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Pathogenesis of Prolactinomas

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Abstract. **In recent years the demonstration that human pituitary adenomas are monoclonal in origin provides further evidence that pituitary neoplasia arise from the replication of a single mutated cell in which growth advantage results from either activation of proto-oncogenes or inactivation of tumor suppressor genes. However, with the exception of one RAS mutation identified in a single unusually aggressive prolactinoma resistant to dopaminergic inhibition that resulted to be lethal, no mutational changes have been so far detected in prolactinomas. In the absence of genetic changes, modifications in the level of expression of oncogenes or tumor suppressor genes have been detected in these tumors, although it is unknown whether these changes have a causative role or are a secondary event. Indeed, our knowledge on the molecular events involved in lactotroph proliferation is even more limited in comparison to the other tumor types, since these tumors are very infrequently surgically removed and therefore available for molecular biology studies. In this respec, it is worth noting that the molecular and biological abnormalities so far described in prolactinomas mainly concern aggressive and atypical tumors and likely do not apply to the typical prolactinomas, that are characterized by good response to medical treatment and a very low growth rate.**

Key Words. **D2 receptor, PRL-omas, growth factors, oncogenes, tumor suppressor genes**

Introduction

The pathogenesis of pituitary tumors has been controversial for many years and the respective role and importance of intrinsic alterations of the pituicytes themselves, hypothalamic dysregulation and locally produced growth factors is still under debate [1–4]. Although the demonstration that the majority of pituitary adenomas is monoclonal in origin indicates that pituitary neoplasia arise from the replication of a single mutated cell, the genetic events able to confer growth advantage to pituicytes are still largely undefined [5,6]. Indeed, the only mutational change so far unequivocally identified in pituitary adenomas, i.e. activating mutations of the guanine nucleotide binding α-subunit 1 gene (GNAS1), termed gsp for Gs protein [7,8], occurs in about 30–40% of GH-secreting adenomas and in 5–10% of other tumor types. In the absence of genetic mutations, changes in the expression of proto-oncogens or tumor suppressor genes (TSG) have been observed. However, it is presently unknown whether these changes have a causative role or represent a secondary event. Moreover, animal models of pituitary tumorigenesis only partially recapitulate the processes occurring in humans. In particular, although pituitary tumors may be induced in the mice by overexpressing or knocking out specific protooncogenes or tumors suppressor genes, respectively, these manipulations generally cause tumor formation almost exclusively in female animals and are preceded by a long-standing phase of cell hyperplasia.

As far as the patogenesis of prolactimonas is concerned, our knowledge on the molecular events involved in lactotroph proliferation is even more limited in comparison to the other tumor types. Indeed, although prolactinomas are the most frequent pituitary tumor, they are very infrequently surgically removed and therefore the few molecular biology studies mainly concern aggressive and atypical prolactinomas. Moreover, several clinical observation suggest the existence of multistep processes in prolactinoma formation. The fact that high resolution neuroradiological imaging "incidentally" detects pituitary microadenomas, that frequently are microprolactinomas, in about 20% of subjects without signs or symptoms of pituitary disorders, a value about 1.000-fold higher than the clinical prevalence of the disease [9,10] suggest that a second hit is required for tumor formation. Finally, the molecular defects responsible for lactotroph proliferation are likely to be heterogenous. Indeed, long term follow up of patients with microprolactinomas suggest that these tumors have a low, if any, tendency to growth over time. The natural history of microprolactinomas is in striking contrast to the rapid growth that characterizes some macroprolactinomas, that appear to be resistant to any therapeutical approach. In this review we will summarize the different molecular alterations that have been proposed to be involved in the formation of human prolactinomas.

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Table 1. *Gain-of-function events in pituitary tumors*

Gene	Defect	Human pituitary adenomas
Cyclin E	Increased expression	ACTH-omas
Cyclin D1	Increased expression	Aggressive adenomas
PTTG	Increased expression	All types (including PRL-omas)
FGFR4	Alternative transcription initiation	All types (including PRL-omas)
Gsα	Somatic mutations	GH-omas
Gi2α	Somatic mutations	NFPA, GH-oma
Ras	Somatic mutations	Pituitary carcinomas metastases; aggressive PRL-oma

FGFR4, fibroblast growth factor receptor 4; PTTG, pituitary tumor transforming gene; NFPA, nonfunctioning pituitary adenoma.

