We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

New Insights in the Pathogenesis of Pituitary Tumours

Marie-Lise Jaffrain-Rea, Sandra Rotondi and
Edoardo Alesse

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/56028>

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 neurohypophysis 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 thyrotrophinomas (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 β FSH promoter develop gonadotroph hyperplasia

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 $\geq 3\%$ 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 $\geq 3\%$ 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 thyrotrophs), 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
<i>Early signalling molecules in pituitary development</i>			
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 <i>Pit-1</i>	Wnt4 : expressed in GH/PRL/TSH-secreting PA
Shh/ <i>Gli2</i>	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
<i>Transcriptional factors that control pituitary development</i>			
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 <i>Hesx1</i>	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 <i>αGSU</i> 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 <i>LHβ</i> , <i>FSHβ</i> , <i>GnRH</i> , <i>PRL</i> and <i>GH</i> gene transcription, while promoting cell differentiation Pitx1 also binds the <i>POMC</i> promoter	
FOXL2	AP	A transcriptional activator of <i>αGSU</i>	Overexpressed in NFPA
<i>Corticotroph differentiation</i>			
NeuroD1	RP	A transcriptional activator of <i>POMC</i> , involved in corticotroph differentiation	Expressed in corticotrophinomas and a subset of NFPA
Tpit	POMC precursor	A transcriptional activator of <i>POMC</i> , essential for the specification and terminal differentiation of corticotrophs and melanotrophs,	Expressed in secreting and silent corticotrophinomas
<i>Differentiation of Pit-1 lineages: somatotroph, lactotroph and thyrotroph differentiation</i>			
Prop1	RP	A transcriptional activator of <i>Pit-1</i> and a trascriptional repressor of <i>Hesx1</i>	Expressed in all PA phenotypes
Gata2	RP	Interacts and cooperates with <i>Pit-1</i> for the activation of <i>TSHβ</i>	Expressed essentially in gonadotroph and thyrotroph PA
Pit-1	Pit-1 lineages	Essential for the terminal differentiation and expansion of GH/PRL/TSH-secreting cells; an enhancer of <i>GH</i> , <i>PRL</i> , <i>TSHβ</i> e <i>GHRHR</i> gene expression and a repressor of <i>Gata2</i>	Expressed in GH/PRL/TSH-secreting PA (including NFPA with silent secretion)
CREB	Pit-1 lineages	Required in somatotrophs	Expressed in all somatotrophinomas
<i>Gonadotroph differentiation</i>			
Gata2	RP/pituitary	A promoter of <i>SF1</i> gene expression	See above
SF1	Gonadotrope	A promoter of <i>αGSU</i> , <i>FSHβ</i> , <i>GnRHR</i> gene expression, it also interacts with <i>Egr1</i> and <i>Pitx1</i> for the activation of <i>LHβ</i>	Expressed in the majority of gonadotroph/ NFPA, not in GH/PRL-secreting PA
Egr1	Gonadotrope	Required for <i>LHβ</i> 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

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 response mediated by Dishevelled (Dsh), the glycogen synthase kinase 3 β (GSK-3 β), Adenomatous Polypoid 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 β 1/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-3 β 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 led 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 detaches 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 Ptc 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

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- α 2-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 cranio-pharyngioma 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 GFR α [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 ER α and PPAR γ and with Smad proteins, involved in TGF β 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 hemizygoty, 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 GH₃ 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. PPAR α , ER α); 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 GH₃ 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, PKC α 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 ascribed to gene amplification or transcriptional upregulation. These include overexpression of growth factors and cytokines (eg. TGF α , VEGF) and their receptors (eg. EGFR), proteins involved in the control of cell cycle and proliferation (eg. cyclins, HMGA, 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. TGF β , BMP4, FGFR2), DNA repair (eg. p53, GADD45 γ) 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 tumorigenesis 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-κB-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 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 amplifies PI3K signalling) [104], whereas upregulation of miR-107 has been recently involved in AIP silencing [105]. Overexpression of PTTG and HMGA 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	Chromosome map (h)	Molecular defect	Histotype	Function	Ref.
miR-128a	2q21.3	Overexpression	NFPA	Target gene: <i>Wee1</i> ($\downarrow$)	109
		Down-regulation	All PA GH	Target gene: <i>BMI1*</i> ($\uparrow$)	103 111
miR-26a/b	3p21 (a)	Overexpression	NFPA (miR-26a)	Potential target genes:	103
	2q35 (b)		GH (miR-26a/b)	<i>Hox-A5</i> , <i>PLAG1</i> Target gene (miR26b): <i>PTEN</i> ($\downarrow$)	111
		Down-regulation	GH/PRL/NFPA (miR 26a)	Target genes: <i>HMGA1</i> , <i>HMGA2</i> ($\uparrow$)	112

MicroRNA	Chromosome map (h)	Molecular defect	Histotype	Function	Ref.
miR-191	3p21	Overexpression	All PA	Cell proliferation	103
miR-145	5q32	Down-regulation	GH	Target genes: <i>c-myc</i> , <i>k-ras</i> , <i>c-fos</i> , <i>cyclin D2</i> , <i>MAPK</i> (↑)	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: <i>VEGFR1</i> and several oncogenes	103
Let7-a	9q22.32	Down-regulation	All PA	Target genes: <i>HMGA1</i> , <i>HMGA2</i> (↑) Potential target genes: <i>Ras</i>	103, 104, 112
miR-126	9q34.3	Down-regulation	GH	Amplification of PI3K signalling; <i>PTTG</i> (↑)	106
miR-107	10q23	Overexpression	GH/ NFPA	Target gene: <i>AIP</i> (↓)	105, 111
miR-326	11q13.4	Down-regulation	GH, some PRL NFPA	Target genes: <i>HMGA1</i> , <i>HMGA2</i> , <i>E2F</i> (↑)	107
miR-141	12p13	Down-regulation	ACTH	Tumor growth and tumor local invasion	103
miR-16-1	13q14	Down-regulation	All PA	Target genes: <i>HMGA1</i> , <i>HMGA2</i> , <i>Bcl2</i> (↑)	102-104, 112
miR-15a	13q14	Down-regulation	All PA, in particular GH/PRL	Target genes: <i>HMGA1</i> , <i>HMGA2</i> (↑)	102, 103, 112
miR-20a	13q31.3	Overexpression	NFPA	Target gene: <i>Wee1</i> (↓)	109
miR-493	14q32.2	Overexpression	ACTH (carcinoma)	Target genes: <i>LGALS-3</i> and <i>RUNX2</i> (↓)	110
miR-381	14q32.31	Down-regulation	GH	Target gene: <i>PTTG</i> (↑)	106
miR-140	16q22.1	Overexpression	NFPA (macroadenomas)	Tumor growth	103
miR-212	17p13.3	Overexpression	All PA	Target gene: <i>DEDD</i> (↓), ↑ 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.
miR-098	Xp11.2	Down-regulation	All PA	<i>Wee1</i> (↓) 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 ($G_s\alpha$), encoded by the *GNAS1* gene, is required for the activation of adenylyl 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 (G_i) inhibits adenylyl 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 adenylyl 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 Gs α protein can be observed in *Gsp*⁻ tumours [86,115]. Accepting the concept that *Gsp* mutations represent an early pathogenic 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 sensitivity 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 intriguing 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 1 α 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 GH₃ 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 TGF β 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 TGF β superfamily includes several members (TGF β 1-3, activin/inhibins and BMPs), which may exert either collaborative or opposing signalling on different pituitary cells and PA histotypes. TGF β signalling is mediated by Smad proteins and generally inhibits cell proliferation. All TGF β isoforms and their receptor TGF β -R-II are expressed in PA, and TGF β 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 TGF β 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 TNF α , 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 GH₄C₁ 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 TGF α has been involved in estrogen-related lactotroph proliferation. On the other hand, EGF is able to induce lactotroph differentiation in GH₃ 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 GH₃ 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 GH₃ 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 GH₃ 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, ER α and ER β play distinct roles and an imbalance between these two isoforms has been reported in PA. Indeed, nuclear overexpression of ER α has been linked to tumour aggressiveness in prolactinomas and NFPA, whereas invasive NFPA were found to express lower ER β [147]. Ablation of ER β 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- β 3 secretion by lactotrophs being proposed to stimulate FGF release by FCS cells, which in turn stimulate lactotroph 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 TGF β 1 and TGF β 2 [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-apoptotic 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 G₁/S and G₂/M transitions, are stimulated by cyclin/CDK complexes and suppressed by their inhibitors, CKIs. The latter are divided into the INK4 family (p16^{Ink4a}, p15^{Ink4b}, p18^{Ink4c}), which negatively regulates the G₁/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 G₀ 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 G₁ 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 G₁/S transition is considered as a mandatory step in tumorigenesis, loss of adequate control of the G₂/M transition represents an important additional event [165]. In particular, DNA damage can induce G₂ 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 G₂/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. GADD45 γ is strongly down-regulated in most PA [168] and GADD45 β 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 HMGA5 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 G₁/S transition but also in G₂/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 GH₄ 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 apparent 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 G₁/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 - HMGA1a/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 G₁/S transition. HMGAs also enhance the transcription of cyclin B2, which is involved in the G₂/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 GH₄C₁ 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, GSK3 β , 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 GSK3 β are

indirect effects of PI3K/Akt activation. The mTOR pathway is also activated by nutrients, cellular energy levels (ATP, O₂) 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 GH₃ 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 TGF β 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]. TNF α 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- or 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 TNF α 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 G₀/G₁ and apoptosis in GH₃, AtT20 and MMQ cells [217]. GH₃ cells also express TNF α and overexpression of TNF α and FasL could induce apoptosis in these cells [218]. In somatotrophs, RET heterodimerizes with its co-receptor GFR α 2 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 *Magma*s 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 alkylating agents may be reversed by the DNA repair enzyme O⁶ 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 tumorigenesis, 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	GF: growth factor	PAP: pituitary adenoma
ACP: adamantinomatous	GH: growth hormone	predisposition
craniopharyngioma	GHRH: growth-hormone-releasing	PDE: phosphodiesterase
ACTH: adrenocorticotrophic hormone	hormone	PI3K: phosphatidylinositol 3-
AHR: aryl hydrocarbon receptor	GNAS1: α-subunit of the stimulatory	kinases
AIP: aryl hydrocarbon receptor	G protein	PIK3CA: phosphatidylinositol-4,5-
interacting protein	GnRH: gonadotropin-releasing	bisphosphate 3-kinase, catalytic
AP: anterior pituitary	hormone	subunit α
BAG1: Bcl2-associated athanogene 1	GPCR: G-protein coupled receptor	PKA: protein kinase A
Bcl2: B-cell lymphoma 2	GSK-3β: glycogen synthase kinase 3β	PKC: protein kinase C
BMP: bone morphogenetic protein	HDAC: hystone deacetylase	PKRAR1A: regulatory subunit type
CDK: cyclin dependent kinase	HIFα: hypoxia-inducible factor α	1A of cAMP-dependent protein
CKI: cyclin dependent kinase	HMGA: high mobility group AT-hook	kinase
inhibitor	protein	POMC: proopiomelanocortin
CNC: Carney complex	IGF1: insulin-like growth factor 1	PPAR: peroxisome proliferator-
CREB: cAMP response element-	IHC: immunohistochemistry	activated receptor
binding protein	IP3: inositol triphosphate	PROP-1: Prophet of Pit1
CRH: corticotrophin-releasing	ISEL: immunogold electron	PRL: prolactin
hormone	microscopy in situ end-labeling	

