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Evaluation of the Biological Behavior of ACTH-Secreting Pituitary Tumors Using Various Methods

A.M. LAPSHINA, E.I. MAROVA

Endocrinology Research Center of the Ministry of Health of the Russian Federation, Moscow, Russia

This literature review is aimed at studying markers obtained by various research methods to assess the biological behavior of ACTH-secreting pituitary tumors. This paper presents the clinical, laboratory, instrumental, histological, and immunohistochemical aspects of ACTH-secreting pituitary tumors. Histological method allows assessment of tumor structure and differential diagnosis from other pathological processes in the sella turcica. Immunohistochemical method enables determining the expression of multiple markers (fibroblast growth factors, matrix metalloproteinases, apoptotic factors, pituitary tumor transforming gene, and vascular markers), which reflect various processes of oncogenesis of studied tumors and, according to the authors, are promising with regard to prediction of biological behavior of corticotropinomas. Epigenetic investigations including the study of microRNA expression, proteomics, and transcriptomics are the novel methods in this area, which are expected to provide additional details regarding the prognosis of corticotropinomas. Therefore, the cumulative assessment using laboratory and instrumental methods and the study of immunoexpression of microRNA-markers of malignant potential in corticotropinoma tissue will allow predicting the further biological behavior of ACTH-secreting pituitary tumors.

Keywords: *ACTH-secreting pituitary tumor, evaluation and markers of malignant potential.*

Pituitary adenomas are neuroendocrine tumors that originate from adenohypophysis cells. Previously, it was believed that pituitary tumors account for about 10% of intracranial tumors. However, significant improvement of radiological and hormonal diagnostic techniques increases the number of diagnosed cases up to 25%, and hormonally active corticotropinomas account for 10—15% of them [1, 2].

The course and clinical presentation of pituitary tumors are diverse (with excessive secretion of various hypophysiotropic hormones or without hormone overproduction). In both cases, extrasellar tumor growth can be accompanied by damage to surrounding structures of the central nervous system (CNS). The vast majority of pituitary adenomas (about 98—99%) are benign tumors. However, determining the prognosis of the further course of pituitary adenomas and effectiveness of various treatments is still an open issue. Thus, over the past few decades, there were many studies aimed at discovering markers that may be associated with pituitary adenomas and increased malignant potential, i.e. invasive growth into the surrounding CNS structures, the development of relapses after various treatments, and the appearance of metastases (in case of pituitary carcinomas). As a result, numerous molecular and immunohistochemical markers were discovered, which are indicative of the aggressive potential of pituitary adenomas.

The analysis of the biological behavior of corticotropinomas would be incomplete if we evaluated only disembodied data from different methods. It is important to evaluate the whole clinical presentation, the data of laboratory and instrumental methods. Assessment of the histological structure and immunophenotype of tissue samples from resected tumors enables the differential diagnosis between pituitary adenomas and other pathological processes in the sella turcica, determining hormonal and proliferative activity, receptor status, etc. To this end, molecular genetic techniques can additionally be used.

The analysis of clinical presentation and blood tests for the level of hypophysiotropic hormones are required to understand the tumor hormone production profile, assess disease activity and the results of treatment (clinical and biochemical remission or tumor progression after surgical, pharmacological, or radiological treatment) [3].

Magnetic resonance imaging (MRI) plays the most important role in assessing pituitary tumor size, growth direction, and involvement of the surrounding CNS structures. T1 MRI with 3 mm slice thickness is and optimal method to diagnose pituitary adenomas. Contrast-enhanced MRI is used for clearer visualization of microadenoma due to different vascularization of tumor tissue and intact adenohypophysis (adenohypophysis tissue accumulates contrast agent more intensively, while tumor tissue demonstrates "accumulation defect") [4]. It is particularly important to note that MRI provides assessment of the presence of invasive growth. J. Trouillas et al. [5] demonstrated that the presence or absence of invasive pituitary tumor growth, as shown by MRI, is the main predictor (using multivariate analysis and ROC-curve) of remission or relapse after neurosurgical resection of the aforementioned tumors. It is also known that MRI is more valuable method to detect invasive growth compared to histological method. Histological examination can detect only 9% of invasive tumors [6].

