β-Catenin stimulates pituitary stem cells to form aggressive tumors

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Activation of β-Catenin in Pituitary Progenitors Causes Craniopharyngiomas

Pituitary adenomas are common in older people, representing approximately 10% of intracranial neoplasias (1). They tend to be slow-growing, and are usually benign, but they can compress the brain and optic chiasm and cause abnormalities in pituitary hormone production. A fraction of pituitary adenomas become invasive and are resistant to treatment with surgery, radiation, and pharmacotherapy. Progress is being made in identification of genes that enhance the risk of pituitary adenoma formation, but the etiology of many adenomas remains unexplained (1, 2). Common adenomas are sporadic and clonal in origin. Craniopharyngiomas differ from most sporadic pituitary adenomas in that they occur in children, tend to be resistant to treatment, and cause significant morbidity. Histopathology analyses suggested that craniopharyngiomas arise from embryonic pituitary tissue and implicated β-catenin as a marker. In PNAS, Gaston-Massuet and colleagues provide proof that craniopharyngiomas arise from activation of β-catenin in pituitary progenitors during embryogenesis (3). Their elegant studies in patient tumor samples and genetically engineered mice lend support to the cancer stem cell hypothesis. They also provide insight into the basic nature of pituitary progenitors, the stem cell niche, and normal regulation of the transition from self-renewal to differentiation. Now the pituitary gland joins a collection of other tissues in which β-catenin signaling can affect this critical decision point (4).

Pituitary Stem Cells Express Signature Transcription Factors and Reside in Rathke's Cleft

Stem cells are defined by their ability to self-renew and to differentiate into multiple types of specialized cells. They are important for tissue maintenance and repair. Regulation of the decision to self-renew or to differentiate is a complex process and a critical aspect of normal development. Expression of signature transcription factors like SOX2 and OCT4 is a common intrinsic control mechanism used by stem cells. Extrinsic control through cell–cell signaling pathways like Notch and Wnt provide an equally important layer of regulation (5). These cell–cell communications typically involve signaling between the cells that constitute the stem cell niche and the stem cells themselves. Groups led by Robinson, Alvarez, Vankelecom, Enikolopov, and Thomas have used different approaches to demonstrate the existence of pituitary "stem cells" with the capacity to self-renew and differentiate into all five hormone-producing cells of the anterior pituitary (6–10). In general, there is agreement that SOX2-expressing progenitors reside around the cleft that remains from the initial formation of the Rathke's pouch from oral ectoderm (11). There are multiple lines of evidence that Notch and Wnt signaling contribute to regulation of the growth and differentiation of the pituitary gland, and future studies may unravel the mechanistic details of how these pathways control the decision to self-renew or differentiate (12–17).

Mouse Model of β-Catenin Activation Accurately Predicts Gene Expression in Human Craniopharyngiomas

In PNAS, Gaston-Massuet et al. report craniopharyngiomas in mice that express degradation-resistant β-catenin in Rathke's pouch (3). They generated these mice by crossing a *Hesx1-cre* knock-in strain to a β-catenin strain that produces degradation-resistant β-catenin upon recombination (*Ctnnb*^{floxex3}). Although all the cells in the pituitary are targeted, only a few form foci that are marked by SOX2 expression. The transcription factor *Hesx1* is expressed in the anterior neural ridge in early embryogenesis, marks all cells of the developing Rathke's pouch, and is critical for normal pituitary development (18). The targeted cells express the stem cell marker SOX2. Most of the mutants die perinatally of unknown cause. Differentiation of the *Pou1f1* lineage is disrupted, which causes growth hormone and thyroid stimulating hormone deficiencies, which can cause neonatal respiratory distress and death because thyroid hormone is required for lung maturation at birth (19). The *Hesx1-cre, Ctnnb*^{floxex3} mutants that survive to weaning are pituitary dwarfs and develop early, lethal pituitary tumors with 100% penetrance. These aggressive tumors kill half the weanlings by young adulthood, and none survive to 6 mo. The tumors resemble human adamantinomatous craniopharyngioma histologically and express the expected genetic markers. Activation of β-catenin is expected to activate expression of a variety of target genes including *Lef1*, *Axin1*, and *CyclinD1*. Gaston-Massuet et al. demonstrate elevated expression of these targets in both the mouse and human craniopharyngiomas (3). Thus, the mouse studies accurately predict diagnostic markers of the human tumors. This provides evidence that β-catenin activation can play a causal role in development of craniopharyngiomas and validates this animal model for testing therapeutic interventions that might benefit affected children.

