REVIEW

Biomarkers of aggressive pituitary adenomas

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Abstract

Pituitary adenomas exhibit a wide range of behaviors. The prediction of aggressive or malignant behavior in pituitary adenomas remains challenging; however, the utility of biomarkers is rapidly evolving. In this review, we discuss potential biomarkers as they relate to aggressive behavior in pituitary adenomas. While detailed histological subtyping remains the best independent predictor of aggressive behavior in the majority of cases, evidence suggests that the additional analyses of FGFR4, MMP, PTTG, Ki-67, p53, and deletions in chromosome 11 may contribute to decisions concerning management of aggressive pituitary adenomas.

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Introduction

With modern imaging methods and hormone assays, the diagnosis of pituitary adenomas has increased. These lesions that were known to be common in autopsy studies are thought to occur in almost 20% of the general population (Ezzat *et al.* 2004a,b); the increase is not just attributed to better detection of microadenomas but also to macroadenomas (Daly *et al.* 2006, Fernandez *et al.* 2010).

These tumors exhibit a wide range of clinical behaviors; some are small and hormonally inactive, others secrete hormones in excess, causing significant morbidity, and some grow rapidly, either by expansion or by infiltration of adjacent tissues (Asa 2011). The terminology ‘aggressive’ has been largely used synonymously with ‘invasive’ when evaluating pituitary adenomas. Sometimes, this terminology has been used to define a high risk of recurrence or lack of therapeutic response. Invasive pituitary adenomas that exhibit relatively higher mitotic activity, a MIB-1 labeling index (LI) > 3%, or extensive p53 immunoreactivity are classified as ‘atypical adenomas’ by the World Health Organization (WHO) (DeLellis *et al.* 2004). While the WHO 2004 classification has promoted this terminology, in our experience, it does not have any biological superiority to the aggressive histological subtypes as determined by the accurate classification

of pituitary adenomas (Mete & Asa 2012). Some authors classify pituitary adenomas into three pathological groups based on radiological findings: non-invasive, invasive, and aggressive–invasive adenomas (Wierinckx *et al.* 2007); in their series, aggressive–invasive adenomas exhibited a Ki-67 index > 1%, mitotic activity more than two per ten high power fields, and p53 expression (Wierinckx *et al.* 2007).

Detailed and comprehensive morphological studies have identified histological subtypes of pituitary adenomas that are much more complex than simple correlation with clinical behavior (Asa 2011, Mete & Asa 2012). This classification remains the best independent predictor of aggressive behavior in the majority of pituitary adenomas (Al-Shraim & Asa 2006). Pituitary adenomas that are associated with aggressive behavior include sparsely granulated somatotroph adenomas, densely granulated lactotroph adenomas, acidophil stem cell adenomas, thyrotroph adenomas, sparsely granulated corticotroph adenomas, Crooke cell adenomas, silent subtype 3 adenomas, and null cell adenomas (Asa & Ezzat 2009, Asa 2011, Mete & Asa 2012). These aggressive adenomas are usually invasive macroadenomas radiographically. Some adenomas, such as silent subtype 3 adenomas and acidophil stem cell adenomas, reveal characteristic preferential downward invasive growth with significant bone invasion and parasellar extension rather than

suprasellar expansion (Asa 2011, Mete & Asa 2012), and complete curative resection cannot be achieved in the majority of these cases due to their invasive nature. Importantly, other less aggressive adenomas can sometimes be invasive; for example, sparsely granulated lactotroph adenomas in men often present as large macroadenomas that exhibit sinonasal invasion (Asa & Ezzat 2009, Asa 2011).

The therapy of aggressive pituitary adenomas is challenging. When surgery and medical therapy fail, radiotherapy becomes the treatment of choice. Conventional chemotherapy is largely ineffective but recent case reports using temozolomide have provided early encouraging results (Colao *et al.* 2011, Mete & Asa 2012).

