INVITED REVIEW

What are the markers of aggressiveness in prolactinomas? Changes in cell biology, extracellular matrix components, angiogenesis and genetics

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Abstract

Prolactinoma is the most common pituitary tumour in adults. Macroprolactinomas, particularly in men, may occasionally exhibit a very aggressive clinical course as evidenced by progressive growth, invasion through bone into the sphenoid sinus, cavernous sinus, suprasellar region or the nasopharynx. Some may even progress to pituitary carcinoma with craniospinal or systemic metastases. Aggressive tumours have low cure rates despite appropriate medical and surgical treatment. The mechanisms underlying this aggressive biological behaviour have not yet been fully clarified. Recent immunohistochemical, molecular and genetic studies have provided some insight in this respect. Invasive prolactinomas may be associated with a high Ki-67/MIB-1 labelling index indicating increased cell proliferation, although this is not a universal finding. The AA polymorphism in the cyclin adenine (A)/guanine (G) gene is more frequently detected in invasive prolactinomas. Increased expression of the polysialylated neural cell adhesion molecule (NCAM) and reduced expression of the E-cadherin/catenin complex implies a contribution of altered cell-to-cell adhesion and cellular migration. Extracellular matrix components (ECM), matrix metalloproteinases (MMPs) and their inhibitors play important roles in the context of angiogenesis and invasion. The induction of fibroblast growth factor and vascular endothelial growth factor via oestrogen-induced overexpression of novel genes (*PTTG*, *hst* and *Edpm*5) enhance cell growth, proliferation and angiogenesis contributing to invasiveness in prolactinomas. Although mutations in proto-oncogenes like *Ras* are uncommon, loss of tumour suppressor genes at loci 11q13, 13q12–14, 10q and 1p seem to be associated with invasiveness. Of the described mechanisms, only reduced E-cadherin/catenin expression and overexpression of *hst* gene seem to be relatively specific markers for prolactinoma invasiveness compared with other pituitary adenomas. Further research is needed to clarify the molecular mechanisms behind the aggressive course of some prolactinomas to predict those with a potentially poor clinical outcome, and to devise treatments that will eventually enable the cure of these challenging tumours.

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Introduction

Prolactinoma is the most common pituitary tumour in adults accounting for 60% of all functioning pituitary adenomas (1). Prolactinomas show various initial presentations, radiological appearances and biological behaviour. In general, prolactinomas in women of childbearing age are indolent microadenomas (< 10 mm in diameter) (2–5). These patients come to medical attention due to the effects of hyperprolactinaemia, for example, galactorrhea, oligomenorrhea, loss of libido and sexual dysfunction (6). In contrast, men and post-menopausal women frequently harbour a macroprolactinoma (> 10 mm in diameter) with or without extrasellar extension (7, 8). Apart from signs of hyperprolactinaemia, patients with macroadenomas

with extrasellar extension generally seek medical attention due to mass effect. These patients may present with headache, visual disturbances, cranial nerve palsies, hypopituitarism (9) or rarely spontaneous cerebrospinal fluid leakage (10), epilepsy due to temporal lobe compression and obstructive hydrocephalus (11).

The mechanisms of aggressive biological behaviour of some prolactinomas, despite their mostly benign histopathological features, have not yet been fully defined. There is inconsistency in the definition of 'aggressiveness' in prolactinomas. From the clinical point of view, the most important factors to take into account are resistance to dopamine agonists and high recurrence rates after surgery. From the radiological point of view, the tumours are considered as aggressive

if they show invasion into adjacent structures such as the cavernous sinus. The lack of any increase in tumour size in the majority of patients with microadenoma is compatible with the frequent incidental finding of these tumours in unselected autopsy cases (12, 13). The risk of progression from a microadenoma to a macroadenoma is estimated to be about 4–7% (1). This observation suggests that aggressive macroadenoma may represent a different and distinct entity (14, 15). There are no clear-cut criteria to predict biological behaviour in prolactinomas (16, 17). As mentioned previously, some prolactinomas are able to invade the surrounding structures and tissues. These are called 'invasive prolactinomas'. Presently, local invasion *per se* is not considered a sign of malignancy. Only rare pituitary tumours with distant metastases are termed 'pituitary carcinomas', indicating truly malignant behaviour. Their pattern of spread may be craniospinal, systemic or both. Traditional histopathological features of malignancy (nuclear polymorphism, high cellularity, cytological atypia, the presence of necrosis and nucleolar prominence) are not generally helpful to discriminate the pituitary carcinomas from benign adenomas (18). When mitotic figures are present, they do provide some insight into the potentially aggressive nature of the tumour, but their discriminating power is somewhat limited (17).

The World Health Organization (WHO) classification of endocrine tumours has defined an 'atypical pituitary adenoma' as one having an elevated mitotic activity, a Ki-67 labelling index $>3\%$, and p53 (a nuclear phosphoprotein whose immunohistochemical accumulation has served as an unfavourable prognostic factor for a wide range of human neoplasms) immunoreactivity $>3\%$ (19, 20). These features are generally lacking in non-invasive adenomas. Nearly, all pituitary carcinomas present the above-mentioned histological features of atypical adenomas (21).

There is also a problem in assessing the invasiveness of a particular tumour, since there are various methods used to define it. For example, the spectrum of invasiveness may encompass radiological invasion, intraoperative observations by the surgeon or microscopical invasion. It has also been proposed that a tumour, which extends into the adjacent structures according to imaging, may not represent an invasive one on microscopy (16). With the advancement of techniques in genetics, molecular biology and immunohistochemistry in the past two decades, more specific markers have been identified which might more precisely indicate an aggressive potential in these tumours.

In this review, after briefly reviewing the biochemical, neuroradiological and clinical aspects of aggressiveness (i.e. tumoural expansion and invasion through the surrounding and even remote structures causing difficulties in management and low cure rates), we will address suggested markers which may help identify these tumours in the context of cell proliferation, cell cycling,

cell-to-cell adhesion, angiogenesis, alterations of extracellular matrix (ECM) components and growth factor signalling. We will also summarise the genetic alterations leading to changes in proliferative capacity and angiogenesis in prolactinomas. The contribution of some of these markers to gender differences in prolactinoma behaviour and the development of resistance to dopaminergic agonists will also be discussed. We should acknowledge, however, that the studies that will be reviewed in this paper are based on surgically removed prolactinomas. As surgical therapy for these tumours is performed in particular subsets of patients only, an inevitable selection bias may be envisioned in all these studies.

Biochemical and radiological assessment of aggressive prolactinomas

Hormone-secreting pituitary adenomas are readily diagnosed by endocrine testing which can be routinely performed. In giant prolactinomas, the diagnosis is generally straightforward, since they present with extreme elevation of prolactin (even $>100\,000$ ng/ml). Prolactinomas are unique in the sense that dopamine agonists usually cause rapid tumour shrinkage with relief in compressive symptoms (22–24). So, it is important to diagnose a prolactinoma patient before considering surgery as a first step treatment modality. Clinicians should also be aware of the 'high dose hook effect' while interpreting a low prolactin level in a patient with giant invasive pituitary adenoma (25, 26).

Calle-Rodrigue *et al.* (27) have shown that preoperative serum prolactin concentrations were significantly higher in invasive prolactinomas compared with the non-invasive ones (median prolactin levels 705 vs 141 ng/ml respectively). When prolactin cut-off was chosen as 3300 ng/ml, the specificity for predicting an invasive prolactinoma was 91% (sensitivity was not calculated). Similarly, Ma *et al.* (28) have reported that prolactinomas invading the cavernous sinus had significantly higher prolactin concentrations and tumour sizes, and furthermore, that preoperative prolactin levels were significantly associated with invasiveness. No comparisons have been done in these studies (27, 28), however, regarding plurihormonal adenomas co-secreting prolactin versus pure prolactin-secreting adenomas in terms of aggressive behaviour as evidenced by invasiveness. These data suggest that prolactin levels correlate with tumour size and that high preoperative prolactin levels indicate the presence of an invasive prolactinoma.

