Endocrine, *vol. 28, no. 1, 43–47, October 2005* 0969–711X/05/28:43–47/$30.00

Genetics and Proteomics of Pituitary Tumors

Sylvia L. Asa and Shereen Ezzat

Endocrine Oncology Site Group, Mount Sinai and Princess Margaret Hospitals, University of Toronto, Toronto, Ontario, Canada

Genetics and proteomics determine structure and function of normal tissues, and the molecular alterations that underlie tumorigenesis result in changes in these aspects of tissue biology in neoplasms. We review the known genetic alterations in pituitary tumors. These include the oncogenic Gsα protein (*GSP*)–activating mutations, and pituitary tumor–derived fibroblast growth factor receptor-4 (ptd-FGFR4), as well as tumor suppressor gene mutations associated with multiple endocrine neoplasia type 1 (MEN1). Other candidates identified from expression profiling include pituitary tumor–transforming gene (PTTG), GADD45, and bone morphogenic protein (BMP)4. Proteomic changes in pituitary tumors include classical alterations identified by immunohistochemistry as well as epigenetic reductions in p27. The underlying mechanisms for dysregulated cell adhesive molecules including cadherins and FGFRs are reviewed. The combined use of genetic and proteomic approaches will enhance novel drug therapeutic development.

Key Words: Pituitary tumor; proteomics; genetics.

Introduction

Genetics and proteomics represent the key determinants of structure and function in normal and abnormal tissues. In the case of tumors, these analyses provide evidence of molecular alterations that underlie the pathogenesis of neoplasia and the resulting functional aspects of the proliferative tissue.

Despite their relatively common frequency and potential for significant morbidity, the etiology of most pituitary tumors remains unknown. Hypotheses regarding their pathogenetic origin have vacillated in the last two decades from hormonal stimulation to genetic alterations. The former was supported by the remarkable biological plasticity that anterior pituitary displays in responding to physiological demands, as exemplified by lactotroph differentiation and proliferation during pregnancy or thyrotroph hyperplasia in primary hypothyroidism. These reversible changes are mediated by an array of signaling events that have supported the role of hormonal stimulation in the pathogenesis of pituitary adenomas *(1)*. However, molecular biology has proven that pituitary adenomas are monoclonal neoplasms. The molecular hypothesis assumes that a somatic defect is necessary and sufficient for pituitary neoplastic transformation. The evidence supporting this genetic concept has been reviewed *(2,3)*. An integrated approach recognizes a role for dysregulated hormonal or growth factor signaling as mediators of proliferation of transformed cells.

Received June 13, 2005; Accepted July 14, 2005.

Author to whom all correspondence and reprint requests should be addressed: Sylvia L. Asa, MD, PhD, 610 University Ave. #4-302, Toronto, Ontario, M5G-2M9, Canada. E-mail: sylvia.asa@uhn.on.ca

Genetics

Oncogenic Factors

The first candidate in the search for genetic alterations was the G-protein alphastimulating activity polypeptide (*GSP*). Activating mutations of this protein that lead to constitutive elevation of adenylyl cyclase activity have been identified in nearly a third of somatotroph adenomas *(4)*. Interestingly, *GSP* mutations are more frequently detected in the maternal allele *(5)*, providing an example of a maternally imprinted gene in pituitary tumorigenesis. The presence of this mutation appears to correlate with a densely granulated ultrastructural morphology of somatotroph tumors *(6,7)* and possibly with greater GH responsiveness to inhibition by the somatostatin analog octreotide *(8)*. Tumors with *GSP* mutations are associated with higher circulating levels of the free glycoprotein α-subunit due to its production by tumor cells *(9)*.

Based on the findings that mutations of Gαq result in constitutive activation of phospholipase C and possess transforming potential, pituitary adenomas have also been screened for mutations in this G protein. No mutations were identified in the conserved GTP-binding and hydrolysis domains of Gαq or the highly similar Gα11 *(10)*. In contrast to the stimulatory effects of the *GSP* mutations, inactivating mutations in the α-subunit of the inhibitory Gi2α coupling protein GIP2 have been identified.

