Bladder Cancer Therapy Related Histopathologic Changes

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Abstract: Various forms of modern therapy of urinary bladder cancer are associated with characteristic changes. Surgical therapy related morphology alterations usually do not present significant differential diagnostic problems. Postoperative spindle cell nodule may resemble a spindle cell sarcoma, but the fact that it arises within 120 days following surgery makes the diagnostic interpretation less cumbersome. Changes induced by BCG include epithelial denudation of the bladder, small superficial granulomas and chronic inflammation. Other forms of immunotherapy are associated with formation of lymphatic follicles or diffuse lymphocytic infiltrates. Radiation induced changes may appear many years after therapy and include atypia, bizarre appearance and multinucleation of urothelial cells. In the late phase, stroma is particularly affected. It is important not to misdiagnose radiation induced changes for CIS or invasive nested carcinoma. Chemotherapy-induced atypia may be recognized cytologically and histologically by the characteristic changes of the nuclear/cytoplasmic ratio, multiple nuclei, nuclear vacuolization, and architectural disturbances in the bladder epithelium. Photodynamic therapy induces sharply demarcated necrosis in the short post-therapy course. Laser treatment destroys the tumor, and may induce atypia in adjacent endothelial cells. Gene therapy-related changes have not been well defined yet.

INTRODUCTION

Urinary bladder cancer is the 7th most common type of cancer in the world [1]. Chemotherapy and radiation therapy are widely used in the treatment of urothelial cancer and thus it is important for pathologists to recognize various therapy-induced changes in these tumors and adjacent normal tissue [1-4]. For one, 70-80% of bladder cancer patients present with localized and low grade (Ta/Tis or T1) tumors, for which the standard of treatment includes transurethral resection (TUR) followed by intravesical application of adjuvant chemotherapeutic or immunotherapeutic agents. Advanced high grade tumors are also treated with radiation and chemotherapy. Other forms of therapy are also being introduced in clinical trials.

Despite the nondisputed efficacy of current multi-modality therapeutic approach, bladder cancer still carries a high risk of recurrence and/or progression, which dictates the need for lifelong follow-up and treatment [2, 3]. This is particularly true for carcinoma-in-situ (CIS), which usually presents in a multifocal form. Furthermore, some authors [4, 5] point out that there may be a subset of patients with muscle-invasive bladder cancers (T2) who are likely to survive without cystectomy when treated by systemic chemotherapy and aggressive endoscopic surgery alone. Despite occasional disagreements [6, 7], bladder sparing approaches could lead to a greater number of patients with partial cystectomy who would undergo follow-up biopsies of the post-chemotherapy bladder. Last, but not least, the results of some pre-clinical trials [8-10] involving gene therapy, which is also known to induce morphological changes [11], make this novel treatment strategy quite promising [12].

In this review article, we will briefly review and illustrate the various therapy related morphologic changes, with special emphasis on changes related to surgery, immunotherapy, chemotherapy, and radiation therapy.

SURGICAL THERAPY

The most frequently encountered pathologic changes that arise in the urinary bladder as complications of previous surgery are:

- Postsurgical necrobiotic granuloma
- Suture granuloma
- Postoperative spindle cell nodule

Postsurgical necrobiotic granuloma can occur following transurethral resection using diathermic cauter-ization and even laser surgery [13-15]. It is composed of a necrotic center surrounded by epithelioid histiocytes, resembling a rheumatoid nodule. Foreign body giant cells may commonly be present.

Suture granuloma may develop in paravesical or intravesical sites following herniorrhaphy [16, 17]. The granuloma is composed of foreign body type giant cells and fibrosis surrounding fragments of suture. Those detected many years after original surgery may be mistaken for a bladder neoplasm.

Postoperative spindle cell nodule (PSCN), a term which was coined by Proppe *et al.* [18], is yet another important surgical therapy-associated entity. It is also known under the name of "inflammatory pseudotumor of the bladder" and "inflammatory myofibroblastic tumor" [19, 20].

