Chapter 2
Signaling Pathways Regulating Pituitary Lactotrope Homeostasis and Tumorigenesis

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Abstract Dysregulation of the signaling pathways that govern lactotrope biology contributes to tumorigenesis of prolactin (PRL)-secreting adenomas, or prolactinomas, leading to a state of pathological hyperprolactinemia. Prolactinomas cause hypogonadism, infertility, osteoporosis, and tumor mass effects, and are the most common type of neuroendocrine tumor. In this review, we highlight signaling pathways involved in lactotrope development, homeostasis, and physiology of pregnancy, as well as implications for signaling pathways in pathophysiology of prolactinoma. We also review mutations found in human prolactinoma and briefly discuss animal models that are useful in studying pituitary adenoma, many of which emphasize the fact that alterations in signaling pathways are common in prolactinomas. Although individual mutations have been proposed as possible driving forces for prolactinoma tumorigenesis in humans, no single mutation has been clinically identified as a causative factor for the majority of prolactinomas. A better understanding of lactotrope-specific responses to intracellular signaling pathways is needed to explain the mechanism of tumorigenesis in prolactinoma.

2.1 Introduction

Prolactin (PRL) is a 23 kDa polypeptide hormone that is a member of the growth hormone (GH) family and is primarily synthesized and secreted from lactotrope cells of the anterior pituitary gland. In mammals, PRL acts at the mammary gland to promote growth and development, milk synthesis, and maintenance of milk secretion [1]. Knockout of PRL or PRL-receptor genes in mice results in impaired growth and development of the mammary gland and absence of milk production [2, 3]. The strongest stimulus for PRL secretion from lactotrope cells is suckling,

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with the duration and intensity of the stimulus corresponding to the amount of PRL secreted into the blood [1, 4, 5].

In addition to its classical actions on the mammary gland, PRL also influences many other physiological systems. The PRL receptor is expressed in the mammary gland, gonads, uterus, brain, pituitary gland, adrenal gland, lung, heart, liver, skeletal muscle, skin, and lymphocytes. Elevated PRL levels act at the gonads to decrease the sensitivity of follicle stimulating hormone (FSH) and luteinizing hormone (LH) receptors. Furthermore, circulating PRL attenuates pulsatile secretion of gonadotropin releasing hormone (GnRH) from the hypothalamus, reducing LH and FSH secretion from the anterior pituitary gland [6]. As a result, increased levels of PRL cause reduced secretion of and sensitivity to LH and FSH, leading to suppression of ovulation. During pregnancy, elevated serum PRL has effects that extend beyond the reproductive system. At the adrenal gland, PRL increases androgen and dihydroepiandrosterone (DHEA) steroidogenesis, and also reduces cortisol and aldosterone secretion [6]. In the liver, PRL increases lipoprotein lipase activity in hepatocytes and increases bile secretion. PRL has osmoregulatory effects in the kidney, reducing renal sodium and potassium excretion, and also increases sodium and chloride excretion in sweat and salt and water absorption in the intestine. Lastly, PRL influences the immune system by inducing proliferation of lymphocytes [6].

As PRL is involved in various different physiological systems, signaling pathways are critical for regulating lactotrope biology from humans to rodents. Pituitary lactotropes have a high-basal PRL secretory activity. To maintain PRL homeostasis, tonic inhibition by dopamine acting via the D2 receptor (D_2R) is required to limit PRL production and secretion, lactotrope proliferation, and growth of PRL-secreting adenomas [7–13]. During pregnancy and lactation, dopaminergic inhibition is diminished by estradiol, allowing local growth factors from folliculostellate support cells to stimulate lactotropes, promoting lactotrope hyperplasia and doubling in pituitary size [7, 14–16]. Circulating PRL levels are elevated during pregnancy and lactation, creating a state of physiological hyperprolactinemia. Dysregulation of the signaling pathways that govern lactotrope biology contributes to tumorigenesis of PRL-secreting adenomas, or prolactinomas [16–18], leading to a state of pathological hyperprolactinemia. Prolactinomas cause hypogonadism, infertility, osteoporosis, and tumor mass effects, and are the most common type of neuroendocrine tumors [19, 20].

In this review, we highlight signaling pathways involved in lactotrope development, homeostasis, and physiology of pregnancy, as well as implications for signaling pathways in pathophysiology of prolactinoma. We review mutations found in human prolactinoma and discuss how such mutations influence signal transduction in lactotrope cells. Lastly, we present a brief review of animal models that are useful in studying pituitary adenoma.

2.2 Signaling Pathways Regulating Pituitary Stem/Progenitor Cells Leading to Lactotrope Development/Ontogeny

During embryogenesis, the pituitary first develops from the anterior neural ridge (ANR) of the neural plate. The actual pituitary organogenesis begins at embryonic day 8.5 (E8.5) with the formation of Rathke's pouch. The ventral diencephalon, which will ultimately become the hypothalamus, develops from neural plate cells posterior to the ANR [21]. The process of pituitary development is dependent upon the homeobox gene Tift1, as well as fibroblast growth factor 8 (FGF8) and bone morphogenic protein 4 (BMP4) signaling from the ventral diencephalon. Knockout of Titf1 results in pituitary aplasia [22]. FGF8 signaling and the resulting expression of the LIM homeodomain transcription factor Lhx3 is required for pituitary development to progress beyond the formation of Rathke's pouch [21]. Without BMP signaling from the ventral diencephalon, pituitary development does not progress beyond E10. Sonic hedgehog (Shh) signaling is required for pituitary patterning and proliferating after E10. Shh works in unison with FGF8 to maintain Lhx3 expression, and it also induces BMP2 expression in the ventral pouch ([21]; Fig. 2.1).