GHRH stimulates somatotroph proliferation *(11)* and results in somatotroph hyperplasia in patients with ectopic GHRH-producing endocrine carcinomas. Older transgenic mice overexpressing GHRH develop pituitary adenomas *(12)*. Moreover, intrapituitary GHRH overexpression has been identified in aggressive somatotroph tumors *(13)*. However, most human GH-producing pituitary adenomas are not

accompanied by any evidence of hyperplasia *(14)*. These data indicate that GHRH stimulation may play a role as a promoter of cell proliferation in transformed somatotrophs. However, no intrinsic constitutively active forms of the GHRH receptor have been identified in human pituitary adenomas. It is unlikely that GHRH plays a principal role in the majority of pituitary tumors.

An interesting observation was the identification of a kinase-containing variant of FGFR4 with an alternative initiation site *(15–17)* that is expressed by pituitary tumors *(18, 19)*. This pituitary tumor-derived (ptd)–FGFR4 isoform (ptd-FGFR4) is transforming in vitro and in vivo and when selectively expressed in the pituitary, results in pituitary tumor formation in transgenic mice *(18)*.

Tumor Suppressor Genes

The identification of the putative tumor suppressor gene encoding the nuclear protein menin was felt to hold the answer to the molecular basis of pituitary tumors. Although genetic analysis has proven that there is germline mutation and loss if the intact allele in tumors associated with the MEN-1 syndrome, disappointingly, there is no evidence that mutations of the *MEN1* gene play a significant role in the sporadic pituitary tumors that form the vast majority of these lesions *(20)* or that downregulation of this gene is implicated *(21)*. Nevertheless, the possibility that a component of the encoded menin signaling cascade plays a role in pituitary tumor pathogenesis remains a possibility.

Approaches to Defining New Candidate Pituitary Oncogenes and Tumor Suppressors: Expression Profiling

After excluding well-characterized oncogenes and tumor suppressors implicated in the genesis of non-endocrine solid neoplasias, it became apparent that a tissue-specific search would be required. Two major strategies have been applied. The first involved differential display techniques. A differential mRNA expression analysis of transformed rat pituitary tumor cells identified the pituitary-tumor transforming gene (*PTTG*) *(22)*. The protein product of *PTTG* has been identified as a securin family member that regulates chromatid separation during mitosis *(23)*. The extent to which *PTTG* contributes to human pituitary tumor formation, therefore, remains unclear.

A cDNA microarray approach singled the folate receptor gene as being significantly overexpressed in non-functioning pituitary adenomas compared with other types of adenomas *(24)*. Subsequently, a cDNA representational differential display study comparing normal human pituitary tissue and clinically non-functioning pituitary adenomas identified GADD45 as a candidate gene that was highly underrepresented *(25)*. The biological plausibility of this finding was supported based on the features of GADD45 as a member of a growth arrest and DNA damage-inducible genes.

mRNA differential display studies also characterized the bone morphogenic protein (BMP) inhibitor noggin as being downregulated and BMP4 as upregulated in lactotroph adenomas from dopamine D2-receptor–deficient mice and in human pituitary tumors. BMP4 was further shown to selectively stimulate lactotroph cell proliferation through a Smad4-dependent pathway *(26)*.

Validation of New Candidates

Establishing causality between genetic alterations and tumorigenesis requires fulfillment of a number of criteria. First, it requires demonstration that the protein of interest is present in the cell type in question with significant deregulation compared to other unaffected cells of the same lineage. Second, experiments of nature must dictate that the deregulated expression of the candidate gene occurs through somatic mutation or epigenetic alteration. Third, gain or loss of function of the putative factor in a controlled cell model should influence some measure of growth including transformation and/or proliferation. Finally, gain or loss of the putative factor in a genetically altered mouse model should yield morphologic features of a pituitary adenoma. Introduction of some of the putative factors discussed here such as the activating mutant GSP allele has been shown in transgenic mice to lead to hyperplasia and adenoma formation in thyroid follicles but not in the pituitary *(27)*. The mouse model of the *MEN1* inactivation displays features of multiple endocrine tumors, including prolactin-producing pituitary adenomas characterized by somatic loss of the wild-type allele reminiscent of patients with MEN1 *(28)*.

