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# Stress-Response Proteins in Human Pituitary Adenomas

## *Expression of Heat-Shock Protein 72 (HSP-72)*

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**The presence of heat-shock protein 72 (HSP-72) was investigated by immunohistochemistry (IHC) in a series of 28 surgically removed pituitary adenomas including six somatotroph, two mammosomatotroph, five lactotroph, six corticotroph, four null cell adenomas, and three oncocytomas. Overall, 25 tumors (90%) were positive for HSP-72. One somatotroph, one lactotroph, and one null cell adenomas each contained only sparse, small HSP-72 immunoreactive granules and were regarded as negative. The expression of HSP-72 was commonly uneven differing in degree from cell to cell and among various tumors. In most adenomas, the immunoreactivity was seen as fine granules of moderate density, distributed throughout the cytoplasm. In some cells, the immunoreactivity was strong and diffuse. In one somatotroph, two corticotroph, one null cell, and one oncocytic adenomas, nearly all tumor cells were strongly positive. Adenoma cells, located adjacent to capillaries and small vessels, commonly showed a selective and strong immunoreactivity for HSP-72. The fragments of nontumorous adenohypophysial parenchyma also contained fine immunoreactive cytoplasmic granules accumulating in scattered hormone-producing cells and in stellate cells. These results show that HSP-72 is expressed in most pituitary adenomas with a mostly focal and less frequently diffuse pattern of overexpression.**

**Key Words:** Heat-shock proteins (HSPs); histology; immunohistochemistry (IHC); microwaves; pituitary adenoma; stellate cells.

## Introduction

Stress-response proteins (SRPs), also known as heat-shock proteins (HSPs), are produced in response to a nonlethal thermal shock or other stressful conditions, in order to resist sudden changes and to repair cell damages *(1,2)*. The role of SRPs in normal cells is connected with protein assembly and disassembly *(3)*, proliferation *(4)*, and differentiation *(1)*, as well as with signal transduction mechanisms *(5)*. In addition, SRPs have been found to interact with c-*myc*, H-*ras*, and p53 oncoproteins in lung tumors, and normal and pathological thyroid tissues in humans *(6–8)*. However, their role in neoplastic lesions remains obscure.

With regard to endocrine function, SRPs are thought to be implicated in regulation of the functional activities of various endocrine glands and target tissues. By binding to steroid receptors, they maintain the receptor stability and mediate the receptor–DNA interactions *(9–11)*. Their functional activities in various endocrine- and hormone-dependent tissues were recently reviewed *(12)*.

The study of SRPs in pituitary adenomas and nontumorous pituitaries is limited. HSP-25 has been localized by immunohistochemistry (IHC) in several cells of the rat adenohypophysis, corresponding to gonadotrophs or thyrotrophs *(13)*. In two separate studies of human brain tumors including pituitary adenomas, HSP-72 was demonstrated by IHC in one of eight tumors, whereas two of five adenomas were immunopositive for HSP-27 *(14,15)*. In addition, 2 of 10 noninvasive and 5 of 10 invasive pituitary adenomas were found to express HSP-27 *(16)*. Lastly, ubiquitin, a nonlysosomal protein involved in chromatin structure and degradation, was localized in 43.5% of pituitary adenomas and in Crooke's cells in 83% of glucocorticoid-treated patients *(17)*.

In the present study the expression of HSP-72 by IHC in a representative series of all common categories of pituitary adenomas was investigated, in order to identify the particular adenoma types that show immunoreactivity and to document sites of overexpression.

## Results

Overall, approx 90% (25 of 28) of adenomas were immunopositive for HSP-72. One somatotroph, one lactotroph, and one null cell adenomas containing only sparse faint immunopositive granules with no evidence of over-

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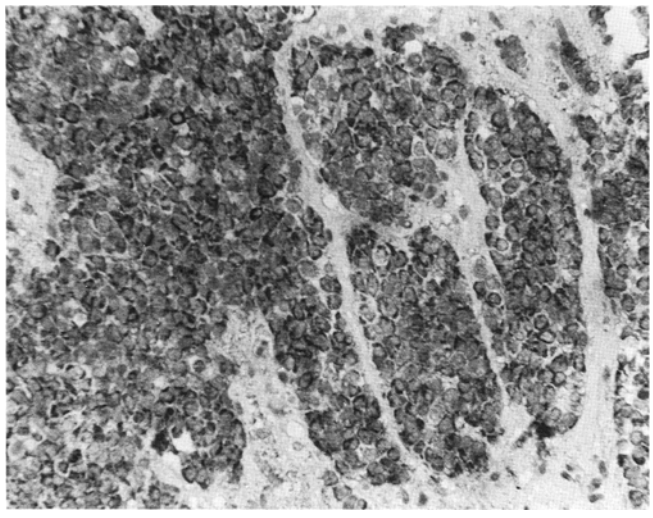

