Journal of Cellular Biochemistry, Supplement 16I:23–29 (1992)

Bladder Cancer From a Perspective of 40 Years

Leopold G. Koss, MD

Department of Pathology, Montefiore Medical Center, Albert Einstein College of Medicine, Bronx, New York 10467

Abstract The key events leading to a better understanding of the natural history of urothelial tumors of the bladder are summarized. These were: the recognition of flat carcinoma *in situ* and related lesions (intraurothelial neoplasia) as principal sources of invasive cancer; identification of the unique structure of the urothelium; analysis of DNA content and the recognition of two pathways of urothelial tumors. A brief comment on the current status of immunologic and molecular genetic markers is appended.

Key words: bladder tumors; carcinoma *in situ*; intraurothelial neoplasia; DNA ploidy; tumor markers

Within the last 40 years significant strides have been made in understanding neoplastic processes in the urinary bladder and, by implication, in the entire lower urinary tract.

In 1952, when this writer became interested in tumors of the urinary bladder, the principal tools used in the assessment and prognostication of this group of diseases were the gross appearance of the tumor on cystoscopic examination; histologic tumor grading, introduced by Broders in 1922 [1]; and anatomic and clinical staging, introduced by Jewett and Strong [2]. Bladder tumors were divided into "papillary" and "solid" and further subdivided into transitional cell carcinoma, squamous carcinoma, and adenocarcinoma [3]. The natural history of these tumors was poorly understood and their origin was obscure. In large series of reported cases terms such as "recurrence" and "progression" were often confused; the data from various centers could not be compared because of lack of acceptable uniform clinical and histologic criteria. The key events that led to progress in this field are briefly discussed below.

EMERGING CONCEPTS OF CARCINOMA *IN SITU*

My own interest in bladder cancer was triggered in 1952 by a case history of a 54 year old male patient seen at Memorial Hospital for Cancer and Allied Diseases. At that time the microscopic examination of cells in the urinary sediment was in its infancy. A paper on this subject published by Papanicolaou and Marshall in 1945 [4] gave equivocal results. Nonetheless the procedure was applied to patients seen in the Urology Service at Memorial by Dr. Willet Whitmore and his associates. The urinary sediment in the patient under discussion revealed unequivocal cancer cells in the absence of any cystoscopic evidence of disease, except for patches of redness. Multiple biopsies of bladder epithelium obtained after a delay of nearly 3 years disclosed an epithelial abnormality that was finally classified as a carcinoma *in situ* with features of Paget's disease. After much hesitation, a cystectomy was finally performed by Dr. Whitmore, perhaps the first such procedure for an invisible lesion. Histologic examination showed extensive carcinoma *in situ* and a single focus of invasive cancer in the dome of the bladder. In spite of this apparently favorable situation, the patient developed a metastasis to the liver that broke down and caused the death of the patient from hemoperitoneum, 2 years after cystectomy. It goes without saying that this sequence of events, *i.e.*, the death of a young patient from an invisible disease of the bladder, was a most impressive event.

At that time, around 1955, very little was known about carcinoma *in situ* of the urinary bladder. The disease was described by Melicow and Hollowell in 1952 as an incidental finding

TABLE I. Characteristics of Non-papillary Carcinoma *in Situ* of Bladder

- The lesion cannot be recognized cystoscopically as a tumor.
- Cystoscopic abnormalities may mimic inflammation; "velvety redness," "cobblestone epithelium" or "interstitial cystitis" were recorded. In other cases there have been no cystoscopic abnormalities whatever.
- The lesion may extend into the ureters.
- In males, the lesion often extends into the prostatic ducts and the penile urethra.
- Because the lesion produces only nonspecific symptoms or may be asymptomatic, diagnosis is based either on cytology of voided urine or on incidental biopsies of bladder epithelium.
- If untreated, carcinoma *in situ* will progress to invasive carcinoma in at least 60% of all patients within 5 years.
- Carcinoma *in situ* may be successfully treated by immunotherapy in approximately 60% of the cases. An extension into the prostatic ducts precludes immunotherapy.

in cystectomy specimens of patients with otherwise advanced cancers [5]. The prognostic significance of carcinoma *in situ* was a complete mystery. In 1957, a large group of industrial workers heavily exposed to the potent bladder carcinogen para-aminodiphenyl was studied in my laboratory by cytologic examination of their urinary sediment. By 1960, there were 22 of these workers whose sediment contained cancer cells in the absence of clinical disease. Four years later, 11 of these workers developed invasive cancer of the bladder [6]. A few additional workers also developed invasive cancer, sometimes after a delay of several years [7].