Proteomics

Although proteomic studies of pituitary adenomas are not reported using novel proteomics technology such as 3D gel electrophoresis, or high-throughput techniques like Maldi- or Seldi-tof, this is a field that has used a targeted approach to proteomics through immunohistochemistry. Since the 1970s, the classification of pituitary adenomas has been based on their hormonal secretory profile. The addition of electron microscopy contributed significantly to the detailed classification, for example, by identifying sparsely and densely granulated variants of hormone-secreting cell types, and experts have rapidly identified immunohistochemical markers, such as keratin fibrous bodies, as surrogate proteomic markers of these variants. The last decade or more has seen a large body of literature emerge concerning the transcription factors that regulate cell differentiation and hormonal activity of adenohypophysial cells *(29,30)*. The best predictive markers remain those that subclassify adenomas accurately based on hormone content and cell structure. For example, among acromegalics who fail surgical resection, response to long-acting somatostatin analogs is best predicted by the subtype of somatotroph adenoma as densely or sparsely granulated *(8,31)*.

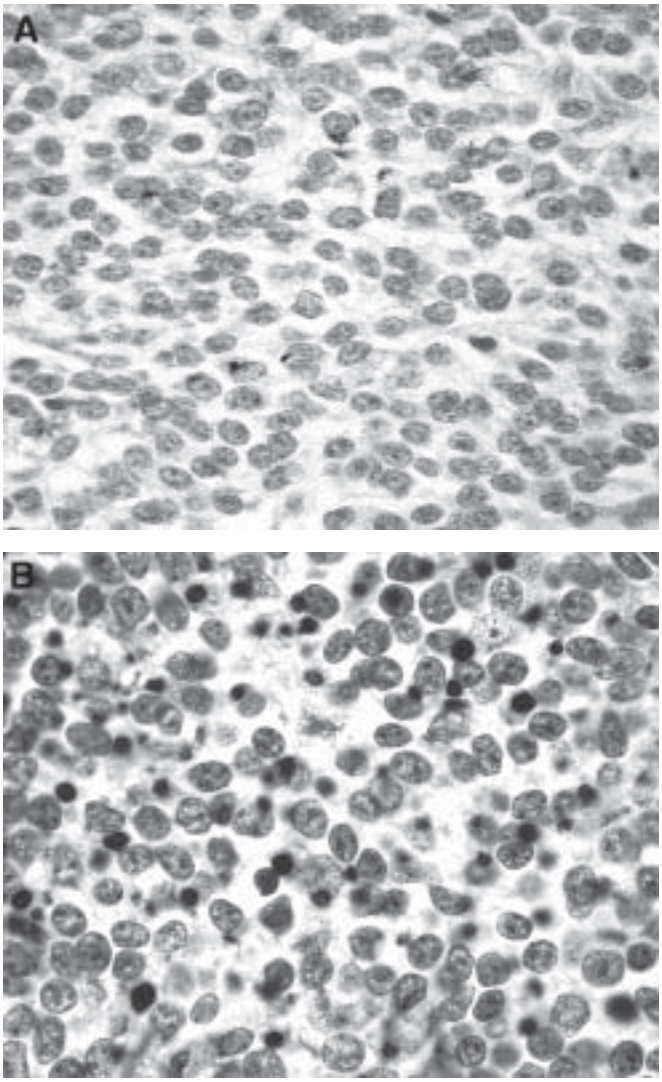

Fig. 1. The value of keratin profiling in somatotroph adenomas. (**A**) A densely granulated somatotroph adenoma has a weak perinuclear staining pattern for low-molecular-weight cytokeratins with the Cam 5.2 antibody. (**B**) In contrast, a sparsely granulated somatotroph adenoma has a characteristic globular juxtanuclear fibrous body that is the hallmark of this tumor type.

This finding renders the value of a Cam 5.2 keratin stain (Fig. 1) more important than almost any other immunostain in this setting. The diagnosis of a densely granulated lactotroph adenoma almost always predicts a bad prognosis, whereas a sparsely granulated lactotroph adenoma is most likely to respond to dopamine agonists. A silent corticotroph adenoma will recur more often and more aggressively than a silent gonadotroph adenoma.

