

“Silent” corticotropinoma

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Abstract

OBJECTIVES: The aim of the study was to evaluate the ACTH-immunopositive pituitary adenomas, especially those without manifestation of Cushing’s disease

MATERIAL AND METHODS: 148 pituitary adenomas removed surgically in years 1994–2007 were studied. The paraffin sections were immunostained with antibodies against the pituitary hormones. In 79 adenomas the immunostaining with anti-ACTH antibody was performed. Additionally, 23 tumors were also immunostained with anti-Ki-67 (MIB-1) antibody. Visualization of reactions was done by means of streptavidin-biotin-peroxidase technique with use of 3,3'-diaminobenzidine as chromogen.

RESULTS: ACTH immunopositivity was found in 34 cases (23%). Fourteen ACTH-immunopositive tumors manifested themselves as Cushing’s disease (including 1 case of Nelson’s syndrome). In the remaining 20 cases in spite of the positive immunostaining for ACTH of the tumor cells, no features of hypercortisolism were observed (in several cases even hypocortisolism was found). Thus, those tumors represented so-called “silent” corticotropinomas. Over one third (37%) of “clinically” nonfunctioning pituitary adenomas, when immunostained with anti-ACTH antibody, showed ACTH immunopositivity. Three adenomas in patients with Cushing’s disease (21.4%) and 7 “silent” corticotropinomas (35%) were recurrent tumors. In contrast, the recurrence rate in the group of ACTH-immunonegative clinically nonfunctioning pituitary adenomas was 14.7%. The “silent” corticotropinomas exhibited a tendency towards the higher expression of a proliferation marker, Ki-67 antigen as compared to the “active” corticotropinomas.

CONCLUSIONS: (i) “Silent” corticotropinomas are rather frequent. (ii) This adenoma type should be considered as aggressive. (iii) It is hypothesized that – like in Nelson’s syndrome – the lack of hypercortisolism or even presence of hypocortisolism favors the exaggerated growth of tumoral corticotrophs.

INTRODUCTION

ACTH-secreting adenoma (corticotropinoma) is a main or even a sole cause of the ACTH-dependent pituitary Cushing syndrome (Cushing disease)[1]. However, it is well known that in several cases the presence of ACTH immunoreactivity

in the adenoma cells is not accompanied by biochemical and/or clinical features of the Cushing disease. Such adenomas were called “silent corticotropinomas” [2,3]. The molecular mechanisms responsible for the lack of hypercortisolemia in spite of the expression of ACTH in pituitary adenoma remain unclear. However, several possibili-

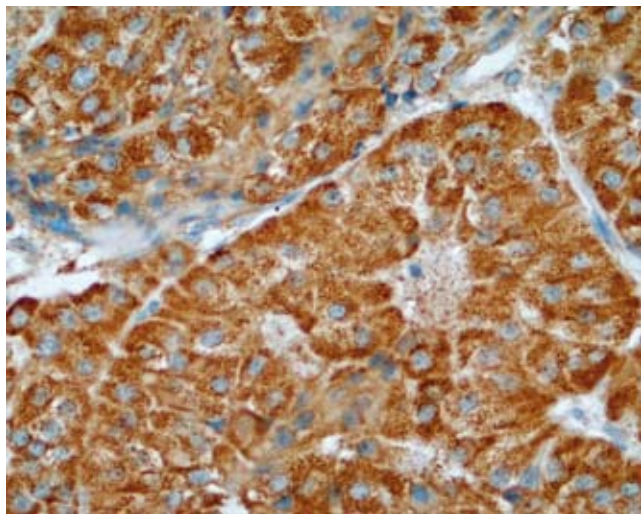


Fig.1. ACTH immunostaining of pituitary adenoma in man JS without symptoms of Cushing's disease

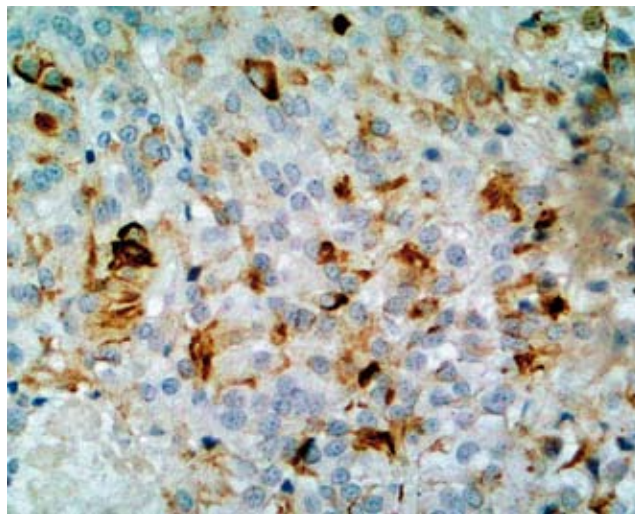


Fig.2. ACTH immunostaining of pituitary adenoma in a woman JW without symptoms of Cushing's disease

