The importance of molecular tumor markers for bladder cancer

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ABSTRACT

A number of urine based tumor markers are available preclinically for bladder cancer. The diagnosis of bladder cancer currently relies on identifying malignant cells in the urine and subsequently visualizing the tumor on cystoscopy. This diagnosis is further confirmed by transurethral resection or biopsy. However their sensitivity rate is highly poor for low-grade bladder neoplasms. Therefore more sensitive tumor markers are needed. On the other hand, easy applicable and cheap molecular diagnostic tools might be better for monitoring illness. In this review new molecular markers are discussed to understand their comparisons easily. [Turk J Cancer 2003;33(4):171-176]

KEY WORDS:

Bladder cancer, BTA, tumor markers, telomerase, BTA Stat, NMP22, EZH2

INTRODUCTION

With its incidence continuing to increase, bladder cancer is now the fifth most common cancer in US, with an estimated 56,500 new cases predicted for 2002, of which 12,600 patients are expected to die of the disease (1). The three main types of cancers that affect the bladder are urothelial carcinoma (transitional cell carcinoma or TCC), squamous cell carcinoma, and adenocarcinoma. TCC, by far the most common form of bladder cancer accounting for more than 90% of these cancers in US, is the second most common malignancy of genitourinary tract and third most common cause of death among genitourinary tumors (2). Bladder cancer is currently diagnosed using cystoscopy and cytology in patients with suspicious signs and symptoms. Most patients with bladder cancer are diagnosed upon a gross or microscopic hematuria (1-3). Approximately 75-90% of newly diagnosed bladder neoplasms are confined to the urothelium or invade the lamina propria (Ta and T1) and can be managed with transurethral resection (TUR) and intravesical therapy (4). The recurrence rate of these neoplasms are high with progression (5). Patients having high-grade disease have a worse prognosis. Approximately half of these patients will have muscle-invasive disease at diagnosis and have distant metastasis within 2 years and 60% die within 5 years despite treatment. Systemic treatment options for bladder cancer include surgery, chemotherapy, radiation, and immunotherapy (1-5).
This situation indicates the importance of exact and sensitive diagnosis at initial level of illness. Current follow-up procedure after initial presentation typically includes flexible cystoscopy and urine cytology every 3 months for 1-2 years, every 6 months for an additional 2-3 years and then annually, assuming no recurrence (1-4, 6,7).

Cystoscopy is a relatively short, minimally traumatic office procedure performed with topical anesthesia that identifies nearly all papillary and sessile lesions (8). However, despite the introduction of the flexible cystoscope, it is still an invasive procedure, and causes some discomfort. Cystoscopy aided by cytology is the mainstay for diagnosing bladder cancer (8, 9). Voided urine cytology has been used since 1945 as a screening test for this condition (8). Cytology has a high sensitivity and specificity for the detection of high-grade tumors. However its sensitivity for low-grade tumor is poor and also expensive. It also requires a highly trained cytopathologist, who may not be available in all areas. On the other hand, cystoscopy is invasive and relatively expensive too, and it may also be inconclusive at times (1-9). Especially in many patients with an indwelling catheter or active inflammation, cystoscopy may not be definitive due to the grossly abnormal appearance of the bladder mucosa. The deficiencies of cytology and the invasiveness of cystoscopy again render each test suboptimal for tumor surveillance. Therefore, a noninvasive method for detecting and monitoring bladder cancer would be objective, noninvasive, and easy to administer and interpret, and have high sensitivity and specificity (1-9).

Other urological malignancies such as prostate cancer have an existing serum based marker that has been well studied or they are not likely to release potential marker substances into the urine, making urine based testing less useful (1,2).

There are many investigations to find the most sensitive, useful for clinics and cheap marker for bladder cancer. However all investigations are preclinical. Some of them have undergone clinical trials and approved by Food and Drug Administration (FDA) for clinical phase studies. In near future there will be many kinds of tumor markers for bladder cancer in clinical usage. In this review, there is literature search for FDA approved and preclinical future candidate molecular markers.

