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Immunohistochemical staining in thyroid carcinoma: has it become a standard?

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Summary

This paper describes results of immunohistochemical studies, used to diagnose primary and metastatic thyroid tumours. We discuss differential diagnosis of medullary thyroid carcinoma and c-cell hyperplasia in patients with RET protooncogene mutation, who had undergone prophylactic thyroidectomy. In particular, we focus on the possible immunohistochemical differentiation of follicular thyroid tumours. We discuss technical issues encountered in applying immunohistochemical methods as well as the commonest errors in the interpretation of results. In conclusion, we indicate areas of thyroid pathology where immunohistochemical staining appears absolutely necessary, and others where such methods can merely assist in reaching final diagnostic decision.
Key words: thyroid, carcinoma, immunohistochemistry.

Badania immunohistochemiczne w raku tarczycy: czy to już standard?

Streszczenie

W pracy przedstawiono sposób wykorzystania badań immunohistochemicznych w diagnostyce guzów pierwotnych i przerzutowych tarczycy. Omówiono diagnostykę różnicową raków rdzeniastych tarczycy i rozrostów komórek u chorych z mutacją protoonkogenu RET operowanych profilaktycznie. Szczególną uwagę zwrócono na możliwości immunohistochemicznego różnicowania guzów tarczycy o budowie pęcherzykowej. Przedstawiono aspekty techniczne immunohistochemii z uwzględnieniem przyczyn najczęstszych błędów w interpretacji wyników reakcji. W podsumowaniu wskazano na działy patologii tarczycy, w których badania immunohistochemiczne są bezwzględnie konieczne oraz te, w których immunohistochemia jest wskazana jako badanie wspomagające podjęcie decyzji diagnostycznej.
Słowa kluczowe: tarczyca, immunohistochemia.

Introduction

Now histopathologic diagnoses of endocrine glands rank among the most difficult since neoplastic processes can coexist here with functional changes. Diagnostic criteria applied to microscopic appearance of thyroid disorders should include distinguishing parameters, for example, low mitotic index and low proliferation activity in cells of differentiated thyroid cancers or nuclear polymorphism in various non-malignant disorders of the gland. These criteria result in relatively poor reproducibility of histopathologic diagnoses of thyroid disorders [1,2] and become particularly significant upon diagnosis of thyroid malignancies. One of the most important purposes of implementing immunostaining methods is to render histopathologic diagnostics more objective and reliable. Until recently, immunohistochemistry has not played a major role in differential diagnostics of thyroid cancer, the exception being medullary thyroid carcinoma [3].

Differential diagnostics of medullary carcinoma

Great variability of morphological patterns seen in medullary carcinoma results in a substantial ratio of incorrect diagnoses. Referral examinations verifying medullary carcinoma cases using immunohistochemistry, carried out

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at the Centre of Oncology in Gliwice between 1993-1995 indicated a very high ratio (nearly 33%) of false positive diagnoses [4]. On the other hand, immunostaining verification of atypical, previously diagnosed follicular, anaplastic or papillary tumours has revealed that they had originated from c-cells. A recommendation adopted at the 1995 Szczyrk Conference [5] states that each diagnosis of medullar carcinoma should be confirmed by immunostaining for calcitonin presence. This procedure becomes indispensable in case of RET protooncogene mutation-bearing patients undergoing prophylactic surgery. It has been often found that in young patients qualified for surgery on the basis of genetic tests the only microscopically observed change involving the thyroid gland is c-cell hyperplasia. Correct diagnosis of the malignant growth of c-cells requires precisely estimated number of such cells using immunohistochemistry [6].

Immunohistochemistry of follicular tumours of the thyroid

Differential diagnosis of follicular tumours has been a major challenge for pathologists for many years [7,8]. Microscopic assessment cannot be based here on the morphology of cell nucleus seen with H-E staining since in highly differentiated follicular cancers the morphology does not essentially differ from that observed for follicular adenomas or proliferative thyroid tumours. The sole criteria allowing to differentiate the encapsulated follicular carcinoma variant from adenoma are angioinvasion and infiltration of the whole tumour capsule wall [9]. Such criteria are highly subjective which results in high ratios (up to 10%) of false positive diagnoses of follicular carcinoma [10,11]. Use of immunohistochemical identification of endothelial cells with antibodies against factor VIII, CD31 or CD34 increases the probability of correctly assessing possible angioinvasion by tumour cells as well as finding foci of vascular occlusion. So far, the attempts to use antibodies for differentiating between benign and malignant follicular lesions have not been successful. An apparent breakthrough was reported in 2001 in Lancet [12]. This paper claimed successful cytological diagnostics of follicular carcinoma based on the expression of galectin-3 and CD44v6 antigen in tumour cells. Subsequent reports have pointed out that the two markers should be used along with other antibodies such as cytokeratin 19, Hbme-1 and anti-leu-7 [13,14] and that the method should be considered as supportive only in assessment of controversial cases. Unfortunately, these antibodies are expressed to some extent also in benign lesions. The group of follicular tumours includes the encapsulated papillary carcinoma variant which involves the highest number of false positive cancer diagnoses [15]. The classic subtype of papillary carcinoma, unambiguously defined based on cell nucleus morphology, does not present a diagnostic problem in examining cytological or histopathological material. Evaluation becomes difficult in case of the follicular type of papillary carcinoma. The characteristic morphological features of cell nuclei, such as grooves and pseudoinclusions can be found in the follicular variant of papillary cancers only in a small number of cells. Typical enlargement of cell nucleus and greater thickness of cell membrane are not observed. In uncertain cases encountered in routine staining with hematoxylin and eosin the presence of Cytokeratin 19 expression in membrane is helpful in reaching cancer diagnosis [16]. Differentiation between follicular and papillary carcinomas remains, however, a major research issue. Some carcinomas defined as papillary, owing to their nuclear morphology, show nonetheless growth and invasion patterns typical of follicular cancers, with tissue texture containing areas of cells having smaller nuclei and coarse chromatin pattern characteristic of follicular carcinomas. The expression of antibodies differentiating between follicular and papillary variants of thyroid cancers, such as cytokeratin 5 and 6, cytokeratin 19, galectin-3 and HBME-1 shows heterogeneity which points to the complex nature of these tumours. This group of carcinomas has been referred to as hybrid thyroid carcinomas [17].