CTNNB1: cadherin-associated protein β 1 (β -catenin)	LOH: loss of heterozygosity	PTAG: pituitary tumor derived apoptosis gene
D2R: D2 dopamine receptor	MAGE-A3: melanoma-associated antigen 3	PTTG: pituitary tumour transforming gene
DA: dopamine agonist	MAPK: mitogen-activated protein kinase	R1 α : regulatory subunit type 1A
DAPK1: death-associated protein kinase 1	MAS: McCune Albright syndrome	RP: Rathke's pouch
EGF: epidermal growth factor	MEG3: maternally expressed gene 3	Shh: Sonic Hedgehog
ER: estrogen receptor	MEN1: multiple endocrine neoplasia type 1	SSA: somatostatin analogues
Erk: extracellular-signal-regulated kinase	MEN4: multiple endocrine neoplasia type 4	SSTR: somatostatin receptor
FasL: Fas ligand	MGMT: methylguanine methyltransferase	TGF: transforming growth factor
FCS: folliculostellate cells	miR: microRNA	TF: transcription factor
FGF: fibroblast growth factor	MMP: matrix metalloproteinase	TNF: tumor necrosis factor
FIPA: familial isolated pituitary adenomas	mTor: mammalian target of rapamycin	TPR: tetratricopeptide repeats
FISH: Fluorescence in situ hybridization	MVD: microvessel density	TRH: TSH-releasing hormone
FSH: follicle-stimulating hormone	NFPA: non-functioning pituitary adenoma	TSG: tumor suppressor gene
GADD45: growth arrest and DNA damage gene	PA: pituitary adenoma	TSH: thyroid-stimulating hormone
		TUNEL: terminal deoxynucleotidyl transferase
		VEGF: vascular endothelial growth factor
		WHO: world health organization

Author details

Marie-Lise Jaffrain-Rea^{1,2}, Sandra Rotondi¹ and Edoardo Alesse¹

¹ Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila, L'Aquila (AQ), Italy

² Neuromed Institute, Pozzilli (IS), Italy

References

- [1] Daly AF, Rixhon M, Adam C, Dempegioti A, Tichomirowa MA, Beckers A. High prevalence of pituitary adenomas: a cross-sectional study in the province of Liege, Belgium. *The Journal of Clinical Endocrinology & Metabolism* 2006;91(12) 4769-75.
- [2] Arafah BM, Nasrallah MP. Pituitary tumors: pathophysiology, clinical manifestations and management. *Endocrine Related Cancer* 2001;8(4) 287-305.
- [3] Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, Burger PC, Jouvet A, Scheithauer BW, Kleihues P. The 2007 WHO classification of tumours of the central nervous system. *Acta Neuropathologica* 2007;114(2) 97-109.

- [4] Mete O, Asa SL. Clinicopathological correlations in pituitary adenomas. *Brain Pathology* 2012;22(4) 443-53.
- [5] Obari A, Sano T, Ohyama K, Kudo E, Qian ZR, Yoneda A, Rayhan N, Mustafizur Rahman M, Yamada S. Clinicopathological features of growth hormone-producing pituitary adenomas: difference among various types defined by cytokeratin distribution pattern including a transitional form. *Endocrine Pathology* 2008;19(2) 82-91.
- [6] Asa SL. Transgenic and knockout mouse models clarify pituitary development, function and disease. *Brain Pathology* 2001;11(3) 371-84.
- [7] Jaffrain-Rea ML, Di Stefano D, Minniti G, Esposito V, Bultrini A, Ferretti E, Santoro A, Faticanti Scucchi L, Gulino A, Cantore G. A critical reappraisal of MIB-1 labelling index significance in a large series of pituitary tumours: secreting versus non-secreting adenomas. *Endocrine Related Cancer* 2002;9(2) 103-13.
- [8] De Lellis RA., Lloyd RV., Heitz PU., Eng C. World Health Organization classification of tumours: tumours of endocrine organs. Lyon: IARC press; 2004.
- [9] Raverot G, Wierinckx A, Dantony E, Auger C, Chapas G, Villeneuve L, Brue T, Figarella-Branger D, Roy P, Jouanneau E, Jan M, Lachuer J, Trouillas J; HYPOPRONOS. Prognostic factors in prolactin pituitary tumors: clinical, histological, and molecular data from a series of 94 patients with a long postoperative follow-up. *The Journal of Clinical Endocrinology & Metabolism* 2010;95(4) 1708-16.
- [10] Galland F, Lacroix L, Saulnier P, Dessen P, Meduri G, Bernier M, Gaillard S, Guibourdenche J, Fournier T, Evain-Brion D, Bidart JM, Chanson P. Differential gene expression profiles of invasive and non-invasive non-functioning pituitary adenomas based on microarray analysis. *Endocrine Related Cancer* 2010;17(2) 361-71.
- [11] Ruebel KH, Leontovich AA, Jin L, Stilling GA, Zhang H, Qian X, Nakamura N, Scheithauer BW, Kovacs K, Lloyd RV. Patterns of gene expression in pituitary carcinomas and adenomas analyzed by high-density oligonucleotide arrays, reverse transcriptase-quantitative PCR, and protein expression. *Endocrine* 2006;29(3) 435-44.
- [12] Clayton RN, Farrell WE. Pituitary tumour clonality revisited. *Frontiers of Hormone Research* 2004;32 186-204.
- [13] Dworakowska D, Grossman AB. The pathophysiology of pituitary adenomas. *Best Practice & Research: Clinical Endocrinology & Metabolism* 2009;23(5) 525-41.
- [14] Melmed S. Pathogenesis of pituitary tumors. *Nature Reviews Endocrinology* 2011;7(5) 257-66.
- [15] Brinkmeier ML, Davis SW, Carninci P, MacDonald JW, Kawai J, Ghosh D, Hayashizaki Y, Lyons RH, Camper SA. Discovery of transcriptional regulators and signaling pathways in the developing pituitary gland by bioinformatic and genomic approaches. *Genomics* 2009;93(5) 449-60.

- [16] Davis SW, Castinetti F, Carvalho LR, Ellsworth BS, Potok MA, Lyons RH, Brinkmeier ML, Raetzman LT, Carninci P, Mortensen AH, Hayashizaki Y, Arnhold IJ, Mendonça BB, Brue T, Camper SA. Molecular mechanisms of pituitary organogenesis: in search of novel regulatory genes. *Molecular and Cellular Endocrinology* 2010;323(1) 4-19.
- [17] Kelberman D, Rizzoti K, Lovell-Badge R, Robinson IC, Dattani MT. Genetic regulation of pituitary gland development in human and mouse. *Endocrine Reviews* 2009;30(7) 790-829.
- [18] De Moraes DC, Vaisman M, Conceição FL, Ortiga-Carvalho TM. Pituitary development: a complex, temporal regulated process dependent on specific transcriptional factors. *Journal of Endocrinology* 2012;215(2) 239-45.
- [19] Perez-Castro C, Renner U, Haedo MR, Stalla GK, Arzt E. Cellular and molecular specificity of pituitary gland physiology. *Physiological Reviews* 2012;92(1) 1-38.
- [20] Fratticci A, Grieco FA, Spilioti C, Giangaspero F, Ventura L, Esposito V, Piccirilli M, Santoro A, Gulino A, Cantore G, Alesse E, Jaffrain-Rea ML. Differential expression of neurogenins and NeuroD1 in human pituitary tumours. *Journal of Endocrinology* 2007;194(3) 475-84.
- [21] Mantovani G, Asteria C, Pellegrini C, Bosari S, Alberti L, Bondioni S, Peverelli E, Spada A, Beck-Peccoz P. HESX1 expression in human normal pituitaries and pituitary adenomas. *Molecular and Cellular Endocrinology* 2006;247(1-2) 135-9.
- [22] Pellegrini-Bouiller I, Manrique C, Gunz G, Grino M, Zamora AJ, Figarella-Branger D, Grisoli F, Jaquet P, Enjalbert A. Expression of the members of the Ptx family of transcription factors in human pituitary adenomas. *The Journal of Clinical Endocrinology & Metabolism* 1999;84(6) 2212-20.
- [23] Nakamura S, Ohtsuru A, Takamura N, Kitange G, Tokunaga Y, Yasunaga A, Shibata S, Yamashita S. Prop-1 gene expression in human pituitary tumors. *The Journal of Clinical Endocrinology & Metabolism* 1999;84(7) 2581-4.
- [24] Egashira N, Minematsu T, Miyai S, Takekoshi S, Camper SA, Osamura RY. Pituitary changes in Prop1 transgenic mice: hormone producing tumors and signet-ring type gonadotropes. *Acta Histochemica and Cytochemica* 2008;41(3) 47-57.
- [25] Egashira N, Takekoshi S, Takei M, Teramoto A, Osamura RY. Expression of FOXL2 in human normal pituitaries and pituitary adenomas. *Modern Pathology* 2011;24(6) 765-73.
- [26] Gueorguiev M, Grossman AB. Pituitary gland and beta-catenin signaling: from ontogeny to oncogenesis. *Pituitary* 2009;12(3) 245-55.
- [27] Sekine S, Shibata T, Kokubu A, Morishita Y, Noguchi M, Nakanishi Y, Sakamoto M, Hirohashi S. Craniopharyngiomas of adamantinomatous type harbor beta-catenin gene mutations. *American Journal of Pathology* 2002;161(6) 1997-2001.

