Chapter 2

New Insights in the Pathogenesis of Pituitary Tumours

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Additional information is available at the end of the chapter

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1. Introduction

Pituitary adenomas (PA) are frequent and typically benign endocrine neoplasia, which clinical prevalence is estimated around 1/1000 inhabitants [1]. The vast majority are sporadic. PA are endowed with significant clinical morbidity related to hormonal hypersecretion, neurological symptoms due to intracranial mass effects or invasion of the surrounding structures and/or secondary hypopituitarism. Their evolution is quite variable, ranging from indolent tumours with an extremely slow growing potential, to recurrent, aggressive, and exceptionally malignant tumours. Their current clinical management is based on pharmacological treatment, mainly dopamine-agonists (DA) and somatostatin analogues (SSA), surgery and radiotherapy [2]. Despite considerable progress in the management of PA, a significant subset of patients are not satisfactorily controlled. Long-term uncontrolled pituitary hormone hypersecretion, leading to potential severe systemic diseases, and tumour recurrence or aggressiveness still represent a difficult clinical challenge. Understanding the mechanisms involved in the pathogenesis of PA is essential for the development of new therapeutic strategies. In this chapter, we will summarize current concepts in pituitary tumorigenesis and focus our attention on the most recent insights and new perspectives in this field.

2. Pituitary tumours

Primary pituitary tumours in human are mainly represented by PA arising from endocrine cells in the anterior lobe and craniopharyngiomas. These latter are divided into adamantinomatous, which derive from the Rathke's pouch, and papillary craniopharyngiomas.

Additional tumour types deriving from non-endocrine cells of the anterior pituitary and the neurophypohysis can be found [3], which pathogenesis is poorly known and will not be considered in this review.

2.1. Classification

PA may be classified according to their macroscopic characteristics into micro- (< 1 cm) or macro-adenomas (≥ 1 cm), and enclosed or invasive adenomas. Invasion of the surrounding structures (cavernous sinuses, bone, sphenoidal sinus) is generally defined according to neuroradiological imaging – especially magnetic resonance imaging -, although intra-operative findings may introduce some correction to the pre-operative radiological classification or reveal macroscopic dural invasion. Of note, microscopic evidence of dural invasion is rarely present on surgical samples. The functional classification of PA is based on their hormone-secreting potential, which may be associated with bio-clinical evidence of hormone hypersecretion or recognized by immunohistochemistry (IHC) for pituitary hormones and/or by specific ultrastructural features. From an epidemiological point of view, prolactinomas are by far the most frequent (50-60%), followed by clinically non-functioning PA (NFPA) (20-30%), somatotrophinomas (10-15%), corticotrophinomas (5-10%) and thyreotrophinomas (1-2%) [1,2,4]. Recruitment bias are frequently encountered in pathological series, since most prolactinomas are treated by DA only. Clinico-pathological correlations in PA have been recently reviewed [4]. In the large majority of PA associated with bio-clinical evidence of hormone secretion, except functional hyperprolactinemia, pathological examination will confirm the diagnosis of PRL, GH, ACTH or TSH-secreting tumours and potentially identify bi- or multi-hormonal secretion. This is especially true for GH-secreting PA, which may also secrete PRL, less frequently glycoprotein hormones, or both (multihormonal). TSH-secreting adenomas are rare and frequently multihormonal. Ultrastructural studies of secreting PA may disclose a “densely granulated” (DG) or “sparsely granulated” (SG) pattern, which may reflect significant differences in hormone secretion and tumour behaviour. This has been well studied in somatotrophinomas, where IHC for cytokeratin can be used to disclose the typical “dot-like” staining pattern of SG adenomas. Although a continuum exists between the SG and DG types, pure SG are typically more aggressive than DG somatotrophinomas [5]. Pathological examination of NFPA may show negative immunostaining for all pituitary hormones (the “null cell” or endocrine inactive histotype), positive immunostaining for FSH and/or LH (gonadotrophinomas) or reveal silent secretion of other pituitary hormones, in particular ACTH and GH (“silent” secreting PA). Cell lineage may also be identified by the expression of specific transcription factors (see “pituitary ontogenesis”) [4]. It is generally accepted that most NFPA derive from the gonadotroph lineage, since data obtained from primary cultures or molecular analysis of these tumours frequently reveal a silent expression of gonadotropins or their subunits, including α–subunit only. Also, transgenic mice overexpressing SV40 under the control of the βFSH promoter develop gonadotroph hyperplasia

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and PA with reduced gonadotropin immunoreactivity and ultrastructural characteristics similar to human null cell PA [6]. Silent corticotroph adenomas are commonly aggressive and different subtypes have been described. Pituitary carcinomas are strictly defined by the presence of extra-pituitary dissemination (see "pituitary carcinomas"). Therefore, no diagnosis of pituitary carcinoma can be made on a surgical pituitary sample. Considerable efforts have been made to recognize the aggressive potential of PA according to pathological criteria. However, mitoses are generally rare and the percentage of cells immunopositive for the Ki67 antigen (which is expressed throughout the cell cycle and detected with the MIB1 monoclonal antibody) is currently considered as the best marker of cell proliferation. A Ki67 index ≥3% is commonly associated with invasiveness [4], although it can be reduced by pre-operative pharmacological treatment in secreting PA [7]. Immunostaining for p53 is also frequently associated with invasiveness and is typically present in carcinomas [4]. For these reasons, the 2004 WHO conference has proposed to define as "atypical adenomas" a subset of invasive PA characterized by Ki67 labelling ≥ 3% and extensive p53 nuclear staining [8]. However, many criticisms remain and search for reliable markers of aggressiveness or malignancy is going on. Gene expression profiling comparing non-invasive adenomas with their invasive counterpart [9, 10] or with pituitary carcinomas [11] represents a promising approach.

2.2. Origin

The pathogenesis of PA is multifactorial. Traditionally, two theories have been proposed: the primary pituitary origin, and the hypothalamic origin of PA or, more generally speaking, the concept that PA may derive from abnormal pituitary regulation. Acromegaly or Cushing's disease resulting from ectopic secretion of GHRH or CRH by neuroendocrine tumours, respectively, or estrogen-induced prolactinomas, represent the main evidence for the second theory. However, the chief lesion in such conditions is hyperplasia, with PA developing in a very minority of cases, whereas hyperplasia is exceptionally observed surrounding the tumoral tissue in human PA. Although hyperplasia may be difficult to identify (reticulin staining is not routinely proposed), there is accumulating evidence supporting the primary pituitary origin of PA. Indeed, PA are essentially monoclonal in origin, and a number of genetic abnormalities have been identified these tumours, which include somatic events and inherited predisposition. The relatively low rate of recurrences following complete surgical removal of enclosed PA, especially microadenomas, also favours a primary pituitary hypothesis. However, polyclonality can also be observed on PA, and different clones may develop in a synchronous or delayed pattern, possibly accounting for tumour progression or regrowth [12]. A unifying view is that pituitary tumorigenesis is a multistep and multifactorial process, which includes early initiating genetic events, growth promotion by extracellular factors (including the extracellular matrix, growth factors, cytokines, neuropeptides and peripheral hormones) and additional genetic events contributing to a further progression of the tumour in terms of invasiveness, recurrences and, exceptionally, metastasis [13,14].

3. Pituitary developmental pathways and their potential alterations in pituitary tumours

Because the expanding knowledge about the molecular mechanisms involved in pituitary ontogenesis is providing new clues in the understanding of pituitary tumorigenesis, relevant findings in this field will be summarized.

3.1. Ontogenesis of the pituitary gland

The anterior pituitary lobe (AP) derives from an invagination of the oral ectoderm forming the Rathke's pouch (RP), which cells proliferate and subsequently undergo progressive terminal differentiation into the 5 adult pituitary cell types (corticotrophs; somatotrophs, lactotrophs, gonadotrophs and thyreotrophs), whereas the posterior lobe (or neurohypophysis) derives from a specialized region of the neuroectoderm, the infundibulum. The intermediate lobe also arises from the RP and contains melanotrophs in rodents but is virtually absent in humans. The molecular mechanisms of pituitary ontogenesis have been mainly studied in the mouse and, in addition to genetic models which have contributed to elucidate the role of single proteins [6], analysis of transcriptomes obtained from cDNA libraries in the developing embryo represents a promising tool to identify new genes involved in this process [15,16]. Complex interactions between signalling molecules (in particular opposite signals coming from the diencephalon and the ventral ectoderm) and pituitary transcriptions factors (TFs) are involved and tightly regulated in a spatially and temporally organised manner. Extensive reviews are available on this topic [17,18]. Genetic defects in pituitary TFs are responsible for inherited abnormalities in pituitary development, spanning from syndromic diseases due to defects in early factors (*eg*. Pitx2, Lhx3/4, Hesx1) to multiple pituitary hormone deficiency (*eg* Prop-1, *Pit-1*) or single hormone deficiency (*eg*. Tpit) [16]. Noteworthy, some of these defects are associated with transient pituitary hyperplasia. On the other hand, abnormal signalling from developmental molecules such as FGFs, BMPs, and master developing pathways (Wnt Hedgehog, Notch) is being increasingly recognized in PA. Table 1 summarizes the potential involvement of developmental TFs and signalling molecules in PA. Overall, the expression of TFs involved in the terminal differentiation of pituitary cells (*eg*. Pit-1, Tpit) is maintained in functioning PA and may contribute to identify the original cell lineage in NFPA [4,19]. However, a phenotypic marker may not be fully specific, as reported for NeuroD1, which is frequently expressed in NFPA in addition to corticotrophinomas [20]. Early to intermediate pituitary TFs such as Hesx1, Pitx1 and PROP1 are expressed in the adult pituitary gland and in all PA [21-23]. PROP1 represses *Hesx1* expression while up-regulating that of *Pit-1* and *SF1* and *Prop-1* transgenic mice develop different PA phenotypes, except corticotrophinomas [24]. Pitx2, which is required at different stages of pituitary development, is expressed in the normal pituitary, in PRL- and TSH-secreting PA and selectively overexpressed in NFPA [22]. FOXL2 is also expressed in the adult pituitary and overexpressed in gonadotroph and NFPA [25].

Gene	Expression during ontogenesis	Function	Expression in pituitary tumours
Early signalling molecules in pituitary development			
BMPs (BMP 2 and 4)	VD	BMP4: essential for the invagination of Rathke's pouch; BMP2: involved in opposing gradients with FGF8 that generate distinct patterns of transcription factors expression	BMP4: overexpressed in prolactinomas, underexpressed in corticotrophinomas
FGFs (FGF 8 and 10)	VD/RP	FGF10 : essential for cell survival FGF8: involved in the maintenance of RP cells proliferation opposing gradients with BMP2	NA
WNT4 and 5A/ β-catenin	VD/RP	Involved in pituitary development and in the induction of *Pit-1*	Wnt4 : expressed in GH/PRL/TSH-secreting PA
Shh/*Gli2*	VD; oral ectoderm except RP	Involved in early proliferation and cell type determination as well as in the control of adult pituitary cell function and proliferation	Shh: underexpressed in PA, including corticotrophinomas
Notch 2 and 3	VD/RP	Required for early lineage commitment and the terminal differentiation of distinct cell lineages	Notch3: overexpressed in NFPA
Sox2	VD/RP	Required for pituitary development and the maintenance or proliferation of pituitary progenitor cells	Expressed in adamantinomatous craniopharyngiomas
Transcriptional factors that control pituitary development			
Hesx1	VD/RP	Involved in early pituitary development	Expressed in all PA phenotypes
Otx2	RP	Involved in RP development, in particular in the expression of *Hesx1*	NA
Lhx3-4	RP	Required for cell survival and prevention of apoptosis	NA
Isl1	RP	Required for the proliferation and differentiation of pituitary progenitors	NA
Six 1, 3 and 6	RP	Six1 regulates cell proliferation; Six6 is required for pituitary development and cell proliferation; Six3 interacts with Hesx1	NA
Pitx 1 and 2	RP	Pitx1 e Pitx2 activate the early transcription of the *αGSU* gene, while promoting cell proliferation, then they	Pitx1: expressed in all PA phenotypes Pitx2: overexpressed in NFPA

Gene	Expression during ontogenesis	Function	Expression in pituitary tumours
		activate *LHβ, FSHβ, GnRH, PRL* and *GH* gene transcription, while promoting cell differentiation Pitx1 also binds the *POMC* promoter	
FOXL2	AP	A transcriptional activator of *αGSU*	Overexpressed in NFPA
Corticotroph differentiation			
NeuroD1	RP	A transcriptional activator of *POMC,* involved in corticotroph differentiation	Expressed in corticotrophinomas and a subset of NFPA
Tpit	POMC precursor	A transcriptional activator of *POMC,* essential for the specification and terminal differentiation of corticotrophs and melanotrophs,	Expressed in secreting and silent corticotrophinomas
Differentiation of Pit-1 lineages: somatotroph, lactotroph and thyreotroph differentiation			
Prop1	RP	A transcriptional activator of *Pit-1* and a trascriptional repressor of *Hesx1*	Expressed in all PA phenotypes
Gata2	RP	Interacts and cooperates with *Pit-1* for the activation of *TSHβ*	Expressed essentially in gonadotroph and thyreotroph PA
Pit-1	Pit-1 lineages	Essential for the terminal differentiation and expansion of GH/PRL/TSH-secreting cells; an enhancer of *GH, PRL, TSHβ e GHRHR* gene expression and a repressor of *Gata2*	Expressed in GH/PRL/TSH-secreting PA (including NFPA with silent secretion)
CREB	Pit-1 lineages	Required in somatotrophs	Expressed in all somatotrophinomas
Gonadotroph differentiation			
Gata2	RP/pituitary	A promoter of *SF1* gene expression	See above
SF1	Gonadotrope	A promoter of *αGSU, FSHβ, GnRHR* gene expression, it also interacts with Egr1 and Pitx1 for the activation of LHβ	Expressed in the majority of gonadotroph/ NFPA, not in GH/PRL-secreting PA
Egr1	Gonadotrope	Required for *LHβ* gene expression	NA

Table 1. Expression of pituitary developmental signalling molecules and TFs

3.2. Developmental pathways in pituitary adenomas

Three master developmental pathways – Wnt, Hedgehog and Notch – have been involved in several human diseases, in particular in cancer. We will attempt to summarize current

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knowledge about their role in pituitary development and tumorigenesis, which is likely to expand significantly in the next years.