Alterations of Proto-oncogenes

Common protooncogenes

Pituitary tumors may originate from either gain of function mutations or overexpression of ubiquitously expressed protooncogenes that are components of common proliferative pathways. The common protooncogenes that have been extensively analyzed in pituitary tumors include proteins involved in signal transduction, growth factors and their receptors, and cell cycle proteins (Table 1). To date very few protooncogenes have been found to be mutated or overexpressed in prolactinomas. The exception is represented by the point mutation (Gly12Val) in the RAS gene, a gene coding for a GTP binding protein mainly involved in the transduction of growth factor signalling. This mutation was identified in one single unusually aggressive prolactinoma resistant to dopaminergic inhibition, that resulted to be lethal [11]. However, although RAS mutations are present with relatively high frequency in human malignancies they are uncommon in pituitary tumors. In fact, subsequent studies on large series of functioning and non functioning pituitary tumors failed to find RAS mutations [12]. Consistent with the view that this mutational change is associated with unusual malignant feature and probably represents a late event, RAS mutations have been detected in metastases of 3 pituitary carcinomas, but not in the primitive tumors [13].

As far as other coupling proteins involved in signal transduction are concerned, no mutations in the heterotrimeric GTP binding proteins have been identified in prolactinomas. The lack of mutations in Gq and G11, two G proteins that participate in growth factor and hormone mediate generation of Ca^{2+}/calmodulin and phospholipid-dependent protein kinase C (PKC) activation is common to all pituitary tumors [14,15]. By contrast, the lack of mutations in the Gs gene seems to be specific of prolactinomas. Indeed, mutations of Gs (the so called *gsp* oncogene) have been deteced in 30–40% of GH-secreting adenomas and 5–10% of nonfunctioning and ACTH-secreting adenomas. Therefore, although somatotrophs and lactotrophs derive from the same cell lineage, the activation of the cAMP pathway resulting from the expression of the *gsp* oncogene seems to be a proliferative signal only for GH-secreting adenomas [16,17]. This observation is consistent with the notion that while in somatototrophs GHRH exerts potent differentiative and proliferative effetcs, that are mediated by cAMP production, no neurohormone signals through this pathway in lactotrophs.

Growth factors and receptors

The normal pituitary and pituitary tumors produce a wide number of substances with secretory, differentiating and proliferative potentials and express specific receptors. Transforming growth factor-α (TGF-α), epidermal growth factor (EGF) and their common tyrosin kinase receptor (EGF-R) are overexpressed in pituitary adenomas, particularly in those with high aggressiveness [18–21]. Moreover, it has been demonstrated that FGF expression is induced by the pituitary tumor transforming gene (PTTG), an estrogen-inducible gene with high transforming properties found in several human neoplasia, including pituitary adenomas [22,23].

Among the different growth factors produced by the pituitary, evidences obtained from both *in vitro* and *in vivo* in experimental animal models suggest that lactotroph cells secrete nerve growth factor (NGF) together with prolactin [24]. Subsequent studies in patients affected with microprolactinomas confirmed that NGF is released in the bloodstream paralleling prolactin secretion, this secretion being modulated by a neurotransmitter-regulated mechanism. In fact, the normalization of prolactin elicited by the D2 dopamine receptor agonist cabergoline was associated with a significant decrease of serum NGF [25]. Interestingly, the same authors demonstrated that cells obtained from prolactinomas resistant to medical treatment do not express D2R receptors and do not secrete NGF [26]. However, NGF administration is able restore the responsiveness to dopamine agonist in "non responder" prolactinomas cells by inducing D2R expression via p75(NGFR) and NF-kappaB [27]. These data seems to suggest that alterations in the expression and function of the NGF-mediated autocrine loop in lactotroph cells could be involved in the development of prolactinomas with different degrees of malignancy.

Among the four receptors mediating FGF signalling, the aberrant expression of a N-terminally truncated FGF receptor-4, that is constitutively phosphorylated and causes transformation *in vitro* and *in vivo*, has been reported in about 40% of pituitary adenomas, including prolactinomas [28]. Moreover, it has been demonstrated that overexpression of the truncated receptor induces prolactinomas in female and

male transgenic mice while the wild type receptor is ineffective [28]. Interestingly, this truncated FGF receptor-4 diminish cell adhesiveness by disrupting neural cell-adhesion molecule/N-cadherin signaling, a mechanism possibly involved in pituitary tumor pathogenesis [29]. It is worth noting that in contrast to previous models of pituitary tumorigenesis, the expression of the truncated receptor in the pituitary of transgenic mice results in tumor formation in the absence of massive hyperplasia, a phenomenon similar to that observed in human pituitary adenomas [28,30].