Transient, intrinsic BMP2 and Wnt4 signaling gradients in the developing pituitary gland promote proliferation and establish a pattern that determines localization of specific pituitary cell types [21]. Somatotrope and lactotrope cells arise within the caudomedial region of the developing pituitary gland. Before each cell type can progress beyond initial proliferation and localization, expression of cell-fate-specific transcription factors is required. For lactotropes, somatotropes, and thyrotropes, expression of paired-like homeodomain factor 1 (Prop1) and Pit-1 POU homeodomain protein is required for terminal differentiation (Fig. 2.1). Prop1 is required for Pit-1 activation, and is expressed only in the developing pituitary gland. Deficiency of Prop1 leads to near complete loss of somatotrope, lactotrope, and thyrotrope cells [23]. After E17.5, cells in the Pit-1 lineage exhibit permanent cell-autonomous commitment and cannot be converted to alternative fates [21]. Hormone secretion from differentiated thyrotropes, somatotropes, and lactotropes is regulated by hypothalamic thyroid-releasing hormone (TRH), GH-releasing hormone (GHRH), and dopamine, respectively (Fig. 2.1).

The Pit-1 transcription factor binds to promoter regions of GH and PRL genes, and is required for their activation. Pit-1 can associate with coactivators and corepressors, and the Pit-1 binding partners required to activate PRL versus GH gene transcription are involved in activation of signaling pathways. Ras-dependent activation of Ets/Pit-1 synergy results in PRL gene transcription [24–26]. Pit-1 is necessary for cell-specific determination, but it is not sufficient; for lactotropes, estrogen receptor (ER), and Ets transcription factors are also required [25].

Until recently, the dogma was that the embryonic ontogeny pathways were also responsible for facultative responses to meet increased pituitary hormonal demand during periods of physiological stress, including lactotrope expansion during pregnancy. However, the identification of pituitary postnatal stem/progenitor

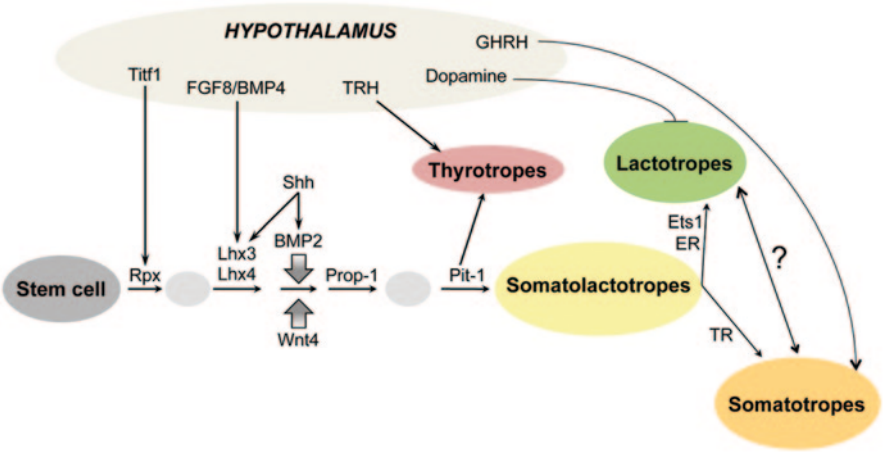

Fig. 2.1 Embryonic ontogeny and *Pit-1* pituitary cell lineage. All hormone-secreting cells in the anterior pituitary gland originate from pituitary stem cells. During embryonic development, *FGF8* and *BMP4* from the hypothalamus stimulate LIM homeodomain transcription factors (Lhx) 3 and 4. Intrinsic gradient signaling of *Wnt4* and *BMP2*, and expression of the *Prop-1* transcription factor, play key roles in determination of pituitary cell fate and localization. *Thyrotropes*, *somatotropes*, and *lactotropes* are derived from the *Pit-1* lineage. Hormone secretion from thyrotropes, somatotropes, and lactotropes is regulated in part by hypothalamic *TRH*, *GHRH*, and *dopamine*, respectively, and the *Pit-1* transcription factor is required cell-specific determination. In lactotropes, *Ets1* and *ER* are also required for prolactin (PRL) production. In *somatotropes*, the thyroid hormone receptor (*TR*) is required for growth hormone (GH) secretion. In rats, a somatolactotrope precursor cell gives rise to PRL secreting lactotropes and GH secreting somatotropes. The contribution of such a precursor cell is well described in rats, but has less of contribution in mice. The existence of a somatolactotrope cell in humans, as well as the possibility that lactotropes and somatotropes may give rise to one another in response to physiological demand, has yet to be confirmed in humans

cells (pSPCs) within the past decade has challenged this dogma. A niche containing pSPCs exists into adulthood in the pituitary gland and is the likely source of facultative organ expansions driven by upstream endocrine tropic hormones and stromal growth factors in response to increased physiological demand (Fig. 2.2). Cells from the anterior pituitary gland are capable of forming "pituispheres," and these cells segregate into the "side population." This side population contains 1–5 % of total pituitary cells, and is a FACS cell fraction known to harbor bona fide stem cells [27]. Further analysis of cells in the side population fraction revealed high expression levels of Sca1, as well as expression of other stem cell markers such as Oct-4, nanog, nestin, CD133, and Bmi-1 [27]. A few years later, three separate studies reported the existence of stem cells in the pituitary gland [28–30]. Together, these studies reveal that the periluminal pSPCs express SSEA-4, Oct4, Sox2, GFRa2, Sca1, nestin, Prop-1, Lhx-3, E-cadherin, and cytokeratins 8 and 18. Importantly, pSPC cells do not express embryonic pituitary stem cell makers Hesx-1 and Lhx-4, distinguishing these cells from embryonic pituitary stem cells. Notch signaling