Although magnetic resonance imaging (MRI) is the best technique for assessing the extent of the tumour, computerised tomography (CT) provides better information about interruption of the sellar floor and intratumoural calcification. From the radiological point

of view, aggressiveness in these tumours is probably best reflected by cavernous sinus invasion.

Recently, Cottier *et al*. (29) defined MRI-based criteria for the diagnosis of cavernous sinus invasion using the surgical findings of 106 patients as standard-of-reference criteria for invasion. Invasion of the cavernous sinus was certain (positive predictive value (PPV) 100%) if the percentage of the encasement of the internal carotid artery (ICA) by tumour was 67% or greater. Invasion was considered to be highly probable if the carotid sulcus venous compartment was not depicted (PPV 95%) or the line joining the lateral wall of the intracavernous and supracavernous ICAs was passed by the tumour (PPV 85%). If the percentage of the encasement of the intracavernous ICA is lower than 25, or the line joining the medial wall of the intracavernous and supracavernous ICAs was not passed by the tumour, cavernous sinus invasion by the tumour is excluded.

Resistance to dopaminergic agonists: not a 'sine qua non' of aggressiveness

There is no consistency in the literature about what constitutes resistance to dopamine agonists. Failure to normalise prolactin levels independently of the baseline value, or failure to reduce prolactin levels below 50% of baseline have been considered as resistance. Similar arguments are valid for tumour size reduction as well. There may be discordance in the response of a given patient with respect to prolactin reduction and tumour size reduction (for review, see 30). While dopamine agonists or surgery cure most non-invasive prolactinomas, invasive ones may not be cured by surgery alone requiring additional treatments like radiotherapy (31). Furthermore, they may have a propensity to be resistant to dopamine agonists. However, dopaminergic agonists should still be considered as the first-line treatment in most patients with giant prolactinomas. Wu *et al*. (26) have recently demonstrated that bromocriptine treatment of invasive giant prolactinomas after a mean follow-up of 37 months results in improvement of clinical symptoms in almost all patients, as well as tumour volume reduction by a mean of 93.3%. Failure to normalise prolactin levels is found in about 25% of patients treated with bromocriptine and in 10–15% of those treated with pergolide or cabergoline in prolactinomas regardless of size or invasiveness (30). Resistance of the tumour to shrinkage with bromocriptine is seen in about 50% of the tumours, while this figure is about 10–15% with cabergoline and pergolide (30). Another important drug, which has been used in bromocriptine-resistant tumours and in patients with intolerance to bromocriptine, is the non-ergot dopamine agonist quinagolide. It is very difficult to verify the incidence of primary quinagolide resistance in prolactinomas. In a recent crossover longitudinal study, Di Sarno *et al*. (32)

have shown that quinagolide treatment normalised prolactin levels in all microprolactinomas, and 87.5% of macroprolactinomas. These figures were almost the same for treatment with cabergoline (32).

Drug resistance is more common in macroadenomas compared with microadenomas. For example, in a retrospective analysis, a group of patients who were initially being treated with bromocriptine was switched to cabergoline (33). Bromocriptine resistance in terms of prolactin normalisation was more frequent in macroprolactinomas (54%) than microprolactinomas (43%). Resistance to cabergoline was 18 and 10% in macro- and microadenomas respectively. The underlying mechanism leading to the resistance in most tumours has been attributed to decreased cellular D2 receptors with lower affinities to their ligands (34).

Macroprolactinomas in men tend to be more frequently resistant to bromocriptine therapy than those in women (8). Delgrange *et al*. (8) have shown significant differences in frequencies of bromocriptine resistance between males (30%) and females (5%). The correlation of drug resistance with other markers of aggressiveness, in particular the ones related with proliferative capacity, will be discussed in the following part to provide an explanation to this gender difference in biological behaviour of these tumours.

Specific markers implicated in aggressiveness in prolactinomas

Implications of proliferative capacity, cell cycling and cell-to-cell adhesion in prolactinoma behaviour

It is well known that tumour expansion and invasion primarily relate to several mechanisms, including increases in cell proliferation, changes in cell cycling and cell-to-cell adhesion. Several markers have been investigated in prolactinomas in an attempt to relate these mechanisms with aggressive tumour biology. Some of the studies that may be relevant are given in Table 1, and are further discussed in the following paragraphs taking into account the inconsistent and controversial issues in the literature.

Markers of proliferation Nuclear proteins, such as Ki-67 and proliferating cell nuclear antigen (PCNA) are often used to assess proliferative activity of tumour cells (16). Ki-67 is recognised by the MIB-1 monoclonal antibody and it is expressed throughout the cell cycle in G1, S, G2 and M phases, but not the quiescent G0 phase (28, 35). The percentage of cells immunostained for this protein (Ki-67/MIB-1 labelling index) represents a reliable marker of proliferative activity (36). PCNA is a protein that accumulates in the nucleus during the cell cycle. It has a higher specificity for the S phase than

Table 1 Detected markers of aggressiveness related to cell proliferation, cell cycling and cell-to-cell adhesion.

Category	Marker	Status of expression and clinical relevance	Ref.
Proliferation	Ki-67/MIB-1 LI	Associated with resistance to DA	(8)
		No association with tumour size	(28)
		Increases may indicate malignant progression	(21)
		If >3%, highly specific for invasiveness	(36)
	PCNA	Increased in parallel with tumour recurrence	(37)
Cell cycling	Cyclin D1	Increased in aggressive pituitary tumours as a whole, no increase in macroadenomas	(41)
	Cyclin E	Increased in macroprolactinomas	(42)
Cell-to-cell adhesion	PNCAM	Highest in invasive rat prolactinomas and metastatic lesions	(53–56)
		No correlation with invasiveness in human prolactinomas	(57)
	E-cadherin/catenin/p120 complex	Decreased in invasive prolactinomas	(63)

LI, labelling index; DA, dopamine agonist; PCNA, proliferating cell nuclear antigen; PNCAM, polysialylated neural cell adhesion molecule.

Ki-67, and labels only those cells that passed the important G1/S boundary (37). A comparison of Ki-67 and PCNA proliferation in pituitary tumours has shown that PCNA overestimates the cell proliferation in these tumours when tumours with a labelling index <0.1% were considered to be negative for proliferation (38). Concerning prolactinomas, DelGrange *et al.* (8) found no significant difference in PCNA index between invasive and non-invasive prolactinomas. Similar to the PCNA index, numerous studies have been performed on the Ki-67/MIB-1 labelling index in pituitary adenomas with inconsistent results in terms of correlation with clinical presentation. With respect to non-functioning adenomas, no significant relationship has been found between the clinical course and the labelling index (39). The predictive ability of the Ki-67/MIB-1 labelling index for aggressive behaviour is greater in functioning pituitary adenomas, including prolactinomas. In a series reported by Thapar *et al.* (36), hormone-secreting adenomas had a significantly higher mean labelling index (3.25%) than non-functioning adenomas (2.06%). The highest labelling indices were in prolactinomas. A cut-off of 3% of labelling as an indicator of invasiveness had a specificity of 97.3% and a sensitivity of 73% (36).

