SOX2 is required independently in both stem and differentiated cells for pituitary tumorigenesis in p27-null mice

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P27, a cell cycle inhibitor, is also able to drive repression of Sox2. This interaction plays a crucial role during development of p27−/− pituitary tumors because loss of one copy of Sox2 impairs tumorigenesis [H. Li et al., Cell Stem Cell 11, 845–852 (2012)]. However, Sox2 is expressed in both endocrine and stem cells (SCs), and its contribution to tumorigenesis in either cell type is unknown. We have thus explored the cellular origin and mechanisms underlying endocrine tumorigenesis in p27−/− mice. We found that pituitary hyperplasia is associated with reduced cellular differentiation, in parallel with increased levels of Sox2 in stem and endocrine cells. Using conditional loss-of-function and lineage tracing approaches, we show that Sox2 is required cell autonomously in p27−/− endocrine cells for these to give rise to tumors, and in SCs for promotion of tumorigenesis. This is supported by studies deleting the Sox2 regulatory region 2 (Smr2), the target of P27 repressive action. Single cell transcriptomic analysis further reveals that activation of a SOX2-dependent MAPK pathway in SCs is important for tumorigenesis. Altogether, our data highlight different aspects of the role of Sox2 following loss of p27, according to cellular context, and uncover an unexpected SOX2-dependent tumor-promoting role for SCs. Our results imply that targeting SCs, in addition to tumor cells, may represent an efficient antitumoral strategy in certain contexts.

Significance

Tumors frequently originate due to dysfunction of genes acting cell autonomously. However, in rarer cases, cells acquiring mutations may not form the tumor themselves, but instead induce others to do so (1, 2). Regardless of their origin, tumors often display considerable cellular heterogeneity, which can include the presence of cancer stem cells (CSCs), a subpopulation of undifferentiated tumoral cells that may originate from resident tissue stem cells (SCs), and fuel growth and recurrence of the tumor (3). Understanding the precise origins and mechanisms of tumorigenesis is important to decipher therapeutic strategies.

The pituitary is a small endocrine gland, located just under the brain. In the mouse, the anterior lobe (AL) is highly vascularized and contains most endocrine cell populations, while the avascular intermediate lobe (IL) is populated by a single endocrine population, the melanotrophs. These secrete melanocyte stimulating hormone (MSH) which is processed from its precursor pro-opio-melanocortin (POMC), whereas corticotrophs in the AL cleave POMC into adrenocorticotropic hormone (ACTH). Expression of Pomp is controlled in both cell types by the T-box factor TBX19 (4). In the embryo, the pioneer transcription factor PAX7 controls acquisition of the melanotroph vs. corticotroph fate (5). Melanotrophs are quiescent, in contrast with AL endocrine cells (6); however, they are particularly sensitive to mutations affecting cell cycle regulators (7). In mice deleted for p27, encoding a protein best known for its negative cell cycle regulatory role (8), tumorigenesis in mature animals is the hallmark of its pleiotropic effects (9–12). In the pituitary, this happens specifically in the IL, while the AL is largely unaffected (12). In the developing murine pituitary, P27, along with P57, is necessary for cell cycle exit in differentiating endocrine cells (13). However, its role is not limited to cell cycle regulation, because in Cyclin D1−/−; p27−/− compound mutants, pituitary hyperplasia is still present (14).

We have previously demonstrated that P27 can recruit cofactors to repress the expression of the transcription factor SOX2 during differentiation of induced pluripotent SCs (iPSCs) (15). SOX2 is an essential factor associated with stemness. It is highly expressed in many cancers and this is associated with poor prognosis (16–18). In vivo, the SOX2-P27 interaction is particularly relevant for the IL because deletion of one copy of Sox2 diminishes tumorigenesis in p27−/− mice (15).

The cellular specificity of this interaction may be explained by the restricted pattern of expression of Sox2 in pituitary endocrine cells; melanotrophs are the only endocrine cell type to express Sox2 (19). However, Sox2 is also expressed in adult pituitary SCs (2, 20, 21). Interestingly, in p27−/− mice, the thickness of the SOX2+ve SC layer is increased (15). Therefore, while the importance of the SOX2-P27 interaction for IL tumorigenesis is apparent, the modalities of this interaction and the roles of SOX2 in SCs and melanotrophs are unknown.
In this study, we have developed complementary genetic approaches to characterize the SOX2-P27 interaction and understand how p27-/- IL tumorigenesis develops. As p27-/- ILs become hyperplastic, melanotrophs tend to lose differentiated features and display increased levels of SOX2 expression in parallel with SCs. To determine the cellular origin of the tumors, and explore SOX2 function, we have performed lineage tracing experiments and deleted the gene separately in melanotrophs and SCs. This allows us to demonstrate that SOX2 is required in melanotrophs, and also, independently, in SCs for tumorigenesis. Deletion of the Sox2 regulatory region 2 (Srr2), the target of P27 repressive action, further supports the protumorigenic role of SOX2 in SCs. Finally, single cell transcriptomic analyses reveal that activation of a SOX2-dependent MAPK pathway in SCs is important for tumorigenesis. In conclusion, our study shows that, following loss of p27, SOX2 orchestrates tumorigenesis both cell autonomously and noncell autonomously, uncovering an unexpected role for SCs in IL tumorigenesis. This suggests that SCs, or the relevant signaling molecules, represent good antitumoral targets, in addition to tumor cells themselves.

Results
Characterization of Intermediate Lobe Tumors in p27-/- Pituitaries. We first examined P27 by immunofluorescence and observed widespread nuclear expression throughout the anterior pituitary with varying levels of expression (Fig. 1A), in agreement with fluctuations of P27 according to the cell cycle. Anterior endocrine and stem cells proliferate rarely (20, 22) and P27 levels are relatively high in these. However, even though IL melanotrophs have been shown to be quiescent (6), P27 immunostaining is much weaker compared with the AL and SCs. We then examined SOX2, which is expressed both in SCs (18), and, at lower levels, in melanotrophs (19) (Fig. 1A). Coexpression of SOX2 and its repressor P27 probably reflects complex regulatory mechanisms controlling Sox2 expression and/or availability of corepressors recruited by P27 mechanisms. In p27-/- pituitaries, the SC layer appears thicker in mutants (15). In agreement with a repressive action of P27 on Sox2 expression (15), SOX2/Sox2 levels are significantly up-regulated both in SCs and melanotrophs in p27-/- pituitaries (Fig. 1 A and B and SI Appendix, Fig. S1 A and B).