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It may arise within 1 to 2 months after surgery or intravesical instrumentation, most frequently following transurethral biopsy or resections of bladder tumors. Grossly PSCN are friable and may have a polypoid appearance. Microscopically these lesions are composed of interlacing spindle cells of varying density (Fig. **1**). Mitoses may be prominent and counts up to 25 per 10 high power fields have been reported [18-20]. There is however no nuclear hyperchromasia or atypia. In between the spindle cells one may see thin walled blood vessels, intercellular edema, and sparse collagen fibers. Foci of inflammation and hemorrhage are usually evident in parts of the lesion. PSCN should not be confused with leiomyosarcoma or other spindle cell malignant tumors [19]. In contrast to sarcomas, PSCN do not show nuclear pleomorphism, or hyperchromasia, and there are no atypical mitoses. In approximately 80% of all PSCN there are cytokeratin positive cells which are only exceptionally present in leiomyosarcomas. By immuno-histochemistry the spindle cells react with antibodies to vimentin, desmin, smooth muscle specific actin. In many cases the cells react with the antibodies to the anaplastic lymphoma kinase (ALK-1) which may support the diagnosis [20]. Nevertheless, perhaps the most important clue to proper diagnosis is the history of recent surgery. PSCN follow a benign course and practically never recur after surgical resection.
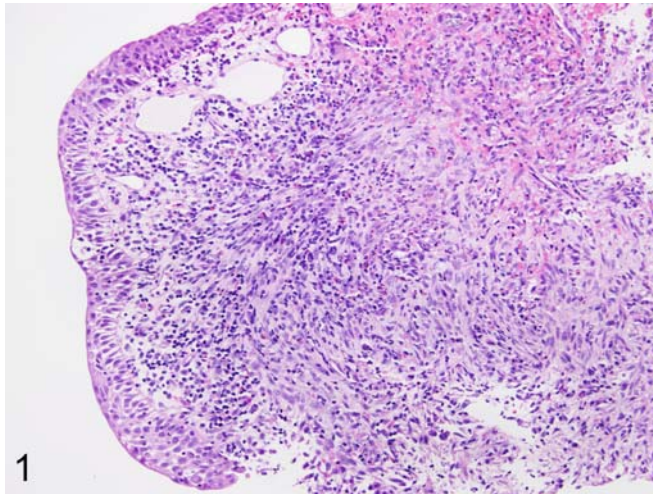

Fig. (1). Postoperative spindle cell nodule. The submucosal nodule consists of dense aggregates of spindle cells. H&E, x 200.

IMMUNOTHERAPY

Bacillus Calmette-Guérin Immunotherapy

Bacillus Calmette-Guérin (BCG) therapy is a form of intravesical immunotherapy most commonly used in patients with Ta/Tis or T1 disease. Typically, it is administered following TUR in order to prevent or at least postpone recurrence/progression of the tumor [21]. BCG, which was originally developed by Calmette and Guérin as a potential vaccine for tuberculosis, is an attenuated strain of *Mycobacterium bovis*. It was first used in the therapy of urinary bladder carcinoma in 1976 [22]. There are several interesting contemporary approaches devised in order to minimize the side effects of this living, albeit attenuated pathogen. These modifications include application of cell wall components [23] or genetically engineered BCG [25],

as well as the combined application of attenuated and killed strains [26]. In its modified form, this BCG treatment seems to be more efficient and produces fewer side effects.

It seems that the BCG binds to the bladder wall at places with a diminished protective glycosaminoglycan layer, such as those with CIS and other "injured" sites [27], thereby exerting a somewhat tumor-specific adhesion. Dendritic cells, macrophages and even tumor cells internalize and process BCG, presenting it to other host cells and thus activating a MHC class II linked cascade of a local immune response.

