Multifocal Pathomorphological Study of Bladder Cancer with the Use of Luminescence Analysis

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Operation material from patients with various forms of urinary bladder cancer was examined. Systemic involvement resulting from multistage and multifocal tumor growth due to previous multicentric changes was demonstrated. Fluorescent study showed that in urinary bladder cancer tumor transformation involves not only adjacent, but also distant mucosa.

Key Words: *bladder cancer; pathomorphology; electron microscopy*

High incidence of bladder cancer relapses (up 75% according to some data [9]) and unsolved problem of urine derivation after radical interventions make early diagnosis of bladder cancer and pretumor changes in the urothelium a pressing problem [7,10,11].

Despite the progress in studies of mechanisms of carcinogenesis, the factors triggering and regulating tumor process and characteristics of pathological states preceding and accompanying malignant transformation remain not quite clear [3]. Therefore it is important to analyze pathological changes leading to malignant transformation and mechanisms of tumor dissemination. Carcinogenesis is regarded as a multistage process of genetic and epigenetic changes leading to disregulation of proliferation, differentiation, and cell death [1,2,4,6].

Modern fiberoptic endoscopic technology, improved laser and electrosurgical devices, new tumor cell photosensitizes essentially extended the sphere of endoscopy application in clinical oncological urology [8]. Photodynamic study can provide the basis for traditional diagnosis of bladder cancer [5]. This method can be used for detecting precancer or cancer lesions in the bladder mucosa and determination of the tumor boundaries.

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Morphological methods play the major role in the diagnosis of bladder cancer. They allow most precise classification of tumor type and evaluation of its dissemination. Therefore the results of examination of the tumor and bladder mucosa outside the tumor focus should be analyzed with consideration for photodynamic and pathomorphological findings.

We carried out a multifocal pathomorphological study of bladder cancer by means of luminescent analysis.

MATERIALS AND METHODS

Material from 20 patients with various forms of bladder cancer (tissue fragments of the tumor and fluorescent and nonfluorescent visually little changed bladder mucosa) was obtained during surgery.

After injection of luminophore to the patient it selectively accumulates in tumor tissues, which fluoresces upon illumination at a certain wavelength. Tetracycline in a daily dose of 0.4 g for 3 days before surgery served as luminophore and for antibacterial therapy of concomitant urinary tract infections. The resected tissue was irradiated at near UV wavelength (λ=360-420 nm) stimulating fluorescence. Mercuric lamp served as the light source; the light was delivered to the bladder mucosa via quartz monofiber. Fluorescence was observed through a JZ-18 optic filter absorbing excitation light and transmitting fluorescence in the visible band of the spectrum.

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Samples for pathomorphological examination were dissected from the tumor node and zones of fluorescent and nonfluorescent visually little changed bladder mucosa. Fragments of bladder wall for light microscopy were fixed in 10% neutral formalin. Paraffin sections were stained with hematoxylin and eosin in combination with Perls reaction, by the method of Van Gieson with post-staining of elastic fibers with Weigert resorcin-fuscine; periodic acid-Schiff reaction was carried out. Samples for electron microscopy were fixed in 4% paraformaldehyde, postfixed in 1% OsO_4, dehydrated, and embedded in epon-araldite. Semithin sections were stained with Schiff reagent and Azur II. Ultrathin sections were contrasted with saturated alcohol solution of uranyl acetate and lead citrate in alkaline sodium vapors and examined under a JEM-1010 electron microscope at accelerating voltage of 80 kV.

Immunohistochemical study of the proliferative potential of the urothelium was carried out on paraffin sections by two-step indirect immunoperoxidase method with streptavidin-biotin system of the reaction products visualization and appropriate controls. Antibodies to the nuclear antigen Ki-67 (NovoCastra Laboratories Ltd.) served as the first antibodies.

RESULTS

Pathomorphological examination of tumor fragments showed transitional-cell bladder carcinoma of different differentiation degree in 17 cases and squamous-cell keratinizing carcinoma in 3 cases. Transitional-cell carcinoma was presented by complexes of transitional epithelium growth separated with stromal layers. In well and moderately differentiated carcinoma transitional epithelium was relatively monomorphic and retained polarity and mitoses (Fig. 1, *a*). There were atypical sites with "dark" cells and complete or partial loss of polarity. In poorly differentiated cancer tumor cells were extremely polymorphic, devoid of polarity, had polygonal shape and hyperchromatic nuclei of different size; pathological mitoses were also seen. Tumor tissue often contained necrotic and hemorrhagic foci. Layers of prickle cells and diskeratosis (zones of squamous-cell metaplasia) along with atypical cells resembling transitional epithelium were seen in 3 cases.