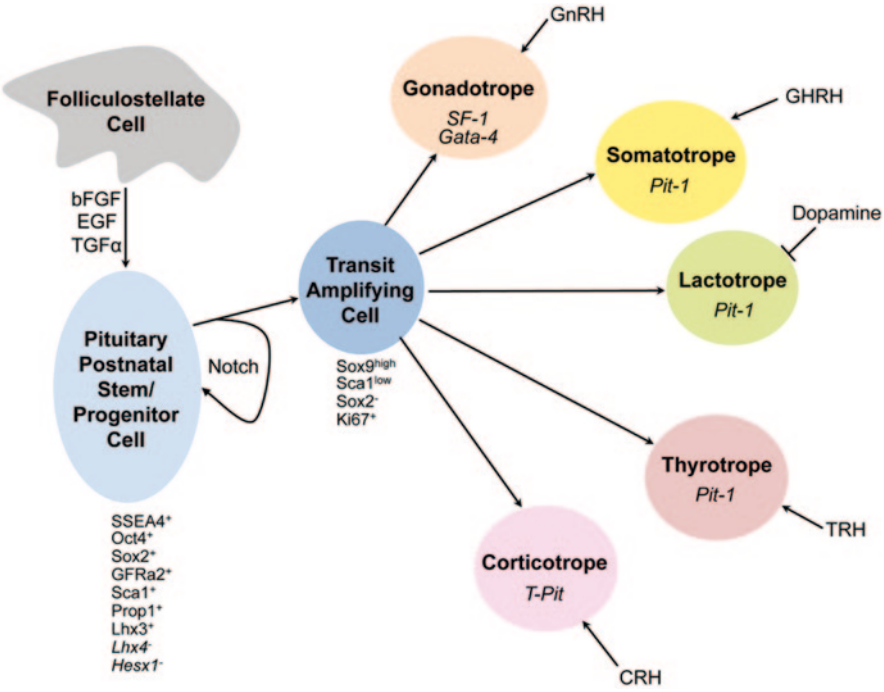

Fig. 2.2 Adult pituitary stem cells, facultative cell expansion, and pituitary tumorigenesis. A niche containing pituitary postnatal stem/progenitor cells (pSPCs) exists into adulthood in the pituitary gland and is the likely source of facultative organ expansions that occur in response to increased physiological demand. Folliculostellate support cells provide growth factors to stimulate pSPCs, and Notch signaling regulates stem cell homeostasis. The pSPCs express *SSEA-4*, *Oct4*, *Sox2*, *GFRa2*, *Sca1*, nestin, *Prop-1*, and *Lhx-3*, but do not express embryonic pituitary stem cell makers Hesx-1 and *Lhx-4*. Transit-amplifying (TAC) cells express *Sox-9* and *Sca1*, but not *Sox-2*, and proliferate more rapidly than pSPCs to allow for prompt cellular expansions in response to physiological demand. The precise signaling events that regulate these expansions remain unknown. Expression of cell-specific transcription factors is required for hormone secretion from each cell type. Hormone secretion from differentiated gonadotropes, somatotropes, lactotropes, thyrotropes, and corticotropes is regulated by gonadotropin releasing hormone (*GnRH*), *GHRH*, *dopamine*, *TRH*, and corticotropin releasing hormone (*CRH*), respectively

functions in pSPC homeostasis [31]. One study also identified putative transit-amplifying (TAC) cells, which express Sox-9, low levels of Sca1, and do not express Sox-2 [28]. The TAC cells are considered to be capable of rapid proliferation, compared to the slow asymmetric doubling of pSPCs, suggesting a role as an important precursor allowing for cellular expansions into differentiated cell types as needed to meet adaptive responses (Fig. 2.2). However, the signaling mechanisms governing these neuroendocrine expansions, the precise role of pSPCs in these adaptive responses, and whether a perturbation in the expansion process leads to prolactinoma tumor formation, all remain unknown [32].

2.3 Signaling Pathways Regulating Lactotrope Homeostasis, Physiological Expansion, and Tumorigenesis

During pregnancy, the mammalian pituitary gland doubles in size, primarily due to expansion of PRL-producing lactotrope cells. However, there is a great deal of debate as to whether this doubling in size is a result of lactotrope hypertrophy or hyperplasia. For obvious reasons, the availability of human pituitary tissue from pregnant women is scarce, and as such many questions remain concerning the morphological changes in the human pituitary gland during pregnancy. Studies in rodents are useful, but are also challenging because human and rodent pituitary physiology is not entirely analogous. In rats, bi-hormonal somatolactotrope precursor cells retain plasticity, allowing for rapid cell differentiation and expansion in response to hormonal need. Somatolactotropes differentiate into lactotropes during pregnancy and into somatotropes in response to exercise [33–36]. No such precursor cell has been identified in humans, and therefore the use of rodent models to study the pituitary during pregnancy becomes convoluted. Additionally, our understanding of the mechanism whereby expanded lactotropes return to the prepregnant state remains unclear. The role of apoptosis, senescence, or simply diminished cell synthesis activity in this process is not understood.

There is an immense capacity for expansion within the lactotrope cell population. During pregnancy, the lactotrope cell population doubles in size. As such, signaling pathways within lactotrope cells are primed to induce rapid cellular expansion. With so much capacity for expansion, there is an increased risk that problems may occur and result in uncontrolled growth. It is very likely that the signaling pathways that are in place to allow lactotropes to undergo recurrent expansions also prime the cell for tumorigenic responses, if one or more oncogenic mutations are present. Here, we will discuss the role of these signaling pathways, and will focus on the pathways that are also known to be involved in mechanisms of tumorigenesis.