3.2.1. The Wnt/beta-catenin pathway

The canonical Wnt/β-catenin signalling pathway plays a role in the specification of pituitary progenitor/stem cells and β-catenin modulates the transcriptional activity of several pituitary TFs, in particular Pitx2, PROP-1 (thereby promoting the activation of *Pit-1* and the repression of *Hesx1*) and SF1 [26]. Wnt4 and Wnt5a have been involved in pituitary development and growth, respectively. Wnt signalling has also been recently involved in the pathogenesis of craniopharyngiomas [27-30] and PA [26]. Briefly, secreted Wnt proteins bind the Frizzled (Fz)/ Lipoprotein low-density receptor (LPR) membrane complex on the cell surface of target cells, inducing an intracellular reponse mediated by Dishevelled (Dsh), the glycogen synthase kinase 3β (GSK-3β), Adenomatous Polypous Coli (APC), axin and β-catenin. In the absence of Wnt ligands, the GSK-3/APC/axin complex drives phosphorylated β–catenin to proteasome-mediated degradation and Wnt signalling is repressed. In the presence of Wnt ligands, the inhibition of β–catenin phosphorylation leads to its cytoplasmic accumulation, nuclear localization and activation of Wnt target genes (*eg cyclin D, c-myc, survivin*). Wnt signalling is modulated by several secreted Wnt inhibitors and further regulated by adhesion molecules (*eg.* E-cadherin) and extra-cellular signalling pathways (*eg.* FGF). Beta-catenin mutants lacking phosphorylation sites are able to activate Wnt signalling in a constitutive manner. Activating mutations of the β–catenin gene (*cadherin-associated protein β1/CTNNB1*) have been reported in several human neoplasias and in adamantinomatous craniopharyngiomas (ACPs) [27]. Although the prevalence of *CTNNB1* mutations has been variably appreciated in craniopharyngiomas, they are specific for the ACP phenotype and consist of amino acid substitution of GSK-3β phosphorylation site or flanking residues [28, 29]. Their causative role in the development of ACPs has been recently proven in a transgenic mice model [30]. *CTNNB1* mutations are exceptional in PA, but several members of the Wnt signalling pathway have been detected at a transcriptional level [26]. Studies on β-catenin expression and localization in PA have lead to conflicting results, some studies reporting a nuclear accumulation in a variable proportion of cases (0-57%) and others a predominant membrane staining with a frequent cytoplasmic immunopositivity [26]. A reduced expression of several Wnt inhibitors has been reported in PA [31]. Among them, WIF1 is a tumour suppressor, which reduced protein expression was predominantly reported in NFPA, although transcriptional down-regulation is common in all histotypes [31].

3.2.2. Sonic hedgehog

Sonic hedgehog (Shh) is involved in pituitary ontogenesis and regulates pituitary hormone release. Shh signalling is mediated by Patched receptors (Ptc1, Ptc2) and the Gli family of TFs. During ontogenesis, Shh is expressed by the ventral diencephalon and by the oral ectoderm immediately adjacent to RP. There is recent evidence that Shh signalling is of particular importance before the RP detachs from the oral ectoderm, and that Gli2 is responsible for the proliferation of early pituitary progenitors and for the diencephalic induction of FGF8 and

BMP4 [32]. This is consistent with reports of human inactivating Gli2 germline mutations associated with severe pituitary developmental defects and different degrees of craniofacial abnormalities [33]. In the adult pituitary Shh/Ptc2/Gli1 signalling is active in corticotrophs and stimulates POMC transcription and ACTH release; Gli1 is necessary for CRF signalling [34]. Contrasting with the corticotroph specificity of Shh, Ptc receptors are also expressed in other pituitary secreting cells, although in a phenotype-specific manner [34]. Shh immunostaining was found low or absent in a large series of PA, including corticotrophinomas, whereas the expression of Ptch receptors was retained [35]. Shh was also found to stimulate ACTH, GH and PRL secretion by normal and tumorous pituitary cells *in vitro* and to inhibit cell proliferation in pituitary cell lines [35]. Thus, Shh may exert differential effects on pituitary cell growth (with stimulating effects on progenitor cells and inhibiting effects in differentiated cells) and is able to differentially modulate secretion and proliferation in PA.

3.2.3. Notch/Hes1

Notch signalling regulates progenitor cell differentiation during embryogenesis. Notch is a cell-to-cell signalling network composed by transmembrane ligands (in mammals Delta-like 1,3,4 and Jagged 1,2), the transmembrane Notch receptors (Notch 1-4), and a transcription factor (in mammals RBPJ), which is activated by the intracellular fragment of Notch after ligand-induced Notch cleavage has occurred. Notch target genes encode beta-helix loop-helix (bHLH) TFs, in particular the *Hairy Enhancer of Split (Hes)* family. A peculiarity of Notch signalling is lateral inhibition, with intracellular signalling being inactive in cells expressing Notch ligands. After the Notch pathway was shown to be oncogenic in haematological malignancies, it has been involved in several solid tumours, with either oncogenic or tumour suppressing functions, depending on the tissue and the expression of the different components of the Notch pathway. Notch is also involved in angiogenesis and participates in tumour angiogenesis through interactions with the VEGF pathway [36]. Genes encoding Notch signalling molecules are temporally and spatially regulated in the developing pituitary. During rodent pituitary ontogenesis, expression of the *Notch2, Notch 3* and *Delta-like 1 (Dll1)* genes was observed in the RP, whereas *Delta-like 3 (Dll3)* gene expression was restricted to the melanotrophs and early corticotrophs [37]. PROP-1 has been shown to specifically induce Notch2, with *Prop1* ablation inducing a dramatic reduction in *Notch2* and a concomitant increase in *Dll1* [37]. Expression of the *Hes1* and *Hes6* genes was also observed in the RP [37]. HES1 was subsequently shown to repress the expression of different cell cycle inhibitors and mediate the balance between pituitary progenitors proliferation and differentiation [38]. At the moment, few data are available on the possible role of the Notch pathway in the pituitary tumorigenesis. Overexpression of Notch3 has been recently reported in NFPA [39], and several elements of the Notch pathways have been identified in the transcriptome of prolactinomas [40] and multihormonal PA [41], suggesting that new data will be available in the future.

3.3. Pituitary stem cells

The pituitary gland is characterized by a high degree of plasticity, which is involved in pituitary function changes through life and adaptation to physiological and pathological

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variations in peripheral hormone feed-back. Several conditions require a re-organization of pituitary cell composition, with an expansion of a specific cell pool (*eg.* lactotrophs during pregnancy and lactation). This may theoretically result from an increased proliferation of already differentiated cells, a transdifferentiation from a pre-existing cell population or a recruitment of putative adult pituitary stem/progenitor cells [42,43]. The presence of post-natal pituitary progenitor/stem cells able to self-renew and differentiate into hormone-secreting cells has now been recognized by several groups. Five potential pituitary stem cells or progenitor cell populations have been characterized in the mouse and reported as side population (SP), pituitary colony-forming cells, nestin-expressing cells, $Sox2^+/Sox9^-$ cells and GFR-α2-Prop1-Stem cell marker-expressing (GPS) cells, respectively [42,43]. The best characterized are SP cells, $Sox2^+/Sox9^-$ and GPS cells [42,43]. These cell populations represent <1% to 3-5% of anterior pituitary cells, are localized mainly along the Rathke's cleft (the proposed pituitary stem cells niche) and are able to form pituispheres *in vitro*. The question arises whether these cells may contribute to pituitary tumorigenesis. Cancer stem cells have been reported in a number of human malignancies and derive their name from their ability to develop tumours, in the same way by which normal stem cells lead to organ development. Their origin is not fully understood and they may derive from normal stem cells, re-differentiation/dedifferentiation of progenitor or differentiated cells, or both [42]. A possible role for pituitary progenitor/stem cells in pituitary tumorigenesis has been recently proposed. Conditional deletion of RB in *Pax7* precursors was sufficient to generate NFPA in mice [44]. In addition, floating clonal spheres have been isolated from two human PA [45]. These putative 'pituitary adenoma stem-like cells' (PASCs) overexpressed several stem cells-related genes, including components of the Notch and Wnt/β-catenin pathways and were tumorigenic *in vivo*. Tumours from the mice craniopharyngioma model cited hitherto also contained cells with phenotypic characteristics of progenitor/stem cells and *Sox2*/*Sox9* expression [30]. Similar findings have been reported in human ACP, with a subset of samples also expressing RET or GFRα [46]. Further work is warranted to clarify the possible contribution of stem cells in PA, especially in those showing an aggressive behavior.

4. Genetics of pituitary tumours

PA are triggered by a variety of genetic abnormalities [13,14,47]. The vast majority occur at a somatic level, but inherited predisposition is being increasingly recognized.

4.1. Inherited predisposition to pituitary tumours

Although inherited predisposition to PAs is often recognized in a familial setting, a sporadic presentation may occur. In addition to their implications for familial screening, genes involved in hereditary tumours may provide important clues in the comprehension of the molecular basis of tumorigenesis. We have recently reviewed this topic and proposed an algorithm for genetic screening in patients with PA [48].

4.1.1. Multiple Endocrine Neoplasia type 1 (MEN1)

MEN1 is an autosomal dominant condition defined by the presence, in a single subject or within a single family, of two or more hyperplastic and/or adenomatous lesions of the parathyroid glands (~90%), the gastro-entero-pancreatic (GEP) tract (30-80%) and/or the anterior pituitary (~40%). The clinical characteristics of MEN1-related PA have been recently reviewed elsewhere [48,49]. Briefly, their phenotypic distribution is unremarkable when compared to sporadic PA, with a predominance of prolactinomas (~60%), but they are more frequently invasive and resistant to pharmacological treatment. Pediatric onset is not uncommon, especially for PRL- and ACTH-secreting PA, and carcinomas have been reported. Peritumoral hyperplasia is rare, but multiple and plurihormonal adenomas are more frequent than in sporadic PA [50]. Since the identification of the *MEN1* gene in 11q13, direct sequencing has been developed in many laboratories worldwide and more than 600 mutations have been identified. Molecular defects in the *MEN1* gene have been reported in details [51] and consist of insertions/deletions with frameshift (40%), non-sense (>20%) and missense (<20%) mutations, scattered throughout the entire coding region and splice sites of the gene. Large deletions (1%) may occur and escape direct sequencing, requiring additional molecular techniques such as Multiple Ligase Probe Amplification (MLPA) for their identification. Most mutations are supposed to lead to a truncated protein (>70%). Although no clear genotype-phenotype association has been established, aberrant familial expression of *MEN1*-related tumours and reports of severe phenotypes associated with mutations that completely abolish menin function suggest that this aspect may currently be underestimated [52]. However, in an increasing proportion of cases, especially in the presence of a sporadic association of *MEN1*–related tumours, no MEN1 gene mutations are found (up to 10-30%). In particular, the relatively common association between PA and primary hyperparathyroidism has been ascribed to MEN1 in <10% of the cases [53]. *MEN1* is a tumour suppressor gene encoding a 610 amino-acid protein, menin, with somatic inactivation of the wild-type allele in *MEN1* tumours occurring mostly through LOH. Different MEN1 mouse models have been generated, including conditional tissue-specific models, which have largely confirming the role of *MEN1* inactivation in endocrine tumorigenesis [51]. Recently, pituitary tumour targeting in a $MEN1^{+/-}$ mice model through *in situ* injection of the *MEN1* gene in adenoviral vectors has proven to restore menin expression and significantly reduce cell proliferation [54]. Menin is an ubiquitous nuclear protein, which has been involved in a complex network of protein-protein interactions and implicated in the regulation of gene transcription, DNA repair and cell division. Menin suppresses PRL transcription and participates in cell cycle control through the induction of the cyclin-dependent kinases inhibitor (CKI) genes encoding $p27^{Kip1}$ and $p18^{Ink4c}$. It also interacts with nuclear receptors involved in the control of pituitary function and/or proliferation such as ERα and PPARγ and with Smad proteins, involved in TGFβ signalling. A role for menin as a general co-regulator of transcription involved in epigenetic changes on histone proteins has emerged during the last decade (see "epigenetics") [55]. Alterations in the *MEN1* gene play a limited role in sporadic pituitary tumorigenesis, and <3% of patients presenting with apparently sporadic PA have germline *MEN1* mutations. However, contrasting with the expression of menin in all normal pituitary cell types, variable degrees of underexpression have been reported in all human PA histotypes, with complete loss occurring

in a PRL-secreting carcinoma [56]. Although LOH in 11q13 may account for reduced menin expression in a subset of PA, post-transcriptional and post-translational mechanisms are also likely.