Another problem with verification of corticotropinomas may be associated with the absence of tumor on MRI. In this situation, blood sampling from the cavernous sinus with desmopressin stimulation is recommended to confirm the ACTH-secreting pituitary tumor [3].

Histological examination of corticotropinomas with hematoxylin staining enables determining the histological structure of tumors and, if necessary, differential diagnosis with other pathological processes in the sella turcica: metastases, craniopharyngiomas, various cysts, inflammatory changes, hyperplasia of the adenohypophysis [7].

ACTH-secreting adenomas are typically characterized by intrasellar location in the middle sector of the pituitary gland. Macroscopically, corticotropinomas tissue is red, soft, and distinctive from a yellowish-brown dense pituitary gland.

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e-mail: nottoforget@yandex.ru

Microscopically, it is sometimes difficult to distinguish between tumor tissue and the surrounding pituitary gland due to poor capsule or its absence [8].

According to electron microscopy data, corticotropinomas are represented by densely granular and sparsely granular tumors. Today, electron microscopy has been replaced by simpler and less time-consuming method of immunohistochemistry using antibodies to low molecular weight keratin CAM 5.2, as well as periodic acid Schiff (PAS) reaction. Densely granulated corticotropinomas consist of monomorphic round basophil cells with pronounced diffuse immunoexpression of ACTH (about 60% of cells are monohormonal, while other can express various hypophysiotropic hormones and CAM 5.2). In these cells, mitoses are rare and Ki-67 index is low (under 1%).

Sparsely granular corticotropinomas consist of chromophobe cells characterized by diffuse growth and formation of sinusoidal structures around the capillaries. Cellular and nuclear polymorphism is often observed, there are signs of apoptosis, and mitoses are very rare. Immunohistochemical reaction with antibodies to ACTH is weak, tumor cell foci are stained, there is pronounced expression of CAM 5.2 and weak PAS reaction.

So-called Crooke's cell adenomas are very rare. They consist of large chromophobes and eosinophilic cells with pronounced Crooke's hyaline changes (accumulation of keratin results in glassy cytoplasm), which occur when pituitary cells are exposed to high levels of cortisol. Keratin deposition in cytoplasm results in shift of ACTH-positive granules towards the periphery of tumor cells or perinuclear space. Staining with antibodies to CAM 5.2 looks like a ring around cell nucleus. These large polymorphic cells must be differentiated from neurons of gangliocytomas or metastatic carcinomas at other sites. Immunoexpression of Tpit (T-box transcription factor) gives evidence of differentiation of corticotroph cells [9].

In patients with Cushing's disease (CD), the size of the tumor and its expansion in the sella turcica is not always associated with the severity of symptoms caused by excessive production of ACTH. Classical course of CD is usually associated with the presence of pituitary microadenomas (70—90%), whose histological examination reveals a densely granular corticotropinoma [9, 10]. Other macro-corticotropinomas have scarcely granular histological structure or represent adenomas with Crooke's changes [11]. Macroadenomas are often characterized by cyclic clinical course, incomplete picture of hypercorticism, or lack of hormonal activity with a tendency to tumor progression after various treatments. [12]

A careful examination of the tissue surrounding the pituitary tumor may provide additional information. In cases where there is no pituitary tumor in surgical material, histological study can reveal the presence of Crooke's changes in pituitary tissue outside the tumor. This gives an evidence of hypercortisolemia, which affects the adenohypophysis cells in this way [9, 13, 14].

Resection of corticotropinomas gives rise to the question of further disease prognosis.

Classification of the WHO in 2004 [8] identifies typical and atypical pituitary adenomas and pituitary carcinomas. Diagnostic criteria of typical adenomas include negative immunoexpression of p53, Ki-67 index below 3%, and low mitotic activity. Atypical adenomas are characterized by excessive immunoexpression of p53, Ki-67 index above 3%, and high mitotic activity. The principal difference between carcinomas and pituitary adenomas lies in the presence of craniospinal and/or distant metastases.