2.3.1 Cyclic 3'-5'-Adenosine Monophosphate (cAMP) and Protein Kinase A (PKA) Signaling

cAMP is a second messenger that regulates a diverse set of cellular events. Upon stimulation from an extracellular ligand, G-protein-coupled receptors (GPCRs) become activated and stimulate an associated G-protein. The resulting downstream signaling events depend upon the alpha subunit of the G-protein. $G_{\alpha s}$ proteins activate adenylate cyclase, an enzyme that catalyzes the conversion of ATP to cAMP, leading to a rapid increase in intracellular cAMP and activation of cAMP-dependent PKA. Activation of the cAMP/PKA pathway stimulates the rPRL promoter via the Pit-1 binding sites of FPI and FPIII [37–39]. $G_{\alpha i}$ proteins inhibit adenylate cyclase

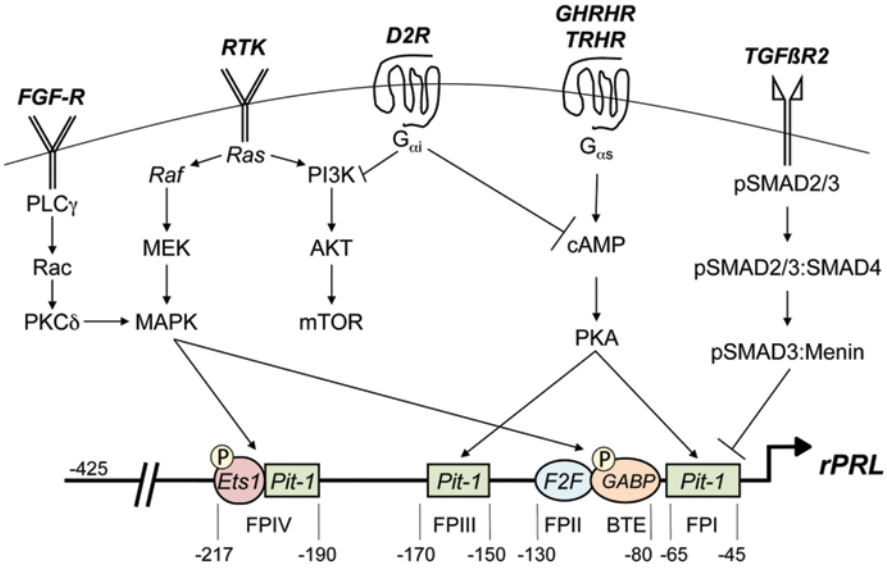

Fig. 2.3 Lactotrope signaling pathways central to pituitary cell proliferation, tumorigenesis, and proximal rat prolactin (PRL) promoter activation. Growth factor receptor tyrosine kinase (*RTK*) and GPCR signaling pathways regulating lactotrope homeostasis and rat PRL (*rPRL*) promoter activation are depicted here. The proximal *rPRL* promoter, with Pit-1 binding sites (*FPI*, *III*, *IV*), *Ets-1* and *GABP* binding sites, and the *F2F* ubiquitous factor binding site are also shown. For further details, see the review by Gutierrez-Hartmann, et al. [39]

activity, resulting in diminished intracellular cAMP levels and reduced PKA activity ([40]; Fig. 2.3).

One of the most studied in the classic regulatory pathways of lactotrope homeostasis is dopaminergic inhibition of lactotrope expansion and PRL secretion. In homeostatic conditions, the secretion of PRL from pituitary lactotropes is inhibited by dopamine. Dopamine binds to the D_2R receptor, which is coupled to a $G_{\alpha i}$ protein, and thus inhibits intracellular cAMP accumulation [7]. Without cAMP, the catalytic subunit of PKA remains sequestered by the regulatory subunit, and cytoplasmic and nuclear target proteins are not phosphorylated, preventing activation of PRL gene transcription and PRL release from the lactotrope cell (Fig. 2.3). GPCR kinases (GRKs) function to desensitize GPCRs that are involved in chemotaxis, and have been shown to play a critical role in cell motility [41]. Another level of homeostatic regulation exists within a short feedback loop between the pituitary and hypothalamus. PRL can bind at the prolactin receptor (PRL-R) on hypothalamic TIDA neurons, increasing dopaminergic release in response to both acute and chronic increases in PRL [42], and further inhibiting cAMP and PKA signaling in lactotrope cells. However, TIDA neurons become refractory when exposed to prolonged hyperprolactinemia during pregnancy or with prolactinoma.

During pregnancy, placental human chorionic gonadotropin (hCG) stimulates production of ovarian estradiol. In response to estradiol, hypothalamic tyrosine hydroxylase, the enzyme that catalyzes the hydroxylation of tyrosine to produce dopamine, is dephosphorylated and inactivated [7, 43, 44]. Similarly, when a suckling stimulus occurs in a lactating mother, dopaminergic inhibition is relieved and PRL is secreted into the blood [45, 46].

Clinically, dopamine agonists such as cabergoline and bromocriptine are used to treat hyperprolactinemia [19]. In many patients, dopamine agonists are successful in halting lactotrope cell proliferation, shrinking prolactinoma size, and reducing PRL secretion. However, a subset of patients are resistant to dopamine agonist therapy [19], likely due to dysfunctional dopamine receptors. Indeed, if dopamine signaling is abolished by dysfunction or knockout of the D_2R receptor in mice, lactotrope homeostasis is lost, resulting in prolactinoma formation [47].

