Biochemically Detectable Tumor Markers in Urine of Bladder Cancer Patients

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Summary

Potential biochemical markers excreted in the urine of bladder cancer patients have been considered, with the conclusion that none alone has yet proven to be useful as a screening procedure for the detection of urothelial cancer. Quantitative fluctuations in urinary levels of several of these markers in combination, such as pseudouridine, β-aminoisobutyric acid, and fibrinogen degradation products, appear to be valuable in the assessment of the treatment of bladder cancer patients and in helping to predict recurrences in these patients.

Introduction

Some of the properties of cells or tissues that might serve as useful indicators of the presence and the biological potential of proliferative lesions of the bladder epithelium will be reviewed. By definition, any substance that is elevated or decreased in the serum or urine of bladder cancer patients or in biopsy specimens and that correlates with the presence or growth rate of the tumor could be considered a tumor marker. Only those markers excreted in urine will be considered in this review. At the outset, it should be pointed out that, despite years of intensive investigation by many investigators, no biochemical marker for any specific type of cancer has yet been found. For convenience of discussion, useful biochemical markers can probably be classified into 4 categories: (a) markers associated with rapid growth or turnover of certain cellular constituents (examples: polyamines, β-aminoisobutyric acid, minor bases of tRNA); (b) biochemical markers associated with, or a consequence of, abnormal leakage of cytoplasmic components through tumor cell membranes (example: fibrinogen degradation products); (c) markers associated with dedifferentiation or the expression of embryonic gene products (example: carinoembryonic antigens); and (d) tumor-associated antigens. Markers falling into the last category are discussed by Reisfeld et al. (21) at this conference. At this present time, current methods for assayimg carinoembryonic antigens in urine are not reliable as a diagnostic test for detection of bladder cancer or in assessing the course of this disease (6, 8, 30). Most of the published work on the use of urinary biochemical markers for the study of bladder cancer falls into the 1st category. Only 1 paper has been published utilizing a marker in the 2nd category.

Pseudouridine and Methylated Nucleosides

Following their synthesis, nucleic acids in mammalian cells are enzymatically modified at the macromolecular level (26), for example, by methylation of bases at specific sites. tRNA in particular is extensively modified after transcription, and over 40 modified bases are known to occur in various tRNA’s (18). Upon degradation of nucleic acids, these modified bases do not enter into purine/pyrimidine salvage pathways to any significant extent, and they are excreted as end products of nucleic acid metabolism, primarily as their nucleosides. Thus, these modified nucleosides can be used as biochemical markers of nucleic acid metabolism, particularly tRNA turnover, and analyses of urinary levels of these compounds in bladder cancer patients could possibly serve as useful indicators of altered growth patterns. Another important factor is that many tumors have abnormally high amounts of methylated bases in their tRNA’s (reviewed in Ref. 4).

Several papers describing elevated excretion of pseudouridine and methylated purine nucleosides in the urine of patients with various types of cancer have appeared in recent years (Refs. 4, 12, and 29; see also Refs. 10, 11, and 28 for recent improvements in methodology). One of the more extensive studies in this area is that by Waalkes et al. (29), who have determined excretion levels for 3 of these nucleosides in 24-hr urine specimens from over 200 patients with various solid tumors. When compared to controls, elevated levels of N2, N2-dimethylguanosine, N1-methylcytosine, and pseudouridine were found for patients in each of several tumor types studied. Unfortunately, bladder cancer was not one of the tumor types listed. In general, for any cancer patient’s urine, if one of the nucleosides was found to be elevated, the levels for one or both other nucleosides usually were also increased. N2,N2-Dimethylguanosine appeared to be somewhat more frequently elevated and to a greater degree than either pseudouridine or N1-methylcytosine. Some patients with diseases other than cancer had elevations in levels of the methylated nucleosides above 2 S.D. of the normal means (12 of 62 for N2,N2-dimethylguanosine and 13 of 62 for N1-methylcytosine), but only 1 of 62 of these patients was found to have a level of urinary pseudouridine greater than 2 S.D. of the normal mean. There has been only 1 study published on the determina-
tion of urinary pseudouridine or methylated purine nucleoside levels in bladder cancer patients (20). This will be described later.

**β-Aminoisobutyric Acid**

β-Aminoisobutyric acid is produced from thymine (7). Degradation of thymine to β-aminoisobutyric acid involves an initial reduction to dihydrothymine, followed by a series of hydrolytic reactions to produce β-aminoisobutyric acid. Thymine from both DNA and tRNA can contribute to urinary β-aminoisobutyric acid (13, 16, 17). Some normal adults show a genetically determined high urinary excretion of β-aminoisobutyric acid (19), and among Japanese high excretors there appears to be a deficiency of the liver enzyme that degrades β-aminoisobutyric acid, β-aminoisobutyrate:pyruvate aminotransferase (27).