To better initiate and develop of the tumors in p27-/- mice, we performed transcriptomic analyses from dissected ILs (Fig. 1 C–E and SI Appendix, Fig. S1C). Before tumorigenesis, at 2 mo of age, we detected 250 differentially expressed genes (DEGs) between p27-/- and control samples. In 7-mo-old animals, when hyperplasia is very clear, there were 3,736 DEGs (Dataset S1). We then performed a pathway analysis (Dataset S1). At both stages, up-regulation of cell cycle markers was observed, in agreement with the CDKI function of P27. We also observed down-regulation of genes associated with the cytoskeleton and cell adhesion which may reflect disruption of the direct interaction between P27 and RhoA (23). Furthermore, down-regulation of POMC and genes associated with its processing suggests an alteration of melanotroph function and/or identity in mutants.

We further investigated whether melanotroph function and/or identity have been altered, something that has not been reported previously (9–11). We observed a significant down-regulation of POMC processing in 2-mo-old mutants, while expression of Pox7 and of the POMC-lineage marker Tnxb (24) are reduced in 7-mo-old mutants (Dataset S1). We then quantified POMC/Pomc expression levels, and detected a down-regulation exclusively in p27-/- melanotrophs, postnatally, but prior to tumorigenesis (Fig. 1 F–H), while corticotrophs appear unaffected. In agreement with these data, radioimmunoassays show that levels of MSH are reduced while ACTH is unaffected in p27-/- pituitaries (Fig. 1H). This shows that the anterior pituitary is affected by loss of p27. TBX19 is a direct upstream regulator required for Pome expression (25). In agreement with a reduction in Pome levels, Thy19 levels are significantly reduced in the IL, while Pax7 levels (5) also appear reduced, although not to a statistically significant degree in 2- to 3-mo-old animals (Fig. 1I).

Moreover, p27-/- melanotrophs are smaller than those in wild-type (WT) pituitaries (Fig. 1J). Altogether, these results suggest that melanotroph identity and/or postnatal maturation are altered in p27-/- mutants, and this occurs before tumors develop.

We then examined cell proliferation by quantifying incorporation of 5-ethyl-2'-deoxyuridine (EdU) in the IL, dorsal (flanking IL), and ventral SC layer (flanking the AL) (Fig. 1 K–N). In E18.5 embryos, proliferation tends to increase in both IL and its flanking SC layer in the mutants; however, this is not statistically significant (Fig. 1K). In contrast, in 1-mo-old mutant animals, we observed a significant increase in proliferation of both melanotrophs and adjacent SCs (Fig. 1 L–N). In 7-mo-old animals, increased proliferation was observed in all compartments of the mutants compared to controls (Fig. 1M), in agreement with the transcriptomic analysis results (Fig. 1 E and F).

To further test the proliferative capacity of SCs, we performed pituisphere assays (20) separately from anterior and intermediate lobe cells and observed an increased sphere-forming ability from p27-/- IL cells (Fig. 1O). Altogether these results suggest that while both SC layers behave abnormally in p27-/- pituitaries, the SCs in direct contact with melanotrophs are more affected, suggesting that there may be some noncell autonomous consequences to the loss of p27.

HIF-1 activation is associated with tumor angiogenesis and its targets are up-regulated in 7-mo-old p27-/- IL transcriptomes (Fig. 1E). Moreover, p27-/- IL tumors are hemorrhagic (9). We thus examined CD31 expression and observed development of ectopic blood vessels in juvenile p27-/- ILs, coincident with the abnormal increase in rates of cell proliferation (Fig. 1P).

Furthermore, p27-/- mice are affected by gigantism, which has been attributed to general loss of cell cycle repression (9–11); however, an additional explanation could be the significant increase in pituitary growth hormone (GH) content we observed (SI Appendix, Fig. S1D). While anterior lobe tumors are not observed in mice (12), in both rats and humans carrying mutations in p27 GH adenomas are observed (26). It would be of interest to examine whether in mice somatotroph numbers are affected.

In conclusion, p27 deletion results in increased cell proliferation and ectopic vascularization in the IL. While P27 starts to be expressed in differentiating embryonic endocrine cells (13), defects consecutive to its loss appear to only become significant postnatally, both in melanotrophs and SCs. In addition, melanotroph identity and/or maturation appears affected in mutants.

Characterization of the SOX2-P27 Interaction in p27-/- Mutants. Deletion of one copy of Sox2 is sufficient to dramatically reduce occurrence of IL tumors (Fig. 2 A and SI Appendix, Fig. S2B) (15); however, hyperplasia is still observed in p27-/-; Sox2-/- IL (Fig. 2A). We further characterized the SOX2-P27 interaction by demonstrating that survival of p27-/- mutants (12.5 mo, n = 28) was significantly improved by Sox2 haploinsufficiency (17.1 mo, n = 10) (SI Appendix, Fig. S2A). However, in agreement with cell-type specificity for the SOX2-P27 interaction, while pituitary and duodenum tumors were sensitive to Sox2 dosage, lung tumor development was unaffected (SI Appendix, Fig. S2B).