Immunohistochemical staining in poorly differentiated and anaplastic carcinomas

The degree of differentiation between follicular and papillary carcinomas often affects the strategy of chosen therapy. Low or negative reaction to the presence of thyreoglobulin is an indicator of tumour differentiation. It is also a predictive factor ruling against treatment involving radioactive iodine. Immunohistochemical diagnostics of poorly differentiated cancers, for example insular thyroid cancer, poses also technical problems. In this group of cancers thyreoglobulin often appears in only trace amounts which necessitates use of very sensitive detection methods, strict adherence to material fixing techniques as well as full standardisation of subsequent steps in specimens' preparation. Cytological features of papillary carcinomas, such as nuclear grooves and pseudoinclusions, are characteristic also for hyalinising trabecular adenomas. Even though routine cytological examination (FNA) of hyalinising trabecular adenoma is doomed nowadays to result in false diagnosis of papillary carcinoma, the examination of tissue material provides, on the other hand, a tool allowing unequivocal differentiation of this rare benign thyroid tumour. The hyalinising trabecular adenoma features a very characteristic expression of Ki-67 membrane antigen [18].

Immunohistochemical diagnostics of metastases to the thyroid

Metastases to the thyroid are relatively common. The pathologist's most common dilemma in cases of clear cell tumours with follicular architectonic is whether one faces

a primary clear cell adenoma, or thyroid follicular carcinoma, or whether is it a metastasis of clear cell kidney carcinoma. Until recently differential diagnostics of these entities has been mostly based on thyreoglobulin presence in tumour cells. One should remember, however, that in some cases of primary clear cell adenomas and thyroid carcinomas immunohistichemical staining reveals very weak expression or even absence of thyreoglobulin. At present, we have at our disposal a relatively specific TTF-1 antibody against thyroid transcription factor (TTF) expressed in both normal thyreocytes and adenomas, as well as in differentiated thyroid carcinomas [19]. By using TTF-1 one can differentiate between primary thyroid lesions and metastatic ones, the only exception being lung adenocarcinoma metastasis. Its cells also show positive reaction with TTF-1. The above antibodies against thyreoglobulin and TTF represent also basic markers that confirm distant metastases to the thyroid.

Interpretation of immunohistochemical staining results and existing problems

Immunohistochemical staining is a very useful tool in differential diagnostics of cancer diseases. One should bear in mind, however, limitations of this method. Papillary carcinomas and oxyphilic thyroid tumours contain large amounts of endogenous biotin and this practically obviates the application of methods based on avidin affinity to biotin from diagnostics of these tumours. Based on our own work (report in preparation), we are of opinion that immunohistochemical staining, albeit very useful in diagnosing thyroid carcinomas, must be used only with the full system of positive and negative control in order to be sure that the staining obtained results indeed from marker presence and its specific reaction with antibody and not from non-specific tissue reaction with visualisation kit reagents. In case of papillary carcinomas and oxyphilic thyroid tumours the assessment is even more difficult since false positive reaction is confined to neoplastic cells only while it is not observed in the vicinity of tumour; this might suggest reaction specificity. It is possible to avoid such non-specific reactions using polymer based visualisation methods.

Immunohistochemical staining in the evaluation of prognostic factors in thyroid cancers

A large number of reports were published recently demonstrating prognostic significance of assessing expression products of certain genes. In the context of thyroid cancer metastases, the significance of nm23-H1 protein expression, a product of suppressor gene bearing the same name, has been brought up [20]. Similar role has been ascribed to the E-cadherin suppressor gene product [21]. In our own studies we observed a prognostically unfavourable (distant metastases of follicular thyroid carcinoma) ex-pression pattern of protein products of p53 *(+)* and p16INK4A *(–)* suppressor genes [22]. Also, immunohistochemically assessed expression level of telomerase has been linked to the aggressiveness of clinical course of papillary and follicular carcinomas [23]. Despite numerous reports of thyroid cancer potential prognostic factors involving expression levels of protooncogenes and suppressor genes, current state of knowledge prevents them to be routinely used in thyroid cancer diagnostics.

Conclusions

In order to answer the question asked in the title of this paper one should concede that immunohistochemical staining provides a foundation for differential diagnostics of medullary, anaplastic and insular thyroid carcinomas as well as for thyroid lymphoma; it is also indispensable or correctly classifying c-cell proliferation in patients with RET protooncogene mutation, who undergo prophylactic surgery. Immunohistochemical studies should form the basis of diagnostics in case of thyroid lymphoma as well as rare primary tumours of and all metastases to the thyroid. The method should be supportive in differentiating benign tumours from follicular thyroid cancers, especially cases of encapsulated variant of follicular papillary carcinoma. Application of immunohistochemistry in the assessment of prognostic factors in thyroid tumours requires further studies.

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