Biomarkers of aggressive behavior in pituitary adenomas

Several biological markers have been investigated in pituitary tumors. They include chromosomal alterations and microRNAs (miRNAs), proliferation markers, oncogenes, tumor suppressor genes, growth factors and their receptors, and factors related to angiogenesis or cell adhesion. No single biomarker has been found to independently predict aggressive behavior in pituitary neoplasms (Thapar *et al.* 1996b, Zhao *et al.* 1999, McCabe *et al.* 2002, Ezzat *et al.* 2004a,b, Asa *et al.* 2007, Gong *et al.* 2008, Wang *et al.* 2008, Asa & Ezzat 2009, Salehi *et al.* 2009, 2010, Asa 2011, Sivapragasa *et al.* 2011, Wierinckx *et al.* 2011, Cornelius *et al.* 2012). In this review, we focus on biomarkers that appear to correlate with aggressive behavior in pituitary adenomas.

Fibroblast growth factor receptor 4

Fibroblast growth factors (FGFs) and their receptors (FGFRs) are a family of ligands and receptors that regulate development, growth, differentiation, migration, and angiogenesis (Ezzat *et al.* 2004a,b). Basic FGF (bFGF; FGF2) was originally described in bovine pituitary folliculostellate cells that regulate pituitary hormone secretion (Ferrara *et al.* 1987, Gospodarowicz *et al.* 1987). Deletion of FGF10 or its receptor, the FGFR2-IIIb isoform, results in failure of primordial pituitary development (De Moerloose *et al.* 2000). While varying levels of FGF mRNA expression have been documented in pituitary adenomas, the highest FGF mRNA and blood levels are associated with the most aggressive pituitary tumors (Ezzat *et al.* 1995a,b).

The 23 FGF ligands signal through four transmembrane tyrosine kinase receptors encoded by independent genes that each generates multiple isoforms. Each prototypic FGFR contains three Ig-like

extracellular domains, a single transmembrane domain, a split tyrosine kinase cytoplasmic domain, and a COOH-terminal tail that typically contains tyrosines that are phosphorylated upon ligand binding and recruit intracellular signaling proteins (Givol & Yayon 1992, Abbass *et al.* 1997, Qian *et al.* 2004). While some FGFs can signal through multiple receptors, the majority have specific affinity for selected receptor isoforms.

FGFRs are expressed in a variety of neoplasms including breast carcinomas, ovarian carcinomas, gastric carcinomas, colorectal carcinomas, prostate carcinomas, pancreatic carcinomas, melanomas, and glial neoplasms (Jaakkola *et al.* 1993, Morrison *et al.* 1994, Ohta *et al.* 1995, Ahmed *et al.* 1997, Yoshimura *et al.* 1998, Giri *et al.* 1999, Shin *et al.* 2000, Yamada *et al.* 2002, Henriksson *et al.* 2011). A single nucleotide polymorphism (SNP) in FGFR4, in which arginine is substituted for glycine at codon 388 in the transmembrane domain (FGFR4-G388R), occurs in up to 50% of the population; the arginine allele has been associated with advanced and treatment-resistant breast carcinoma, colorectal carcinoma, prostate carcinoma, sarcomas, and head and neck carcinomas (Bange *et al.* 2002, Morimoto *et al.* 2003, da Costa Andrade *et al.* 2007, Frullanti *et al.* 2011). The FGFR4-R388 allele has also recently been linked with mitochondrial STAT3 serine phosphorylation that facilitates pituitary growth hormone (GH) cell tumorigenesis (Tateno *et al.* 2011).

Pituitary adenomas have altered FGFR subtype and isoform expression (Abbas *et al.* 1997). The normal human pituitary expresses mRNAs for FGFR1, 2, and 3, including both Ig-like extracellular domains as well as transmembrane and kinase domains; by contrast, transcripts of only the first Ig-like domain of FGFR4 were found in the normal gland (Abbas *et al.* 1997). Pituitary adenomas show two major alterations: loss of FGFR2, with resultant upregulation of MAGEA3 (Zhu *et al.* 2008), and an N-terminally truncated cytoplasmic isoform of FGFR4, known as pituitary tumor derived (ptd-FGFR4) (Ezzat *et al.* 2002, Qian *et al.* 2004).

