THE FOLLICULOSTELLATE CELLS IN THE PITUITARY GLAND

D. C. Danila
Massachusetts General Hospital and Harvard Medical School, Boston

The pituitary cells are regulated by numerous endocrine, paracrine and autocrine feed-back pathways, and their hormone secretion exerts major control over the function of several endocrine glands as well as a wide range of physiologic states. In the anterior pituitary, secretory cells are in close interconnection with folliculostellate cells, agranular functional units of an interactive endocrine networking. First described in 1953 by Rinehart *et al*. (1), accounting for 5-10% of cells in the anterior pituitary, the role of the folliculostellate cells in the pituitary has been increasingly recognized and further characterized by recent studies. It has been suggested that the folliculostellate cells, derived from neuroectoderm, form a three dimensional network, supporting and modulating endocrine cells by intercellular communication in a paracrine manner (2, 3). In the pituitary, it has been shown that a subset of pituitary adenomas has significant numbers of folliculostellate cells (4, 5) and, furthermore, pituitary tumors consisting only of folliculostellate cells have been described (4).

Folliculostellate cells also produce a number of bioactive peptides including basic fibroblast growth factor (6), vascular epithelial growth factor (7), IL-6 (8), and neuronal nitric oxide synthesis (9). It has been demonstrated that folliculostellate cells also produce lipocortin-1 (10), a key inhibitory mediator of glucocorticoids on corticotrophin secretion, at both the hypothalamic and pituitary levels (11). The regulatory interactions between folliculostellate and lactotroph cells (12, 13) and the role of folliculostellate cells in immune system modulation (14, 15) have also been reported. Activin belongs to the transforming growth factor-ß family of cytokines and function as both growth and differentiation factors in a variety of cell types. Locally secreted activin in the normal pituitary is a potent factor in controlling hormone biosynthesis and secretion (16, 17). Follistatin, a glycosylated monomeric protein that binds to activin, has been shown to be

Correspondence to: Daniel C. Danila, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114, ddanila@partners.org

Acta Endocrinologica (Buc), vol. I, no. 1, p. 86-93, 2005

expressed in gonadotropes and folliculostellate cells in the pituitary (18, 19) and therefore regulates FSH biosynthesis and secretion indirectly, mostly through paracrine effects (20).

Human pituitary tumors *in vitro* studies using short-term primary cell culture have significantly advanced the learning of hormone regulation and tumor genesis (19, 21). However, technical difficulties and the limited viability and progressive loss of differentiated functions of these cells, together with the paucity of well characterized human cell lines of pituitary lineage have hindered the advancement in studying pituitary functions (22). Cell lines originating from anterior pituitary secretory cells that apparently developed spontaneously or that were immortalized using a temperature-sensitive mutant of simian virus 40 large T antigen have been described. These human epithelial-like endocrine tumor cells could provide models for the studies of cell proliferation and regulation of hormonal secretion (22).

To better understand the function of pituitary FS cells, a spontaneously transformed human cell line of folliculostellate origin, PDFS, derived from a pituitary gonadotroph adenoma was characterized (23). Tumor fragments from a 71-yr-old man with a clinically nonfunctioning pituitary macroadenoma were obtained after transsphenoidal surgery, tissue was enzymatically dispersed and nonfibroblastic cells were maintained in culture for over 1 yr and in excess of 70 passages.

The folliculostellate origin of this cell line was demonstrated by the lack of Pit-1 or any of the anterior pituitary hormones mRNA expression, and the presence of folliculostellate specific vimentin, and S-100 protein. These cells show an epithelial spindle-like morphology with large nuclei with multiple nucleoli and numerous mitotic figures, with abundant cytoplasm. Electron microscopy demonstrated intercellular junctions, desmosomes, and multiple filopodia, and no evidence of secretory granules (Fig. 1). As previously seen with spontaneously transformed cell lines, cytogenetic analysis revealed that all cells were hyperdiploid as a result of multiple clonal trisomies and tetrasomies. Structural clonal aberrations were present, including rearrangements of a chromosome X short arm, chromosome 1 long arm and chromosome 7 short arm.