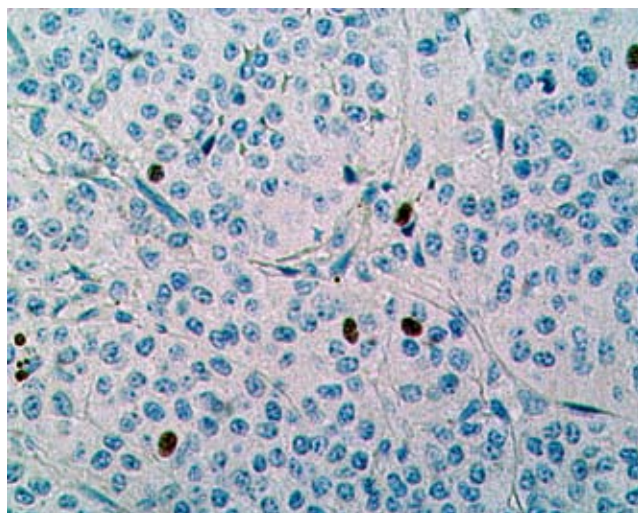


Fig.3. Ki-67 immunostaining in the recurrent "silent" corticotropinoma in a woman AS.

ties have been indicated, like the incomplete processing of proopiomelanocortin (POMC) in the tumoral corticotrophs, leading to secretion of the larger molecular forms devoid of corticotropic action [4,5]. Such a possibility is supported by the recent studies showing the reduced expression of prohormone convertase 1/3, an enzyme involved in POMC processing, in silent corticotropinomas [6]. The normal cortisol levels in spite of the enhanced ACTH levels in blood at least in some cases of silent corticotropinomas may also be explained by the above mentioned abnormality [2]. However, a decreased responsiveness of the adrenal cortex to ACTH could be also taken into consideration. Recently, some other differences in genes expression between adenomas with active Cushing's disease and "silent" corticotropinomas are described. It concerns the higher expression of genes for corticotropin releasing hormone receptor, vasopressin 1b receptor and 11beta hydroxysteroid dehydrogenase 2 in "active" Cushing's disease as compared to "silent" corticotropinomas [7]. Irrespec-

tive of the knowledge of molecular mechanisms leading to the lack of hypercortisolism in some corticotropinomas, the questions concerning their frequency and clinical significance are still unanswered.

The aim of the study was to evaluate the incidence and features of ACTH-immunopositive pituitary adenomas, especially those without manifestation of Cushing's disease.

MATERIAL AND METHODS

One hundred forty eight pituitary adenomas removed surgically were immunostained on paraffin sections with antibodies against the pituitary hormones, including the immunostaining with anti-ACTH polyclonal antibody (Sigma) in 79 tumors. Twenty three tumors were also immunostained with anti-Ki-67 antigen (MIB-1) antibody (Dako-Cytomation, Denmark). Visualization of reactions was done by means of streptavidin-biotin-

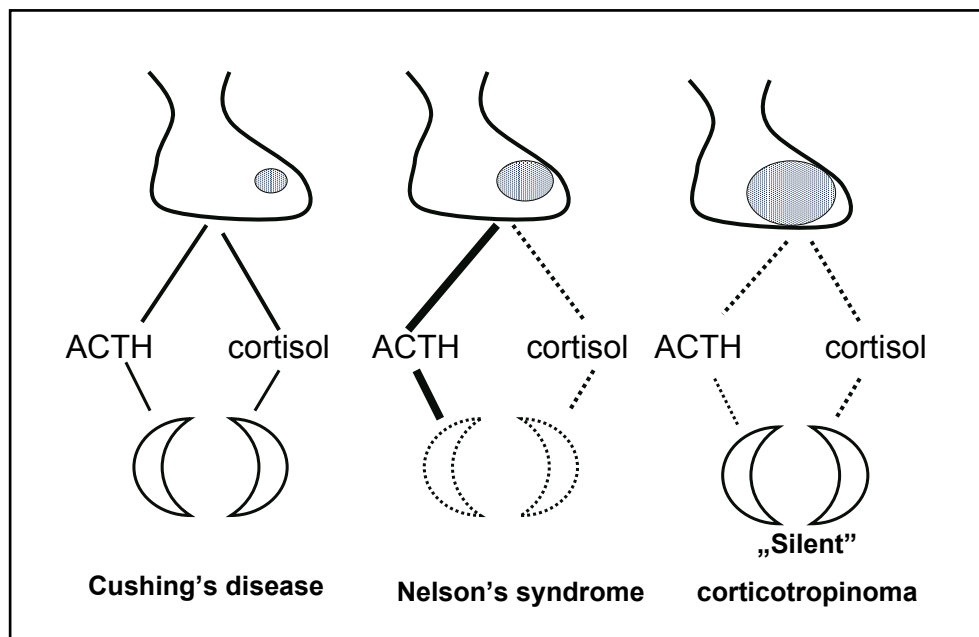


Fig.4. Schematic illustration of hypothesis explaining the enhanced growth of "silent" corticotropinomas.

peroxidase technique with use of 3,3'-diaminobenzidine as chromogen.