Whereas WT FGFR4 (FGFR4-G388) is a 110 kDa membrane-anchored protein that maintains affinity for the extracellular matrix of normal adenohypophyseal cells, ptd-FGFR4 is a 65 kDa cytoplasmic protein that is constitutively activated by phosphorylation on tyrosine residues. Intact FGFR4 is known to interact with neural cell adhesion molecule (NCAM) and N-cadherin (Cavallaro *et al.* 2001). The altered expression of FGFR4, thought to be due to Ikaros-induced silencing of the 5'-*FGFR4* promoter (Yu *et al.* 2002, Ezzat *et al.* 2003) and unmasking of a cryptic intronic promoter (Yu *et al.* 2003), interferes with the triprotein complex, displacing N-cadherin into the cytoplasm (Ezzat *et al.* 2004a,b). Expression of ptd-FGFR4 induces invasive growth of pituitary tumor cells *in vivo* with marked loss of membranous N-cadherin expression

Figure 1 The integrity of FGFR4/NCAM/N-cadherin/β-catenin complexes is necessary to maintain normal neuroendocrine cell phenotype and interactions with the extracellular matrix. Intact NCAM association with FGFR4 maintains N-cadherin at the cell membrane that links β-catenin to the cell surface. Altered protein interactions induced by cytoplasmic ptd-FGFR4 and/or the polysialylated form of NCAM (PSA-NCAM) result in disruption of N-cadherin residence at the cell membrane and loss of β-catenin-mediated cytoskeletal integrity. Expression of ptd-FGFR4 induces invasive growth of pituitary tumor cells *in vivo* with marked loss of membranous N-cadherin expression, and the PSA-NCAM also correlates with pituitary tumor growth and invasiveness (Adapted from Asa SL & Ezzat S 2009 The pathogenesis of pituitary tumors. *Annual Review of Pathology* 4 97–126.).

(Ezzat *et al.* 2004a,b, Morita *et al.* 2008; Figs 1 and 2). The polysialylated form of NCAM (PSA-NCAM) also correlates with pituitary tumor growth and invasiveness (Daniel *et al.* 2000). Evidence suggests that the signaling properties of N-cadherin, with particular emphasis on its cross talk with cell surface partners such as FGFR4 and NCAM, are important in pituitary tumorigenesis (Ezzat *et al.* 2004a,b, 2006, Asa & Ezzat 2009). Intact NCAM association with FGFR4 maintains N-cadherin at the cell membrane that links β-catenin to the cell surface (Fig. 1). The integrity of FGFR4/NCAM/N-cadherin/β-catenin complexes is necessary to maintain normal neuroendocrine cell phenotype and interactions with the extracellular matrix (Ezzat *et al.* 2004a,b, 2006, Asa & Ezzat 2009). Altered protein interactions induced by cytoplasmic ptd-FGFR4 and/or PSA-NCAM (Fig. 1) result in disruption of N-cadherin residence at the cell membrane and loss of β-catenin-mediated cytoskeletal integrity (Ezzat *et al.* 2006, Asa & Ezzat 2009). Reduced β-catenin expression is a recognized feature of invasive pituitary adenomas (Asa & Ezzat 2005). Furthermore, pharmacological inhibition of WT FGFR4 results in N-cadherin displacement and impaired cell-matrix adhesiveness, whereas pharmacological inhibition of ptd-FGFR4 can restore acinar architecture (Ezzat *et al.* 2006).

Matrix metalloproteinases

Matrix metalloproteinases (MMPs) are a family of single-chain zinc-containing proteolytic enzymes that

regulate the extracellular matrix in both physiological and pathological conditions including neoplasia. Eight different classes that encompass at least 24 functional types of MMPs have been described (Arakaki *et al.* 2009). They cleave extracellular matrix molecules including collagens, laminin, fibronectin, vitronectin, and proteoglycans. While the expression of MMP1, MMP2, MMP7, and MMP14 is regulated by transcription factors such as SMAD interacting protein-1 (SIP1) and Snail, various polymorphisms in *MMP* promoters and oncogenic signal transduction can induce transcription of MMPs (Miyoshi *et al.* 2004, Arakaki *et al.* 2009, Ota *et al.* 2009, González-Arriaga *et al.* 2012). Invasive tumor cells secrete MMPs, and mesenchymal cells, especially fibroblasts, represent an important source of these enzymes in the tumor microenvironment.