Etiology of Craniopharyngiomas Supports Cancer Stem Cell Hypothesis

Pituitary stem cell research suggests a spatial and temporal progression from multipotent progenitors located near Rathke's cleft through a transit-amplifying intermediate, and finally to differentiated cells with the ability to reenter the cell cycle at a low rate. The earliest progenitors express SOX2 and progress to a SOX2, SOX9 double-positive transit-amplifying cell. These cells are enriched near Rathke's cleft remnants in mouse and man (6, 7). Rathke's cleft is a zone of active cell proliferation during embryogenesis and is thought to be the main stem cell niche. Rodent studies reveal that there is an intermediate zone just outside the niche that expresses Notch2 and cyclin E in fetuses (17, 20). These cells have apparently left the cell cycle and are ready to undergo differentiation. Cells that express hormone markers characteristic of individual cell types lie outside this intermediate zone, even further away from the cleft. In late gestation, proliferating cells are still enriched around the cleft, but there are progenitors scattered throughout the anterior lobe that reenter the cell cycle (21, 22). A few of these are SOX2-, SOX9-positive cells that have the potential to differentiate into all five anterior pituitary hormone-producing cells (6, 7). Cells that have undergone lineage commitment, i.e., expressing *Pou1f1*, reenter the cell cycle during neonatal life to expand the population of growth hormone, thyroid-stimulating hormone, and prolactin cells, probably in response to mitogenic stimulation by hypothalamic releasing hormones.

Gaston-Massuet et al. demonstrate that the fraction of pituitary cells with the ability to form colonies steadily increases from late gestation to mouse postnatal day 10 to 13 (~7%) and decreases precipitously after that, with very few colony-forming cells present in adults (<0.1%) (3). At almost all stages, there are approximately threefold more colony-forming cells in the

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Hesx1-cre, *Ctnnb*^{floxex3} mutant pituitaries, and their proliferation rate is higher. These cells have the ability to self-renew through eight passages. The *Hesx1-cre*, *Ctnnb*^{floxex3} mutant pituitaries have more cells expressing SOX2, SOX9, cyclin E, p57, and the cell proliferation marker Ki67. Craniopharyngioma cells in mouse and man accumulate β-catenin and the cells around them express SOX9, consistent with the idea of progression from stem cell to SOX9 expression in pituitaries of both species. The expression of activated β-catenin in committed *Pou1f1*-positive progenitors or in growth hormone-expressing cells is not sufficient to produce adenomas of any type. All these data are consistent with the idea that pituitary stem cells are the only susceptible target of transformation by excess β-catenin, supporting the cancer stem cell hypothesis as an origin for craniopharyngiomas.

Study Raises Several Questions About Regulation of Pituitary Stem Cells and Differentiation

The work of Gaston-Massuet et al. (3) raises several questions. First, why are so few cells accumulating β-catenin even though *Hesx1-cre* lineage studies mark each of the hormone-producing cell types and virtually all the cells in the organ besides the blood vessels? Second, is β-catenin normally a regulator of pituitary progenitor proliferation, and, if so, which WNT regulates its expression? Third, why is the *Pou1f1* lineage blocked by activated β-catenin?