We then performed comparisons of the transcriptomes of wild type, p27-/-, and p27-/-; Sox2-/- IL (Fig. 2B and SI Appendix, Fig. S2C). To pinpoint genes underlying IL tumor impairment in compound mutants, and conversely those associated with higher levels of SOX2 and tumorigenesis, we focused our analyses on those that were exclusively differentially expressed in p27-/-, and
pituitaries. (SOX2;PAX7 double positive in WT (132
± 0.20) while in p27−/− it becomes similar (0.91 ± 0.16); *P = 0.019, n = 4–6 in each group). (C and D) Bulk RNAseq selected pathway analysis in 2-mo-old IL (p27−/− n = 2 vs. WT n = 3, adjusted P value <0.05) (C), and in 7-mo-old hyperplastic IL (p27−/− n = 4 vs. WT n = 2) (D). Blue tones show down-regulated pathways while red-yellow represent up-regulation. (E) Heatmap of representative genes expression from pathways shown in C and D. (F) POMC immunofluorescence in adult pituitaries. (G) Quantification of POMC intensity in melanotrophs in relation to corticotrophs at E18.5 in WT (1.01 ± 0.17, n = 5) and p27−/− (1.10 ± 0.19, n = 5) and in 2- to 4-mo-old animals. Levels are significantly reduced in mutants (WT: 2.42 ± 0.76, n = 3; p27−/−: 1.1 ± 0.07, n = 4, *P = 0.0286). (H) Radioimmunoassays for MSH and ACTH. p27−/− pituitaries contain less MSH (*P = 0.0306, n = 4 in each group) (Left graph); ACTH levels are not affected (Right graph). (I) RT-qPCR in 2- to 3-mo-old p27−/− and WT. Pomc (WT: 1.03 ± 0.27, n = 3; p27−/−: 0.32 ± 0.09, n = 3, **P = 0.0013) and Tbx19 levels (WT: 0.94 ± 0.08, n = 3; p27−/−: 0.30 ± 0.10, n = 3, **P = 0.0071) were reduced in mutants. (J) Average cell size of melanotrophs. p27−/− melanotrophs (99 ± 5) are smaller compared to WT (132 ± 10, n = 3, ***P = 0.0007). (K–M) Analysis of cell proliferation. EdU incorporation was quantified in melanotrophs (EdU;SOX2;PAX7 triple positive/ SOX2;PAX7 double positive in L or EdU;SOX2 double positive; SOX9 negative/ SOX2 positive; SOX9 negative positive cells in M and N) and SCs (EdU;SOX2 double positive; PAX7 negative/ SOX2 positive; PAX7 negative cells in L or EdU;SOX2;SOX9 triple positive/ SOX2; SOX9 double positive in M and N). SCs were distinguished as ventral (flanking AL) or dorsal (flanking IL). In 1-mo-old mice (M), cell proliferation increases in p27−/− melanotrophs and dorsal SCs (n = 3–4 mice/ genotype, melanotrophs *P = 0.0235, dorsal cleft *P = 0.0419). In 7-mo-old mice, (N) cell proliferation increases in melanotrophs and both dorsal and ventral SCs (n = 3 mice/ genotype, melanotrophs *P = 0.0187, dorsal cleft *P = 0.0085, ventral cleft *P = 0.0229). (O) SOX2; SOX9, and EdU triple staining in 1-mo-old pituitaries. Immunofluorescence for SOX2 on IL pituispheres from 2- to 3-mo-old animals. The proportion of pituispheres is increased in mutant IL (**P < 0.0001, n = 6–8 in each group). (P) Immunofluorescence for CD31 in 1-mo-old pituitaries. p27−/− ILs show ectopic blood vessels (arrows). ns, nonsignificant.

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obtained a list of pathways comparable in our two analyses (Figs. 1C and D and Datasets S1 and S2). This implies that in the IL, the different processes affected by P27 loss are restored to normal levels upon deletion of one copy of Sox2. This thus suggests that SOX2 is not required for a specific aspect of tumorigenesis in p27−/− mice, but rather that it has a broad and therefore probably early role in tumor formation. Reduced Sox2 dosage is associated with a decrease in proliferation and HIF1-target gene expression, in agreement with less angiogenesis in these samples, suggesting a broader role of SOX2 than observed for IL tumorigenesis in p27-null mice.

Requirement for SOX2 in p27−/− Melanotrophs. Conditional deletion of p27 in melanotrophs results in formation of tumors; therefore, transformation is cell autonomous in this context (29). To better characterize the role of SOX2 for tumorigenesis, we deleted one copy of the gene exclusively in melanotrophs in p27−/− mutants. We initially used Pomc-CreERT2 which displays a mosaic pattern of recombination in the IL (SI Appendix, Fig. S4A). To look at heterozygosity for Sox2, we induced Cre activity in 4-wk-old Pomc-CreERT2; Sox2+/-; p27−/− animals and controls, which were then examined 6 to 8 mo later. Tumor development was unaffected by the mosaic deletion of one copy of Sox2 in p27−/− melanotrophs (Fig. 4A). In agreement with this observation, proliferation was unaffected by Sox2 reduction, as we observed a similar increase in EdU incorporation in Pomc-CreERT2; Sox2+/-; p27−/− and Pomc-CreERT2; p27−/− IL compared to controls (Fig. 4A and B). To analyze more directly the effect of the loss of one copy of Sox2 on melanotrophs' proliferative ability, we analyzed EdU incorporation in Pomc-CreERT2; Sox2+/-; p27−/−; Rosa26RFP/VP samples. We compared the percentages of eYFP; PAX7; EdU triple positive and eYFP negative, PAX7; EdU double positive melanotrophs, assuming that Rosa26RFP/VP recombination also reflected Sox2 heterozygous deletion. We observed a significant reduction of EdU incorporation in eYFP-positive vs. negative cells (Fig. 4 C and D). Therefore, reduction of Sox2 dosage in p27−/− melanotrophs diminishes their proliferative capacity.

We then hypothesized that a more efficient Cre-driver may block tumorigenesis; we therefore generated Pomc-Cre; Sox2+/-; p27−/− animals. We showed previously that Sox2 is required for acquisition of melanotroph identity; however, homozygous deletion of the gene once melanotrophs have up-regulated PAX7 does not have an apparent effect on cell fate acquisition (19). There were no IL tumors or signs of hyperplasia in any 4- to 7-mo-old Pomc-Cre; Sox2−/−; p27−/− animals (Fig. 4 E–G and SI Appendix, Fig. S4B). Moreover POMC staining intensity was increased in Pomc-Cre; Sox2−/−; p27−/− melanotrophs compared to p27−/+ samples (Fig. 4H), indicating restoration of a normal melanotroph phenotype. This demonstrates that Sox2 is required in melanotrophs for P27 deletion to result in tumorigenesis. Strikingly, the morphology of IL appears completely normal in Pomc-Cre; Sox2−/−; p27−/− (Fig. 4 E and F) compared to p27−/−; Sox2−/− IL where hyperplasia is clear (Fig. 2A). This difference in phenotype is unexpected, because in both mutants the genotype of melanotrophs is the same: only one allele of Sox2 is active while p27 is deleted. However, different regulatory mechanisms may affect the levels of expression of the remaining copy of Sox2 in Pomc-Cre; Sox2+/-; p27−/− vs. p27−/− Sox2+/- melanotrophs. We thus quantified Sox2 levels and observed an up-regulation in p27−/−, and to a lesser extent in p27−/−; Sox2+/- samples (Fig. 4 I and J). In agreement with the absence of hyperplasia, we observed a further reduction in Sox2 expression in Pomc-Cre; Sox2−/− vs. p27−/−; Sox2−/− samples, suggesting that regulation of Sox2 is indeed modulated according to the spatiotemporal pattern of its deletion. Furthermore we also observed, albeit to a lesser extent, reduction of Sox2 expression in Pomc-Cre; Sox2−/−; p27−/− SCs.

In conclusion, while cell transformation in p27−/− melanotrophs is known to be cell autonomous (29), we demonstrate here that SOX2 is required in these cells for tumor development in p27−/− mutants. Moreover, reduction of Sox2 expression in SCs in Pomc-Cre; Sox2−/−; p27−/− compared to p27−/− suggests a noncell autonomous role and implies an interaction between melanotrophs and SCs.