Proteins involved in cell cycle progression

Previous studies investigated changes of early immediate genes, such as JUN, FOS and MYC in pituitary adenomas, including prolactinomas [31,32]. More recent studies, carried out to evaluate the expression of proteins regulating cell progression through G1 of the cell cycle, reported overexpression of cyclin D1 in pituitary adenomas, that was mainly related to the aggressiveness of the tumor, while cyclin E was preferentially detected in corticotroph adenomas [33]. No specific pattern of expression has been observed in prolactinomas. However, it is worth noting that surgically removed prolactinomas are likely to be not representative of the prolactinoma *per se*, particularly when indices of cell cycle progression are considered.

All pituitary adenoma subtypes, including prolactinomas and particularly invasive hormone-secreting ones, express PTTG (Pituitary Tumor-Transforming Gene), that was first isolated from rat GH-secreting cells by differential RNA display [34–36]. The human PTTG homolog (PTTG1) is a member of a gene family, maps on chromosome 5q33 and causes *in vitro* cell transformation and *in vivo* tumor induction [37,38]. It is expressed at low levels in most normal human tissues while it is highly expressed in malignant human cell lines and in pituitary and non pituitary tumors [34,39,40]. Structural characterization has identified PTTG as a member of the securin family that regulates the separation of sister chromatids during mitosis [23,41]. Due to the critical role of PTTG in participating in cellular responses to DNA damage and in maintaining genomic stability, it has been proposed that PTTG overexpression may be, at least in part, responsible for the aneuploidism frequently observed in pituitary tumors [42–45]. Moreover, PTTG regulates endocrine tumor cell division and survival [46].

The role of PTTG in pituitary tumorigenesis has been investigated particularly in rat prolactinoma development [45]. In this model, that emphasizes the importance of estrogen in lactotroph proliferation and therefore does not necessary apply to human proactinomas, estrogen induced PTGG overexpression results in the stimulation of fibroblast growth factor-2 (bFGF) expression, that in turn modulates angiogenesis, tumor formation and progression [47–53]. Accordingly PTTG induction, that occurs early in pituitary transformation, correlate with bFGF expression and secretion [21,22]. As estrogens inducs PTTG and PTTG expression coincides with early lactotrophic hyperplasia, angiogenesis and prolactinoma development, a paracrine growth factor-mediated mechanism for pituitary tumor pathogenesis can be envisaged.

Subsequent studies investigating the direct effects of PTTG on hormonal phenotypes of pituitary tumor cells showed that overexpression of PTTG1 C-terminal peptide in rat PRL- and GH-secreting GH3 cells silences PRL gene expression. In contrast, mutations at the PTTG1 C-terminal peptide inactivate PRL gene suppression, suggesting that targeted inhibition of PTTG1 action may be a potential subcellular tool for therapy of prolactinomas [54].

Overexpression of high-mobility group A nonhistone chromosomal proteins (HMGA), that play a role in determining chromatin structure, has been found to be overexpressed in several human carcinomas such as thyroid, prostate and pacreatic carcinomas. As far as the possible involvement of HMGA2 in pituitary tumor pathogenesis is concerned, it has been demonstrated that overexpression of HMGA2 induces GH- and PRL-secreting adenomas in 80% of female transgenic mice by 6 month of age, while the transgenic males develop the same phenotype with a lower penetrance and a longer latency period [55]. Consistent with these observations, high levels of HMGA2 protein have been found in human prolactinomas, the highest expression being observed in those tumors not responsive to dopamine agonist therapy [56].

Pituitary specific proto-oncogenes

Pituitary function is under the control of hypothalamic neurohormones that are required for pituitary cell commitment and growth as well as hormone synthesis and release. Therefore, they may be considered pituitary specific growth factors. As fas as lactotrophs are concerned, it is well established that dopaminergic tone is the most important regulator of prolactin secretion. However, other neuropeptides, such as TRH, may have a stimulatory role on lactotroph function, as indicated by the presence of mild hyperprolactinemia in patients with primary hypothyroidism due to the upregulation of TRH gene expression induced by thyroid hormone defect.

Previous studies carried out on large series of functioning and nonfunctioning adenomas indicate that genes encoding receptors for stimulatory hormones are normal in almost all tumors. In particular, TRH receptor gene has been found unaltered in a large series of secreting and nonsecreting adenomas while prolactinomas generally express a truncated TRH receptor characterized by a reduced binding to the ligand [57]. Although TRH administration typically fails to increase serum prolactin levels in patients with prolactinomas,

TRH receptors are overexpressed in these tumors [58,59]. These data are consistent with the observation that TRH is able to increase intracellular Ca^{2+} levels in adenomatous lactotrophs, suggesting other defects responsible for the *in vivo* unresponsiveness to TRH [60].

