The human pituitary master gland stripped to single-cell resolution

*Emma Laporte 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), Leuven, Belgium

*Corresponding author: hugo.vankelecom@kuleuven.be

Orcids:
Hugo Vankelecom: 0000-0002-2251-7284
Emma Laporte: 0000-0003-0799-3116

Stem cells in the pituitary master gland remain mystifying, moreover barely approached in humans. A new study applied transcriptome and chromatin accessibility profiling at single-cell resolution on human postmortem pituitaries of different ages, thereby providing novel insights into heterogeneity and regulatory mechanisms of the projected human pituitary stem cell population.

Refers to Zhang, Z. et al. Single nucleus transcriptome and chromatin accessibility of postmortem human pituitaries reveal diverse stem cell regulatory mechanisms. *Cell Rep.* **38**, 110467 (2022). https://doi.org/10.1016/j.celrep.2022.110467.[1]

The pituitary "master" gland positions at the regulatory summit of our endocrine system, punctiliously orchestrating fundamental physiological processes including growth, stress, metabolism and reproduction. In addition to specialized hormone-producing cell types, the (mouse) gland contains a population of stem cells whose biological passport remains highly blurred, despite their discovery almost 20 years ago[2]. Current knowledge on pituitary biology and stem cells primarily derives from experimental animals. Indeed, the human pituitary is hidden from detailed portraying due to its challenging accessibility. A new study[1] started to tackle this lacuna by bioinformatically profiling postmortem human pituitaries at single-cell (sc) resolution. The exploration especially zoomed in on the population of cells prospectively designated as stem cells based on mouse findings, and started to strip their cellular and regulatory complexity. Such endeavours are essential to fully grasp the biology of this key endocrine gland, and to eventually reach (stem cell) regenerative tactics for treating pituitary defects, which cause burdening endocrine impacts.

Zhang and colleagues[1] granularly profiled both transcriptome and chromatin accessibility of human pituitary by applying single-nucleus (sn) RNA- and ATAC-sequencing on flash-frozen glands, archived for several years (Fig. 1). Their enterprise entirely fits in the contemporary striving of organ sc atlasing. Also the pituitary lately receives a burst of interest, not only in rodents[3–5] but also in humans with the recent sc portraying of fetal pituitary[6]. The present study for the first time profiled *postnatal* human pituitaries, representing different ages (pediatric, adult, aged), at one sample of each gender per age. Although capturing a broad view, the approach at once entails some caveat about the robustness of the age- and gender-related findings. In particular, the pediatric ages (2 vs. 8 years) are quite different as well as ethnicity and cause of death, while medication history is mainly undefined. Endocrine glands and axes are known to be highly sensitive to (patho-)biological parameters. Moving now to a proactive approach in which patients are included using well-defined criteria will help to gain in robustness and

depth. Moreover, instead of analyzing only one fragment per pituitary[1], whole-gland pulverization (as done for one pituitary[1]) will strengthen the outcome generalizability.