Thus the concept of a flat, clinically invisible lesion, carcinoma *in situ*, as a potentially life-threatening disease emerged from these studies. The summary of the principal features of this disease is shown in Table I.

STRUCTURE OF THE UROTHELIUM

During the ensuing years further insight was also gained from histological and ultrastructural studies of the epithelium of origin of bladder tumors. Studies by Hicks [8] and Koss [9] disclosed that the epithelium lining the bladder, the ureters and the renal pelvis of rats and mice had a unique structure, particularly in reference to the superficial cells. These unique cells, called umbrella cells because of their large, flat configuration, have surface plaques composed of a specialized membrane known as the asymmetric unit membrane (AUM). The presence of a virtually identical structure in human epithelium was documented shortly thereafter [10]. Normal human urothelium was shown to be composed of approximately 7 layers of cells overlaid by umbrella cells. The latter often contained multiple nuclei of different sizes. These studies also brought into focus the unique characteristics of bladder epithelium, justifying the term "urothelium" and "urothelial tumors" as a replacement of the previous term "transitional epithelium" and "transitional cell tumors" [11].

CLASSIFICATION AND GRADING OF UROTHELIAL TUMORS

Once the normal structure of the urothelium became known, it was possible to classify tumors derived from this epithelium against a normal reference point [11]. Using the number of cell layers, the presence of umbrella cells as a guide to epithelial differentiation, and the

nuclear abnormalities as evidence of malignant transformation, papillary tumors could be classified into 3 groups:

1. Tumors lined by normal or nearly normal urothelium were classified as papillomas or papillary tumors grade I;
2. Tumors without similarity to normal urothelium, characterized by highly abnormal nuclei, were classified as grade III*;
3. Tumors intermediate to the two groups in reference to epithelial structure and level of nuclear abnormalities were classified as grade II.

The classification of non-papillary, solid tumors had to be approached in a different way; these tumors were usually recognized when located within the wall of the bladder, and hence were invasive. Such tumors retained little of the original structure of the urothelium and contained no umbrella cells. Their classification was based mainly on patterns of growth, size and configuration of component cells and nuclear abnormalities [11]. Well-differentiated tumors of this type, resembling grade I papillary tumors, were distinctly rare. Most solid non-papillary tumors were grade II and III. According to Jewett [2], the outcome of surgical treatment of such tumors depended on their level of penetration into the bladder wall. The bladder wall is composed of several layers: the epithelium (discussed above), a layer of connective tissue known as the lamina propria, and two thick layers of muscle surrounded on the outside by an outer layer of connective tissue, the serosa. Solid tumors invading muscle (or beyond) were shown to have a much less satisfactory treatment outcome than more superficial tumors.

Carcinoma *in situ* was also recognized as a part of the spectrum of solid tumors; such lesions lined the surface of the bladder without evidence of invasion and were, by definition, composed of cancer cells, hence cells with marked nuclear abnormalities.

THE RELATIONSHIP OF CARCINOMA *IN SITU* TO INVASIVE CARCINOMA

Several observers [12,13] noted that about 80% of patients with invasive solid carcinoma had no prior history of papillary tumors, and concluded that most solid, invasive tumors were derived from the clinically invisible and often asymptomatic carcinomas *in situ*. In the remaining 20% of the patients in whom papillary tumors and solid invasive cancers were observed, mapping studies [14–16] disclosed that foci of carcinoma *in situ* in various areas of the bladder, often remote from the papillary tumors, were the source of the invasive cancer. Thus, as suspected in the early 1950s, carcinoma *in situ* assumed a pivotal role in our understanding of the sequence of events in invasive bladder cancer. Similar observations were made by mapping the renal pelvis [17].

ANALYSIS OF DNA CONTENT OF BLADDER TUMORS

The search for prognostic parameters in bladder tumors led to studies of their DNA content, first by cytophotometry [18], then by flow cytometry [19] and subsequently by image analysis [20]. A summary of data based on Tribukait's DNA flow cytometry work [19] is shown in Table II.

It may be noted that grade I papillary tumors are virtually always diploid. Tumors grade III and above, whether papillary or solid, are virtually always aneuploid. The intermediate grade II tumors are almost equally divided between diploid and aneuploid tumors. When studied by stage of disease, nearly all deeply invasive tumors (T2, T3, T4) are aneuploid, as are all carcinomas *in situ*. Thus ploidy studies confirmed the relationship between carcinoma *in situ* and invasive cancer. These studies also offer a very strong support for the value of aneuploidy as a factor in poor prognosis of urothelial tumors [21].