**Fig. 1.** Densely granulated somatotroph adenoma showing strong and extensive immunoreactivity for HSP-72 (ABC H&E, ×40).

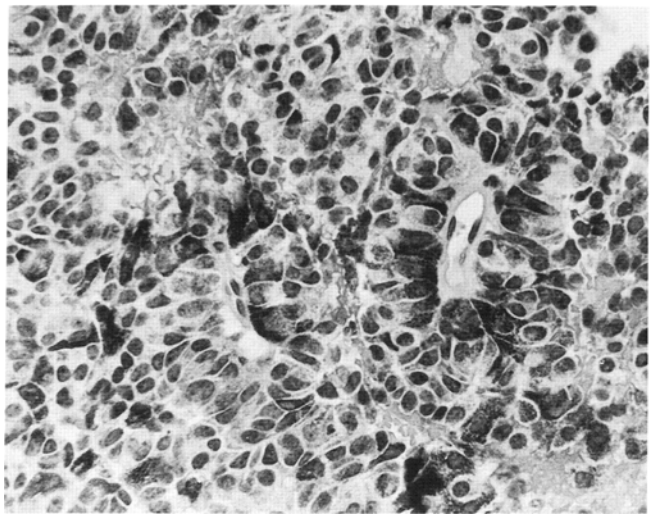

**Fig. 3.** Characteristic selective overexpression of HSP-72 of adenoma cells arranged around two small vessels (ABC H&E, ×100).