The study of tissue samples of 121 pituitary adenomas by G. Zada et al. [15] revealed 15% of atypical adenomas: hormonally active (somatotropinomas, n= 32, 5 of them were atypical, corticotropinomas, n=12, 2 of them were atypical, prolactinomas, n= 18, 2 of them were atypical) and inactive tumors (n=58, 9 of them were atypical). However, there was no dynamic follow-up of patients after tumor resection.

The study of W. Saeger [7] revealed significantly lower amount of atypical adenomas. A total of 451 pituitary adenomas have been studied, 12 (2.7%) of them were atypical. Atypical adenomas were represented by 3 somatotropinomas, 1 prolactinoma, 3 corticotropinomas, 1 gonadotropinoma, 3 null cell adenomas, and 1 plurihormonal adenoma. This discrepancy can be explained by the fact that different criteria were used to assess the results.

Some experts suggest adjustments to the classification of pituitary adenomas, which would takes into account the clinical presentation and the data of laboratory and instrumental studies, the results of surgical treatment, and further course of the disease.

G. Raverot et al. [6] suggest more detailed clinical and pathological classification of pituitary tumors, which is based on the following characteristics.

1. Tumor size on MRI: microadenomas (less than 10 mm), macroadenomas (more than 10 mm), and giant tumors (more than 40 mm)

2. Hormonal profile of the tumor according to the immunohistochemical studies: CTH-, PRL-, ACTH-, LH/FSH-, and TSH-secreting adenomas.

3. The stage of tumor development according to the following criteria:

- invasive tumor growth into the cavernous or sphenoidal sinuses according to histological examination and MRI;
- proliferative activity based on the presence of two of the three attributes: mitoses (2 in 10 fields of view); Ki-67 index not less than 3%; positive P53 (more than 10 stained nuclei in 10 fields of view);

Based on these characteristics, three are three stages of tumor development:

1a — non-invasive;
1b — non-invasive and proliferative;
2a — invasive;
2b — invasive and proliferative
3 — metastatic, with the development of cerebrospinal or distant metastases (pituitary carcinoma).

The proliferative activity of pituitary adenomas can be assessed based on the most common markers (number of mitoses, Ki-67, and p53). Given the contradictory significance of these markers, especially Ki-67, underspecified methodological standards and P53 cut-off values, the authors of this classification suggest the use of at least two of the three criteria of proliferative activity: more than two mitoses per 10 fields of view and Ki-67 index not less than 3%, similarly to endocrine pancreatic tumors [16].

The authors believe that this classification has weighty prognostic value regarding the postoperative period for all types of pituitary adenomas. If a patient has invasive and proliferating tumor (2b stage), the probability of tumor progression increased 12-fold compared to non-invasive and non-proliferative tumor (1a stage) during 8 years [17].

Diagnosis of pituitary carcinomas posed some problems both previously and currently. It is believed that pituitary

carcinoma is a tumor derived from the adenohypophysis cells with proliferation of metastases into the central nervous system and distant organs (WHO classification, 2004). According to various reports, malignant pituitary tumors occur in 0.2—1% of cases with confirmed cranial and/or spinal metastases. In total, the literature [6, 18] describes 132 cases of pituitary carcinomas during 1961—2009. Most of them are hormonally active carcinomas: 36% of prolactinomas and 30% of corticotropinomas. Hormonally inactive carcinomas account for about 23%.

Macroscopic view of most pituitary carcinomas do not differ from that of invasive macroadenomas. The main features of carcinomas include the continued growth in the form of one or more subarachnoid nodules with invasion into the underlying brain tissue or dura mater.

The majority of malignant pituitary tumors demonstrate invasive growth into sella turcica structures (dura mater, bones, cavernous sinuses, cranial nerves) and hypothalamus. The tumor spreads to the central nervous system through the cerebrospinal fluid. Systemic metastases spread through the hematogenous way via cavernous sinuses and jugular vein, which is accompanied by involvement of the liver, lungs, bones, adrenal glands, heart, and even ovaries. In the case of invasive growth into the skull base and soft tissues, the tumor is disseminated through the cervical lymph nodes.

