

ANGIOGENIC FACTORS AND BLADDER CANCER

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1. ABSTRACT

Angiogenesis is required for the growth as well as expansion of a solid tumor. It has been reported that tumor angiogenesis may be triggered after an increase in the level of angiogenic factors and a concomitant decrease in the level of angiogenic inhibitors. Of all potential pro-angiogenic mediators of bladder cancer, VEGF and bFGF appear to be most relevant in terms of physiology. To date, it appears that the angiogenic factors and inhibitors may play an important role in the diagnosis and prognosis of bladder cancer.

2. INTRODUCTION

Angiogenesis is required for the growth as well as expansion of a solid tumor (1-2). The initiation of this vascular phase is marked by a period of more rapid growth, local invasion and ultimately metastasis of epithelial neoplasms (3-4). It has been reported that tumor angiogenesis may be triggered after an increase in the level of angiogenic factors and a concomitant decrease in the level of angiogenic inhibitors (5). Several factors that enhance angiogenesis in various malignant diseases, such as bladder cancer, have been identified, including vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), platelet-derived endothelial cell growth factor (PD-ECGF), and angiogenin (6-8). Of all potential pro-angiogenic mediators of bladder cancer, VEGF and bFGF appear to be most relevant in terms of physiology. To date, it appears that the angiogenic factors and inhibitors may play an important role in the diagnosis and prognosis of bladder cancer.

3. VEGF IN BLADDER CANCER

Vascular endothelial growth factor is a crucial growth factor mediating tumor angiogenesis. Serum VEGF in patients with bladder cancer with clinical parameters has been reported (9-10). Bernardini et al has found that significant differences in serum VEGF are observed in healthy controls and patients with bladder cancer (9). The serum VEGF level is significantly associated with tumor stage, grade, vascular invasion and carcinoma in situ. Patients with metastasis have significantly higher levels than those with localized disease. The level of VEGF may be a valuable angiogenic marker for identifying metastatic bladder cancer. It may be used as a new predictor of disease.

The expression of VEGF in bladder cancer samples has a clinical significance as well (11-12). Chow et al has reported that there is a positive association of VEGF expression with histological grading by using immunohistochemistry in 185 cases of pTa/pT1 transitional cell bladder cancers (11). Inoue et al also demonstrated that VEGF expression and microvessel density (MVD) in the biopsy specimens are significant predictors of disease recurrence. By multivariate analysis, they found that only VEGF expression is an independent prognostic factor. Their results indicate that the expression levels of VEGF as indicated by in situ hybridization can identify patients with muscle-invasive transitional cell carcinoma (TCC) who are at high risk of developing metastasis after aggressive therapy with systemic Methotrexate, Vinblastine, Doxorubicine and Cisplatin (M-VAC) chemotherapy and radical cystectomy (12).

VEGF has been shown to be excreted in the urine of bladder cancer patient (13-14). Jeon et al investigated the urinary VEGF levels of patients with superficial bladder transitional cell carcinoma (TCC) to determine its predictive value for recurrence (13). They found that the urinary VEGF levels are significantly higher in recurrent vs. non-recurrent patients. However, there is no statistical correlation between VEGF levels and tumor stage (Ta or T1), tumor size or tumor grade. High pre-operative urinary VEGF levels are associated with a risk of recurrence in patients with superficial bladder TCC. Quantitation of urinary VEGF may prove to be a valuable, non-invasive indicator of carcinoma recurrence in patients with superficial bladder TCC. Urinary VEGF may be a therapeutic target for intravesical therapy. Jones et al also has demonstrated significantly elevated levels of urinary VEGF in patients with active bladder cancer. Their finding shows that the sensitivity and specificity of urinary VEGF for diagnosing primary or recurrent bladder cancer are superior to that of urine cytology, which remains the most widely used noninvasive diagnostic test. Their results strongly implicate VEGF in the pathogenesis of bladder cancer recurrence and progression. The potential exists for anti-VEGF strategies in the treatment of, or prophylaxis against, recurrent superficial bladder cancer (14).

