), as well as other SC markers, has been proposed to form part of a SC niche located in the MZ of the pituitary lumen and it has been suggested they may play a role in PSC regulation, including cell survival or structural guidance of these cells (Garcia-Lavandeira et al., 2009). This putative SC population was identified in both human and mouse pituitaries and expresses progenitor markers SOX2, SOX9 and OCT4. Half of the GPS population also expressed S100β. Additionally, when GFRα2+ cells were isolated from mouse pituitaries they could be cultured as spheres and could be differentiated into hormone-producing cell types. Furthermore, an appealing proposal involving the GPS population was recently introduced by Garcia-Lavendeira et al., and concerns a mechanism for the regulation of pituitary cell turnover (a largely unexplored subject) through the apoptosis of differentiated cell types by the RET/Pit-1/Arf/p53 pathway (Garcia-Lavandeira et al., 2015).

SOX2 expression has been shown to be exclusively expressed in both adherent and non-adherent PSC colonies in clonal culture (Andoniadou et al., 2012; Fauquier et al., 2008). SOX2 is a pluripotency-associated factor expressed in embryonic stem cells, induced pluripotent cells and shown to be important for the maintenance of stem cells in various adult tissues. A subset of SOX2 PSCs of the postnatal AP resides in the MZ and are thought to be part of the presumptive progenitor/stem cell niche. SOX2+ cells are also expressed throughout the AP parenchyma, where SOX2 expression does not overlap with differentiated hormonal cells. However, only a small proportion of postnatal SOX2+ cells retain self-renewing clonogenic potential when sorted and maintained in clonal culture (2.5–5%) (Andoniadou et al., 2013). This could suggest that not all SOX2+ cells of the AP retain stem cell capacity. Furthermore, lineage tracing of SOX2+ cells in the postnatal AP revealed their long-term persistence and multipotent differentiation capability, as they were shown to give rise to all AP hormone-producing cell types throughout life (Andoniadou et al., 2013; Rizzoti et al., 2013). Interestingly, SOX2 expression mostly overlaps with SOX9, and partially overlaps with S100β in the AP marginal zone, while it was shown that sorted S100β+ cells possess increased clonogenic potential (Rizzoti et al., 2013). Therefore, current evidence suggests SOX2+/S100β+ cells represent a type of adult PSCs.

Finally, there is evidence supporting the existence of PSCs in humans. In one study, isolation and culture of pituispheres derived from 5 patients after autopsy was reported. The resulting pituispheres were shown to express NESTIN, pituitary-specific markers *LHX3* and *PITX2* after several passages, as well as expressing all six pituitary hormones (Weiss et al., 2009). In another study, authors identified a GPS population similar to that shown to exist in mouse pituitaries. This population was located in the MZ and showed the expression of stemness-associated factors SOX2, SOX9, OCT4, KLF4 and GFRA3 (Garcia-Lavandeira et al., 2011).

In summary, there has been extensive characterization of candidate PSC populations which express stem cell associated markers and display stem cell related features. However, "stemness" marker expression alone, is not sufficient for defining a SC population. For example, S100β+ cells appear to be a heterogeneous population encompassing folliculostellate cells and also subsets of stem cells, committed progenitors and pituicytes of the postnatal pituitary (Sato et al., 2005; Soji et al., 1997; Yoshida et al., 2016). Furthermore, expression of NESTIN is not restricted to PSCs, but has been shown to be expressed in pericytes and non-hormonal pituitary cell types (Galichet et al., 2010; Krylyshkina et al., 2005; Vankelecom, 2007). Therefore, further experiments are needed in order to properly assert putative S100 + and NESTIN + subsets as PSCs. Ultimately, long-term lineage tracing *in vivo* of these populations would demonstrate their persistence and their ability to give rise to differentiated progeny.

1.2. Cancer stem cells and the cancer stem cell model

Similar to the presence of tissue specific stem cells, experimental evidence has revealed the presence of multipotent cells thought to continuously propagate cancer cells within tumors (Kreso and Dick, 2014; Nakanishi et al., 2013; Schepers et al., 2012; Zomer et al., 2013). These cancer stem cells (CSCs) possess many similarities to normal tissue specific stem cells, as they retain the capability to self-renew, give rise to cancer cell progenitors with multipotent differentiation potential, can be slow-cycling/