Even though all aspects of the immune response following intravesical instillation of BCG have not been completely elucidated, there is sufficient knowledge of the morphologic alterations induced by BCG therapy [28]. Typically, the superficial lamina propria contains round or ovoid small loosely textured granulomas composed of epitheloid histiocytes and lymphocytes (Figs. **2A**, **B**). Acid-fast stains only rarely reveal the presence of mycobacteria. The overlying urothelium is frequently denuded as a result of intense inflammation (Fig. **2C**). Patients may exhibit dysuria or in rare cases even systemic symptoms accompanied by granulomatous lymphadenitis. In rare instances, nephrogenic adenomas may develop following BCG therapy [29].

Other Forms of Immunotherapy

Interferon gamma (IFN-γ) is one of the putative mediators of BCG-induced immune response. As such, it has been used in several studies as a replacement for BCG [30, 31]. Morphological features encountered in one of the studies [32], evaluated 6 months after the beginning of recombinant human IFN-γ therapy, included numerous well-formed lymphocytic follicles composed of both B and T cells. Interestingly, such follicles were not present in any of the patients who experienced tumor recurrence during the follow-up period.

Other forms of immunotherapy include instillation of recombinant human interferon alpha (IFN-α), the vaccinia virus, interleukins 2 and 12 and other alternative agents [33]. Most of these treatments are experimental and the morphologic changes caused by these agents have not been very well documented so far. Non-specific inflammatory alterations have been occasionally encountered, such as lamina propria edema and perivascular collections of inflammatory cells.