No histological, immunohistochemical, and ultrastructural features, providing reliable diagnosis of malignant pituitary tumor, especially in cases where the tumor does not expand beyond the sella turcica, have been discovered so far [19].

Microscopic structure of pituitary carcinomas is more or less similar to that of adenomas; the only distinctive feature is the presence of metastases. Mitotic activity may also vary and it is found in 3.9% of noninvasive adenomas, 21.4% of invasive adenomas, and 66.7% of carcinomas.

Both carcinomas and adenomas are immunopositive to various pituitary hormones (they typically express ACTH and PRL with development of corresponding clinical presentation), neuroendocrine markers (synaptophysin and chromogranin A).

When comparing the expression of tumor suppressor gene (TP53), adenomas were found to lack the expression of this marker, while 15% of invasive adenomas and 100% of carcinomas and their metastases demonstrated pronounced immunoexpression. In many carcinomas, the presence of p53, p27, topoisomerase 2a (which regulates DNA replication), VEGF, and HER-2/neo was observed. Cell-cycle inhibitors are rarely observed in carcinomas compared to normal adenohypophysis. Ki-67 index is the highest in carcinomas (12%) compared to invasive (4.5%) and non-invasive (1%) adenomas [8].

The average survival of patients is 2 years and it is reduced to 1 year and below when metastases are detected. Continued growth of the tumor on MRI, growth of hypopituitarism symptoms or significant increase in hormone levels, disappearance of sensitivity to a dopamine agonists or somatostatin analogues are unfavorable prognostic factors.

In recent years, numerous investigations were conducted studying a large number of biological markers associated with unfavorable course of pituitary adenomas (invasive properties, tumor progression). However, no marker that could be used alone to determine the prognosis of the behavior of pituitary tumors has been discovered so far.

This literature review presents a number of biological markers (fibroblast growth factors, matrix metalloproteinases, apoptotic factors, pituitary tumor transforming gene, vascular markers), which can be detected by means of immunohistochemical studies and are considered as the most accurate prognostic markers with respect to biological behavior of pituitary adenomas.

Expression of the second and fourth types of fibroblast growth factors (FGF2, FGF4), regulating the growth, differentiation, cell migration, and angiogenesis, were found in the pituitary gland [21]. The highest expression of these factors was observed in corticotropinomas compared to somatotropinomas and prolactinomas. This may be due to the involvement of FGFs in the pathogenesis of ACTH-secreting tumors. The loss of FGF2 in the tumor results in activation of MAGE-A3 (melanoma-associated A3 antigen) [49] and modified FGF4 isoform, which lead to invasive growth of tumor cells in vivo and the loss of N-cadherin expression at the cell membrane. Moreover, modified FGF4 isoform interacts with NCAM (neuronal cell adhesion molecules), which is also associated with invasive properties of pituitary adenomas.

Matrix metalloproteinases (MMPs) are the family of single-stranded zinc-containing proteolytic enzymes that regulate the state of the extracellular matrix in physiological and pathological conditions. Eight various classes of metalloproteinases have been described, which include 24 functional types. MMPs penetrate into the extracellular matrix structures, such as collagen fibers, laminin, fibronectin, vitronectin, and proteoglycans. MMPs are produced by invasive tumor cells, fibroblasts, macrophages, etc. [20]. MMPs, in particular type 9 ones, destroy type 4, 5 and 10 collagen fibers, elastin, fibronectin, and proteins associated with proteoglycans. MMP2 and MMP9 are expressed by vascular endothelial cells and stromal cells. MMP9 expression was found in breast and lung malignant tumors. In pituitary adenomas, MMP expression is significantly higher than in invasive tumors [21—23].

Taking into account the aforementioned properties of fibroblast growth factor and matrix metalloproteinases, the study of these markers is believed to be promising with a view to determining the unfavorable prognosis of corticotropinomas.