- [28] Buslei R, Nolde M, Hofmann B, Meissner S, Eyupoglu IY, Siebzehnriibl F, Hahnen E, Kreutzer J, Fahlbusch R. Common mutations of beta-catenin in adamantinomatous craniopharyngiomas but not in other tumours originating from the sellar region. *Acta Neuropathologica* 2005;109(6) 589-97.
- [29] Oikonomou E, Barreto DC, Soares B, De Marco L, Buchfelder M, Adams EF. Beta-catenin mutations in craniopharyngiomas and pituitary adenomas. *Journal of Neuro-Oncology* 2005;73(3) 205-9.
- [30] Gaston-Massuet C, Andoniadou CL, Signore M, Jayakody SA, Charolidi N, Kyeyune R, Vernay B, Jacques TS, Taketo MM, Le Tissier P, Dattani MT, Martinez-Barbera JP. Increased Wntless (Wnt) signaling in pituitary progenitor/stem cells gives rise to pituitary tumors in mice and humans. *Proceedings of the National Academy of Sciences of the United States of America* 2011;108(28) 11482-7.
- [31] Elston MS, Clifton-Bligh RJ. Identification of Wnt family inhibitors: a pituitary tumor directed whole genome approach. *Molecular and Cellular Endocrinology* 2010;326(1-2) 48-54.
- [32] Wang Y, Martin JF, Bai CB. Direct and indirect requirements of Shh/Gli signaling in early pituitary development. *Developmental Biology* 2010;348(2) 199-209.
- [33] Roessler E, Du YZ, Mullor JL, Casas E, Allen WP, Gillessen-Kaesbach G, Roeder ER, Ming JE, Ruiz i Altaba A, Muenke M. Loss-of-function mutations in the human GLI2 gene are associated with pituitary anomalies and holoprosencephaly-like features. *Proceedings of the National Academy of Sciences of the United States of America* 2003;100(23) 13424-9.
- [34] Vila G, Papazoglou M, Stalla J, Theodoropoulou M, Stalla GK, Holsboer F, Paez-Pereida M. Sonic hedgehog regulates CRH signal transduction in the adult pituitary. *FASEB Journal* 2005;19(2) 281-3.
- [35] Vila G, Theodoropoulou M, Stalla J, Tonn JC, Losa M, Renner U, Stalla GK, Paez-Pereda M. Expression and function of sonic hedgehog pathway components in pituitary adenomas: evidence for a direct role in hormone secretion and cell proliferation. *The Journal of Clinical Endocrinology & Metabolism* 2005;90(12) 6687-94.
- [36] Thurston G, Kitajewski J. VEGF and Delta-Notch: interacting signalling pathways in tumour angiogenesis. *British Journal of Cancer* 2008;99(8) 1204-9.
- [37] Raetzman LT, Ross SA, Cook S, Dunwoodie SL, Camper SA, Thomas PQ. Developmental regulation of Notch signaling genes in the embryonic pituitary: Prop1 deficiency affects Notch2 expression. *Developmental Biology* 2004;265(2) 329-40.
- [38] Monahan P, Rybak S, Raetzman LT. The notch target gene HES1 regulates cell cycle inhibitor expression in the developing pituitary. *Endocrinology* 2009;150(9) 4386-94.
- [39] Miao Z, Miao Y, Lin Y, Lu X. Overexpression of the Notch3 receptor in non-functioning pituitary tumours. *Journal of Clinical Neuroscience* 2012;19(1) 107-10.

- [40] Evans CO, Moreno CS, Zhan X, McCabe MT, Vertino PM, Desiderio DM, Oyesiku NM. Molecular pathogenesis of human prolactinomas identified by gene expression profiling, RT-qPCR, and proteomic analyses. *Pituitary* 2008;11(3) 231-45.
- [41] Jiang Z, Gui S, Zhang Y. Analysis of differential gene expression in plurihormonal pituitary adenomas using bead-based fiber-optic arrays. *Journal of Neuro-Oncology* 2012;108(3) 341-8.
- [42] Rizzoti K. Adult pituitary progenitors/stem cells: from in vitro characterization to in vivo function. *European Journal of Neuroscience* 2010;32 2053–2062.
- [43] Florio T. Adult pituitary stem cells: from pituitary plasticity to adenoma development. *Neuroendocrinology* 2011;94(4) 265-77.
- [44] Hosoyama T, Nishijo K, Garcia MM, Schaffer BS, Ohshima-Hosoyama S, Prajapati SI, Davis MD, Grant WF, Scheithauer BW, Marks DL, Rubin BP, Keller C. A postnatal Pax7 progenitor gives rise to pituitary adenomas. *Genes Cancer* 2010;1(4) 388-402.
- [45] Xu Q, Yuan X, Tunici P, Liu G, Fan X, Xu M, Hu J, Hwang JY, Farkas DL, Black KL, Yu JS. Isolation of tumour stem-like cells from benign tumours. *British Journal of Cancer* 2009; 101(2) 303-11.
- [46] Garcia-Lavandeira M, Saez C, Diaz-Rodriguez E, Perez-Romero S, Senra A, Dieguez C, Japon MA, Alvarez CV. Craniopharyngiomas express embryonic stem cell markers (SOX2, OCT4, KLF4, and SOX9) as pituitary stem cells but do not coexpress RET/GFRA3 receptors. *The Journal of Clinical Endocrinology & Metabolism* 2012;97(1) E80-7.
- [47] Vandeva S, Jaffrain-Rea ML, Daly AF, Tichomirowa M, Zacharieva S, Beckers A. The genetics of pituitary adenomas. *Best Practice & Research: Clinical Endocrinology & Metabolism* 2010;24(3) 461-76.
- [48] Jaffrain-Rea ML, Daly AF, Angelini M, Petriossians P, Bours V, Beckers A. Genetic susceptibility in pituitary adenomas: from pathogenesis to clinical implications. *Expert Review of Endocrinology & Metabolism* 2011;(2) 195-214.
- [49] Agarwal SK, Ozawa A, Mateo CM, Marx SJ. The MEN1 gene and pituitary tumours. *Hormone Research* 2009;71 Suppl 2 131-8.
- [50] Trouillas J, Labat-Moleur F, Sturm N, Kujas M, Heymann MF, Figarella-Branger D, Patey M, Mazucca M, Decullier E, Vergès B, Chabre O, Calender A, Groupe d'études des Tumeurs Endocrines. Pituitary tumors and hyperplasia in multiple endocrine neoplasia type 1 syndrome (MEN1): a case-control study in a series of 77 patients versus 2509 non-MEN1 patients. *The American Journal of Surgical Pathology* 2008;32(4) 534-43.
- [51] Thakker RV. Multiple endocrine neoplasia type 1 (MEN1). *Best Practice & Research: Clinical Endocrinology & Metabolism* 2010;24(3) 355-70.

- [52] Lips CJ, Dreijerink KM, Höppener JW. Variable clinical expression in patients with a germline MEN1 disease gene mutation: clues to a genotype-phenotype correlation. *Clinics* 2012;67 Suppl 1 49-56.
- [53] Ellard S, Hattersley AT, Brewer CM, Vaidya B. Detection of a MEN1 gene mutation depends on clinical features and supports current referral criteria for diagnostic molecular genetic testing. *Clinical Endocrinology* 2005;62(2) 169-75.
- [54] Walls GV, Lemos MC, Javid M, Bazan-Peregrino M, Jeyabalan J, Reed AA, Harding B, Tyler DJ, Stuckey DJ, Piret S, Christie PT, Ansorge O, Clarke K, Seymour L, Thakker RV. MEN1 gene replacement therapy reduces proliferation rates in a mouse model of pituitary adenomas. *Cancer Research* 2012;72(19) 5060-8.
- [55] Dreijerink KM, Lips CJ, Timmers HT. Multiple endocrine neoplasia type 1: a chromatin writer's block. *Journal of Internal Medicine* 2009;266(1) 53-9.
- [56] Theodoropoulou M, Cavallari I, Barzon L, D'Agostino DM, Ferro T, Arzberger T, Grübler Y, Schaaf L, Losa M, Fallo F, Ciminale V, Stalla GK, Pagotto U. Differential expression of menin in sporadic pituitary adenomas. *Endocrine Related Cancer* 2004;11(2) 333-44.
- [57] Rothenbuhler A, Stratakis CA. Clinical and molecular genetics of Carney complex. *Best Practice & Research: Clinical Endocrinology & Metabolism* 2010;24(3) 389-99.
- [58] Bertherat J, Horvath A, Groussin L, Grabar S, Boikos S, Cazabat L, Libe R, René-Corail F, Stergiopoulos S, Bourdeau I, Bei T, Clauser E, Calender A, Kirschner LS, Bertagna X, Carney JA, Stratakis CA. Mutations in regulatory subunit type 1A of cyclic adenosine 5'-monophosphate-dependent protein kinase (PRKAR1A): phenotype analysis in 353 patients and 80 different genotypes. *The Journal of Clinical Endocrinology & Metabolism* 2009;94(6) 2085-91.
- [59] Yin Z, Williams-Simons L, Parlow AF, Asa S, Kirschner LS. Pituitary-specific knockout of the Carney complex gene *Prkar1a* leads to pituitary tumorigenesis. *Molecular Endocrinology* 2008;22(2) 380-7.
- [60] Veugelers M, Bressan M, McDermott DA, Weremowicz S, Morton CC, Mabry CC, Lefaiivre JF, Zunamon A, Destree A, Chaudron JM, Basson CT. Mutation of perinatal myosin heavy chain associated with a Carney complex variant. *The New England Journal of Medicine* 2004;351(5) 460-9.
- [61] Libé R, Horvath A, Vezzosi D, Fratticci A, Coste J, Perlemoine K, Ragazzon B, Guillaud-Bataille M, Groussin L, Clauser E, Raffin-Sanson ML, Siegel J, Moran J, Drori-Herishanu L, Faucz FR, Lodish M, Nesterova M, Bertagna X, Bertherat J, Stratakis CA. Frequent phosphodiesterase 11A gene (PDE11A) defects in patients with Carney complex (CNC) caused by PRKAR1A mutations: PDE11A may contribute to adrenal and testicular tumors in CNC as a modifier of the phenotype. *The Journal of Clinical Endocrinology & Metabolism* 2011;96(1) E208-14.