2.3.2 Mitogen-Activated Protein Kinase (MAPK) Signaling

The MAPK signaling pathways connect a wide variety of extracellular signals to intracellular outcomes, including proliferation, differentiation, and apoptosis. The MAPK pathways consist of a three-level kinase cascade, where a MAPK is phosphorylated by a mitogen-activated protein kinase kinase (MAPKK), which must first be phosphorylated by a mitogen-activated protein kinase kinase kinase (MAPKKK). The ERK pathway is the best studied of the MAPK signaling pathways, as dysregulation of ERK signaling is associated with many human cancers. In the ERK signaling pathway, extracellular growth factors and mitogens bind to receptor tyrosine kinases, activating the GTPase Ras, which leads to recruitment and activation the MAPKKK Raf, phosphorylation of the MAPKK Mek, and stimulation of ERK, which ultimately results in phosphorylation of a wide variety of effector proteins including other kinases, phosphatases, and transcription factors ([48]; Fig. 2.3).

The D_2S receptor, the short isoform of D_2R, functions to upregulate MAPK signaling in lactotropes upon stimulation by dopamine [47], suggesting that basal activation of the MAPK pathway does not promote proliferation, but instead maintains lactotrope homeostasis. Furthermore, key regulators of lactotrope biology, such as thyrotropin releasing hormone (TRH) and vasoactive intestinal peptide (VIP), act via Ras to activate MAPK in somatolactotrope cells [49–51].

The duration of MAPK signaling is critical in dictating cellular response [52]. In this review, we will use the following terms: short-term (minutes to hours), long-term (hours to days), and persistent (many days or constitutive activation). Estrogen-induced PRL expression is MAPK-regulated [53], and importantly, the estrogenic effect on lactotropes during pregnancy is persistent, lasting for many months. Estrogen stimulates folliculostellate support cells to produce growth factors such as fibroblast growth factor (FGF) that act via the MAPK pathway [14]. The Ras/MAPK pathway regulates the PRL promoter via a composite Ets1/Pit-1 site [24, 25, 39, 54], and via a BTE ([55, 56]; Fig. 2.3). The precise role of MAPK signaling

in lactotrope proliferation versus differentiation has been somewhat controversial. In vitro studies using rat pituitary somatolactotrope or lactotrope cell lines have shown that short-term (24–96 h) MAPK pathway activation mediates cellular proliferation [14, 57, 58]. By contrast, long-term treatment of GH3 or GH4 rat pituitary tumor cells over 4–7 days with epidermal growth factor (EGF), fibroblast growth factor-4 (FGF4), or thyrotropin-releasing hormone (TRH) result in decreased GH4 cell proliferation and enhanced differentiation to the lactotrope phenotype [59–63]. A persistent pattern of pMAPK activation has been shown to play a pivotal role in cellular differentiation in other endocrine tumors including thyroid carcinoma and pheochromocytoma [64, 65]. The inconsistency in the reported effects of MAPK on lactotrope proliferation or differentiation suggests that the duration of MAPK activation is also critical in dictating the response of lactotrope cells.

The specific role of MAPK signaling in durable lactotrope proliferation and differentiation, and whether activated pMAPK is sufficient for lactotrope proliferation and tumor formation remains unknown. Ras mutations and persistently activated pMAPK are found in human tumors [66, 67], including prolactinomas and other pituitary tumors [18, 68, 69]. Uncontrolled activation of growth factor signaling pathways, such as the Ras/MAPK pathway, results in lactotrope hyperplasia with very delayed adenoma formation in transgenic mice [17, 70]. Transforming growth factor α (TGFα) activates the epidermal growth factor receptor (EGFR) to stimulate the Ras/Raf/MAPK pathway. TGFα is expressed in lactotropes, and upon overexpression promotes proliferation, suggesting a role for TGFα and MAPK signaling in prolactinoma formation [71].

2.3.3 Phosphatidylinositol-3-Kinase (PI3K) Signaling

The PI3K family of lipid kinases functions to activate signaling cascades that regulate diverse intracellular processes such as cell survival, cell cycle progression, and cell growth. Extracellular growth factors bind to receptor tyrosine kinases, which are associated with an intracellular PI3K. When growth factor binds, the receptor is autophosphorylated and PI3K binds to the receptor. The catalytic subunit of PI3K is allosterically activated, resulting in the conversion of phosphatidylinositol-4,5-bisphosphate (PI-4,5-P2 or PIP2) to the second messenger phosphatidylinositol-4,5-trisphosphate (PI-4,5-P3 or PIP3). PIP3 anchors Akt near the membrane via its plekstrin homology (PH) domain, where Akt is phosphorylated by 3′phosphoinositide-dependent kinase 1 (PDK1), which also has a PH domain. Akt is also phosphorylated by the mammalian target of rapamycin (mTOR) 2 complex, mTORC2. Once phosphorylated, Akt activates and inhibits several targets to ultimately influence cell survival, growth, and proliferation. PTEN, a PI-3,4,5-P3 phosphatase, can dephosphorylate PIP3 to negatively regulate PI3K/Akt signaling [72].

The D_2L receptor, a long isoform of D_2R, inhibits PI3K/Akt signaling in lactotropes upon activation by dopamine ([47]; Fig. 2.3), suggesting that inhibition of the PI3K pathway is necessary to inhibit lactotrope proliferation. Inhibition of Akt

results in decreased GH3 somatolactotrope cell viability, likely due to decreased NF-κB activity [73]. Further studies revealed that the proliferative effects of constitutively activated Akt were diminished by the mTOR inhibitor rapamycin as a result of G1 growth arrest [74]. Pharmacological inhibition of PI3K or AKT in GH4C1 somatolactotrope cells results in increased phosphorylation of ERK1/2, as well as Raf1 kinase activity [75]. However, these effects of PI3K/AKT inhibition were diminished upon cotreatment with IGF-1 [75], suggesting that the MAPK and PI3K pathways regulate lactotrope physiology through a delicate balance of intracellular signaling. Preclinical data suggest that increased Ras/MAPK and/or increased PI3K/Akt pathway activity may contribute to pituitary tumorigenesis [76].