In addition to the hypothalamic neurohormones, alteration in the peripheral milieaux may influence lactotroph function and eventually proliferation. In particular, it has been suggested that estrogens may play a role in promoting lactotroph growth. This hypothesis mainly derives from the observation that estrogen administration rapidly causes the appearance of huge prolactinomas in female rats. However, although prolactinomas are more frequent in women than in men, no direct relationship between the exposure to estrogens, even at high doses, and the occurrence of prolactinomas seems to be present in humans. Indeed, lactotroph hyperplasia in estrogen treated male-to-female transsexual patients seems to be a rare event [49]. Moreover, the sex difference in prolactinoma incidence mainly concerns microprolactinomas. As far as the expression of estrogen receptor in pituitary tumors is concerned, *in vitro* studies demonstrate that the expression of estrogen receptor alpha and several of its messenger ribonucleic acid alternate splice variants is restricted to prolactinomas and gonadotroph tumors [61]. However, little is known about the impact of these variants on cell growth.

Alterations of Tumor Suppressor Genes

Pituitary cell proliferation may results from the inactivation of either common tumor suppressor genes or specific inhibitors of pituitary cell function and growth. Contrary to oncogenes that cause the tumoral phenotype also when present in only one allele, tumor suppressor genes are recessive and the inactivation of both alleles is believed to be required to cause the loss of antitumoral action [62,63].

Common tumor suppressor genes

The role of loss of tumor suppressor genes in causing pituitary tumors has been clearly demonstrated in rodents, in which knocking out retinoblastoma gene (RB) or p27Kip1, a cyclin-dependent kinase inhibitor that induces G1 arrest by RB hypophosphorylation, results in intermediate lobe hyperplasia and ACTH-secreting adenoma by few months of age in the mouse [64–66]. However, data in human pituitary tumors are not conclusive. Indeed, although loss of heterozygosity (LOH) on chromosome 13q, where RB gene is located, is a relatively frequent event particularly in invasive or malignant tumors [67,68], no mutational changes in RB or p27Kip1 genes have been identified while the reduced expression at the protein levels seem to be restricted to p27Kip1 in ACTH-secreting adenomas, recurrent pituitary tumors and pituitary carcinomas [69,70]. A similar reduced expression affects p16INK4a, another cyclin-dependent kinase inhibitor that prevents RB phosphorylation [71,72] (Table 2).

Table 2. *Loss-of-function events in sporadic pituitary tumors*

Gene	Defect	Human pituitary tumor
RB	Promoter methylation	Aggressive adenomas
p16INK4a	Promoter methylation	All types (including PRL-omas)
p27Kip1	Reduced expression	ACTH-omas
TRβ	Inactivating mutations	TSH-omas
GR	LOH	ACTH-omas
D2R	Reduced expression	Resistant PRL-omas
Sst2	Reduced expression	Resistant GH-omas

RB, retinoblastoma; LOH, loss of heterozygosity; D2R, dopamine receptor type 2; sst2, somatostatin receptor type 2; TRβ, thyroid hormone receptor β; GR, glucocorticoid receptor.

Finally, no mutations have been found in the gene encoding p53, a proapoptotic signal that is frequently altered in human neoplasia while the significance of p53 overexpression detected by immunohistochemistry in invasive nonfunctioning pituitary tumors and corticotropinomas remains elusive [73–75]. As far as prolactinomas are concerned, it has been recently demonstrated by using conformation-specific antibodies and immunocytochemistry that in bromocriptine-resistant prolactinoma cells p53 adopts a mutant conformation that precludes its nuclear translocation and transcriptional activity. Interestingly, this phenotype is reverted by NGF administration which results in p53 refolds into wild-type tertiary structure, promoting its nuclear translocation, and restoring its DNA-binding activity [76].

Since pituitary tumor is part of the multiple endocrine neoplasia type I (MEN-I) syndrome, the gene responsible for the disease has been thought to be implicated also in the genesis of sporadic pituitary adenomas [77]. The gene whose mutation is responsible for MEN1 encodes a 610 amino acid protein, menin, which is able to interct with several proteins and transcription factors involved in the control of cell proliferation [78]. Accordingly, mice heterozygous for MEN1 knockout develop parathyroid, pancreatic ß-islet and pituitary tumors, largely recapitulating human MEN1 syndrome [79–84]. Although LOH in the region 11q13, the region where MEN1 gene is located, is present in 10–20% of sporadic pituitary adenomas [74,85] subsequent studies failed to find either mutations in MEN 1 gene in the retained allele or reduced mRNA levels in most pituitary tumors [86–89]. The LOH in several other loci in addition to 11q13 and 13q, such as 10q26, 11p, 22q13, suggest the involvement of other still unknown tumor suppressor genes in the genesis of human pituitary adenomas [2].