- [62] Dumitrescu CE, Collins MT. McCune-Albright syndrome. *Orphanet Journal of Rare Diseases* 2008;3 12.
- [63] Lumbroso S, Paris F, Sultan C; Activating Gsalpha mutations: analysis of 113 patients with signs of McCune-Albright syndrome. A European collaborative study. *The Journal of Clinical Endocrinology & Metabolism* 2004;89(5) 2107-13.
- [64] Pellegata NS, Quintanilla-Martinez L, Siggelkow H, Samson E, Bink K, Höfler H, Fend F, Graw J, Atkinson MJ. Germ-line mutations in p27Kip1 cause a multiple endocrine neoplasia syndrome in rats and humans. *Proceedings of the National Academy of Sciences of the United States of America* 2006;103(50) 19213.
- [65] Pellegata NS. MENX and MEN4. *Clinics (Sao Paulo)* 2012;67 Suppl 1 13-8.
- [66] Agarwal SK, Mateo CM, Marx SJ. Rare germline mutations in cyclin-dependent kinase inhibitor genes in multiple endocrine neoplasia type 1 and related states. *The Journal of Clinical Endocrinology & Metabolism* 2009;94(5) 1826-34.
- [67] Ozawa A, Agarwal SK, Mateo CM, Burns AL, Rice TS, Kennedy PA, Quigley CM, Simmonds WF, Weinstein LS, Chandrasekharappa SC, Collins FS, Spiegel AM, Marx SJ. The parathyroid/pituitary variant of multiple endocrine neoplasia type 1 usually has causes other than p27Kip1 mutations. *The Journal of Clinical Endocrinology & Metabolism* 2007;92(5) 1948-51.
- [68] Xekouki P, Pacak K, Almeida M, Wassif CA, Rustin P, Nesterova M, de la Luz Sierra M, Matro J, Ball E, Azevedo M, Horvath A, Lyssikatos C, Quezado M, Patronas N, Ferrando B, Pasini B, Lytras A, Tolis G, Stratakis CA. Succinate dehydrogenase (SDH) D subunit (SDHD) inactivation in a growth-hormone-producing pituitary tumor: a new association for SDH? *The Journal of Clinical Endocrinology & Metabolism* 2012;97(3) E357-66.
- [69] Daly AF, Jaffrain-Rea ML, Ciccarelli A, Valdes-Socin H, Rohmer V, Tamburrano G, Borson-Chazot C, Estour B, Ciccarelli E, Brue T, Ferolla P, Emy P, Colao A, De Menis E, Lecomte P, Penfornis F, Delemer B, Bertherat J, Wémeau JL, De Herder W, Archambeaud F, Stevenaert A, Calender A, Murat A, Cavagnini F, Beckers A. Clinical characterization of familial isolated pituitary adenomas. *The Journal of Clinical Endocrinology & Metabolism* 2006;91(9) 3316-23.
- [70] Vierimaa O, Georgitsi M, Lehtonen R, Vahteristo P, Kokko A, Raitila A, Tuppurainen K, Ebeling TM, Salmela PI, Paschke R, Gündogdu S, De Menis E, Mäkinen MJ, Launonen V, Karhu A, Aaltonen LA. Pituitary adenoma predisposition caused by germline mutations in the AIP gene. *Science* 2006;312(5777) 1228-30.
- [71] Daly AF, Vanbellinghen JF, Khoo SK, Jaffrain-Rea ML, Naves LA, Guitelman MA, Murat A, Emy P, Gimenez-Roqueplo AP, Tamburrano G, Raverot G, Barlier A, De Herder W, Penfornis A, Ciccarelli E, Estour B, Lecomte P, Gatta B, Chabre O, Sabaté MI, Bertagna X, Garcia Basavilbaso N, Stalldecker G, Colao A, Ferolla P, Wémeau JL, Caron P, Sadoul JL, Oneto A, Archambeaud F, Calender A, Sinilnikova O, Montaña-

- na CF, Cavagnini F, Hana V, Solano A, Delettieres D, Luccio-Camelo DC, Basso A, Rohmer V, Brue T, Bours V, Teh BT, Beckers A. Aryl hydrocarbon receptor-interacting protein gene mutations in familial isolated pituitary adenomas: analysis in 73 families. *The Journal of Clinical Endocrinology & Metabolism* 2007;92(5) 1891-6.
- [72] Trivellin G, Korbonits M. AIP and its interacting partners. *Journal of Endocrinology* 2011;210(2) 137-55.
- [73] Tichomirowa MA, Barlier A, Daly AF, Jaffrain-Rea ML, Ronchi C, Yaneva M, Urban JD, Petrossians P, Elenkova A, Tabarin A, Desailoud R, Maiter D, Schürmeyer T, Cozzi R, Theodoropoulou M, Sievers C, Bernabeu I, Naves LA, Chabre O, Montañana CF, Hana V, Halaby G, Delemer B, Aizpún JI, Sonnet E, Longás AF, Hagelstein MT, Caron P, Stalla GK, Bours V, Zacharieva S, Spada A, Brue T, Beckers A. High prevalence of AIP gene mutations following focused screening in young patients with sporadic pituitary macroadenomas. *European Journal of Endocrinology* 2011;165(4) 509-15.
- [74] Cazabat L, Bouligand J, Chanson P. AIP mutation in pituitary adenomas. *New England Journal of Medicine* 2011;364(20) 1974-5.
- [75] Daly AF, Tichomirowa MA, Petrossians P, Heliövaara E, Jaffrain-Rea ML, Barlier A, Naves LA, Ebeling T, Karhu A, Raappana A, Cazabat L, De Menis E, Montañana CF, Raverot G, Weil RJ, Sane T, Maiter D, Neggers S, Yaneva M, Tabarin A, Verrua E, Eloranta E, Murat A, Vierimaa O, Salmela PI, Emy P, Toledo RA, Sabaté MI, Villa C, Popelier M, Salvatori R, Jennings J, Longás AF, Labarta Aizpún JI, Georgitsi M, Paschke R, Ronchi C, Valimaki M, Saloranta C, De Herder W, Cozzi R, Guitelman M, Magri F, Lagonigro MS, Halaby G, Corman V, Hagelstein MT, Vanbellinghen JF, Barra GB, Gimenez-Roqueplo AP, Cameron FJ, Borson-Chazot F, Holdaway I, Toledo SP, Stalla GK, Spada A, Zacharieva S, Bertherat J, Brue T, Bours V, Chanson P, Aaltoonen LA, Beckers A. Clinical characteristics and therapeutic responses in patients with germ-line AIP mutations and pituitary adenomas: an international collaborative study. *The Journal of Clinical Endocrinology & Metabolism* 2010;95(11) E373-83.
- [76] Raitila A, Lehtonen HJ, Arola J, Heliövaara E, Ahlsten M, Georgitsi M, Jalanko A, Paetau A, Aaltoonen LA, Karhu A. Mice with inactivation of aryl hydrocarbon receptor-interacting protein (Aip) display complete penetrance of pituitary adenomas with aberrant ARNT expression. *American Journal of Pathology* 2010;177(4) 1969-76.
- [77] Khoo SK, Pendek R, Nickolov R, Luccio-Camelo DC, Newton TL, Massie A, Petillo D, Menon J, Cameron D, Teh BT, Chan SP. Genome-wide scan identifies novel modifier loci of acromegalic phenotypes for isolated familial somatotropinoma. *Endocrine Related Cancer* 2009;16(3) 1057-63.
- [78] Villa C, Lagonigro MS, Magri F, Koziak M, Jaffrain-Rea ML, Brauner R, Bouligand J, Junier MP, Di Rocco F, Sainte-Rose C, Beckers A, Roux FX, Daly AF, Chiovato L. Hyperplasia-adenoma sequence in pituitary tumorigenesis related to aryl hydrocarbon

- receptor interacting protein gene mutation. *Endocrine Related Cancer* 2011;18(3) 347-56.
- [79] Kasuki Jomori de Pinho L, Vieira Neto L, Armondi Wildemberg LE, Gasparetto EL, Marcondes J, de Almeida Nunes B, Takiya CM, Gadelha MR. Low aryl hydrocarbon receptor-interacting protein expression is a better marker of invasiveness in somatotropinomas than Ki-67 and p53. *Neuroendocrinology* 2011;94(1) 39-48.
- [80] Chahal HS, Trivellin G, Leontiou CA, Albani N, Fowkes RC, Tahir A, Igreja SC, Chapple JP, Jordan S, Lupp A, Schulz S, Ansorge O, Karavitaki N, Carlsen E, Wass JA, Grossman AB, Korbonits M. Somatostatin analogs modulate AIP in somatotroph adenomas: the role of the ZAC1 pathway. *The Journal of Clinical Endocrinology & Metabolism* 2012;97(8) E1411-20.
- [81] Kasuki L, Vieira Neto L, Wildemberg LE, Colli LM, de Castro M, Takiya CM, Gadelha MR. AIP expression in sporadic somatotropinomas is a predictor of the response to octreotide LAR therapy independent of SSTR2 expression. *Endocrine Related Cancer* 2012;19(3) L25-9.
- [82] Finelli P, Giardino D, Rizzi N, Buiatitot S, Virduci T, Franzin A, Losa M, Larizza L. Non-random trisomies of chromosomes 5, 8 and 12 in the prolactinoma sub-type of pituitary adenomas: conventional cytogenetics and interphase FISH study. *International Journal of Cancer* 2000;86(3) 344-50.
- [83] Larsen JB, Schröder HD, Sørensen AG, Bjerre P, Heim S. Simple numerical chromosome aberrations characterize pituitary adenomas. *Cancer Genetics and Cytogenetics* 1999;114(2) 144-9.
- [84] Kontogeorgos G, Kapranos N, Tzavara I, Thalassinou N, Rologis D. Monosomy of chromosome 11 in pituitary adenoma in a patient with familial multiple endocrine neoplasia type 1. *Clinical Endocrinology* 2001;54(1) 117-20.
- [85] Bates AS, Farrell WE, Bicknell EJ, McNicol AM, Talbot AJ, Broome JC, Perrett CW, Thakker RV, Clayton RN. Allelic deletion in pituitary adenomas reflects aggressive biological activity and has potential value as a prognostic marker. *The Journal of Clinical Endocrinology & Metabolism* 1997;82(3) 818-24.
- [86] Lania A, Spada A. G-protein and signalling in pituitary tumours. *Hormone Research* 2009;71 Suppl 2 95-100.
- [87] De Martino I, Fedele M, Palmieri D, Visone R, Cappabianca P, Wierinckx A, Trouillas J, Fusco A. B-Raf mutations are a rare event in pituitary adenomas. *Journal of Endocrinological Investigation* 2007;30(1) RC1-3.
- [88] Lin Y, Jiang X, Shen Y, Li M, Ma H, Xing M, Lu Y. Frequent mutations and amplifications of the PIK3CA gene in pituitary tumors. *Endocrine Related Cancer* 2009;16(1) 301-10.

