Immunocytochemistry of folliculo-stellate cells of normal and neoplastic human pituitary gland

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SUMMARY Five normal human pituitaries and 20 pituitary neoplasms were investigated by immunocytochemical methods. Glial fibrillary acidic protein and S100 have been shown in the anterior lobe of the pituitary. Both these markers were present in the folliculo-stellate cell. Evidence is presented for the presence of a transitional folliculo-stellate cell which is immunoreactive for S100. The role of the folliculo-stellate cell is discussed.

The characterisation of hormone secreting cells within pituitary neoplasms by immunocytochemical methods is well established and has contributed to our understanding of the biology of such tumours. It is surprising that exploration of the characteristics of these cells in response to neural or glial cell markers has received so little attention.

Of the six recognisable cell types within the human pituitary gland, five are hormone secreting and the sixth is a less well defined cell type variously referred to as a follicular cell, a stellate cell, an agranular stellate cell, and folliculo-stellate cell. About 3–4% of the total pituitary cell population comprises stellate cells with one or more processes passing to the perivascular space and to the central pituitary follicle. Bergland and Torack have described the electron microscopic appearance of these structures in detail. Fakuda points out that the cell changes its properties throughout life: in fetal stages it is follicular but it progressively differentiates into a stellate form. On these grounds he refers to them as agranular stellate cells. The term folliculo-stellate cell, however, appears to be the preferable term.

Although glial fibrillary acidic protein (GFAP) is regarded as a characteristic and specific glial marker, it is also present in the pituitary gland: it has been found in large amounts in rat pars nervosa and, recently, it has also been shown in human pars distalis. The presence of GFAP in pars distalis can be attributed to cells originating from the neural tube, unlike the hormone secreting cells which originate from the epithelium of the stomatodeum. Two other markers, S100 and neurone specific enolase (NSE), have been extensively studied in relation to purely nervous structures. S100 is found in glial cells and also in neurones and has been shown in the pituitary gland of the adult rat. There it has been found in stellate cells of the pars distalis and tuberalis, in the marginal cells lining the hypophyseal cleft, and in the glial like pituicytes of the neural lobe. It has therefore been suggested that all these cells are of neuroectodermal origin. S100, however, has a wider distribution and has been shown in melanocytes of the skin and sweat glands, salivary glands, and the breast and a number of other non-nervous neoplasms such as carcinomas of the lung and teratomas of the ovary. Thus a wide range of tissues contain S100. Although a predominantly neuroectodermal marker, its presence in a wide range of other tissues makes it a less valuable determinant than GFAP.

NSE has been found in a number of neural tumours including neuroblastomas, oligodendrogliomas, astrocytomas, ependymomas, acoustic neuromas, choroid plexus papillomas, and pituitary adenomas. It has also been found in other tumours of non-neuroectodermal origin such as carcinoma of the breast and chordoma. Its major use has been as a marker of the amine precursor uptake and decarboxylation (APUD) system.

There is good evidence for the presence of these three common neural markers within the pituitary gland, which we have investigated in other immunocytochemical studies on the pituitary.

Material and methods

The material comprised five normal human pituitaries and 20 pituitary neoplasms. These were...
all fixed in formalin, embedded in paraffin, and serially sectioned. Immunocytochemistry was performed by the peroxidase-antiperoxidase (PAP) immunoperoxidase technique.17,18

Commercially available antisera (Dako, Mercia Brocades, Byfleet, Surrey) were used for the GFAP, S100, and NSE determinations. The antisera supplied were of high titre and after titration were used at their optimal dilutions of 1/300 for anti-GFAP, 1/400 for anti-S100, and 1/500 for anti-NSE.

Preliminary studies showed that the results were more reliable and reproducible when the tissue sections were pretreated with trypsin. Normal pituitaries obtained at necropsy or surgical specimens of pituitary neoplasms were often heavily contaminated by blood and it was essential to block endogenous peroxidase. The optimal incubation times in trypsin and hydrogen peroxide solutions were determined empirically.