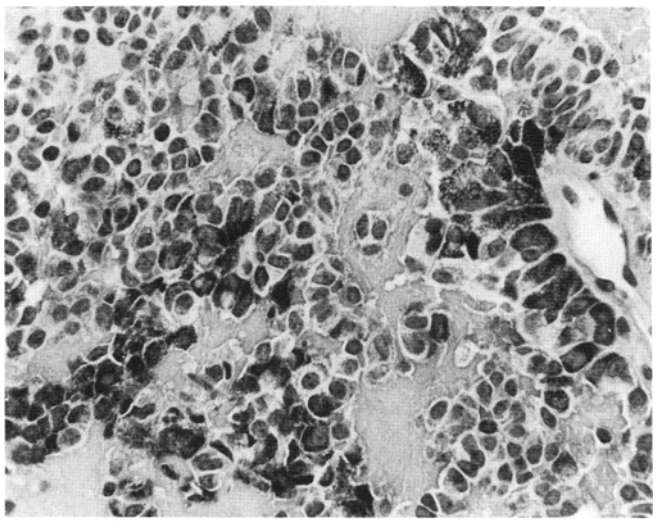

**Fig. 2.** Variable immunoreactivity for HSP-72 with focal overexpression in a corticotroph adenoma (ABC H&E, ×100).

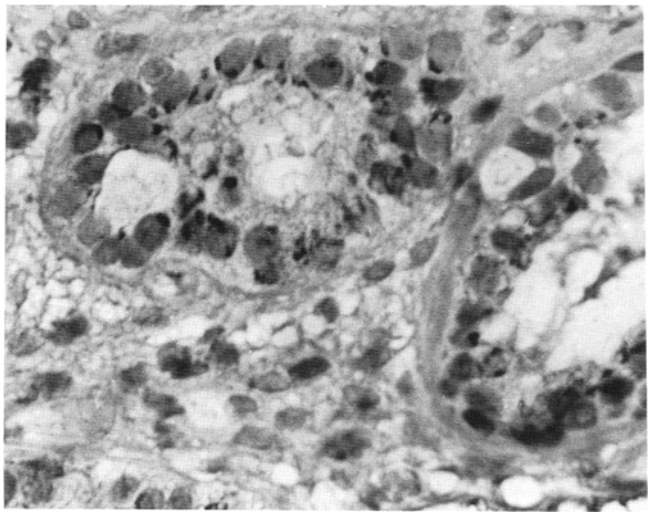

**Fig. 4.** Scattered cells of nontumorous adenohypophysial parenchyma immunoreactive for HSP-72 (ABC H&E, ×160).

expression were regarded as immunonegative for HSP-72. The degree and intensity of immunoreactivity were uneven, differing from cell to cell in the same tumor and among various adenomas. Dispersed fine granules of moderate density, diffusely distributed throughout the cytoplasm, represented a common pattern of immunoreactivity. Some adenoma cells, scattered or focally arranged in groups, exhibited strong and diffuse immunopositivity (Figs. 1 and 2). In 5 of 28 adenomas (one somatotroph, two corticotrophs, one null cell, and one oncocytoma), nearly all cells were strongly immunopositive. Selective and strong immunopositivity was commonly noted in adenoma cells adjacent to capillaries and small vessels (Fig. 3). Even in adenomas exhibiting strong and diffuse HSP-72 immunopositivity, the cells adjacent to capillaries were more prominently immunoreactive. In addition, several, scattered hormone-producing cells were encountered within fragments of nontumorous adenohypophysis containing various amounts of immunoreactive cytoplasmic granules (Fig. 4), which were also accumulated in S-100 protein immunopositive stellate cells forming follicles (Fig. 5). The latter exhibited characteristic dot-like, paranuclear pattern of immunoreactivity, probably corresponding to the prominent Golgi apparatus. Paranuclear localization of HSP-72 was also encountered in scattered stellate cells in one corticotroph adenoma (Fig. 6).

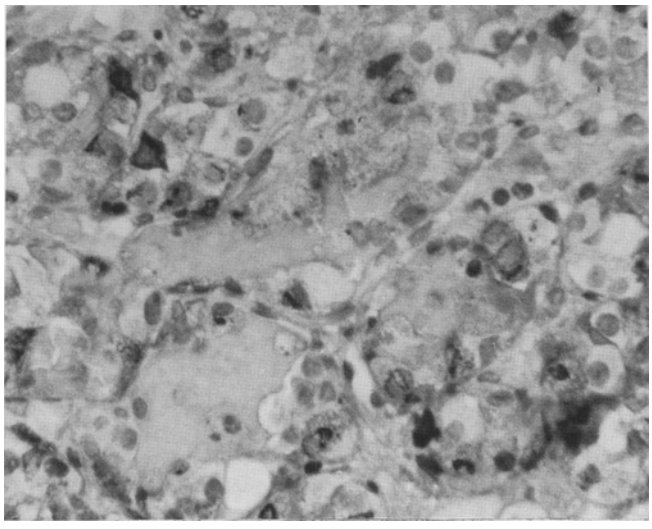

**Fig. 5.** Stellate cells forming follicles in nontumorous pituitary parenchyma and exhibiting a dot-like, paranuclear pattern of HSP-72 immunoreactivity (ABC-H&E ×160).

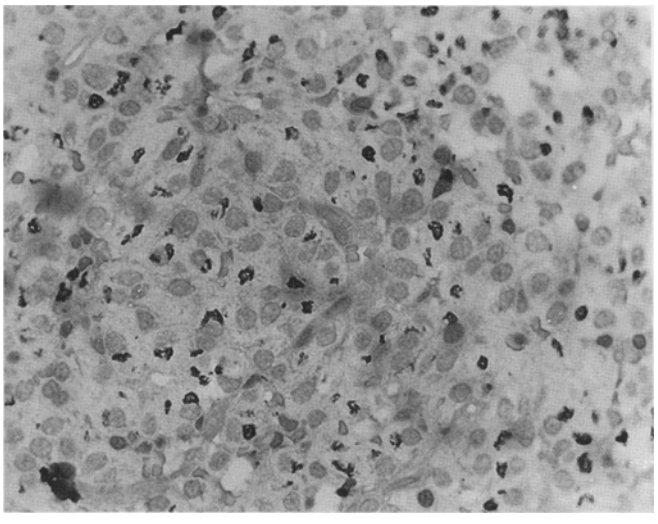

**Fig. 6.** Paranuclear localization of HSP-72 in a corticotroph adenoma (ABC-H&E ×100).

## Discussion

HSP-72 belongs to the HSP-70 family. Members of this family share high sequence homology of approx 95%. Among them, HSP-72 is thought to be constitutively expressed being overproduced in response to shock or stress *(18)*.

