Folliculo-stellate Cells of the Human Pituitary: A Type of Adult Stem Cell?

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Ultrastructural and immunocytochemical observations of pituitary folliculo-stellate cells (FSC) in a large series of adenomatous and nontumorous human pituitaries led to the following conclusions: (1) The endocrine cells of both the nontumorous and the adenomatous pituitary are capable of transforming into FSC while changing from endocrine to nonendocrine phenotype. (2) As shown on consecutive sections in prolactin cell adenomas with FSC-rich areas including microcyst formation, S-100 protein and glial fibrillary acidic protein (GFAP) immunoreactivities are strongest in the smallest newly formed follicles. The 2 immunoreactivities do not overlap. The epithelium of older microcysts is immunonegative, implying that expression of the 2 markers is restricted to the early phase of FSC formation. (3) Transformation of endocrine cells into FSC may signify retrodifferentiation into their Rathke's pouch derived precursors as suggested by occasional presence of ciliated and/or mucin producing cells in the lining of microcysts. (4) In lymphocytic hypophysitis a marked activation as well as increase of number and size of FSC are evident in areas of ongoing immune destruction supporting their immune role. (5) Considering the multifaceted nature of FSC, it is suggested that they represent a type of pluripotent adult stem cell.

Keywords: adenohypophysis, folliculo-stellate cells, immunocytochemistry, pituitary adenoma, ultrastructure

The folliculo-stellate cells (FSC) of the pituitary were first described at the dawn of electron microscopy by Farquhar [1]. Since then many aspects of FSC have been investigated, yet the deceptively simple cells have eluded precise definition so far. This article provides a short account of the morphologic aspects of FSC in the nontumorous and adenomatous human pituitary. The data presented herein are based on our extensive experience over the last 30 years, gained in the course of routine ultrastructural examination of more than 8,500 pituitary biopsies [2–4]. The overwhelming majority of these represented adenomas, but nontumorous adenohypophysial tissue was present in at least 1,500 biopsies.

The usually central follicle is an essential part of every acinus in the human pituitary. They consist of FSC [5–7] joined at their apical surfaces by terminal bars, which may be incomplete. They extend slender processes among the hormone-producing cells. Desmosomal attachments are present along their lateral cell borders and between FSC and glandular cells as well. If they have no content, the human follicles may be inconspicuous. The nuclei of FSC vary in shape determined by the spaces left for them by the much larger glandular cells. The small cytoplasm contains scanty RER, small Golgi complex, and few mitochondria. Lysosomal bodies, vimentin-type filaments, and, rarely, glycogen particles may be present. In 1974 [8] we reported that necrosis of hormone-producing cells initiated by rupture of plasmalemma triggers transformation of adjacent glandular cells into FSC. Presumably, the first step of the process is the formation of junctional complexes (terminal bars) between the affected cell(s) and the adjacent glandular cells or already existing FSC, accompanied or followed by rapid reorganization of cytoplasm from endocrine to nonendocrine phenotype (Figure 1). Fully granular FSC are encountered only occasionally (Figure 2). In the early phase of transformation secretory granules may still be detectable and accumulation of lysosomes may occur as well. The cell debris, at first containing...
recognizable cellular constituents, then turning into a deeply osmophilic mass, is confined to the lumen. Cell death by necrosis may be common in peritumoral areas [8, 9], but cell types subjected to sustained stimulation (e.g., gonadal deficiency/castration cells in postmenopausal or ovariectomized women, hyperplastic thyrotrophs) are also prone to rupture. As a result of accumulating debris, in affected areas of the pars distalis marked expansion of follicular lumina may ensue. The constant if sporadic formation of FSC notwithstanding, widespread undue accumulation of the FSC is not known, which by inference signifies that

follicle formation/ expansion is not only ubiquitous but also reversible. It is of note that other forms of cell death, the most common gradual involution ("dark cell") or the rare apoptosis, do not induce formation of FSC.

Follicle formation is also evident in pituitary adenomas [8, 10–13]. It occurs at variable frequency in different types, being most common in gonadotroph adenomas in our material (Figures 2, 3). Prolactin (PRL)-producing adenomas deserve special attention, since in these tumors follicle formation may be focally rampant, leading to development of structures of variable sizes sometimes containing several lumina (Figure 4). Some follicles grow into microcysts, others reach even larger sizes, resulting in what is known by neurosurgeons and imaging specialists as the "cystic prolactinoma." The histologic, immunocytochemical, and ultrastructural features of follicles in the normal

**FIG. 1** Part of a follicle detected in the nontumorous adenohypophysis adjacent to an adenoma. One FSC (star) still harbors small secretory granules many of which display markedly reduced electron density. Magnification, ×6,750.