MMP1 is one of the most common interstitial collagenases and degrades mainly type I collagen (Arakaki *et al.* 2009). MMP1 expression has been linked to a poor prognosis in several cancers including oral carcinoma (Nishizawa *et al.* 2007, Shimizu *et al.* 2008), nasopharyngeal carcinoma (Nasr *et al.* 2007), and colorectal cancer (Woo *et al.* 2007). The *MMP1* gene is located on chromosome 11q22 (Arakaki *et al.* 2009). Both the proximal and distal regions of the *MMP1* promoter recognize the Jun/Fos dimer accompanied by the polyomavirus enhancer A binding protein 3 (PEA3), which in turn binds members of the electron transport system (ETS) transcription factor family (Waslyk *et al.* 1991, Sharrocks *et al.* 1997). Higher levels of ETS transcription factors induce MMP1 expression, thereby increasing degradation of the extracellular matrix to promote motility or invasion of tumor cells (Buttice *et al.* 1996, Westermarck *et al.* 1997). A SNP in the *MMP1* gene promoter, which inserts a guanine (G) at position 1607 (Arakaki *et al.* 2009), gives rise to a new ETS recognition site (5'-GGA-3')

Figure 2 A gonadotroph adenoma that shows extensive sinonasal mucosal invasion (A) exhibits strong cytoplasmic positivity for FGFR4, consistent with ptd-FGFR4 (B).

(Rutter *et al.* 1998). Altaş *et al.* (2010) investigated the impact of this polymorphism in pituitary adenomas and demonstrated that 90% of invasive pituitary adenomas occur in patients who are homozygous for this *MMP1* SNP.

MMP9 is located on chromosome 20q12-13 and degrades collagens (types IV, V, and X), elastin, gelatin, fibronectin, and proteoglycan-link protein (Chakraborti *et al.* 2003). MMP9 is activated by several factors including other MMPs such as MMP2, MMP3, and MMP13 (Chakraborti *et al.* 2003). Its secretion appears at an early stage of tumor cell migration (Paez-Pereda *et al.* 2005). Both MMP2 and MMP9 are widely expressed by endothelial cells and stromal cells. Their expression has been investigated in several malignancies including breast and lung cancers. MMP9 expression is significantly higher in invasive pituitary adenomas (Kawamoto *et al.* 1996, Liu *et al.* 2005, Hussaini *et al.* 2007, Gong *et al.* 2008) and MMP2 is also elevated (Liu *et al.* 2005). Moreover, there may be a correlation between activation of protein kinase C (PKC) and increased levels of MMP9, which can be antagonized by PKC inhibitors (Hussaini *et al.* 2007).

The *FGFR4* SNP has also been linked to MMP expression (Sugiyama *et al.* 2010). MT1-MMP (MMP14) is known to regulate tissue remodeling and cell invasiveness and serves as an activator for secreted MMPs, especially MMP2 and MMP13 (Page-McCaw *et al.* 2007, Rowe & Weiss 2009). While *FGFR4*-G388 downregulates MT1-MMP, the *FGFR4*-R388 variant induces MT1-MMP activity and collagen invasion. Although the role of *FGFR4*-R388 with respect to MMP14 has not been largely investigated in invasive pituitary adenomas, the association of increased levels of MMP9 and MMP2 in invasive adenomas may suggest a potential link to the *FGFR4* polymorphism. Further investigations are warranted to clarify the interactions between these biomarkers.