Hesx1-cre targets efficient excision of multiple floxed alleles in all the cells in Rathke's pouch. Gaston-Massuet et al. have convincing proof that *Hesx1-cre* effectively excises the floxed exon 3 of β-catenin (3). Despite the strong penetrance, only a few cells in the anterior pituitary gland accumulate cytoplasmic β-catenin and become transformed. Is a second hit required? If the second hit were genetic, all the cells would be expected to express cytoplasmic β-catenin, and only a few that received the second hit would be transformed. Perhaps the missing step is actually loss of suppression. If the pituitary gland is programmed to silence β-catenin epigenetically, and a few cells escape the suppression, those rare cells will accumulate the degradation-resistant β-catenin and become transformed. In support of this idea, epigenetic silencing by polycomb proteins has been implicated in suppression of stem cell differentiation in other systems (23).

The balance between self-renewal and differentiation is regulated by β-catenin in many tissues. Thus, there are precedents for the idea that pituitary progenitor self-renewal is regulated by Wnt signaling and β-catenin stabilization (4). Two observations are difficult to reconcile with this theory. First, deletion of β-catenin early in pituitary organogenesis by a *Pitx1-cre* transgene blocks the *Pou1f1* lineage and elevates *Hesx1* expression, while activation of degradation resistant β-catenin also blocks the *Pou1f1* lineage. Second, there is little or no activation of a β-catenin–dependent reporter gene in pituitary gland and broad activation would be expected if the theory were correct (3, 24). If β-catenin stabilization is a natural regulator of pituitary stem cell proliferation, what Wnts are responsible? Many Wnts are expressed in the developing pituitary gland, but those tested have modest effects on pituitary morphology and differentiation (12). Resolving these issues is an important area for future study.

Finally, it is not obvious why abnormal activation of β-catenin in the stem cell niche should result in blockage of the *Pou1f1* lineage. β-Catenin has been proposed to interact with the homeodomain PROP1 to activate *Pou1f1* expression, and there are conflicting reports about the importance of LEF1 and β-catenin for *Pou1f1* activation (24, 25). Gaston-Massuet et al. clearly show that mutants do not express *Pou1f1* or the target genes growth hormone and thyroid stimulating hormone at the appropriate time, and *Pou1f1* expression is blocked in mutant pituispheres (3). Further studies will reveal the complete mechanism of *Pou1f1* regulation, including the role of corepressors, epigenetic regulation, and microRNAs (25–27).

Conclusion

Gaston-Massuet et al. (3) have clearly demonstrated that β-catenin influences expansion of the pituitary progenitor pool and the decision to differentiate. Their studies provide a mechanism underlying a common, problematic intracranial neoplasm affecting children, and the animal model they developed accurately predicted markers in human craniopharyngiomas. This work represents an important step in understanding the etiology of pituitary adenomas and sets the stage for exploring the role of epigenetics and tumor suppressors in normal and abnormal pituitary development (27).