### BLADDER TUMOR MARKERS

Cytology is a noninvasive marker for comparison. It has some limitations such as trained cytopathologist and low sensitivity rate for grade 1 tumors. This test performed on voided urine samples or bladder wash (requires catheterization). Wiener et al (10) indicated that there is no significant difference in urine cytology between bladder wash and voided urine samples. Its usage is recommended for carcinoma in situ which was not able to be detected by cystoscopy. It is still an adjunct to cystoscopy (10,11).

### FDA Approved bladder tumor markers

**BTA tests (BTA stat and BTA TRAK)**

These tests detect human complement factor H related protein. This protein can be isolated from urine samples of bladder cancer patients. BTA stat is a spectrochromatographic assay and it is really rapid and single step test. Its sensitivity rate is 75% for carcinoma in situ patients and its specificity is over 85%. This test takes only 5-7 min for each specimen. Test is available in 30 specimens package for US 480$ (2,5,12).

BTA TRAK test is a sandwich immunoassay test and it requires trained personal and sufficient laboratory conditions. This test measures the H related protein in urine samples. Its cut off level is 14 U/ml. The sensitivity rate is over 65%. As with BTA stat test, benign genitourinary conditions, particularly hematuria, may yield false positive results (13,14).

Both of these tests have sensitivity comparable to cytology for high-grade tumors and better for low-grade tumors (Table 1). FDA approves these tests for use as an

| Table 1 |
| BTA stat and BTA TRAK sensitivity rates for different grades |

<table>
<thead>
<tr>
<th></th>
<th>BTA stat (%)</th>
<th>BTA TRAK (%)</th>
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<tbody>
<tr>
<td>Grade 1</td>
<td>30,9</td>
<td>48</td>
</tr>
<tr>
<td>Grade 2</td>
<td>55,3</td>
<td>59</td>
</tr>
<tr>
<td>Grade 3</td>
<td>88,3</td>
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aid in the management of bladder cancer in combination with cystoscopy. However, they are not sufficiently accurate to be used for screening or diagnosis (2,5,12-14).

**Nuclear matrix protein 22 (NMP22)**

It was first described in 1947. This test detects a nuclear mitotic apparatus protein that is a component of nuclear matrix. These proteins play an important role in gene expression (2,15). NMP22 protein settles down in the spindles during mitosis and regulates chromatids and daughter cell separation (15,16). Its expression rate highly increases in bladder cancer patient’s bladder epithelium. It is a quantified sandwich enzyme-linked immunoassay test and uses two antibodies of which recognizes different epitopic regions of the nuclear in the mitotic apparatus (5). This test takes US 29$ in USA. Its cut off level is 10 U/ml. NMP22 test sensitivity and specificity is 69 and 72% for this cut off level (10,11,17). If cut off level is chosen as 8 U/ml, this rates change to 81 and 61%. Unlike BTA tests, there is only a slight increase in sensitivity with increase in tumor grade. All analysis for the data clearly exhibit that NMP22 test is superior to cytology for detection of low-grade bladder tumors. However, its specificity is lower than others and cystitis can cause false positive results. This test is the only one approved by the FDA for screening bladder cancer. It may be useful for diagnosing bladder cancer in patients under high-risk carcinogens (2,18).

**ACCU-Dx**

Fibrin fibrinogen degradation (FDP) products are protein fragments generated by the action of the plasminogen on fibrin and fibrinogen (1,2,19). Since the mid 1970s, the identification of FDP in the urine has been evaluated as a test for bladder cancer (3,19). This test detects fibrinogen-fibrin degradation products in urine samples of bladder cancer patients. Bladder neoplasms cause the production of vascular endothelial growth factor that increases plasma protein lackage (3,20). Accu-Dx test was approved by the FDA as an adjunct to cystoscopy in the diagnosis of malign bladder specimens (20). It is performed as an immunoassay detecting fibrin-fibrinogen degradation product in urine samples of bladder cancer patients (20,21). Accu-Dx test was approved by the FDA as an adjunct to cystoscopy in the diagnosis of malign bladder specimens (20,21). It is a rapid test and lasts only 10 minutes. Its sensitivity and specificity is recorded as 68 and 86% (3,20,21). Cytology and test results are similar to BTA test. However patients with hematuria and prostate cancer can be positive for this test. Although it is thought as a good marker with high sensitivity and specificity there are still some studies to make it more efficient (3,20,21).