GH receptor mutations

Hypothalamic adenohypophysiostrophic hormones regulate their target cells through their receptors in complex feedback mechanisms (Asa 1991). In somatotrophs, GHRH is known to stimulate GH secretion and somatotroph proliferation through its receptor that binds the G_{α} to increase cAMP levels. Densely granulated somatotroph adenomas have high cAMP levels, often due to somatic activating *gsp* mutations in the gene encoding G_{α} . These tumors are usually responsive to somatostatin analogs that typically activate inhibitory G proteins, reducing cAMP levels (Bhayana *et al.* 2005, Asa & Ezzat 2009, Asa 2011, Mete & Asa 2012). However, the potential role of GH in auto-regulation was not appreciated until it was shown that mice with GH receptor (GHR) disruption develop

somatotroph hyperplasia (Asa *et al.* 2000). Germline mutations in exon 4 of the GHR result in Laron-type dwarfism with GH resistance (Putzolu *et al.* 1997, Chen *et al.* 2003, Shevah *et al.* 2004). Sparsely granulated adenomas, which are by definition aggressive GH-producing adenomas, are thought to lack the high levels of cAMP that predict a response to somatostatin analogs (Ezzat *et al.* 1995a,b, Bhayana *et al.* 2005); instead, they have altered STAT signaling that, in some cases, has been attributed to a somatic GHR mutation resulting in a histidine-to-leucine substitution in the extracellular domain in exon 4, codon 49. This mutation impairs glycosylation-mediated receptor processing and signaling (Asa *et al.* 2007, Mete & Asa 2012). Altered GHR signaling is also associated with a morphological change resulting in the formation of paranuclear keratin aggregates (Fig. 3), also known as ‘fibrous bodies’ (Asa *et al.* 2007). This marker of altered GH autoregulation allows pathologists to distinguish sparsely granulated somatotroph adenomas from their densely granulated counterparts using immunohistochemistry for low-molecular-weight keratin (CAM5.2) (Fig. 4). The discrimination of this entity is not only of prognostic interest, as sparsely granulated somatotroph adenomas are known to be more aggressive than their densely granulated counterparts, but also has major implications for treatment decisions, as Pegvisomant, a GHR antagonist, is used in patients with sparsely

Figure 3 Sparsely granulated adenomas, which are by definition aggressive GH-producing adenomas, are thought to lack the high levels of cAMP that predict a response to somatostatin analogs; instead, they have altered STAT signaling that in some cases has been attributed to a somatic GHR mutation resulting in a histidine-to-leucine substitution in the extracellular domain in exon 4, codon 49. Altered GHR signaling is associated with a morphological change resulting in the formation of paranuclear keratin aggregates, also known as ‘fibrous bodies’ (Adapted from Asa SL & Ezzat S 2009 The pathogenesis of pituitary tumors. *Annual Review of Pathology* 4 97–126.).

Figure 4 Low-molecular-weight keratin (CAM5.2) immunohistochemistry serves as a diagnostic and prognostic tool for pathologists to distinguish densely granulated somatotroph adenomas (A) from sparsely granulated somatotroph adenomas that contain fibrous bodies (B).

granulated somatotroph adenomas that do not respond to somatostatin analogs (Bhayana *et al.* 2005, Asa *et al.* 2007, Asa 2011, Mete & Asa 2012).

Loss of chromosome 11p and/or 11q

Genomic imbalance has been reported to occur frequently in pituitary tumors (Weil *et al.* 1998, Pack *et al.* 2000, 2005). Allelic deletions at 11q13, 13q12-14, 10q, and 1p have been reported in invasive pituitary adenomas (Bates *et al.* 1997). Loss of the 11p region has been described in several familial and sporadic neoplasms including those arising from breast, ovary, thyroid, and bladder (Kiechle-Schwarz *et al.* 1993, Voorter *et al.* 1996, Kitamura *et al.* 2000). Some of these studies also highlighted that loss of 11p correlates with tumor progression and metastasis. In addition to a previously reported panel of seven genes (*CRMP1*, *ADAMTS6*, *PTTG1*, *CCNB1*, *AURKB*, *ASK* (*DBF4*), and *CENPE*) that correlate with recurrence or progression in PRL-producing tumors (Raverot *et al.* 2010), Wierinckx *et al.* (2011) highlighted the impact of deregulation of five genes on 11p, *CD44*, *DGKZ*, *TSG101*, *GTF2H1*, and *HTATIP2*, that are potentially responsible for aggressive and malignant behavior in PRL-producing tumors. Based on their results, Wierinckx *et al.* (2011) proposed a model for progression wherein genomic instability of 11q in association with 11p loss can be responsible for an aggressive and malignant phenotype in PRL-producing tumors. In fact, 11q is implicated in somatotroph and lactotroph adenomas; both the aryl hydrocarbon receptor interacting protein and multiple endocrine neoplasia (*MEN1*) genes are located at 11q13 (Tahir *et al.* 2010, Newey & Thakker 2011).