TWO PATHWAYS OF BLADDER TUMORS

Studies of the behavior of papillary and non-papillary tumors and their DNA ploidy led to the formulation of the concept of two separate,

---

Footnote:

* In some institutions and laboratories grade III tumors are subdivided by cell size and configuration into grade III (larger cells) and grade IV (smaller and spindly cells).

TABLE II. Distribution of DNA Values in 277 Untreated Bladder Tumors†

Distribution by Grade

Grade	No. in Group	Diploid	Aneuploid*
0	2	2	0
I	30 (100%)	24 (80%)	6 (20%)
II	107 (100%)	56 (52%)	51 (48%)
III	130 (100%)	6 (5%)	124 (95%)
Adenocarcinoma	8	1	7
Total	277	89	188

Distribution by Stage

Stage	No. in Group	Diploid	Aneuploid
T0	42 (100%)	32 (76%)	10 (24%)
T1	118 (100%)	50 (42%)	68 (58%)
T2, T3, T4	93 (100%)	7 (7.5%)	86 (92.5%)
TIS	24 (100%)	0	24 (100%)
Total	277	89	188

† Modified from Tribukait [19].
* Includes tetraploid-aneuploid tumors.

albeit overlapping, pathways of bladder tumors [22], as summarized in Table III.

Several other factors besides morphology and ploidy are also listed in this table; however, they cannot be discussed in detail in this summary. The interested reader is referred to the original sources, listed in the references [23–25].

THE CONCEPT OF INTRAUROTHELIAL NEOPLASIA

Although the concept of flat carcinoma *in situ* is well-established, patterns of epithelial abnormality do not always fulfill the criteria of a fully developed lesion. Such abnormalities have been variously named "atypical hyperplasia" [11] or "dysplasia" [26]. The morphologic separation of these entities as well as their grading as "mild," "moderate," and "severe" are not reproducible. Although the natural history of these lesions is still poorly understood because of lack of follow-up data, there is evidence that the presence of such atypical lesions in the bladder epithelium is an important risk factor in the development of invasive cancer [27,28]. The term intraurothelial neoplasia, suggested some years ago [29] may help to avoid the controversy created by the grading of "dysplasia."

MONITORING PATIENTS WITH BLADDER TUMORS

Cytology

Cytologic diagnosis of grade III cancers and carcinoma *in situ* is secure in competent hands [29,30]. However, the cytologic diagnosis of grade I tumors is nearly impossible because of the similarities of tumor cells to normal urothelial cells. For grade II tumors, the results vary and are related to the DNA ploidy of these tumors: diploid tumors cannot be recognized

TABLE III. Characteristics of 2 Groups of Urothelial Tumors

Feature	Low-Grade Papillary Tumors	High-Grade Papillary Tumors and Invasive Carcinomas
Epithelial abnormality or origin	Hyperplasia	Flat grade carcinoma *in situ* and related abnormalities (intraurothelial neoplasia, high grade)
Invasive potential	Low	High
Urine cytology	Negative or atypical	Positive
DNA ploidy pattern (see Table II)	Predominantly diploid	Predominantly aneuploid
Density of nuclear pores [22]	Normal	Increased
Expression of Ca antigen (Epitectin) [23]	As in normal urothelium	Increased
Blood group isoantigen expression [24]	Usually present	Usually absent

and aneuploid grade II tumors shed "atypical" cells that are often referred to as "suspicious" [30].

Cytologic diagnosis of bladder tumors has several important pitfalls, listed in Table IV. The reader is referred to another source [30] for a detailed analysis. The presence of cancer cells in treated patients is of major prognostic significance even in the absence of clinical disease. Such patients are prone to the development of invasive cancer, often from foci hidden within the prostatic ducts.

TABLE IV. Principal Sources of Error in Urinary Cytology

- Effects of instrumentation
- Human polyomavirus infection
- Effect of certain drugs (cyclophosphamide, myeleran)
- Effect of radiotherapy

DNA Analysis of Bladder Washings or Barbotage

It has been suggested [31] that a DNA analysis of cells in bladder washings or barbotage is a reliable means of monitoring patients with bladder tumors. Although impressive data have provided support for this thesis [32–34] the reality is less persuasive. Inflammatory events, regenerative events, and prostatic hyperplasia may cause aneuploid histograms despite the absence of bladder tumors [35,36]. In some instances, no obvious reasons for aneuploidy can be found. Further, the interpretation of DNA histograms is difficult if the histogram is not "clean," as is often the case.

Supplementing flow cytometry with DNA image analysis of Feulgen-stained cell sediment, particularly in situations where flow cytometric histograms are difficult to interpret, may clarify obscure findings [20].