RADIATION THERAPY

Radiation therapy is an important modality in most bladder-sparing protocols for the treatment of muscle-invasive tumors [34-36]; it is most frequently combined with chemotherapy and its cytotoxic effects are enhanced if combined with cyclophosphamide [37]. Histopathologic alterations of urinary bladder morphology induced by radiation may also be observed whenever the bladder is included in the radiation field during treatment of other pelvic tumors [38]. The mechanism by which radiation exerts anti-tumor effect in the urinary bladder is not completely elucidated, but one of the feasible explanations is that X-Rays damage the blood supply of tumors. It is important to keep this in mind, as some of the morphologic alterations result from damage to the blood vessels.
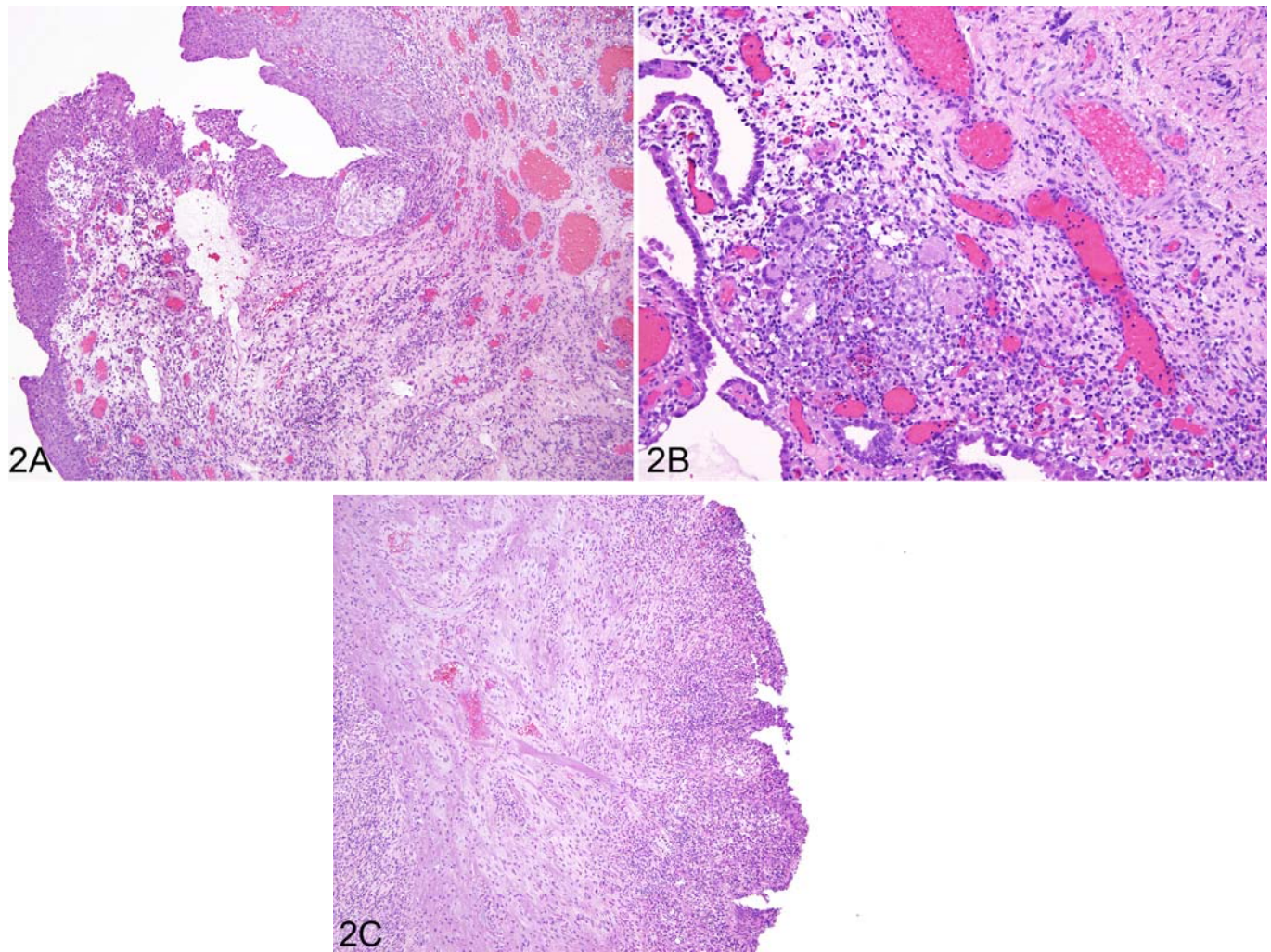
Fig. (2). Bladder treated with BCG. (**A**) The bladder wall is infiltrated with inflammatory cells and a few epithelioid granulomas. H&E x 120. (**B**) The lamina propria contains a loosely structured granuloma containing epithelioid histiocytes, multinucleated cells and lymphocytes. There is prominent dilatation and congestion of blood vessels. H&E x 200. (**C**) The bladder wall denuded of the surface epithelium is densely infiltrated with inflammatory cells. H&E, x 120.