Two studies have shown higher levels of the labelling indices in males with macroprolactinomas when compared with similar tumours in female patients (8, 28). Regarding the association of Ki-67/MIB-1 labelling index with tumour behaviour, these studies (8, 28) did not find consistent differences between invasive and non-invasive macroprolactinomas except in a small group of males (n = 4) with dopamine agonist-resistant invasive prolactinomas. The mean labelling index was 4.7% compared with <1% in all tumours that are responsive (8). PCNA labelling yielded similar results (8). On the other hand, one recent study by Ma *et al.* (28), conducted in 123 Japanese prolactinoma patients, found no association between Ki-67 labelling index and clinical parameters. In this study, half of the cases were pure prolactin-producing tumours while the other half was plurihormonal prolactinomas. No significant

association could be detected between proliferative capacities and tumour size. The impact of patient age and gender in both plurihormonal tumours and pure prolactinomas was not assessed (28). Recently, Scheithauer *et al.* (21) found no statistically significant difference between invasive and non-invasive adenomas (including prolactinomas) with respect to Ki-67 and PCNA labelling indices. The level of p53 expression was also similar between the groups. Ki-67 labelling and p53 were not statistically significant in determining adenomas prone to recur. It should be emphasised that the difference between the Ki-67 results of Scheithauer *et al.* (21) and Thapar *et al.* (36) may relate to different techniques used for the interpretation of immunohistochemical stainings (manual cell counting versus digital image analysis respectively). The clinical value of the recently introduced 'atypical adenoma' category in predicting recurrence or malignant progression, therefore, remains to be determined.

Proteins implicated in cell cycling Cyclins and cyclin-dependent kinases (CDK) are essential for cell cycle regulation in eukaryotes. Active cyclin–CDK complexes drive cells through cell cycle phases by phosphorylating the protein substrates that are essential to achieve transition to the next phase. The levels of cyclins are regulated at the level of transcription and also by targeted degradation via the ubiquitin pathway (40). Jordan *et al.* (41) have found that non-functioning adenomas and functioning invasive tumours, including prolactinomas significantly express cyclin D1 to a greater extent than non-invasive tumours and the normal pituitary gland. Pituitary carcinomas did not appear to be different from the benign tumours in terms of cyclin expression (41). We have previously observed that it was only cyclin E expression that was higher in macroprolactinomas than microprolactinomas (42). Since cyclin E is expressed maximally at G1/S transition of the cell cycle and thought to be important in the initiation of DNA replication, it may have an implication in growth and expansion of prolactinomas (43). This warrants further analysis in

larger prolactinoma groups categorised according to their invasiveness or recurrence rate.

The cyclin D1 gene contains a frequent A/G polymorphism within the splice donor region of exon 4/intron 4 (44). The cyclin D1 genotype is associated with clinical outcome, particularly the survival after diagnosis, in a significant number of neoplasms (44, 45). In a cohort of 294 (20% were prolactinomas) pituitary adenomas, Simpson *et al.* (46) examined the distribution of cyclin D1 A/G polymorphisms across the Hardy grade of the tumours. Grade 3 and 4 tumours were considered as invasive. When the data were analysed separately in the prolactinoma subgroup, the authors observed a progressive increase in AA genotype from grade 1 (11%) through grade 4 (66%), while the GG genotype progressively decreased from grade 1 (43%) through grade 4 (0%) (46). Two patients out of eleven in the invasive group showed recurrence after the operation, while none of the grade 1 patients recurred. Therefore, AA genotype in cyclin D1 gene may be a marker for invasiveness of prolactinomas, and may also contribute to tumour recurrence. Overexpression of high-mobility group A non-histone chromosomal proteins (HMGA) play a significant role in the determination of chromatin structure. These proteins have been found overexpressed in several cancer types (47). It has previously been demonstrated that transgenic mice overexpressing HMGA2 develop growth hormone (GH) and prolactin-secreting tumours (48). With respect to human prolactinomas, aggressive tumours with unresponsiveness to dopamine agonists are associated with higher expression level of this protein (49).

Role of adhesion molecules The neural cell adhesion molecules (NCAMs), member of the immunoglobulin superfamily, contribute to cell-to-cell or cell substrate adhesion through homophilic and heterophilic mechanisms (50, 51). They can be post-translationally modified by polysialylation giving rise to PNCAMs that are implicated in cell growth and migration (52). PNCAM expression has been noted in aggressive tumours, such as small-cell lung carcinomas, and also some neuroendocrine tumours (53–55). The expression of PNCAM has been investigated both in animal models and in human pituitary adenomas. Daniel *et al.* (56) have investigated the PNCAM expression in four lineages of rat pituitary transplantable tumours (SMtTW) primarily secreting prolactin. They observed the highest PNCAM expression in the lineage SMtTW4 that was characterised by invasion and metastasis. The expression level was correlated with growth rate in different tumour lineages. Embarking from this observation, Trouillas *et al.* (57) have recently investigated the expression of PNCAMs in a variety of human pituitary tumours in relation to extrasellar invasion

using immunohistochemistry, western blot analyses and ELISA. They have observed a positive correlation between tumour invasiveness and PNCAM expression, particularly in the GH-secreting adenomas. This was not the case in the prolactinoma subgroup ($n = 14$), although some of them expressed PNCAM (57). The discrepancy between animal and human prolactinomas may be related to differences in tumour invasion mechanisms between species, and the small number of prolactinomas studied in humans.

Tumour cell invasion is a multistep process that requires complex changes in adhesive interactions among cells. The detachment of tumour cells from the primary lesion is thought to be an important initiative step leading to invasion. E-cadherin is an important molecule implicated in tumoural invasion processes (58). The functions of E-cadherin are mediated through its cytoplasmic linkage to the actin cytoskeleton via certain cytoplasmic plaque proteins known as the catenins (α, β and γ) (59, 60). P120, by binding to E-cadherin as a regulatory factor, enables it to function to promote intercellular adhesion (61). It is believed that the cadherin/catenin/p120 complex acts as an 'invasion suppressor system'. There are several observations in various cancers indicating its abnormal or reduced expression (62). Qian *et al.* (63) have shown that expression of E-cadherin and β-catenin seems to be significantly lower in invasive prolactinomas compared with non-invasive ones, and the complex is significantly less expressed in macroadenomas than microadenomas. The tumours derived from men showed a significantly lower level of expression than tumours from women. The decrease in cadherin/catenin expression predicted increased MIB-1 labelling indicative of high proliferative activity. The results of this study indicate that low cadherin/catenin expression may be a useful marker in predicting tumour aggressiveness in prolactinomas (63). In contrast, Kawamoto *et al.* (64) have found no significant difference in E-cadherin expression between invasive and non-invasive, non-functioning and GH-secreting pituitary adenomas. It seems therefore that loss of E-cadherin expression may be a marker of invasiveness for prolactinomas but not GH-producing or non-functioning adenomas. The molecular mechanisms responsible for loss of cadherin/catenin expression in invasive prolactinomas are not presently known.

Role of extracellular matrix components (ECM) and degradation of ECM

Components of the extracellular matrix are implicated in pituitary tumourigenesis (65). Laminin, for example, inhibits the production of prolactin and cell proliferation. Differential expression of laminin has been observed in D2 receptor knockout mice that spontaneously develop aggressive prolactinomas (66). From the initial formation of hyperplasia, a dramatic reduction occurs in

laminin expression with the advancement of tumour progression. The expression of laminin is confined to basal membranes surrounding blood vessels despite the fact that it is abundantly expressed in the normal adenohypophysis parenchyma (66). The significance of laminin remains to be investigated in human prolactinoma behaviour.