Bladder Tumor Markers

Recently a number of immunologic markers and markers based on molecular biology has been introduced. The earliest such group of markers is blood group antigens, as first proposed by Kovarik *et al.* [37]. Variants of the original procedure, particularly Lewis blood group antigens, will be discussed elsewhere in this volume (see Sheinfeld, these proceedings).

Monoclonal antibodies to various components of the urothelium will also be discussed (see Fradet, these proceedings).

Markers based on tumor inhibitory genes, such as Rb and p53, will also be presented (see Reuter, von Eschenbach, and Benedict, these proceedings). Our own group has been studying the modifications of the Ha-*ras* gene in retrospective and prospective studies. A change in codon 12 of the exon of the *ras* gene has not proven to be a good prognostic marker, although it occurs more often in aneuploid and invasive tumors [38]. A synchronous modification of codon 12 and the intron of the *ras* gene, however, appears to have considerable prognostic value as it has been observed in carcinoma *in situ* several years before the occurrence of invasive cancer with the same genetic characteristics [39].

Still, the issue of molecular biological markers in human tumors is still in its infancy. With a few notable exceptions such as the deficiency of the Rb gene in retinoblastoma or an increase in the *myc* gene expression in neuroblastoma, no markers of a major significance have so far emerged. In fact, most genetic abnormalities thus far described are limited to subgroups of malignant tumors and fail to fulfill diagnostic or prognostic expectations. It may be that genetic changes in human cancers do not follow a logical sequence of events. It is also quite likely that several different pathways may lead to the development and progression of human cancer. So far the sequence of events has eluded our understanding.

REFERENCES

1. Broders AC: Epithelioma of the genitourinary organs. Ann Surg 75:574–604, 1922.
2. Jewett HJ, Strong GH: Infiltrating carcinoma of bladder: Relation of depth of penetration of bladder wall to incidence of local extension and metastases. J Urol 35:366–372, 1946.
3. Friedman NB, Ash JE: Tumors of the urinary bladder. In "Atlas of Tumor Pathology," Fascicle 31A. Washington DC: Armed Forces Institute of Pathology, 1959.
4. Papanicolaou GN, Marshall VF: Urine sediment smears as diagnostic procedure in cancers of urinary tract. Science 101:519–520, 1945.
5. Melicow MM, Hollowell JW: Intraurothelial cancer: Carcinoma *in situ*, Bowen's disease of the urinary system: Discussion of thirty cases. J Urol 68:763–772, 1952.
6. Koss LG, Melamed MR, Ricci A, Melick WF, Kelly RE: Carcinogenesis in the human urinary bladder. Observations after exposure to para-aminodiphenyl. N Engl J Med 272:767–770, 1965.
7. Koss LG, Melamed MR, Kelly RE: Further cytologic and histologic studies of bladder lesions in workers exposed to para-aminodiphenyl: Progress report. J Natl Cancer Inst 43:233–243, 1969.
8. Hicks RM: Fine structure of the transitional epithelium of rat ureter. J Cell Biol 26:25–48, 1966.
9. Koss LG: The asymmetric unit membrane of the epithelium of the urinary bladder of the rat. An electron microscopic study of a mechanism of epithelium maturation and function. Lab Invest 21: 154–168, 1969.
10. Koss LG: Some ultrastructural aspects of experimental and human carcinoma of the bladder. Cancer Res 37:2824–2835, 1977.
11. Koss LG: Tumors of the urinary bladder. In "Atlas of Tumor Pathology," Second Series, Fascicle 11. Washington DC: Armed Forces Institute of Pathology, 1975.
12. Brawn PN: The origin of invasive carcinoma of the bladder. Cancer 50:515–519, 1982.
13. Kaye KW, Lange PH: Mode of presentation of invasive bladder cancer: Reassessment of the problem. J Urol 128:31–33, 1982.
14. Koss LG, Tiamson EM, Robbins MA: Mapping cancerous and precancerous bladder changes. A study of the urothelium in ten surgically removed bladders. JAMA 227:281–286, 1974.
15. Koss LG, Nakanishi I, Freed SZ: Nonpapillary carcinoma *in situ* and atypical hyperplasia in cancerous bladders. Further studies of surgically removed bladders by mapping. Urology 9:442–455, 1977.
16. Koss LG: Mapping of the urinary bladder: Its impact on the concepts of bladder cancer. Hum Pathol 10:533–548, 1979.
17. Mahadevia PS, Karwa GL, Koss LG: Mapping of urothelium in carcinomas of the renal pelvis and ureter. A report of nine cases. Cancer 51:890–897, 1983.
18. Lederer B, Mikuz G, Gutter W, zur Neiden G: Zytophotometrische untersuchungen von tumoren des ubergangsepithels der harnblase. Vergleich zytophotometrischer untersuchungsergebnisse mit dem histologischen grading. Beitr Pathol 147:379–389, 1972.
19. Tribukait B: Flow cytometry in surgical pathology and cytology of tumors of the genito-urinary tract. In Koss LG, Coleman DV (eds): "Advances in Clinical Cytology," Vol. 2. New York, NY: Mason Publishing, 1984, pp 163–189.
20. Koss LG, Wersto RP, Simmons DA, Deitch AD, Herz F, Freed SZ: Predictive value of DNA measurements in bladder washings. Comparison of flow cytometry, image cytophotometry, and cytology in