Proteomic approaches have led to new insights in pituitary tumor pathogenesis. The role of cell cycle inhibitors in pituitary tumorigenesis has been emphasized by the marked pituitary alterations identified in mouse models of gene deletion. $p27^{kip1}$–null mice develop multiorgan neoplasia, including pituitary tumors *(32–34)*. Mice lacking both $p18^{ink4}$ and $p27^{kip1}$ succumb to lethal pituitary adenomas by 3 mo of age *(35)*. The expression of p27 is reduced in pituitary adenomas (Fig. 2), mainly in ACTH-producing tumors *(36–39)*. Hypermethylation resulting in decreased protein expression of p16 is detected in adenomas of gonadotroph lineage *(40)*. In contrast to these models, another mouse model of pituitary tumorigenesis, deficiency of the retinoblastoma (Rb) gene, does not seem to have human application. While the mice develop pituitary corticotroph adenomas arising in the intermediate lobe *(41)*, human tumors have no mutation or loss of heterozygosity (LOH) at the Rb locus *(42,43)*. This may be attributed to unique features of intermediate lobe corticotrophs that do not apply in humans.

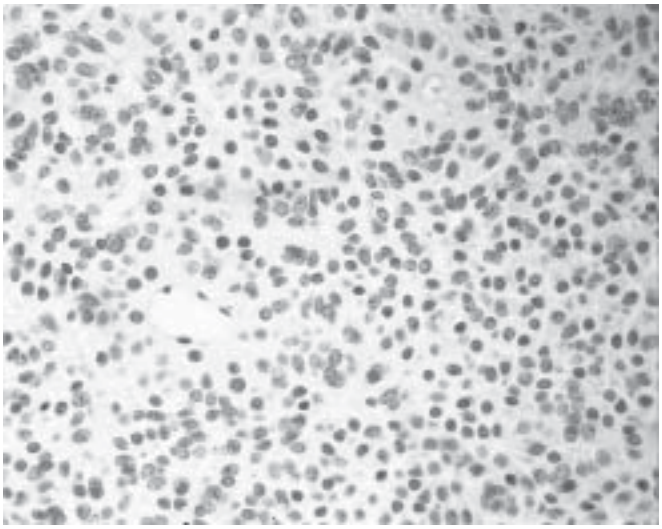

Fig. 2. A corticotroph adenoma shows almost total loss of nuclear p27 immunoreactivity.

Recently, it was shown that distinct FGFR4 isoforms display markedly different effects on endocrine cell adhesiveness by altering a multiprotein cell adhesion complex involving NCAM and N-cadherin *(44)*. In contrast to wild-type FGFR4, which maintains affinity for the extracellular matrix, ptd-FGFR4 results in marked loss of affinity to this extracellular matrix and disrupts normal cell adhesiveness *(45)*. This information sheds light on the previously published observation that the polysialated form of NCAM (PSA-NCAM) is highly expressed in pituitary adenomas where it correlates with tumor growth and invasiveness *(46)*. Polysialation has been proposed to involve steric inhibition of membrane–membrane apposition and cell adhesiveness, based on PSA's biophysical properties *(47)*. The disruption of NCAM/FGFR4 pro-adhesive complexes by ptd- FGFR4 represents an alternative mechanism for interruption of NCAM-mediated cell adhesive functions. ptd-FGFR4 expression is associated with diminished and ectopic cytoplasmic expression of N-cadherin *(45)*. Moreover, uncoupling of cadherins from the cytoskeleton disrupts β-catenin stability. Interestingly, ptd-FGFR4, but not FGFR4 in the absence or presence of FGF stimulation, is effective at destabilizing β-catenin from

N-cadherin with resultant diminished β-catenin expression *(45)*. Again, reduced β-catenin expression has been a well-recognized feature of human pituitary adenomas that correlates with tumor invasiveness *(48)*. The altered stromal adhesiveness of cells expressing ptd-FGFR4 explains a key feature of pituitary neoplasia. Loss of the reticulin network represents the morphological hallmark of the transition from hyperplasia to adenoma and is a diagnostic marker for neoplasia *(49)*. Neoplastic pituitary cells are characterized by their ability to form solid nests or trabecula in the absence of a stromal support or framework. Disruption of distinct NCAM/N-cadherin and possibly other proadhesive complexes by ptd-FGFR4 provides a novel tumorigenic mechanism that explains the pathobiology of proliferative and infiltrative but non-metastasizing pituitary neoplasms.