Radiation cystitis usually presents as hematuria. It cannot be predicted whether or when it will occur; cases presenting with symptoms as late as 10 years following therapy have been reported [39]. Acute symptoms appear as early as 4 to 6 weeks after initiation of therapy. Microscopic changes that accompany the symptoms are not diagnostic [40]. They include marked edema and congestion of the lamina propria or hemorrhage which may be accompanied by a loss of the urothelium (Fig. **3A**, **B**). Chronic inflammation may be prominent (Fig. **3C**). At the same time, epithelial proliferation may take place changes resembling full thickness atypia may appear (Fig. **4A**). Occasionally these epithelial changes may be indistinguishable from CIS (Fig. **4B**). However, epithelial cells altered by radiation typically exhibit more bizarre, sometimes multiple nuclei whose chromatin appears smudged, lacking the crisp detail of CIS nuclei. Other useful features that may distinguish radiologic from carcinomatous atypia are nuclear and cyto-plasmic vacuoles, karyorrhexis and a normal nuclear/cytoplasmic ratio [41].

Pseudocarcinomatous epithelial hyperplasia is yet another radiation-induced change and its significance lies in the fact that it sometimes mimicks the nested variant of invasive urothelial carcinoma. There are epithelial proliferations of small urothelial infiltrative nests with either rounded or irregular, jagged border (Fig. **5A**, **B**). A characteristic feature is the presence of pseudoinvasive urothelial nests wrapping around blood vessels associated with fibrin deposition. These epithelial proliferations do not extend into the muscularis propria. In a series reported by Chan and Epstein [38], two of the twenty reported patients had only systemic chemotherapy, while others had a history of pelvic irradiation. None of the followed patients developed bladder cancer. According to Zhaoli and Epstein [42], pseudocarcinomatous epithelial hyperplasia does not always occur in the setting of irradiation or chemotherapy; they identified 8 patients with pseudocarcinomatous hyperplasia without prior history of radiation/chemotherapy. In seven of the eight patients there were factors that could have resulted in localized ischemia or injury to the urothelium.
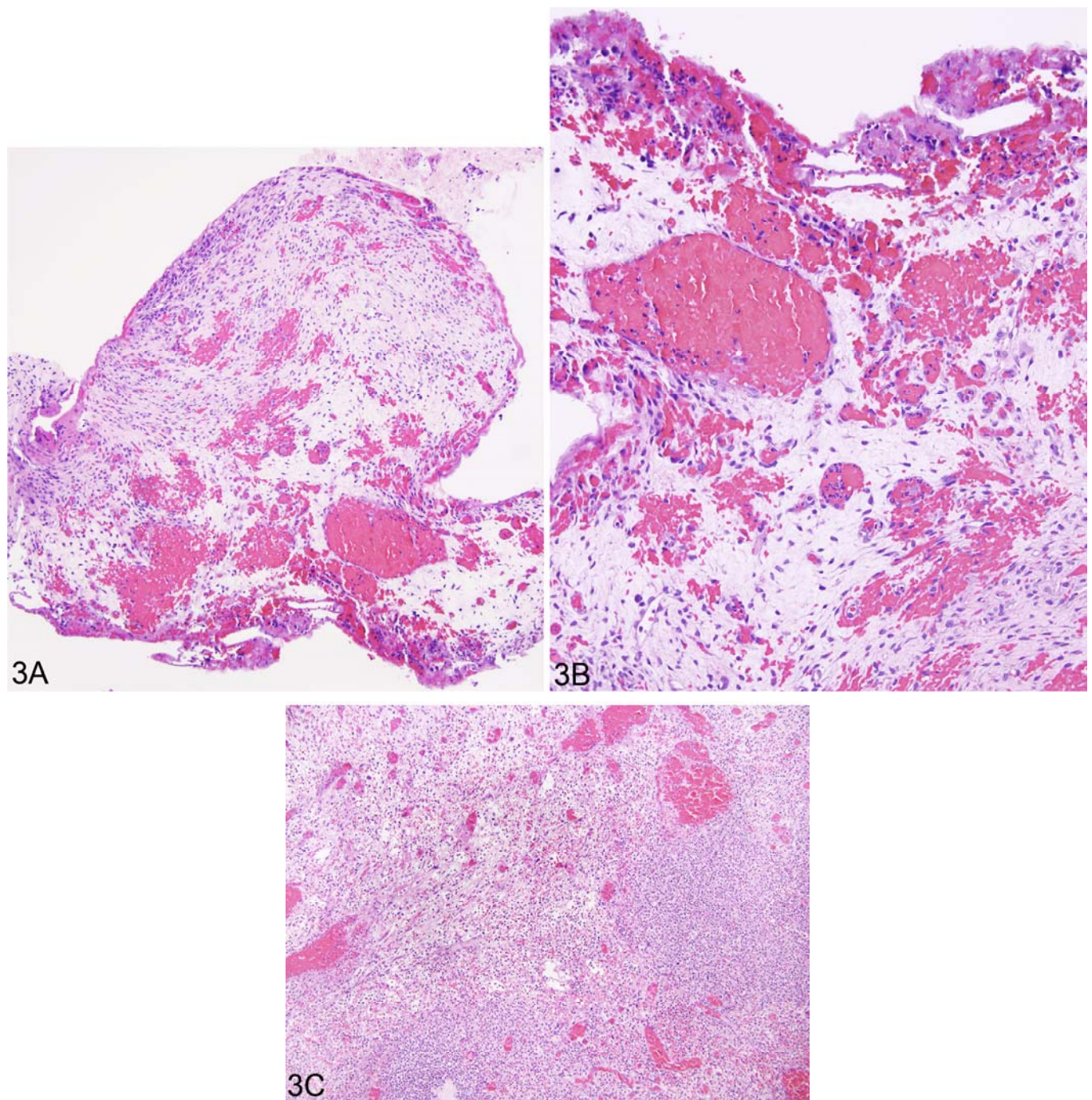
Fig. (3). Radiation related changes. (**A**) Marked edema and congestion are seen. H&E, 120. (**B**) Congestion and hemorrhage and edema are accompanied by a loss of the surface epithelium. H&E, x200. (**C**) Dense cellular infiltrate permeates the entire wall. H&E, 120.