Different studies have investigated the presence of differences in prolactinoma behavior in MEN1 patients

vs non-MEN1, and it was assumed that there were no clinical or histopathological differences between the two groups [90–94]. However, a recent study reporting the first large systematic analysis of pituitary tumors in patients with MEN1 vs patients without suggests that pituitary tumors in MEN1 patients may be more aggressive as assessed by size and responsivity to treatments [95]. The tumor size difference was evidenced by a doubled prevalence of macroadenoma (85% vs 42%) and the greater resistance to treatment was deduced because only 42% of tumors in MEN1 cases reduced prolactin levels within the normal range during dopaminergic treatment, compared with 90% without MEN1. These unexpected differences, if confirmed by further investigations, have clinical implication and raise interesting issues about tumorigenesis mechanisms in prolactinomas.

Pituitary specific tumor suppressor genes

Genes coding for membrane and nuclear receptors that physiologically inhibit pituitary hormone secretion may be considered as possible targets for inactivating mutations leading to hormone hypersecretion and eventually tumor growth. This is the case of the dopaminergic D2 receptor (D2R) that mediates the inhibitory effect of dopamine on prolactin secretion at the pituitary level. In fact, it has been demonstrated that D2R-deficient mice present hyperprolactinemia and massive lactotroph hyperplasia at 9 to 12 months of age. Moreover, the same D2R-deficient mice subsequently develop pituitary lactotroph adenomas that are often 50-fold larger than normal glands with marked suprasellar extension and invasion of brain [96,97]. Although this model indicates that loss of dopamine inhibition induces neoplastic transformation, its relevance to the human situation is questionable. In fact, up to now no mutation in the D2R gene has been reported, although it is worth noting that the number of screened tumors is low since patients with this tumor type rarely undergo pituitary surgery [98]. However, little is known about the possible involvement of D2R polymorphisms in the pathogenesis of prolactinomas. In particular, it has been demonstrated that the TaqI A DRD2 minor (A1) allele, which has been found to be linked to alcohol, cocaine, nicotine and opioid dependence [99,100], is associated with a reduced brain dopaminergic function. Interestingly, a reduction of D2R mRNA, and particularly of the shortest isoform that is more efficiently coupled to phospholipase, has been frequently demonstrated in prolactinomas resistant to dopamine agonist treatment [101].

In addition to the defect in D2R splicing and expression, the absence of D2R protein despite the retention of D2 transcript has been observed in metastases of a malignant prolactinoma resistant to different dopamine agonists [102], suggesting that alterations in protein stability or degradation may contribute to the failure of medical therapy and eventually to lactotroph growth.

Conclusion

In the past years several candidate factors have been implicated in the genesis and progression of prolactinomas. To date, the only mutational change so far unequivocally identified in pituitary adenomas is the gsp oncogene, that has been identified in about 30–40% of GH-secreting adenomas, in 5–10% of non-functioning pituitary adenomas and ACTH-secreting adenomas, but in none prolactinoma. Overexpression of protooncogenes, that include cell cycle progression molecules, growth factors or receptors, such as PTTG, HMGA2, FGF-R type 4, has been observed in prolactinomas, although it is presently unknown whether these changes have a causative role or represent a secondary event. Similarly, the low expression of tumor suppressor genes has been implicated in lactotroph proliferation, although no genetic mutations of candidate genes have been so far identify, despite the frequent presence of LOH in several loci.

Several questions arise when studying the pathogenesis of human prolactinomas. In particular, animal models of prolactinomas only partially recapitulate the processes occurring in humans. In fact, estrogens, that are the most potent stimulus for lactotroph proliferation in the rat, have a poor, if any, relevance for human pituitary tumorigenesis. Moreover, overexpressing or knocking out specific protooncogenes or tumors suppressor genes in the mice cause tumor that are preceded by a long-standing phase of cell hyperplasia, a phenomenon typically absent in human prolactinomas. Finally, although prolactinomas are the most frequent pituitary tumor, they are very infrequently surgically removed and therefore few molecular biology studies are available and, most importantly, they mainly concern aggressive and atypical prolactinomas.

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