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 (Abbass 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 (Abbass 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.
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 (Wasylyk 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′).

Matrix metalloproteinases

Matrix metalloproteinases (MMPs) are a family of single-chain zinc-containing proteolytic enzymes that
(Rutter et al. 1998). Altas 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 MMP14SNP.

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 malignancies, including breast and lung cancers. MMP9 expression is significantly higher in invasive pituitary adenomas (Kawamoto 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 and 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 adenohypophysiotrophic 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 Gsα to increase cAMP levels. Densely granulated somatotroph adenomas have high cAMP levels, often due to somatic activating gsp mutations in the gene encoding Gsα. These tumors are usually responsive to somatostatin analogs that typically activate inhibitory G proteins, reducing cAMP levels (Bhayana et al. 2005, Asa and Ezzat 2009, Asa 2011, Mete and Asa 2012). However, the potential role of GH in autoregulation 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 defined as 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 and Asa 2012). Altered GHR signaling is also associated with a morphological change resulting in the formation of paranuclear keratin aggregosomes (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...

**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±3.15%) than macroadenomas (1.12±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 adenohypophyseal 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.
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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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