As discussed previously, activating mutations in the Ras/MAPK signaling pathway are not sufficient to promote tumorigenesis of lactotrope cells. Transgenic mice studies targeting growth factors (nerve growth factor, TGFα, and FGF-R4) to pituitary lactotropes resulted in early hyperplasia, occurring within approximately 4 months, followed by delayed adenoma formation at approximately 10 months, but these pituitary cells were resistant to true carcinogenesis [71, 77–79]. Activating mutations in an additional pathway, often PI3K, must also occur to promote tumorigenesis [70, 80–82]. Transgenic mice studies targeting oncogenic Ras to thyroid and ovarian endocrine cells show that activated MAPK is necessary, but not sufficient, to mediate proliferative and tumorigenic responses, and that the PI3K pathway is essential [83–86]. These findings support the notion that the MAPK and PI3K signaling pathways work in unison to drive lactotrope differentiation and hyperplasia during pregnancy or prolactinoma formation.

2.3.4 Transforming Growth Factor β (TGFβ) Signaling

TGFβ signaling is important in a wide variety of cellular events, including proliferation, differentiation, and apoptosis. The TGFβ ligand binds to the heterodimerized TGFβ receptor (TGFβ-R), consisting of type I and type II receptor serine/threonine kinases. Upon dimerization, the type II receptor phosphorylates the kinase domain of the type I receptor, ultimately resulting in the phosphorylation of Smad effector proteins. Activated Smad protein complexes are translocated to the nucleus and regulate transcription of target genes [87].

Under basal conditions, TGFβ1 acts on lactotropes to inhibit the effects of estradiol on cell proliferation [12, 88]. Dopamine stimulates TGFβ1 secretion and mRNA expression, resulting in inhibited cell proliferation, suggesting that TGFβ1 mediates the inhibitory action of dopamine on lactotropes [13]. TGFβ1 also inhibits activity of the rat PRL promoter in GH4 cells [89]. Lactotropes do not express the TGFβ2 isoform, and the effect of TGFβ3 on lactotrope proliferation is negligible in the absence of high levels of estrogen [15]. Activin, a member of the TGFβ family, negatively regulates PRL production in lactotropes by repressing transcription of Pit-1. Activin also stimulates phosphorylation of Smad3, which interacts with the tumor suppressor menin to inhibit PRL transcription ([90]; Fig. 2.3).

However, upon exposure to increased estrogen concentration, TGFβ3 indirectly increases lactotrope proliferation by simulating production of growth factors from folliculostellate cells, suggesting that TGFβ3 mediates the mitogenic effects of estrogen [15]. Furthermore, this reveals that TGFβ1 and TGFβ3 have opposing actions on lactotrope cell proliferation [88]. Together these data suggest that a balance of TGFβ signaling is required for lactotrope homeostasis, and a substantial shift in this balance in favor of TGFβ3 is required for physiological lactotrope proliferation in pregnancy and lactation.

2.3.5 Hippo Signaling

The Hippo signaling pathway regulates the growth of tissues during development and regeneration, and also plays a role in cancer. The core kinase cassette of the Hippo pathway consists of mammalian sterile 20 (STE-20) like protein kinases MST1 and MST2, large tumor suppressor proteins LATS1 and LATS2, and adaptor proteins Salvador homologue 1 (SAV1), and MOB kinase activator proteins MOB1A and MOB1B. In the absence of upstream signaling, LATS1 and LATS2 phosphorylate Yes-associated protein (YAP) and transcriptional coactivator with PDZ-binding motif (TAZ), repressing the activity of YAP and TAZ by stimulating ubiquitin-mediated proteolysis. In the presence of upstream signaling, the activity of the core kinase cassette is altered and YAP and TAZ are no longer degraded. Ultimately, Hippo signaling promotes tissue growth and cell viability by regulating the activity of transcription factors such as SMADs and TEADs [91].

In mice, YAP1 activation results in increased liver size [92]. Together with data from human colorectal cancers overexpressing YAP1, it appears that activation of Hippo signaling results in dysplastic growth that promotes increased organ size. Specifically, YAP1 acts to expand multipotent undifferentiated progenitor cells, promoting organ growth in cancer [92]. Activated Hippo signaling is required for mammary gland expansion during pregnancy [93]. Transgenic mice deficient in LATS1 are infertile, have severely impaired mammary gland development, and pituitary hyperplasia [94].

The doubling of pituitary size during pregnancy presents a potential role for the Hippo pathway, although this pathway has yet to be specifically described in the pituitary gland during pregnancy or in prolactinoma.

2.3.6 Casein Kinase 2 (CK2) Signaling

CK2, a serine/threonine protein kinase, is activated by Wnt signaling and is involved in cell cycle control as well as DNA repair. Expression of CK2 is positively correlated with tumor phenotype in various cancers [95]. As of yet, little is known regarding CK2, but it embodies a good candidate for altered regulation of lactotrope cell proliferation in physiological and/or pathological conditions.

2.4 Mutations in Signaling Pathways Associated with Prolactinoma and Useful Mouse Models of Pituitary Adenoma

Neuroendocrine tumors are characterized by excessive secretion of tumor-derived hormone(s), which then inhibit upstream tropic hormones. Despite reductions in tropic hormone levels, the tumor continues to secrete hormone, creating a severely blunted endocrine-feedback mechanism. Prolactinomas are the most common type of neuroendocrine tumor. These tumors secrete excessive amounts of PRL, leading to hypogonadism, infertility, as well as tumor mass effects [19, 20].