4.1.2. Carney Complex (CNC)

The "complex of myxomas, spotty skin pigmentation, and endocrine overactivity" is a rare and heterogeneous condition, characterized by the association of endocrine overactivity and tumors - Primary Pigmented Nodular Adrenocortical Disease (PPNAD), acromegaly, thyroid and gonadal tumors – with cardiac myxomas, schwannomas and skin pigmented lesions [57]. Primary pituitary presentation is rare and pituitary abnormalities are mainly represented by an hyperplasia of GH/PRL-secreting cells, which frequently translates into mild hyperprolactinemia and/or subclinical alterations of GH/IGF1 secretion. Early onset GH/IGF1 hypersecretion may induce gigantism, but acromegaly and somatotrophinomas develop in a minority of patients (15%). Up to 70% of CNC patients present in a familial setting, with an autosomal dominant transmission. Germline heterozygote mutations in the *regulatory subunit type 1A of cAMP-dependent protein kinase* (*PKRAR1A*) gene in 17q22-24 can be identified in ~60% of the cases, especially in familial forms (80%), with recent evidence for some genotype-phenotype correlation [58]. Most *PKRAR1A* mutations are distributed through the coding sequence, 20% are intronic and affect splicing and two short deletions have been identified as hot spots [57,58]. LOH at the corresponding locus may be observed in CNC-related PA and conditional pituitary heterozygous knockout of the *PKRAR1A* gene is associated with an increased prevalence of PA arising from the Pit-1 dependent lineage [59], supporting a tumour suppressor function for PKRAR1A. CNC tumours show increased protein kinase A (PKA) activity and abnormal activation of the cAMP pathway. A second locus for Carney complex (CNC2) has been mapped in 2p16 by linkage analysis in CNC kindreds and confirmed by FISH analysis. Because most alterations were amplifications, undisclosed oncogenes may be involved [57,58]. A missense mutation in the *MYH8* gene, encoding the myosin heavy polypeptide 8, has been exceptionally reported in a CNC variant co-segregating with the trismus-pseudocamptodactyly syndrome [60]. An increased prevalence of point mutations in the phosphodiesterase *PDE11A* gene has also been reported in CNC patients affected by *PKRAR1A* mutations [61]. However, these variants were associated with an increased prevalence of adrenal and testicular tumours but did not impact the prevalence of PA.

4.1.3. McCune Albright Syndrome (MAS)

MAS is a rare sporadic disease due to post-zygotic activating mutations in the α *subunit of the stimulatory G protein* (*GNAS1)* gene on 20q13 leading to mosaicism. It is typically characterized by the clinical triad of café-au-lait skin spots, polyostotic fibrous dysplasia and autonomous endocrine overactivity syndromes, including peripheral precocious puberty, hyperthyroidism and GH/IGF-1 and/or PRL hypersecretion [62]. Due to mosaicism, *GNAS1* sequencing on leukocyte DNA is disappointing, since less than 50% can be detected even in the presence of a typical MAS triad [63]. Pituitary abnormalities are similar to those observed in CNC patients, with a typical hyperplasia of GH/PRL-secreting cells and somatotrophinomas developing in

a minority of patients. This can be explained by constitutive activation of the cAMP pathway as a common molecular hallmark of MAS-and CNC-related cellular abnormalities. In MAS patients, increased cAMP signalling only occurs in cells affected by activating mutations of *GNAS1*, which typically affect the Arginine 201 residue [63].

4.1.4. MEN-4 and other CDKI-related disorders

Inactivating mutations in the *CDKN1B* gene encoding $p27^{Kip1}$ have been involved in atypical multiple endocrine neoplasia syndromes called MENX in the rat and MEN4 in humans, respectively, where a familial association of acromegaly, hyperparathyroidism, testicular cancer and angiomyolipomas was reported [64]. In this kindred, both the proband and his father had acromegaly and a germline heterozygous W76X truncating mutation was found. Subsequently, additional *CDKN1B* mutations were reported in MEN1-like patients by different groups, with hyperparathyroidism appearing as a constant feature and the presence of additional PA phenotypes being confirmed in some cases (one ACTH-secreting and one micro-NFPA) [65]. Associated tumours in these patients included gastro-entero-pancreatic endocrine tumours, carcinoids and papillary thyroid cancer. In most cases, functional studies *in vitro* have revealed decreased protein expression, cellular mislocalization in the cytoplasm, or a defective interaction with protein partners [65]. Search for mutations in *CDKN1B* and genes encoding additional CKIs has also been performed in 196 patients with MEN1-like syndromes. In addition to common benign polymorphisms, mutations in the *CDKN1B*/ $p27^{Kip1}$ gene were the most frequently encountered (1.5%), followed by genes encoding $p15^{Ink4b}$ (1.0%), $p18^{Ink4c}$ (0.5%) and $p21^{WAF1}$ (0.5%) [66]. However, PA were associated only with mutations in the *CDKN1B*/$p27^{Kip1}$ and *CDKN1A*/$p21^{Cip1}$ genes [66]. Of note, the association between primary hyperparathyroidism and PA does not appear to be explained by *CDKN1B*/ $p27^{Kip1}$mutations [67]. Overall, human *CDKI*-related conditions are very rare but appear to be inherited in a dominant manner.

4.1.5. The pituitary adenoma-paraganglioma/pheochromocytoma association: A new syndrome?

Succinate dehydrogenase (SDH) is a mitochondrial enzyme composed of four functionally different subunits A,B,C,D. Mutations in genes encoding the SDH B,C and D subunits predispose to pheochromocytomas/ paragangliomas and additional tumours, including thyroid cancer. Recently, a germline mutation in the *SDHD* gene leading to a truncated protein was reported in an acromegalic patient affected by a pheochromocytoma occurring in a familial context. LOH at the corresponding locus in 11q23 was found in the pituitary GH-secreting macroadenoma, suggesting a role for SDHD loss in pituitary tumorigenesis [68]. Because the association between PA and pheochromocytomas/paragangliomas has been reported in the literature, further search for inactivating mutations in *SDH* genes is warranted.

4.1.6. Familial Isolated Pituitary Adenomas (FIPA) and the Aryl hydrocarbon receptor Interacting Protein (AIP) gene

Familial Isolated Pituitary Adenomas (FIPA) are defined by the familial presentation of PA in the absence of syndromic features. In 2006, 64 European FIPA kindreds were reported,

including patients affected by prolactinomas (55%), somatotrophinomas (~30%), non-secreting PA (~15%) and corticotrophinomas (<5%) [69]. The prevalence of FIPA among PA was estimated about 2-3% and kindreds were almost equally divided into homogeneous and heterogeneous, as defined by the familial association of PA with a single or multiple phenotypes, respectively. Familial homogeneous somatotrophinomas, previously reported as "Isolated Familial Somatotrophinomas", accounted for ~20% of the whole series. In the same year, the *Aryl hydrocarbon receptor Interacting Protein (AIP)* gene was identified as a predisposition gene in two large Finnish pedigrees with GH- and/or PRL-secreting adenomas [70]. The *AIP* gene was located in 11q13.3 and its causative role in FIPA was soon supported by the identification of germline *AIP* mutations in 11/73 FIPA kindreds (15%), including 50% of those presenting with homogeneous somatotrophinomas and ~10% of heterogenous kindreds [71]. Since this date, *AIP*-related FIPA have been further identified worldwide [48]. These findings and the expanding knowledge about the implications of germline *AIP* mutations and the potential role of AIP in the pathogenesis of PA have been recently reviewed [48,72] and will be summarized and updated. Because *AIP* was originally reported as a "Pituitary Adenoma Predisposition" (PAP) gene with a low penetrance, and on the basis of accumulating evidence for an incomplete penetrance of PA in AIP^{mut}-FIPA kindreds, screening for *AIP* mutations in patients with an apparently sporadic presentation of the disease was then performed by several groups. Although *AIP* mutations were very rarely observed in unselected patients, they could be identified in 12% of patients presenting with pituitary macroadenomas before the age of 30 [73] and up to 23% of pediatric PA patients [74]. The highest prevalence was found in patients presenting with gigantism and/or early onset GH/PRL-secreting PA. The phenotype of AIP^{mut} PA has now been well characterised. Most AIP^{mut} PA are somatotrophinomas (up to 80%), followed by prolactinomas (13.5%) and any other phenotype, with a male predominance and a frequent early onset of the disease [75]. Compared to their non-AIP^{mut} counterpart, AIP^{mut} somatotrophinomas are more aggressive and resistant to SSA [74]. AIP^{mut} prolactinomas are also frequently aggressive and resistant to DA. From a molecular point of view, more than 50 *AIP* mutations have been identified so far and distributed through the entire coding sequence. Most are truncating (60-70%) - including the Finnish AIP^{Q14X} founding mutation –, more than 20% are missense and a few hotspots have been reported. Large deletions (10%) and exceptional promoter mutations may also be encountered, in addition to changes of uncertain biological significance (polymorphisms and non-splicing intron variants). The molecular pathways involved in AIP-related pathogenesis have not been elucidated yet, but an $AIP^{+/-}$ mice model has been developed [76]. Interestingly, the wild type strain of mice used for the generation of the $AIP^{+/-}$ model spontaneously develops prolactinomas, and heterozygous $AIP^{+/-}$ ablation lead to a full penetrance of PA, with a shift toward a GH-secreting phenotype and an earlier onset of the disease. While further supporting the tumour suppressing function of AIP in somatotrophs, these findings highlight the importance of the genetic background and the contribution of genetic modifiers in the development of AIP^{mut} PA, which have also been recognized in humans [77]. Pituitary tissue surrounding AIP^{mut} PA has been rarely described, but hyperplasia was recently reported [78]. The *AIP* gene encodes a 330 amino-acids cytoplasmic protein (AIP/XAP2/ARA9), which is abundantly expressed by normal somatotrophs and to a lesser extent by lactotrophs. It is frequently down-regulated in AIP^{mut} PA, due to somatic

AIP hemizygosity, but also in invasive sporadic somatotrophinomas and in prolactinomas, whereas it appears to be upregulated in a subset of NFPA [reviewed in 48]. Loss of AIP expression has been recently proposed as a marker of aggressiveness in sporadic somatotrophinomas [79]. Accordingly GH_3 cell proliferation was increased by *AIP* gene silencing or inactivation and decreased by overexpression of wild-type *AIP*, respectively [reviewed in 48,72]. The AIP protein presents a N-terminal immunophilin-like domain (FKBP-52), but its functional properties appear to be mediated essentially through three tetratricopeptide repeats (TPR) and a C-terminal α-helix, which are involved in multiple protein-protein interactions. Identified partners of AIP include: 1) the Aryl Hydrocarbon Receptor (AHR or "dioxin receptor"), a transcription factor involved in cell response to polycyclic aromatic hydrocarbons but also in developmental processes and the regulation of cell cycle and differentiation, which is stabilized in the cytoplasm in a multimeric AIP/AHR/Hsp90 complex; 2) the phosphodiesterases PDE4A5 and PDE2A, both implicated in cAMP signalling ; 3) the anti-apoptotic factor survivin and RET, which prevents the stability of the AIP/survivin complex; 4) members of the steroid receptor superfamily (*eg.* PPARα, ERα); 5) viral proteins [48,72]. Most *AIP* mutations reported so far may theoretically disrupt one or more functional interactions of AIP and defective interactions with PDE4A5 have been proven *in vitro* [72]. An emerging field of research is the potential role of AIP as a mediator of SSA in the treatment of somatotrophinomas, which would explain the frequent pharmacological resistance of AIP^{mut} tumours. Indeed, AIP immunostaining was recently reported to be higher in somatotrophinomas treated with SSA pre-operatively than in untreated tumours [80] and proposed as a predictive factor for the post-operative response to SSA in these tumours [81] Accordingly, octreotide treatment was found to increase AIP expression in GH_3 cells [80].

4.2. Somatic events in pituitary tumours

Somatic events in pituitary tumours include genetic and epigenetic changes. Intragenic mutations are less frequently encountered than in other solid tumours. Indeed, oncogene activation is mainly triggered by the overexpression of genes involved in extracellular signalling and cell cycle progression, with a few gain-of-function mutations and occasional rearrangements being reported. Inactivation of tumour suppressor genes is very common and occurs through epigenetic changes more frequently than through loss-of-function mutations and/or allelic deletions. MicroRNAs (miRs) have also recently emerged as important regulators of gene/protein expression in pituitary tumours.

4.2.1. Chromosome and DNA alterations in PA

Several chromosome abnormalities have been reported in PA, although on limited series of tumours, and include aneuploidy and evidence for intrachromosomal gain or loss of DNA. For example, trisomies involving chromosomes 5, 8 and 12 appear to be very frequent in prolactinomas [82], whereas trisomies involving chromosomes 7, 9 and 20 were reported in NFPA [83]. Conversely, monosomy of chromosome 11 was observed in an aggressive MEN1-related prolactinoma [84] and LOH with single or multiple allelic deletions at different loci have been reported, which also appear to be more frequent in invasive PA. For instance, the

frequency of LOH in 11q13, 13q12-14 and/or 10q26 was found to increase from <10% in low grade to nearly 75% in high grade PA, according to a modified Hardy's classification [85]. As compared to other solid tumours, classical somatic oncogenic mutations are relatively rare in PA [reviewed in 13,47]. The most frequent oncogenic mutation is represented by activating missense mutations in the *GNAS1* gene, the so-called *Gsp* oncogene, which has been reported in up to 40% of somatotrophinomas and occasionally in other phenotypes (<10% NFPA, <5% corticotrophinomas) [86]. A single B-Raf mutation (V600E) was reported in a NFPA sample [87]. Other activating mutations have been reported in aggressive tumours, such as activating H-Ras point mutations in pituitary carcinomas, PKCα in invasive PA and more recently PIK3CA in pituitary carcinomas and invasive secreting PA [88]. A pituitary specific truncated form of the FGFR4 (ptFGFR4) endowed with constitutive phosphorylation was also reported in prolactinomas, and a truncated activin receptor (Alk4), devoided of growth suppressing activity, has been observed in NFPA [19]. More frequently, overexpression of oncogenic proteins has been reported in the absence of mutations and ascrìbed to gene amplification or transcriptional upregulation. These include overexpression of growth factors and cytokines (*eg.* TGFα,VEGF) and their receptors (*eg*. EGFR), proteins involved in the control of cell cycle and proliferation (*eg.* cyclins, HMGAs, PTTG, c-myc), cell survival (*eg.*Bcl2, survivin, BAG1), intracellular signalling (*eg.* GNAS1, PIK3CA, Akt) and tumour invasion (*eg.* MMPs). Somatic inactivating mutations in TSGs are even less common. Although p53 frequently accumulates in aggressive PA and especially in pituitary carcinomas, rare mutations have been reported in carcinomas only [89]. Homozygous deletions in the protein-binding pocket of pRB have been reported in a minority of PA showing a complete loss of pRb expression [90]. However, downregulation of TSGs occurs very frequently in PA, due to LOH, epigenetic silencing or microRNAs dysregulation. Pituitary TSGs have been recognized among proteins involved in cell cycle control (*eg.* CKIs, pRb, Zac1), extracellular signalling (*eg.* TGFβ, BMP4, FGFR2), DNA repair (*eg.* p53, GADD45γ) or apoptosis (*eg.* PTAG, DAPK1), which will be discussed further on. Current studies on gene expression profiling in PA are giving an important contribution to the identification of new genes, which expression is dysregulated in PA [10,13,91,92]. It should be kept in mind, however, that a subset of proteins involved in pituitary tumorigenesis may be downregulated regardless of gene expression, since increased protein degradation through the proteasome pathway may in some cases play a prevalent role, as reported for $p27^{Kip1}$ or Reprimo [93] and miRs may impact the stability and translation of target mRNAs (see "microRNAs").