CHEMOTHERAPY

Intravesical Chemotherapy

Over the decades, many agents have been used for topical therapy of bladder cancer. Among these drugs thiotepa and mitomycin were probably used most frequently [44]. Both agents that inhibit DNA synthesis by slightly different mechanisms [45, 46], cause identical morphologic alterations. They induce extensive denudation followed by an atypical appearance of the remaining cells, resembling CIS. However, several cytological and architectural clues are helpful in determining the true pseudoneoplastic nature of the chemotherapy-induced process. Unlike CIS, which is characterized by atypia involving the whole thickness of the epithelium, in thiotepa/mitomycin induced changes, atypia is limited to the layer of superficial umbrella cells. Hyperchromatic nuclei with irregular borders represent a cytologic similarity between CIS and the above mentioned reactive processes. The features that distinguish thiotepa/mytomicin changes from CIS include common multinucleated and vacuolated cells (similar to those in radiation cystitis), a normal nuclear/cytoplasmic ratio and the marked increase in cell size, a feature absent or minimally present in carcinoma.
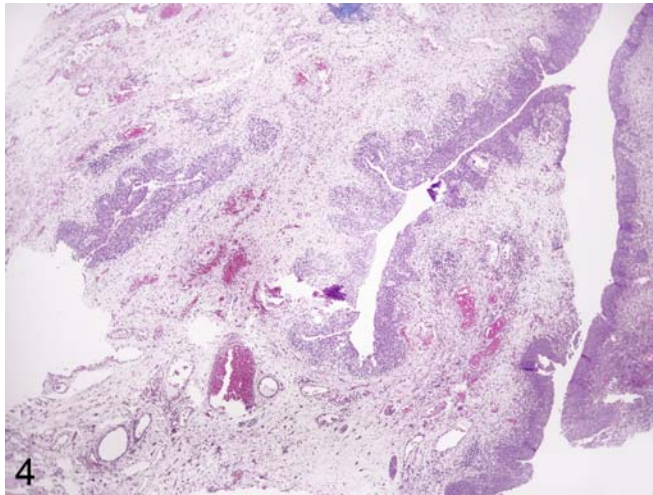
Fig. (4). Radiation related changes. Congestion, stromal fibrosis and surface epithelial proliferation are seen. Surface changes resemble carcinoma *in situ*. H&E, 200.

Late radiation-induced changes resemble those seen in other parts of the body. These changes include scarring of lamina propria and collagenization of muscle fibers, hyalinization of blood vessels and, sometimes, superficial ulceration with an abundant fibrinous exudate [43]. In the lamina propria there are atypical hyperchromatic fibroblasts (Fig. **6**). Similar nuclear changes may be seen in the vascular cells.

Several other agents, including gemcitabine [47], epirubicin [48], cisplatin [49] and others have been documented as effective in the treatment of bladder cancer. It has been shown that some of them induce morphologic changes that mimic carcinoma.

Systemic Chemotherapy

Systemic chemotherapy has recently received more attention as a part of multimodal treatment aimed at preserving the urinary bladder in selected cases of muscle invasive disease. Several drugs such as cisplatine, methotrexate, vinblastine and some other agents have been proposed [50]. There is, however, limited data on the morphological changes induced by this kind of treatment.

