Advances in Our Understanding of Pituitary Adenoma

a report by

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Pituitary adenomas are common benign monoclonal neoplasms – accounting for 15% of intracranial neoplasms – that may be clinically silent or secrete anterior pituitary hormones such as prolactin, growth hormone (GH), adrenocorticotrophic hormone (ACTH) or, rarely, thyroid-stimulating hormone (TSH) or gonadotrophins. Radiological studies for other reasons using high-resolution computed tomography (CT) or magnetic resonance imaging (MRI) detect incidental pituitary adenomas in approximately 20% of asymptomatic patients.[1] The incidence of the various types of adenoma varies;[2] prolactinomas are the most common pituitary adenomas. Clinically non-functioning pituitary adenomas (NFPAs), which do not secrete hormones, often cause local mass symptoms and represent one-third of pituitary adenomas. GH- and ACTH-producing adenomas each account for 10–15% of pituitary adenomas, while TSH-producing adenomas are rare. Pituitary adenomas are infrequent in childhood: fewer than 10% of pituitary adenomas are diagnosed before 20 years of age.[3] These adenomas can be either micro- or macroadenomas. The natural course of microadenomas is that a few tumours enlarge over a period of more than eight years.

Although several genes and signalling pathways have been identified as important factors in the development of pituitary tumours, our understanding of pituitary tumorigenesis remains incomplete and is the focus of current research. The reason for this is that current treatment modalities fail to completely control this disorder and prevent the associated morbidity and mortality. This article reviews the advances in our understanding of pituitary adenoma, especially in the field of pathogenesis of pituitary tumours, and the possibility of new therapeutic approaches.

Why Do Pituitary Microadenomas Fail to Grow?

Pituitary microadenomas, which have a diameter of less than 1cm, are exceedingly common, with a prevalence of 25% at autopsy and pituitary imaging.[4] Most microadenomas remain clinically occult and stable in size, without an increase in tumour cells and without local mass effects. Recent studies have attempted to explain this cessation of growth, which would not be expected to occur in such tissue. It was thought that apoptosis might play a role in curtailing outgrowth, but this did not explain the stable size for several years. The lack of induction of vascular stroma was thought to be relevant, but again this was an insufficient explanation. The proliferative activity of microadenomas is low, which indicates that the growth of tumour cells is arrested.

The hypothesis is that after a certain number of cell divisions cells display a change in cell phenotype, i.e. they enter into a senescence-like state.[5] Currently, the likely factor in growth arrest in pituitary microadenomas is thought to be oncogene-induced cellular senescence.[6] The likelihood that the majority of pituitary adenomas are monoclonal in origin suggests that neoplasia arises either by oncogene activation or by inactivation of tumour-suppressor genes.[7] Upregulation of oncogenes plays a role in the pathogenesis of pituitary tumours, and oncogene-induced senescence results from the activation of powerful antiproliferative signalling networks (activated cell-cycle-progression inhibitors), which prevent the outgrowth of early neoplastic lesions driven by oncogenes.[8] One of the upregulated oncogenes is the pituitary tumour-transforming gene (Pttg), which facilitates cell-cycle progression, maintains chromosomal stability and mediates tumorigenesis.[9,10]

In a transgenic model, an overexpression of Pttg causes cell transformation and induces aneuploidy and tumorigenesis.[11] On the other hand, mice lacking Pttg have pituitary hypoplasia and the endocrine glands are senescent; these Pttg-null mice are protected from tumorigenesis.[12] Pttg abundance correlates with pituitary tumour invasiveness, recurrence and prognosis.[13] Furthermore, Pttg deletion selectively activates the pituitary pathway with decreased pituitary-cell proliferation, which may be a future target to restrain tumour growth. Nowadays, it seems that the premature proliferative arrest of pituitary adenomas underlies their failure to enlarge and progress to malignancy.