Pituitary tumor transforming gene

Pituitary tumor transforming gene (*PTTG*) is a member of the securin family, which regulates sister chromatid separation during mitosis (Zou *et al.* 1999). *PTTG* was initially isolated and described in rat pituitary GH4 cells (Pei & Melmed 1997). The human *PTTG* family consists of at least three genes: *PTTG1*, *PTTG2*, and *PTTG3*. Evidence suggests tissue-specific expression of the three *PTTG* genes and potential roles for each of them in tumorigenesis, cell transformation, DNA repair, angiogenesis, and gene regulation (Salehi *et al.* 2008). As securins are required for cell division, it is not surprising that loss of *PTTG* suppresses cell proliferation, diminishing the development of pituitary tumors in Rb-deficient animals (Chesnokova *et al.* 2005). It is also expected that *PTTG* expression is increased in tumors of all types. It has been shown that *PTTG* upregulates vascular endothelial growth factor and bFGF expression, both of which are elevated in pituitary tumors (Ishikawa *et al.* 2001, McCabe *et al.* 2002).

Hunter *et al.* (2003) showed statistically higher levels of *PTTG* mRNA in somatotroph adenomas compared with nonfunctioning tumors. However, no statistical differences were observed when comparing corticotroph, lactotroph, and somatotroph adenomas. In some series, *PTTG* expression was found to be higher in hormone-secreting invasive pituitary adenomas than their noninvasive counterparts (Pei & Melmed 1997, Zhang *et al.* 1999). Filippella *et al.* (2006) reported that a *PTTG*/Ki-67 score higher than 2.9% predicts a biologically aggressive behavior in pituitary adenomas. *PTTG* expression is also high in malignant rat PRL-producing tumors (Wierinckx *et al.* 2007). However, as is the case for other markers of proliferation, such as Ki-67 and PCNA, it remains to be seen whether these results provide valuable information that can be used for clinical management.

Ki-67

Nuclear Ki-67 (identified with the MIB-1 antibody) is a marker of cell division that is usually counted to determine a proliferation index in neoplasms (Fig. 5). The Ki-67 LI is of prognostic significance in the assessment of neuroendocrine tumors, especially those originating from the gastrointestinal tract and pancreas (Bosman *et al.* 2010). Ki-67 has also been studied extensively in pituitary tumors. The Ki-67 LI has been reported to vary from <1% to as high as 23% in a series (Salehi *et al.* 2009). A significant association of Ki-67 LI with invasion was found in several studies (Landolt *et al.* 1987, Daita & Yonemasu 1996, Thapar *et al.* 1996a, Zhao *et al.* 1999, Iuchi *et al.* 2000, Jaffrain-Rea *et al.* 2002, Wolfsberger *et al.* 2004). Thapar *et al.* (1996a) established a threshold LI of 3% to

Figure 5 Nuclear Ki-67 (identified with the MIB-1 antibody) expression can be variable in pituitary tumors; some pituitary adenomas can exhibit <1% positive cells (A), while others have higher labeling index as illustrated in this lactotroph adenoma (B).