Cyclophosphamide, a drug used in organ transplantation and a number of autoimmune and myeloproliferative diseases is a well known cause of hemorrhagic cystitis. Clinically, a well-developed hemorrhagic cystitis can be accompanied by massive hematuria [51], which is nowadays an uncommon finding, as it is prevented by extensive therapeutic hydration. The occurrence of hemorrhagic cystitis is not dose-dependent [52].

Microscopic changes are characterized by marked hemorrhage and edema of the lamina propria. Urothelial surface is ulcerated and covered by a fibrinopurulent exudate. Following the acute stage, urothelium becomes thickened and may form papillae. Cytological changes similar to those induced by intravesical chemotherapy may ensue. Sometimes, in cases of high doses and repeated exposure, the bladder wall becomes fibrotic and the bladder contracted [53]. There have also been reports of both mesenchymal and epithelial neoplasms several years following prolonged cyclophosphamide therapy [54, 55].

PHOTODYNAMIC AND LASER THERAPY

Photodynamic therapy is a form of treatment in which a photosensitizing drug that preferentially accumulates in tumor cells is locally activated by light, thereby inducing tumor-specific necrosis [56, 57]. The effect is exerted 1 to 2 days post-therapy [58] in the form of coagulation necrosis sharply demarcated from the surrounding tissue. Sometimes, there may be hemorrhagic necrosis, spindle cell transformation of urothelial cells, or dystrophic calcification [59, 60].

Laser therapy is used in urothelial neoplasms that are considered indolent, i.e. not in neoplasms showing higher malignant potential than low grade Ta papillary urothelial carcinoma [61]. Following laser treatment practically no tissue or just a limited amount of it is available for histology, showing coagulative necrosis [62]. However, endothelial cells surrounding the neoplasm frequently may show atypia [63], which should not be interpreted as residual cancer.
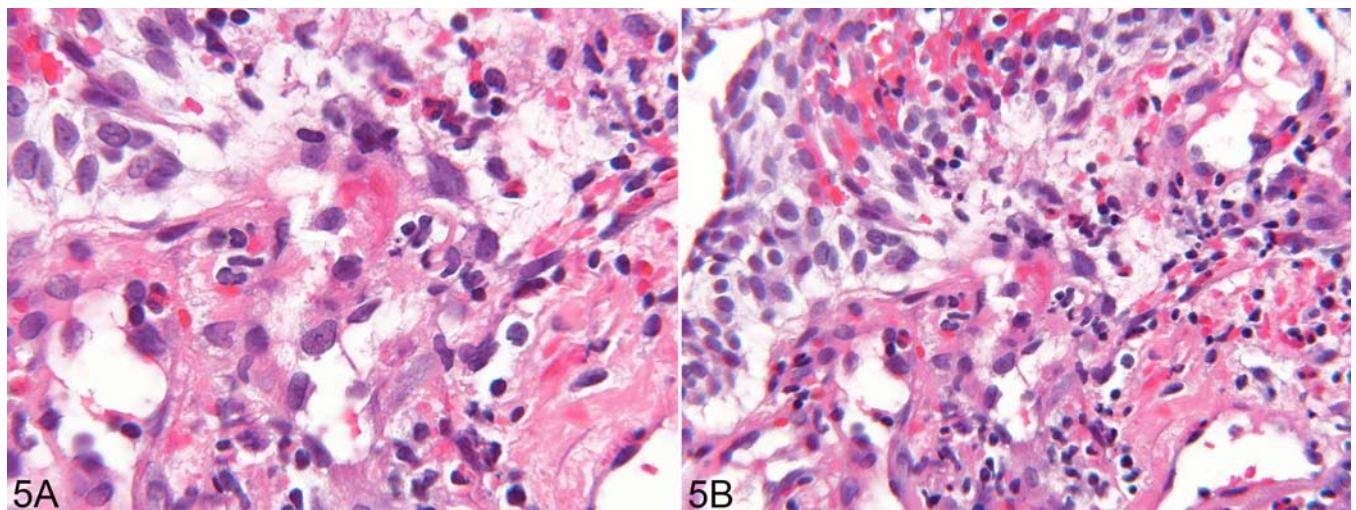