The ECM can be degraded and reorganised by specific enzymes from the family of matrix metalloproteinases (MMPs). The activities of various MMPs are balanced by tissue inhibitors of metalloproteinases (TIMPs) (67). MMP-2 and MMP-9 are both type IV collagenases that are able to promote tumour invasion by breaking down the basement membrane (68). The secretion of MMP is also an important early step contributing to endothelial cell migration through degraded ECM enabling angiogenesis to occur (69). Serine proteases and their modulators (plasminogen activators and inhibitors) are also involved in ECM remodelling by initiating a cascade of events, involving MMP-9 proteolytic activity (70).

In the context of the biological behaviour of pituitary adenomas, studies investigating the contribution of MMP-9 have yielded conflicting results. Our group has previously demonstrated that MMP-9 expression by macrophages in invasive macroprolactinomas was significantly higher than in non-invasive macroprolactinomas, while expression levels of MMP-9 in pituitary carcinomas was similar to that of invasive adenomas (71). We have also found a positive correlation between MMP-9 expression and angiogenesis. However, more recently, Knappe *et al.* (70) found contradictory results with respect to MMP-9 expression and invasiveness in pituitary adenomas. Contrary to our observations, they could not demonstrate a relationship between aggressive behaviour and MMP-9 expression (70). However, they identified that TIMP-2 was overexpressed in non-invasive as compared with invasive adenomas. These observations suggest that inhibitors of MMPs may play an important role in the suppression of aggressive behaviour in pituitary tumours. The discrepancies in MMP-9 data among the cited studies above may have resulted from different techniques used, diversity of tumour subtypes, selected criteria for aggressiveness, and different number of cases studied.

Local growth factor expression In the pituitary, various growth factors are expressed and released into the extracellular matrix (65). Fibroblast growth factors (FGF) and vascular endothelial growth factor (VEGF) are implicated in angiogenesis. Another local factor that may be operative in aggressive prolactinoma behaviour is epidermal growth factor (EGF). Anterior pituitary cells normally express EGF, and it has binding sites mainly confined to lactotroph and somatotroph cells (72, 73). In pituitary tumours, EGF-binding sites are present in 76.5% of prolactinomas and 62.5% of gonadotrophinomas (74). EGF binding is more frequent in the invasive

than non-invasive pituitary adenoma group which includes prolactinomas (exact number not specified), especially in adenomas invading the cavernous sinus. In addition, adenomas including prolactinomas with supra/extrasellar extension show higher EGF-binding sites compared with those without (74). It seems that EGF is a key factor contributing to pituitary tumour aggressiveness possibly by its ability to modulate cell proliferation. However, since the correlation of EGF-binding sites with prolactinoma invasiveness has not been specifically addressed in this study (74), more data in prolactinomas are clearly needed to state that EGF receptor expression levels reflect the invasiveness in this particular type of pituitary adenomas.

Role of tumour angiogenesis

Angiogenesis is the process of new vessel development from existing ones and this has been shown to be involved in tumour growth and spreading. In many cancers, increased vascularisation of the tumour has been correlated with poor prognosis and metastatic spread (75).

Investigations related to angiogenesis in normal pituitary tissues and pituitary adenomas have opened new avenues to understand the biology of aggressiveness. Contrary to expectations, normal pituitary gland is more vascular than tumours as exemplified by low microvessel counts in immunohistochemistry for CD31 and ulex europaeus agglutinin I (UEAI) (76, for review, see 77). It seems possible that pituitary tumours may induce the development of new blood vessels directly from the systemic circulation (77). Although pituitary tumours are less vascular than normal pituitary tissue, there is a distinction between specific tumour types in respect of vascular density according to tumour size. We have demonstrated that micro- and macroadenomas that secrete GH or ACTH have comparable vascular densities, whereas macroprolactinomas are significantly more vascular than microprolactinomas (76). The relationship of angiogenesis with pituitary tumour behaviour has also been studied by our group (78). We have noticed that preoperative serum hormone levels were directly proportional to microvessel density in prolactinomas, but not in GH-secreting tumours. A significantly increased microvessel density was observed in invasive prolactinomas compared with non-invasive ones (78). These data support the view that microvessel density as assessed by CD31 and UEAI staining is a marker of aggressive behaviour in prolactinomas affecting the clinical outcome (78). In another study, we have identified a positive relationship between anti-apoptotic protein Bcl-2 expression and microvessel density in a group of pituitary adenomas, including prolactinomas (79). Thus, prevention of programmed cell death and switch to an angiogenic phenotype seem to be associated with tumour progression and invasion.

Genetic alterations leading to aggressiveness

A considerable number of genetic alterations have been implicated in the pathogenesis of prolactinomas. Those that may be associated with aggressive behaviour, are summarised in Table 2, and discussed below.

The Ras proto-oncogene Pituitary tumours arise from either gain of function mutations, or the overexpression of proto-oncogenes that function in proliferative pathways (80). The *Ras* gene codes for a GTP-binding protein that is important in cellular proliferation and differentiation and involved particularly in early stages of tumourigenesis (81). A case has been reported with a point mutation (Gly12Val) in the *Ras* gene presenting with recurrent invasive prolactinoma with dopamine agonist resistance (82). Despite the fact that Ras mutations have been implicated in various human malignancies, they are uncommon in pituitary adenomas (83) and probably not relevant as genetic markers of tumour progression or malignancy.

Common and pituitary-specific tumour suppressor genes The role of tumour suppressor genes in the pathogenesis of pituitary tumours in animal models is well known. In rodent models, knockout of the retinoblastoma gene (*RB*) or *p27Kip1* gene (a gene involved in cell cycle arrest at G1 stage) has been identified as causing pituitary adenomas (84, 85). In humans, loss of heterozygosity (LOH) on chromosome 13q, the location of the *RB* gene, has been identified in a small number of pituitary carcinomas (86). Bates *et al.* (87) assessed the impact of allelic deletion of various genes by LOH studies. LOH was found at loci 11q13 (the site for the tumour suppressor gene of multiple endocrine neoplasia type 1, MEN-1), 13q12-14, 10q and 1p. About half of the invasive tumours, which included prolactinomas, had at least one allelic deletion at these sites. Of the six tumours with only one deletion, five involved the 11q13 locus suggesting that this is an early event taking place in transition from a non-invasive state into an invasive one (87). Although the

loss of alleles on chromosome 11q13 has been identified in about 10–20% of both sporadic GH-secreting adenomas as well as prolactinomas (88, 89), subsequent studies have failed to reproduce similar results in terms of mutations in the *MEN-1* gene (90, 91). In a recent study, MEN-1-associated prolactinomas have been found to be more aggressive compared with the sporadic ones (92), although other studies have previously failed to show significant differences in this regard (93, 94). Since mutations and deletions thus far identified account for only a small percentage of pituitary tumours, including prolactinomas, it is presently difficult to infer that there is a generalised genetic mechanism for aggressive tumour behaviour.

Genes related to growth factor expression, angiogenesis and tumour progression The relevance of the locus 11q13 with respect to tumourigenesis and tumour progression in the pituitary gland may also be related to the *hst* gene that was initially identified as a transforming gene in solid malignant tumours (95). DNA derived from human prolactinomas express transforming activity in heterologous cells and had sequences in close resemblance with those of *hst* gene (96). Overexpression of *hst* gene leads to increased production of FGF-4 (97). Shimon *et al.* (97) demonstrated the function of the *hst* gene in rat lactotroph tumour formation and prolactin secretion. They were able to show that lactotrophs in 5 of 14 prolactinomas stained strongly with anti-FGF-4 monoclonal antibodies. Immunoreactive hst product in adenoma cells was observed in three of five invasive prolactinomas, whereas only two of nine non-invasive ones were positive. Interestingly, most of the non-functioning adenomas and other types of functioning adenomas did not present any staining for *hst* gene product. Furthermore, immunostaining for proliferative marker Ki-67 showed a greater proliferative status in hst-positive adenomas compared with those that are immunonegative for hst (97). These findings imply a role of *hst* gene, and its product FGF-4, in cellular proliferation, growth and aggressive behaviour in prolactinomas.