**Tumor markers for bladder cancer under preclinical investigations**

**Telomerase**

This enzyme helps the synthesis of telomeres (the terminal ends of eucaryotic chromosomes and nucleotide sequences at the 3’ end of the lagging (5’ to 3’) strand of DNA that remained uncopied after each cycle of DNA replication) and thus maintains chromosomal ends (3,22). This enzyme is first described in ovarian carcinoma cells by demonstrating the extension of primers with hexameric repeat sequences. During cell division activity, telomeres decrease in size. This causes chromosomal instability, and eventually cell death. In malign situations telomerase activity is increased and this enzyme is found in urine samples of bladder cancer patients. It is determined with telomeric repeat amplification protocol assay (TRAP). This assay finds TTAGGG nucleotides with PCR in urine specimens. In comparative studies urinary telomerase activity has shown better sensitivity than cytology with slightly lower specificity (2,3,23-25).

This test needs trained personnel and sufficient laboratory conditions. Therefore wide usage is difficult. In most of the studies its sensitivity is showed as 80%. The disadvantage of the test is that it shows wide spread sensitivity rate. When compared with other tests, telomerase test has a relatively high sensitivity for low grade TCC and the sensitivity rate increases for higher-grade cancers. RT-PCR results for mRNA of telomerase enzyme show same range sensitivity with TRAP assay. RT-PCR can lead to false positive result in patients with mild inflammatory conditions. This test needs more investigation to be useful in clinic for monitoring or diagnosis of bladder cancer patients (23,26).

**Cytokeratins**

Normal urothelial and bladder tumor cells express Cytokeratin 19. The immunoradiometric assay and enzyme linked immunosorbent assay detect the soluble fragments of Cytokeratin 19 (27). The researchers noted 96% sensitivity and 74% specificity at the 4 ng/ml cut off level. The
corresponding sensitivity of cytopathological evaluation was only 43%.

Cytokeratin 20 is detected mainly in the urothelial cells of patients with bladder carcinoma. It is detected by RT-PCR in the cell of urine specimens of patients. The sensitivity of this method was 91% and specificity was 67% (28). Cytokeratin based tests have the disadvantage of being somewhat nonspecific for bladder cancer and they may also be positive in other types of epithelial cancer. Besides these, the usage in clinics is highly difficult for patients with risk factors such as smoking (2,3,28).

**Hyaluronic acid and hyaluronidase**

Hyaluronic acid is a nonsulphated glycosaminoglycan, which is known to promote tumor cell adhesion and migration. It is known as a component of tissue matrix and fluids. It generally binds cell surface receptors such as CD44 and, thereby, regulates cell adhesion, proliferation and migration. Bladder tumor cells have been shown to induce hyaluronic acid production by fibroblasts in co-cultures. Using an enzyme-linked immunosorbent assay with a hyaluronic acid binding protein instead of an antibody for detecting hyaluronic acid in urine of bladder carcinoma patients established reliable results. At a cut off level of 100 ng/ml urine hyaluronic acid had 92% sensitivity for detecting bladder cancer and 93% specificity. Interestingly the sensitivity of hyaluronic acid for detecting grade 1 disease was higher than for higher-grade tumors (29-31).

Hyaluronidases are endoglycosidases that cleave hyaluronic acid. Also it is established that hyaluronidase secretion by bladder tumor cells correlates with invasiveness. At a cut off of 10 milliunits per mg hyaluronidase was 2.6 to 10 fold higher in patients with grade 2 or 3 disease than in normal control or patients with benign urological conditions and grade 1 tumors. However in contrast to hyaluronic acid, the sensitivity of hyaluronidase for detecting grade 1 tumors is under debate. Its widespread applicability may be somewhat limited by the fact that the accuracy of this test for detecting low-grade tumor is poor and may even be less than that of routine voided urine cytology. Another problem may be false-negative results in patients with hyaluronidase, which would cleave hyaluronic acid into smaller fragments that may not be detected by the ELISA for hyaluronic acid. More investigations are needed to make this marker in clinics available (1-3, 29-31).