The doubling time of the cells in optimum culture media was approximately 24 hours. In an anchorage-independent growth assay, PDFS cells formed colonies in soft agar similar to the human carcinoma cell line U2OS (Fig. 2), demonstrating that the cells had undergone immortalization. Cell malignant transformation is usually caused by oncogene activation or inactivation of tumor suppressor genes such as p53. p53 gene is upregulated in response to cellular stresses to induce G1 arrest in cells with functional Rb protein, whereas in cells lacking functional Rb, p53 induces apoptosis. Although it has been previously demonstrated that mutations in p53 gene are not involved in the pathogenesis of the majority of human pituitary tumors (24), during establishment of immortalized cell lines, p53 was found to be frequently mutated. In the PDFS cells, a point mutation in p53 gene caused protein

conformation changes, preserving the protein size and the nuclear localization, but likely interfering in the clearance of the protein and resulting in its accumulation within the cell (13). PDFS cells lack the expression of MEG3, a novel growth suppressor shown to play an important role in the development of human pituitary adenomas (25). *GADD45*, a member of growth arrest and DNA damage-inducible gene family, has been shown to be present in normal pituitary tissue and absent in the majority of human pituitary tumors. In colony formation assays, transfection of human *GADD45* cDNA into PDFS cells results in a dramatic decrease in cell growth (26).