Table 2 Detected genetic alterations in invasive prolactinomas.

Affected gene (locus)	Detected alteration	Biological effect	Ref.
Retinoblastoma (*RB*) (11q13)	Deletion (LOH)	Transition to an invasive state	(87)
hst (11q13)	Oestrogen-induced overexpression	Induction of FGF-4, ↑ growth, ↑ proliferation, ↑ angiogenesis	(97)
PTTG (5q33)	Oestrogen-induced overexpression	↑ bFGF expression, ↑ VEGF expression, tumour progression, ↑ angiogenesis	(101)
Edpm5	Variability between rat strains	Change in angiogenic switch, alterations in tumour angiogenesis upon oestrogen stimulation	(106)

LOH, loss of heterozygosity; bFGF, basic fibroblast growth factor; VEGF, vascular endothelial growth factor; PTTG, pituitary tumour-transforming gene.

Oestrogen promotes tumourigenesis in the pituitary gland via induction of a novel pituitary tumour-transforming gene (*PTTG*) that is located on chromosome 5q33 (98). PTTG has been shown to be tumourigenic *in vivo*, by regulating basic fibroblast growth factor (bFGF) secretion and inhibiting chromatid separation (99, 100). bFGF modulates angiogenesis, tumour formation and progression in several tissues, including the pituitary (101). Oestrogen induces over-expression of rat PTTG leading to increased bFGF and VEGF production (VEGF) in the pituitary gland (101). Moreover, bFGF itself has an ability to modulate VEGF expression (101). This finding implies that oestrogen is important in pituitary adenoma formation and progression via a paracrine mechanism involving angiogenesis. In accordance with these effects of oestrogen, lactotroph tumours tend to enlarge considerably during pregnancy (102). As noted earlier, prolactinomas in men tend to exhibit a more aggressive course. This seems to be counterintuitative concerning the oestrogen–PTTG pathway, since men have lower oestrogen levels than women. However, this discrepancy may be explained by the observation that although men have lower oestrogen levels, their tumours express more oestrogen receptors than those in women (103). It should be noted, however, that PTTG overexpression is not specific for prolactinomas, and seen in all classes of hormone-secreting pituitary adenomas (104, 105). Recently, a novel gene locus named *Edpm*5 has been shown to prevent the angiogenic switch in prolactinomas after chronic oestrogen treatment in rats (106). The contribution of *Edpm*5 gene to angiogenesis and aggressive behaviour in human prolactinomas, however, is yet to be defined.

Genes implicated in resistance to dopamine agonists It is well known that dopaminergic receptor-2 (*D2R*) gene-deficient mice develop lactotroph cell hyperplasia and tumours capable of massive invasion (107). No mutation in the *D2R* gene has been reported in 79 invasive and dopamine agonist-resistant prolactinomas (108). However, the impact of dopamine receptor gene polymorphisms on prolactinoma behaviour warrants further investigations.

Summary and conclusions

Aggressive prolactinomas represent a group presenting with invasion into surrounding and remote structures and causing challenges in treatment. Invasive tumours are usually associated with lower cure and higher recurrence rates as compared with non-invasive ones. Prolactin levels at diagnosis are higher in these tumours in correlation with their sizes, and naïve or acquired resistance to dopamine agonists are more frequently encountered. Invasive prolactinomas may be associated

with increased Ki-67/MIB-1 labelling indices indicating increased cell proliferation, although this is not a universal finding. Increased expression of polysialylated neural cell adhesion molecule and reduced expression of E-cadherin/catenin complex may imply a contribution of changes in cell-to-cell adhesion and cellular migration. The reduced E-cadherin/catenin expression seems to be relatively specific for invasive prolactinomas, but not other functioning and non-functioning pituitary tumours. ECM and MMPs with their inhibitors play important roles in the context of angiogenesis, a key switch factor leading to invasion. The induction of local FGF and VEGF activity via the oestrogen-induced overexpression of novel genes (*PTTG*, *hst* and *Edpm*5) enhance prolactin cell growth, proliferation and tumoural angiogenesis in prolactinomas. *hst* gene overexpression is relatively specific for invasive prolactinomas. Loss of tumour suppressor genes at various loci, including 11q13 has been found in invasive pituitary adenomas, including prolactinomas. Since only a few specific markers have been identified thus far, more investigations into the mechanisms of aggressive behaviour in prolactinomas are needed to eventually predict cases with poor clinical outcome. New treatment modalities specifically targeting these mechanisms may promise a cure for these challenging tumours.

Acknowledgements

References

1 Ciccarelli A, Daly AF & Beckers A. The epidemiology of prolactinomas. *Pituitary* 2005 **8** 3–6.
2 Drange MR, Fram NR, Herman-Bonert V & Melmed S. Pituitary tumor registry:a novel clinical resource. *Journal of Clinical Endocrinology and Metabolism* 2000 **85** 168–174.
3 Ramot Y, Rapoport MJ, Hagag P & Wysenbeek AJ. A study of the clinical differences between women and men with hyperprolactinemia. *Gynecological Endocrinology* 1996 **10** 397–400.
4 Choudhury SD & Goswami A. Hyperprolactinemia and reproductive disorders – a profile from north east. *Journal of the Association of Physicians of India* 1995 **43** 617–618.
5 Batrinos ML, Panitsa-Faflia C, Tsiganou E & Liapi C. Incidence and characteristics of microprolactinomas (3–5 mm) in 4199 women assayed for prolactin. *Hormone and Metabolic Research* 1992 **24** 384–391.
6 Arafah BM & Nasrallah MP. Pituitary tumors: pathophysiology, clinical manifestations and management. *Endocrine-Related Cancer* 2001 **8** 287–305.
7 Colao A, Sarno AD, Cappabianca P, Briganti F, Pivonello R, Somma CD, Faggiano A, Biondi B & Lombardi G. Gender differences in the prevalence, clinical features and response to cabergoline in hyperprolactinemia. *European Journal of Endocrinology* 2003 **148** 325–331.

8 Delgrange E, Trouillas J, Maiter D, Donckier J & Tourniaire J. Sex-related difference in the growth of prolactinomas: a clinical and proliferation marker study. *Journal of Clinical Endocrinology and Metabolism* 1997 **82** 2102–2107.

9 Ozgen T, Oruckaptan HH, Ozcan OE & Acikgoz B. Prolactin secreting pituitary adenomas: analysis of 429 surgically treated patients, effect of adjuvant treatment modalities and review of the literature. *Acta Neurochirurgica* 1999 **141** 1287–1294.

10 Ohtakara K, Matsubara T, Kojima T, Taki W & Waga S. Cerebrospinal fluid rhinorrhea associated with untreated pro-lactinoma – case report. *Neurologia Medico-chirurgica* 2000 **40** 413–418.

11 Scarone P, Losa M, Mortini P & Giovanelli M. Obstructive hydrocephalus and intracranial hypertension caused by a giant macroprolactinoma. Prompt response to medical treatment. *Journal of Neuro-oncology* 2006 **76** 51–54.

12 Clayton RN. Sporadic pituitary tumours: from epidemiology to use of databases. *Bailliere's Best Practice and Research. Clinical Endocrinology and Metabolism* 1999 **13** 451–460.

13 Buurman H & Saeger W. Subclinical adenomas in postmortem pituitaries: classification and correlations to clinical data. *European Journal of Endocrinology* 2006 **154** 753–758.