SOX2 Is Required Independently in SCs for Induction of Melanotroph Tumors in p27−/− Animals. Because deletion of p27 induces up-regulation of SOX2 in SCs, we asked whether the SCs flanking the IL directly give rise to tumor cells or induce tumorigenesis. Given that, in the context of the pituitary, SOX9 is expressed...
Fig. 2. Deletion of one copy of Sox2 in p27−/− animals improves survival and results in reduction of cell proliferation and impairment of tumorigenesis. (A) Brightfield pictures taken at the same magnification of WT, Sox2+/−, p27−/−, and p27−/−; Sox2+− pituitaries (Left). Histological analysis of 10- to 12-mo-old WT, p27−/−, and p27−/−; Sox2+− pituitaries (Right). In p27−/−; Sox2+− IL tumorigenesis is impaired, but hyperplasia is observed. (B) Comparison modalities for RNAseq analysis of wild-type, p27−/−, and p27−/−; Sox2+− 2- to 3-mo-old IL samples (WT n = 4, p27−/− n = 3, p27−/−; Sox2+− n = 3, adjusted P value <0.05) (Left). Selected enriched pathways are represented (Center) and heatmap of representative gene expression from affected pathways (Right). (C) Analysis of cell proliferation. EdU incorporation was quantified in melanotrophs (EdU; SOX2 double positive; SOX9 negative/SOX2 positive; SOX9 negative cells) and SCs (EdU; SOX2; SOX9 triple positive/SOX2; SOX9 double positive cells). SCs were further distinguished as ventral or dorsal, respectively, as flanking the anterior or intermediate lobe. Deletion of one copy of Sox2 results in a reduction of cell proliferation compared to p27−/− samples in dorsal cleft SCs (n = 3 to 4 mice/ genotype, *P = 0.0208), melanotrophs. (D) SOX2 and EdU double staining in 2- to 3-mo-old WT, p27−/−, and p27−/−; Sox2+− animals. There is a clear reduction in SOX2 levels in p27−/−; Sox2+− ILs. Cleft and IL are outlined. (E) Proportion of pituispheres obtained from AL and IL WT, Sox2+−, p27−/−, and p27−/−; Sox2+− 2- to 3-mo-old animals. There is a reduction in the proportion of pituitaries formed in p27−/−; Sox2+− ILS compared to p27−/− samples. WT (1 ± 0.11, n = 3), Sox2+− (0.2 ± 0.08, n = 2), p27−/− (2.09 ± 0.31, n = 4), and p27−/−; Sox2+− (1 ± 0.52, n = 3), ****P < 0.0001 and **P = 0.0002; the reduction in Sox2+− samples in agreement with the slightly hypomorphic pituitaries observed in Sox2 heterozygous mice (25). (F) Quantitative analysis of melanotroph marker expression levels by RT-qPCR in 2- to 3-mo-old WT, Sox2+−, p27−/−, and p27−/−; Sox2+− IL. In p27−/−; Sox2+− animals, Tbx19 expression levels are restored to WT levels. Pomc; WT (n = 3) vs. p27−/−; Sox2+− (n = 3), *P = 0.03 and WT vs. p27−/− (n = 3), **P = 0.0022, Tbx19, WT vs. p27−/−; Sox2+− (n = 3, P = nonsignificant [ns]) and WT vs. p27−/− (n = 3, *P = 0.0169).
uniquely in the SCs (20), we first performed lineage tracing experiments using Sox9iresCreERT2 (30). Cre activity was induced in 4-wk-old Sox9iresCreERT2/+; Rosa26tdTomato reporter animals and pituitaries were harvested 6 to 11 mo later. In controls, the vast majority of eYFP-positive cells are SOX9-positive SCs (21) (Fig. 5A). In agreement with a low turnover of melanotrophs (6), very few eYFP-positive cells are present in the IL (Fig. 5A); these are negative for SOX9 and PAX7, where the latter indicates they are not melanotrophs. Their identity is unclear; they may originate from the pituitary SC layer or the posterior lobe where SOX9 is also expressed. In p27−/− animals we also observed that most eYFP-positive cells are SOX9 positive SCs. However, in contrast with Sox9iresCreERT2/+; Rosa26tdTomato/+ controls, we observed some rare eYFP; PAX7-positive melanotrophs. These results clearly demonstrate that SCs are not at the origin of the melanotroph tumors in p27−/− animals. However, tumorigenesis and/or lack of p27 in SCs, drives commitment of rare SCs toward the melanotroph lineage.

To investigate the role of SOX2 in SCs during tumorigenesis, one allele of the gene was removed exclusively in SCs in Sox9iresCreERT2/+; Sox2−/+; p27−/− animals. Cre activity was induced in 4-wk-old animals and pituitaries harvested when the animals were 6 to 12 mo old. Strikingly, only one among seven 12-mo-old Sox9iresCreERT2/+; Sox2−/+; p27−/− animals developed an IL tumor, while all p27−/− controls were affected (Fig. 5B, Upper and SI Appendix, Fig. S5A). The IL is however consistently hyperplastic in Sox9iresCreERT2/+; Sox2−/+; p27−/− animals, similarly to p27−/−; Sox2−/− mice. These results unequivocally demonstrate that SOX2 is required independently in SCs for tumors to develop in p27−/− IL. The thickness of the SOX2-positive SC layer appeared reduced in Sox9iresCreERT2/+; Sox2−/+; p27−/− compared to Sox9iresCreERT2/+; p27−/− (Fig. 5B, Lower), and there were fewer

Fig. 3. Deletion of Srr2 in p27−/− animals results in reduction of proliferation in SCs and impairment of tumorigenesis. (A) Schematic representation of the Sox2 murine locus and Srr2 deletions. Two pairs of sgRNA (pairs 1 and 2, protospacer adjacent motif sequence in orange) were designed. The primers used to genotype Srr2-deleted animals are marked by red arrows. Three founders deleted for Srr2 were used to generate stable lines (founders 1 through 3); the size of the deletion is indicated for each line. (B) Gel electrophoresis showing mosaic loss of Srr2 in founder mice. The number above each lane corresponds to a particular founder. (C) Brightfield pictures of WT, Srr2−/−, p27−/−, and p27−/−; Srr2−/− animals. Cre activity was induced in 4-wk-old animals and pituitaries harvested when the animals we also observed that most eYFP-positive cells are SOX9 positive SCs. However, in contrast with Sox9iresCreERT2/+; Rosa26tdTomato/+ controls, we observed some rare eYFP; PAX7-positive melanotrophs. These results clearly demonstrate that SCs are not at the origin of the melanotroph tumors in p27−/− animals. However, tumorigenesis and/or lack of p27 in SCs, drives commitment of rare SCs toward the melanotroph lineage.