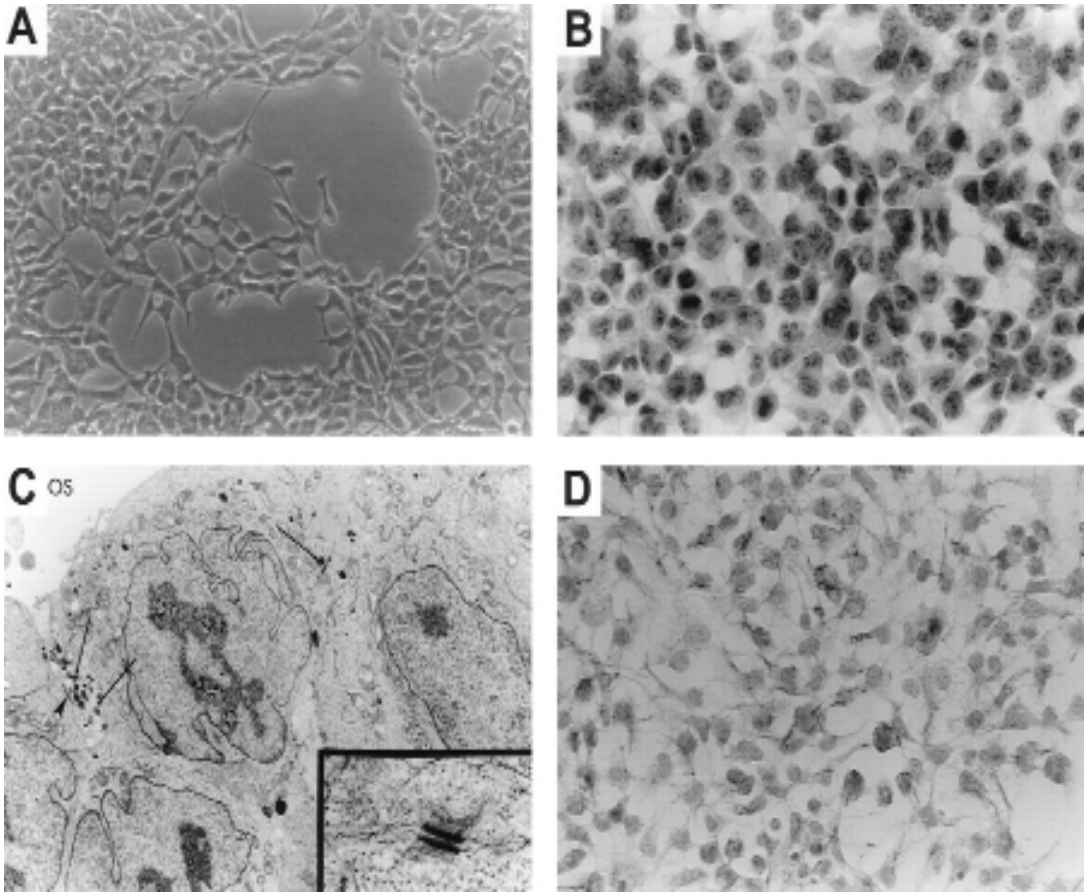

Figure 1. The morphology of PDFS cells. A, Phase contrast micrograph of PDFS cells in monolayer culture. Note the long cytoplasmic processes formed by cells in monolayer (magnification, ×100). B, Hematoxylin- and eosin-stained PDFS cells showing pleomorphism, coarse chromatin, multiple nucleoli, and numerous mitotic figures (magnification, ×400). C, Electron micrograph of a group of PDFS cells showing many primary and secondary lysosomes (arrows), intercellular junctions (arrowheads), and filopodia along the cells free surfaces projecting into the open space (OS; original magnification, ×6600). Inset, A desmosome at high magnification (×62,000). D, Immunohistochemistry staining for vimentin of PDFS cells showing that the individual cells express vimentin in their cytoplasm (counterstained with hematoxylin; magnification, ×400). (From Danila DC et al. J Clin Endocrinol Metab 85:1180-7 Copyright 2000, The Endocrine Society).

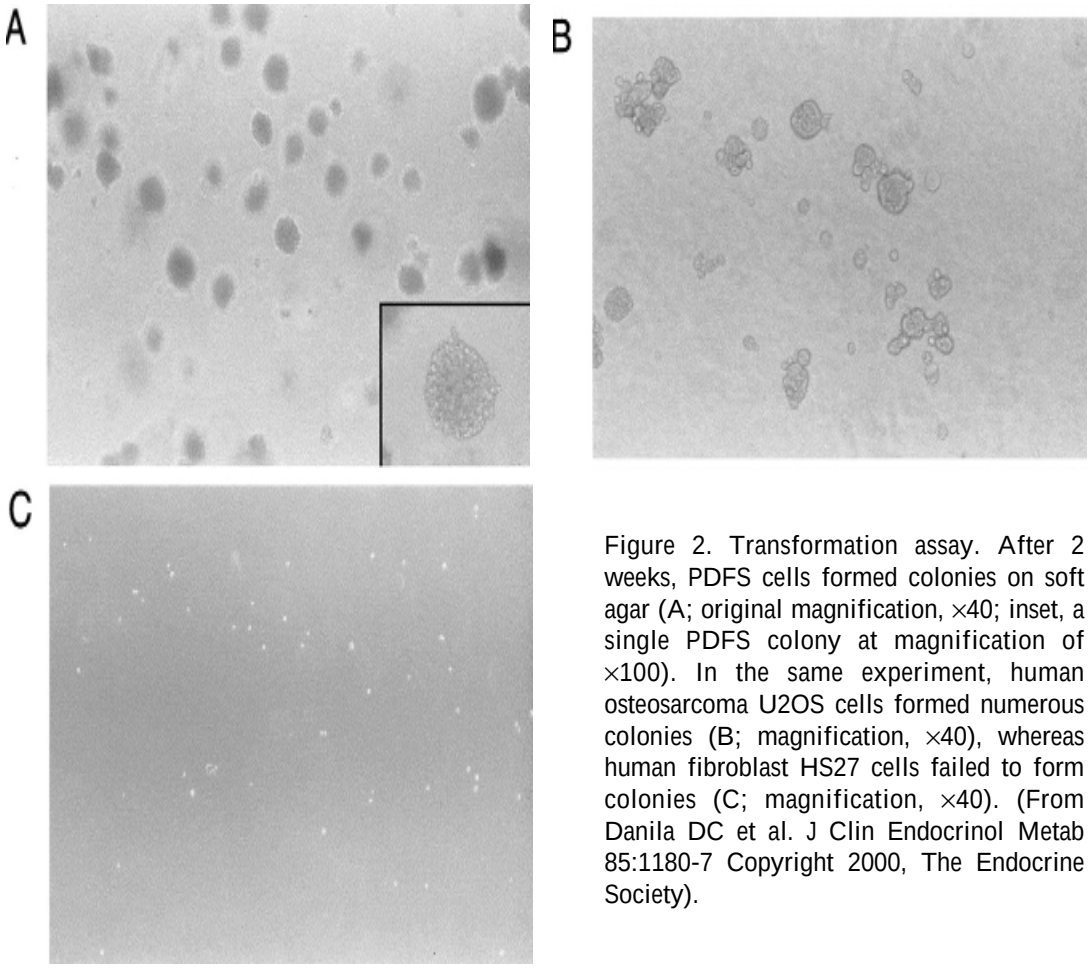

Figure 2. Transformation assay. After 2 weeks, PDFS cells formed colonies on soft agar (A; original magnification, ×40; inset, a single PDFS colony at magnification of ×100). In the same experiment, human osteosarcoma U2OS cells formed numerous colonies (B; magnification, ×40), whereas human fibroblast HS27 cells failed to form colonies (C; magnification, ×40). (From Danila DC et al. J Clin Endocrinol Metab 85:1180-7 Copyright 2000, The Endocrine Society).