Elevated levels of the angiogenic peptide basic fibroblast growth factor have also been significantly correlated with the status and extent of disease in bladder cancer (8, 15-18). We have measured the levels of bFGF in the urine of 39 bladder cancer patients. We found that patients with metastatic active disease have the highest levels of urine bFGF and patients with local active disease also have elevated levels of urine bFGF. Our results suggest that the association between the levels of urine bFGF and the extent and the status of disease in bladder cancer patients is statistically significant. In our study, urine bFGF determination appears to be more sensitive than urine cytology for diagnosing bladder cancer (18). O'Brien et al also has reported that median urinary bFGF is higher in patients with active bladder cancer than in those with a clear cystoscopy (8). Median urinary bFGF is also elevated in patients about to undergo transurethral resection of the prostate (TURP). Like VEGF, urine bFGF may be a potentially useful test for bladder cancer (8).

Hyaluronic acid (HA), a glycosaminoglycan, is known to promote tumor cell adhesion and migration, and its small fragments can stimulate angiogenesis. The levels of HA in the urine are significantly different between the normal individuals and patients with bladder cancer (19-20). It has been shown that the urinary HA levels of bladder cancer patients with G1 (255 :t 41.7 ng/mg), G2 (291.8 :t 68.3 ng/mg) and G3 (428.4 :t 67 ng/mg) tumors are 4-9-fold elevated as compared to those of normal individuals (44.7 :t 6.2 ng/mg) and patients with other genitourinary conditions (69.5 :t 6.8 ng/mg; $p < 0.001$) among the 144 specimens analyzed (19). Urinary HA measurement by the ELISA-like assay shows a sensitivity of 91.9% and specificity of 92.8% to detect bladder cancer. Urinary HA measurement may be a simple, noninvasive yet highly sensitive and specific method for bladder cancer detection. The profiles of urinary HA species of normal individuals and bladder cancer patients are different. Henri et al has demonstrated that the small angiogenic HA fragments are present in the urine of high-grade bladder cancer patients (20). Among the 139 specimens analyzed, the urinary hyaluronidase levels in patients with G2/G3 tumors (33.4 :I: 4.5 milliunits/mg protein) are 5-8-fold higher than those in normal individuals (4.2 :I: 1.2 milliunits/mg protein) and those in patients with G1 tumors (6.5 :I: 1.7 milliunits/mg protein) or other genitourinary conditions (7.4 :I: 1.4 milliunits/mg protein). Urinary hyaluronidase measurement shows a sensitivity of 100% and a specificity of 88.8% to detect high-grade bladder (G2/G3) tumors. Thus urinary hyaluronidase measurement may be a simple, noninvasive yet highly specific and sensitive method for high-grade bladder cancer detection (20).

Angiogenin (ANG) is also an important angiogenic factor (21-22). Miyake et al evaluated the expression of ANG in the tumor tissue and serum of patients with urothelial carcinoma (21). They found that the ANG mRNA transcripts are detected in all of the bladder carcinoma cell lines, urothelial carcinomas, and normal tissues. The mean level of ANG expression in invasive urothelial carcinomas is 4-fold higher than in superficial carcinomas and 5-fold higher than in normal tissues. The

mean serum ANG concentration in invasive urothelial carcinoma is significantly higher than in superficial urothelial carcinoma patients and healthy volunteers. The overall survival rate of patients with elevated serum levels of ANG is significantly lower than that of patients with normal levels. Moreover, among the 47 patients with advanced urothelial carcinoma who underwent complete resection, the disease free survival rate of patients with elevated serum levels of ANG is significantly lower than that of patients with normal levels. Their results indicate that ANG is strongly expressed in the tumor tissue and is present in high levels in the serum of patients with invasive urothelial carcinoma compared with superficial carcinoma patients, and that elevation of serum ANG level could be used as a novel predictor of the prognosis of patients with urothelial carcinoma.

Other angiogenic factors or inhibitors that may be of utility include aFGF (acidic fibroblast growth factor, 23), scatter factor (24), midkine (25), and thrombospondin (26). Future studies require large numbers of patients in order to determine whether continual and intermittent measurements of angiogenic growth factors add significant and clinically useful information to the management of patients with bladder cancer. In addition, we need to determine whether a complete profile with all known angiogenic growth factors is important, or whether a certain growth factor is more appropriate for bladder tumors. With the advent of multiple clinical trials involving angiogenic inhibitors as anti-tumor agents, we may find that determination of the level of angiogenic factor(s) is particularly appropriate in order to monitor therapeutic progress (27).

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