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BRF1-Mediated Paracrine Signalling by a Subset of SOX2-Expressing Stem Cells is Required for Normal Development of the Stem Cell Compartment and Terminal Differentiation of Pituitary Committed Progenitors

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Introduction: Hormone-producing pituitary cell lineages are derived from a population of embryonic precursors expressing SOX2. These cells maintain multipotency into early postnatal life, acting as the resident population of pituitary stem cells (PSCs) and contributing extensively to all the endocrine cell lineages. In addition to this direct contribution to pituitary turnover, paracrine signalling from PSCs has been shown to be important for cell proliferation of neighbouring progenitors (PMC7803373). It is not known if SOX2+ PSCs are involved in the regulation of additional cell attributes during normal physiology and if there is functional heterogeneity among the SOX2+ PSC population.

Experimental Methods: We have carried out single-cell RNA-Sequencing of SOX2+ PSCs from Sox2Egfp/+ mouse pituitaries at three postnatal stages from P3 to P56 and used computational approaches to analyse their molecular signatures. A novel conditional mouse model expressing a constitutively active mutant form of the RNA binding factor BRF1 (R26stop-mBRF1) has been used to attenuate the expression of several cytokines and chemokines in SOX2+ cells embryonically and postnatally (PMC4589897).

Results: We show that the SOX2+ PSC population consists of three subgroups (SC1, SC2 and SC3). We reveal that SC1-SC2 express abundant cytokines and secreted factors, suggesting paracrine function. In contrast, SC3 is characterised by robust expression of Lef1, is identified as a committing PSC cluster, and its presence diminishes with age. Key markers of PSC clusters SC1-SC2 include the RNA binding factor BRF1. We show that BRF1 is highly expressed in PSCs and validate its expression by immunohistochemistry in both mouse and human pituitaries. Secondly, we show that the dysregulation of BRF1 in embryonic SOX2+ cells using the Hesx1-Cre driver (PMC3461924) results in pituitary hypoplasia and severe hypopituitarism due to a failure of the PIT1 and SF1 cell-lineage committed progenitors to terminally differentiate into hormone-producing cells. Additionally, there is a significant reduction of the stem cell compartment, manifested by lower numbers of SOX2/SOX9+ stem cells. This phenotype is recapitulated when using a Sox2-CreERT2 driver (PMID24094324). The differentiation failure can be rescued in vitro through co-culture of mutant cells with wild-type stem cells, as well as in vivo, in mutant pituitaries where activation of constitutively active BRF1 is restricted to few SOX2+ PSCs in a mosaic manner. Finally, we identify key ligands underlying this differentiation phenotype and demonstrate a partial restoration of terminal

differentiation in the mutant, when cultured in the presence of these ligands.

Conclusion: We provide evidence indicating the presence of functionally distinct groups of SOX2+ pituitary stem cells and reveal a critical role for a PSC subset in the development of the stem cell compartment and in driving terminal differentiation of committed progenitors.

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