14 Schlechte J, Dolan K, Sherman B, Chapler F & Luciano A. The natural history of untreated hyperprolactinemia: a prospective analysis. *Journal of Clinical Endocrinology and Metabolism* 1989 **68** 412–418.

15 Weiss MH, Teal J, Gott P, Wycoff R, Yadley R, Apuzzo ML, Giannotta SL, Kletzky O & March C. Natural history of microprolactinomas: six-year follow-up. *Neurosurgery* 1983 **12** 180–183.

16 Turner HE & Wass JA. Are markers of proliferation valuable in the histological assessment of pituitary tumours? *Pituitary* 1999 **1** 147–151.

17 Pernicone PJ, Scheithauer BW, Sebo TJ, Kovacs KT, Horvath E, Young WF Jr, Lloyd RV, Davis DH, Guthrie BL & Schoene WC. Pituitary carcinoma: a clinicopathologic study of 15 cases. *Cancer* 1997 **79** 804–812.

18 Scheithauer BW, Kovacs KT, Laws ER Jr & Randall RV. Pathology of invasive pituitary tumors with special reference to functional classification. *Journal of Neurosurgery* 1986 **65** 733–744.

19 DeLellis RA, Lloyd RV, Heitz PU & Eng C Tumour of the pituitary gland. In *World Health Organization Classification of Tumours: Pathology and Genetics of Tumours of Endocrine Organs.* Ch 1, pp 10–47. Eds RA DeLellis, RV Lloyd, PU Heitz & C Eng, Lyon: IARC Press 2004.

20 Al-Shraim M & Asa SL. The 2004 World Health Organization classification of pituitary tumors: what is new? *Acta Neuropatho-logica* 2006 **111** 1–7.

21 Scheithauer BW, Gaffey TA, Lloyd RV, Sebo TJ, Kovacs KT, Horvath E, Yapicier O, Young WF Jr, Meyer FB, Kuroki T, Riehle DL & Laws ER Jr. Pathobiology of pituitary adenomas and carcinomas. *Neurosurgery* 2006 **59** 341.

22 Bevan JS, Webster J & Burke CW. Dopamine agonists and pituitary tumour shrinkage. *Endocrine Reviews* 1992 **13** 221–235.

23 Liuzzi A, Dallabonzana D, Oppizzi G, Verde GG, Cozzi R, Chiodini P & Luccarelli G. Low dose of dopamine agonists in the long-term treatment of macroprolactinomas. *New England Journal of Medicine* 1985 **313** 656–659.

24 Wass JA, Moult PJ, Thorner MO, Dacie JE, Charlesworth M, Jones AE & Besser GM. Reduction of pituitary-tumour size in patients with prolactinomas and acromegaly treated with bromocriptine with or without radiotherapy. *Lancet* 1979 **2** 66–69.

25 Fleseriu M, Lee M, Pineyro MM, Skugor M, Reddy SK, Siraj ES & Hamrahian AH. Giant invasive pituitary prolactinoma with falsely low serum prolactin: the significance of 'hook effect'. *Journal of Neuro-oncology* 2006 **79** 41–43.

26 Wu ZB, Yu CJ, Su PZ, Zhuge QC, Wu JS & Zheng WM. Bromocriptine treatment of invasive giant prolactinomas invol-ving the cavernous sinus: results of a long-term follow up. *Journal of Neurosurgery* 2006 **104** 54–61.

27 Calle-Rodrigue RD, Giannini C, Scheithauer BW, Lloyd RV, Wollan PC, Kovacs KT, Stefaneanu L, Ebright AB, Abboud CF & Davis DH. Prolactinomas in male and female patients: a comparative clinicopathologic study. *Mayo Clinic Proceedings* 1998 **73** 1046–1052.

28 Ma W, Ikeda H & Yoshimoto T. Clinicopathologic study of 123 cases of prolactin-secreting pituitary adenomas with special reference to multihormone production and clonality of adeno-mas. *Cancer* 2002 **95** 166–258.

29 Cottier J-P, Destrieux C, Brunereau L, Bertrand P, Moreau L, Jan M & Herbreteau D. Cavernous sinus invasion by pituitary adenoma: MR imaging. *Radiology* 2000 **215** 463–469.

30 Molitch ME. Pharmacologic resistance in prolactinoma patients. *Pituitary* 2005 **8** 43–52.

31 Yildiz F, Zorlu F, Erbas T & Atahan L. Radiotherapy in the management of giant pituitary adenomas. *Radiotherapy and Oncology* 1999 **52** 233–237.

32 Di Sarno A, Landi ML, Marzullo P, Di Somma C, Pivonello R, Cerbone G, Lombardi G & Colao A. The effect of quinagolide and cabergoline, two selective dopamine receptor type 2 agonists, in the treatment of prolactinomas. *Clinical Endocrinology* 2000 **53** 53–60.

33 Di Sarno A, Landi ML, Cappabianca P, Di Salle F, Rossi FW, Pivonello R, Di Somma C, Faggiano A, Lombardi G & Colao A. Resistance to cabergoline as compared with bromocriptine in hyperprolactinemia: prevalence, clinical definition, and thera-peutic strategy. *Journal of Clinical Endocrinology and Metabolism* 2001 **86** 5256–5261.

34 Caccavelli L, Feron F, Morange I, Rouer E, Benarous R, Dewailly D, Jaquet P, Kordon C & Enjalbert A. Decreased expression of the two D2 dopamine receptor isoforms in bromocriptine-resistant prolactinomas. *Neuroendocrinology* 1994 **60** 314–322.

35 Thapar K, Yamada Y, Scheithauer B, Kovacs K & Stefaneanu L. Assessment of mitotic activity in pituitary adenomas and carcinomas. *Endocrine Pathology* 1996 **7** 215–221.

36 Thapar K, Kovacs K, Scheithauer BW, Stefaneanu L, Horvath E, Murray D & Laws ER Jr. Proliferative activity and invasiveness among pituitary adenomas and carcinomas: an analysis using the MIB-1 antibody. *Neurosurgery* 1996 **38** 99–106.

37 Hsu DW, Hakim F, Biller BMK, de la Monte S, Zervas NT, Klibanski A & Hadley-Whyte ET. Significance of proliferating cell nuclear antigen index in predicting pituitary adenoma recur-rence. *Journal of Neurosurgery* 1993 **78** 753–761.

38 Atkin SL, Green VL, Hipkin LJ, Landolt AM, Foy PM, Jeffreys RV & White MC. A comparison of proliferation indices in human anterior pituitary adenomas using formalin fixed tissue and *in vitro* cell culture. *Journal of Neurosurgery* 1997 **87** 85–88.

39 Honegger J, Prettin C, Feuerhake F, Ptrick M, Schulte-Monting J & Reincke M. Expression of Ki-67 antigen in nonfunctioning pituitary adenomas:correlation with growth velocity and invasiveness. *Journal of Neurosurgery* 2003 **99** 674–679.

40 Sherr CJ. Mammalian G1 cyclins. *Cell* 1993 **73** 1059–1065.

41 Jordan S, Lidhar K, Korbonits M, Lowe DG & Grossman AB. Cyclin D and cyclin E expression in normal and adenomatous pituitary. *European Journal of Endocrinology* 2000 **143** R1–R6.

42 Turner HE, Nagy ZS, Sullivan N, Esiri MM & Wass JAH. Expression analysis of cyclins in pituitary adenomas and the normal pituitary gland. *Clinical Endocrinology* 2000 **53** 337–344.

43 Pines J. Cyclins and cyclin-dependent kinases;theme and variations. *Advances in Cancer Research* 1995 **66** 181–212.