4.2.2. Epigenetics in PA

Epigenetic changes are mainly characterized by DNA methylation and histone modifications, leading to chromatin remodelling and regulation of gene expression through a modulation of DNA accessibility to TFs. Epigenetic changes may coexist with genetic events (*eg.* inactivating mutations, LOH). Methylation of cytosine residues in CpG islands of gene promoters is associated with gene silencing and is inheritable through successive divisions, with *de novo* methylation being carried out by the DNA methyltransferase DNMT3, whereas histone modifications, such as methylation, acetylation, or phosphorylation, are reversible upon specific enzymatic modifications and their effect on gene transcription can be predicted

through a "histone code" [94]. For instance, acetylation and trimethylation on specific Lysine residues (K) on histone 3 (H3K9Ac and H3K4me3, respectively) are associated with an open chromatin configuration and active gene transcription, whereas other modifications on histones 3 and 4 (*eg.* H3K9me3, H3K27me3, H4K12Ac) are associated with a closed chromatin configuration and gene silencing. Aberrant gene expression due to epigenetic changes can be involved at all stages of tumourigenesis and is very frequently encountered in PA [95]. An early event in pituitary tumorigenesis is silencing of the *CDKN2A/p16*^{INK4a} gene by promoter methylation, which has been reported in all PA histotypes, though it may be less frequent in somatotrophinomas. Down-regulation of additional TSGs may be at least partially explained by gene promoter methylation, including other cell cycle regulators (*eg.* p15^{INK4b}, pRB, p14^{ARF}, GADD45γ, neuronatin/NTTA, *MEG3*), developmental and growth factor signalling molecules (*eg.* FGFR2, WIF1, RASSF1A), pro-apoptotic genes (*eg.* PTAG, DAP kinase, Zac1), genes involved in cell adhesion (*eg.* E-cadherin, H-cadherin) and TFs (*eg.* Ikaros 6) [13,47,95]. Of note, a minority of these genes are normally imprinted (*eg. MEG3, Zac1*, *NNTA*). In contrast, promoter hypomethylation can induce overexpression of pituitary oncogenes. For instance, loss of imprinting of the *GNAS1* gene may be responsible for an overexpression of the wild-type gene, mimicking the effects of the *Gsp* oncogene. Abnormal expression of MAGE-A3, an X-chromosome linked gene silenced by thorough promoter methylation in normal pituitary cells, has been explained by promoter hypomethylation in PA [96]. Further characterization of DNA methylome by specific array techniques appears as a promising tool for the identification of new genes modified by abnormal methylation patterns [97]. Histone modifications may contribute either to transcriptional repression, as reported for *FGFR2* [96] and *BMP4* [98], or to increased gene expression, as reported for the estrogen-induced *MAGE-3A* expression [96] or for *DNMT3* [95]. An interesting function of menin as a co-regulator of transcription though histone changes has also been proposed to explain several alterations in gene expression observed in *MEN1*-related tumours [55]. Menin is able to tether histone deacetylase activity to genes, thereby repressing gene expression, as reported for JunD- or NF-kB-dependent transcription. On the other hand, as a part of Mixed Lineage Leukemia (MLL) protein-containing complexes which specifically triggers H3K4me3 trimethylation, menin has been involved in the activation of several genes, including homeobox genes *(Hox)*, *CDKN1B/p27*^{Kip1}, *CDKN2C/p18*^{Ink4} and nuclear hormone receptor sensitive genes. These functions of menin and their final effects on cell proliferation depend on the cellular context and may explain why menin acts as a TSG in endocrine cells and a co-oncogenic protein in leukemias [99].

4.2.3. The emerging role of microRNAs and other non-coding RNAs

MicroRNAs (miRs) are small non-coding RNA molecules (18-25 nucleotides) involved in the post-transcriptional regulation of mRNAs stability. More than 600 miRs have been reported in the human genome and up to 30% of genes are believed to be regulated by miRs. Briefly, miRs derive from the intracellular processing of precursors called pri- and pre-miRNAs, and mature miRs bind to the 3' untranslated sequence of mRNA target molecules, leading to the formation of miRs duplexes. MiRs duplexes are incorporated into protein containing RNA-induced silencing complexes (RISC) and, according to their degree of complementarity with

miRs, target mRNAs can be cleaved (perfect complementarity) or undergo partial degradation and translational inhibition (imperfect complementarity, which is the most common figure). MiRs have been involved in the control of pituitary development [100] and function [101]. Alterations in miRs expression profile, including loss or gain of expression, have been increasingly involved in pituitary tumorigenesis. Since the first report on miR15a and miR16-1 downregulation in GH- and PRL-secreting macroadenomas [102], more than 100 dysregulated miRs have been identified in PA, with phenotype-specific expression profiles being reported in GH- and/or PRL-secreting, ACTH-secreting or NFPA [103-112]. Although in most cases their biological significance is not fully understood, several miRs have been involved in the control of pituitary cell cell proliferation, differentiation, apoptosis, cell adhesion and metabolism, and a subset has been linked to a more aggressive behavior or even to malignant transformation [103, 104, 110]. Target mRNAs are also being increasingly recognized. For instance, *Bcl2* mRNA is a target for miR-16-1 and the expression of *Bcl2* can be upregulated as a consequence of miR-16-1 down-regulation, contributing to promote cell survival. In somatotrophinomas, loss of miR-126 contributes to cell proliferation through an upregulation of the PI3K regulatory subunit β (which amplificates PI3K signalling) [104], whereas upregulation of miR-107 has been recently involved in AIP silencing [105]. Overexpression of PTTG and HMGAs in PA may also be partially explained by specific miRs dysregulation [104, 106,107]. Another interesting aspect is the potential role of LOH in the dysregulation of miRs expression, as reported for miR-15a and miR-16-1 which may be underexpressed as a consequence of LOH in 13q14. There is also recent evidence that the miRs profile in PA may be influenced by their pre-operative pharmacological treatment, as reported in somatotrophinomas treated by lanreotide [106] and in prolactinomas treated by bromocriptine [108]. A non exhaustive list of miRs involved in pituitary tumorigenesis is shown in Table 2. Other non-coding RNAs may also be implicated. The *Maternally Expressed Gene 3 (MEG3)*, a large non-coding RNA molecule expressed by normal pituitary cells and selectively lost in NFPA, suppresses cell proliferation through p53-mediated/p21 independent and pRB-mediated pathways [113]. *MEG3* is part of the imprinted *DLK1/MEG* locus, where maternally expressed genes (*eg MEG3*) are non-coding RNAs and paternally expressed genes (*eg DLK1*) encode proteins. Interestingly, silencing of the *DLK1/MEG* locus in NFPA is responsible for the underexpression of additional non-coding RNAs, including several miRs [114].

MicroRNA	Cromosome map (h)	Molecular defect	Histotype	Function	Ref.
miR-128a	2q21.3	Overexpression	NFPA	Target gene: *Wee1* (↓)	109
		Down-regulation	All PA GH	Target gene: *BMI1** (↑)	103 111
miR-26a/b	3p21 (a) 2q35 (b)	Overexpression	NFPA (miR-26a) GH (miR-26a/b)	Potential target genes: *Hox-A5, PLAG1* Target gene (miR26b): *PTEN* (↓)	103 111
		Down-regulation	GH/PRL/NFPA (miR 26a)	Target genes: *HMGA1, HMGA2* (↑)	112

MicroRNA	Cromosome map (h)	Molecular defect	Histotype	Function	Ref.
miR-191	3p21	Overexpression	All PA	Cell proliferation	103
miR-145	5q32	Down-regulation	GH	Target genes: *c-myc, k-ras, c-fos, cyclin D2, MAPK* (↑)	106
miR-30a/b/c/d	6q13 (c); 8q24.2 (b)	Overexpression	ACTH	-	103
miR-320	8p21.3	Overexpression	GH	-	107
miR-24-1	9q22.1	Down-regulation	All PA	Predicted target genes: *VEGFR1* and several oncogenes	103
Let7-a	9q22.32	Down-regulation	All PA	Target genes: *HMGA1*, *HMGA2* (↑) Potential target genes: *Ras*	103, 104, 112
miR-126	9q34.3	Down-regulation	GH	Amplification of PI3K signalling; *PTTG* (↑)	106
miR-107	10q23	Overexpression	GH/ NFPA	Target gene: *AIP* (↓)	105, 111
miR-326	11q13.4	Down-regulation	GH, some PRL NFPA	Target genes: *HMGA1, HMGA2, E2F (↑)*	107
miR-141	12p13	Down-regulation	ACTH	Tumor growth and tumor local invasion	103
miR-16-1	13q14	Down-regulation	All PA	Target genes: *HMGA1, HMGA2, Bcl2* (↑)	102-104, 112
miR-15a	13q14	Down-regulation	All PA, in particular GH/PRL	Target genes: *HMGA1*, *HMGA2* (↑)	102, 103, 112
miR-20a	13q31.3	Overexpression	NFPA	Target gene: *Wee1*(↓)	109
miR-493	14q32.2	Overexpression	ACTH (carcinoma)	Target genes: *LGALS-3* and *RUNX2* (↓)	110
miR-381	14q32.31	Down-regulation	GH	Target gene: *PTTG* (↑)	106
miR-140	16q22.1	Overexpression	NFPA (macroadenomas)	Tumor growth	103
miR-212	17p13.3	Overexpression	All PA	Target gene: *DEDD* (↓), ↑ apoptosis	103
miR-132	17p13.3	Down-regulation	All PA	-	103
miR-152	17q21	Overexpression	All PA	Cell proliferation	103
miR-122	18q21.31	Overexpression	ACTH (carcinoma)	-	110
miR-23a	19p13.13	Overexpression	GH/PRL	-	103
miR-24-2	19p13.13	Overexpression	GH/PRL	See miR-24-1	103
		Down-regulation	ACTH/ NFPA	See miR-24-1	103
miR-155	21q21.3	Overexpression	GH/PRL/NFPA	Target gene:	109

MicroRNA	Cromosome map (h)	Molecular defect	Histotype	Function	Ref.
				Wee1 (↓)	
miR-098	Xp11.2	Down-regulation	All PA	Predicted target genes involved in cell progression, cytoskeleton and vesicle organization	103

*a transcriptional repressor of PTEN

Table 2. Differential expression of microRNAs in pituitary tumours

5. Alterations in neuropeptide signalling in pituitary tumours

Hypothalamic peptides play an essential role in normal pituitary cells and their biological effects are mediated by G-protein coupled receptors (GPCRs) [19]. In addition to their dynamic control on pituitary hormone secretion and release, they may exert trophic effects on target cells - such as GHRH and CRH on somatotrophs and corticotrophs, respectively – or limit their proliferation – such as dopamine in lactotrophs. The expression of neuropeptide receptors is generally conserved in pituitary tumours and represents the molecular basis for their pharmacological treatment with DA and SSA [2]. However, abnormal expression of these receptors may be involved in paradoxical responses or in pharmacological resistance. Neuropeptides may also be produced by the pituitary gland or ectopically by neuroendocrine tumours, and their ectopic secretion may lead to pituitary hyperplasia and/or adenoma. Finally, abnormal intracellular signalling may occur in PA, contribute to pituitary tumorigenesis and potentially influence the response to pharmacological treatment. An extensive review of such processes would be beyond the scope of this work, so we will focus on the most relevant and recent findings in this field.