The study zoomed in on the projected stem cell population, thereby epitomizing the booming interest in these mysterious cells[3-6]. Authors designated the stem cell population mainly by *SOX9* expression, whereas in rodents, pituitary stem cells are primarily assigned through the overarching SOX2[3–5] while SOX9 expression may indicate a further developed (progenitor) stage[7], although not clearly settled yet. Then, authors subclustered the projected stem cell compartment into eight subpopulations (Fig. 1). Unfortunately, the subgrouping foundation (i.e. discriminating differentially expressed genes) is not provided, making it tricky to understand the authors' functional interpretation. Sc transcriptome-based pituitary stem cell heterogeneity was already reported earlier in mouse, revealing two major subclusters diverging in *SOX2* and *SOX9* expression levels and displaying specific markers (e.g. TROP2)[4,5]. It would be informative to map the human subclusters on the reported mouse subpopulations. Interestingly, the present study proposed new stem cell markers genes[1]. Their functional annotation was not included which would rationally back translational efforts from *in silico* to real-life implications (e.g. using mouse or organoid models)[4,5]. Remarkably, the projected stem cell population also contained "committing" or "likely committing" progenitor cells, marked by *GATA3* and *POU1F1/POMC* expression, respectively, which suggests that clustering based on *SOX9* expression may have expanded the genuine (i.e. uncommitted) stem cell population with further evolved progenitor cells. Pseudotime trajectory analysis between uncommitted and committing cells would further support the authors' functional interpretations. A surprising finding is that the "(likely) committing" progenitor cells increase from pediatric to older age, which feels counterintuitive and is different from mouse pituitary in which cell remodeling activity contrarily declines from neonatal to adult age[4,5,8,9]. Caution is warranted when extracting quantitative data from very low abundance cells (and limited sample numbers), and one should keep in mind that hormone expression increases toward adulthood, entailing higher risks of ambient transcripts. It is also puzzling why *GATA3* and not *GATA2* was considered, the latter being much more supported as lineage commitment driver in pituitary development[10]. Nonetheless, the finding of "(likely) committing" progenitor cells is appealing, and further deep exploration will open up the still enigmatic developmental process from pituitary stem to progenitor cells. Further puzzling findings, not understood yet, are the completely different abundance and cluster profile of uncommitted stem cells between males and females, and the absence of proliferation in the stem cell population, even at very young age which is different from (neonatal) mouse[5,9]. Pseudotime trajectory analysis, while generating expected projections from pediatric to adult and aged stem cells, revealed large gaps between early and later age, pointing to the need for intermediate stages. Integration with fetal pituitary data[6] would give insight into the earliest stem cell paths. Also, puberty, entailing active pituitary cell remodeling to support body maturation, would represent an attractive transitional stage. Finally, aging-associated stem cell changes are already observed at middle-age in mice[4], a phase which could also be included. Interestingly, using an advanced bioinformatic tool, the authors identified a specific chromatin module with decreased accessibility during aging in all pituitary cell types, among others associated with cell cycle genes. The same tool uncovered a stem cell-specific gene expression module over the ages and sexes, interestingly not advancing "usual suspect" but novel genes (Fig. 1). This original computational tool will now allow to deeply infer cell type- and age-specific determinative modules, which would be interesting to benchmark with common validated bioinformatic tools such as regulon identification by SCENIC[4,5]. Through another innovative *in silico* analysis tool, novel insights were gained in the mechanisms (i.e. transcription factors and chromatin state) that regulate stem/progenitor cell gene

expression (Fig. 1). Linear framework modelling inferred that distinct regulatory mechanisms could play, either driven by specific transcription factors (e.g. for stem cell-specific *SOX2* expression), by chromatin accessibility of specific regulatory sites (e.g. for *GATA3* expression within the "committing" cells), or a combination of both. Application of this original tool will generate a wealth of novel insights into the regulatory mechanisms underlying pituitary stem/progenitor (and endocrine) cell identity. For instance, authors inferred a new candidate transcriptional regulator of POMC lineage commitment (*MNX1*), and located previously unexplored regulatory chromatin domains of stem/progenitor cell genes.

Taken together, the present study represents a technical tour de force and milestone in pituitary research, providing the first-ever single-cell atlas of postnatal human gland, as well as inventive (although intricate) computational tools. An important strength is that authors open up their rich resource to the research community in an interactive web portal (snpituitaryatlas.princeton.edu) which will allow to granularly strip the human pituitary cell by cell. Functional translation of the *in silico* findings is now essential to move the pituitary (stem cell) field forward to deep basic insights and clinical perspectives. From this[1] and recent studies[4,5,8], it is surfacing that "*the*" pituitary stem cell does not exist but that several (fluidic?) subtypes or states with distinct functions in turnover, repair and regulatory signaling may exist. The present study is considered an important starting point to comprehensively portray human pituitary stem cell biology across life including conditions of active tissue remodeling (like puberty), and of disruption following trauma or tumorigenesis, highly prevalent disorders.

Fig. 1. Graphical summary of setup and consideration of the human pituitary sn profiling study[1]. (sn, single nucleus; sc, single cell; TF, transcription factor; G, gene). Created with BioRender.com