Recent Advances in Understanding Somatotroph Tumorigenesis

The only oncogenes associated with GH-secreting adenomas are mutations of GNAS1, the gene encoding for the α subunit of the stimulatory G protein (Gs), i.e. the *gsp* oncogene.[14] The stimulatory Gs is a ubiquitously expressed protein belonging to the family of heterotrimeric G proteins. At the pituitary level, Gs is required for the activation of

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adenylyl cyclase and the generation of cyclic adenosine monophosphate (cAMP) in somatotroph and corticotroph cells in response to GH-releasing hormone (GHRH) and corticotroph-releasing hormone, respectively. Mutations in Gs are characterised by extremely high adenylyl cyclase activity and no further stimulation of cAMP levels. cAMP represents a mitogenic signal in somatotrophs. Despite the growth potential of this mutation (*gsp* oncogene) demonstrated *in vitro*, patients carrying this mutation have a similar clinical and biochemical phenotype to those who do not carry it.

It has been demonstrated that tumours expressing the *gsp* oncogene are highly sensitive to somatostatin.[7,15,16] These results are consistent with *in vitro* studies showing increased sensitivity to somatostatin analogues in cells obtained from tumours with the *gsp* oncogene and the occurrence of resistance to somatostatin among the negative adenomas only. Consistent with a potential role of the cAMP pathway in pituitary tumorigenesis, particular attention has been paid to the gene that encodes the type 1 α regulatory subunit of protein kinase A (PRKAR1), which is crucial for intracellular protection against unrestrained enzyme activity. Inactivating mutations of this gene have been identified in the multiple neoplasia syndrome: the Carney complex, a syndrome including GH-secreting adenomas.[17–19] PRKAR1 expression has been found to be reduced in both secreting and non-secreting sporadic pituitary tumours. Although mutational changes in molecules involved in the cAMP-dependent pathways have been associated with somatotroph proliferation, patients harbouring these mutations do not differ from those who do not, meaning that there is a complex cross-signalling between as yet undiscovered proliferative and antiproliferative inputs. Somatotroph tumorigenesis will be discussed in the section regarding familial-isolated pituitary adenomas (FIPA).

Pituitary Tumours that Occur in a Hereditary Setting

Multiple endocrine neoplasia type 1 (MEN1) is defined as a hereditary endocrine tumour syndrome. MEN1 is an autosomal-dominant condition associated with the occurrence of parathyroid, enteropancreatic and pituitary tumours.[20] The gene that causes MEN1 is localised to chomosome 11q13, identified as MEN1, a gene that encodes a protein called menin.[21,22] Over 1,000 mutations in the MEN1 gene have been reported. MEN1 acts as a tumour-suppressor gene, MEN1 syndrome can occur in families or sporadically and MEN-1-associated pituitary adenomas are more aggressive than sporadic and most are macroadenomas. The most common subtype of pituitary adenomas are prolactinomas.

MEN1-like syndrome (MEN4) relates to mutations in the CDKN1B gene that encodes p27 protein, which is the intracellular antiproliferative pathway protein.[23,24] This syndrome consists of GH-secreting pituitary tumour, hyperparathyroidism and renal and testicular cancer. Other combinations are also possible, such as Cushing's disease, cervical carcinoid tumour and hyperparathyroidism. The clinical relevance of MEN1 screening is that the MEN1 carrier is periodically followed in order to recognise the tumour early. As for whether a young child at risk of mutation should undergo sequencing and then periodic monitoring, the consensus recommends periodic surveillance for tumours in known MEN1 carriers beginning at five years of age.[25] Research in these hereditary pituitary tumours is important since most investigators consider that the gene for a rare hereditary disorder also has important roles in common tumours of similar types. Menin pathways could be a potential drug target in the future.

Familial-isolated Pituitary Adenomas

FIPAs are isolated pituitary tumours that occur in families and are in a MEN-like state, in which GH is often oversecreted. Familial acromegaly has been described in the literature, and in an international study performed by Daly et al. 64 FIPA families were identified.[26,27] Tumours in these families are macroadenomas and occur at a young age. Recently, genetic linkage studies pointed to a region of chromosome 11q13 that differs from the MEN1 gene region as the area for the idiopathic familial somatotrophinoma gene.[28] In 2006, Vierimaa et al. reported that mutations in the aryl hydrocarbon receptor-interacting protein gene (AIP) occurred in association with familial acromegaly.[29] Today, we know that FIPA is not limited to acromegaly, but includes all pituitary adenoma subtypes.[29–31] The 330-amino-acid AIP is a molecular chaperone protein involved in the functional maturation of aryl hydrocarbon receptor (AhR), the nuclear receptor for the environmental toxin dioxin.[32,33] AIP has been shown to both increase and decrease the function of AhR. Loss of heterozygosity at the AIP locus implies germline mutations of AIP in FIPA families.[34] Not all of the families tested positive for this mutation. Those patients with AIP mutations were significantly younger than patients with FIPA without AIP mutations.