- [89] Tanizaki Y, Jin L, Scheithauer BW, Kovacs K, Roncaroli F, Lloyd RV. P53 gene mutations in pituitary carcinomas. *Endocrine Pathology* 2007;18(4) 217-22.
- [90] Simpson DJ, Hibberts NA, McNicol AM, Clayton RN, Farrell WE. Loss of pRb expression in pituitary adenomas is associated with methylation of the RB1 CpG island. *Cancer Research* 2000;60(5) 1211-6.
- [91] Wierinckx A, Raverot G, Nazaret N, Jouanneau E, Auger C, Lachuer J, Trouillas J. Proliferation markers of human pituitary tumors: contribution of a genome-wide transcriptome approach. *Molecular and Cellular Endocrinology* 2010; 326(1-2) 30-9.
- [92] Tong Y, Zheng Y, Zhou J, Oyesiku NM, Koeffler HP, Melmed S. Genomic characterization of human and rat prolactinomas. *Endocrinology* 2012;153(8) 3679-91.
- [93] Xu M, Knox AJ, Michaelis KA, Kiseljick-Vassiliades K, Kleinschmidt-DeMasters BK, Lillehei KO, Wierman ME. Reprimo (RPRM) is a novel tumor suppressor in pituitary tumors and regulates survival, proliferation, and tumorigenicity. *Endocrinology* 2012;153(7) 2963-73.
- [94] Jenuwein T, Allis CD. Translating the histone code. *Science* 2001;293(5532) 1074-80.
- [95] Dudley KJ, Revill K, Clayton RN, Farrell WE. Pituitary tumours: all silent on the epigenetics front. *Journal of Molecular Endocrinology* 2009;42(6) 461-8.
- [96] Ezzat S. Epigenetic control in pituitary tumors. *Endocrine Journal* 2008;55(6) 951-7.
- [97] Duong CV, Emes RD, Wessely F, Yacub-Usman K, Clayton RN, Farrell WE. Quantitative, genome-wide analysis of the DNA methylome in sporadic pituitary adenomas. *Endocrine-Related Cancer* 2012;19(6) 805-16.
- [98] Yacub-Usman K, Richardson A, Duong CV, Clayton RN, Farrell WE. The pituitary tumour epigenome: aberrations and prospects for targeted therapy. *Nature Reviews Endocrinology* 2012;8(8) 486-94.
- [99] Wu X, Hua X. Menin, histone H3 methyltransferases, and regulation of cell proliferation: current knowledge and perspective. *Current Molecular Medicine* 2008; 8(8) 805-15.
- [100] Zhang Z, Florez S, Gutierrez-Hartmann A, Martin JF, Amendt BA. MicroRNAs regulate pituitary development, and microRNA 26b specifically targets lymphoid enhancer factor 1 (Lef-1), which modulates pituitary transcription factor 1 (Pit-1) expression. *The Journal of Biological Chemistry* 2010;285(45) 34718-28.
- [101] Yuen T, Ruf F, Chu T, Sealfon SC. Microtranscriptome regulation by gonadotropin-releasing hormone. *Molecular and Cellular Endocrinology* 2009;302(1) 12-7.
- [102] Bottoni A, Piccin D, Tagliati F, Luchin A, Zatelli MC, degli Uberti EC. miR-15a and miR-16-1 down-regulation in pituitary adenomas. *Journal of Cellular Physiology* 2005;204(1) 280-5.

- [103] Bottoni A, Zatelli MC, Ferracin M, Tagliati F, Piccin D, Vignali C, Calin GA, Negrini M, Croce CM, Degli Uberti EC. Identification of differentially expressed microRNAs by microarray: a possible role for microRNA genes in pituitary adenomas. *Journal of Cellular Physiology* 2007;210(2) 370-7.
- [104] Sivapragasam M, Rotondo F, Lloyd RV, Scheithauer BW, Cusimano M, Syro LV, Kovacs K. MicroRNAs in the human pituitary. *Endocrine Pathology* 2011;22(3) 134-43.
- [105] Trivellin G, Butz H, Delhove J, Igreja S, Chahal HS, Zivkovic V, McKay T, Patócs A, Grossman AB, Korbonits M. MicroRNA miR-107 is overexpressed in pituitary adenomas and inhibits the expression of aryl hydrocarbon receptor-interacting protein in vitro. *American Journal of Physiology – Endocrinology and Metabolism* 2012;303(6) E708-19.
- [106] Mao ZG, He DS, Zhou J, Yao B, Xiao WW, Chen CH, Zhu YH, Wang HJ. Differential expression of microRNAs in GH-secreting pituitary adenomas. *Diagnostic Pathology* 2010;5 79.
- [107] D'Angelo D, Palmieri D, Mussnich P, Roche M, Wierinckx A, Raverot G, Fedele M, Croce CM, Trouillas J, Fusco A. Altered microRNA expression profile in human pituitary GH adenomas: down-regulation of miRNA targeting HMGA1, HMGA2, and E2F1. *The Journal of Clinical Endocrinology & Metabolism* 2012;97(7) E1128-38.
- [108] Wang C, Su Z, Sanai N, Xue X, Lu L, Chen Y, Wu J, Zheng W, Zhuge Q, Wu ZB. microRNA expression profile and differentially-expressed genes in prolactinomas following bromocriptine treatment. *Oncology Reports* 2012;27(5) 1312-20.
- [109] Butz H, Likó I, Czirják S, Igaz P, Khan MM, Zivkovic V, Bálint K, Korbonits M, Rácz K, Patócs A. Down-regulation of Wee1 kinase by a specific subset of microRNA in human sporadic pituitary adenomas. *The Journal of Clinical Endocrinology & Metabolism* 2010;95(10) E181-91.
- [110] Stilling G, Sun Z, Zhang S, Jin L, Righi A, Kovács G, Korbonits M, Scheithauer BW, Kovacs K, Lloyd RV. MicroRNA expression in ACTH-producing pituitary tumors: up-regulation of microRNA-122 and -493 in pituitary carcinomas. *Endocrine* 2010;38(1) 67-75.
- [111] Palumbo T, Faucz FR, Azevedo M, Xekouki P, Iliopoulos D, Stratakis CA. Functional screen analysis reveals miR-26b and miR-128 as central regulators of pituitary somatotomotropic tumor growth through activation of the PTEN-AKT pathway. *Oncogene* 2012 [Epub ahead of print].
- [112] Palmieri D, D'Angelo D, Valentino T, De Martino I, Ferraro A, Wierinckx A, Fedele M, Trouillas J, Fusco A. Downregulation of HMGA-targeting microRNAs has a critical role in human pituitary tumorigenesis. *Oncogene* 2012;31(34) 3857-65.
- [113] Zhou Y, Zhang X, Klibanski A. MEG3 noncoding RNA: a tumor suppressor. *Journal of Molecular Endocrinology* 2012; 48(3) R45-53.

- [114] Cheunsuchon P, Zhou Y, Zhang X, Lee H, Chen W, Nakayama Y, Rice KA, Tessa Hedley-Whyte E, Swearingen B, Klibanski A. Silencing of the imprinted DLK1-MEG3 locus in human clinically nonfunctioning pituitary adenomas. *American Journal of Pathology* 2011;179(4) 2120-30.
- [115] Pertuit M, Barlier A, Enjalbert A, Gérard C. Signalling pathway alterations in pituitary adenomas: involvement of Gsalpha, cAMP and mitogen-activated protein kinases. *Journal of Neuroendocrinology* 2009;21(11) 869-77.
- [116] Lania AG, Mantovani G, Ferrero S, Pellegrini C, Bondioni S, Peverelli E, Braidotti P, Locatelli M, Zavanone ML, Ferrante E, Bosari S, Beck-Peccoz P, Spada A. Proliferation of transformed somatotroph cells related to low or absent expression of protein kinase a regulatory subunit 1A protein. *Cancer Research* 2004;64(24) 9193-8.
- [117] Peverelli E, Ermetici F, Filopanti M, Elli FM, Ronchi CL, Mantovani G, Ferrero S, Bosari S, Beck-Peccoz P, Lania A, Spada A. Analysis of genetic variants of phosphodiesterase 11A in acromegalic patients. *European Journal of Endocrinology* 2009;161(5) 687-94.
- [118] Asa SL, Scheithauer BW, Bilbao JM, Horvath E, Ryan N, Kovacs K, Randall RV, Laws ER Jr, Singer W, Linfoot JA, Thorner MO, Vale W. A case for hypothalamic acromegaly: a clinicopathological study of six patients with hypothalamic gangliocytomas producing growth hormone-releasing factor. *The Journal of Clinical Endocrinology & Metabolism* 1984;58 796-803.
- [119] Garby L, Caron P, Claustrat F, Chanson P, Tabarin A, Rohmer V, Arnault G, Bonnet F, Chabre O, Christin-Maitre S, du-Boullay H, Murat A, Nakib I, Sadoul JL, Sassolas G, Claustrat B, Raverot G, Borson-Chazot F; GTE Group. Clinical characteristics and outcome of acromegaly induced by ectopic secretion of growth hormone-releasing hormone (GHRH): a French nationwide series of 21 cases. *The Journal of Clinical Endocrinology & Metabolism* 2012;97(6) 2093-104.
- [120] Senovilla L, Núñez L, de Campos JM, de Luis DA, Romero E, Sánchez A, García-Sancho J, Villalobos C. Multifunctional cells in human pituitary adenomas: implications for paradoxical secretion and tumorigenesis. *The Journal of Clinical Endocrinology & Metabolism* 2004;89(9) 4545-52.
- [121] Molitch ME. Pharmacologic resistance in prolactinoma patients. *Pituitary* 2005;8(1) 43-52.
- [122] Peverelli E, Mantovani G, Vitali E, Elli FM, Olgiati L, Ferrero S, Laws ER, Della Mina P, Villa A, Beck-Peccoz P, Spada A, Lania AG. Filamin-A is essential for dopamine D2 receptor expression and signaling in tumorous lactotrophs. *The Journal of Clinical Endocrinology & Metabolism* 2012;97(3) 967-77.
- [123] Lania A, Mantovani G, Spada A. Genetic abnormalities of somatostatin receptors in pituitary tumors. *Molecular and Cellular Endocrinology* 2008;286(1-2) 180-6.

- [124] Durán-Prado M, Saveanu A, Luque RM, Gahete MD, Gracia-Navarro F, Jaquet P, Dufour H, Malagón MM, Culler MD, Barlier A, Castaño JP. A potential inhibitory role for the new truncated variant of somatostatin receptor 5, sst5TMD4, in pituitary adenomas poorly responsive to somatostatin analogs. *The Journal of Clinical Endocrinology & Metabolism* 2010;95(5) 2497-502.
- [125] Denef C. Paracrinicity: the story of 30 years of cellular pituitary crosstalk. *Journal of Neuroendocrinology* 2008;20(1) 1-70.
- [126] Perumal P, Vrontakis ME. Transgenic mice over-expressing galanin exhibit pituitary adenomas and increased secretion of galanin, prolactin and growth hormone. *Journal of Endocrinology* 2003;179(2) 145-54.
- [127] Shimon I, Hinton DR, Weiss MH, Melmed S. Prolactinomas express human heparin-binding secretory transforming gene (hst) protein product: marker of tumour invasiveness. *Clinical Endocrinology* 1998;48(1) 23-9.
- [128] Ezzat S, Zheng L, Zhu XF, Wu GE, Asa SL. Targeted expression of a human pituitary tumor-derived isoform of FGF receptor-4 recapitulates pituitary tumorigenesis. *The Journal of Clinical Investigation* 2002;109(1) 69-78.
- [129] Mukdsi JH, De Paul AL, Petiti JP, Gutiérrez S, Aoki A, Torres AI. Pattern of FGF-2 isoform expression correlated with its biological action in experimental prolactinomas. *Acta Neuropathologica* 2006;112(4) 491-501.
- [130] Zhu X, Lee K, Asa SL, Ezzat S. Epigenetic silencing through DNA and histone methylation of fibroblast growth factor receptor 2 in neoplastic pituitary cells. *American Journal of Pathology* 2007;170(5) 1618-28.
- [131] Haedo MR, Gerez J, Fuertes M, Giacomini D, Páez-Pereda M, Labeur M, Renner U, Stalla GK, Arzt E. Regulation of pituitary function by cytokines. *Hormone Research* 2009;72(5) 266-74.
- [132] Lebrun JJ. Activin, TGF-beta and menin in pituitary tumorigenesis. *Advances in Experimental Medicine and Biology* 2009;668 69-78.
- [133] Danila DC, Zhang X, Zhou Y, Haidar JN, Klibanski A. Overexpression of wild-type activin receptor alk4-1 restores activin antiproliferative effects in human pituitary tumor cells. *The Journal of Clinical Endocrinology & Metabolism* 2002;87(10) 4741-6.
- [134] Giacomini D, Acuña M, Gerez J, Nagashima AC, Silberstein S, Páez-Pereda M, Labeur M, Theodoropoulou M, Renner U, Stalla GK, Arzt E. Pituitary action of cytokines: focus on BMP-4 and gp130 family. *Neuroendocrinology* 2007;85(2) 94-100.
- [135] Barbieri F, Bajetto A, Stumm R, Pattarozzi A, Porcile C, Zona G, Dorcaratto A, Ravetti JL, Minuto F, Spaziante R, Schettini G, Ferone D, Florio T. Overexpression of stromal cell-derived factor 1 and its receptor CXCR4 induces autocrine/paracrine cell proliferation in human pituitary adenomas. *Clinical Cancer Research* 2008;14(16) 5022-32.