The PAP immunoperoxidase technique was carried out as follows: the sections were deparaffinised in three changes of xylene and ethanol and then rinsed in two changes of phosphate buffered saline for 5 min each before trypsinisation. All sections were pretreated with trypsin (0-1% trypsin in 0-1% CaCl₂ solution, pH 7-8) for 20 min at 37°C. They were subsequently rinsed three times for 5 min each in PBS containing 0-2% Triton X-100. All subsequent rinsing was similarly performed. Endogenous peroxidase was blocked by incubation in 3% aqueous hydrogen peroxide for 15 min at room temperature. After rinsing the sections for 5 min in distilled water and in two changes of PBS for 5 min each, a standard PAP technique was followed. The final immunoreactive product was visualised with 3-amino-9-ethylcarbazole. The sections were counterstained with Celestin blue and Mayer's haematoxylin and then mounted in glycerol gelatin (Sigma Chemical Company, Poole, Dorset, product code GG-1).

The following controls were included in all assays: endogenous peroxidase control; substitution of primary antiserum with normal non-immune serum; omission of the bridge antiserum; omission of the PAP complex. All negative controls failed to show any immunoreactivity. In addition, positive controls of suitable normal tissues were included for each antiserum.

The sections were then viewed by each author independently and graded according to intensity of staining on a four point scale; the regions of maximum staining were noted. Folliculo-stellate cells were identified on the basis of morphology and distribution. The England Finder* was used to identify particular cells and distinguish their individual stain-

* A stage graticule with reference guide permitting repositioning of any standard (76 × 26 mm) slide allowing re-examination of a given field. Carefully matched serial sections can thus be aligned on consecutive slides permitting location of identical fields of interest.

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Fig. 1 Normal pituitary. Glial fibrillary acidic protein positive folliculo-stellate cells lining a Rathke’s cyst. ×300.

Fig. 2 Normal pituitary. Glial fibrillary acidic protein positive folliculo-stellate cells in pars distalis. Stellate form with several processes radiating between granulated cells. ×500.
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Fig. 3 Normal pituitary. S100 positive folliculo-stellate cells in pars distalis in close association with hormone secreting cells. ×500.

ing properties, and comparisons were made in selected areas and occasionally in selected cells.

Results

In general, normal human pituitary gland and pituitary neoplasms showed strong reactivity to all three agents. There was a greater proportion of GFAP positive cells than S100 positive cells in normal pituitary, but the reverse was true in pituitary neoplasms. Pars nervosa, intermedia, and distalis were carefully examined in the normal pituitaries and in pituitary tumours; a general survey of the whole section was made.

Normal Pituitary

Pars nervosa showed strong staining for NSE, a lesser amount for S100, and sparse GFAP reactivity. In the residual Rathke's cysts a variety of reactions were seen (Fig. 1). Some cysts were lined with cells which were either GFAP or S100 or NSE positive, but many were negative. Occasionally, cysts were seen lined completely by reactive cells. More usually both positive and negative cells were present around the cysts.

GFAP positive folliculo-stellate cells were scattered throughout the pars distalis, but they tended to occur in greater numbers closer to the capsule, where they often stained intensely. Within the substance of the pars distalis their location appeared to be more within the stroma than within the acinar structures. The cells were often pear shaped, but frequently had a distinct stellate form with four to five processes with broad bases (Fig. 2). In some sections we were able to see these prolongations continue between secretory cells with an appearance almost of investment. Other cells were spindle shaped and then were often seen close to capillaries. These cells were compared with the hormone secreting cells and in no instance were they recognisably the same and appeared morphologically quite distinct.

S100 positive folliculo-stellate cells were scattered throughout the pars distalis, usually in small groups of between five and ten cells and often closely associated with hormone secreting cells (Fig. 3). In stromal areas there was also staining similar to that shown by GFAP positive cells.

NSE positive cells were scattered throughout the gland but again tended to concentrate closer to the capsule. They differed from folliculo-stellate cells, however, in being large, plump, and granular and
corresponded with the corticotrophs (Fig. 4). Not all granular cells were stained and it is probable that the remainder were accounted for by gonadotrophs, lactotrophs, thyrotrophs, and somatotrophs.