Regarding the central nervous system, induction of HSP-72 was found in animal experiments, in response to various stressful conditions, such as hyperthermia *(19)*, ischemia, and injury *(20)*. Increased expression of HSP-72 was demonstrated in neuritic plaques and neurofibrillary tangles in brains of patients with Alzheimer's disease *(21)*. In addition, HSP-72 was demonstrated by IHC in brain tumors, including pituitary adenomas *(14)*.

In the authors' studies, up to 90% of pituitary adenomas were immunoreactive for HSP-72. An uneven distribution of immunoreactivity differing in degree and intensity was commonly encountered. Most adenoma cells exhibited a microgranular pattern of immunoreactivity, whereas scattered cells or small groups of cells were strongly positive. These findings suggest a constitutive expression of HSP-72 variably associated with focal induction.

Pituitary adenomas are affected by changes in the endocrine milieu including hypothalamic factors and steroid hormones. Since SRPs couple steroid receptors in the absence of ligand, it seems that heterogeneity of HSP-72 expression may represent a variable receptor status, depending on differences in ligand–receptor complex levels among adenoma cells. Cells adjacent to capillaries are directly exposed to regulatory hypothalamic and other circulating factors. Thus, they might be most likely prone to overexpress HSP-72 by producing large amounts of SRPs in response to frequent alteration in their microenvironment.

Previous studies have confirmed the expression of estrogen receptor mRNA by *in situ* hybridization in all hormone secreting cells of the nontumorous pituitary, and in all adenoma types *(22)*. In addition, lactotroph and mammosomatotroph adenomas exhibited the strongest hybridization signal; however, the signal was weak or absent in bromocriptine-treated tumors *(22)*. The presence of estrogen receptors in all types of adenomas is in keeping with the HSP-72 expression by the substantial majority of tumors, and with the weak expression in most of lactotroph adenomas of these series, which were all treated with bromocriptine.

The presence of SRPs in stellate cells reveals an additional role of this particular cell type *(17)*. However, the pattern of paranuclear localization of HSP-72 in stellate cells, probably corresponding to the prominent Golgi complex, requires additional morphologic studies. In conclusion, our results suggest a constitutive expression of HSP-72 by most pituitary adenomas, which is variably associated with focal or diffuse, probably inducible overexpression.

## Materials and Methods

Twenty surgically removed pituitary adenomas including five somatotroph, two mammosomatotroph, six lactotroph, six corticotroph, and four null cell adenomas, and three oncocytomas were studied. The patients with lactotroph adenomas were preoperatively treated with bromocriptine. All tumors were diagnosed and classified by histology, IHC for anterior pituitary hormones and electron microscopy (Table 1). DG-SM adenomas were focally immunoreactive for PRL, α- and one or more β-subunits in addition to GH. This type of adenomas represents the most common among plurihormonal tumors, constituting approx 50% of all patients associated with acromegaly *(23,24)*. Three of the

**Table 1**
Morphological and Immunohistochemical Data[a]

| Case | EM diagnosis | Pituitary hormone immunoreactivity[b] | HSP-72 expression | |
|---|---|---|---|---|
| | | | Density | Extent |
| 1 | DG-SM | GH, *PRL, β-TSH, β-LH, α-SU* | ++++ | ++++ |
| 2 | DG-SM | GH, *PRL, β-TSH, β-LH, α-SU* | ++ | ++ |
| 3 | DG-SM | GH, *PRL, β-TSH, β-LH, α-SU* | ++ | ++ |
| 4 | DG-SM | GH, *PRL, β-TSH, β-LH, α-SU* | – | – |
| 5 | SG-SM | GH | ++++ | + |
| 6 | SG-SM | GH, *β-FSH* | + | + |
| 7 | MIXED | GH, PRL | + | + |
| 8 | MSM | GH, PRL | + | + |
| 9 | MSM | GH, PRL | + | + |
| 10 | SG-PRL | PRL | + | + |
| 11 | SG-PRL | PRL | + | + |
| 12 | SG-PRL | PRL | + | + |
| 13 | SG-PRL | PRL | ++ | ++ |
| 14 | SG-PRL | PRL | – | – |
| 15 | SG-PRL | PRL | + | + |
| 16 | CORT | ACTH | + | + |
| 17 | COTR | ACTH | + | + |
| 18 | CORT | ACTH | + | + |
| 19 | CORT | ACTH | ++ | ++++ |
| 20 | CORT | ACTH | + | + |
| 21 | CORT | ACTH | ++++ | ++++ |
| 22 | NULL-GLY | *β-LH, α-SU* | ++ | + |
| 23 | NULL | *β-TSH* | ++++ | ++++ |
| 24 | NULL-GLY | *β-TSH, β-FSH, β-LH, α-SU* | – | – |
| 25 | NULL-GLY | *GH, PRL, ACTH, β-FSH, β-LH, α-SU* | + | + |
| 26 | ONCO | – | ++++ | ++++ |
| 27 | ONCO-GLY | *β-FSH, α-SU* | ++ | + |
| 28 | ONCO-GLY | *β-TSH, β-LH, α-SU* | + | + |