Conclusions

The individual contributions of components involved in the complex cascade of events leading to human neoplasia is finally being identified by genomic and proteomic approaches. In the pituitary, it is clear that there is a delicate balance maintained by many different factors, including hormones, growth factors, adhesion molecules, and cell cycle regulators. It is important to emphasize that no single factor can effectively explain the many facets of the tumorigenic process. In some tumors, the genetic alterations resulting from mutation represent a primary event; in others the hormonal environment may be the trigger. Other epigenetic factors may result in altered proteomic profiles that may play permissive or modulatory roles. A clearer understanding of the various genetic and proteomic changes in pituitary tumors, will lead to therapies aimed at specific interruption of their signaling cascades, permitting more effective strategies in the management of pituitary tumors.

References

1. Ezzat, S. (2001). *Brain Pathol.* **11,** 356–370.
2. Asa, S. L. and Ezzat, S. (1998). *Endocr. Rev.* **19,** 798–827.
3. Asa, S. L. and Ezzat, S. (2002). *Nat. Rev. Cancer* **2,** 836–849.
4. Spada, A., Vallar, L., and Faglia, G. (1992). *Trends Endocrinol. Metab.* **3,** 355–360.
5. Hayward, B. E., Barlier, A., Korbonits, M., et al. (2001). *J. Clin. Invest.* **107,** R31–R36.
6. Landis, C. A., Harsh, G., Lyons, J., Davis, R. L., McCormick, F., and Bourne, H. R. (1990). *J. Clin. Endocrinol. Metab.* **71,** 1416–1420.
7. Spada, A., Arosio, M., Bochicchio, D., et al. (1990). *J. Clin. Endocrinol. Metab.* **71,** 1421–1426.
8. Ezzat, S., Kontogeorgos, G., Redelmeier, D. A., Horvath, E., Harris, A. G., and Kovacs, K. (1995). *Eur. J. Endocrinol.* **133,** 686–690.
9. Harris, P. E., Alexander, J. M., Bikkal, H. A., et al. (1992). *J. Clin. Endocrinol. Metab.* **75,** 918–923.
10. Oyesiku, N. M., Evans, C.-O., Brown, M. R., Blevins, L. S., Tindall, G. T., and Parks, J. S. (1997). *J. Clin. Endocrinol. Metab.* **82,** 4184–4188.
11. Sano, T., Asa, S. L., and Kovacs, K. (1988). *Endocr. Rev.* **9,** 357–373.
12. Asa, S. L., Kovacs, K., Stefaneanu, L., et al. (1992). *Endocrinology* **131,** 2083–2089.
13. Thapar, K., Kovacs, K., Stefaneanu, L., et al. (1997). *Am. J. Pathol.* **151,** 769–784.
14. Ezzat, S., Asa, S. L., Stefaneanu, L., et al. (1994). *J. Clin. Endocrinol. Metab.* **78,** 555–560.
15. Abbass, S. A. A., Asa, S. L., and Ezzat, S. (1997). *J. Clin. Endocrinol. Metab.* **82,** 1160–1166.
16. Yu, S., Asa, S. L., Weigel, R. J., and Ezzat, S. (2003). *J. Biol. Chem.* **278,** 19597–19602.
17. Ezzat, S., Yu, S., and Asa, S. L. (2003). *Am. J. Pathol.* **163,** 1177–1184.
18. Ezzat, S., Zheng, L., Zhu, X. F., Wu, G. E., and Asa, S. L. (2002). *J. Clin. Invest.* **109,** 69–78.
19. Qian, Z. R., Sano, T., Asa, S. L., et al. (2004). *J. Clin. Endocrinol. Metab.* **89,** 1904–1911.
20. Zhuang, Z., Ezzat, S., Vortmeyer, A. O., et al. (1997). *Cancer Res.* **57,** 5446–5451.