**FIG. 2** Follicle in a growth hormone producing adenoma. One of the FSC (star) still has secretory granules in and around the Golgi region. The cell is also oncocytic, as many cells in this adenoma. Magnification, ×7,350.
Most reports found S-100 protein immunoreactive cells more numerous than those positive for GFAP, but, when studying corresponding areas of consecutive sections, the reverse may occur as well.

In our attempt to determine whether S-100 protein and GFAP positivities are reliable and specific markers of FSC, we tested PRL cell adenomas in which a large number of follicles/microcysts were present in various phases of their development. Our results clearly demonstrated that the smallest, and probably the youngest, follicles contain the largest number of cells positive for S-100 protein and GFAP (Figure 8).

However, as shown on consecutive sections, there was no overlap in either the number or the position of cells immunoreactive for the 2 markers. The epithelial lining of larger, and by all probability older, structures was negative except for sporadic cells positive for S-100 protein and/or GFAP (Figure 8). The hypothesis best explaining the above staining pattern is that S-100 protein and GFAP are expressed in the early phase of follicle formation, but not necessarily at the same time and only temporarily. The immunoreactivities appear to be linked to the phase of transformation from endocrine to nonendocrine phenotype. Established FSC of the microcysts are negative except for occasional S-100 protein/GFAP positivity. The latter has a simple explanation supported by numerous ultrastructural observations. At any given time, single cells in the lining epithelium of microcysts may undergo gradual involution transforming into a "dark cell." Such cells are eventually sloughed off into the lumen (Figure 5), exposing the adenoma cell(s) underneath. The tumor cells thereby affected become part of the cyst lining and are compelled to undergo the process of transformation into nonendocrine FSC.

Based on these observations S-100 protein and GFAP are markers of human FSC only in a limited context: the immunopositive cells are FSC, the opposite of which is not valid. Consequently, neither of the markers is suitable for quantification. The sequence of expression of the 2 markers cannot be unequivocally decided by morphology. It is suggestive, however, that many S-100-positive cells have the same size and polyhedral shape as the surrounding adenoma cells, whereas most GFAP-positive cells have long processes indicative of changing into stellate morphology. The role of the 2 substances is unclear. S-100 protein has been suggested to serve as neurotrophic and glial maturation factor in the CNS and was also detected in some pituitary cell types during fetal growth [19].

**SPECIFICITY OF IMMUNOCYTOCHEMICAL MARKERS**

Observations demonstrating immunoreactivities for S-100 protein [14, 15], and glial fibrillary acidic protein (GFAP) [16] were hailed as being of major importance. Especially, S-100 protein immunoreactivity became accepted as a specific marker of FSC [17, 18]. Several studies using cell counts followed, but they were ridden with inconsistencies and led to no real progress concerning the nature and physiologic role of FSC.

**CELL BIOLOGICAL SIGNIFICANCE**

The lining of microcysts consists of simple epithelial cells joined by terminal bars at their apical portion and by desmosomes along their lateral cell borders. The structures are never delimited by basal lamina; the cells of the lining directly join the surrounding adenoma cells forming also infrequent adherent junctions. The lining epithelium of larger cysts often acquire a highly visible function: the secretion of a variably electron-dense
granular or flaky substance into the lumen. The ultrastructural appearance of this product is similar to that of the content of Rathke’s cleft cyst. The cell biological significance of follicle/cyst formation and the associated phenotypical changes is unclear. An unexpected ultrastructural observation may hold the answer. In a few cases we noted the appearance of ciliated and/or mucin-producing cells in the lining of cysts (Figure 6). The association of simple epithelial, ciliated, and mucin-producing cells is the well-known triad of typical Rathke’s cysts [20]. In this context the formation of FSC from adenoma cells may represent retrodifferentiation into their Rathke’s pouch-derived precursor.