The PDFS cells, the first human pituitary-derived line, have been used to investigate the autocrine/paracrine associations in the human pituitary. PDFS cells synthesize and secrete bioactive follistatin and activin A, express functional activin receptor types I and II, which signal through an intact transduction pathway. Therefore, this cell line could also be very useful for studying the molecular events underlying the regulation of cell proliferation and hormone secretion by activin in the pituitary (Fig. 3) (19).

Lipocortin-1, also called annexin-1, implicated in the regulation of many physiological and physiopathological functions related to glucocorticoids action on pituitary and hypothalamus (27) is present in the folliculostellate cells. Using PDFS cells, Solito et al. demonstrated that glucocorticoids upregulate lipocortin-1 mRNA synthesis, cause translocation of a serine-phosphorylated species of lipocortin-1 on the cell surface by PKC and MAPK dependent mechanisms, and therefore exert paracrine/autocrine regulatory actions on the release of ACTH and other pituitary hormones in the pituitary (28).

Prolactin-releasing peptide is the first described hypothalamic peptide hormone that specifically stimulates prolactin production from the pituitary gland,

in addition to its actions on metabolic homeostasis, stress responses, cardiovascular regulation, gonadotropin secretion, GH secretion and sleep regulation. It is also known that folliculostellate cells have been implicated in the cell growth regulation of the lactrotroph cell in response to the estrogens (6, 13). PDFS cells express prolactin-releasing peptide (29), which may explain the paracrine regulation of prolactin production in the anterior pituitary gland in response to multiple factors, such as glucocorticoids.