The process of apoptosis in pituitary tumors is poorly known. Pituitary adenomas are tumors with low proliferative activity (a small number of mitoses, low Ki-67 index) and low level of apoptosis. Apoptosis and mitotic activity are inherently opposed processes. In tumors located at other sites, the balance between anti-proliferative activity and proliferation of tumor cells is usually shifted towards the latter. However, these processes are like-directed in pituitary adenomas. Pituitary carcinomas demonstrate high level of proliferation and apoptosis [24].

Due to low activity of apoptosis in pituitary adenomas, it is difficult to detect tumor cells with apoptotic changes using routine histological examination. In the early stages of apoptosis, pycnosis of nuclei and subsequent condensation of chromatin with irregular contours is observed. Cell size is reduced and cytoplasm becomes compact. The cells gradually lose contact with the surrounding structures. Then, nucleus fragmentation into small particles occurs accompanied by formation of pycnotic apoptotic bodies.

The process of apoptosis can also be studied by electron microscopy, immunohistochemistry, cytometry, and TUNEL-method.

Immunohistochemical study shows that about 30% of various pituitary adenomas express Bcl-2. Bcl-2 is an intracellular protein factor that inhibits apoptosis in many cell

systems, including lymphohematopoietic and neuronal cells and regulates cell death by controlling the permeability of the mitochondrial membrane. Almost all hormonally active and significant proportion of hormonally inactive tumors are immunopositive with respect to the pro-apoptotic bax protein. Disturbance of Bcl-2 and bax expression is observed in the pituitary tumors and plays an important role in regulation of apoptotic mechanisms. Among various pituitary adenomas, apoptotic changes are most often detected in corticotropinomas by electron microscopy. According to various studies, there is no significant difference in the level of apoptosis in pituitary microadenomas and macroadenomas. Several studies [25—27] have shown that expression of proapoptotic molecules bax and bad is reduced in invasive pituitary adenomas compared to non-invasive ones.

Interesting results were obtained by C. Tanase [28] in the study of functional proteins involved in apoptosis process (apoptotic protease activating factor 1 (APAF-1) and cathepsin-B) by immunohistochemistry with specified antibodies, western blot, and chemiluminescence. Cathepsin-B is a lysosomal protease, which contributes to disintegration of the extracellular matrix due to activation of MMPs and is also involved in activation of apoptotic genes and suppression of antiapoptotic ones. The study included tissue samples of 30 pituitary adenomas (16 of them were hormonally active and 22 had invasive growth) to compare APAF-1 and cathepsin-B expression in the groups of invasive and non-invasive adenomas. Invasive adenomas demonstrated reduced expression of APAF-1, which was associated with high Ki-67 index and positive expression of p53. Expression of APAF-1 and cathepsin-B demonstrated inverse mutual correlation. Increased expression of cathepsin-B was observed in invasive adenomas and correlated with high Ki-67 index and positive expression of p53.

In recent papers, another marker reflecting apoptosis process was described. Survivin is a member of the apoptosis inhibitor family. In these studies, the results of comparing survivin expression in normal pituitary tissue, invasive and non-invasive hormonally active pituitary adenomas using immunohistochemistry and PCR methods are reported. Overexpression of survivin in pituitary adenomas compared to normal pituitary tissue, as well as in invasive adenomas compared to non-invasive ones, was shown [29, 30].

Therefore, the study of extended marker panel will lead to better understanding of apoptosis and proliferation in pituitary tumors and will allow using them as predictors of poor prognosis.

Pituitary tumor-transforming gene (PTTGE) is another important marker. Expression of pituitary tumor-transforming gene is observed in many tumors derived from the endocrine system organs (including pituitary tumors) and other systems. PTTG is involved cellular and tissue processes such as proliferation, DNA repair, stimulation and inhibition of apoptosis, activation of angiogenesis, invasion, and induction of genetic instability [31].