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p27−/− animals. However, tumorigenesis and/or lack of p27 in SCs, drives commitment of rare SCs toward the melanotroph lineage.

To investigate the role of SOX2 in SCs during tumorigenesis, one allele of the gene was removed exclusively in SCs in Sox9iresCreERT2/+; Sox2−/+; p27−/− animals. Cre activity was induced in 4-wk-old animals and pituitaries harvested when the animals were 6 to 12 mo old. Strikingly, only one among seven 12-mo-old Sox9iresCreERT2/+; Sox2−/+; p27−/− animals developed an IL tumor, while all p27−/− controls were affected (Fig. 5B, Upper and SI Appendix, Fig. S5A). The IL is however consistently hyperplastic in Sox9iresCreERT2/+; Sox2−/+; p27−/− animals, similarly to p27−/−; Sox2−/− mice. These results unequivocally demonstrate that SOX2 is required independently in SCs for tumors to develop in p27−/− IL. The thickness of the SOX2-positive SC layer appeared reduced in Sox9iresCreERT2/+; Sox2−/+; p27−/− compared to Sox9iresCreERT2/+; p27−/− (Fig. 5B, Lower), and there were fewer
Fig. 4. Deletion of one copy of Sox2 in p27−/− melanotrophs prevents IL tumorigenesis. (A) Sox2 and EdU double staining in 6- to 8-mo-old Pomc-CreERT2, Pomc-CreERT2; p27−/−, and Pomc-CreERT2; Sox2fl/+, p27−/− pituitaries. (B) Analysis of cell proliferation. EdU incorporation was quantified in melanotrophs (EdU; SOX2 low; POMC triple positive/DAPI nuclei in IL). EdU levels are similarly elevated in both p27−/− samples. Pomc-CreERT2; Sox2fl/+, p27−/− (7.53 ± 2.92, n = 4, **P = 0.0023) and Pomc-CreERT2; p27−/− (7.68 ± 1.20, n = 8, ***P = 0.0007) vs. Pomc-CreERT2 (controls), (1.60 ± 0.55, n = 3). (C and D) EdU incorporation was quantified in Sox2−/− melanotrophs (PAX7−/−; eYFP−/−) (2.40 ± 1.31, n = 6) and in Sox2−/− melanotrophs (PAX7; eYFP double positive) (0.78 ± 0.86, n = 6) in 2.5- to 6-mo-old Pomc-CreERT2; Sox2fl/++; p27−/−; Rosa26RFP mice, assuming that Rosa26RFP recombination reflects Sox2 heterozygous deletion. In Sox2−/− melanotrophs EdU incorporation is significantly reduced compared to Sox2−/− ones (**P = 0.0210), demonstrating that loss of one copy of Sox2 results in reduced proliferation in p27−/− melanotrophs. (D) EdU, eYFP, and PAX7 triple staining. Most EdU, PAX7 positive cells (arrows) do not express eYFP in Pomc-CreERT2; Sox2fl/++; p27−/−; Rosa26RFP IL. (E) Brightfield pictures of Pomc-Cre, Pomc-Cre; p27−/−, and Pomc-Cre; Sox2fl/++; p27−/− pituitaries. Deletion of one copy of Sox2 using Pomc-Cre prevents IL tumorigenesis. (F) Double immunofluorescence for POMC and SOX9 in 6- to 10-mo-old animals, illustrating reduced IL size in Pomc-Cre; Sox2fl/++; p27−/−. (G) Measurement of IL area in Pomc-Cre; p27−/− (1.472,054 ± 808,382, n = 3) and Pomc-Cre; Sox2fl/++; p27−/− (495,612 ± 185,861, n = 3). IL area in Pomc-Cre; Sox2fl/++; p27−/− pituitaries represents a third of IL area in p27−/− mutants (**P = 0.0041). (H) Quantification of POMC intensity in melanotrophs in relation to corticotrophs in 6- to 10-mo-old Pomc-Cre; p27−/− (1.13 ± 0.11, n = 3) vs. Pomc-Cre; Sox2fl/++; p27−/− (1.89 ± 0.29, n = 3) animals, where levels are significantly increased, *P = 0.0127. (I) In situ hybridization for Sox2 in 6.25- to 10-mo-old wild-type, p27−/−, p27−/−, Sox2−/−, and Pomc-Cre; Sox2−/−; p27−/− animals. (J) Quantification of Sox2 levels following in situ hybridization. Sox2 expression levels are reduced in both p27−/−; Sox2−/− and Pomc-Cre; Sox2−/−; p27−/− melanotrophs and SCs compared to p27−/−. Quantification of Sox2 levels in melanotrophs: p27−/− (12.17 ± 2.15, n = 4), WT (3.5 ± 2.5, **P < 0.003), p27−/−: Sox2−/− (5.4 ± 1, *P = 0.0276), Pomc-Cre; Sox2−/−: p27−/− (2.56 ± 0.1, ***P = 0.0038). Quantification of Sox2 levels in SCs: p27−/− (28 ± 6, n = 4), WT (11.4 ± 8, n = 5, ****P < 0.0001), p27−/−: Sox2−/− (11.3 ± 1.4, n = 3, ***P < 0.0001), Pomc-Cre; Sox2−/−: p27−/− (19 ± 3, n = 3, ***P = 0.0039). IL is outlined in A. IL and stem cell cleft are outlined in I. ns, nonsignificant.
SCs upon Sox2 reduction (SI Appendix, Fig. S5B). SOX2 staining intensity in SCs was also reduced (Fig. S5 and SI Appendix, Fig. S5C). Furthermore, we observed a significant reduction of proliferation in both SCs and melanotrophs in 6-mo-old Sox9\textsuperscript{iresCreERT2/+/csk\textsuperscript{−/−}}; Sox2\textsuperscript{fl/+}; p27\textsuperscript{−/−}IL compared to Sox9\textsuperscript{iresCreERT2/+/csk\textsuperscript{−/−}}; Sox2\textsuperscript{fl/+}; p27\textsuperscript{−/−} samples (Fig. S5 D, and F). In addition, formation of ectopic vascular structures is significantly decreased (Fig. 5 E and F).

Altogether these results indicate that, while SCs are not themselves giving rise to melanotroph tumors in p27\textsuperscript{−/−} IL, SOX2 activity in the SCs and consequently the SCs themselves are required for tumorigenesis in p27\textsuperscript{−/−} IL.