In this section we will review mutations in signaling pathways that have been clinically identified in prolactinoma (Table 2.1). While each of these genetic mutations accounts for only small proportion of clinical prolactinomas, they provide valuable insight into which signaling pathways contribute to prolactinoma formation, as well as those that are most important in regulation of lactotrope homeostasis. We will also provide a brief review of animal models that are useful in studying prolactinoma. A great deal has been learned from rodent models and can be applied to human pituitary physiology with awareness that not all facets are equivalent. Prolactinoma is a malady of signal transduction, and attempts to identify a single-key oncogene responsible for lactotrope tumorigenesis have been unsuccessful. Nevertheless, mutations that are identified clinically, as well as mutations that yield prolactinoma in rodents, highlight the fact that alterations in signaling pathways are common in prolactinomas.

2.4.1 Ras

Ras is a small GTPase protein that activates signaling pathways that regulate cellular processes such as proliferation, differentiation, and survival, including the MAPK and PI3K signaling pathways. In humans, there are three Ras genes: HRAS, NRAS, and KRAS. Oncogenic mutations allow Ras to remain in its GTP-bound state, resulting in constitutive activation of Ras signaling. These oncogenic Ras mutations are commonly found in human cancers, with mutations most commonly occurring in *KRAS*. An unusually invasive human prolactinoma was identified to have an HRAS G12V point mutation, and was lethal [96]. Despite the frequency with which Ras is mutated in human cancer, Ras mutations are rare in pituitary adenomas [97–100].

2.4.2 Menin

Multiple endocrine neoplasia type I (MEN1; menin) is a tumor suppressor protein that regulates transcription of cyclin kinase inhibitors such as p27 and p18 by promoting histone methylation [101]. Menin serves to regulate pregnancy-associated islet β-cell expansion [102], suggesting a pivotal role of menin in regulating pSPC-

Table 2.1 Clinically identified mutations in human prolactinoma

Gene	Defect	Signaling abnormality	Phenotype	Reference
RAS	G12V;GOF mutation	Persistent MAPK, PI3K signaling	Invasive prolactinoma	[96]
MENIN	LOF mutation	Fails to induce p18 and $p27^{kip1}$	Multiple endocrine neoplasia type 1	[101]
HST	Overexpression	Induces FGF4 signaling	Invasive prolactionoma	[99]
PTTG	Overexpression	Estrogen-induced; Stimulates FGF2 signaling	Lactotrope hyperplasia; angiogenesis	[18, 99]
AIP	LOF mutation	Decreased PDE4A5 activity resulting in persistent cAMP signaling	Benign adenoma (GH and PRL cosecretion)	[111]
GNAS	GOF mutation (Gsp oncogene)	Persistent $G_{\alpha s}$ signaling	McCune–Albright syndrome (GH and PRL cosecretion)	[18]

GOF gain of function, *LOF* loss of function

mediated expansions during pregnancy or prolactinoma tumorigenesis. Menin-null mice develop late-onset pituitary and β-cell tumorigenesis [103, 104]. An inactivating mutation on chromosome 11q13, the site of the MEN1 gene, has been reported in sporadic human prolactinoma [97], and 60% of MEN1-associated pituitary tumors secrete PRL [105]. However, a separate study reported that somatic MEN1 mutations do not significantly contribute to prolactinoma tumorigenesis [106], suggesting that mutations in other genes may be necessary for prolactinoma formation.

2.4.3 Heparin Secretory Transforming (hst) Gene

The hst gene was originally identified to function as a transforming gene in malignant stomach cancers [107], and encodes for fibroblast growth factor 4 (FGF4). Expression of *hst* mRNA was later identified in human prolactinomas [108], and has been shown to be a marker of invasive prolactinoma [109]. Overexpression of *hst* in rat lactotropes results in increased FGF4 production, as well as increased cell proliferation [109].

2.4.4 Pituitary Tumor Transforming Gene (PTTG)

PTTG is found in all classes of human pituitary adenomas, including prolactinoma. PTTG is expressed at low levels in normal human tissues, but shows increased expression in some human tumors and malignant cell lines. PTTG functions to regulate the separation of sister chromatids during mitosis [99], and has been shown to regulate cell division and survival in endocrine tumors [18]. PTTG was first isolated from rat GH-secreting adenoma cells, and has been shown to be induced by estro-

gen and stimulate FGF2 signaling, resulting in prolactinoma tumor formation and progression in rats [18]. Expression of PTTG is associated with lactotrope hyperplasia, angiogenesis, and prolactinoma development [99], and increased expression level correlates with tumor invasiveness [110]. However, as of yet, a clear correlation between PTTG and tumorigenesis in human adenoma remains unclear [99].

2.4.5 Aryl Hydrocarbon Interacting Protein (AIP)

AIP associates with the cytoplasmic aryl hydrocarbon receptor (AHR), which is a transcription factor that interacts with cell cycle regulators such as retinoblastoma protein (Rb). AIP directly interacts with AHR to regulate its subcellular localization and nuclear cytoplasmic shuttling. AIP also regulates the localization and activity of phosphodiesterase 4A5 (PDE4A5), an enzyme responsible for the hydrolysis of intracellular cAMP. Mutations in AIP can alter the interactions with AHR and PDE4A5, providing a potential role for AIP to regulate signaling pathways that control tumorigenesis [111]. However, the precise mechanisms by which AIP acts as a tumor suppressor in pituitary tumorigenesis have not been specifically identified. Germ-line mutations in AIP have been reported in some familial types of pituitary adenoma, including prolactinomas [111–113]. AIP is considered a pituitary adenoma predisposition (PAP) gene [111]. Many patients with mutations in AIP have pituitary adenomas that secrete both GH and PRL [111], underscoring the shared ontogeny of pituitary lactotropes and somatotropes.