Immunostaining of normal pituitary with a monoclonal antibody revealed AIP staining in the cytoplasm of somatotroph and prolactin cells. Familial somatotroph tumours also stained positive for AIP, which is found in association with secretory granule.[34] Although an absence of AIP protein on immunostaining is likely with mutations, Leontiou and colleagues suggested that using AIP staining for screening for AIP mutations is not appropriate because AIP staining was observed in some tumours with AIP mutations. It is not clear how this molecular chaperone is involved in tumorigenesis. Leontiou and colleagues overexpressed AIP in GH3 pituitary cell lines, which dramatically reduced cell proliferation, whereas mutant AIP lost this ability.[34] Thus, AIP suppresses cell proliferation. AIP mutations occur in 15% of familial pituitary adenoma syndromes. AIP was found to be a candidate gene in patients with familial acromegaly. AIP mutations are thought to cause the loss of interactions with other proteins such as heat shock protein 90 (hsp90) and AhR.[35–37] AIP activity is associated with the modulation of phosphodiesterase (PDE4A5), and changes in this enzyme may potentially be related to tumorigenesis. The question remains unanswered regarding the genetic cause for familial acromegaly in 16 of the 22 familial cases in the study of Leontiou and colleagues, in whom they have failed to find an exonic mutation of AIP. In a recent study, Daly et al. showed that 50% of pure acromegalic familial cases had no mutation in the coding region of the AIP.

These studies have revealed that pituitary tumours may be more common than previously thought and that they occur in a familial setting. Families bearing AIP mutations have more aggressive pituitary tumours seen at a younger age; therefore, it is valuable to test for AIP mutations in families with FIPAs. FIPAs are thought to be rare, although these recent findings have raised awareness and FIPAs are becoming recognised with increased frequency. At this stage, AIP mutations are thought not to play an important role in the pathogenesis of sporadic pituitary tumours – at least in Canadian and US patients.[38,39]

Recent Advances in Non-functioning Pituitary Adenomas

One of the most common forms of pituitary adenomas, usually diagnosed incidentally with increased frequency, are NFPAs. Following immunostaining, these tumours show hormone synthesis and most are of gonadotroph origin, although they can be corticotroph or

somatotroph. As they do not release hormones and do not cause clinical symptoms, they are known as silent adenomas and are usually large in size (macroadenomas). There are several hypotheses why silent adenomas do not release hormones. This may be due to incorrect packaging in the Golgi apparatus, preferential secretion of inactive molecules and translational and post-translational abnormalities.

Previously clinically silent hormone-positive NFPAs can emerge as clinically hormone-releasing lesions at a later recurrence of the tumour. This has been described in cases of ACTH, GH and thyroid-stimulating-hormone-secreting adenomas.[40] NFPAs are usually macroadenomas, and some of these tumours have a more aggressive clinical course and a higher recurrence rate. The oncogenic pathways activated in NFPA are the Ras-BRAF-mitogen-activated protein kinase (MAPK) pathway and the Wnt-signalling pathway. In an elegant study, Korbonits et al. observed significant upregulation of the BRAF gene in NFPA, but did not observe any mutations in the BRAF gene, which is commonly mutated in both melanomas and papillary thyroid carcinomas.[41] Mutations are not a frequent finding in pituitary adenomas. Others found that changes in the Wnt pathways are implicated in pituitary tumorigenesis.[42–45]