In squamous-cell keratinizing cancer tumor tissue formed solid and tubular structures from immature cells often with signs of degeneration and necrobiosis. Foci of keratoformation (epithelial pearls) surrounded by foreign body giant multinuclear cells were seen in the tumor tissue (Fig. 1, *b*). Necrosis of the surface epithelium with degeneration of multilamellar squamous epithelium, diskeratosis, and acanthosis was observed. Tumor stroma was often sclerosed and infiltrated with plasma cells and lymphocytes. Tumor emboli were found in some vessels.

A total of 40 fragments of the urinary bladder from fluorescent zones located at different distance from macroscopic border of the tumor were examined. Photo-optical analysis revealed tumors with histological structure corresponding to the primary (visually seen) tumor focus (Fig. 1, *c*) or individual cells and epithelial pearls (in squamous-cell carcinoma) in 28 fragments. Tumor cells were also found in capillary lumens.

More or less pronounced local immune reactions in the bladder mucosa were detected in virtually all fragments at a distance from the tumor focus; these reactions manifested by mononuclear infiltration of the lamina propria and formation of lymphoid follicles in the basal part of the mucosa. These reactions were caused by local exogenous damaging factors and by propagation of antigenic stimulation from malignant tumor.

No tumor cells were detected in 28 control samples of the bladder wall from non-fluorescent zones. Degenerative changes in the transitional epithelium were detected in these fragments collected in patients with transitional-cell cancer; in squamous-cell cancer we found squamous-cell metaplasia, hornification, and acanthosis of the surface epithelium in these zones (Fig. 1, *d*). Polymorphocellular infiltration of the stroma with predominance of mononuclear cells was detected in all samples; focal accumulations of lymphocytes and lymphoid follicles were often seen. Sclerosis and granulation were often seen in the mucosa.

The most pronounced expression of Ki-67 nuclear antigen was detected in tumor growth zones irrespective of the histological type of the tumor. The product of immunohistochemical reaction was present equally frequently at all levels of the tumor tissue, in contrast to samples from the perifocal zone (visually intact bladder mucosa). Ki-67 was detected in sites of urothelial hyperplasia in the nuclei of scanty basal and solitary intermediate cells, while in squamous-cell metaplasia it was found mainly in the basal cells.

Electron microscopy of the bladder mucosa from the tumor zone showed ultrastructure characteristic of mainly poorly differentiated cells, irrespective of the tumor histology. The majority of cells were oval, with large euchromatic nuclei and several nucleoli, a high nuclear-cytoplasmatic ratio, numerous free ribosomes and polysomes, and small compact mitochondria; apoptotic transformation of the nuclei was detected in some cells (Fig. 2, *a*). Accumulations of cells with cytoplasm heterogeneous by electron density, with numerous filamentous structures and protein-lipid incorporations were seen in transitional-cell well differentiated cancer (Fig. 2, *b*).