FUNCTIONAL ASPECTS OF FSC

From early on, FSC were perceived as sustentacular elements similar to those observed in other endocrine organs and neoplasms [5, 21]. Their morphology, the stellate shape and long cytoplasmic processes forming desmosomal attachments with adjacent FSC as well as
glandular cells, renders them eminently suitable for this role. The suggested phagocytic role of FSC appears to be plausible as well, since follicles often have a content of cell debris and lysosomes may be present in the cytoplasm [5, 21]. However, although carefully searched for, we have never observed evidence of uptake of lumenar content by FSC. It may be, that the debris breaks down by autodigestion. Alternatively, the FSC may secrete enzymatic substances to aid decomposition of follicular content. It is relevant to note the Giometto et al. [22], who proposed that FSC belong to the macrophage lineage and may represent “resider pituitary macrophages.” were unable to show expression of any macrophage markers in S-100- and GFAP-positive cells. The lysosomal bodies in FSC ma
be attributable to autophagy; it could signify the removal of the endocrine machinery of the cell in the phase of transformation from the endocrine to the nonendocrine phenotype.

Starting in the late 1970s, the outstanding Belgian group of Denef and colleagues [23–27] revolutionized the in vitro investigation of FSC. They succeeded obtaining an FSC-enriched population from dispersed pituitary cells by gradient sedimentation at unit gravity. The studies of FSC that followed led to an astounding variety of information [23–25]. The findings assigned many, often unrelated, functions to the unassuming simple FSC, ranging from forming an intercellular messenger system for local inhibitory control of hormone secretion [23–25] and ion regulation [28] to production of cytokines, such as IL-6 [29], growth factors, such as basic fibroblast growth factor (bFGF) [30], vascular endothelial growth factor (VEGF) [31, 32], leukemia inhibiting factor (LIF) [33], hormonal substances, such as leptin [34] and follistatin [31, 35], as well as receptors for the neupeptide PACAP [36, 37], tumor necrosis factor (TNF) [38], and recently TSH [39]. FSC also express nitric oxide synthase [40, 41], which, by generating NO, is involved in the regulation of many functions. Laminin [42], a component of basal lamina, is also produced by FSC.

It is of special significance that a contingent of FSC expresses MHC class I and II determinants, several lymphoid markers, as well as lymphoid dendritic cell markers [43, 44]. Bodey et al. [44] consider FSC as part of the “professional” antigen presenting dendritic cell network. Such cells are capable of initiating immune responses and act as part of a neuroendocrine immune regulation system [44].

Many of the in vitro findings cannot be tested by presently available morphologic methods in routine surgical or autopsy material, but we certainly can lend support to the presumed immune role of FSC based on our ultrastructural findings in 7 cases of lymphocytic hypophysitis. In these lesions marked activation of FSC takes place expressed as enlargement of the nucleus and appearance of conspicuous nuclear inclusions, expansion of the cytoplasm associated with increased content of RER, free polyribosomes, and sometimes intermediate filaments and/or clusters of glycogen particles. These changes did not appear consistent in the small biopsies of 6 earlier cases, rendering interpretation difficult. One recent case of a chronic, unusually massive lymphocytic hypophysitis associated with disappearance of prolactin cells shed light on the phenomenon [45]. In the fibrotic areas of the lesion, where the inflammation had already run its course, the FSC displayed normal features in the surviving acini, whereas in areas of ongoing immune destruction the FSC became, by size and number, the most prominent cell type in many of the affected acini (Figure 7). This finding clearly indicates the involvement of FSC in the immune response, the exact nature of which is still unclear. It is noteworthy that in this unusually advanced lesion the S-100 protein and GFAP immunoreactivities were not extensive and lacked synchrony (Figure 8), attesting again to the fact that the positivity of these markers in the human adenohypophysis is determined not by the number of the FSC, but by the phase of their life cycle.

**DISCUSSION AND CONCLUSIONS**

Considering the multifaceted nature of FSC, it is not surprising that several investigators assumed the heterogeneous or even nonpituitary origin of FSC [17, 26, 27, 44, 46]. Bodey et al. [44] perceived FSC as a part of the lymphatic dendritic cell network originating in the thymic endodermal epithelial anlage and migrating during embryonal development to various parts of the endocrine and immune system. They considered FSC as part of the “professional” antigen presenting dendritic cell network expressing MHC class I and II antigens as well as other lymphoid markers. Allaerts et al. [27] distinguish S-100-positive pituitary FSC and monocyte-derived dendritic cells carrying MHC class II determinants. However, they conclusively showed overlaps between the 2 categories [26] and demonstrated that a proportion of S-100 immunopositive cells display morphologic, immunocytochemical, and ultrastructural characteristics of both FSC and dendritic cells, a finding difficult to reconcile with the presumed dual derivation.