Pituitary tumors demonstrated overexpression of PTTG as compared to normal pituitary tissue, which is characterized by low expression or lack thereof [32]

Immunohistochemical study shows cytoplasmic reaction in most pituitary tumor cells, while nuclear reaction is observed more rarely. Several studies have shown that nuclear immunohistochemical reaction with anti-PTTG antibodies (along with mitotic activity, Ki-67 index, and p53) in pituitary tumor cells is a valuable prognostic marker related to biological behavior. X. Zhang [33, 34] and A. Wierinckx [35] discovered overexpression of PTTG micro-RNA in invasive hormonally active pituitary tumors. There was no correlation between PTTG expression level and the presence of invasion in hormonally inactive pituitary adenomas. M. Fillippella et al. [36] discovered nuclear immunoexpression of PTTG in 89% various hormonally active pituitary adenomas, which was significantly higher in recurrent tumors and correlated with Ki-67 index. Breakdown point for PTTG expression in recurrent and non-recurrent adenomas was 3.3% (60% sensitivity and 76% specificity). There was no significant correlation between PTTG immunoexpression and tumor size, radiological picture, age, gender, or treatment of patients.

Some studies [30, 34, 37] focused on the relationship between PTTG expression and pituitary adenoma type. In the study of 40 pituitary adenomas (12 somatotropinomas, 5 prolactinomas, 5 corticotropinomas, and 18 hormonally inactive adenomas), significant increase in the expression of PTTG microRNAs was found in somatotropinomas compared to prolactinomas and corticotropinomas. It is believed that different level of PTTG expression depends on different molecular mechanisms triggering initiation and/or progression of a tumor, caused by PTTG.

In vitro studies showed the relationship between the expression of PTTG and fibroblast growth factors. Overexpression of these markers was found specifically in invasive pituitary adenomas [38].

C. McCabe [39, 40] showed significant positive correlation between PTTG and VEGF microRNA in 111 different types of pituitary adenomas. T. Minematsu et al. [41, 48] revealed the relationship between vascular density (determined using CD34 immunoexpression) and PTTG in somatotropinomas. Based on these data, the authors concluded that PTTG is involved in angiogenesis of pituitary tumors through activation of VEGF. Interesting results were reported by E. Hunter et al. [37]. The correlation between expression of VEGF and S100, which is a Sertoli cell marker (alleged source of VEGF secretion) was detected.

The study of A. Heaney et al. [42] on four pituitary adenomas revealed decreased expression of PTTG, which was associated with decreased proliferative activity (PCNA) and Bcl-2.

Thus, pituitary tumor-transforming gene is an integrative marker of the most important processes of carcinogenesis in pituitary tumors.

In tumors derived from various organs (mammary gland, ovaries, bladder, thyroid, and pituitary gland), chromosomal abnormalities associated with loss of 11q region of chromosome 13, which were associated with tumor progression and metastatic spread, were reported. Allelic deletions 11q13, 13q12—14, 10q and 1p were found in invasive pituitary adenomas. This information is to a greater degree related to prolactinomas and somatotropinomas rather than corticotropinomas. The lack of information on chromosomal disorders in corticotropinomas causes the need for further study in this direction [43—45].

Investigations of microRNA are promising with respect to prognostic factors of the biological behavior of corticotropinomas. MicroRNAs are non-coding endogenous RNAs regulating gene expression at the post-transcriptional level. MicroRNAs may act as a tumor gene suppressors or as prooncogenes. These proteins can be determined by quantitative real-time PCR and microarray techniques.

Various studies demonstrated both overexpression and hypoexpression of microRNA in ACTH-secreting pituitary adenomas. Reduced expression of miR-145, miR-21, and miR-141 was reported in corticotropinomas, which was associated with poor prognosis [43]. In somatotropinomas and prolactinomas, decreased expression of miR-15a and miR-16-1 correlated with tumor size as opposed to corticotropinomas [46]. F. Amaral et al. [43] revealed hypoexpression of miR-145, miR-21, miR-141, and let-7a in corticotropinomas compared to normal pituitary gland and found no relationship between the size of these adenomas and the results of neurosurgical treatment. Decreased expression of miR-141, which is associated with the development of CD remission after prostatectomy, exempted from this rule. Overexpression of a number of microRNAs, such as 122, 10b, 208a/b, and 592, which may be associated with tumorous progression of corticotropinoma [24, 47], was reported in the literature.

