REVIEW

Current perspectives on the pathogenesis of clinically non-functioning pituitary tumours

N J L Gittoes

Department of Medicine, Queen Elizabeth Hospital, Edgbaston, Birmingham B15 2TH, UK

Introduction

Pituitary tumours are classified by the characteristic clinical syndromes that accompany tumour hormone production. All of the constituent hormone-secreting cell types of the anterior pituitary are capable of undergoing neoplastic change, although it is evident from histochemical analysis that adenomas derived from pure, functional lactotrophs, corticotrophs, somatotrophs, gonadotrophs and thyrotrophs are the exception rather than the rule. Tumours frequently express more than one hormone (Scheithauer *et al.* 1986, Kovacs & Horvath 1988), most frequently growth hormone (GH) and prolactin (PRL), although immunohistochemical detection of hormones is not a marker of true secretory function. Pituitary tumours that secrete intact hormones result in clinical syndromes of hormone hypersecretion – PRL causing clinical features of hyperprolactinaemia, GH causing acromegaly or gigantism, adrenocorticotrophic hormone (ACTH) causing Cushing's disease and thyroid-stimulating hormone (TSH) causing secondary hyperthyroidism. Approximately 25–30% of all anterior pituitary neoplasms, however, do not cause clinical syndromes of hormone hypersecretion and are termed non-functioning tumours (NFTs).

The term non-functioning is strictly a clinical term, as it is now clear from the results of immunohistochemistry that the majority of these tumours do indeed synthesise, and sometimes secrete, hormones (Arafah 1986), predominantly subunits of the glycoprotein hormones (GPHs; luteinizing hormone (LH), follicle-stimulating hormone (FSH) and TSH) or, occasionally, intact GPHs (Snyder *et al.* 1979, 1984, Snyder 1985, Black *et al.* 1987, Jameson *et al.* 1987, Klibanski 1987, Warnet *et al.* 1994). Figure 1 shows typical immunostaining properties of an NFT.

Tumour characterisation

The application of the technique of immunohistochemistry has revolutionised the classification of pituitary tumours in terms of their hormone expression. It has also become apparent, however, that the clinical entity of NFT encompasses a marked heterogeneity of neoplastic

pituitary cell types. By histopathological convention, NFTs are sometimes subdivided into pure null cell adenomas (17%) and oncocytomas (6%; Thapar *et al.* 1995). The distinction is purely morphological, and relates to the intracellular accumulation of mitochondria in oncocytomas to greater than 10% of the cell volume. There is evidence of non-neoplastic null cells scattered throughout the normal pituitary cytoarchitecture (Kovacs *et al.* 1980, Kontogeorgos *et al.* 1991) that probably represent transitional, undifferentiated or precursor cells, capable of shifting from a hormonally inactive resting state to a hormonally active differentiated state. Null cell adenomas may therefore be envisaged as neoplastic derivatives of such cells. No clinical or prognostic differences exist between null cell adenomas and oncocytomas.

'Silent' corticotroph adenomas represent a subcategory of clinically non-functioning tumours and account for up to 8% of NFTs (Kovacs 1985). These tumours have emerged as distinct clinicopathological entities (Horvath *et al.* 1980, 1988), but bear striking morphological and immunohistochemical resemblance to hormonally active corticotroph adenomas. They are not, however, accompanied by clinical or biochemical evidence of ACTH excess. Silent somatotroph adenomas have also been described (Warnet *et al.* 1994) and exhibit positive immunostaining for GH, but result in no clinical features of acromegaly. These tumours account for approximately 3% of all NFTs (Kovacs 1985).

In vitro studies have demonstrated expression of intact gonadotrophins or subunits of these GPHs in approximately 80% of NFTs (Samuels & Ridgway 1995). Pure α -subunit secreting pituitary adenomas have also been recognised, and represent approximately 7% of all NFTs (Snyder 1985). In view of the propensity of NFTs to express gonadotrophin subunits, the cell type of origin of these tumours is likely to be gonadotrophs in the majority of cases.