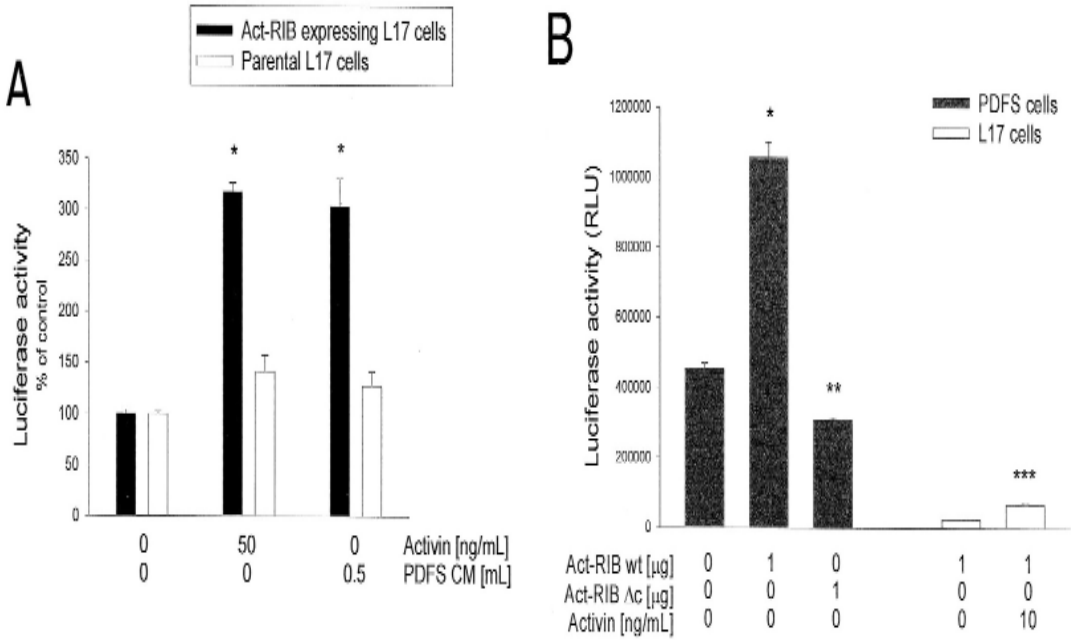

Figure 3. A, Effects of PDFS-conditioned medium (CM) on 3TPLux reporter activity in L17 cells. Cells (2×10^5) were transfected with 3TPLux in the presence (filled bars) or absence (open bars) of Act-RIB, then cells were incubated in presence of 0.5 mL PDFS CM or 50 ng human recombinant activin A. Significant induction of luciferase activity was observed only in the presence of Act-RIB (*, $P < 0.001$). B, p3TPLux reporter activity in PDFS cells (filled bars). Cotransfection with wt Act-RIB further enhances the reporter activity, whereas cotransfection of truncated Act-RIB (Δc) decreases reporter activity [*, $P = 0.0001$; **, $P = 0.0003$ (compared to p3TPLux transfection alone)]. In parallel, L17 cells (open bars) were transfected with p3TPLux and pCI-Act-RIB wt, and treated with 10 ng/mL activin A (***, $P = 0.001$, compared to L17 cells that were not treated with activin A). Results are presented as relative light units of luciferase activity. (From Danila D. C. et al. J Clin Endocrinol Metab 85:1180-7 Copyright 2000, The Endocrine Society).

In conclusion, PDFS cells are the first transformed human pituitary cell line, derived from a gonadotroph adenoma of the pituitary and expressing folliculostellate cell characteristics. This line expresses many novel peptides with growth factor or cytokine activity that play a vital roles in the cross-talk in the autocrine/paracrine regulation of anterior pituitary cells. PDFS cell line may serve as an indispensable *in vitro* system to further study the mechanisms of folliculostellate regulation in cell growth and hormone secretion in normal and neoplastic human pituitary.

References

1. Rinehart JF, Farquhar MG. Electron microscopic studies of the anterior pituitary gland. J Histochem Cytochem 1953;1:93-113.
2. Soji T, Mabuchi Y, Kurono C, Herbert DC. Folliculo-stellate cells and intercellular communication within the rat anterior pituitary gland. Microsc Res Technol. 1997;39:138-149.
3. Allaerts W, Denef C. Regulatory activity and topological distribution of folliculo-stellate cells in rat anterior pituitary cell aggregates. Neuroendocrinology. 1989;49(4):409-18.
4. Lach B, Rippstein P, Benott BG, Staines W. Differentiating neuroblastoma of pituitary gland: neuroblastic transformation of epithelial adenoma cells. Case report. J Neurosurg. 1996;85:953-960.
5. Giometto B, Miotto D, Botteri M, et al. Folliculo-stellate cells of human pituitary adenomas: immunohistochemical study of the monocyte/macrophage phenotype expression. Neuroendocrinology. 1997;65:47-52.
6. Hentges S, Boyadjieva N, Sarkar DK. Transforming growth factor-beta3 stimulates lactotrope cell growth by increasing basic fibroblast growth factor from folliculo-stellate cells. Endocrinology. 2000;141:859-67.
7. Lohrer P, Gloddek J, Hopfner U, et al. Vascular endothelial growth factor production and regulation in rodent and human pituitary tumor cells in vitro. Neuroendocrinology. 2001;74:95-105.
8. Tichomirowa M, Theodoropoulou M, Lohrer P, et al. Bacterial Endotoxin (lipopolysaccharide) Stimulates Interleukin-6 Production and Inhibits Growth of Pituitary Tumour Cells Expressing the Toll-Like Receptor 4. J Neuroendocrinol. 2005;17:152-60.
9. Ceccatelli S, Hulting AL, Zhang X, Gustafsson L, Villar M, Hokfelt T. Nitric oxide synthase in the rat anterior pituitary gland and the role of nitric oxide in regulation of luteinizing hormone secretion. Proc Natl Acad Sci U S A. 1993;90:11292-6.
10. Johnson MD, Gray ME, Pepinsky RB, Stahlman MT. Lipocortin-1 immunoreactivity in the human pituitary gland. J Histochem Cytochem. 1990;38:1841-1845.
11. Loxley HD, Cowell AM, Flower RJ, Buckingham JC. Modulation of the hypothalamo-pituitary adrenocortical response to cytokines in the rat by lipocortin 1 and glucocorticoids: a role for lipocortin 1 in the feedback inhibition of CRF-41 release? Neuroendocrinology. 1993;57:801-813.
12. Oomizu S, Chaturvedi K, Sarkar DK. Folliculostellate cells determine the susceptibility of lactotropes to estradiol's mitogenic action. Endocrinology. 2004;145:1473-80.
13. Hentges S, Sarkar DK. Transforming growth factor-beta regulation of estradiol-induced prolactinomas. Front Neuroendocrinol. 2001;22:340-63.
14. Kobayashi H, Fukata J, Murakami N, et al. Tumor necrosis factor receptors in the pituitary cells. Brain Res. 1997;758:45-50.
15. Herkenham M. Folliculo-stellate (FS) cells of the anterior pituitary mediate interactions between the endocrine and immune systems. Endocrinology. 2005;146:33-4.
16. Alexander JM, Jameson JL, Bikkal HA, Schwall RH, Klibanski A. The effects of activin on follicle-stimulating hormone secretion and biosynthesis in human glycoprotein hormone-producing pituitary adenomas. J Clin Endocrinol Metab. 1991;72:1261-7.
17. Billestrup N, Gonzalez-Manchon C, Potter E, Vale W. Inhibition of somatotroph growth and growth hormone biosynthesis by activin *in vitro*. Mol Endocrinol. 1990;4:356-62.
18. Alexander JM, Swearingen B, Tindall GT, Klibanski A. Human pituitary adenomas express endogenous inhibin subunit and follistatin messenger ribonucleic acids. J Clin Endocrinol Metab. 1995;80:147-52.
19. Danila DC, Inder WJ, Zhang X, et al. Activin effects on neoplastic proliferation of human pituitary tumors. J Clin Endocrinol Metab. 2000;85:1009-15.
20. Kaiser UB, Lee BL, Carroll RS, Unabia G, Chin WW, Childs GV. Follistatin gene expression in the pituitary: localization in gonadotropes and folliculostellate cells in diestrous rats. Endocrinology. 1992;130:3048-56.
21. Danila DC, Haidar JN, Zhang X, Katznelson L, Culler MD, Klibanski A. Somatostatin receptor-