5.1. Abnormalities in the cAMP-PKA pathway

The cAMP/PKA pathway is essential in pituitary cells, especially in somatotrophs and in corticotrophs. Briefly, the α-subunit of the stimulatory G protein (Gsα), encoded by the *GNAS1* gene, is required for the activation of adenyl cyclase and the generation of cAMP in somatotroph and corticotroph cells in response to GHRH and CRH, respectively, whereas the α-subunit of the inhibitory G protein (Gi) inhibits adenylate cyclase activity and decreases cAMP signalling in response to dopamine, somatostatin and their pharmacological analogues. As already reported, somatic activating mutations in the *GNAS1* gene – the *Gsp* oncogene – are frequent in somatotrophinomas whereas post-zygotic mutations are associated with the MAS syndrome. The *GNAS1* locus is under a complex imprinting control and *GNAS1*/*Gsp* mutations are exclusively found on the maternal allele. Gsp^+ somatotrophinomas were first characterized by high adenyl cyclase activity and high intracellular cAMP concentration. However, conflicting results concerning the phenotype of Gsp^+ somatotrophinomas arised from large studies comparing Gsp^+ and Gsp^- tumours [86]. In particular, no significant differences were

found concerning their hormone secretion profile and tumour aggressiveness. This could be partially explained by the development of molecular mechanisms able to counteract a sustained increase in cAMP concentration, such as an increased phosphodiesterase (PDE) activity, in particular PDE4, in *Gsp*$^+$ tumours. In addition, the *Gs* protein is instable and barely detectable in *Gsp*$^+$ tumours, whereas an overexpression of the wild-type Gsα protein can be observed in *Gsp*$^-$ tumours [86,115]. Accepting the concept that *Gsp* mutations represent an early pathogenetic event, long-standing *Gsp*$^+$ tumours may also have accumulated additional molecular abnormalities able to modify their early phenotype. However, most studies indicate that *Gsp*$^+$ somatotrophinomas are significantly smaller than their *Gsp*$^-$ counterpart and show a better response to SSA. This could be explained *in vitro* by a greater effect of cAMP decrease in the presence of high basal cAMP concentrations. The presence of a cAMP-responsive element (CRE) in the SSTR2 promoter also suggested that constitutive activation of the cAMP/PKA pathway might result in an increased sentivity to SSA, but no difference in SSTR2 expression has been found between *Gsp*$^+$ and *Gsp*$^-$ adenomas. However, considering the proliferative effects of the cAMP/PKA pathway in somatotrophs, the smaller volume of *Gsp*$^+$ tumours is intringuing and suggests that cAMP signalling is also linked to somatotroph differentiation. A few additional mutations in G proteins have been reported in PA, among which rare mutations involved in IP3/calcium signalling [86]. Additional abnormalities in cAMP signalling are represented by dysregulated protein kinase A (PKA) activity. In its inactive form, PKA is composed of two regulatory and two catalytic subunits. These latter are released when the intracellular cAMP concentration increases and bind the regulatory subunits. The inactivating mutations in the *PRKAR1A* gene encoding the type 1α regulatory subunit of PKA reported in patients affected by CNC is associated with increased cAMP signalling, probably through an altered equilibrium between regulatory and catalytic PKA subunits [57]. No somatic *PRKAR1A* mutations have been observed in PA. However, a marked reduction in PRKAR1A protein expression was reported in PA of different histotypes, due to an increase in proteasome-mediated protein degradation [116]. This was found to result in an imbalance between the R1 and R2 isoforms of the PKA regulatory subunit, which in turn was associated with an increased cell proliferation in GH_3 cells and increased Cyclin D1 expression in somatotroph PA. Following the discovery of missense and inactivating mutations in adrenal and testicular tumours, variants in the *PDE11A* gene have been reported in 17% of acromegalic patients [117]. Due their high frequency in the control population and the absence of phenotypic changes in the corresponding tumours, these variants were unlikely to be pathogenic. Accordingly, *PDE11* variants in CNC patients were not found to increase the prevalence of PA [61]. However, a single truncating mutation was observed and LOH at the corresponding locus was found in an additional tumour, with decreased PDE11A immunoreactivity in both cases, suggesting that genetic alterations in *PDE11A* may occasionally contribute to pituitary tumorigenesis [117].

5.2. Neuropeptides, neuropeptide receptors and their implications in pituitary tumour pathogenesis and treatment

Abnormal hypothalamic neuropeptide signalling has long been involved in the pathogenesis of PA. GHRH stimulates somatotroph proliferation and causes somatotroph hyperplasia in mice, with PA developing in old transgenic GHRH animals. Hypothalamic acromegaly

has been exceptionally reported in patients with gangliocytomas [118] and ectopic secretion of GHRH by neuroendocrine tumours has been well characterized. In a retrospective analysis of 21 patients with ectopic acromegaly [119], most patients showed radiological evidence of pituitary enlargement, but 5 had normal pituitary imaging and 6 had suspected PA, respectively. Out of the four cases who underwent pituitary surgery, all had somatotroph hyperplasia and 2 had concomitant GH/PRL-secreting PA. Noteworthy, a MEN1 context was identified in 8/11 cases. Ectopic secretion of CRH by neuroendocrine tumours may cause ACTH-dependent hypercortisolism, but this is an exceptional condition and ectopic secretion of ACTH and other POMC-derived peptides is by far most frequent. TRH stimulates thyrotrophs and lactotrophs, mainly through the IP3/calcium pathway, and patients with long-standing, severe, primary hypothyroidism may develop pituitary thyrotroph and lactotroph hyperplasia due to an altered feedback on TRH/TSH secretion. Similar mechanisms are involved in gonadotroph hyperplasia secondary to long-standing hypogonadism (of note, pituitary hyperplasia is not a feature of menopause). However, true "feed-back" PA are rare and are likely to require additional somatic events. Abnormal/ectopic, expression of neuropeptide receptors may also occur in PA and account for some "paradoxical" responses observed *in vivo* or *in vitro* (*eg.* GH increase after TRH or GnRH stimulation in acromegalic patients) [120]. Loss of hormonal inhibition might also be involved in pituitary cell dysregulation. Dopamine maintains a tonic inhibition on PRL secretion and cell proliferation in lactotrophs through the dopaminergic receptor D2R. In prolactinomas, neovascularization by-passing the hypothalamus has been proposed to allow tumor cells to escape dopamine inhibition. D2R-deficient mice develop lactotroph hyperplasia and late-onset adenomas, especially in females [6]. However, no mutations in the *D2R* gene have been identified in human prolactinomas and most of them respond to DA in terms of prolactin normalization and tumour shrinkage. Reduced D2R expression, in particular of its short isoform, is frequently observed in resistant prolactinomas [121]. An essential role for the cytoskeleton-associated protein filamin A in D2R expression and signalling has been recently shown in MMQ cells and reduced filamin-A expression has been associated with low D2R expression and pharmacological resistance in human prolactinomas [122]. Similarly, hypothalamic somatostatin inhibits GH secretion and somatostatin receptors (SSTRs) are expressed on somatotrophinomas. Among the five SSTRs subtypes, SSTR2 and SSTR5 mediate most of the therapeutic effects of SSA in these tumours. Although there is no evidence that loss of somatostatin inhibition plays a role in pituitary tumorigenesis, abnormal somatostatin signalling may impact the outcome of pharmacological treatment [123]. Resistance to SSA in somatotrophinomas has been associated with low SSTR2 expression and a few genetic abnormalities in SSTR5, including an inactivating SSTR5 missense mutation in the third intracellular loop and LOH at the SSTR5 gene locus [117]. The truncated isoform of SSTR5, sst5TDM4, may also reduce the efficacy of SSA [124]. Finally, the pituitary gland produces neuropeptides with paracrine effects on pituitary cells (*eg.* galanin, bombesin, VIP, PACAP) [19,125]. Some of these peptides may also play a role in pituitary tumorigenesis, as suggested by animal models such as transgenic mice overexpressing galanin under the control of the GH promoter, which develop pituitary hyperplasia and adenomas [126].

6. Dysregulated cell growth and survival in pituitary tumours

Alterations in cell cycle control and imbalance between cell proliferation and apoptosis are general mechanisms in tumours. Such alterations may be driven by abnormal extracellular signalling and/or abnormal intracellular responses to extracellular signals, including constitutive activation of proliferative pathways and/or escape to normal regulatory signals.

6.1. Extracellular signalling in PA

In addition to neuropeptides, pituitary cells depend on extracellular signalling from the extracellular matrix, a variety of growth factors (GFs) and peripheral hormones secreted by target glands. Extracellular signalling molecules are involved in a complex network of paracrine and autocrine pathways, which are tightly regulated in the developing and adult pituitary [125]. Cell-to-cell signalling regulates pituitary hormone secretion, cell differentiation, growth, survival and plasticity, as well as angiogenesis. Abnormal signalling may therefore participate in pituitary tumorigenesis.

6.1.1. Growth factors and cytokines

The pituitary gland is an abundant source of GFs, in particular the FGF, EGF and VEGF families, and cytokines (the TGFβ family, interleukins and chemokines) [19,125]. Due to the abundant literature in this field, we will present an overview of the best studied GFs and related pathways in PA. FGFs play an important role in pituitary development. FGFs include >20 members and FGF signalling is mediated by 4 FGF receptors (FGFR1-4), with different isoforms (cell-bound, secreted, truncated) generated by alternative splicing. FGFs and FGFRs are involved in proliferative and anti-proliferative signals. The *heparin-binding secretory transforming (hst)* gene encoding FGF4, was first identified in prolactinomas, with strong FGF4 immunostaining being correlated with tumour invasiveness [127]. A tumour specific N-terminally truncated FGFR4 (ptdFGFR4), characterized by an exclusive intracellular localization and constitutive phosphorylation, was also identified in PA of different histotypes and proven to be tumorigenic *in vitro* and *in vivo* [128]. FGF2 has been involved in the pathogenesis of estrogen-induced experimental prolactinomas [129]. In contrast, signalling through the FGFR2 by FGF7 has anti-proliferative effects and down-regulation of FGFR2 has been reported in a subset of PA, in particular prolactinomas [130]. The TGFβ superfamily includes several members (TGFβ1-3, activin/inhibins and BMPs), which may exert either collaborative or opposing signalling on different pituitary cells and PA histotypes. TGFβ signalling is mediated by Smad proteins and generally inhibits cell proliferation. All TGFβ isoforms and their receptor TGFβ-R-II are expressed in PA, and TGFβ inhibits estrogen-induced lactotroph proliferation and PRL secretion [131]. Activin normally increases the synthesis of FSH while inhibiting that of PRL, GH and ACTH and exerts anti-proliferative effects on lactotrophs and prolactinoma cells [132]. Truncated activin receptors (Alk4) have been reported in PA, with a dominant effect opposing the antiproliferative effects of activin [133]. Inactivation of menin also blocks the anti-proliferative effects of TGFβ and activin on lactotrophs [132]. In contrast, BMP4 promotes the proliferation of GH/PRL-secreting cells and the development of prolactinomas, while inhibit-

ing the proliferation of corticotrophs [134]. Accordingly, BMP4 is up-regulated in prolactinomas and down-regulated in corticotrophinomas [19]. Interestingly, BMP4 is down-regulated by DA in prolactinomas [108]. Other cytokines produced by the pituitary gland include interleukin 1 (IL1) 1 and TNFα, leukemia inhibitory factor (LIF), macrophage migration inhibitory factor (MIF), interferon γ, interleukins 2 (IL2) and 6 (IL6), which potential role in PA has not been extensively studied [131]. An increasing attention has been paid to IL6, which is normally produced by FCS cells but is secreted by tumour cells in PA, where it has been involved in cell proliferation, angiogenesis and oncogene-induced senescence [131]. Chemokines have also been increasingly involved in ontogenesis and cancer. The chemokine receptor CXCR4 is expressed in the normal pituitary and its ligand, the Stromal-cell Derived Factor SDF1/CXCL12, was found to increase GH/PRL secretion and cell proliferation in GH_4C_1 cells. An overexpression of CXCR4 and, to a lower extent, SDF1, has been reported in somatotrophinomas and NFPA [135]. EGF expression has been observed at all stages of pituitary development and in the adult pituitary, and the EGFR pathway contributes to pituitary physiology and tumorigenesis [136]. It is involved in the control of corticotroph and gonadotroph functions at both hypothalamic and pituitary levels and modulates hormone secretion and proliferation in GH/PRL-secreting cells. More than 60% of PA, in particular the secreting histotypes, have been shown to express EGFR by different techniques, whereas ErB2 was found in ~30% of PA, especially in NFPA [136]. Both have been positively correlated with tumour invasiveness. The EGFR pathway is of particular importance for corticotroph tumorigenesis. Overall, 75% of corticotrophinomas express EGFR [136]. The strongest positivity for EGFR and its phosphorylated form was found in corticotrophinomas and associated with p27 down-regulation [137]. Recently, nuclear localization of EGFR was observed in human and canine corticotrophinomas and associated with increased POMC transcription, whereas inhibition of EGFR tyrosine kinase activity by gefitinib decreased hormone secretion and cell proliferation *in vitro* and *in vivo* [138]. More than 50% of GH- and/or PRL-secreting PA also express EGFR [136]. However, the role of EGFR signalling in these tumours is less clearcut. EGF enhances the proliferation of lactotrophs and PRL transcription and TGFα has been involved in estrogen-related lactotroph proliferation. On the other hand, EGF is able to induce lactotroph differentiation in GH_3 cells, with a decrease in GH secretion, an increase in PRL secretion and an induction of D2R expression, with conflicting data reported on cell proliferation. Gefitinib was found to decrease cell proliferation of GH_3 cells, with a reduction in PRL and an increase in GH secretion, respectively [139]. The dual EGFR/HER2 tyrosine kinase inhibitor lapatinib showed similar or stronger effects on GH_3 cells, attenuated the effects of estrogens in estrogen-induced prolactinomas *in vivo* and suppressed PRL secretion from human prolactinomas *in vitro* [138]. Interestingly, EGFR expression was positively correlated with p27 immunostaining in somatotrophinomas [137]. The role of EGFR signalling in NFPA has not been extensively studied yet. NGF is the best characterized neurotrophin in the pituitary and NGF signalling is mediated by TrkA and $p75^{NTR}$, a member of the TNF receptor superfamily. All Trk receptors have been observed in the normal pituitary and in PA [140]. NGF plays a role in lactotroph differentiation and in the stress-related stimulation of corticotroph function, and escape to NGF control has been involved in the pathogenesis of prolactinomas. NGF induces lactotroph differentiation of GH_3 cells similarly to EGF, exerts autocrine anti-proliferative actions on

prolactinoma cells and loss of NGF expression in prolactinomas has been linked to low D2R expression and pharmacological resistance [141]. The VEGF is a family of angiogenic factors composed by 6 members, including VEGFA (also known as VEGF), which effects are mediated by two tyrosine kinase receptors (VEGFR1 and KDR/Flk-1/VEGFR2). VEGF and its receptors are differentially expressed in normal pituitary cells and in PA [142]. In particular, VEGF and KDR are markedly upregulated in NFPA. Overexpression of *VEGF* and *KDR* mRNAs in PA was recently associated with extrasellar growth and a shorter recurrence-free survival, respectively [143].