Pathogenesis

The pathogenesis of NFTs, like the majority of other pituitary tumour types, remains elusive. The potential

Figure 1 Typical immunostaining characteristics for anterior pituitary hormone expression in an NFT. Brown cytoplasmic colouration represents positive immunostaining. Panels A–D show results for GPH subunits (A, common α -subunit; B, FSH β ; C, LH β ; D, TSH β). Negative (blue) immunostaining is noted for other anterior pituitary hormones (E, ACTH; F, GH; G, prolactin).

Figure 2 Diagrammatic representation of possible aetiological factors involved in the genesis of NFTs. EGFR, epidermal growth factor receptor; LOH, loss of heterozygosity; ER, oestrogen receptor; RXR, retinoid X receptor; TR, thyroid hormone receptor; FGF, fibroblast growth factor; PKC, pyruvate kinase C.

mechanisms of oncogenesis can, however, be grouped into broad categories. Abnormalities of genes regulating growth and hormone secretions may be implicated, abnormalities of tumour suppressor genes that normally inhibit growth and proliferation may also be involved in tumorigenesis, as may alterations in genes involved in the induction of apoptosis. Figure 2 provides a summary of the possible aetiological factors that may be important in the development of NFTs.

Influence of hypothalamic trophic hormones on tumorigenesis

Hypothalamic releasing factors evoke specific hormonal responses in anterior pituitary cells, but also affect pituitary cell proliferation (Asa *et al.* 1984b, 1992, Wehrenberg *et al.* 1984, Gertz *et al.* 1987, Cella *et al.* 1990, Burton *et al.* 1991, Struthers *et al.* 1991, Stenzel-Poore *et al.* 1992). In this way, disordered hypothalamic control has been implicated in the aetiology of pituitary adenomas (reviewed by Faglia & Spada (1995)). The feedback tumour that rarely develops in patients with congenital adrenal hyperplasia (an ACTH-secreting pituitary tumour arising secondary to the absence of normal negative feedback effect of cortisol on ACTH secretion) provides evidence that unopposed hypothalamic stimulation can lead to tumour progression, although there are a number of factors against a primary

hypothalamic defect being the initiating step in pituitary tumorigenesis. Hyperplasia of somatotrophs or corticotrophs surrounding an adenoma is rarely seen in acromegaly and Cushing's disease, implying a more focal origin of the tumours as opposed to the release of a generalised trophic factor (Molitch 1987). Patients with an ectopic source of GH-releasing hormone hypersecretion (secondary to bronchial carcinoids, small cell lung carcinomas and pancreatic islet cell tumours) exhibit consequent somatotroph hyperplasia and GH hypersecretion, but rarely develop true somatotroph adenoma formation (Sano *et al.* 1988). Similarly, patients with Cushing's syndrome secondary to ectopic CRH hypersecretion do not develop corticotroph adenomas (Asa *et al.* 1984a, Carey *et al.* 1984). Furthermore, surgical resection of functioning pituitary tumours results in a definitive cure of excess hormone secretion in a significant proportion of tumours. These observations strongly suggest that pituitary adenomas are derived from an intrinsic defect of a single progenitor cell, leading to tumour formation, rather than from excessive polyclonal expansion of cells as a result of hypersecretion of hypothalamic trophic factors. Despite the unlikely role of hypothalamic factors in initiating the majority of pituitary tumours, it is likely that progression of tumour formation in a susceptible subtype of pituitary cells may be due to hypothalamic dysregulation (Faglia & Spada 1995).

Figure 3 Diagrammatic representation of the cAMP signalling cascade. Receptor (Rec) occupation results in activation of coupled G proteins, which serve to activate adenylate cyclase, causing conversion of ATP to cAMP. Activated cAMP-dependent protein kinases phosphorylate cAMP response element binding protein (CREB) which translocates to the nucleus and, via occupation of cAMP response elements (CRE), regulates transcription of genes involved in cellular growth and differentiation.