specific analogs: effects on cell proliferation and growth hormone secretion in human somatotroph tumors. J Clin Endocrinol Metab. 2001;86:2976-81.
22. Ooi GT, Tawadros N, Escalona RM. Pituitary cell lines and their endocrine applications. Mol Cell Endocrinol. 2004;228:1-21.
23. Danila DC, Zhang X, Zhou Y, et al. A human pituitary tumor-derived folliculostellate cell line. J Clin Endocrinol Metab 2000;85:1180-1187.
24. Asa SL, Ezzat S. The cytogenesis and pathogenesis of pituitary adenomas. Endocr Rev. 1998;19:798-827.
25. Zhang X, Zhou Y, Mehta KR, et al. A pituitary-derived MEG3 isoform functions as a growth suppressor in tumor cells. J Clin Endocrinol Metab, 2003;88:5119-26.
26. Zhang X, Sun H, Danila DC, et al. Loss of Expression of *GADD45*, a Growth Inhibitory Gene, in Human Pituitary Adenomas: Implications for Tumorigenesis. J. Clin. Endocrinol. Metab., 2002;873:1262-7.
27. Buckingham JC, Solito E, John C, et al. Annexin 1: a paracrine/juxtacrine mediator of glucorticoid action in the neuroendocrine system. Cell Biochem Funct. 2003;21:217-21.
28. Solito E, Mulla A, Morris JF, Christian HC, Flower RJ, Buckingham JC. Dexamethasone induces rapid serine-phosphorylation and membrane translocation of annexin 1 in a human folliculostellate cell line via a novel nongenomic mechanism involving the glucocorticoid receptor, protein kinase C, phosphatidylinositol 3-kinase, and mitogen-activated protein kinase. Endocrinology. 2003;144:1164-74.
29. Zhang X, Danila DC, Katai M, Swearingen B, Klibanski A. Expression of prolactin-releasing peptide and its receptor messenger ribonucleic acid in normal human pituitary and pituitary adenomas.J Clin Endocrinol Metab. 1999;84:4652-5.