21. Asa, S. L., Somers, K., and Ezzat, S. (1998). *J. Clin. Endocrinol. Metab.* **83,** 3210–3212.
22. Pei, L. and Melmed, S. (1997). *Mol. Endocrinol.* **11,** 433–441.
23. Zou, H., McGarry, T. J., Bernal, T., and Kirschner, M. W. (1999). *Science* **285,** 418–422.
24. Evans, C. O., Young, A. N., Brown, M. R., et al. (2001). *J. Clin. Endocrinol. Metab.* **86,** 3097–3107.
25. Zhang, X., Sun, H., Danila, D. C., et al. (2002). *J. Clin. Endocrinol. Metab.* **87,** 1262–1267.
26. Paez-Pereda, M., Giacomini, D., Refojo, D., et al. (2003). *Proc. Natl. Acad. Sci. USA* **100,** 1034–1039.
27. Michiels, F. M., Caillou, B., Talbot, M., et al. (1994). *Proc. Natl. Acad. Sci. USA* **91,** 10488–10492.
28. Crabtree, J. S., Scacheri, P. C., Ward, J. M., et al. (2001). *Proc. Natl. Acad. Sci. USA* **98,** 1118–1123.
29. Asa, S. L. and Ezzat, S. (1999). *Pituitary* **1,** 159–168.
30. Asa, S. L. and Ezzat, S. (2004). *Front. Horm. Res.* **32,** 1–19.
31. Bhayana, S., Booth, G., Kovacs, K., Asa, S. L., and Ezzat, S. (2005). *J. Clin. Endocrinol. Metab.,* in press.
32. Nakayama, K., Ishida, N., Shirane, M., et al. (1996). *Cell* **85,** 707–720.
33. Kiyokawa, H., Kineman, R. D., Manova-Todorova, K. O., et al. (1996). *Cell* **85,** 721–732.
34. Fero, M. L., Rivkin, M., Tasch, M., et al. (1996). *Cell* **85,** 733–744.
35. Franklin, D. S., Godfrey, V. L., Lee, H., et al. (1998). *Genes Develop.* **12,** 2899–2911.
36. Jin, L., Qian, X., Kulig, E., et al. (1997). *Am. J. Pathol.* **151,** 509–519.
37. Bamberger, C. M., Fehn, M., Bamberger, A. M., et al. (1999). *Eur. J. Endocrinol.* **140,** 250–255.
38. Dahia, P. L., Aguiar, R. C., Honegger, J., et al. (1998). *Oncogene* **16,** 69–76.
39. Lidhar, K., Korbonits, M., Jordan, S., et al. (1999). *J. Clin. Endocrinol. Metab.* **84,** 3823–3830.
40. Simpson, D. J., Bicknell, J. E., McNicol, A. M., Clayton, R. N., and Farrell, W. E. (1999). *Genes Chromosomes Cancer* **24,** 328–336.
41. Jacks, T., Fazeli, A., Schmitt, E. M., Bronson, R. T., Goodell, M. A., and Weinberg, R. A. (1992). *Nature* **359,** 295–300.
42. Zhu, J., Leon, S. P., Beggs, A. H., Busque, L., Gilliland, D. G., and Black, P. M. (1994). *J. Clin. Endocrinol. Metab.* **78,** 922–927.
43. Simpson, D. J., Hibberts, N. A., McNicol, A. M., Clayton, R. N., and Farrell, W. E. (2000). *Cancer Res.* **60,** 1211–1216.
44. Cavallaro, U., Niedermeyer, J., Fuxa, M., and Christofori, G. (2001). *Nat. Cell Biol.* **3,** 650–657.

45. Ezzat, S., Zheng, L., and Asa, S. L. (2004). *Mol. Endocrinol.* **18,** 2543–2552.
46. Daniel, L., Trouillas, J., Renaud, W., et al. (2000). *Cancer Res.* **60,** 80–85.
47. Fujimoto, I., Bruses, J. L., and Rutishauser, U. (2001). *J. Biol. Chem.* **276,** 31745–31751.
48. Qian, Z. R., Li, C. C., Yamasaki, H., et al. (2002). *Mod. Pathol.* **15,** 1357–1365.
49. Asa, S. L. (1998). *Tumors of the pituitary gland. Atlas of tumor pathology*, Third Series, Fascicle 22. Armed Forces Institute of Pathology: Washington, DC.