Activating genetic mutations

Clonality studies have revealed that, like the majority of pituitary tumour types, NFTs are monoclonal in origin (Alexander *et al.* 1990, Herman *et al.* 1990). Search for rearrangements or mutations of known candidate oncogenes in NFTs has largely been unfruitful, however. Of a total of 68 NFTs examined for oncogene (*myc*, *ras*, *bcl*, *Hst1*, *sea* and *fos*) amplifications or rearrangements, none was found to harbour these anomalies (Karga *et al.* 1992, Herman *et al.* 1993, Boggild *et al.* 1994). Mutations of the gene encoding the α -subunit of the membrane-bound stimulatory GTP-binding protein ($G_s\alpha$) transduction molecule (Lyons *et al.* 1990), more commonly associated with a subgroup of GH-secreting pituitary adenomas (Spada *et al.* 1990, Masters *et al.* 1990), have been described in approximately 10% of NFTs (Tordjman *et al.* 1993, Williamson *et al.* 1994). Mutations in the gene encoding $G_s\alpha$ result in formation of the *gsp* oncogene, a constitutively active form of $G_s\alpha$ that results in unabated activation of adenylate cyclase, increased cyclic AMP (cAMP) generation, increased intracellular calcium and activation of cAMP-dependent protein kinases leading to phosphorylation of the cAMP response element-binding protein (CREB), which mediates the transcriptional effects of cAMP (Fig. 3). This signalling pathway transduces messages that dictate cellular growth and

differentiation, and thus changes in the intrinsic activity of this mechanism result in enhanced proliferative capacity of tumour cells. Interestingly, Bertherat *et al.* (1995) have demonstrated increased concentrations of activated CREB in all GH-secreting tumours (compared with concentrations in NFTs), irrespective of the presence of the *gsp* oncogene. This finding suggests that mechanisms other than *gsp* mutations can lead to constitutive activation of CREB and thus to tumour formation.

Abnormal signal transduction pathways

The trophic effects of factors derived from the hypothalamus are mediated in cells of the anterior pituitary by cell surface receptors that are differentially expressed in a cell-type-specific pattern. A number of studies have looked for evidence of mutated cell surface receptors for hypothalamic releasing or inhibitory factors in pituitary tumours. As a result of the observation that a proportion of NFTs respond to administration of a bolus of thyrotrophin-releasing hormone (TRH) by a brisk increase in GPH subunit secretion (MacFarlane *et al.* 1982, Snyder 1985, Daneshdoost *et al.* 1991), the TRH receptor gene has been investigated for evidence of mutations. Of seven NFTs examined, none was found to harbour TRH receptor mutations (Faccenda *et al.* 1996). The dopamine

type 2 receptor gene has also been analysed in a series of NFTs and no mutations were detected (Friedman *et al.* 1994). Differential expression of somatostatin receptor subtypes has been demonstrated in NFTs, although no consistent pattern of subtype expression has been determined (Greenman & Melmed 1994, Miller *et al.* 1995, Panetta & Patel 1995, Murabe *et al.* 1996).

The membrane-bound protein kinase C (PKC) enzyme family regulates many cellular processes, including cell proliferation and differentiation, via serine or threonine phosphorylation of substrate proteins. Increased levels of expression and enhanced activity of PKC have been detected in NFTs compared with the findings in normal pituitaries (Alvaro *et al.* 1992). Furthermore, invasive tumours demonstrated higher levels of expression of PKC than did non-invasive tumours (Alvaro *et al.* 1992), suggesting a possible role for this important growth regulating enzyme in the genesis of NFTs and other pituitary adenomas.

Activins are members of the transforming growth factor- β (TGF β) cytokine family that act as pituitary cell mitogens and as modulators of hormone biosynthesis via cell surface receptors with transmembrane serine/threonine kinase activity (Mathews 1994). *In vitro*, exogenous activin has been shown to regulate pituitary cell proliferation (Billestrup *et al.* 1990, Bilezikjian *et al.* 1991), but, in addition, endogenous activin subunit expression has been demonstrated in pituitary adenomas (Haddad *et al.* 1994, Alexander *et al.* 1995), raising the possibility of an autocrine action for these growth factors. Alexander *et al.* (1996) determined the expression of activin/TGF β receptor subtype mRNAs in a series of pituitary adenomas, including nine NFTs and normal pituitaries. There was a difference in the pattern of activin/TGF β receptor expression between normal glands and tumours that may convey a growth advantage upon pituitary tumour cells.