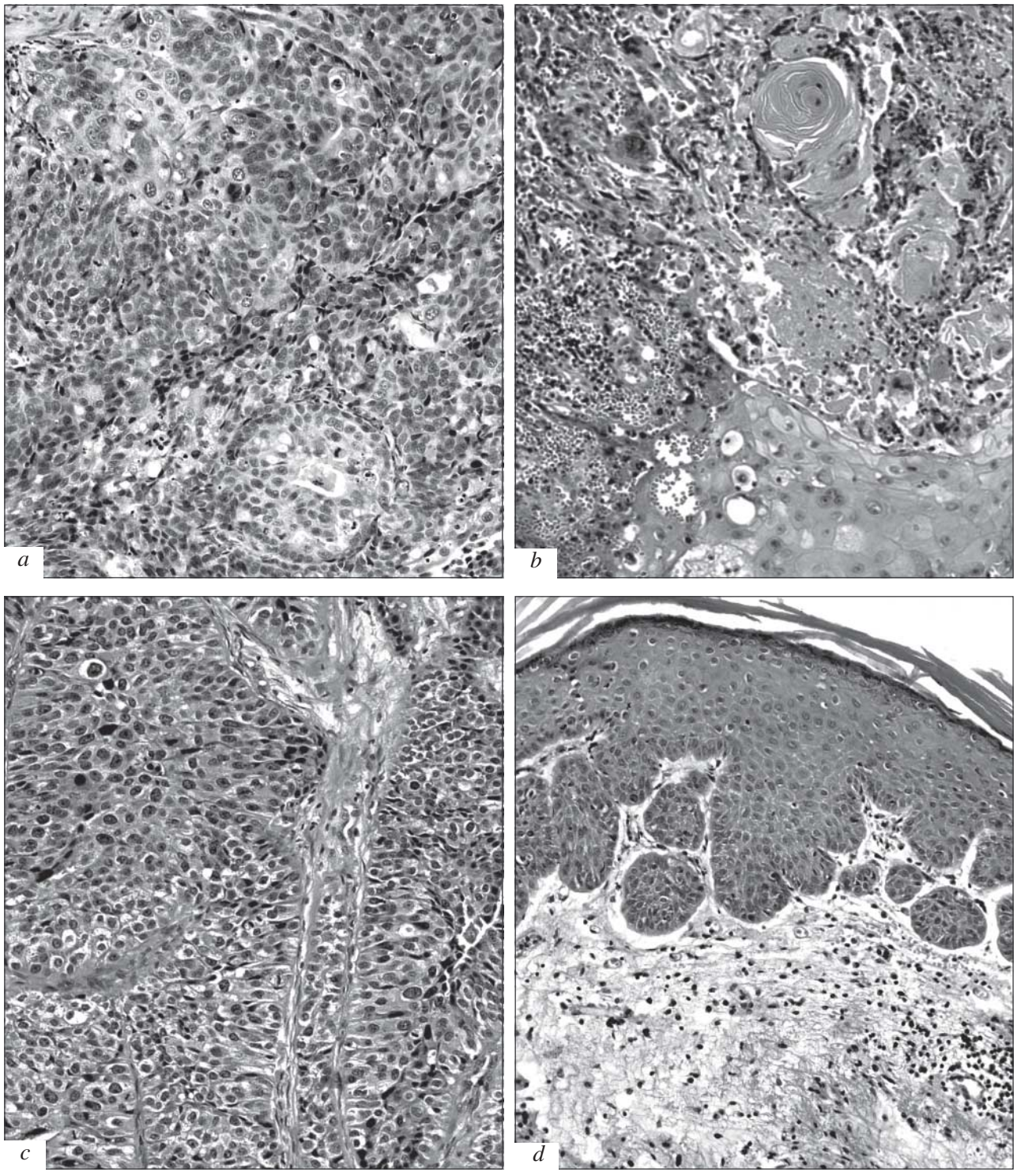

Fig. 1. Pathohistological changes in the urinary bladder in various forms of cancer. Paraffin sections. Hematoxylin and eosin staining, ×400. *a*) moderately differentiated transitional-cell carcinoma of the urinary bladder: complexes of monomorphic tumor cells in the tumor zone; *b*) squamous-cell bladder carcinoma. Foci of keratoformation (horny pearls), foci of necrosis and hemorrhages, degeneration and necrobiosis of surface epithelium in the tumor zone; *c*) transitional-cell bladder cancer: complexes of tumor cells in the zone of active fluorescence of macroscopically intact mucosa; *d*) squamous-cell bladder cancer. Sites of mucosa outside the zone of tumor involvement, no fluorescence: acanthosis, squamous-cell metaplasia, hornification of surface epithelium, edema, and polymorphocellular infiltration of the stroma.