2.4.6 Guanine Nucleotide Activating Subunit (GNAS)

Gain-of-function somatic mutations typically occur in GPCR genes expressed in a tissue-restricted manner, and can lead to neuroendocrine adenoma formation and glandular hyperfunction. The stimulatory G protein, $G_{\alpha s}$, is a product of the *GNAS* gene and regulates activation of adenylate cyclase to produce intracellular cAMP. Activating mutations in *GNAS*, resulting in the expression of the *gsp* oncogene, are associated with somatotrope growth as well as the development of PRL and GH co-secreting adenomas in McCune–Albright syndrome [114]. An invasive prolactinoma that was resistant to dopamine agonists was observed to transition into a GH-secreting adenoma while simultaneously acquiring a de novo mutation in *GNAS* [115].

2.4.7 Unknown/Unidentified Mutations

The aforementioned mutations have been identified clinically in humans. There are many more candidate genes that have been shown to have the potential to promote prolactinoma tumorigenesis, but that have yet to be identified clinically. The majority of patients that present with prolactinoma can be successfully treated with

Table 2.2 Animal models of prolactinoma

Gene	Mutation or altered expression	Phenotype	Reference
Drd2(D2R)	KO	Delayed lactotrope hyperplasia (after 8 months); prolactinoma formation (after 16 months)	[118]
$p27^{kip1}$	KO	Spontaneous anterior pituitary tumor formation	[119]
Retinoblastoma (Rb)	+/−	Pituitary tumor formation in intermediate and posterior lobes (after 8 months)	[120, 121]
Men1	+/−	Anterior pituitary adenoma or carcinoma (after 16 months)	[104, 122]
TGFα	Targeted overexpression in lactotropes via PRL promoter	Lactotrope hyperplasia; prolactinoma formation (after 6 months)	[71]
Estrogen (treatment)	Long-term elevation of serum estrogen in Fischer-344 rats	Lactotrope hyperplasia; Prolactinoma formation	[17]
CDK4	KO	Lactotrope hypoplasia; Diminished serum PRL	[123]

KO knockout

medical therapy, thus surgical resection of tumor tissue is not necessary. As such, prolactinoma tissue is not abundantly available for genetic and molecular analyses. Unfortunately, from the tissue that is available, state-of-the-art immunohistochemical, microarray, and proteomic expression analysis, oncogenic mutation studies, and DNA epigenetic approaches have been mostly unproductive. Novel candidate oncogenes are frequently proposed for tumorigenesis of prolactinomas and other neuroendocrine tumors, but minimal progress has been made to implicate a specific oncogene or tumor suppressor, or markers of proliferation, senescence, dormancy, or antiapoptosis, in pituitary tumorigenesis [116]. The difficulty in identifying candidate oncogenes may be a result of a transient phosphorylation event that cannot be detected with traditional proteomics. Correlative studies have provided only modest information and have failed to give insights as to cause. To date, the best clues about the mechanism of pituitary tumorigenesis come from familial pituitary tumor disorders and mouse models, where mutations in conserved signaling pathways and factors that govern the cell cycle are critical in pituitary tumor formation [112, 117].

2.4.8 Useful Animal Models of Pituitary Adenoma (Table 2.2)

In Table 2.2, we have assembled a list of animal models that have proven useful for studying pituitary adenoma; for more details, see the following references: [17, 71, 104, 118–123]. While rodent models have understandable limitations, a great deal

has been learned from these models and they provide significant insights into the intracellular pathways that may be altered in abnormal human pituitary and lactotrope physiology. It is important to emphasize that although certain genetic alterations can yield PRL-secreting pituitary tumors in mice, adenoma formation is very delayed and thus is not fully accurate in representing the human disease state. This demonstrates that a single gene mutation or deletion is not sufficient, and that an additional mutation is likely required for true prolactinoma tumorigenesis.

2.5 Discussion

Although individual mutations have been proposed as possible driving forces for prolactinoma tumorigenesis in humans, no single mutation has been clinically identified as a causative factor for the majority of prolactinomas. Data collected from individual cases, genomic sequencing, and molecular arrays provide valuable insights into which signaling pathways contribute to prolactinoma formation, as well as those that are most important in the regulation of lactotrope homeostasis. The clinically identified oncogenic V12Ras mutation has been reported in one human prolactinoma that was particularly invasive. However, the same oncogene has antiproliferative and antitumorigenic properties when expressed in GH4 somatolactotrope cells (Booth and Gutierrez-Hartmann, unpublished data), suggesting that Ras signaling is antagonized in lactotrope cells, allowing for evasion of oncogenic Ras signaling and tumorigenesis. It is possible that the invasive prolactinoma with the V12Ras mutation also had an additional mutation in another protein or signaling pathway that resulted in loss of the antagonistic signal, thus allowing Ras signaling to proceed and contribute to the invasiveness of the tumor. Furthermore, recent data demonstrate that the dopamine receptor, D_2R, oppositely regulates MAPK and PI3K signaling [47], indicating that a delicate balance of these signaling pathways may be required to maintain lactotrope homeostasis. The PI3K and MAPK signaling pathways have been shown to act synergistically to promote tumorigenesis in other cancers [70, 81, 82]. Thus, it may be that deregulated MAPK signaling in lactotrope cells that results from oncogenic V12Ras is not tumorigenic as long as PI3K signaling remains in check. Concurrent mutations in MAPK and PI3K pathways may be required for full prolactinoma tumorigenesis. A better understanding of lactotrope-specific responses to Ras/MAPK and PI3K signaling is needed to explain the mechanism of tumorigenesis in prolactinoma. As such, as we move forward in our attempts to elucidate the mechanism(s) of prolactinoma tumorigenesis, it is important to consider the malady of signal transduction that occurs within lactotrope cells. It is unlikely that one sole oncogene responsible for prolactinoma will be identified; instead we must use our knowledge of signaling pathways and the interplay of signals from a cell-specific perspective to make sense of the data we acquire from arrays and clinically identified mutations.

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