Growth factors in pituitary tumorigenesis

The effects of many growth factors, including insulin like growth factor-I (IGF-I), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF) and epidermal growth factor (EGF) have been implicated in many human neoplasms (Zumkeller & Schofield 1995). The cellular effects of growth factors are mediated by high-affinity receptor tyrosine kinases that share a common intracellular signalling pathway. Ligand binding to the extracellular domain of this class of receptor results in activation of a cascade of downstream signalling molecules. Autophosphorylation of the liganded receptor results in recruitment of adapter proteins such as Grb2, which in turn serve to activate the guanine-nucleotide exchange factors *sos*, *ras*, mitogen activated protein (MAP) kinase kinase, MAP kinase kinase, MAP kinase and, ultimately, the nuclear transcription factors *jun* and *fos* (Fig. 4). Growth factor receptors may also influence gene

transcription via a more direct route. After ligand binding, the tyrosine kinase receptors can activate specific cytoplasmic proteins, which translocate to the nucleus and mediate transcriptional regulation (Montminy 1993). From the preceding description, it can be seen that a number of intermediary elements are involved in transducing the effects of growth factors, and abnormalities within any element of the activating cascade may be responsible for constitutive activation and unabated cell growth. Experimentally derived mutants of MAP kinase have been shown to exhibit such constitutive activity (Mansour *et al.* 1994). Furthermore, the MAP kinase signal transduction pathway has also been shown to regulate the expression of the GPH common α -subunit (Roberson *et al.* 1995) and the specific FSH β -subunit *in vitro* (Strahl *et al.* 1997), findings that may be pertinent to the frequent observation of GPH subunit expression in NFTs (Samuels & Ridgway 1995).

EGF and EGF receptor (EGFR) expression have been studied in a series of pituitary adenomas, and over-expression of EGFR was detected in 80% of NFTs examined, although EGFR was virtually undetectable in all functioning pituitary tumours (Chaidarun *et al.* 1994a). The response of tumour cells *in vitro* to EGF administration was also examined and showed accelerated tumour growth and up-regulation of EGFR mRNA expression (Chaidarun *et al.* 1994a), implicating a role for EGF and its receptor in the development, progression, or both, of NFTs.

FGF-4 (or heparin-binding secretory transforming gene, *hst*) is normally expressed exclusively in embryonic tissues, although it has been detected in a number of human neoplasms (Sakamoto *et al.* 1986). *hst* transfected rat pituitary cells have aggressive and invasive characteristics when injected subcutaneously (Shimon *et al.* 1996) and the observation of frequent expression of *hst* in prolactinomas suggests that it has a significant role in the pathogenesis of PRL-secreting adenomas (Gonsky *et al.* 1991). The gene product of *hst* has also been detected in 5% of other pituitary tumours, including NFTs (unpublished data in Shimon & Melmed 1997), suggesting a possible aetiological role of *hst* in a minority of NFTs.

Tumour suppressor genes

Much attention has focused on the importance of the role of tumour suppressor genes in down-regulating cellular growth. Tumour suppressor gene abnormalities promote neoplasia through loss of function of a protein normally involved in restricting cell growth. They usually act in a recessive manner and require that both alleles must be mutated (usually in the form of a gross chromosomal deletion) for the transformed phenotype to be fully expressed. Studies of loss of heterozygosity have demonstrated allelic deletions on chromosome 11q13 in 20% of NFTs (Boggild *et al.* 1994), indicating the possibility of a

Figure 4 Diagrammatic representation of the MAP kinase signal transduction pathway. Receptor (Rec) autophosphorylation (P) occurs upon ligand (L) binding. Recruitment of Grb2 and *sos* results in activation of *ras* and perpetuation of the cascade of MAP kinases (MAPK), ultimately causing activation of the transcription factors *jun* and *fos*, which serve to regulate important cellular activities, including growth and differentiation.