44 Betticher D, Thatcher N, Altermatt HJ, Hoban P, Ryder WD & Heighway J. Alternate splicing produces a novel cyclin D1 transcript. *Oncogene* 1995 **11** 1005–1011.

45 Matthias C, Branigan K, Jahnke V, Leder K, Haas J, Heighway J, Jones PW, Strange RC, Fryer AA & Hoban PR. Polymorphism in the cyclin D1 gene is associated with prognosis in patients with squamous cell carcinoma of the head and neck. *Clinical Cancer Research* 1998 **4** 2411–2418.

46 Simpson DJ, Fryer AA, Grossman AB, Wass JA, Pfeifer M, Kros JM, Clayton RN & Farrell WE. Cyclin D1 (CCND1) genotype is associated with tumour grade in sporadic pituitary adenomas. *Carcinogenesis* 2001 **22** 1801–1807.

47 Spada A, Mantovani G & Lania A. Pathogenesis of prolactinomas. *Pituitary* 2005 **8** 7–15.

48 Fedele M, Battista S, Kenyon L, Baldassarre G, Fidanza V, Klein-Szanto AJ, Parlow AF, Visone R, Pierantoni GM, Outwater E, Santoro M, Croce CM & Fusco A. Overexpression of the *HMGA2* gene in transgenic mice leads to the onset of pituitary adenomas. *Oncogene* 2002 **21** 3190–3198.

49 Finelli P, Pierantoni GM, Giardino D, Losa M, Rodeschini O, Fedele M, Valtorta E, Mortini P, Croce CM, Larizza L & Fusco A. The High Mobility Group A2 gene is amplified and overexpressed in human prolactinomas. *Cancer Research* 2002 **62** 2398–2405.

50 Edelman GM. Cell adhesion molecules in the regulation of animal form and tissue pattern. *Annual Review of Cell Biology* 1986 **2** 81–116.

51 Williams A & Barclay AN. The immunoglobulin superfamily, domains for cell surface recognition. *Annual Review of Immunology* 1988 **6** 381–405.

52 Sadoul R, Hirn M, Deagostini-Bazin H, Rougon G & Goridis C. Adult and embryonic mouse neural cell adhesion molecules have different binding properties. *Nature* 1983 **304** 347–349.

53 Jin L, Hemperly JJ & Lloyd RV. Expression of neural cell adhesion molecule in normal and neoplastic human neuroendocrine tissues. *American Journal of Pathology* 1991 **138** 961–969.

54 Figarella-Branger DF, Durbec PL & Rougon GN. Differential spectrum of expression of neural cell adhesion molecule isoforms and L1 adhesion molecules on human neuroectodermal tumors. *Cancer Research* 1990 **50** 6364–6370.

55 Komminoth P, Roth J, Lackie PM, Bitter-Suermann D & Heitz PU. Polysialic acid of the neural cell adhesion molecule distinguishes small cell lung carcinoma from carcinoids. *American Journal of Pathology* 1991 **139** 297–304.

56 Daniel L, Trouillas J, Renaud W, Chevallier P, Gouvernet J, Rougon G & Figarella-Branger D. Polysialylated-neural cell adhesion molecule expression in rat pituitary transplantable tumors (spontaneous mammotropic transplantable tumor in Wistar-Furth rats) is related to growth rate and malignancy. *Cancer Research* 2000 **60** 80–85.

57 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** 1084–1093.

58 Vleminckx K, Vakaet L Jr, Mareel M, Fiers W & van Roy F. Genetic manipulation of E-cadherin expression by epithelial tumor cells reveals an invasion suppressor role. *Cell* 1991 **66** 107–119.

59 Ozawa M, Baribault H & Kemler R. The cytoplasmic domain of the cell adhesion molecule uvomorulin associates with three independent proteins structurally related in different species. *EMBO Journal* 1989 **8** 1711–1717.

60 Gumbiner BM & McCrea PD. Catenins as mediators of the cytoplasmic functions of cadherins. *Journal of Cell Science* 1993 **17** 155–158.

61 Shibamoto S, Hayakawa M, Takeuchi K, Hori T, Miyazawa K, Kitamura N, Johnson KR, Wheelock MJ, Matsuyoshi N & Takeichi M. Association of p120, a tyrosine kinase substrate, with E-cadherin/catenin complexes. *Journal of Cell Biology* 1995 **128** 949–957.

62 Van Aken E, De Wever O, Correia da Rocha AS & Mareel M. Defective Ecadherin/catenin complexes in human cancer. *Virchows Archiv* 2001 **439** 725–751.

63 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** 1357–1365.

64 Kawamoto H, Mizoue T, Arita K, Tominaga A, Eguchi K & Kurisu K. Expression of epithelial cadherin and cavernous sinus invasion in human pituitary adenomas. *Journal of Neuro-Oncology* 1997 **34** 105–109.

65 Paez-Pereda M, Kuchenbauer F, Arzt E & Stalla GK. Regulation of pituitary hormones and cell proliferation by components of the extracellular matrix. *Brazilian Journal of Medical and Biological Research* 2005 **38** 1487–1494.

66 Kuchenbauer F, Theodoropoulou M, Hopfner U, Stalla J, Renner U, Tonn JC, Low MJ, Arzt E, Stalla GK & Paez-Pereda M. Laminin inhibits lactotroph proliferation and is reduced in early prolactinoma development. *Molecular and Cellular Endocrinology* 2003 **207** 13–20.

67 Murphy G & Docherty AJ. The matrix metalloproteinases and their inhibitors. *American Journal of Respiratory Cell and Molecular Biology* 1992 **7** 120–125.

68 Stetler-Stevenson WG, Liotta LA & Kleiner DE Jr. Extracellular matrix 6: role of matrix metalloproteinases in tumor invasion and metastasis. *FASEB Journal* 1993 **7** 1434–1441.

69 Sang QXA. Complex role of matrix metalloproteinases in angiogenesis. *Cell Research* 1998 **8** 171–177.

70 Knappe UJ, Hagel C, Lisboa BW, Wilczak W, Ludecke DK & Saeger W. Expression of serine proteases and metalloproteinases in human pituitary adenomas and anterior pituitary lobe tissue. *Acta Neuropathologica* 2003 **106** 471–478.

71 Turner HE, Nagy Z, Esiri MM, Harris AL & Wass JA. Role of matrix metalloproteinase 9 in pituitary tumor behavior. *Journal of Clinical Endocrinology and Metabolism* 2000 **85** 2931–2935.

72 Fan X & Childs GV. Epidermal growth factor and transforming growth factor-alpha messenger ribonucleic acids and their receptors in the rat anterior pituitary: localization and regulation. *Endocrinology* 1995 **136** 2284–2293.

73 Chabot JG, Walker P & Pelletier G. Distribution of epidermal growth factor binding sites in the adult rat anterior pituitary gland. *Peptides* 1986 **7** 45–50.

74 Jaffrain-Rea ML, Petrangeli E, Lubrano C, Minniti G, Di Stefano D, Sciarra F, Frati L, Tamburrano G, Cantore G & Gulino A. Epidermal growth factor binding sites in human pituitary macroadenomas. *Journal of Endocrinology* 1998 **158** 425–433.

75 Folkman J. What is the evidence that tumors are angiogenesis dependent? *Journal of the National Cancer Institute* 1990 **82** 4–6.

76 Turner HE, Nagy Z, Gatter KC, Esiri MM, Harris AL & Wass JA. Angiogenesis in pituitary adenomas and the normal pituitary gland. *Journal of Clinical Endocrinology and Metabolism* 2000 **85** 1159–1162.

77 de la Torre NG, Turner HE & Wass JA. Angiogenesis in prolactinomas: regulation and relationship with tumour behaviour. *Pituitary* 2005 **8** 17–23.