Another interesting feature of NFPA is the lack of response to somatostatin analogues despite the presence of somatostatin receptors. What is even more puzzling is that these tumours do respond to somatostatin analogues *in vitro* (octreotide, paseriotide).[46] In an attempt to answer the question of why NFPAs do not respond to somatostatin analogues, Korbonits et al. observed *in vitro* significant inhibition of proliferative cell pathways (ERK) and upregulation of p27, an antiproliferative cellular pathway.[41] The lack of *in vivo* effects of somatostatin analogues is thought to be due to the induction of vascular endothelial growth factor (VEGF) by stimulation of somatostatin receptor 5 (SSTR5).[47] Thus, somatostatin receptor analogues, through activation of SSTR5, stimulate VEGF expression and cell proliferation. VEGF and VEGF receptors have been shown to be overexpressed in NFPA.[48] Another reason why somatostatin analogues are not effective may be due to increased seladin expression in NFPAs, as seladin is known to prevent apoptosis.[49] Furthermore, Zac1, a Zn- finger protein that regulates apoptosis, is downregulated in most pituitary adenomas. Somatostatin analogues increase Zac1 gene expression, and if Zac1 is downregulated, cells are unresponsive to somatostatin analogues.[50]

As some NFPAs demonstrate an aggressive and invasive clinical course and somatostatin analogue therapy is ineffective, fortunately new treatments have recently become available. Temozolomide is a novel drug that was originally used in the treatment of cerebral gliomas and metastatic melanomas. It is an alkylating agent and inhibits angiogenesis in tumour tissue.[51] Temozolomide was first used in a patient with pituitary carcinoma in 2006 and subsequently in prolactinomas.[52–55] Some patients responded to treatment and others did not, depending on the DNA methyltransferase (MGMT) expression.[56]

Conclusion

Small but significant advances in the field of pathogenesis of sporadic, familial and inherited pituitary tumours allow us to better understand the clinical presentation, and new pathophysiology brings novel therapeutic targets. ■