recessively acting tumour suppressor gene in this region. The recently cloned gene for multiple endocrine neoplasia (MEN)-type 1 has been found to reside at this chromosomal location (Chandrasekharappa *et al.* 1997), hence the so-called 'menin' gene product is also a likely candidate in the pathogenesis of sporadic pituitary tumours. Mutations of the *p53* tumour suppressor gene are common events in many human cancers (Hollstein *et al.* 1991, 1996), although in three series with a total of 186 pituitary tumours investigated, including 72 NFTs, no *p53* gene mutations were detected (Herman *et al.* 1993, Levy *et al.* 1994, Boggild *et al.* 1994).

The retinoblastoma (Rb) gene has been the focus of much attention as a candidate for pituitary tumorigenesis via loss of heterozygosity. The Rb gene product has important regulatory properties in the cell cycle, controlling differentiation and survival. Homozygous inactivation of the Rb alleles results in the formation of retinoblastomas, and loss of heterozygosity of the Rb gene in mice results in pro-opiomelanocortin-expressing pituitary tumours (Jacks *et al.* 1992, Hu *et al.* 1994). Search for loss of heterozygosity in the chromosomal region (13q) of the Rb gene in human pituitary tumours (secretory and non-secretory)

has revealed interesting results. One group identified no loss of either Rb allele (Woloschak *et al.* 1994), although other groups have detected loss of heterozygosity in the region of the Rb gene (Pei *et al.* 1995, Pearce *et al.* 1996). Further studies revealed, however, that Rb protein was detectable by immunohistochemistry in those tumours with loss of heterozygosity on chromosome 13, indicating that a novel (non-Rb) candidate tumour suppressor gene may be present at this location (Pei *et al.* 1995).

Cyclin-dependent kinase (CDK) inhibitors have a pivotal role in controlling cell cycle progression. CDK4 phosphorylates the product of the Rb gene which results in inactivation of its ability to regulate the cell cycle (Weinberg 1995). The complexity of this system is further enhanced by the presence of a specific inhibitor of CDK4, p16. p16 acts to maintain the integrity and function of the Rb protein, but has been detected at lower levels in some pituitary tumours, including 14 NFTs (Woloschak *et al.* 1996), than in normal pituitaries. Although no structural abnormalities of the p16 gene or loss of heterozygosity at its chromosomal location have been identified, p16 remains of possible aetiological significance in pituitary tumorigenesis.

The so-called metastasising suppressor gene, nm23, is a purine binding factor that has been detected in reduced quantities in highly metastatic cancers (Bevilacqua *et al.* 1989). nm23 expression has been determined in a number of pituitary tumours, including ten NFTs (Takino *et al.* 1995). Reduced expression of the H2 isoform mRNA and protein was detected in invasive tumours and there was an inverse correlation between nm23 mRNA concentrations and the tendency for cavernous sinus invasion. There was no evidence of mutations in the nm23 gene in any of the invasive tumours, however.

Abnormal expression of nuclear steroid/thyroid hormone receptors

Although mutations leading to altered expression of oncogene or tumour suppressor gene products may have a pathogenic role in NFTs, a change in hormonal regulation of pituitary gene expression may contribute to uncontrolled tumour cell proliferation. Recently, a number of reports of abnormal expression of nuclear steroid/thyroid hormone receptors have been described in NFTs. We have documented reduced expression of isoforms of thyroid hormone receptors and retinoid X receptors in NFTs (Gittoes *et al.* 1997a,b) compared with the findings in normal pituitaries. Absent, or reduced, expression of oestrogen receptor (α) protein has also been demonstrated in NFTs (Nakao *et al.* 1989, Friend *et al.* 1994, Stefaneanu *et al.* 1994, Zafar *et al.* 1995, Gittoes *et al.* 1997a). The latter observation is of particular relevance, as NFTs are believed to be derived from cells of the gonadotroph lineage and thus would be expected to express oestrogen receptor (Friend *et al.* 1994, Zafar *et al.* 1995). Reduced expression of oestrogen receptor in this cell type may therefore be expected to mediate inadequate negative feedback, perhaps leading to autonomous secretory and growth characteristics. Recently, tumour-specific expression of oestrogen receptor mRNA splice variants has been described in some NFTs and other pituitary tumours (Chaidarun *et al.* 1997).