6.1.2. Steroid hormones

Steroid hormones may play an important role in pituitary tumorigenesis. The best characterized model is represented by estrogen-induced lactotroph hyperplasia and prolactinomas. Estrogen-induced lactotroph hyperplasia occurs physiologically during pregnancy, but sustained estrogen exposure may lead to prolactinomas in some strains of rats. Although evidence for estrogen-induced prolactinomas in humans is very poor, prolactinoma growth and/or intra-tumoral hemorrhage may occur during pregnancy, especially in macroprolactinomas [144]. Among PA, prolactinomas express the higher concentration of ER [145] and estrogens can increase PA cell proliferation in primary culture [146]. Importantly, ERα and ERβ play distinct roles and an imbalance between these two isoforms has been reported in PA. Indeed, nuclear overexpression of ERα has been linked to tumour aggressiveness in prolactinomas and NFPA, whereas invasive NFPA were found to express lower ERβ [147]. Ablation of ERβ in mice was also associated with the development of invasive gonadotrophinomas in females [148]. Estrogens exert multiple effects on pituitary cells, especially on lactotrophs. They enhance the transcription of the *PRL* gene, as well as *PTTG* and genes encoding other growth-promoting proteins, such as *c-myc* and GFs [149]. Based on the comparison of microarray profiles obtained in human prolactinomas and in estrogen-induced rat prolactinomas, a common set of genes has been identified, supporting a major role for *E2F1*, *c-myc* and *Igf1* in their pathogenesis [92]. GFs have been involved in an interplay between FCS and lactotrophs, with the estrogen-induced increase in TGF-β3 secretion by lactotrophs being proposed to stimulate FGF release by FCS cells, which in turn stimulate latotroph proliferation. Estrogens also increase the secretion of VEGF and FGF2, thereby enhancing angiogenesis. Indeed, estrogen-induced prolactinomas are strongly vascularised, spontaneous hemorrhage may occur, and high ER concentrations were found in hemorrhagic PA [145]. In contrast, estrogens inhibit the secretion of the anti-proliferative TGFβ1 and TGFβ2 [150]. Finally, estrogens contribute to reduce the inhibitory effects of dopamine on lactotrophs, in part by favouring the expression of the long form of D2R [150]. Noteworthy, estrogens are also involved in pituitary plasticity, and a pro-apoptic role of estrogens through membrane ERs and TNF/TNFR1 has been involved in lactotroph cells renewal during the estrous cycle [151,152]. Other gonadal steroids may play a role in PA, since receptors for androgens and progesterone may also be expressed in PA and associated with the modulation of their proliferation by their relative agonists *in vitro* [145,146]. Lack of steroid feed-back on pituitary cells can also be involved in pituitary tumorigenesis, but as already mentioned, true "feed-

back" gonadotroph PA are very rare. The potentially aggressive Nelson's syndrome, defined by corticotrophinoma growth after bilateral surrenalectomy for Cushing's disease, provides indirect evidence that glucocorticoid excess previously contributed to control cell proliferation. However, different degrees of impaired glucocorticoid feedback are observed in Cushing's and Nelson's tumours. A glucocorticoid-receptor (GR) mutation that diminished glucocorticoid inhibition has been reported in a single Nelson's tumour, but GRs are generally overexpressed in corticotrophinomas, and at the moment the best candidates for glucocorticoid resistance are Bgr1 and HDAC2, which are involved in the GR-dependent repression of POMC and are deficient in about 50% of corticotroph PA [153]. Bgr1 is a tumour suppressor and loss of nuclear Bgr1 has been associated with loss of p27Kip1 expression [153].

6.1.3. Adhesion molecules

Adhesion molecules are important to maintain cell-to-cell contact and a normal cell morphology and tissue architecture. Similarly to other epithelial tumours, PA may develop some degree of epithelial-to-mesenchymal transition, which is associated with loss of cell adherence and tumour aggressiveness. The N- and C-cadherins are involved in pituitary development and in the organization of the adult pituitary into a functional network involving FCS cells [154]. E-cadherin has been largely recognized as a TSG in the pituitary. Reduced E-cadherin expression was first associated with an aggressive behavior in prolactinomas [155]. Subsequently, downregulation of the E-cadherin gene (*CDH1*) and decreased E-cadherin expression has been reported in different PA phenotypes. The reduced expression of E-cadherin was associated with its redistribution from the cell membrane to the nucleus in invasive tumours [156]. Similar findings were recently reported in a large series of somatotrophinomas [157]. In this latter study, pre-operative treatment with SSA was associated with increased E-cadherin expression, although its nuclear localization correlated negatively with tumour shrinkage [157]. A positive association between reduced E-cadherin expression, increased nuclear E-cadherin and tumour aggressiveness has also been reported in corticotrophinomas, with a gradual decrease from micro- to macro-adenomas and Nelson's tumours [158]. Neural adhesion molecules (NCAMs) can be modified by polysialation. Polysialated NCAMs, which are implicated in cell proliferation and migration, have been involved in pituitary tumour invasiveness [91,159].

6.2. Abnormalities in cell cycle control

The main positive regulators of the cell cycle are the cyclin dependent kinases (CDKs), which are activated by specific associations with cyclins (A, B, D, E). The progression through the different phases of the cell cycle, in particular the G_1/S and G_2/M transitions, are stimulated by cyclin/CDK complexes and suppressed by their inhibitors, CKIs. The latter are divided into the INK4 family ($p16I^{nk4a}$, $p15^{Ink4b}$, $p18^{Ink4c}$), which negatively regulates the G_1/S transition through a direct interaction with the CDK4/6 containing complexes, and the so-called "universal" Cip/Kip family ($p21^{Cip1}$, $p27^{Kip1}$, $p57^{Kip2}$), which is involved at various phases of the cell cycle by interacting with different cyclin/CDK complexes. Down-regulation of cell cycle

inhibitors (*eg* CKIs, pRB) and overexpression of molecules involved in cell cycle progression (*eg.* cyclins, PTTG and HMGA proteins) are among the best characterised mechanisms of pituitary tumorigenesis [160]. The pituitary phenotype associated with genetic alterations in cell cycle regulators has been studied in several mouse models, including double mutants [160]. Some of the best characterized molecules involved in the dysregulation of cell cycle in PA are illustrated in Figure 1

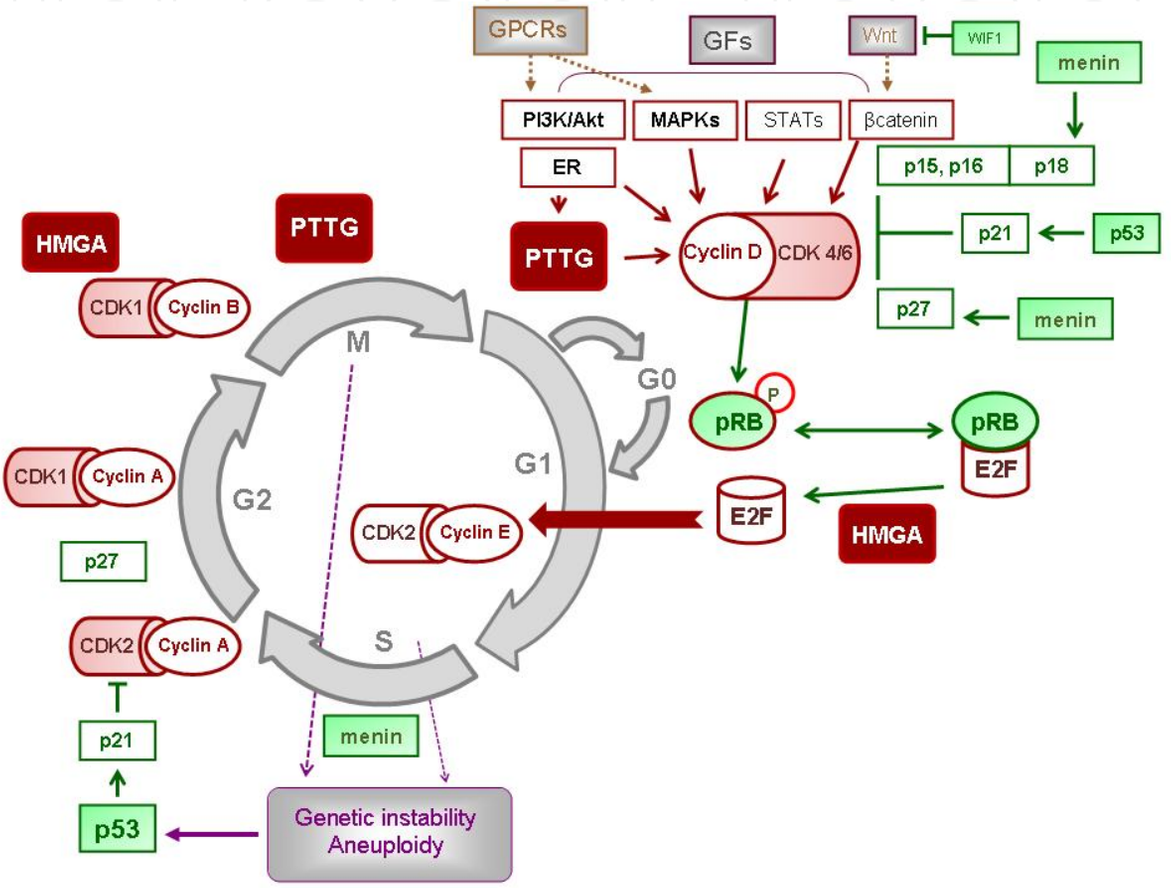

Figure 1. Cell cycle dysregulation in pituitary tumours

6.2.1. Abnormalities in cell cycle progression

Increased expression of D-type cyclins is a key event in the exit from the quiescent G_0 state under mitogenic stimulation by GFs. They activate CDK4 and CDK6, which phosphorylate pRB and therefore indirectly induce the transcription of S-phase genes by releasing TFs of the E2F family from their interaction with pRB. Transition from late G_1 to the S phase is also driven by Cyclin E/ CDK2 complexes. Overexpression of cyclins D1 and D3 has been reported in PA of different histotypes [160]. In particular, Cyclin D1 is overexpressed in ~70% of NFPA and 40% of somatotrophinomas, especially in invasive PA, with allelic imbalance suggesting gene amplification in 25% of the cases [161]. Increased GFs and Wnt/β-catenin signalling is also likely to contribute to *CCDN1*/*cyclinD1* expression in PA. In contrast, cyclin E overexpression

has been mostly reported in corticotrophinomas, where it has recently been correlated with the loss of Bgr1 expression [162]. Overexpression of Cyclin E under the control of the POMC promoter in transgenic mice was shown to promote re-entry in the cell cycle and centrosome instability [162]. CDK4 is involved in pituitary ontogenesis. Although PA may develop in the anterior lobe of transgenic mice expressing a *CDK4* missense mutation (R24C), which inhibits its interaction with INK4 proteins, it does not appear to play a major role in human PA. The tumour suppressor function of pRB in the pituitary has long been recognized in rodents, with $pRB^{+/-}$ mice developing tumours of the intermediate lobe. In humans, loss of pRB expression in PA mainly occurs through LOH in 13q14 or promoter methylation, especially in invasive PA, though homozygous deletions in the protein-binding pocket can be found [90]. Reduced expression of the INK4 family members of CKIs - $p16^{Ink4a}$ and, to a lesser extent, $p15^{Ink4b}$, which are encoded by adjacent genes in 9p21 - has been frequently reported in PA and methylation of the *CDKN2A/p16 Ink4a* gene promoter is considered as a precocious event [160]. Overall, abnormalities of the cyclinD/pRb/$p16^{Ink4a}$ pathway have been reported in 93% of NFPA and 56% of somatotrophinomas [163]. The role of $p18^{In4c}$ in pituitary tumorigenesis has been shown in mouse models. A reduced expression of $p18^{In4c}$ has been observed in a subset of PA, due to promoter methylation or, less frequently, LOH at the corresponding locus [164]. The *CDKN2C/ $p18^{In4c}$* gene is a target of menin and $p18^{In4c}$ collaborates with menin to suppress pituitary tumorigenesis. Although failure to adequately control cell cycling at G_1/S transition is considered as a mandatory step in tumorigenesis, loss of adequate control of the G_2/M transition represents an important additional event [165]. In particular, DNA damage can induce G_2 arrest as a result of the activation of ATR and ATM kinases, which activate p53-dependent and -independent intracellular cascades [165]. A reduced expression of Wee1, which induces an inhibiting phosphorylation of Cdk1 and therefore contributes to prevent G_2/M progression, has been reported in somatotrophinomas and NFPA [109]. An increased expression of Cyclin A has been reported in PA of different histotypes [160] and similar findings were reported for Cyclin B1 and B2, especially in prolactinomas [166,167]. Genes induced by p53 upon genotoxic stress include GADD45, p21 and Reprimo. GADD45γ is strongly down-regulated in most PA [168] and GADD45β has been identified as a TSG in gonadotrophinomas [169]. Reprimo has also been recently characterized as a pituitary TSG, especially in gonadotroph and somatotroph tumours [93]. Down-regulation of universal CKIs and upregulation of PTTG and HMGAs may contribute to cell cycle dysregulation at different stages.