78 Turner HE, Nagy Z, Gatter KC, Esiri MM, Harris AL & Wass JA. Angiogenesis in pituitary adenomas – relationship to endocrine function, treatment and outcome. *Journal of Endocrinology* 2000 **165** 475–481.

79 Turner HE, Nagy Z, Gatter KC, Esiri MM, Wass JA & Harris AL. Proliferation, bcl-2 expression and angiogenesis in pituitary adenomas:relationship to tumour behaviour. *British Journal of Cancer* 2000 **82** 1441–1445.

80 Spada A, Mantovani G & Lania A. Pathogenesis of prolactinomas. *Pituitary* 2005 **8** 7–15.

81 Barbacid M. Ras genes. *Annual Review of Biochemistry* 1987 **56** 779–827.

82 Karga HJ, Alexander JM, Hedley-Whyte ET, Klibanski A & Jameson JL. Ras mutations in human pituitary tumors. *Journal of Clinical Endocrinology and Metabolism* 1992 **74** 914–919.

83 Cai WY, Alexander JM, Hedley-Whyte ET, Scheithauer BW, Jameson JL, Zervas NT & Klibanski A. Ras mutations in human prolactinomas and pituitary carcinomas. *Journal of Clinical Endocrinology and Metabolism* 1994 **78** 89–93.

84 Jacks T, Fazeli A, Schmitt EM, Bronson RT, Goodell MA & Weinberg RA. Effects of an Rb mutation in the mouse. *Nature* 1992 **359** 295–300.

85 Nakayama K, Ishida N, Shirane M, Inomata A, Inoue T, Shishido N, Horii I, Loh DY & Nakayama K. Mice lacking p27(Kip1) display increased body size, multiple organ hyperplasia, retinal dysplasia, and pituitary tumors. *Cell* 1996 **85** 707–720.

86 Pei L, Melmed S, Scheithauer B, Kovacs K, Benedict WF & Prager D. Frequent loss of heterozygosity at the retinoblastoma susceptibility gene (RB) locus in aggressive pituitary tumors: evidence for a chromosome 13 tumor suppressor gene other than RB. *Cancer Research* 1995 **55** 1613–1616.

87 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. *Journal of Clinical Endocrinology and Metabolism* 1997 **82** 818–824.

88 Herman V, Drazin NZ, Gonsky R & Melmed S. Molecular screening of pituitary adenomas for gene mutations and rearrangements. *Journal of Clinical Endocrinology and Metabolism* 1993 **77** 50–55.

89 Weil RJ, Vortmeyer AO, Huang S, Boni R, Lubensky IA, Pack S, Marx SJ, Zhuang Z & Oldfield EH. 11q13 allelic loss in pituitary tumors in patients with multiple endocrine neoplasia syndrome type 1. *Clinical Cancer Research* 1998 **4** 1673–1678.

90 Poncin J, Stevenaert A & Beckers A. Somatic *MEN1* gene mutation does not contribute significantly to sporadic pituitary tumorigenesis. *European Journal of Endocrinology* 1999 **140** 573–576.

91 Wenbin C, Asai A, Teramoto A, Sanno N & Kirino T. Mutations of the MEN1 tumor suppressor gene in sporadic pituitary tumors. *Cancer Letters* 1999 **142** 43–47.

92 Verges B, Boureille F, Goudet P, Murat A, Beckers A, Sassolas G, Cougard P, Chambe B, Montvernay C & Calender A. Pituitary disease in MEN type 1 (MEN1): data from the France-Belgium MEN1 multicenter study. *Journal of Clinical Endocrinology and Metabolism* 2002 **87** 457–465.

93 Burgess JR, Shepherd JJ, Parameswaran V, Hoffman L & Greenaway TM. Prolactinomas in a large kindred with multiple endocrine neoplasia type 1:clinical features and inheritance pattern. *Journal of Clinical Endocrinology and Metabolism* 1996 **81** 1841–1845.

94 Carty SE, Helm AK, Amico JA, Clarke MR, Foley TP, Watson CG & Mulvihill JJ. The variable penetrance and spectrum of manifestations of multiple endocrine neoplasia type 1. *Surgery* 1998 **124** 1106–1113.

95 Sakamoto H, Mori M, Taira M, Yoshida T, Matsukawa S, Shimizu K, Sekiguchi M, Terada M & Sugimura T. Transforming gene from human stomach cancers and a noncancerous portion of stomach mucosa. *PNAS* 1986 **83** 3997–4001.

96 Gonsky R, Herman V, Melmed S & Fagin J. Transforming DNA sequences present in human prolactin-secreting pituitary tumors. *Molecular Endocrinology* 1991 **5** 1687–1695.

97 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** 23–29.

98 Prezant TR, Kadioglu P & Melmed S. An intronless homolog of human protooncogene hPTTG is expressed in pituitary tumors: evidence for hPTTG family. *Journal of Clinical Endocrinology and Metabolism* 1999 **84** 1149–1152.

99 Zhang X, Horwitz GA, Prezant TR, Valentini A, Nakashima M, Bronstein MD & Melmed S. Structure, expression, and function of human pituitary tumor-transforming gene (PTTG). *Molecular Endocrinology* 1999 **13** 156–166.

100 Zou H, McGarry TJ, Bernal T & Kirschner MW. Identification of a vertebrate sister-chromatid separation inhibitor involved in transformation and tumorigenesis. *Science* 1999 **285** 418–422.

101 Heaney AP, Horwitz GA, Wang Z, Singson R & Melmed S. Early involvement of estrogen-induced pituitary tumor transforming gene and fibroblast growth factor expression in prolactinoma pathogenesis. *Nature Medicine* 1999 **5** 1317–1321.

102 Toffle RC, Webb SM, Tagatz GE, Taylor S, Nagel TC, Campbell B, Phipps W & Okagaki T. Pregnancy-induced changes in prolactinomas as assessed with computed tomography. *Journal of Reproductive Medicine* 1988 **33** 821–826.

103 Stefaneanu L, Kovacs K, Horvath E, Lloyd RV, Buchfelder M, Fahlbusch R & Smyth H. *In situ* hybridization study of estrogen receptor messenger ribonucleic acid in human adenohypophysial cells and pituitary adenomas. *Journal of Clinical Endocrinology and Metabolism* 1994 **78** 83–88.

104 Gillam MP, Molitch ME, Lombardi G & Colao A. Advances in the treatment of prolactinomas. *Endocrine Reviews* 2006 **27** 485–534.

105 Zhang X, Horwitz GA, Heaney AP, Nakashima M, Prezant TR, Bronstein MD & Melmed S. Pituitary tumor transforming gene (PTTG) expression in pituitary adenomas. *Journal of Clinical Endocrinology and Metabolism* 1999 **84** 761–767.

106 Pandey J, Bannout A & Wendell DL. The Edpm5 locus prevents the 'angiogenic switch' in an estrogen-induced rat pituitary tumor. *Carcinogenesis* 2004 **25** 1829–1838.

107 Kelly MA, Rubinstein M, Asa SL, Zhang G, Saez C, Bunzow JR, Allen RG, Hnasko R, Ben-Jonathan N, Grandy DK & Low MJ. Pituitary lactotroph hyperplasia and chronic hyperprolactinemia in dopamine D2 receptor-deficient mice. *Neuron* 1997 **19** 103–113.

108 Friedman E, Adams EF, Hoog A, Gejman PV, Carson E, Larsson C, De Marco L, Werner S, Fahlbusch R & Nordenskjold M. Normal structural dopamine type 2 receptor gene in prolactin-secreting and other pituitary tumors. *Journal of Clinical Endocrinology and Metabolism* 1994 **78** 568–574.