Nielsen et al. (14) first reported in 1970 that patients with bladder tumors had an increased urinary excretion of β-aminoisobutyric acid compared to controls without any urological complaints. The elevated urinary β-aminoisobutyric acid was not associated with genetic factors, and it appears that the excess β-aminoisobutyric acid in urine of these cancer patients may be related to an increased turnover of tRNA. The clinical significance of changes in urinary β-aminoisobutyric acid in patients with bladder cancer has been studied and followed up rather extensively by the Danish group (15, 16, 19). They have found that urinary β-aminoisobutyric acid is significantly increased in patients with invasive tumors compared to controls and patients with noninvasive urothelial tumors. For example, the incidences of increased urinary α-aminoisobutyric acid in the controls and in patients with noninvasive tumors were 15 and 10%, respectively, whereas in patients with invasive tumors the incidence of increased urinary β-aminoisobutyric acid was 32 to 46%. Furthermore, increased urinary β-aminoisobutyric acid appeared to be correlated with high-grade tumor cell dysplasia (Grade III), and recurrences in these patients with elevated urinary β-aminoisobutyric acid were preceded by a decrease in the excretion of β-aminoisobutyric acid. The incidence of increased urinary β-aminoisobutyric acid was independent of tumor stage. Although it appears that urinary β-aminoisobutyric acid alone cannot be used as a general screening procedure for bladder cancer, determinations of urinary β-aminoisobutyric acid may be helpful in a prognostic evaluation of the bladder cancer patient. In the most recent communication from the Danish group (20), it has been demonstrated that in patients treated for low-grade urothelial tumors (Grades I and II), recurrences were likely to appear more often if the patient had a low urinary β-aminoisobutyric acid. The highest incidence of recurrences in these patients was seen in individuals with a low urinary β-aminoisobutyric acid accompanied by an elevated urinary pseudouridine excretion; 14 of 20 (70%) of the patients with low-grade urothelial tumors displaying this excretion pattern developed a recurrence within 6 months.

**Polyamines**

There are many studies in the literature indicating that the naturally occurring polyamines putrescine, spermidine, and spermine are synthesized at greatly elevated rates and accumulate in mammalian cells during periods of rapid growth (1, 22, 23). There appears to be a high positive correlation between tumor growth rate and the cellular pseudouridine:spermine ratio (9).

In 1971, Russell et al. (24) reported that patients with various types of cancer excreted elevated levels of polyamines in the urine. They suggested that a measurement of urinary polyamines might be useful in the diagnosis of cancer and in predicting the efficacy of various treatments (24). The use of polyamines as biochemical markers of cancer was recently reviewed by Bachrach (2), who concluded that the use of polyamines as a diagnostic tool for the early detection of cancer had several limitations. One limitation was that the available analytical procedures are rather time consuming or too complex for routine use. Another limitation was that polyamine levels are also high in some noncancerous tissues.

There are no published studies dealing specifically with the determination of urinary polyamine levels in bladder cancer patients. Such studies might prove to yield useful data. A radioimmunoassay for polyamines in serum, recently described by Bartos et al. (3), would appear to be the method of choice for such studies, if it could be adapted to urine.

Based on some studies by Russell et al. in various types of cancer patients (23–25), determination of urinary polyamine levels might also be useful for monitoring the bladder cancer patient during the course of his disease.

**Fibrinogen Degradation Products**

Tumor cells tend to leak certain cytoplasmic enzymes, among which is plasminogen activator (31). This probably accounts for the generally prominent fibrinolytic activity of tumor cells (30, 31). Wajsman et al. (30) recently reported results of a study on the levels of fibrinogen degradation products (determined by immunoassay) in urines of a group of 66 bladder cancer patients. Forty-six of these patients had no evidence of disease, but they did have a positive tissue diagnosis of bladder cancer in the 2 years preceding the study. There was evidence of active disease in 20 of the patients. A high degree of accuracy (90%) was found in correlating the levels of urinary fibrinogen degradation products combined with urine cytology with the activity of the disease. Further studies would appear to be warranted for use of urinary levels of fibrinogen degradation products for screening and follow-up of bladder cancer patients.

**Conclusions**

None of the biochemical markers considered is valuable alone as a general screening procedure for the detection of urothelial cancer. Future emphasis should be given to studies on the use of 2 or 3 of these markers in combination with one another. The most promising biochemical markers for further study would appear to be the use of pseudouridine and β-aminoisobutyric acid, in combination with the assay for fibrinogen degradation products. Urinary polyamine lev-
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els in bladder cancer patients is an alternative which needs to be explored.

Use of these markers for the evaluation of the bladder cancer patient during the course of his disease should be especially valuable for the assessment of treatment and predicting the probability of recurrences in patients with low-stage disease. These markers might also be of some benefit in helping to select patients with more advanced disease who might benefit by adjuvant drug therapy.

Addendum

The reader is referred to a recent report by S. S. Cohen (5) on the "Conference on Polyamines in Cancer." This report contains an interesting discussion of the history of biochemical markers in cancer, as well as specific remarks on polyamine levels in cancer patients.

References

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