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Morphology

Practical Use of Immunochistochemical Investigation in Diagnosis, Differential Diagnosis, Grading and Staging of Urothelial Carcinomas of Urinary Bladder

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Abstract

Urothelial carcinomas are the most common type of bladder tumors. They are the subject of a number of invasive and surgical interventions with a diagnostic and / or therapeutic purpose. In most cases their diagnosis is not a problem for the pathologist but there are also those when histological judgment is difficult and an immunohistochemical investigation is required. It may be useful in determining tumors with low differentiation, in situ carcinomas, in differential diagnosis, and assessment of invasion in order to correct staging and grading of the neoplasm.

Key words: urothelial carcinoma, urinary bladder, immunohistochemical investigation.

Introduction

Urothelial tumors represent 80% of bladder tumors. The most common malignances among them are carcinomas [2]. Diagnosis of urothelial carcinomas (UC) does not require immunohistochemical (IHC) investigation. But in everyday practice there are cases that demand such investigation and pathologist needs to be able to interpret the result accurately in order to make the correct diagnosis.

In the past years IHC become a routine method in the pathology units. It is used in differential diagnosis of many tumours. In other cases histopathological diagnoses are considered as incomplete if there is no phenotypic assessment of the morphological type of the neoplasm. This is important for lung and breast cancers in relation with

their subsequent therapy. Such investigations also take place in the diagnosis of UC. For these reasons, as well as the lack of sufficient information in the literature, the aim of this article is to highlight the possibilities of IHC in diagnosis of urothelial cancer of bladder.

Materials and Methods

Biopsy cases were selected from the daily practice associated with the routine diagnostics of bladder materials obtained from the urology units of University Hospital "St. George", Plovdiv and Grand Hospital de l'Est Francilien Jossigny, France.

Immunohistochemistry for cytokeratin (CK) 7, CK 20, Prostate Specific Antigen (PSA), p504S (Alpha-methylacyl-CoA racemase – AMACR), CDX2, p53 and Ki-67 was performed on 4–5μm formalin-fixed, paraffin-embedded tissue sections that were cut from the TMAs. The immunostainings are made in DAKO "AutostainerLink 48" under routine procedure.

Results and Discussion

Modern European criteria and protocols [1, 2, 5], as well as our own experience, take place in the development of the current study which is dedicated to the use of IHC in the diagnostics of the UC.

The use of IHC for diagnosis, of urothelial bladder carcinomas is required in the following cases [2, 6]:

I. For diagnosis and differential diagnosis (DD) of UC.

II. For grading and staging of invasive UC.

III. For diagnosis and DD of noninvasive urothelial tumors with flat appearance.

I. The use of IHC in diagnosis and DD of UC of the bladder.

The normal immune phenotype of the urothelium as well as the tumor cells of the UC is GATA3 (+), p63 (+), CK 7 (+), (**Fig. 1**), CK AE1/AE3 (+), CK 20 (+/-)p (**Fig. 2**) independently of the stage and grading of carcinoma.

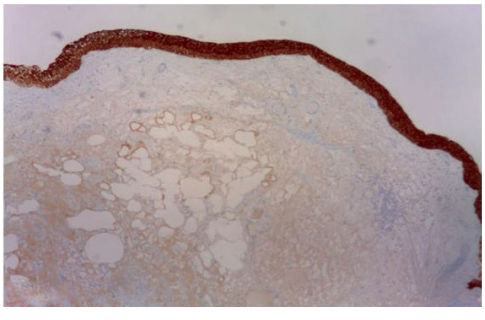

Fig. 1. CK 7, normal mucose. × 50

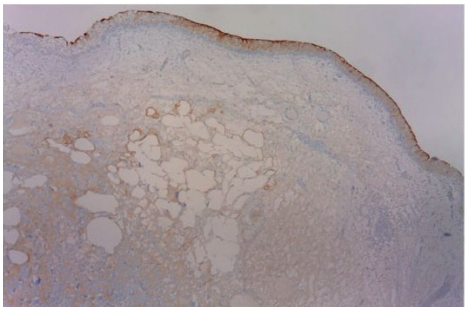

Fig. 2. CK 20, normal mucose. × 50

Commonly use of IHC may be necessary in:

1. DD of urothelial bladder carcinoma with prostate carcinoma:

a) in the cases of endovesical papillary type tumor located near the bladder neck in a patient treated from prostate adenocarcinoma;

b) in poorly differentiated adenocarcinoma of the prostate gland, spreading into the bladder neck. In these cases it should be considered that prostate adenocarcinoma is PSA (+), p504S (AMACR) (+) (**Figs. 3–5**), GATA3 (-), CK 7 (-).

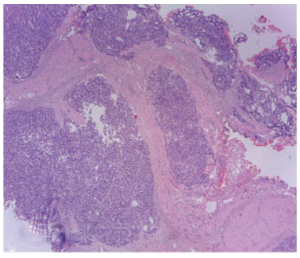

Fig. 3. Prostate adenocarcinoma, Hematoxylin-Eosin. × 50

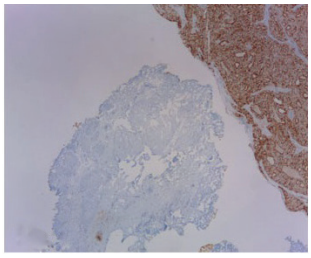

Fig. 4. Prostate adenocarcinoma, p504 S (AMACR). × 50

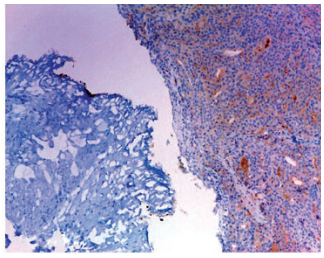

Fig. 5. Prostate adenocarcinoma, PSA. × 100

2. In bladder tumor presented by spindle cells, where the diagnostic problem is whether it is a sarcomatoid carcinoma, leiomyosarcoma or myofibroblast proliferation. Sarcomatoid carcinoma expresses the epithelial markers CK AE1/AE3, CK7, EMA, while leiomyosarcoma and myofibroblast tumors are negative and have positive expression for muscle and connective tissue markers – actin, caldesmon, desmin.

3. Bladder tumor with glandular architecture; in these cases, the dilemma is whether it is a UC with glandular structure or an adenocarcinoma – a primary bladder adenocarcinoma or secondary adenocarcinoma, originating from the adjacent organs (prostate, colon, endometrium). Primary bladder adenocarcinoma is most often of intestinal type and is CK 20 (+), CEA (+) but is CDX2 (-) in contrast to colorectal carcinoma; the latter have positive expression of CK20 (+) and CDX2 (+) (**Figs. 6–8**); beta-catenin is also a useful marker in these cases, it shows a positive nuclear staining in the colorectal adenocarcinoma and also a positive but membrane staining in the primary bladder adenocarcinoma;

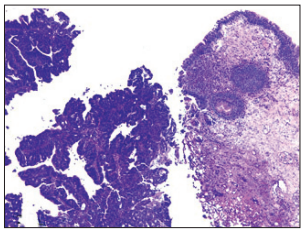

Fig. 6. Colorectal adenocarcinoma, Hematoxylin-Eosin. × 50,

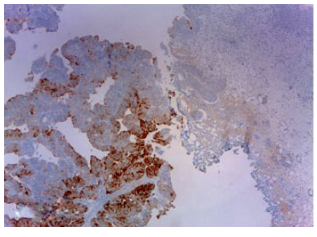

Fig. 7. Colorectal adenocarcinoma, CK 20. × 50

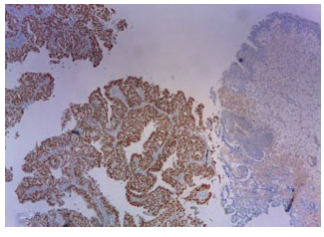

Fig. 8. Colorectal adenocarcinoma, CDX-2. × 50

4. Undifferentiated bladder tumor; in these cases, a melanoma must be excluded because is S100 pr. (+), HMB 45 (+) and Melan-A (+) and secondly rhabdomyosarcoma that express myogenin (+) and desmin (+); UC is negative for the listed markers but have possitive expression for GATA3, CK 7 and p63.