- [136] Cooper O, Vlotides G, Fukuoka H, Greene MI, Melmed S. Expression and function of ErbB receptors and ligands in the pituitary. *Endocrine Related Cancer* 2011;18(6) R197-211.
- [137] Theodoropoulou M, Arzberger T, Gruebler Y, Jaffrain-Rea ML, Schlegel J, Schaaf L, Petrangeli E, Losa M, Stalla GK, Pagotto U. Expression of epidermal growth factor receptor in neoplastic pituitary cells: evidence for a role in corticotropinoma cells. *Journal of Endocrinology* 2004;183(2) 385-94.
- [138] Fukuoka H, Cooper O, Ben-Shlomo A, Mamelak A, Ren SG, Bruyette D, Melmed S. EGFR as a therapeutic target for human, canine, and mouse ACTH-secreting pituitary adenomas. *The Journal of Clinical Endocrinology* 2011;121(12) 4712-21.
- [139] Vlotides G, Siegel E, Donangelo I, Gutman S, Ren SG, Melmed S. Rat prolactinoma cell growth regulation by epidermal growth factor receptor ligands. *Cancer Research* 2008;68(15) 6377-86.
- [140] Assimakopoulou M, Zolota V, Chondrogianni C, Gatzounis G, Varakis J. p75 and TrkC neurotrophin receptors demonstrate a different immunoreactivity profile in comparison to TrkA and TrkB receptors in human normal pituitary gland and adenomas. *Neuroendocrinology* 2008;88(2) 127-34.
- [141] Missale C. Nerve growth factor, D2 receptor isoforms, and pituitary tumors. *Endocrine* 2012;42(3) 466-7.
- [142] Lloyd RV, Vidal S, Horvath E, Kovacs K, Scheithauer B. Angiogenesis in normal and neoplastic pituitary tissues. *Microscopy Research and Technique* 2003;60(2) 244-50.
- [143] Sánchez-Ortiga R, Sánchez-Tejada L, Moreno-Perez O, Riesgo P, Niveiro M, Picó Alfonso AM. Over-expression of vascular endothelial growth factor in pituitary adenomas is associated with extrasellar growth and recurrence. *Pituitary* 2012 [Epub ahead of print].
- [144] Molitch ME. Prolactinoma in pregnancy. *Best Practice & Research Clinical Endocrinology & Metabolism* 2011;25(6) 885-96.
- [145] Jaffrain-Rea ML, Petrangeli E, Ortolani F, Fraioli B, Lise A, Esposito V, Spagnoli LG, Tamburrano G, Frati L, Gulino A. Cellular receptors for sex steroids in human pituitary adenomas. *Journal of Endocrinology* 1996; 151(2) 175-84.
- [146] Caronti B, Palladini G, Bevilacqua MG, Petrangeli E, Fraioli B, Cantore G, Tamburrano G, Carapella CM, Jaffrain-Rea ML. Effects of 17 beta-estradiol, progesterone and tamoxifen on in vitro proliferation of human pituitary adenomas: correlation with specific cellular receptors. *Tumour Biology* 1993;14(1) 59-68.
- [147] Zhou W, Song Y, Xu H, Zhou K, Zhang W, Chen J, Qin M, Yi H, Gustafsson JA, Yang H, Fan X. In nonfunctional pituitary adenomas, estrogen receptors and slug contribute to development of invasiveness. *The Journal of Clinical Endocrinology & Metabolism* 2011;96(8) E1237-45.

- [148] Fan X, Gabbi C, Kim HJ, Cheng G, Andersson LC, Warner M, Gustafsson JA. Gonadotropin-positive pituitary tumors accompanied by ovarian tumors in aging female ERbeta-/- mice. *Proceedings of the National Academy of Sciences of the United States of America* 2010;107(14) 6453-8.
- [149] Heaney AP, Fernando M, Melmed S. Functional role of estrogen in pituitary tumor pathogenesis. *The Journal of Clinical Investigation* 2002;109(2) 277-83.
- [150] Sarkar DK. Genesis of prolactinomas: studies using estrogen-treated animals. *Frontiers of Hormone Research* 2006;35 32-49.
- [151] Zaldivar V, Magri ML, Zárate S, Jaita G, Eijo G, Radl D, Ferraris J, Pisera D, Seilicovich A. Estradiol increases the expression of TNF- α and TNF receptor 1 in lactotropes. *Neuroendocrinology* 2011;93(2) 106-13.
- [152] Zárate S, Jaita G, Ferraris J, Eijo G, Magri ML, Pisera D, Seilicovich A. Estrogens induce expression of membrane-associated estrogen receptor α isoforms in lactotropes. *PLoS One* 2012;7(7) E41299.
- [153] Dworakowska D, Grossman AB. The molecular pathogenesis of corticotroph tumours. *European Journal of Clinical Investigation* 2012;42(6) 665-76.
- [154] Chauvet N, El-Yandouzi T, Mathieu MN, Schlernitzauer A, Galibert E, Lafont C, Le Tissier P, Robinson IC, Mollard P, Coutry N. Characterization of adherens junction protein expression and localization in pituitary cell networks. *Journal of Endocrinology* 2009;202(3) 375-87.
- [155] Qian ZR, Li CC, Yamasaki H, Mizusawa N, Yoshimoto K, Yamada S, Tashiro T, Horiguchi H, Wakatsuki S, Hirokawa M, Sano T. Role of E-cadherin, alpha-, beta-, and gamma-catenins, and p120 (cell adhesion molecules) in prolactinoma behavior. *Modern Pathology* 2002;15(12) 1357-65.
- [156] Elston MS, Gill AJ, Conaglen JV, Clarkson A, Cook RJ, Little NS, Robinson BG, Clifton-Bligh RJ, McDonald KL. Nuclear accumulation of e-cadherin correlates with loss of cytoplasmic membrane staining and invasion in pituitary adenomas. *The Journal of Clinical Endocrinology & Metabolism* 2009;94(4) 1436-42.
- [157] Fougner SL, Lekva T, Borota OC, Hald JK, Bollerslev J, Berg JP. The expression of E-cadherin in somatotroph pituitary adenomas is related to tumor size, invasiveness, and somatostatin analog response. *The Journal of Clinical Endocrinology & Metabolism* 2010;95(5) 2334-42.
- [158] Evang JA, Berg JP, Casar-Borota O, Lekva T, Kringen MK, Ramm-Pettersen J, Bollerslev J. Reduced levels of E-cadherin correlate with progression of corticotroph pituitary tumours. *Clinical Endocrinology* 2011;75(6) 811-8.
- [159] Trouillas J, Daniel L, Guigard MP, Tong S, Gouvernet J, Jouanneau E, Jan M, Perrin G, Fischer G, Tabarin A, Rougon G, Figarella-Branger D. Polysialylated neural cell

adhesion molecules expressed in human pituitary tumors and related to extrasellar invasion. *Journal of Neurosurgery* 2003; 98(5) 1084-93.

- [160] Quereda V, Malumbres M. Cell cycle control of pituitary development and disease. *Journal of Molecular Endocrinology* 2009;42(2) 75-86.
- [161] Hibberts NA, Simpson DJ, Bicknell JE, Broome JC, Hoban PR, Clayton RN, Farrell WE. Analysis of cyclin D1 (CCND1) allelic imbalance and overexpression in sporadic human pituitary tumors. *Clinical Cancer Research* 1999;5(8) 2133-9.
- [162] Roussel-Gervais A, Bilodeau S, Vallette S, Berthelet F, Lacroix A, Figarella-Branger D, Brue T, Drouin J. Cooperation between cyclin E and p27(Kip1) in pituitary tumorigenesis. *Molecular Endocrinology* 2010;24(9) 1835-45.
- [163] Simpson DJ, Frost SJ, Bicknell JE, Broome JC, McNicol AM, Clayton RN, Farrell WE. Aberrant expression of G(1)/S regulators is a frequent event in sporadic pituitary adenomas. *Carcinogenesis* 2001;22(8) 1149-54.
- [164] Kirsch M, Mörz M, Pinzer T, Schackert HK, Schackert G. Frequent loss of the CDKN2C (p18INK4c) gene product in pituitary adenomas. *Genes Chromosomes Cancer*. 2009;48(2) 143-54.
- [165] Foijer F, te Riele H. Check, double check: the G2 barrier to cancer. *Cell Cycle* 2006;5(8) 831-6.
- [166] Wierinckx A, Auger C, Devauchelle P, Reynaud A, Chevallier P, Jan M, Perrin G, Fèvre-Montange M, Rey C, Figarella-Branger D, Raverot G, Belin MF, Lachuer J, Trouillas J. A diagnostic marker set for invasion, proliferation, and aggressiveness of prolactin pituitary tumors. *Endocrine Related Cancer* 2007;14(3) 887-900.
- [167] De Martino I, Visone R, Wierinckx A, Palmieri D, Ferraro A, Cappabianca P, Chiappetta G, Forzati F, Lombardi G, Colao A, Trouillas J, Fedele M, Fusco A. HMGA proteins up-regulate CCNB2 gene in mouse and human pituitary adenomas. *Cancer Research* 2009;69(5) 1844-50.
- [168] Zhang X, Sun H, Danila DC, Johnson SR, Zhou Y, Swearingen B, Klibanski A. Loss of expression of GADD45 gamma, a growth inhibitory gene, in human pituitary adenomas: implications for tumorigenesis. *The Journal of Clinical Endocrinology & Metabolism* 2002;87(3) 1262-7.
- [169] Michaelis KA, Knox AJ, Xu M, Kiseljak-Vassiliades K, Edwards MG, Geraci M, Kleinschmidt-DeMasters BK, Lillehei KO, Wierman ME. Identification of growth arrest and DNA-damage-inducible gene beta (GADD45beta) as a novel tumor suppressor in pituitary gonadotrope tumors. *Endocrinology* 2011;152(10) 3603-13.
- [170] St Croix B, Sheehan C, Rak JW, Flørenes VA, Slingerland JM, Kerbel RS. E-Cadherin-dependent growth suppression is mediated by the cyclin-dependent kinase inhibitor p27(KIP1). *Journal of Cellular Biology* 1998;142(2) 557-71.