Preliminary attempts at double labelling for GFAP plus S100, GFAP plus NSE, and S100 plus NSE supported the hypothesis that there may be more than one population of folliculo-stellate cells. Some cells were GFAP positive, others S100 positive, and a small population appeared to be reactive for both. NSE reactivity was restricted to granulated cells morphologically identical to hormone containing cells, and none of the folliculo-stellate cells was double labelled.

**PITUITARY TUMOURS**

These tumours comprised a variety of hormone types and included prolactinomas, bi-hormonal and multi-hormonal tumours.

GFAP positive folliculo-stellate cells were less prominent in tumour tissue than in normal pituitary. They were scattered throughout the tumour mass with little tendency to accumulate in any site other than in the stroma (Fig. 5). In some instances they juxtaposed and interdigitated with the hormone producing cells. There was great variation in the number of GFAP positive folliculo-stellate cells for different tumours, but there appeared to be no correlation between this variation and hormone type. In some tumours the GFAP positive cells were diffusely scattered through the tumour mass, with considerable variation in staining intensity between different tumours and different sites within the same tumour. It was noticeable that these cells stained less intensely when closely related to secretory cells. In contrast, GFAP positive cells within the stroma often showed intense staining. Similar intense reactivity could often be seen in areas immediately below the capsule. Folliculo-stellate cells appeared to assume a more stellate shape when located close to the secretory cells, compared with their appearance in stromal areas. Occasionally, they were located around microcysts and capillaries.

In general more cells were S100 positive than GFAP positive. S100 positive cells showed a similar variation in reactivity and distribution to GFAP positive cells and, similarly, no correlation with hormone type. S100 positive cells, however, were more profuse and more intensely staining than GFAP positive cells and appeared in larger groups.

![Fig. 4 Normal pituitary. Neurone specific enolase positive cells in pars distalis. Their morphology resembles that of granulated hormone secreting cells and they are seen in large groups. ×500.](image-url)
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(Fig. 6). These cells seemed to have a more intimate relation with secretory cells and interdigitations were particularly prominent. There was no difference in staining intensity between cells within the stroma and those among secretory cells. They were commonly associated with microcysts and follicular structures (Fig. 7).

NSE positive cells were often numerous and correlated with the type of hormone secreting cells distinguished by other means and were clearly not folliculo-stellate cells. Double labelling with NSE and S100 and with NSE and GFAP in one tumour showed that none of the folliculo-stellate cells could be double labelled; this confirms the results for the normal pituitary.

Conclusions and discussion

This investigation has clearly shown a pituitary cell which is not hormone secreting but which bears a close relation to those cells with which it often interdigitates. Similar cells have been found in animals and man and identified as folliculo-stellate cells. To date, S100 positive cells have been found only in rat pituitary. 5611 GFAP positive pituitary cells have been found in both rat 19 and man, 8 but primarily these were pituicytes of the neural lobe. There is only one reference to the demonstration of GFAP in the anterior lobe of man 8 and none has reported S100. This investigation has shown that the folliculo-stellate cells stain for GFAP and S100, although not all cells stained for S100 are GFAP positive.

Cocchia and Miani 11 showed S100 protein within the stellate cells of the pars distalis and tuberalis of
the rat and also in the hypophyseal cleft and the pituicytes of the neural lobe. The latter are regarded as glial like cells. These authors pointed to the common properties of these cells, which are satellite cells to the secretory axons of the neural lobe and hormone secretory cells of the anterior lobe. They therefore suggest that all S100 positive cells in the pituitary are of neuroectodermal origin and glial like. They note that all pituicytes contain S100 and the protein distribution is the same in all cells.

Nakajima et al. also showed S100 protein within the nucleus and cytoplasm of folliculo-stellate cells and agreed that they were probably of neuroectodermal origin. They particularly noted the extension of cytoplasmic processes between granular cells, a characteristic feature which we have also observed.