distinguish invasive from noninvasive adenomas with 97% specificity and 73% sensitivity, and this was associated with positive and negative predictive values of 96% and 80% respectively. With some exceptions, invasive pituitary tumors exhibit significantly higher growth fractions (Thapar *et al.* 1996a). Pizarro *et al.* (2004) showed that invasive pituitary adenomas had a significantly higher Ki-67 index ($2.01 \pm 3.15\%$) than macroadenomas ($1.12 \pm 1.87\%$); however, the Ki-67 index was not significantly different in the subgroup of adenomas with invasion of the cavernous sinus compared with groups with other types of invasion. Nevertheless, the ability of the Ki-67 index to predict tumor invasiveness is somewhat controversial as others have found no difference in Ki-67 expression in invasive pituitary adenomas (Yonezawa *et al.* 1997, Lath *et al.* 2001, Paek *et al.* 2005, Pan *et al.* 2005, Wierinckx *et al.* 2007).

p53

p53 is a tumor suppressor protein encoded by the *TP53* gene. It plays an important role in cell proliferation, apoptosis, and genomic stability. p53 expression has been linked to aggressive tumor behavior in pituitary tumors. Thapar *et al.* (1996b) demonstrated that noninvasive and invasive adenomas and pituitary carcinomas revealed p53 expression in 0%, 15.2%, and 100% of cases respectively. While some studies have suggested that p53 correlates with local relapse in pituitary adenomas (Ozer *et al.* 2003), ‘aggressive–invasive’ PRL-producing tumors were associated with higher p53 expression (Wierinckx *et al.* 2007). Again, similar to Ki-67 LI, other papers did not observe this significant correlation with invasive growth (Suliman *et al.* 2001, Hentschel *et al.* 2003, Scheithauer *et al.* 2006). These conflicting results suggest that p53 is not an

independent prognostic factor to determine the aggressive behavior of pituitary tumors.

miRNAs

miRNAs are small endogenous noncoding RNAs that regulate gene expression at the posttranscriptional level by direct cleavage of a mRNA or by inhibition of protein synthesis; they can also act as tumor suppressor genes or oncogenes (Sivapragasam *et al.* 2011). Aberrant miRNA expression has been linked to neoplasia in the pituitary gland. Stilling *et al.* (2010) reported differential expression of miR-122 in corticotroph adenomas compared with corticotroph carcinomas. Underexpression of miR-145, miR-21, miR-141, let-7a, miR-150, miR-15a, miR-16, and miR-145 has been reported in ACTH-producing adenomas. Although miRNA expression did not correlate with tumor size in one study, lower miR-141 expression correlated with postoperative remission in patients with corticotroph adenomas (Amaral *et al.* 2009). Reduced expression of miR-15a and miR-16-1 has been linked to tumor size in GH- and PRL-producing adenomas (Bottoni *et al.* 2004); however, this was not the case in corticotroph adenomas (Amaral *et al.* 2009).

Recently, a significant link between *HMGA2* oncogene and *let-7* miRNA has been shown in pituitary tumorigenesis (Qian *et al.* 2009). In this series, high levels of expression of *HMGA2* correlated with the extent of invasion, tumor size, and the Ki-67 proliferation index in pituitary adenomas. In addition, loss or reduction of *let-7* expression contributes to increased *HMGA2* protein expression in pituitary adenomas. Higher expression of *HMGA2* and lower *let-7* expression were also noted in invasive pituitary adenomas (Qian *et al.* 2009).

Conclusion

Despite emerging evidence, there is still little consensus about what constitutes an aggressive pituitary adenoma. The diagnosis of pituitary carcinoma is still restricted to adeno-hypophyseal proliferations that exhibit cerebrospinal and/or systemic metastasis (DeLellis *et al.* 2004, Asa 2011, Mete & Asa 2012). Thus, no morphological criteria appear to distinguish aggressive adenomas from carcinomas. Although many of the molecular events underlying pituitary tumorigenesis have been elucidated, reliable biological prognostic markers remain to be identified. We propose that detailed and comprehensive histological subtyping should be integrated with a panel of biomarkers. Of these, FGFR4, MMP, PTTG, Ki-67 LI, p53, miRNA profile, and deletions in chromosome 11p currently represent promising candidates with the potential to guide the management of patients with aggressive pituitary adenomas.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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