Characterization of the Transcriptional Consequences of Altering Sox2 Levels in p27\textsuperscript{−/−} IL SCs. To analyze the role of SOX2 in SCs, we performed single cell RNA sequencing (scRNAseq) on ILs from 3-mo-old Sox9\textsuperscript{iresCreERT2/+, Sox9\textsuperscript{iresCreERT2/+/csk\textsuperscript{−/−}}; Sox2\textsuperscript{fl/+}; p27\textsuperscript{−/−}} and Sox9\textsuperscript{iresCreERT2/+, Sox2\textsuperscript{fl/+}; p27\textsuperscript{−/−}} animals, where Cre activity had been induced at birth. Dataset clustering was performed by generating uniform manifold approximation and projection (UMAP) plots (Fig. 6A and SI Appendix, Fig. S6A). The three datasets were integrated and clusters identified according to the expression of known DEGs, and by comparison with published SC types (24, 31). The major cell cluster corresponds to melanotrophs as expected (Tbx19, Pnoc, and Pax7 positive). The SC cluster (Sox2 and Sox9 positive) is proportionally larger in our IL dataset in comparison with studies performed on the whole pituitary (24, 31).

We independently reanalyzed the SC (Fig. 6 B and C) and melanotroph clusters (Fig. 6 F and G). Within each cell type we observed that cells were mostly, but not perfectly, clustered according to their genotype. We thus aimed at examining DEGs between the clusters to characterize the direct effect of Sox2 dosage on SCs, and the consequences on melanotrophs. More precisely, we analyzed DEGs in Sox9\textsuperscript{iresCreERT2/+, Sox9\textsuperscript{iresCreERT2/+/csk\textsuperscript{−/−}}; Sox2\textsuperscript{fl/+}; p27\textsuperscript{−/−}} compared to Sox9\textsuperscript{iresCreERT2+/+} and Sox9\textsuperscript{iresCreERT2/+, Sox2\textsuperscript{fl/+}; p27\textsuperscript{−/−}}.

In SCs, (Fig. 6 B and C) 36 DEGs were identified by comparing subcluster 1 (which has the highest proportion of p27\textsuperscript{−/−} cells) to the two other subclusters (Dataset S3). Thirty of the DEGs were up-regulated in subcluster 1, and among them, 14 have been associated with tumorigenesis (Fig. 6D). Among these, 5 are immediate early response genes (Jr2, Fos, Jund, Jun, and Egr7) that can be activated by the MAPK pathway. This pathway was also activated in our p27\textsuperscript{−/−} bulk RNAseq dataset (Fig. 1D). In embryonic pituitary progenitors, activation of the MAPK pathway is associated with expansion of this compartment (32). We thus performed immunohistochemistry for phosphorylated ERK and observed a clear and specific up-regulation of the signal in p27\textsuperscript{−/−} SCs, in contrast with both control and Sox9\textsuperscript{iresCreERT2+/+, Sox2\textsuperscript{fl/+}; p27\textsuperscript{−/−}} samples (Fig. 6E).

This suggests that Sox2 positively regulates activity of the pathway in SCs, either directly or indirectly, and this correlates with tumorigenesis.

Subclustering of the melanotrophs, which were more numerous than the SCs, was better associated with the different genotypes (Fig. 6 F and G). In agreement with the absence of tumorigenesis, there was a reduction in melanotroph numbers in Sox9\textsuperscript{iresCreERT2+/+, Sox2\textsuperscript{fl/+}; p27\textsuperscript{−/−}} compared to Sox9\textsuperscript{iresCreERT2+/+, Sox2\textsuperscript{fl/+}; p27\textsuperscript{−/−}} , but these were still more numerous than in wild type, in agreement with the hyperplasia observed in control mutants (Fig. 6F). Furthermore, while melanotrophs are genetically equivalent in Sox9\textsuperscript{iresCreERT2+/+, p27\textsuperscript{−/−}} and Sox9\textsuperscript{iresCreERT2+/+, Sox2\textsuperscript{fl/+}; p27\textsuperscript{−/−}} samples, their transcriptionome is clearly different, further confirming the SOX2-dependent effect of SCs on melanotrophs.

There were 597 DEGS in cluster 0 (Sox9\textsuperscript{iresCreERT2+/+, p27\textsuperscript{−/−}} melanotrophs compared to clusters 2 (Sox9\textsuperscript{iresCreERT2+/+}) and 1 (Sox9\textsuperscript{iresCreERT2+/+, Sox2\textsuperscript{fl/+}; p27\textsuperscript{−/−}}) (Fig. 6H). Pathway analysis of the 573 down-regulated genes in Sox9\textsuperscript{iresCreERT2+/+, p27\textsuperscript{−/−}} melanotrophs revealed that vesicular and endosomal traffic, and cytoskeleton remodeling, as we observed in our bulk analysis (Figs. 1 C and D and 2B), were predominantly affected, suggesting that the differentiated, secretary phenotype of melanotrophs was restored upon reduction of SOX2 in SCs (Fig. 6 I, Upper). In agreement with this hypothesis, Tbx19 was among the down-regulated genes in Sox9\textsuperscript{iresCreERT2+/+, p27\textsuperscript{−/−}} melanotrophs. Recovery of normal expression levels of Tbx19 upon Sox2 down-regulation in SCs was further validated by quantitative PCR (Fig. 6I). Finally, pairwise comparison between clusters 0 and 1, comprising Sox9\textsuperscript{iresCreERT2+/+, p27\textsuperscript{−/−}} and Sox9\textsuperscript{iresCreERT2+/+, Sox2\textsuperscript{fl/+}; p27\textsuperscript{−/−}} cells, respectively, revealed a significant enrichment in process networks associated with translation and transcription (Fig. 6 I, Bottom), suggesting reduced levels of both transcription and translation in p27\textsuperscript{−/−} cells, restored in melanotrophs upon reduction of Sox2 levels in SCs. SCs and CSCs are known to have reduced levels of translation. Moreover, SOX2 was recently shown to be directly involved in alterations in the rate of protein synthesis in a mouse model of squamous tumor initiating cells (33). This suggests that SOX2 may play a similar role in p27\textsuperscript{−/−} melanotrophs, acting as a repressor of translation and transcription of genes related to differentiation.

Altogether these data show that SOX2 up-regulation in p27\textsuperscript{−/−} SCs results in the induction of a dedifferentiated, protumoral phenotype in melanotrophs. In SCs, SOX2 overexpression leads to increased MAPK pathway activation, which correlates with and may mediate the role of SOX2 in tumorigenesis, at least in p27\textsuperscript{−/−} IL.