As judged by our experience, we see no reason to assume either heterogeneous or nonpituitary derivation of FSC. In agreement with Fukuda [47], we also found that an average pituitary follicle in the normal adult human gland, consisting of small cells with no appreciable lumen, is very similar to those seen in the fetal pituitary. The fetal gland does not have the acinar architecture of the adult adenohypophysis as early as 12 weeks of gestation; it consists of sheets of epithelial cells interspersed at fairly regular intervals by follicles. Their FSC displayed some variability in terms of quantity of membranous organelles and presence or absence of intermediate filaments and glycogen, but fetal glands exhibit no immunoreactivity for S-100 protein GFAP. Some of the FSC may contain immature secretory granules regarded as an early sign of functional differentiation. We presume, that the FSC, similarly to the rest of adenohypophyseal cells, derive from Rathke’s pouch and that they remain pluripotent, capable of assuming numerous functions from endocrine regulation to angiogenesis and immune response. The finding that the pituitary transcription factor Pit1 is expressed not only in progenitors of hormone producing cells, but in FSC as well [48], also supports their common origin.

In his recent review, Itoe [49] suggested that FSC may be “a kind of stem cell.” The remarkable plasticity of FSC is similar to that seen in the so-called adult stem cells present in many tissues, such as epidermis, intestines, the hematopoietic system, and even the central nervous system [50]. By definition, “adult stem cells are often relatively slow-cycling cells able to respond to specific environmental signals and either generate new stem cells or select a particular
FIG. 7 Enormous follicle in a case of lymphocytic hypophysitis. Several of the FSC still contain secretory granules. Note the prominent nuclear inclusions (arrowheads). Magnification, ×4,240.

differentiation program” [50]. As opposed to the omnipotent, morphologically undifferentiated embryonal stem cells, adult stem cells display morphologic and biochemical features of differentiated cell types of the tissues, they reside in. As adult stem cells, FSC would completely fit into the community of hormone-producing cells. As opposed to the earlier perception (“one cell—one hormone” theory), the pituitary emerged as a remarkably plastic organ, where reversible transdifferentiation, i.e., change of hormonal function and morphologic phenotype in response to functional demand, is a proven reality [51–53].

If we accept FSC as a pluripotent cell type, the ultimate question is whether FSC contribute to cell renewal in the normal and hyperplastic gland and whether they can give rise to adenomas. Due to the static nature of morphologic investigation, we have only circumstantial evidence. In hypothyroid pituitaries with marked, sustained stimulation of thyrotrophs we regularly observed small cells having small 100 to 200-nm secretory granules, which, by immunoelectron microscopy, were immunoreactive for either TSH or growth hormone [52]. Such cells could not have derived from either the enormous, highly stimulated thyroid deficiency cells or mature, fully granulated somatotrophs. The functional differentiation of TSH and GH cells from an undifferentiated cell type, FSC, is explained partly by the sustained thyrotropin-releasing hormone (TRH) stimulation and partly by the transdifferentiation of somatotrophs to thyrotrophs, leading to continuous reduction of somatotroph population. As far as tumorigenesis is concerned, FSC could be the ideal parent cell of null cell adenomas, hormonally nonfunctioning tumors displaying morphologic signs of endocrine differentiation, but no markers of differentiation into any of the pituitary cell types.

If FSC are indeed adult stem cells, the only troubling aspect is not the multiplicity of their functions, but the lack of evidence of their multiplication. Mitoses are admittedly very rare in the pituitary, even in cases of hyperplasia, with the notable exception of the enlarged gland in late pregnancy [51]. During our 30 years of experience, we have not observed a mitotic FSC and have found only 1 article reporting cell division in the cell type. Gon et al. [54] autotransplanted pituitaries of rats under the renal capsule. At 3 days they observed an increased number of S-100 protein immunoreactive FSC in the autografts. At 5 days mitoses were also
FIG. 8 A large group of follicles in a prolactin producing adenoma, immunostained for S-100 protein (A) and GFAP (B), as shown on consecutive sections. The positivities overlap only partially. The lining of a large follicle (star) is nearly negative. Original magnification, ×250. C,D Follicles of various sizes, immunostained for GFAP, are shown in two prolactin cell adenomas. The lining of the largest structures (stars) contains few positive cells. Original magnification, ×400. E,F Lymphocytic hypophysisis. Only one FSC shows strong immunoreactivity for S-100 protein (E), whereas several follicles stain well for GFAP (F) on the consecutive section. Stellate morphology is evident in several GFAP-positive cells (arrow). Original magnification, ×400.
evident in glandular cells and, to a lesser extent, in FSC. The apparent contradiction between the manifold potentials and dynamic nature of human FSC and virtually undetectable multiplication could only be approximated by studies of cell cycle kinetics.

REFERENCES