6.2.2. The universal Cip/kip CDKI family

Both $p27^{Kip1}$ and $p21^{Cip1/Waf1}$ have been involved in pituitary tumorigenesis. The $p27^{Kip1}$ protein is normally localized in the nucleus of quiescent cells and undergoes rapid degradation upon mitogenic stimulation. Phosphorylation of $p27^{Kip1}$ is involved in its degradation through the ubiquitin/proteasome pathway. Loss of nuclear $p27^{Kip1}$ due to a reduced expression or an abnormal cytoplasmic localization has a negative prognostic value in a variety of human neoplasia. *CDKN1B/ $p27^{Kip1}$* is a haploinsufficient gene. In mice, both heterozygous and homozygous ablation of the *CDKN1B* gene induce pituitary tumours of the intermediate lobe and a collaborative effect with cyclin E up-regulation has been reported [162]. As reported hitherto, germline *CDKN1B* gene mutations are associated with MEN1-like syndromes (MEN4

in humans) [64], but this is a very rare condition. Down-regulation of $p27^{Kip1}$ has been reported in human PA, especially in corticotrophinomas and in pituitary carcinomas, due to an increased protein phosphorylation and degradation, with no significant alterations in *CDKN1B* transcripts. Of note, $p27^{Kip1}$ expression may be enhanced by other pituitary TSGs, such as E-cadherin [170] or menin [51], and by SSA in somatotrophinomas [171]. Another member of the Cip/Kip family, $p21^{Cip1/Waf1}$, is a target of p53 and induces growth arrest, essentially at the G_1/S transition but also in G_2/S. It may also be induced by oncogenes such as PTTG and Ras. The role of $p21^{Cip1/}$ in tumorigenesis is complex, since it may also have anti-apoptotic properties, depending on its subcellular localization [172]. Indeed, nuclear $p21^{Cip1}$ represses the transcriptional activity of E2F1, STAT3 and c-myc, whereas cytoplasmic $p21^{Cip1}$ binds to and inhibits pro-apoptotic molecules such as pro-caspase 3 and caspase 8 [172]. In the pituitary, the best characterized effects of $p21^{Cip1}$ are growth arrest and senescence. Using double and triple knock-out mice models for $pRb^{+/-}$, $PTTG^{-/-}$ and/or $p21^{-/-}$, p21 ablation was shown to restore tumorigenesis – induced by $pRb^{+/-}$ and abolished by $PTTG^{-/-}$ knock-out - and accelerate S-phase entry [173]. As already reported, germline *CDKN1A*/ $p21^{Cip1}$ mutations have been occasionally involved in MEN1-like syndromes [66]. Nuclear 21^{Cip1} immunostaining has been reported in secreting PA, especially somatotrophinomas [174,175]. Similar data were obtained in a recent study, which also reported a frequent cytoplasmic localization in pituitary tumours, especially NFPA and pituitary carcinomas, but not in somatotrophinomas [176].

6.2.3. PTTG

Although PTTG1 – the most abundant and widely studied member of the PTTG family, commonly referred to as PTTG - was first identified in the rat pituitary GH_4 cells, it has important oncogenic properties in a number of additional endocrine and non-endocrine neoplasia and cells overexpressing PTTG are tumorigenic *in vivo*. The biological functions of PTTG are highly complex and derive from important functional domains among which DNA-binding, SH3-binding and transactivating domains. Both nuclear and cytoplasmic localizations can be observed, nuclear shuttling being enhanced by the ubiquitous PTTG-binding protein (PBF) and by MAP kinases [177]. As a nuclear protein, PTTG has a dual role: it acts as a global TF - enhancing gene transcription either directly through binding DNA at specific promoter sites or indirectly through an interaction with other TFs - and as a securin protein - inhibiting premature separation of sister chromatids during mitosis –. PTTG functions, partners and target genes have been recently reviewed [177,178]. The securin function of PTTG is in appearent contrast with its oncogenic properties, and the multifaceted aspects of PTTG are illustrated by accumulating evidence for PTTG involvement in organ development, cell proliferation, survival and/or apoptosis, according to the experimental model and conditions. PTTG interacts with Sp1 to control progression through the G_1/S phase of the cell cycle and to suppress $p21^{Cip1}$ transcription by binding its promoter. The expression of PTTG may be increased by estrogens and GFs (EGF, FGF2), thereby creating positive feedback loops on cell proliferation [177]. PTTG expression is cell-cycle-dependent, with protein degradation occurring at anaphase and, as an estrogen inducible factor, varies according to the estrous cycle in rodents. PTTG is also involved in the control of genetic stability and angiogenesis. Detailed reviews are available on this topic [177-179]. In the normal pituitary, PTTG is expressed at low

levels and is typically undetectable by IHC [177]. PTTG is necessary to pituitary development and a large body of evidence supports its dual involvement in the initiation and progression of PA [177,179]. Targeted pituitary overexpression of PTTG under the control of the α-subunit promoter in transgenic mice induced multihormonal pituitary hyperplasia with focal gonadotroph and somatotroph PA [180]. PTTG overexpression has been consistently shown to occur at both transcriptional and protein levels in PA, though conflicting data have been reported about its subcellular localization. Predominant cytoplasmic localization was first reported in PA cells [177]. Subsequently, using a monoclonal antibody, nuclear PTTG immunostaining was observed in 90% of PA of different histotypes and strongly correlated with the Ki67 labeling index ($P<0.001$), although the percentage of PTTG immunopositive nuclei was highly variable from case to case [177]. PTTG expression was also found to correlate with invasiveness in secreting PA, in particular in somatotrophinomas [177,179]. Yet, no genetic alterations have been found to explain PTTG overexpression and epigenetic changes [179] or miRs dysregulation [104] have been proposed.

6.2.4. HMGA proteins

The HMGA family of nuclear proteins - HMGA1*a*/*b*/*c* and HMGA2 - is composed of DNA-binding molecules involved in the architecture of chromatin and modulates the transcriptional activity of several TFs. They are widely expressed during embryogenesis, strongly down-regulated or silenced in normal adult tissues, and commonly re-expressed in solid tumours, including PA [181,182]. HMGAs are involved in different biological processes such as cell proliferation, cell differentiation, DNA repair [181] and interfere with the cell cycle in different ways. HMGA2 interacts with pRB and facilitates the expression of E2F1 target genes through histone modifications and acetylation of E2F1 itself, thereby promoting the G_1/S transition. HMGAs also enhance the transcription of cyclin B2, which is involved in the G_2/M transition. The critical role of HMGAs in pituitary tumorigenesis has been proven by transgenic HMGA models, which develop GH/PRL-secreting PA and additional neoplasia (eg, lipomas, fibroadenomas, T-cell lymphomas). HMGA1b and HMGA2 have been recently shown to enhance *Pit1* gene transcription and interact with the Pit1 protein [183]. Overexpression of HMGA2 was reported by IHC in 39% of human PA, in particular in FSH/LH- and ACTH-secreting PA (>60%), to a lesser extent in prolactinomas (~30%), and significantly associated with tumor invasion [184]. In contrast, HMGA2 was not detected in the normal pituitary [184]. Similarly, nuclear immunostaining for HMGA1 was observed in 62% of PA of all histotypes, with a prevalence ranging from 37% in corticotrophinomas to 100% of null cell PA, but not in the normal pituitary [185]. Overexpression of HMGA2 in prolactinomas has been explained by gene amplification and rearrangements of the HMGA2 locus in 12q14-15, with a frequent polysomy of chromosome 12, but this rarely occurs in NFPA [182,185]. Alternatively, overexpression of HMGA genes could be explained by the down-regulation of *let-7* [182] and an additional set of miRNAs [107, 112], which enforced expression was found to decrease cell proliferation in pituitary cells [107, 112].

6.3. Abnormal proliferative pathways in pituitary tumours

The Raf/MEK/ERK and PI3K/Akt/mTor pathways are typically activated by GFs but crosstalks with neuropeptide/GPCRs signalling pathways are being increasingly recognized [186]. Crosstalks between the Raf/MEK/ERK and PI3K/Akt/mTor pathways result in the modulation of ERK1/2 activity, which is involved in the regulation of cell growth and differentiation, depending on the cellular context, and represents an important proliferative pathway in cancer. Secondary activation of mTOR signalling represents an important link between cell proliferation and metabolism [187]. These pathways are essential in oncology, many drugs have been designed to target their effector molecules at different steps [188] and similar strategies may be of interest in selected PA [189,190].

6.3.1. The Ras/Raf/MEK/ERK pathway

The Ras/Raf/MEK/ERK pathway originates at the cell membrane with receptors for GFs or cytokines, which activate the GTPAse Ras protein family through the coupling complex Shc/Grb2/SOS. The active, GTP bound, form of Ras recruits Raf proteins, which in turn activate a cascade of phosphorylations on cytoplasmic MAP kinases (MEKs and ERKs). A large number of cytoplasmic and nuclear proteins have been recognized as ERK1/2 targets, including the ribosomal S6 kinase (which in turns phosphorylates CREB), TFs (*eg.* c-myc, c-fos) and *Cyclin D1*. Activation of the Ras/Raf/MEK/ERK pathway in PA may result from increased stimulation by GFs/cytokines, overexpression or constitutive activation of their receptors, overexpression of B-Raf [191], exceptionally *H-Ras* [88] or *B-Raf* mutations [87]. Noteworthy, overexpression of B-Raf and a V600E mutation were found only in NFPA [87,191]. In an extensive western blot study, an over-phosphorylation of MEK1/2 and ERK1/2 was observed in all PA histotypes, although CyclinD1 was overexpressed in NFPA only [192]. GPCRs may activate Raf through the cAMP/PKA or the DAG/PKC pathways and therefore induce ERK1/2 phosphorylation. There is accumulating evidence that such mechanisms are relevant in different pituitary cells: ERKs have been involved in CRH-induced POMC transcription in AtT20 cells [193], gender-specific regulation of gonadotrophs by GnRH [194], GHRH induced expression of Cyclin D1 in somatotrophs [189] and the stimulation of PRL promoter activity by the *Gsp* oncogene in GH_4C_1 cells [195]. In contrast, reduced ERK1/2 activation has been involved in SSA signalling in somatotrophinomas [172].

6.3.2. The PI3K/Akt/mTOR pathway

The PI3K/AKT/mTOR pathway also initiates at the cell membrane in response to a variety of GFs and hormones, including insulin. PI3K phosphorylates phosphoinositides, resulting in the production of phosphoinositide 3-phosphate which in turn regulates the activity and intracellular localization of a number of target proteins, among which the best characterized is Akt (also known as Protein Kinase B/PKB). PI3K is negatively regulated by the tumour suppressor PTEN. The activation of Akt/PKB results in a cascade of phosphorylations, including mTOR, GSK3β, crosstalks with the Raf/MEK/ERK pathway at different levels, and has been involved in cell proliferation and motility in a number of cancers. Moreover, repression of the tumour suppressor Zac1 and activation of β-catenin through GSK3β are

indirect effects of PI3K/Akt activation. The mTOR pathway is also activated by nutrients, cellular energy levels (ATP, O_2) and stress conditions; it is a major regulator of ribosomal biogenesis and protein synthesis, in particular through the activation of the ribosomal S6 kinase p70S6K and 4EBP1, which enhances the translation of *c-myc* and *Cyclin D1* mRNA [186]. Mutations and amplifications of the *PIK3CA* gene, encoding the p110 subunit of PI3K, has been reported in a large series of PA [88]. In this study, *PIK3CA* mutations were present in nearly 10% of invasive PA, whereas gene amplification, associated with protein immunostaining in a subset of cases, was found in ~30% of PA, with the highest prevalence in NFPA and no significant correlation with tumour invasiveness. Both Akt1 and Akt2 isoforms were found over-expressed and over-phosphorylated in PA, especially in NFPA, with no change in PTEN expression or sequence [196]. Increased phosphorylation of Akt, mTOR and p70S6K was reported in a genetic model of TSH-oma [197]. However, no change in mTOR and S6K expression or phosphorylation status was observed in PA [192]. Zac1, a zinc finger TF, is widely expressed in normal tissues, down-regulated in a variety of human neoplasia, and induces cell cycle arrest and apoptosis, at least in part through the induction of $p21^{Kip1}$ and $p57^{Kip2}$ [198]. It is involved in pituitary development and normally expressed by all pituitary cell types, but is strongly down-regulated in NFPA, where a complete loss of expression can be observed, especially in the null cell histotype [198]. No *Zac1* mutations have been found but epigenetic silencing is frequent and LOH at the corresponding locus (6q24-25) may occur. Interestingly, the expression of *Zac1* in somatotrophinomas is increased by SSA and correlates with the therapeutic response [198]. Data obtained in GH_3 cells indicate that this effect depends on the inhibition of PI3K/Akt signalling [198]. Zac1 appears as an essential mediator of SSA [198] and may be related to AIP [80].

6.4. Angiogenesis and hypoxia-pathways

Although angiogenesis is involved in the progression of many solid tumours, its pathogenetic role in PA is still not well defined. The normal pituitary gland is already highly vascularised and different studies on microvessel density (MVD) in PA have provided evidence for a reduced vascularity as compared to the normal tissue. On the other hand, increased vascularity can occur, as reported in estrogen-induced prolactinoma models and in some human pituitary tumours [142]. Despite conflicting results about potential factors associated with an increased vascularity (*eg.* age, male gender), most studies suggest that, in contrast to other solid tumours, there is no correlation between increased vascularity and the proliferative activity of PA [142,199]. Angiogenesis results from the balance between angiogenic and anti-angiogenic factors and requires extracellular matrix remodelling to allow the migration of endothelial cells. Among pituitary pro-angiogenic factors, VEGF and FGFs are the best characterized. Both VEGF and FGF2 are upregulated by estrogens and PTTG in PA, and correlations have been reported between the expression of PTTG and both FGF2 and VEGF-A [179].Vascular density was also associated with PTTG expression in somatotrophinomas [179]. Estrogens down-regulate thrombospondin, an anti-angiogenic factor also involved in TGFβ signalling and apoptosis [125], and thrombospondin analogues have recently proven useful in the treatment of estrogen-induced prolactinomas [200]. In contrast, with the possible exception of gonadotrophinomas, the endocrine-gland derived VEGF (EG-VEGF) was found to be down-regulated

in PA [201]. Hypoxia pathways also stimulate angiogenesis, the Hypoxia-Inducible Factor (HIF)-α being the most important TF involved in the cellular response to hypoxia. Nuclear HIF-α immunostaining was reported in PA but not in the normal pituitary [202]. HIF-α protects HP75 cells from apoptosis under hypoxic conditions [203] and is stabilised by RSUME, a sumoylation factor, which is over-expressed in PA and plays an important role in VEGF-A induction under hypoxic conditions in pituitary tumour cells [204]. Angiogenesis may favour hemorrhage, and tumour apoplexy may occur, especially in pituitary macroadenomas. Hemorrhagic PA were reported to express high levels of VEGF [205, 206] and high ER concentrations [143]. Overexpression of HIF-α in MMQ cells was found to induce VEGF and the pro-apoptotic BNIP3 gene and to promote hemorrhagic transformation in MMQ cells xenografts [207]. TNFα was also found to promote VEGF and MMP-9 expression as well as tumour hemorrhage in the same model [206]. Interestingly, endoglin (CD105), a marker of endothelial proliferating cells, was found to be lower in secreting PA treated by SSA or DA than in untreated PA, indicating that inhibition of angiogenesis contributes to their therapeutic effect [208]. Anti-VEGF therapy (bevacizumab) has been proposed in experimental models of estrogen-induced [209] and dopamine-resistant [210] prolactinomas, and has been successfully used in a pituitary corticotroph carcinoma [211].