Discussion

The regulation of Sox2 expression is extremely complex (34) and variation of its expression levels has contrasting consequences in cancers (16). Here we have further dissected the role of SOX2, and also demonstrated the importance of Sox2 for the SOX2–P27 interaction. We have shown that SOX2 is required independently both in melanotrophs and SCs for IL tumors to develop in p27\textsuperscript{−/−} animals (see model, Fig. 7). The pleiotropic, protumorigenic effects of SOX2 in distinct cell types in the absence of P27 demonstrate that it is a crucial mediator of tumorigenesis and hence, a good target for antitumoral treatments. However, we also show that SOX2 only appears to be a relevant target in a subset of tissues when P27 is lost or its expression decreased, such as the pituitary and duodenum.

Understanding the reasons behind the tissue specificity for the SOX2–P27 interaction could help to define preexisting factors favoring tumorigenesis. Our results suggest that derepression of Sox2 in the absence of P27 leads to IL tumors. However, this happens only in some cell types, while expression of p27 is ubiquitous, likely illustrating the multiple antiproliferative roles of P27 that are distinct from Sox2 repression (35, 36). Differential expression of P27 interactors and/or targets, and of other cell cycle repressors, may explain tissue-specific features of the p27\textsuperscript{−/−} phenotype.

In pituitary endocrine cells, SOX2 expression appears to be a prerequisite for tumoral development in absence of p27, since melanotrophs are the only such cell type where SOX2 is detectable (19). While melanotrophs are also uniquely characterized by their nonproliferative status (6), levels of P27 are unexpectedly relatively low in melanotrophs. This suggests that in p27\textsuperscript{−/−} mutants, an active Sox2 locus normally under P27 control, coupled with a relaxed control of the cell cycle renders melanotrophs particularly prone to tumorigenesis. In agreement, independent deletion of genes encoding for different negative cell cycle regulators show that the IL is particularly sensitive to tumoral development (7). Furthermore derepression of Sox2 is also observed in pRb\textsuperscript{+/−} pituitary tumors (37). It would be interesting to examine whether SOX2 is required for IL tumorigenesis in other cell cycle regulator mutants (7). Conversely we can now screen for cell types satisfying the relevant criteria, including low
cell turnover, low negative cell cycle regulator levels, and presence of SOX2, and examine for tumorigenic potential.

To further characterize the molecular mechanisms involved in the SOX2-P27 interaction, we have deleted Srr2. SOX2 itself, c-myc, and members of the POU transcription factor family bind to Srr2 to promote expression of the gene (38).

Fig. 5. SOX2 is required in SCs for tumorigenesis in p27−/− IL. (A) eYFP, SOX9 (Middle), and eYFP, PAX7 (Right) double stainings in 7- to 12-mo-old Sox9iresCreERT2/+;Rosa26ReYFP/+ and Sox9iresCreERT2/+;p27−/−;Rosa26ReYFP/+ animals. eYFP, SOX9 double positive cells are localized in the epithelium lining the cleft in both genotypes (arrows). Very few eYFP, SOX9, and PAX7 cells are present in Sox9iresCreERT2/+;Rosa26ReYFP/+ animals (arrows). In Sox9iresCreERT2/+;Rosa26ReYFP/+ pituitaries some rare eYFP, PAX7 double-positive cells are present, demonstrating low levels of differentiation into melanotroph (Left). (B) Brightfield pictures (Upper) and SOX2 immunohistochemistry (Bottom) on sections of Sox9iresCreERT2/+;Rosa26ReYFP/+;p27−/−, and Sox9iresCreERT2/+;Sox2fl/+;p27−/− pituitaries. In Sox9iresCreERT2/+;Sox2fl/+;p27−/− pituitaries tumorigenesis is impaired (1-yr-old 14% tumor incidence, n = 7) compared to Sox9iresCreERT2/+;p27−/− (1-yr-old, n = 5, 100% tumor incidence). The thickness of the SOX2 positive SC epithelium resembles that of control, demonstrating that reduction of Sox2 dosage in SCs impairs p27−/− proliferation in tumorigenesis. (C, D, and F) Analysis of cell proliferation. EdU incorporation was quantified in SCs and melanotrophs in 7- to 12-mo-old Sox9iresCreERT2/+;Sox9iresCreERT2/+;p27−/−, and Sox9iresCreERT2/+;Sox2fl/+;p27−/− IL. In fact, proliferation in Sox9iresCreERT2/+;Sox2fl/+;p27−/− samples (0.23 ± 0.35, n = 3, **P = 0.0011) compared to Sox9iresCreERT2/+;p27−/− ones (3 ± 0.68, n = 3). In melanotrophs (POMC positive cells in IL/DAPI positive nuclei), there is a significant reduction of proliferation in Sox9iresCreERT2/+;Sox2fl/+;p27−/− samples (1.88 ± 0.72, n = 6, **P = 0.0003) compared to Sox9iresCreERT2/+;p27−/− (3.7 ± 0.76, n = 4). Again proliferation in Sox9iresCreERT2/+;Sox2fl/+;p27−/− is similar to Sox9iresCreERT2/+;Sox2fl/+;p27−/− controls (0.60 ± 0.4, n = 4, *P = 0.03) while proliferation is increased in Sox9iresCreERT2/+;p27−/− vs Sox9iresCreERT2/+;Sox2fl/+;p27−/− controls (***P = 0.0001). (E) Quantification of the vascular structures ectopic development in 7 to 12 mo old. Sox9iresCreERT2/+;Sox9iresCreERT2/+;p27−/−, and Sox9iresCreERT2/+;Sox2fl/+;p27−/− animals. Deletion of one copy of Sox2 in SC leads to a reduction in the development of ectopic blood vessels in IL. IL vascular structures area: Sox9−/−, p27−/− (0.32 ± 0.28, n = 3), Sox9−/−, p27−/− (2.56 ± 1.26, n = 3, *P < 0.03), and Sox9−/−, Sox2fl/+;p27−/− (1.15 ± 0.43, n = 3). (F) EdU, SOX2 double staining in 7- to 12-mo-old Sox9iresCreERT2/+;Sox9iresCreERT2/+;p27−/− and in absence of p27−/−, Sox2fl/+;p27−/− pituitary glands. Right, immunofluorescence for CD31 showing ectopic blood vessel formation reduction in Sox9iresCreERT2/+;Sox2fl/+;p27−/− sample. SC layer is outlined in A, IL in F. ns, nonsignificant.