Proteomics and transcriptomics are among the most innovative methods to study various human diseases, including pituitary tumors. These methods are based on determining the extensive profile of proteins in various media (blood, tissues, etc.) using gel electrophoresis, mass spectrometry (for proteomics), and microarray studies (for transcriptomics). Transcriptomics and proteomics can detect a large number of protein compounds, facilitating understanding of carcinogenesis of different types of pituitary adenomas at the molecular level. These data can be used as markers of the biological behavior of pituitary adenomas [24].

As can be seen from the above, the knowledge on clinical presentation, histological structure, and immunophenotype required to assess the biological behavior of ACTH-secreting pituitary tumors, has been accumulated. However, the true proportion of typical and atypical corticotropinomas is not known so far. There are no results of long-term studies related to outcomes of neurosurgical treatment of corticotropinomas, depending on diagnostic categories of these tumors (typical and atypical adenomas). At the same time, the relationship between the histological structure of corticotropinomas, their immunophenotype, and outcome after prostatectomy is being studied. Results obtained by many international research teams have shown that functional activity of tumor cells (proliferative and antiproliferative processes), stromal component, and angiogenesis are of certain importance for understanding of the biological behavior of corticotropinomas. However, these data are disembodied and there is no comprehensive understanding of these processes. In this regard, the molecular genetic aspects of corticotropinoma carcinogenesis is being extensively studies all over the world, which probably will enable discovering reliable predictors of poor prognosis of pituitary adenomas and, in particular, corticotropinomas, in the future.

Authors declare no conflict of interest.

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Commentary

Evaluation of biological behavior of pituitary adenomas, including corticotropinomas, is extremely important to find out the causes of pathogenesis and progression of pituitary tumors, as well as to develop effective methods of diagnosis and treatment of this complex and diverse group of tumors.

Until recently, pituitary adenomas were divided into acidophilic, basophilic, chromophobic, and mixed ones. However, this classification was of little importance for diagnosis of tumors, since there was no sufficient correlation with clinical presentation of the disease.

In 1995, E. Horvath and K. Kovacs proposed a modified classification based on morphological, immunohistochemical, and ultrastructural features of different types of adenomas. According to this classification, there are somatotropic, lactotropic, mammo-somatotropic, corticotropic, tireotropic, gonadotropic, plurihormonal, "silent", and other types of adenomas.

The latter morphological classification of pituitary tumors has been revised by the WHO over 10 years ago (in 2004).

However, in recent years, numerous studies focusing on pituitary adenomas (including ACTH-secreting ones) were published, using modern morphological methods.

The authors analyzed a large bulk of modern literature on morphology and molecular biology of pituitary adenomas.

Interestingly, the article was written by prof. E.I. Marova, one of the pioneer researchers of Cushing's disease in our country, and candidate of medical sciences A.M. Lapshina, an expert in the modern morphological diagnosis of pituitary adenomas, which enabled comprehensive assessment of the results provided by laboratory and instrumental methods together with clinical presentation of the disease.

The review presents an interesting analysis of histological and immunohistochemical aspects of ACTH-secreting pituitary tumors compared to other morphological types of pituitary adenomas. Morphological classifications of pituitary adenomas and criteria of pituitary adenocarcinomas are shown. The authors demonstrated the possibilities of widely used histological and immunohistochemical diagnostic methods for pituitary adenomas, as well as the prospects of epigenetic research including the study of microRNA expression, proteomics, and transcriptomics.

This paper presents the best known biomarkers playing an important role in the pathogenesis of pituitary adenomas, in particular corticotropinomas. The article will be extremely useful for neurosurgeons, endocrinologists, and morphologists, who deal with the problem of pituitary adenomas. The data reported in this article can be taken as a basis for planning prospective studies, including the study of the morphological features of pituitary tumors with subsequent follow-up study and identifying the factors that play the most important role in the pathogenesis and biological behavior of tumors, including prediction of relapses.

L.I. Astaf'eva (Moscow, Russia)