6.5. Apoptosis in pituitary tumours

As in other tissues, apoptosis is a physiological event during pituitary ontogenesis [212]. It is also believed to contribute to pituitary plasticity and may occur in PA, either spontaneously or in response to pharmacological treatment. Apoptosis is generally low in normal pituitaries and in PA, but is increased in pituitary carcinomas [213]. Apoptotic cells in PA can be detected on routine examination on the basis of their morphological changes, though they can be missed even by experienced pathologists, so that specific assays are more suitable for the definition of apoptotic indexes [214]. The ISEL and TUNEL assays, which are based on the visualization of DNA breaks, can be used on paraffin-embedded sections, but should be combined with morphological criteria to minimize artefacts [214]. Immunohistochemical detection of proteins involved in the apoptotic process such as activated caspase 3, cleaved cytokeratins or annexin-5 are also useful. As a general rule, apoptosis can be triggered by extra-cellular signalling by Fas ligand (FasL) or TNF interacting with "death receptors" or by endogenous signalling following mitochondrial or DNA damage, which is able to induce apoptosis in a p53-dependent manner. No significant relationship between p53 expression and apoptosis have been reported in PA, with the exception of nuclear p53 in corticotrophinomas [214]. An important determinant of apoptosis is the cellular expression of the Bcl2 family of proteins, which contains pro-apoptotic (*eg*. Bax, Bad) and anti-apoptotic/pro-survival (*eg.* Bcl2, Bcl-X_L) proteins, which can homo- o heterodimerize and influence cell fate. Several members of the Bcl2 family are expressed in PA, although some discrepancies have been reported about their distribution according to tumour histotype and behaviour [213-215]. Pro-apoptotic signals may be phenotype-dependent. Fas and FasL have been observed in the rat pituitary, especially in lactotrophs and somatotrophs, where they have been involved in the increased rate of apoptosis during proestrous [216], although an estrogen-dependent increase in TNFα and its receptor TNFR1 have also been implicated [151]. FasL is expressed by pituitary tumour cell lines of different

lineages and Fas signalling was found to induce an arrest in cell proliferation at G_0/G_1 and apoptosis in GH_3, AtT20 and MMQ cells [217]. GH_3 cells also express TNFα and overexpression of TNFα and FasL could induce apoptosis in these cells [218]. In somatotrophs, RET heterodimerizes with its co-receptor GFRα2 and induces apoptosis *in vivo* and *in vitro* [219]. Pit-1 and $p19^{ARF}$ ($p14^{ARF}$ in humans) activation have been involved, leading to p53-induced apoptosis [220]. RET is a dependent receptor and both RET and its ligand GDNF are normally expressed by somatotrophs [221]. Both have also been reported in all somatotrophinomas and, to a lesser extent, in other-secreting PA [221]. Interestingly survivin, an anti-apoptotic protein of the IAP family, interacts with AIP, which enhances its stability *in vitro* and assists its mitochondrial import [222], but RET prevents survivin from binding to AIP [223]. Although ablation of RET induces somatotroph hyperplasia in mice [219], no mutation in *c-RET* has been reported in PA yet. Either, no *AIP* mutant was found to disrupt AIP-survivin interaction [223]. Studies on survivin expression in PA has lead to conflicting results [224,225]. TIM16 is a mitochondrial protein encoded by the *Magmas* gene and overexpressed in the majority of PA, in particular in corticotroph PA and cell lines, where it has been involved in cell progression and protection from apoptotic stimuli [226]. The Pituitary Tumour Apoptosis Gene (PTAG), identified by random PCR analysis of methylated genes in PA, was involved in bromocriptine-induced apoptosis in AtT20 cells [227]. Pitx2, a developmental pituitary TF involved in Wnt/β-catenin signalling, is an anti-apoptotic factor in gonadotrophs and is overexpressed in NFPA [228]. Although PTTG overexpression has been shown in different tumour cell lines to promote apoptosis, in part through p53-mediated mechanisms, it may also inhibit the transcriptional activity of p53 [177]. The potential role of PTTG in the control of apoptosis in pituitary cells remains to be further clarified. Several p53-inducible genes have been involved in cell cycle arrest and apoptosis in PA(*eg.* GADD45, Reprimo). Drug-induced apoptosis may be part of the therapeutic response to SSA and DA in PA. DA may induce apoptosis in lactotrophs through the short isoform of D2R and this effect can be sensitized by estrogens [229]. The apoptotic response to SSA has been mainly associated with SSTR2 and SSTR3, although the alternatively spliced variants of SSTR2 may have opposite effects [230].

6.6. Senescence

Senescence is an alternative tumour-suppressive cell faith to apoptosis in benign neoplasia. It is characterised by an irreversible arrest in cell proliferation and accompanied by an increase in cell cycle inhibitors such as p53, $p19^{ARF}$, $p21^{Cip1}$ and $p16^{Ink4a}$. Because PA remain typically benign, even in the presence of invasive features, it has been proposed that a senescence buffer in PA cells exerts a protective effect against malignancy. This could in particular explain the very low prevalence of GH-secreting carcinomas and NFPA. Indeed, beta-galactosidase, a marker of senescence, was recently found overexpressed in somatotrophinomas and NFPA [176]. Interestingly, this could be explained by different molecular mechanisms. Somatotrophinomas show intranuclear p21 accumulation (possibly induced by aneuploidy and/or p53), which is able to restrain cell proliferation [176,231]. In gonadotroph PA, which express low nuclear $p21^{Cip1}$, high levels of clusterin have been proposed to restrain cell proliferation by triggering the CKIs $p15^{Ink4b}$/$p16^{Ink4a}$/$p27^{Kip1}$ [232]. However, in both cases overexpression of PTTG and DNA damage are present [231,232]. Because senescence may also be activated by

oncogenes, as originally described for Ras, this may apply to PTTG in pituitary tumours. Oncogene-induced senescence (OIS) is a protective mechanism against cancer which may also involve cytokines. Due to its role in pituitary development and its frequent expression in PA, IL6 is an attractive candidate for OIS in PA [131].

7. Pituitary carcinomas

Pituitary carcinomas represent about 0.2% of symptomatic primary pituitary tumours and are defined exclusively by the presence of metastases. Their prevalence may be somewhat underestimated, since metastases can be discovered post-mortem and the number of reported cases has been significantly increasing during the last 15 years [233-235]. The current interest in pituitary carcinomas certainly reflects the recent improvements in their diagnosis and therapeutic management. Their clinical characteristics have been reviewed in details elsewhere [233-236]. Briefly, most pituitary carcinomas are secreting (>80%), with malignant prolactinomas and corticotrophinomas being the most frequently encountered. They usually present as recurrent invasive macroadenomas, with an increasing degree of pharmacological resistance. Noteworthy, silent ACTH-secreting PA may become functional as malignant transformation occurs. Metastases may develop in the central nervous system (with intracranial and/or spinal localizations) or present as systemic secondary tumours, in particular in the bones, lungs or liver. Therefore, malignant transformation is a late event, which typically complicates the evolution of an aggressive PA, although exceptions have been reported. Yet, there is no specific biological marker of pituitary carcinoma and no reliable prognostic marker of potential malignant transformation in PA. The primary pituitary tumour often displays a high mitotic index and extensive p53 immunostaining, the apoptotic index is typically higher than in PA, but none of these features is invariably present and no threshold value of Ki67 or p53 immunopositivity can be defined. Several molecular abnormalities are encountered more frequently in pituitary carcinomas, such as chromosome gains, *H-Ras* mutations, overexpression of c-myc, HER2, galectin-3, loss of pRB, p27 and menin expression [233,234]. Active research is ongoing, aiming to identify molecular markers of aggressiveness and/or malignancy, which may differ according to the functional phenotype [110,237,238]. Understanding the molecular pathways of malignant transformation and progression of pituitary carcinomas should provide new tools for targeted therapies, as recently proposed for anti-VEGF or anti-EGFR therapy [138,210,211]. In addition, there is a need for reliable predictive markers of chemotherapy efficacy. Temozolomide (TMZ) has recently proven to be a very efficient tool for the treatment of pituitary carcinomas and highly aggressive PA, in particular prolactinomas [239-241]. Because changes in DNA induced by alkalylating agents may be reversed by the DNA repair enzyme O'6 methylguanine methyltransferase (MGMT), it has been proposed that, as reported in glioblastomas, MGMT status could be used as a predictive marker of TMZ efficacy in pituitary tumours. Although the first reports appeared to confirm such hypothesis [239], recent data indicate that neither MGMT immunostaining nor *MGMT* methylation status are sufficiently predictive; instead, a 3-

months clinical trial is worth regardless of MGMT status and predictive of long-term response [240,241]. In addition, the absence of correlation between MGMT gene methylation and MGMT immunostaining argues for a complex regulation of MGMT in pituitary tumours and further highlights the need for better prognostic tools [241, 242].

8. Conclusion

Pituitary tumours are very heterogeneous and their pathogenesis is multifactorial. During the last two decades, increasing knowledge about factors involved in pituitary ontogenesis, physiology and genetics have provided significant new information concerning dysregulated pathways in pituitary tumorigenesis. The rapid development of new methodological approaches allowing to explore hundreds of genes and proteins simultaneously (genomics/epigenomics/proteomics) has become an essential tool to unravel new players in pituitary tumourigenesis, although data obtained with such screening methods need to be validated on large series of pituitary tumours and integrated with functional studies. Molecular signatures of functional PA phenotypes are emerging and may provide significant information in terms of pathogenesis, prognosis and treatment. The identification of reliable markers of aggressiveness remains a priority for a better understanding and management of secreting PA resistant to conventional pharmacological treatment, NFPA and pituitary carcinomas.

Glossary

αGSU= α-glycoprotein subunit
ACP: adamantinomatous craniopharyngioma
ACTH: adrenocoticotropic hormone
AHR: aryl hydrocarbon receptor
AIP: aryl hydrocarbon receptor interacting protein
AP: anterior pituitary
BAG1: Bcl2-associated athanogene 1
Bcl2: B-cell lymphoma 2
BMP: bone morphogenetic protein
CDK: cyclin dependent kinase
CKI: cyclin dependent kinase inhibitor
CNC: Carney complex
CREB: cAMP response element-binding protein
CRH: corticotrophin-releasing hormone
GF: growth factor
GH: growth hormone
GHRH: growth-hormone-releasing hormone
GNAS1: α-subunit of the stimulatory G protein
GnRH: gonadotropin-releasing hormone
GPCR: G-protein coupled receptor
GSK-3β: glycogen synthase kinase 3β
HDAC: hystone deacetylase
HIFα: hypoxia-inducible factor α
HMGA: high mobility group AT-hook protein
IGF1: insulin-like growth factor 1
IHC: immunohystochemistry
IP3: inositol triphosphate
ISEL: immunogold electron microscopy in situ end-labeling
PAP: pituitary adenoma predisposition
PDE: phosphodiesterase
PI3K: phosphatidylinositide 3-kinases
PIK3CA: phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit α
PKA: protein kinase A
PKC: protein kinase C
PKRAR1A: regulatory subunit type 1A of cAMP-dependent protein kinase
POMC: proopiomelanocortin
PPAR: peroxisome proliferator-activated receptor
PROP-1: Prophet of Pit1
PRL: prolactin

CTNNB1: cadherin-associated protein β1 (β-catenin)
D2R: D2 dopamine receptor
DA: dopamine agonist
DAPK1: death-associated protein kinase 1
EGF: epidermal growth factor
ER: estrogen receptor
Erk: extracellular-signal-regulated kinase
FasL: Fas ligand
FCS: folliculostellate cells
FGF: fibroblast growth factor
FIPA: familial isolated pituitary adenomas
FISH: Fluorescence in situ hybridization
FSH: follicle-stimulating hormone
GADD45: growth arrest and DNA damage gene
LOH: loss of heterozigosity
MAGE-A3: melanoma-associated antigen 3
MAPK: mitogen-activated protein kinase
MAS: McCune Albright syndrome
MEG3: maternally expressed gene 3
MEN1: multiple endocrine neoplasia type 1
MEN4: multiple endocrine neoplasia type 4
MGMT: methylguanine methyltransferase
miR: microRNA
MMP: matrix metalloproteinase
mTor: mammalian target of rapamycin
MVD: microvessel density
NFPA: non-functioning pituitary adenoma
PA: pituitary adenoma
PTAG: pituitary tumor derived apoptosis gene
PTTG: pituitary tumour trasforming gene
RIα: regulatory subunit type 1A
RP : Rathke's pouch
Shh: Sonic Hedgehog
SSA: somatostatin analogues
SSTR: somatostatin receptor
TGF: trasforming growth factor
TF: transcriptions factor
TNF: tumor necrosis factor
TPR: tetratricopeptide repeats
TRH: TSH-releasing hormone
TSG: tumor suppressor gene
TSH: thyroid-stimulating hormone
TUNEL: terminal deoxynucleotidyl transferase
VEGF: vascular endothelial growth factor
WHO: world health organization

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