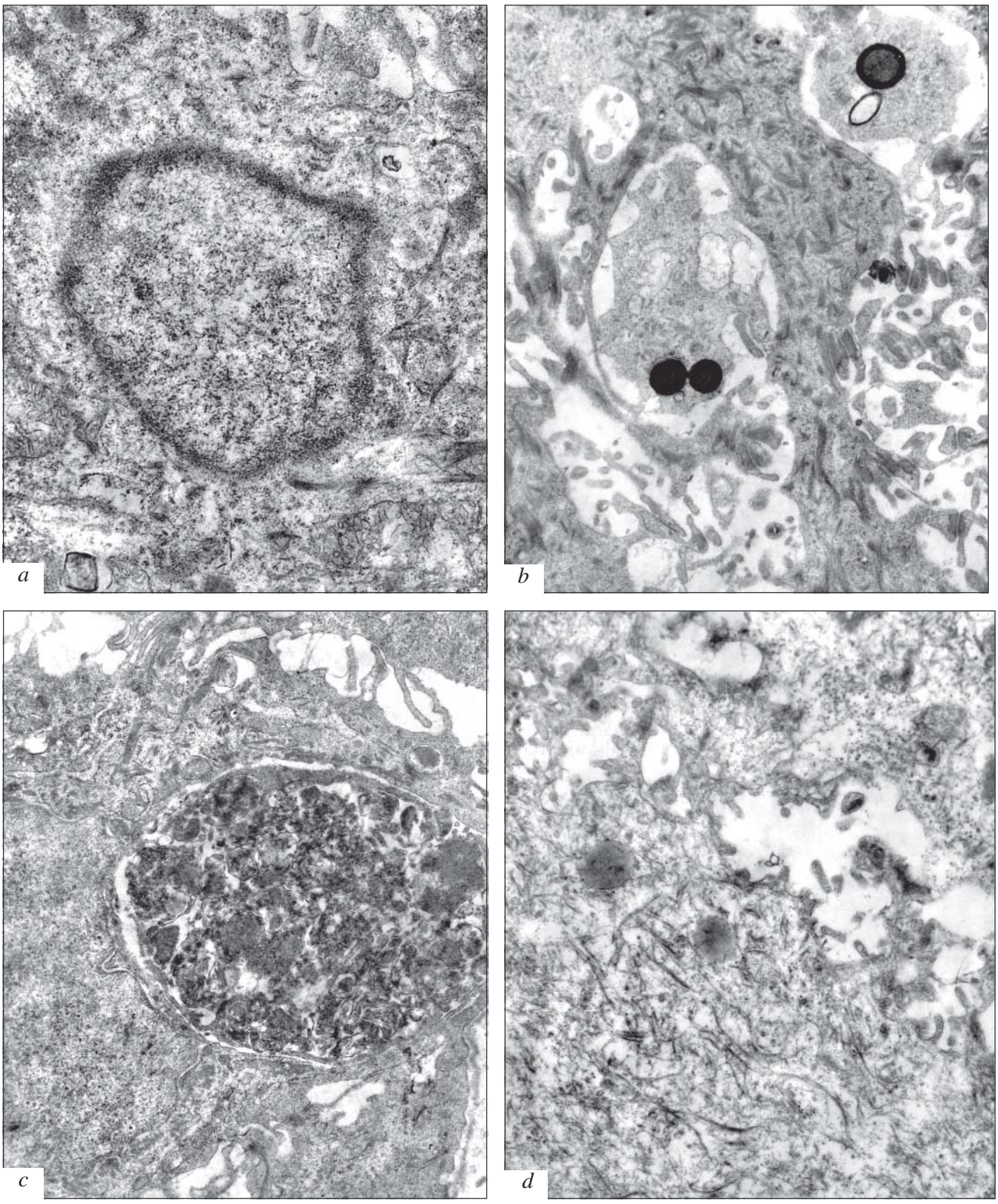

Fig. 2. Ultrastructural changes in bladder epithelium in various forms of cancer. Electronograms. *a*) apoptotic transformation of the nucleus in a tumor cell, ×10,000; *b*) fragments of tumor cells with lipid incorporations and filamentous structures, ×8000; *c*) apoptotic death of an urotheliocyte outside the tumor zone, ×8000; *d*) fragments of keratinizing urotheliocytes outside tumor zone with numerous filamentous structures, ×8000. *a*, *b*: transitional-cell well differentiated cancer; *c*: transitional-cell poorly differentiated cancer; *d*: squamous-cell keratinizing cancer.

Ultrastructural changes in urotheliocytes outside the tumor zone were mosaic, which was due to polymorphism of precancer and paraneoplastic changes. Many cells had degenerating cytoplasmatic organelles, some cells underwent apoptosis (Fig. 2, *c*). Signs of epitheliocyte keratinization were seen in specimens of visually intact mucosa in squamous-cell keratinizing cancer (Fig. 2, *d*).

Hence, the use of pathomorphological analysis with multifocal evaluation of bladder status in cancer patients extended our knowledge on structural reactions of the urinary tract under conditions of developing neoplastic process. The systemic nature of involvement, *i. e.* multistage and multifocal tumor growth, is worthy of note: cancer develops as a result of previous multicentric changes. It was shown that malignant transformation involves the mucosa not only directly inside or near the focus, but also the sites distant from the focus, which can be visualized by fluorescent analysis.

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