Potentialities of Differential Immunohistochemical Diagnosis of Some Follicular Tumors of the Thyroid Gland

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Suppl. 1, pp. 49-51, 2008
Original article submitted July 29, 2008

Differential diagnosis of thyroid follicular adenomas, follicular cancer, and papillary cancer with follicular structure was carried out. Proliferation coefficients, probability of atypia, and tumor growth activity were evaluated on the basis of quantitative proportions of proliferation and apoptosis genes expression, derived from indexes of labeled nuclei expressing Ki-67, bcl2, p53 and cytoplasm with expression of bcl2, p53 in cells of these tumors. Significant differences between all the studied tumors of the thyroid grand with follicular structure were detected. The proposed criteria supplement the postoperative morphological differential diagnosis of thyroid tumors.

Key Words: *thyroid; follicular tumors; immunohistochemistry*

Tumor growth and its type along with tumor atypia are largely determined by the proportion of factors regulating the proliferation and apoptosis processes [1,3,5,8]. The method of immunohistochemical study of the expression of genes controlling proliferation and apoptosis processes [4-6,10] together with cytological and histological methods provide just tentative conclusions on the benign or malignant type of the studied tumors. On the other hand, in difficult cases more accurate differential diagnosis is needed in the majority of patients in order to solve the problems of treatment strategy.

We made an attempt of differential diagnosis of thyroid follicular adenomas, follicular cancer, and papillary cancer with follicular structure on the basis of estimated parameters derived from the labeling indexes of the nuclei and cytoplasm expressing some genes in cells of these tumors.

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MATERIALS AND METHODS

Tumor specimens were collected from 266 patients operated for thyroid tumors and studied by the blind method. The diagnosis was made by pathomorphological methods in independent histological studies in all patients. Tumor specimens were fixed in 10% neutral aqueous solution of formalin, dehydrated in ascending alcohols, and embedded in paraffin. The sections (5 μ) were routinely stained with hematoxylin and eosin. Immunophenotyping of tumors was carried out using monoclonal antibodies to proteins Ki-67, bcl2, and p53 (Novocastra). Diaminobenzidine providing brown staining served as the chromogen.

Label index in the sections was evaluated in 30 visual fields using a system of image analysis, consisting of Pentium IV IBM PC, AxioStar+ optic microscope, Axiocam digital videocamera, and AxioVision 3.1 image analysis software (Carl Zeiss). The number of specifically stained (brown) cell nuclei and cytoplasms was evaluated. The objects for estimation were chosen automatically by the color using a 24 bite color depth scale. The index

0007-4888/08/1466-0794

of labeled nuclei was determined by the proportion of stained nuclei to 100, cytoplasm label index by proportion of cells with stained cytoplasm to 100 [2].

The proliferation coefficient (PC) was calculated as the proportion of indexes of labeled nuclei with bcl2 and p53 expression, multiplied by the index of labeled nuclei expressing Ki-67. The probable atypia coefficient (PAC) was calculated as the proportion of indexes of labeled cytoplasm (mitochondria) with bcl2 and p53 expression. Tumor growth activity coefficient (TGAC) was calculated as the product of coefficient of probable atypia and proliferation coefficient.

The criterion of sufficient precision of the mean at 95% correct judgment level was used. The probability of reliable differences between the means was evaluated using the Kolmogorov—Smirnov test.

RESULTS

Proliferation coefficients calculated for follicular tumors of different types differed significantly, characterizing the cells belonging to different general totalities (Table 1), in other words, to different tumors, this confirming the rightfulness of distinguishing atypical follicular adenoma as a special tumor type.

Each normal cell has a peculiar mitochondrial morphotype determined by its DNA, while malignant cell is characterized by signs of atypia manifesting, among other things, at the subcellular level and most demonstratively in the mitochondria [9]. That is why we studied the expression of anti- and proapoptotic genes in mitochondria by immunohistochemical methods. Since elimination of defective cells and their mitochondria depends on the balance of genes regulating the proliferation processes (bcl2) and suppressor genes "cutting out" these defective types (p53), we hypothesized that the proportion of these factors would indicate mitochondrial atypia (will help us to evaluate the degree of probable atypia of tumor cells at the subcellular level).

The differences in PAC values (Table 1) confirm the appurtenance of the studied tumors to different types. The mitochondrial PAC helps to differentiate between the follicular tumors, which is particularly valuable when tumor cell nuclear atypia is poorly expressed, including cases virtually without atypical mitoses [9,11,12].

Hence, the degree of cell atypia at the subcellular level can serve as an accessory criterion in the absence of atypical mitoses, but with great differences in the proliferative potential of follicular and papillary cancers.

The TGAC values demonstrated the differences in all types of the studied tumors, the minimum value being obtained for follicular adenomas with, accordingly, the minimum proliferation and atypia values. The TGAC of atypical follicular adenomas surpassed 8-fold the value in adenomas, but was 2.5 times lower than in follicular cancers. The TGAC of papillary cancers with follicular structure was 4.3 times higher than that of follicular cancer. So great difference in TGAC values of follicular and papillary cancers can be explained by high proliferative potential of papillary cancer, whereas by atypia these two types of malignant tumors are close. Presumably, this explains different biological behavior of follicular and papillary cancers; the latter is characterized by local tumor growth, gradual invasion into the adjacent glandular tissue, predominant metastases to the regional lymph nodes, and absence of distant hematogenic metastases.

Hence, immunohistochemical study and the proposed coefficients formalizing biological status of thyroid follicular tumors can be useful for their postope-

TABLE 1. Immunohistochemical Diagnosis of Follicular Tumors of the Thyroid Gland

Coefficients and expressed genes		Follicular adenoma	Atypical follicular adenoma	Follicular cancer	Papillary cancer
Nuclear label index	Ki-67	8.2±0.2[4]	7.4±0.1	6.60±0.19	34.1±1.3[1,2,3]
	bcl2	8.4±0.4[2,3,4]	12.6±1.1[1,4]	16.8±1.2[1,4]	32.0±1.1[1,2,3]
	p53	31.4±1.2[2,3]	13.6±0.9[1,4]	12.8±0.7[1,4]	33.8±1.4[2,3]
	PC	2.20±0.06[2,3,4]	6.8±0.1[1,3,4]	8.6±0.3[1,2,4]	32.2±1.0[1,2,3]
Cytoplasm label index	bcl2	4.4±0.2[2,3,4]	10.2±1.2[1,4]	13.6±1.1[1,4]	24.0±1.1[1,2,3]
	p53	30.5±1.1[2,3]	27.2±1.0[3,4]	18.8±0.8[1,2,4]	28.4±1.2[2,3]
	PAC	0.14±0.01[2,3,4]	0.37±0.02[1,3,4]	0.72±0.02[1,2,4]	0.84±0.01[1,2,3]
	TGAC	0.30±0.01[2,3,4]	2.50±0.02[1,3,4]	6.20±0.02[1,2,4]	27.0±1.0[1,2,3]

Note. $p<0.01$ compared to: [1]follicular adenoma, [2]atypical follicular adenoma, [3]follicular cancer, [4]follicular variant of papillary cancer.

rative morphological study, more reliable differential diagnosis, and planning of treatment protocols.

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