Beside P27 (15), other negative regulators of Srr2 have been characterized such as P21 in neural SCs (39) and pRb in the pituitary (37). Analysis of p27−/−; Srr2−/− (SI Appendix, Fig. S3E).
Fig. 6. Characterization of the transcriptomic effects of Sox2 dosage reduction in SCs in p27/− IL by single cell analysis. (A) Identification of clusters in integrated UMAP. (B) Subclustering of the stem cell fraction (0.9 resolution) shows that segregation only partially correlates with genotype (Right). (C) Representation of the proportion of cells of the indicated genotype in each cluster, Sox9iresCreERT2+/+ (n = 137 cells), Sox9iresCreERT2+/−; p27−− (n = 104 cells), and Sox9iresCreERT2−− (n = 82 cells). (D) Heatmap showing expression of 14 genes selected out of 36 differentially expressed genes (adjusted P value <0.05) in Sox9iresCreERT2+/+, p27−− compared with Sox9iresCreERT2+/+ or Sox9iresCreERT2+/−; Sox2+/−, p27−−. (E) Immunohistochemistry for p-ERK on Sox9iresCreERT2+/+, Sox9iresCreERT2+/−, p27−−, and Sox9iresCreERT2−−; Sox2+/+, p27−− pituitaries. While the MAPK/ERK pathway seems overactive in p27−− SCs, p-ERK levels return to normal levels upon deletion of one copy of Sox2 in SCs. (F) Subclustering of the melanotroph fraction (0.2 resolution) shows that segregation correlates with genotype (Right). (G) Representation of the proportion of cells of the indicated genotype in the most abundant cluster, Sox9iresCreERT2+/+ (n = 1,172 cells), Sox9iresCreERT2+/−; p27−− (n = 3,561 cells), and Sox9iresCreERT2−−; Sox2−− (n = 3,006 cells). (H) Strategy followed for scRNAseq analysis of Sox9iresCreERT2+/+, p27−− vs. Sox9iresCreERT2+/− or and Sox9iresCreERT2−−; Sox2−− melanotrophs. (I, Upper) Pathway analysis on genes differentially expressed (adjusted P value <0.05) in melanotrophs following the strategy delineated in I. Pathways associated with secretory function and cytoskeleton are down-regulated in Sox9iresCreERT2+/−; p27−− cells. (Bottom) Process network analysis performed on genes differentially expressed between Sox9iresCreERT2+/+, p27−−, and Sox9iresCreERT2−−; Sox2−−; p27−− melanotrophs (adjusted P value <0.05). Processes associated with translation and transcription are down-regulated in Sox9iresCreERT2+/−; p27−− melanotrophs, while genes promoting cell cycle progression are up-regulated. (J) RT-qPCR analysis of Tbx19 levels in 5- to 12-mo-old Sox9iresCreERT2+/+, p27−−, Sox9iresCreERT2−−; Sox2−−, p27−−, and p27−−; Sox2−− IL. Tbx19 levels are increased in Sox9iresCreERT2−−; Sox2−−; p27−− (0.99 ± 0.20, n = 3, *P = 0.02) and p27−−; Sox2+/− (1.07 ± 0.15, n = 3, **P = 0.0044) vs. Sox9iresCreERT2−−; p27−− (0.58 ± 0.28, n = 4).

Notably, loss of Sir2 affects cell proliferation exclusively in p27−− SCs, suggesting that the role of the enhancer is more important in SCs, in agreement with a role for these in promoting the melanotroph tumors.

Our study highlights common and cell-specific roles of SOX2 during tumorigenesis. Both in p27−− melanotrophs and SCs, SOX2 up-regulation is associated with overproliferation. The association of SOX2 with dedifferentiation and overproliferation has been observed in many tumors (16), as we also observe here in melanotrophs. In pituitary SCs, our results suggest that this overproliferation is linked to MAPK overactivation, something that has been shown in embryonic pituitaries expressing an activated form of Braf (32). How SOX2 derepression induces MAPK activation is less clear; MAPK pathway activation has been linked to induction of Sox2 expression (40), but the molecular mechanisms underlying the converse have not been characterized. It would be of interest to determine whether this SOX2-MAPK interaction is relevant in other contexts. While SOX2 has been associated with CSC properties in some other tumors (41), we report here its requirement in SCs for these to promote tumorigenesis in neighboring melanotrophs. Manipulation of MAPK in
SCs in p27−/− animals is now required to characterize their effect on SCs and melanotroph transformation.

In conclusion, our study reveals how Sox2 derepression, independently in two adjacent cell types, underlies tumorigenesis in one of them. Moreover, our work reveals the existence of reciprocal interactions between melanotrophs and flanking SCs to orchestrate tumor development. This highlights the complexity of the mechanisms triggering tumorigenesis and the central role of SOX2 in this process. Furthermore, pituitary SCs, which are not tumoral, but have a tumor-inducing activity, represent good anti-tumoral treatment targets, highlighting the importance of detailed characterization of mechanisms underlying tumorigenesis to decipher possible antitumor strategies.

Materials and Methods

All experiments carried out on mice were approved under the UK Animal (scientific procedures) Act (project licenses 802405 and 708560). Detailed protocols and mouse strains can be found in SI Appendix, Supplementary Material and Methods.

Data Availability. RNA sequencing datasets have been deposited in Gene Expression Omnibus (GSE152010). All study data are included in the article and/or supporting information.

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Fig. 7. Model recapitulating the consequences of Sox2 dosage modulation during IL p27−/− tumorigenesis and its proposed roles. In normal IL, SOX2 is expressed at high levels in SCs and at low levels in melanotrophs. In p27−/− IL, melanotrophs develop with increased SC proliferation. SOX2 levels are increased in both cell types, while expression of TBX19 and POMC is reduced in melanotrophs. Removal of one copy of Sox2 in p27−/− animals (p27−/−; Sox2+/−) results in impairment of tumorigenesis, while hyperplasia is still observed. Conditional deletion of Sox2 in melanotrophs (Pomc-Cre; Sox2fl/−; p27−/−) prevents hyperplasia and tumor formation; therefore, SOX2 is required cell autonomously for tumor formation (Fig. 4). Furthermore, modulation of SOX2 levels in SCs in this model suggests that melanotrophs interact with SCs (dotted arrow). Conditional deletion of Sox2 in SCs (Sox9creERT2; p27+/−; Sox2−−/−) also impairs tumorigenesis, reduction of Tbx19 levels, and SC overproliferation but hyperplasia is still observed (Figs. 5 and 6). Therefore SCs promote tumor formation and loss of differentiated features, while not giving rise to tumorigenic cells themselves. This tumor-promoting role of SCs (arrow) depends on SOX2. Therefore SOX2 is required both in melanotrophs and SCs for respectively cell-autonomous and tumor-promoting functions following loss of P27. This model was created with BioRender.com.
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