The functional significance of abnormalities of nuclear steroid/thyroid hormone receptor expression is not as yet determined, although the ligands for these receptors (for example thyroid hormone, retinoic acid and oestrogens) are known to be important regulators of growth and development in various tissues, including the pituitary (Shupnik *et al.* 1989, Lloyd *et al.* 1991, Koga *et al.* 1992, Chomczynski *et al.* 1993, Chaidarun *et al.* 1994b, Weiss & Refetoff 1996). Abnormal expression of nuclear steroid/thyroid hormone receptors in NFTs may therefore be expected to influence the growth pattern, and thus the overall biological behaviour of these tumours.

Pituitary tumour transforming genes

Recently, a potent transforming gene has been isolated from a rat GH-secreting pituitary tumour cell line,

designated pituitary tumour-transforming gene (PTTG; Pei & Melmed 1997). PTTG is not expressed in normal pituitaries but when over-exposed in 3T3 fibroblasts, induces cellular transformation *in vitro*. Injection of transfected 3T3 cells into athymic nude mice also results in rapid formation of tumours. It remains to be determined whether this gene or related genes have a pathogenic role in human pituitary tumours.

Summary and future research directions

The heterogeneity of pituitary tumour subtypes that are clinically manifest as NFTs, makes it highly unlikely that there is a single common aetiological event. Multiple molecular events, possibly acting independently, or more probably synergistically, are likely to be necessary for tumour initiation and progression (illustrated in Fig. 2). Early events in tumorigenesis are likely to involve chromosomal mutations, resulting perhaps in oncogene activation or loss of tumour suppressor genes. Subsequently, permissive factors, such as aberrant hypothalamic hormone receptor signals and dysregulated control of growth and differentiation, are likely to be important in allowing clonal expansion of the transformed pituitary cell.

In view of the probable multifactorial nature of pituitary tumorigenesis, the scope of future research is likely to remain diverse. In terms of initiating events in tumorigenesis, however, considerable interest will focus upon the recently characterised gene responsible for MEN-type 1 (Chandrasekharappa *et al.* 1997). A significant proportion of sporadic pituitary tumours harbour loss of heterozygosity of chromosome 11q13 (Boggild *et al.* 1994), and it is therefore likely that loss of this gene product (menin, which bears no apparent sequence homology with any previously described proteins) has a crucial role in sporadic pituitary tumorigenesis. An alternative strategy, recently successfully applied by Pei & Melmed (1997) to isolate the PTTG gene from a rat GH-secreting cell line, involves differential display RT-PCR to delineate pituitary tumour-specific genes. This approach is appealing with respect to human pituitary tumours, although the cellular heterogeneity often observed in pituitary adenomas significantly reduces the feasibility of this approach.

To determine the pathogenesis of NFTs, it may be pertinent to elucidate a characteristic feature of this tumour type, such as GPH subunit expression. Expression of GPH subunits is known to be regulated by gonadal steroids and thyroid hormone, and our own work has shown that receptors for these ligands are present in reduced quantities in NFTs (Gittoes *et al.* 1997a). For the future, it will be interesting to investigate the effects of transfecting these receptors into primary cultures of NFTs to determine whether there is any significant change in tumour growth or secretory capacity. The MAP kinase signal transduction pathway has also been shown to be

involved in transcriptional regulation of GPH subunits (Roberson *et al.* 1995, Strahl *et al.* 1997). To date there has been no 'systematic' analysis of this pathway in NFTs, and abnormalities along this complex chain of intermediaries may therefore be involved in altered GPH synthesis and, possibly, cellular growth.

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