**Lewis X antigen**

Lewis elated antigens are cell surface molecules divided into four subclasses, of which only the Lewis X group is associated with bladder cancer (2,12). This blood group antigen is present only on umbrella cells within TCC epithelium. It is determined with immunostaining test for bladder tissue. P12 antibody is used to determine Lewis X antigen with avidin-biotin immunohistochemical technique (32,33). Its established sensitivity rate is 80% and specificity is 86% (34). The testing of Lewis X antigen has shown high sensitivity for bladder cancer, particularly when two samples from each patient are tested. However, testing on more heterogeneous populations of patients is needed to determine the true specificity (2,33,34).

**Microsatellite analysis**

Highly polymorphic DNA repeats called as microsatellites which are found in human genome. Some mutations occur in gene specific areas and they can be used as marker of neoplasms. Chromosomes 4p, 8p, 9p, 11p, and 17p also often display loss of heterogeneity in bladder cancer. Molecular methods are needed to identify microsatellite changes in patient’s bladder tissue gene profiles. Therefore trained personnel and sufficient laboratory conditions are needed for this identification. Researchers identify approximately 20 voided urine microsatellite markers. Its specificity rate is 90% and sensitivity is 85% in average. If this test is automated and replicated by others it may have a role for bladder cancer screening follow-up (35,36).

**Mucin 7 gene expression**

Mucins (MUCs) are glucoproteins with a high molecular weight that are synthesized by glandular epithelial cells of gastrointestinal, respiratory and urogenital tracts. Nine human MUC genes have been identified in the international nomenclature (MUC 1, 2, 3, 4, 5ac, 5b, 6, 7, and 8). Their proposed functions include forming, protecting and lubricating the selective barrier between urothelial cells protecting against changes in urinary pH and osmolarity. There are many preclinical investigations to understand specificity and sensitivity by RT-PCR of mRNA mucins. It has a possibility of using urine to detect TCC cells, even in the early stages of disease when tumor is still regarded as a superficial lesion. More than 90% of all bladder cancer
cases are TCC, emphasizing the importance of the method as a noninvasive tool for diagnosis, screening and possible prognosis (37-39).

CONCLUSION

Based on analysis of the published literature, tumor markers for bladder cancer is not exact solution for diagnosis and monitoring of the illness. The low sensitivity of urine cytology has prompted the development of numerous tests for invasively detecting and monitoring bladder cancer. Although many markers have been targeted as a replacement for cytology, the goal of marker technology is to replace cystoscopy, at least in the content of identifying bladder tumors. Another potential use for bladder tumor markers is in the diagnosis of bladder cancer, either as an adjunct or a replacement for current standard tests. Some invasive tumor markers such as BTA stat, BTA TRAK, NMP22, telomerase have equal or higher sensitivity for bladder cancer than cytology, even in high-grade cancers. The physician can chose either one of the currently available bladder tumor markers such as BTA stat, BTA TRAK, ImmunoCyt, NMP22, or cytology as adjunctive tests to cystoscopy in the follow-up process. Another advantage of bladder tumor markers are to decrease the necessity of invasive testing to improve patient comfort and to decrease cost. Besides, cystoscopy is performed in the office with local anesthesia and small caliber flexible endoscopes, which is minimally invasive in men and even less invasive in women. One of the disadvantages of molecular markers is the need for trained laboratory personal and conditions. If strip tests can be evaluated based on molecular markers or automated systems can be found in every place, cheap and easy usage of clinical markers can be the best solution for bladder carcinoma patients. With regard to prognostic urine based markers are still studied. Instead of this, their professional usage history began 5 years ago. They need more research and heterogeneous population groups to test their sensitivity rates in different conditions.

ACKNOWLEDGEMENT

I thank to E. Damla BUYUKTUNCER for valuable advices and critics.

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