- [171] Hubina E, Nanzer AM, Hanson MR, Ciccarelli E, Losa M, Gaia D, Papotti M, Terreni MR, Khalaf S, Jordan S, Cziráj S, Hanzély Z, Nagy GM, Góth MI, Grossman AB, Korbonits M. Somatostatin analogues stimulate p27 expression and inhibit the MAP kinase pathway in pituitary tumours. *European Journal of Endocrinology* 2006;155(2) 371-9.
- [172] Abbass T, Dutta A. p21 in cancer: intricate networks and multiple activities. *Nature Review in Cancer* 2009; 9(6) 400-14.
- [173] Chesnokova V, Zonis S, Kovacs K, Ben-Shlomo A, Wawrowsky K, Bannykh S, Melmed S. p21(Cip1) restrains pituitary tumor growth. *Proceedings of the National Academy of Sciences of the United States of America* 2008;105(45) 17498-503.
- [174] Hiyama H, Kubo O, Kawamata T, Ishizaki R, Hori T. Expression of cyclin kinase inhibitor p21/WAF1 protein in pituitary adenomas: correlations with endocrine activity, but not cell proliferation. *Acta Neurochirurgica* 2002;144(5) 481-8.
- [175] Neto AG, McCutcheon IE, Vang R, Spencer ML, Zhang W, Fuller GN. Elevated expression of p21 (WAF1/Cip1) in hormonally active pituitary adenomas. *Annals of Diagnostic Pathology* 2005;9(1) 6-10.
- [176] Alexandraki KI, Munayem Khan M, Chahal HS, Dalantaeva NS, Trivellin G, Berney DM, Caron P, Popovic V, Pfeifer M, Jordan S, Korbonits M, Grossman AB. Onco-gene-induced senescence in pituitary adenomas and carcinomas. *Hormones* 2012;11(3) 297-307.
- [177] Vlotides G, Eigler T, Melmed S. Pituitary tumor-transforming gene: physiology and implications for tumorigenesis. *Endocrine Reviews* 2007;28(2) 165-86.
- [178] Tong Y, Eigler T. Transcriptional targets for pituitary tumor-transforming gene-1. *Journal of Molecular Endocrinology* 2009;43(5) 179-85.
- [179] Salehi F, Kovacs K, Scheithauer BW, Lloyd RV, Cusimano M. Pituitary tumor-transforming gene in endocrine and other neoplasms: a review and update. *Endocrine Related Cancer* 2008;15(3) 721-43.
- [180] Abbud RA, Takumi I, Barker EM, Ren SG, Chen DY, Wawrowsky K, Melmed S. Early multipotential pituitary focal hyperplasia in the alpha-subunit of glycoprotein hormone-driven pituitary tumor-transforming gene transgenic mice. *Molecular Endocrinology* 2005;19(5) 1383-91.
- [181] Cleynen I, Van de Ven WJ. The HMGA proteins: a myriad of functions (Review). *International Journal of Oncology* 2008;32(2) 289-305.
- [182] Fedele M, Fusco A. Role of the high mobility group A proteins in the regulation of pituitary cell cycle. *Journal of Molecular Endocrinology* 2010;44(6) 309-18.
- [183] Palmieri D, Valentino T, De Martino I, Esposito F, Cappabianca P, Wierinckx A, Vitiello M, Lombardi G, Colao A, Trouillas J, Pierantoni GM, Fusco A, Fedele M. PIT1

upregulation by HMGA proteins has a role in pituitary tumorigenesis. *Endocrine Related Cancer* 2012;19(2) 123-35.

- [184] Qian ZR, Asa SL, Siomi H, Siomi MC, Yoshimoto K, Yamada S, Wang EL, Rahman MM, Inoue H, Itakura M, Kudo E, Sano T. Overexpression of HMGA2 relates to reduction of the let-7 and its relationship to clinicopathological features in pituitary adenomas. *Modern Pathology* 2009;22(3) 431-41.
- [185] Wang EL, Qian ZR, Rahman MM, Yoshimoto K, Yamada S, Kudo E, Sano T. Increased expression of HMGA1 correlates with tumour invasiveness and proliferation in human pituitary adenomas. *Histopathology* 2010;56(4) 501-9.
- [186] May LT, Hill SJ. ERK phosphorylation: spatial and temporal regulation by G protein-coupled receptors. *The International Journal of Biochemistry & Cell Biology* 2008;40(10) 2013-7.
- [187] Pópulo H, Lopes JM, Soares P. The mTOR Signalling Pathway in Human Cancer. *International Journal of Molecular Sciences* 2012;13(2) 1886-918.
- [188] McCubrey JA, Steelman LS, Franklin RA, Abrams SL, Chappell WH, Wong EW, Lehmann BD, Terrian DM, Basecke J, Stivala F, Libra M, Evangelisti C, Martelli AM. Targeting the RAF/MEK/ERK, PI3K/AKT and p53 pathways in hematopoietic drug resistance. *Advances in Enzyme Regulation* 2007;47 64-103.
- [189] Suojun Z, Feng W, Dongsheng G, Ting L. Targeting Raf/MEK/ERK pathway in pituitary adenomas. *European Journal of Cancer* 2012;48(3) 389-95.
- [190] Cakir M, Grossman AB. Targeting MAPK (Ras/ERK) and PI3K/Akt pathways in pituitary tumorigenesis. *Expert Opinion on Therapeutic Targets* 2009;13(9) 1121-34.
- [191] Ewing I, Pedder-Smith S, Franchi G, Ruscica M, Emery M, Vax V, Garcia E, Czirják S, Hanzély Z, Kola B, Korbonits M, Grossman AB. A mutation and expression analysis of the oncogene BRAF in pituitary adenomas. *Clinical Endocrinology* 2007;66(3) 348-52.
- [192] Dworakowska D, Wlodek E, Leontiou CA, Igreja S, Cakir M, Teng M, Prodromou N, Góth MI, Grozinsky-Glasberg S, Gueorguiev M, Kola B, Korbonits M, Grossman AB. Activation of RAF/MEK/ERK and PI3K/AKT/mTOR pathways in pituitary adenomas and their effects on downstream effectors. *Endocrine Related Cancer* 2009;16(4) 1329-38.
- [193] Kovalovsky D, Refojo D, Liberman AC, Hochbaum D, Pereda MP, Coso OA, Stalla GK, Holsboer F, Arzt E. Activation and induction of NUR77/NURR1 in corticotrophs by CRH/cAMP: involvement of calcium, protein kinase A, and MAPK pathways. *Molecular Endocrinology* 2002;16(7) 1638-51.
- [194] Bliss SP, Navratil AM, Xie J, Roberson MS. GnRH signaling, the gonadotrope and endocrine control of fertility. *Frontiers in Neuroendocrinology* 2010;31(3) 322-40.

- [195] Pertuit M, Romano D, Zeiller C, Barlier A, Enjalbert A, Gerard C. The gsp oncogene disrupts Ras/ERK-dependent prolactin gene regulation in gsp inducible somatotroph cell line. *Endocrinology* 2011;152(4) 1234-43.
- [196] Musat M, Korbonits M, Kola B, Borboli N, Hanson MR, Nanzer AM, Grigson J, Jordan S, Morris DG, Gueorguiev M, Coculescu M, Basu S, Grossman AB. Enhanced protein kinase B/Akt signalling in pituitary tumours. *Endocrine Related Cancer* 2005;12(2) 423-33.
- [197] Lu C, Willingham MC, Furuya F, Cheng SY. Activation of phosphatidylinositol 3-kinase signaling promotes aberrant pituitary growth in a mouse model of thyroid-stimulating hormone-secreting pituitary tumors. *Endocrinology* 2008;149(7) 3339-45.
- [198] Theodoropoulou M, Stalla GK, Spengler D. ZAC1 target genes and pituitary tumorigenesis. *Molecular and Cellular Endocrinology* 2010;326(1-2) 60-5.
- [199] Pizarro CB, Oliveira MC, Pereira-Lima JF, Leães CG, Kramer CK, Schuch T, Barbosa-Coutinho LM, Ferreira NP. Evaluation of angiogenesis in 77 pituitary adenomas using endoglin as a marker. *Neuropathology* 2009;29(1) 40-4.
- [200] Recouvreux MV, Camilletti MA, Rifkin DB, Becu-Villalobos D, Díaz-Torga G. Thrombospondin-1 (TSP-1) analogs ABT-510 and ABT-898 inhibit prolactinoma growth and recover active pituitary transforming growth factor- β 1 (TGF- β 1). *Endocrinology* 2012;153(8) 3861-71.
- [201] Raica M, Coculescu M, Cimpean AM, Ribatti D. Endocrine gland derived-VEGF is down-regulated in human pituitary adenoma. *Anticancer Research* 2010;30(10) 3981-6.
- [202] Vidal S, Horvath E, Kovacs K, Kuroki T, Lloyd RV, Scheithauer BW. Expression of hypoxia-inducible factor-1alpha (HIF-1alpha) in pituitary tumours. *Histology and Histopathology* 2003;18(3) 679-86.
- [203] Yoshida D, Kim K, Noha M, Teramoto A. Anti-apoptotic action by hypoxia inducible factor 1-alpha in human pituitary adenoma cell line, HP-75 in hypoxic condition. *Journal of Neuro-oncology* 2006;78(3) 217-25.
- [204] Shan B, Gerez J, Haedo M, Fuertes M, Theodoropoulou M, Buchfelder M, Losa M, Stalla GK, Arzt E, Renner U. RSUME is implicated in HIF-1-induced VEGF-A production in pituitary tumour cells. *Endocrine Related Cancer* 2012;19(1) 13-27.
- [205] Jin Kim Y, Hyun Kim C, Hwan Cheong J, Min Kim J. Relationship between expression of vascular endothelial growth factor and intratumoral hemorrhage in human pituitary adenomas. *Tumori* 2011;97(5) 639-46.
- [206] Xiao Z, Liu Q, Mao F, Wu J, Lei T. TNF- α -induced VEGF and MMP-9 expression promotes hemorrhagic transformation in pituitary adenomas. *International Journal of Molecular Sciences* 2011;12(6) 4165-79.