patients with a past history of urothelial tumors. Cancer 64:916–924, 1989.
21. Tribukait B: Flow cytometry in assessing the clinical aggressiveness of genito-urinary neoplasms. World J Urol 5:108–122, 1987.
22. Koss LG: Supplement to Atlas of Tumors of the Urinary Bladder, Second Series, Fascicle 11. Washington DC: Armed Forces Institute of Pathology, 1985.
23. Czerniak B, Koss LG, Sherman A: Nuclear pores and DNA ploidy in human bladder carcinomas. Cancer Res 44:3752–3756, 1984.
24. Czerniak B, Koss LG: Expression of Ca antigen on human urinary bladder tumors. Cancer 55:2380–2383, 1985.
25. Weinstein RS, Alroy J, Farrow GM, Miller AW III, Davidsohn I: Blood group isoantigen detection in carcinoma *in situ* of the urinary bladder. Cancer 43:661, 1979.
26. Murphy WM, Soloway MS: Developing carcinoma (dysplasia) of the urinary bladder. Pathol Annu 17:197–217, 1982.
27. Althausen AF, Prout GR Jr, Daly JJ: Noninvasive papillary carcinoma of bladder associated with carcinoma *in situ*. J Urol 116:575–580, 1976.
28. Wolf H, Hjogaard K: Urothelial dysplasia concomitant with bladder tumours as a determinant factor for future new occurrences. Lancet 2:134–136, 1983.
29. Koss LG, Deitch AD, Ramanathan R, Sherman AB: Diagnostic value of cytology of voided urine. Acta Cytol 29:810–816, 1985.
30. Koss LG: "Diagnostic Cytology and Its Histopathologic Bases," 4th Edition. Philadelphia: JB Lippincott, 1992.
31. Melamed MR, Traganos F, Sharpless T, Darznkiewicz Z: Urinary cytology automation: Preliminary studies with acridine orange stain and flow-through cytofluorometry. Invest Urol 13:333–338, 1976.
32. Klein FA, Herr HW, Sogani PC, Whitmore WF Jr, Melamed MR: Detection and follow-up of carcinoma of the urinary bladder by flow cytometry. Cancer 50:389–395, 1982.
33. Badalament RA, Hermansen DK, Kimmel M, Gay H, Herr HW, Fair WR, Whitmore WF Jr, Melamed MR: The sensitivity of bladder wash flow cytometry, bladder wash cytology, and voided cytology in the detection of bladder carcinoma. Cancer 60: 1423–1427, 1987.
34. Badalament RA, Gay H, Whitmore WF Jr, Herr HW, Fair WR, Oettgen HF, Melamed MR: Monitoring intravesical bacillus Calmette-Guerin treatment of superficial bladder carcinoma by serial flow cytometry. Cancer 58:2751–2757, 1986.
35. deVere White RW, Olsson CA, Deitch AD: Flow cytometry: Role in monitoring transitional cell carcinoma of bladder. Urology 8:15–20, 1986.
36. Murphy WM, Emerson LD, Chandler RW, Moinuddin SM, Soloway MS: Flow cytometry versus urinary cytology in the evaluation of patients with bladder cancer. J Urol 136:815–819, 1986.
37. Kovarik S, Davidsohn I, Stejkal R: ABO antigen in cancer: Detection with the mixed cell agglutination reaction. Arch Pathol 86:12–21, 1968.
38. Czerniak B, Deitch AD, Simmons H, Etkind P, Koss LG: Ha-*ras* gene codon 12 mutations and DNA ploidy in urinary bladder carcinoma. Br J Cancer 62:762–763, 1990.
39. Czerniak B, Cohen G, Etkind P, Deitch AD, Simmons H, Herz F, Koss LG: Concurrent mutations of coding and regulatory sequences of the Ha-*ras* gene in urinary bladder carcinomas. Hum Pathol 23:1199–1204, 1992.