Fig. (5). Radiation related pseudocarcinomatous epithelial hyperplasia. (**A**) Nests of hyperchromatic epithelial cells are invaginating into the bladder wall. H&E, x200. (**B**) Epithelial cell nests have round borders. H&E, 200. Courtesy of Dr. Rodolfo Montironi, Ancona, Italy.
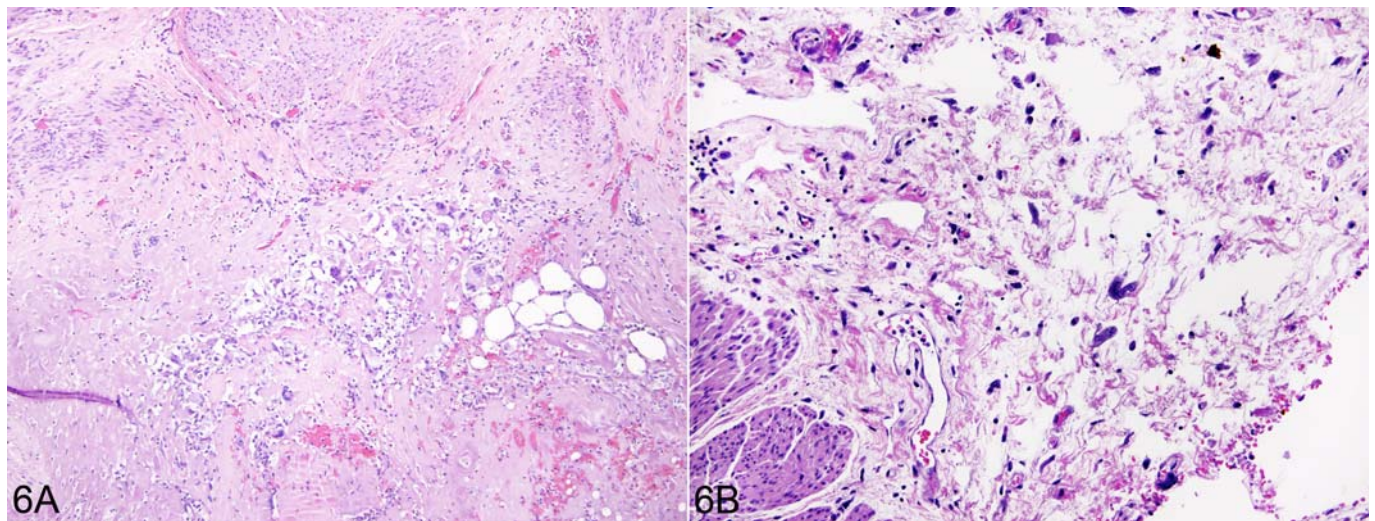
Fig. (6) Radiation related changes. (**A**) Stromal cells have enlarged and atypical nuclei. H&E, x120. (**B**) Enlarged nuclei of stromal cells appear hyperchromatic. H&E, x200.

GENE THERAPY

There are many experimental mouse models [64, 65] in which various viral vectors are used to deliver desired genetic material to tumor cells and prevent their growth. However, the descriptions of the morphologic effects of gene therapy are scarce [66] and a thorough review remains yet to be written. It seems that there is an admixture of necrosis and nuclear changes ranging from a loss of chromatin detail and nucleoli to pyknosis and spindle-like elongation in later stages.

ABBREVIATIONS

ALK   =   Anaplastic lymphoma kinase

BCG   =   Bacillus Calmette- Guérin

CIS   =   Carcinoma in situ

H&E   =   Hematoxylin and eosin

INF   =   Interferon

PSCN   =   Postoperative spindle cell nodule

TUR   =   Transurethral resection

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