[a]Abbreviations: CORT, corticotroph adenoma; DG-PRL, densely granulated lactotroph adenoma; DG-SM, densely granulated somatotroph adenoma; GLY, signs of glycoprotein differentiation (gonadotroph adenoma); MIXED, mixed somatotroph–lactotroph adenoma; MSM, mammosomatotroph adenoma; NULL, null cell adenoma; ONCO, oncocytoma; SG-PRL, sparsely granulated lactotroph adenoma; SG-SM, sparsely granulated somatotroph adenoma.
[b]Italics indicate focal hormone immunoreactivities, in small groups or scattered adenoma cells.

null cell adenomas and two oncocytomas exhibited immunohistochemical and electron microscopic signs of glycoprotein differentiation and thus, could be regarded as gonadotroph adenomas *(25)*. Antibodies were directed toward the following hormones: growth hormone (GH; dilution 1:4000, AFP-163102481), prolactin (PRL; dilution 1:1500, AFP-55781789), adrenocorticotropic hormone (ACTH; dilution 1:2000, AFP-173-P), β-thyroid-stimulating hormone (β-TSH; dilution 1:2000, AFP-55741789), β-follicle-stimulating hormone (β-FSH; dilution 1:1500, AFP-891891), β-luteinizing hormone (β-LH; dilution 1:2000, AFP-54372), and α-subunit of LH (α-SU; dilution 1:2000, AFP-#2). Before application of the primary antiserum, sections were pretreated with 5 mg/10 mL pronase E (Sigma, St. Louis, MO) for 10 min at room temperature. Incubations with the primary antibodies were carried out overnight at 4°C. All antibodies for pituitary hormones of IHC grade were donated by the National Hormone and Pituitary Program (NHPP, Rockville, MD).

For HSP-72 IHC, deparaffinized sections were rehydrated in graded alcohols, and transferred in a plastic jar containing Tissue Unmasking Fluid (TUF; Kreatech Diagnostics, Amsterdam, Holland) diluted 1:3 with distilled water. The jar was placed in the center of the turntable of a microwave oven and was exposed three times to irradiation at high power (650 W) for 5 min each, with a 5 min intervals. To avoid tissue drying, the evaporated content was replaced with warm distilled water. After the last heating, the sections remained in the oven for 20 min, then washed with tap water for 10 min and after rinsing with phosphate-buffered saline (PBS) proceeded to IHC. Subsequently, the sections were incubated overnight at 4°C with a monoclonal antibody specific for HSP-72 (dilution 1:200, Amersham, Bucks, England). For detection of antigen–

antibody binding sites, the avidin–biotin–peroxidase complex (ABC) detection system was employed (elite Vectastain kit, Vector Labs, Burlingame, CA) using the chromogen 3'3'-diaminobenzidine (DAB, Sigma).

The specificity of HSP-72 immunoreactivity was tested using positive control sections of lymph nodes containing metastases from breast carcinoma. Sections incubated with PBS or normal horse serum served as negative controls.

To identify stellate cells, IHC with a monoclonal S-100 protein antibody (dilution 1:1000, Dako, Copenhagen, Denmark) that specifically recognizes these cells was performed. IHC for S-100 protein and HSP-72 was employed on serial sections and the results were compared. The primary antibody was applied after incubation with microwaves.

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