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1. Elster AD, *Radiology*, 1993;187:1–14.
2. Klibanski A, Zervas NT, *N Engl J Med*, 1991;324:822–31.
3. Kane LA, Leinung MC, Scheithauer BW, et al., *J Clin Endocrin Metab*, 1994;79:1135–40.
4. Burrow GN, Wortzman G, Rewcastle NB, et al., *N Engl J Med*, 1981;304:156–8.
5. Serrano M, Lin A, McCurrach M, et al., *Cell*, 1997;88:593–602.
6. Mooi WJ, Peeper DS, *N Engl J Med*, 2006;355:1037–46.
7. Lania A, Mantovani G, Spada A, *Eur J Endocrinol*, 2001;145:543–59.
8. Asa SL, Ezzat S, *Nat Rev Cancer*, 2002;2:836–49.
9. Zhang X, Howitz GA, Heaney AP, et al., *J Clin Endocrinol Metab*, 1999;84:761–7.
10. Musat M, Korbonits M, Kola B, et al., *Endocr Relat Cancer*, 2005;12(2):423–33.
11. Yu R, Lu W, Chen J, et al., *Endocrinology*, 2003;144:4991–8.
12. Chesnokova V, Zonis S, Rubinek T, et al., *Cancer Res*, 2007;67:10564–72.
13. Filippella M, Galland F, Kujas M, et al., *Clin Endocrinol (Oxf)*, 2006;65:536–43.
14. Vallar L, Spada A, Giannattasio G, *Nature*, 1987;330:566–8.
15. Spada A, Arosio M, Bochicchio D, et al., *J Clin Endocrinol Metab*, 1990;71:1421–6.
16. Barlier A, Gunz G, Zamora AJ, et al., *J Clin Endocrinol Metab*, 1998;83:1604–10.
17. Casey M, Vaughan CJ, He J, et al., *J Clin Invest*, 2000;106:R31–8.
18. Kirschner LS, Carney JA, Pack SD, et al., *Nat Genet*, 2000;26:89–92.
19. Stratakis CA, Kirschner LS, Carney JA, *J Clin Endocrinol Metab,* 2001;86:4041–6.
20. Brandi ML, Gagel RF, Angeli A, et al., *J Clin Endocrinol Metab*, 2001;86:5658–71.
21. Larsson C, Skogseid B, Oberg K, Nakamura Y, Nordenskjold M, *Nature*, 1988;332:85–7.
22. Chandrasekharappa SC, Guru SC, Manickam P, et al., *Science*, 1997;276:404–7.
23. Fritz A, Walch A, Piotrowska K, et al., *Cancer Res*, 2002;62: 3048–51.
24. Pellegata NS, Quintanilla-Martinez L, Siggelkow H, et al., *Proc Natl Acad Sci U S A*, 2006; 103:15558–63.
25. Brandi ML, Gagel RF, Angeli A, et al., *J Clin Endocrinol Metab*, 2001;86:5658–71.
26. Daly AF, Jaffrain-Rea ML, Ciccarelli A, et al., *J Clin Endocrinol Metab*, 2006;91:3316–23.
27. Beckers A, Daly AF, *Eur J Endocrinol*, 2007;157:371–82.
28. Soares B, Eguchi K, Frohman L, *J Clin Endocrinol Metab*, 2005;90:6580–87.
29. Vierimaa O, Georgitsi M, Lehtonen R, et al., *Science*, 2006;312:1228–30.
30. Daly AF, Vanbellinghen JF, Khoo SK, et al., *J Clin Endocrinol Metab*, 2007;92:1891–6.
31. Toledo RA, Lourenco DM Jr, Liberman B, et al., *J Clin Endocrinol Metab*, 2007;92:1934–7.
32. Carver LA, LaPres JJ, Jain S, et al., *J Biol Chem*, 1998;273: 33580–87.
33. Meyer BK, Perdew GH, *Biochemistry*, 1999;38:8907–17.
34. Leontiou CA, Gueorguiev M, van der Spuy J, et al., *J Clin Endocrinol Metab*, 2008;93:2390–2401.
35. Bell DR, Poland A, *J Biol Chem*, 2000;275:36407–14.
36. Meyer BK, Petrulis JR, Perdew GH,*Cell Stress Chaperones*, 2000; 5:243–54.
37. Petrulis JR, Perdew GH, *Chem Biol Interact*, 2002;141:25–40.
38. Di Giovanni R, Serra S, Ezzat S, Asa SL, *Endocr Pathol*, 2007;18:76–8.
39. Yu R, Bonert V, Saporta I, et al., *J Clin Endocrinol Metab*, 2006;91:5126–9.
40. Daems T, Verhelst J, Michotte A, et al., *Pituitary*, 2008; Epub ahead of print.
41. Ewing I, Pedder-Smith S, Franchi G, et al., *Clin Endocrinol*, 2007;66:348–52.
42. Tziortzioti V, Ruebel KH, Kuroki T, et al., *Endocr Pathol*, 2001;12:125–36.
43. Xu B, Samo T, Yoshimoto K, Yamada S, *Endocr Pathol*, 2002;13:341–51.
44. Sano T, Rong QZ, Kagawa N, Yamada S, *Front Horm Res*, 2004;32:127–32.
45. Oikonomou E, Barreto DC, Soares D, et al., *J Neurooncol*, 2005;73:205–9.
46. Hubina E, Nanzer AM, Hanson MR, et al., *Eur J Endocrinol*, 2006;155:371–9.
47. Zatelli MC, Piccin D, Vignali C, et al., *Endocr Relat Cancer*, 2007; 14:91–102.
48. McCabe CJ, Boelaert K, Tannahill LA, et al., *J Clin Endocrinol Metab*, 2002;87:4238–44.
49. Luciani P, Gelmini S, Ferrante E, et al., *J Clin Endocrinol Metab*, 2005;90:6156–61.
50. Theodoropoulou M, Zhang J, Laupheimer S, et al., *Cancer Res*, 2006;66: 1576–82.
51. Kurzen H, Schmitt S, Naher H, Mohler T, *Anticancer Drugs*, 2003;14:515–22.
52. Fadul CE, Kominsky AL, Meyer LP, et al., *J Neurosurg*, 2006;105:621–6.
53. Syro LV, Uribe H, Penagos LC, et al., *Clin Endocrinol*, 2006;65:552–3.
54. Kovacs K, Horvath E, Syro LV, et al., *Hum Pathol*, 2007;38: 185–9.
55. Neff LM, Weil M, Cole A, et al., *Pituitary*, 2007;10:81–6.
56. Kovacs K, Scheithauer BW, Lombardero M, et al., *Acta Neuropathol*, 2008;115:261–2.