- [207] Xiao Z, Liu Q, Zhao B, Wu J, Lei T. Hypoxia induces hemorrhagic transformation in pituitary adenomas via the HIF-1 α signaling pathway. *Oncology Reports* 2011;26(6) 1457-64.
- [208] Rotondo F, Sharma S, Scheithauer BW, Horvath E, Syro LV, Cusimano M, Nassiri F, Yousef GM, Kovacs K. Endoglin and CD-34 immunoreactivity in the assessment of microvessel density in normal pituitary and adenoma subtypes. *Neoplasma* 2010;57(6) 590-3.
- [209] Miyajima K, Takekoshi S, Itoh J, Kakimoto K, Miyakoshi T, Osamura RY. Inhibitory effects of anti-VEGF antibody on the growth and angiogenesis of estrogen-induced pituitary prolactinoma in Fischer 344 Rats: animal model of VEGF-targeted therapy for human endocrine tumors. *Acta Histochemica Cytochemica* 2010;43(2) 33-44.
- [210] Luque GM, Perez-Millán MI, Ornstein AM, Cristina C, Becu-Villalobos D. Inhibitory effects of antivascular endothelial growth factor strategies in experimental dopamine-resistant prolactinomas. *Journal of Pharmacology and Experimental Therapeutics* 2011;337(3) 766-74.
- [211] Ortiz LD, Syro LV, Scheithauer BW, Ersen A, Uribe H, Fadul CE, Rotondo F, Horvath E, Kovacs K. Anti-VEGF therapy in pituitary carcinoma. *Pituitary*. 2012;15(3) 445-9.
- [212] Saraga-Babic M, Bazina M, Vukojevic K, Bocina I, Stefanovic V. Involvement of pro-apoptotic and anti-apoptotic factors in the early development of the human pituitary gland. *Histology and Histopathology* 2008;23(10) 1259-68.
- [213] Kulig E, Jin L, Qian X, Horvath E, Kovacs K, Stefaneanu L, Scheithauer BW, Lloyd RV. Apoptosis in nontumorous and neoplastic human pituitaries: expression of the Bcl-2 family of proteins. *American Journal of Pathology* 1999;154(3) 767-74.
- [214] Kapranos N, Kontogeorgos G, Horvath E, Kovacs K. Morphology, molecular regulation and significance of apoptosis in pituitary adenomas. *Frontiers of Hormone Research* 2004;32 217-34.
- [215] Sambaziotis D, Kapranos N, Kontogeorgos G. Correlation of bcl-2 and bax with apoptosis in human pituitary adenomas. *Pituitary* 2003;6(3) 127-33.
- [216] Jaita G, Zárate S, Ferrari L, Radl D, Ferraris J, Eijo G, Zaldivar V, Pisera D, Seilicovich A. Gonadal steroids modulate Fas-induced apoptosis of lactotropes and somatotropes. *Endocrine* 2011;39(1) 21-7.
- [217] Chen L, Zhuang G, Li W, Liu Y, Zhang J, Tian X. RGD-FasL induces apoptosis of pituitary adenoma cells. *Cellular & Molecular Immunology* 2008;5(1) 61-8.
- [218] Candolfi M, Jaita G, Pisera D, Ferrari L, Barcia C, Liu C, Yu J, Liu G, Castro MG, Seilicovich A. Adenoviral vectors encoding tumor necrosis factor-alpha and FasL induce apoptosis of normal and tumoral anterior pituitary cells. *Journal of Endocrinology* 2006;189(3) 681-90.

- [219] Cañibano C, Rodriguez NL, Saez C, Tovar S, Garcia-Lavandeira M, Borrello MG, Vidal A, Costantini F, Japon M, Dieguez C, Alvarez CV. The dependence receptor Ret induces apoptosis in somatotrophs through a Pit-1/p53 pathway, preventing tumor growth. *EMBO Journal* 2007;26(8) 2015-28.
- [220] Diaz-Rodriguez E, García-Lavandeira M, Perez-Romero S, Senra A, Cañibano C, Palmero I, Borrello MG, Dieguez C, Alvarez CV. Direct promoter induction of p19Arf by Pit-1 explains the dependence receptor RET/Pit-1/p53-induced apoptosis in the pituitary somatotroph cells. *Oncogene* 2012;31(23) 2824-35.
- [221] Japón MA, Urbano AG, Sáez C, Segura DI, Cerro AL, Diéguez C, Alvarez CV. Glial-derived neurotrophic factor and RET gene expression in normal human anterior pituitary cell types and in pituitary tumors. *The Journal of Clinical Endocrinology & Metabolism* 2002;87(4) 1879-84.
- [222] Kang BH, Xia F, Pop R, Dohi T, Socolovsky M, Altieri DC. Developmental control of apoptosis by the immunophilin aryl hydrocarbon receptor-interacting protein(AIP) involves mitochondrial import of the survivin protein. *The Journal of Biological Chemistry* 2011;286(19) 16758-67.
- [223] Vargiolu M, Fusco D, Kurelac I, Dirnberger D, Baumeister R, Morra I, Melcarne A, Rimondini R, Romeo G, Bonora E. The tyrosine kinase receptor RET interacts in vivo with aryl hydrocarbon receptor-interacting protein to alter survivin availability. *The Journal of Clinical Endocrinology & Metabolism* 2009;94(7) 2571-8.
- [224] Wasko R, Waligorska-Stachura J, Jankowska A, Warchol JB, Liebert W, Sowinski J. Coexpression of survivin and PCNA in pituitary tumors and normal pituitary. *Neuro Endocrinology Letters* 2009;30(4) 477-81.
- [225] Formosa R, Gruppette M, Falzon S, Santillo G, DeGaetano J, Xuereb-Anastasi A, Vassallo J. Expression and clinical significance of Wnt players and survivin in pituitary tumours. *Endocrine Pathology* 2012;23(2) 123-31.
- [226] Tagliati F, Gentilin E, Buratto M, Molè D, degli Uberti EC, Zatelli MC. Magmas, a gene newly identified as overexpressed in human and mouse ACTH-secreting pituitary adenomas, protects pituitary cells from apoptotic stimuli. *Endocrinology* 2010;151(10) 4635-42.
- [227] Bahar A, Simpson DJ, Cutty SJ, Bicknell JE, Hoban PR, Holley S, Mourtada-Maaraouni M, Williams GT, Clayton RN, Farrell WE. Isolation and characterization of a novel pituitary tumor apoptosis gene. *Molecular Endocrinology* 2004;18(7) 1827-39.
- [228] Acunzo J, Roche C, Defilles C, Thirion S, Quentien MH, Figarella-Branger D, Graillon T, Dufour H, Brue T, Pellegrini I, Enjalbert A, Barlier A. Inactivation of PITX2 transcription factor induced apoptosis of gonadotroph tumoral cells. *Endocrinology* 2011;152(10) 3884-92.

- [229] Radl DB, Ferraris J, Boti V, Seilicovich A, Sarkar DK, Pisera D. Dopamine-induced apoptosis of lactotropes is mediated by the short isoform of D2 receptor. *PLoS One* 2011;6(3) e18097.
- [230] Grozinsky-Glasberg S, Shimon I, Korbonits M, Grossman AB. Somatostatin analogues in the control of neuroendocrine tumours: efficacy and mechanisms. *Endocrine Related Cancer* 2008;15(3) 701-20.
- [231] 223. Chesnokova V, Melmed S. Pituitary senescence: the evolving role of Pttg. *Molecular and Cellular Endocrinology* 2010;326(1-2) 55-9.
- [232] Chesnokova V, Zonis S, Zhou C, Ben-Shlomo A, Wawrowsky K, Toledano Y, Tong Y, Kovacs K, Scheithauer B, Melmed S. Lineage-specific restraint of pituitary gonadotroph cell adenoma growth. *PLoS One* 2011;6(3) e17924.
- [233] Ragel BT, Couldwell WT. Pituitary carcinoma: a review of the literature. *Neurosurgical Focus* 2004;16(4) E7.
- [234] Kaltsas GA, Nomikos P, Kontogeorgos G, Buchfelder M, Grossman AB. Clinical review: Diagnosis and management of pituitary carcinomas. *The Journal of Clinical Endocrinology & Metabolism* 2005;90(5) 3089-99.
- [235] Colao A, Ochoa AS, Auriemma RS, Faggiano A, Pivonello R, Lombardi G. Pituitary carcinomas. *Frontiers of Hormone Research* 2010;38 94-108.
- [236] van der Klaauw AA, Kienitz T, Strasburger CJ, Smit JW, Romijn JA. Malignant pituitary corticotroph adenomas: report of two cases and a comprehensive review of the literature. *Pituitary* 2009;12(1) 57-69.
- [237] Ruebel KH, Leontovich AA, Jin L, Stilling GA, Zhang H, Qian X, Nakamura N, Scheithauer BW, Kovacs K, Lloyd RV. Patterns of gene expression in pituitary carcinomas and adenomas analyzed by high-density oligonucleotide arrays, reverse transcriptase-quantitative PCR, and protein expression. *Endocrine* 2006;29(3) 435-44.
- [238] Sav A, Rotondo F, Syro LV, Scheithauer BW, Kovacs K. Biomarkers of pituitary neoplasms. *Anticancer Research* 2012;32(11) 4639-54.
- [239] Syro LV, Ortiz LD, Scheithauer BW, Lloyd R, Lau Q, Gonzalez R, Uribe H, Cusimano M, Kovacs K, Horvath E. Treatment of pituitary neoplasms with temozolomide: a review. *Cancer* 2011;117(3) 454-62.
- [240] Raverot G, Sturm N, de Fraipont F, Muller M, Salenave S, Caron P, Chabre O, Chanson P, Cortet-Rudelli C, Assaker R, Dufour H, Gaillard S, François P, Jouanneau E, Passagia JG, Bernier M, Cornélius A, Figarella-Branger D, Trouillas J, Borson-Chazot F, Brue T. Temozolomide treatment in aggressive pituitary tumors and pituitary carcinomas: a French multicenter experience. *The Journal of Clinical Endocrinology & Metabolism* 2010;95(10) 4592-9.

- [241] Raverot G, Castinetti F, Jouanneau E, Morange I, Figarella-Branger D, Dufour H, Trouillas J, Brue T. Pituitary carcinomas and aggressive pituitary tumours: merits and pitfalls of temozolomide treatment. *Clinical Endocrinology* 2012;76(6) 769-75.
- [242] Salehi F, Scheithauer BW, Kros JM, Lau Q, Fealey M, Erickson D, Kovacs K, Horvath E, Lloyd RV. MGMT promoter methylation and immunoexpression in aggressive pituitary adenomas and carcinomas. *Journal of Neuro-oncology* 2011;104(3) 647-57.