II. The use of IHC in staging and grading of bladder invasive UC.

1. Staging.

The common between clinical and pathological assessment of bladder UC is that they are divided into non-invasive and invasive.

The difference is that for urologists, the invasiveness criteria are determined by tumor muscle infiltration (from pT2 to pT4), while for the pathologist the invasiveness of urothelial tumor begins when the basal membrane and mucosal chorion is infiltrated (from pT1 to pT4) [6]. This difference has its therapeutic logic and explanation. The unfavorable prognosis of invasive UC requires to be treated with cysto-prostatectomy whenever it's possible, with or without chemotherapy (adjuvant or non-adjuvant).

In other words, the visualization of a muscle layer in biopsy specimen or in transurethral resection (TUR) of bladder cancer is a key moment responsible for taking a strategic therapeutic decision.

The presence of an initial tumor infiltration in lamina propria in a UC (pT1) can be visualized using cytokeratins (CK AE1/AE3 or CK7). Isolated single or small groups of cytokeratin-positive cells are observed in superficial lamina propria, which is particularly important for TUR material [1].

In pathology practice, the visualization of a muscle layer is not always an easy task. Especially when there are artefacts caused by electrocoagulation or because the TUR material is cross-cut. In these cases the use of IHC is appropriate. Smooth-muscle actin (SMA) is a useful marker. It is positive in the bladder musculature (superficial and deep) and helps the assessment of the tumor infiltration in it. To proof the latter is possible if there are cytokeratin positive (CK AE1 / AE3) elements, indicating their epithelial origin. The ideal option is to use more specific muscle marker – smoothelin, which has positive expression in the muscular fibres of detrusor (muscularis propria) and is negative in the fine muscle bundles – muscularis mucosae of lamina propria [4]. Unfortunately this variant is expensive and difficult to apply.

Sometimes, when there is a suspicion for tumor vascular invasion, endothelium markers may also be used. The most commonly used markers are D2-40 (podoplanin) which have a positive expression of lymph capillary endothelium or CD 31, which is expressed in endothelial cells of blood vessels [2]; the latter are well-stained by CD 34, as our experience shows.

2. Grading.

In cases where the morphology of a UC is not typical and the pathologist hesitates whether the UC is of low or high malignancy, it is recommended to use Ki 67 – a proliferative marker that is positive in over 20% and is positive in the upper cell lines in a high grade lesions.

III. The use of IHC in diagnosis and DD of noninvasive bladder UC with flat appearance.

In these cases, it is mostly about urothelial carcinoma in situ (UCIS) (pTis), which is defined as a flat, non-papillary urothelial proliferation with different thickness, that is made up by cytologically malignant cells [2]. Histological criteria for diagnosis of UCIS (architectural disorganization of tumor urothelium, cellular atypism, mitosis) are sometimes insufficient, and diagnosis is not easy, especially when there is a differential diagnosis with a wide range of bladder mucosal lesions that are characterized by reactive urothelial atypia; or in small-size materials; cross-cut materials or in electrocoagulated tissues.

DD of UCIS with other flat mucosal lesions [2, 3, 5] :

– *urothelial proliferation with uncertain malignant potential;*
– *urothelial dysplasia;*
– *reactive urothelial atypia (inflammatory, after treatment);*
– *urothelial hyperplasia without dysplasia.*

In these cases the pathologist use IHC. It is recommended to be done a panel of several markers performed on consecutive serial histological sections [2]:

1. Ki 67 (proliferative marker) – two major differences in the proliferative index of the UC are observed, as opposed to benign flat lesions - there is nuclear staining in over 20% of tumor cells throughout the whole thickness of the mucosal proliferation; in reactive urothelial atypia IHC signal for Ki 67 is with basal localization (**Fig. 9, Fig. 10**).