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1. Vandeva S, et al. (2010) The genetics of pituitary adenomas. *Best Pract Res Clin Endocrinol Metab* 24:461–476.
2. Hosoyama T, et al. (2010) A postnatal Pax7 progenitor gives rise to pituitary adenomas. *Genes Cancer* 1: 388–402.
3. Gaston-Massuet C, et al. (2011) Increased Wingless (*Wnt*) signaling in pituitary progenitor/stem cells gives rise to pituitary tumors in mice and humans. *Proc Natl Acad Sci USA* 108:11482–11487.
4. Nusse R (2008) Wnt signaling and stem cell control. *Cell Res* 18:523–527.
5. Morrison SJ, Spradling AC (2008) Stem cells and niches: Mechanisms that promote stem cell maintenance throughout life. *Cell* 132:598–611.
6. Fauquier T, Rizzoti K, Dattani M, Lovell-Badge R, Robinson IC (2008) SOX2-expressing progenitor cells generate all of the major cell types in the adult mouse pituitary gland. *Proc Natl Acad Sci USA* 105:2907–2912.
7. Garcia-Lavandeira M, et al. (2009) A GRFa2/Prop1/stem (GPS) cell niche in the pituitary. *PLoS ONE* 4:e4815.
8. Chen J, et al. (2009) Pituitary progenitor cells tracked down by side population dissection. *Stem Cells* 27: 1182–1195.
9. Gleiberman AS, et al. (2008) Genetic approaches identify adult pituitary stem cells. *Proc Natl Acad Sci USA* 105:6332–6337.
10. Lepore DA, et al. (2005) Identification and enrichment of colony-forming cells from the adult murine pituitary. *Exp Cell Res* 308:166–176.
11. Castinetti F, Davis SW, Brue T, Camper SA (2011) Pituitary stem cell update and potential implications for treating hypopituitarism. *Endocr Rev* 10.1210/er.2010-0011.
12. Potok MA, et al. (2008) WNT signaling affects gene expression in the ventral diencephalon and pituitary gland growth. *Dev Dyn* 237:1006–1020.
13. Kioussi C, et al. (2002) Identification of a Wnt/Dvl/beta-Catenin —> Pitx2 pathway mediating cell-type-specific proliferation during development. *Cell* 111:673–685.
14. Salisbury TB, Binder AK, Grammer JC, Nilson JH (2009) GnRH-regulated expression of Jun and JUN target genes in gonadotropes requires a functional interaction between TCF/LEF family members and beta-catenin. *Mol Endocrinol* 23:402–411.
15. Kita A, et al. (2007) Hes1 and Hes5 control the progenitor pool, intermediate lobe specification, and posterior lobe formation in the pituitary development. *Mol Endocrinol* 21:1458–1466.
16. Zhu X, et al. (2006) Sustained Notch signaling in progenitors is required for sequential emergence of distinct cell lineages during organogenesis. *Genes Dev* 20: 2739–2753.
17. Raetzman LT, et al. (2004) Developmental regulation of Notch signaling genes in the embryonic pituitary: *Prop1* deficiency affects *Notch2* expression. *Dev Biol* 265:329–340.
18. Kelberman D, Rizzoti K, Lovell-Badge R, Robinson IC, Dattani MT (2009) Genetic regulation of pituitary gland development in human and mouse. *Endocr Rev* 30:790–829.
19. Nasonkin IO, et al. (2004) Pituitary hypoplasia and respiratory distress syndrome in *Prop1* knockout mice. *Hum Mol Genet* 13:2727–2735.
20. Bilodeau S, Roussel-Gervais A, Drouin J (2009) Distinct developmental roles of cell cycle inhibitors p57Kip2 and p27Kip1 distinguish pituitary progenitor cell cycle exit from cell cycle reentry of differentiated cells. *Mol Cell Biol* 29:1895–1908.
21. Ward RD, Stone BM, Raetzman LT, Camper SA (2006) Cell proliferation and vascularization in mouse models of pituitary hormone deficiency. *Mol Endocrinol* 20: 1378–1390.
22. Davis SW, Mortensen AH, Camper SA (2011) Birthdating studies reshape models for pituitary gland cell specification. *Dev Biol* 352:215–227.
23. Clarke MF, Fuller M (2006) Stem cells and cancer: Two faces of eve. *Cell* 124:1111–1115.
24. Olson LE, et al. (2006) Homeodomain-mediated beta-catenin-dependent switching events dictate cell-lineage determination. *Cell* 125:593–605.
25. Zhang Z, Florez S, Gutierrez-Hartmann A, Martin JF, Amendt BA (2010) MicroRNAs regulate pituitary development, and microRNA 26b specifically targets lymphoid enhancer factor 1 (Lef-1), which modulates pituitary transcription factor 1 (*Pit-1*) expression. *J Biol Chem* 285:34718–34728.
26. Carvalho LR, Brinkmeier ML, Castinetti F, Ellsworth BS, Camper SA (2010) Corepressors TLE1 and TLE3 interact with HESX1 and PROP1. *Mol Endocrinol* 24: 754–765.
27. Dudley KJ, Revill K, Clayton RN, Farrell WE (2009) Pituitary tumours: All silent on the epigenetics front. *J Mol Endocrinol* 42:461–468.