References

1. Zhang, Z. *et al.* Single nucleus transcriptome and chromatin accessibility of postmortem human pituitaries reveal diverse stem cell regulatory mechanisms. *Cell Rep.* **38**, 110467 (2022).
2. Chen, J. *et al.* The adult pituitary contains a cell population displaying stem/progenitor cell and early-embryonic characteristics. *Endocrinology* **146**, 3985–3998 (2005).
3. Ruf-Zamojski, F. *et al.* Single nucleus multi-omics regulatory landscape of the murine pituitary. *Nat. Commun.* **12**, 2677 (2021).
4. Vennekens, A. *et al.* Interleukin-6 is an activator of pituitary stem cells upon local damage, a competence quenched in the aging gland. *Proc. Natl. Acad. Sci. U. S. A.* **118**, e2100052118 (2021).
5. Laporte, E. *et al.* Decoding the activated stem cell phenotype of the vividly maturing neonatal pituitary. Preprint at bioRxiv https://doi.org/10.1101/2022.01.18.476723 (2022).
6. Zhang, S. *et al.* Single-cell transcriptomics identifies divergent developmental lineage trajectories during human pituitary development. *Nat. Commun.* **11**, 5275 (2020).
7. Fauquier, T., Rizzoti, K., Dattani, M., Lovell-Badge, R. & Robinson, I. C. A. F. SOX2-expressing progenitor cells generate all of the major cell types in the adult mouse pituitary gland. *Proc. Natl. Acad. Sci.* **105**, 2907–2912 (2008).
8. Russell, J. P. *et al.* Pituitary stem cells produce paracrine WNT signals to control the expansion of their descendant progenitor cells. *Elife* **10**, e59142 (2021).
9. Zhu, X., Tollkuhn, J., Taylor, H. & Rosenfeld, M. G. Notch-dependent pituitary SOX2+ stem cells exhibit a timed functional extinction in regulation of the postnatal gland. *Stem Cell Reports* **5**, 1196–1209 (2015).
10. Zhu, X., Gleiberman, A. S. & Rosenfeld, M. G. Molecular physiology of pituitary development:

signaling and transcriptional networks. *Physiol. Rev.* **87**, 933–963 (2007).

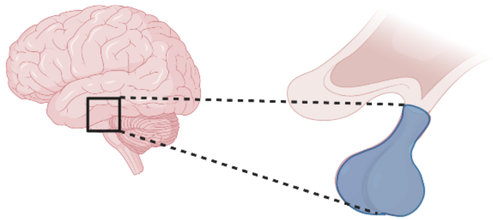
Acknowledgement (optional)
We thank Lara Hemeryck for her major help in creating the figure.

Competing interests
The authors declare no competing interests.

Related links (optional)
snpituitaryatlas.princeton.edu

Study setup

postmortem human pituitary

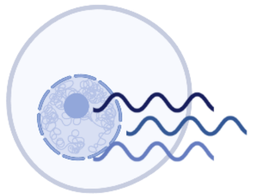


pediatric

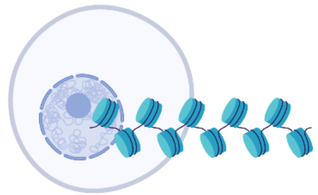
adult

aged

gene expression (snRNA-seq)



chromatin accessibility (snATAC-seq)



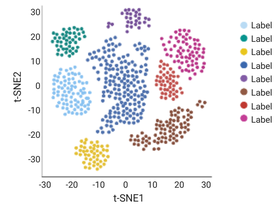
Innovation sequencing of human postnatal pituitary at sc resolution

Strength sn data provided in interactive web portal

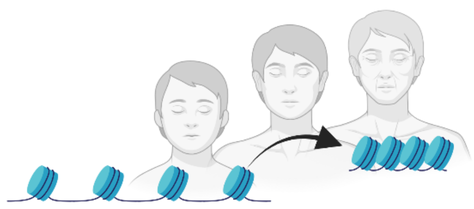
Limitation low number and unwell defined or matching samples

Innovative findings

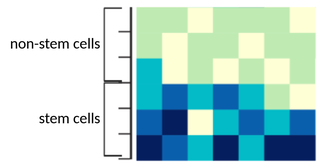
subclustering of (projected) stem cells



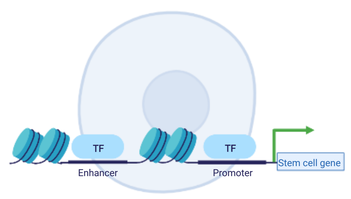
age-specific chromatin module



stem cell-specific gene expression module

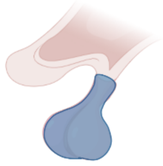


regulation of stem cell gene expression



Future needs

advanced sample selection



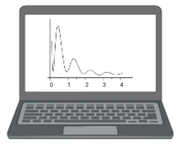
well-defined inclusion criteria

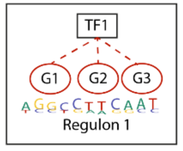
increased number/age

intermediate stages

bioinformatic validation

example: regulon analysis





functional validation

mouse models

pituitary organoids