Speculating on the role of these cells, Yoshimura et al. considered the folliculo-stellate cell to be related to the renewal cell system of the rat anterior pituitary. Ohtsuka et al. cultured rat folliculo-stellate cells with corticotrophin releasing hormone and suggested that they differentiated into both acidophils and basophils. There is clearly a relation between folliculo-stellate cells and hormone secreting cells. Shirasawa et al. found that castrated rats developed numerous folliculo-stellate cells with cytoplasmic processes surrounding the gonadotrophs. They failed to show any topographic affinity for thyrotrophs after thyroidectomy. Yoshimura and Nogami presented some evidence for the presence of adrenocorticotropic hormone and thyroid stimulating hormone in stellate cells of the rat, though Shirasawa et al. showed the independence of these hormone secreting cells from folliculo-stellate cells. Other postulated functions of the folliculo-stellate cells have been listed and include a supportive function and phagocytic scavenger activity.

We have shown intense staining for GFAP, S100, and NSE within normal pituitary follicular structures in man. We have also confirmed the intense immunoreactivity of the walls of the pars intermedia cysts, the so called Rathké's cysts. We have found intense reactivity for GFAP, S100, and NSE within the cells lining these cysts, as have others in man and in rat. The multiple reactivity of these cells suggests that they may be pluripotential. The folliculo-stellate cells in the anterior lobe may be derived from these cyst areas and transform on reaching their destination to GFAP or S100 positive cells or they may even produce both proteins. Whether there is any additional pluripotential function is debatable. As yet there is little strong evidence to support the theory that folliculo-stellate cells may differentiate into hormone secreting cells other than the work on rats of Ohtsuka et al. The evidence against transformation into a hormone secretory cell is the failure to show any transitional cell with immune reactivity for S100, GFAP, and hormones. On some occasions we have seen follicular structures with a definite lumen, which have stained positively for S100 (Fig. 8) and, rarely, similar structures with a predominance of GFAP positive cells. It is possible that these represent the transitional cell, but we have not yet attempted to show by double labelling whether these cells are also secretory. Neither the GFAP positive cells nor the S100 positive cells in these follicular structures contain any obvious granules.

It is noteworthy that the folliculo-stellate cells are NSE negative and the likelihood is then that they
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are of glial type and neuroectodermal in origin. Their negative staining for NSE indicates that they are unlikely to be part of the APUD system as NSE is regarded as a specific marker for neurones and cells of the APUD system.\textsuperscript{14,16} Our failure to show NSE in folliculo-stellate cells using anti-\(\gamma\) \(\gamma\) enolase antiserum supports the concept that these are glial like cells which contain only the \(\alpha\)-isoenzyme.\textsuperscript{23,24}

Our limited experience with the use of this technique on cultures suggests that this hypothesis is correct since we have been able to show within a pituitary tumour culture a population of cells which are strongly positive to GFAP but only weakly staining to S100. These differences may be due to a maturation process and differential expression of S100.

We have attempted to explore further the possible neuroectodermal origin of the folliculo-stellate cell using the monoclonal antibody U113A (the generous gift of Dr JT Kemshead), which is a neuroectodermal marker.\textsuperscript{25} In two cultures derived from pituitary adenomas with a high proportion of folliculo-stellate cells we were able to label these cells, which confirms their neuroectodermal origin (unpublished observations).

In speculating on the reason for the different staining properties of the folliculo-stellate cell at different sites and especially the striking intensity of staining within tumours we tentatively suggest an explanation based on a maturation process and a differential expression of both S100 and GFAP. We have noted that GFAP staining in stromal areas is more intense than in follicular areas, which suggests changes in the intermediate filaments of the folliculo-stellate cells. No such differences have been noted in S100 reactivity, and it is possible that these reflect metabolic changes and cell function appropriate to their site, the S100 protein serving as a source for a variety of follicular activities requiring a carrier protein function. These changes are particularly prominent in adenomas, where both number and reactivity is greater than in the normal gland, and are similar to the changes described after hormone manipulation in the rat.\textsuperscript{6}

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