RESULTS

ACTH immunopositivity was found in 34 cases (23% of all examined pituitary adenomas). Fourteen ACTH-immunopositive tumors (41.2% of ACTH-immunopositive adenomas) manifested themselves as Cushing's disease (including 1 case of Nelson's syndrome). In the remaining 20 cases (58.8%) in spite of the positive immunostaining for ACTH of the tumor cells (Fig.1 and 2), no features of hypercortisolism were observed. Thus, those tumors represented the so-called "silent" corticotropinomas. In 13 patients with "silent" corticotropinomas the morning ACTH and cortisol concentrations in blood serum were available. In none of them the elevation of neither ACTH nor cortisol was found. Interestingly, 4 of them presented ACTH and/or cortisol levels below the lower limit of the normal values.

In patients with Cushing disease, as expected, a prevalence of the female sex was found (11 women and 3 men). In contrast, this prevalence was not observed in the group of "silent" corticotropinomas (10 women and 10 men).

All "silent" corticotropinomas were diagnosed before surgery as nonfunctioning. The frequency of these tumors in the group of "clinically" nonfunctioning pituitary adenomas was estimated in our material as 27.7% (20/54). In 10 cases ACTH was the only pituitary hormone detected in the tumoral cells, while in the remaining cases ACTH was co-expressed with prolactin

(18 cases), FSH (3 cases), LH (3 cases), GH (1 case), TSH (1 case) or free alpha-subunit (2 cases). Three adenomas in the group with Cushing's disease, including a patient with Nelson's syndrome (21.4%) and 7 "silent" corticotropinomas (35%) were recurrent tumors. In contrast, the recurrence rate in the group of ACTH-immunonegative clinically nonfunctioning pituitary adenomas was 5/34 (14.7%).

Ten adenomas from patients with Cushing's disease and 13 "silent" corticotropinomas were immunostained with anti-Ki-67 antibody (Fig.3). The values of Ki-67 indexes (% of Ki-67-positive nuclei) were very variable in both investigated groups. However, a tendency towards higher Ki-67 values in "silent" corticotropinomas were noticed (3,77 +/- 1,67% vs 1,48 +/- 0,6%, means +/- SEM). Atypical adenomas (Ki-67 > 3%) seemed also more frequent in the group of "silent" corticotropinomas (4/13) than in Cushing's disease (2/10).

DISCUSSION

The data presented above indicate that "silent" corticotropinomas are rather frequent. They are at least as frequent as the active corticotropinomas manifesting the Cushing's disease and represent approximately one third of all "clinically" nonfunctioning pituitary adenomas. Our data roughly corroborate with observation of Tateno et al. [6] who found 6 silent corticotropinomas among 30 nonfunctioning adenomas immunostained for ACTH. The answer why those tumors do not produce Cushing's disease in spite of enhanced intratumoral ACTH expression still remains unclear and needs further studies at the molecular level. Tateno et

al.[6] showed the lack of immunopositivity for pro-hormone convertase 1/3 (PC 1/3) in silent corticotropinomas, in contrast to the expression of this enzyme in active tumors. Since PC 1/3 is responsible for excision of ACTH molecule from proopiomelanocortin (POMC), its defect may lead to the deficiency of ACTH secretion. However, the defect of PC 1/3 in silent corticotropinomas was not confirmed by other authors [3]. The data presented in this study suggest that “silent” corticotropinomas should be considered as more aggressive in comparison to the “active” corticotropinomas in Cushing’s disease or ACTH-immunonegative clinically nonfunctioning pituitary adenomas.

This suggestion is compatible with the earlier observations of Bradley et al [8] who found the higher invasiveness of “silent” corticotropinomas as compared to ACTH-immunonegative tumors. However, in contrast to our data they do not observed a higher recurrence rate of the former. In an earlier paper we found that “silent” corticotropinomas exhibited high expression of the another proliferation marker, PCNA [9]. The PCNA index calculated for “silent” corticotropinomas was significantly higher than the indexes estimated for all other types of pituitary adenomas including corticotropinomas in patients with Cushing’s disease. A question arises what may be a cause of the higher aggressiveness of silent corticotropinomas in comparison of active corticotropinomas in patients with Cushing’s disease as well as with ACTH-immunonegative hormonally nonfunctioning pituitary adenomas. It is well known that corticotropinomas in patients with Cushing’s disease become more aggressive after adrenalectomy. This exaggeration of corticotropinoma growth in the patients suffering from Cushing’s disease was called Nelson’s syndrome and is certainly evoked by the failure of glucocorticoid feedback action [10,11,12]. Such a feedback action is also impaired in “silent” corticotropinomas as compared with active tumors in Cushing’s disease. We hypothesize that in silent corticotropinomas –like in Nelson’s syndrome – the lack of hypercortisolism or even presence of hypocortisolism favors the exaggerated growth of tumoral corticotrophs (see Fig.4.). Some recent papers show that “silent” corticotropinomas differ from the “active” corticotropinomas by galectin-3 (Gal-3) expression, which seems to be defective in “silent” adenomas [13,14]. Since Gal-3 is a protein involved in a variety of biological functions, including cell proliferation and apoptosis, its alteration may contribute to the enhanced growth of the latter type of adenomas. This presumption does not stay in opposition to our hypothesis, because it was recently shown that glucocorticoids modulates Gal-3 expression [15].

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