2. CK 20 – in normal and non-tumor urothelial mucosa, the expression of this marker is restricted to the superficial layer of cells, whereas in the UCIS, the expression is of the so-called aberrant type, i.e. in the entire thickness of the epithelium (**Fig. 11**). In reactive urothelial atypia, the IHC signal is localized only in the superficial umbrella cells.

3. p53 – it is observed a diffuse expression of the marker through the entire thickness of proliferation and in all tumor cells (60% of the UCIS cases) (**Fig. 12**).

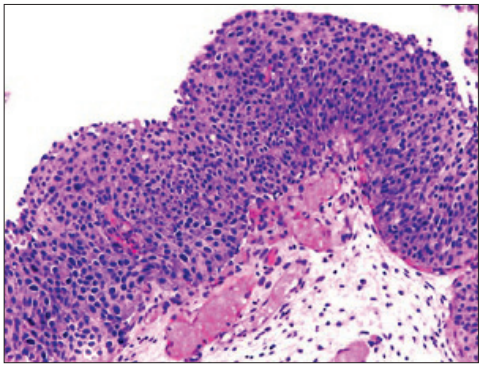

Fig. 9. Carcinoma in situ, Hematoxylin-Eosin. × 200

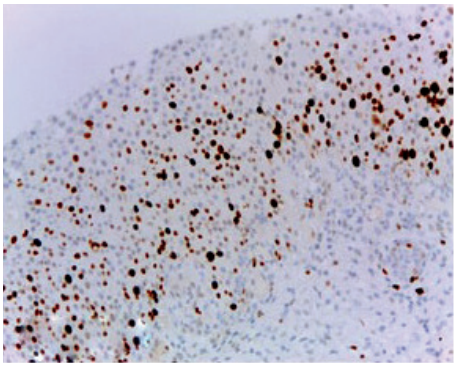

Fig.10. Carcinoma in situ, Ki 67. × 200

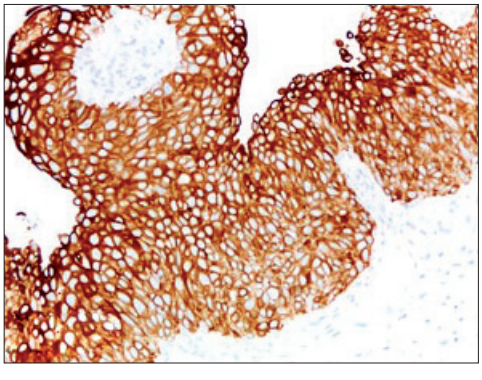

Fig. 11. Carcinoma in situ, CK 20. × 200

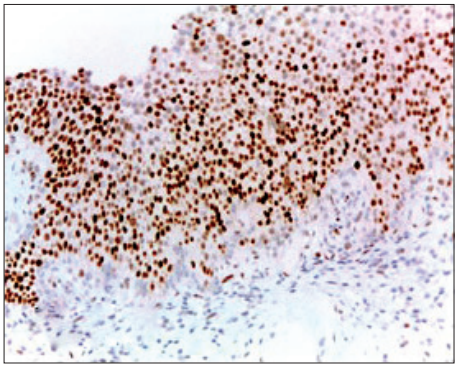

Fig. 12. Carcinoma in situ, p53. × 200

Conclusion

IHC investigation is not used in UCs as often as in other tumors, but in some cases it finds its application. Such are those with problems with differential diagnosis or grading and staging of the tumor, as well as the assessment of the presence of UCIS. Knowing the possibilities of IHC is a prerequisite for an adequate diagnosis.

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