Thymidine Phosphorylase Activity in Human Bladder Cancer: Difference between Superficial and Invasive Cancer

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ABSTRACT

The activity of thymidine phosphorylase (TdR-Pase), which is identical to platelet-derived endothelial cell growth factor (a potent angiogenic factor), was analyzed in primary human bladder cancer tissues. TdR-Pase activity in tissues was determined from the conversion rate of 5'-deoxy-5-fluorouridine to 5-fluorouracil. The mean activity in 37 bladder cancers and in 8 samples of normal bladder epithelial tissue was 108.5 and 19.2 pg 5-fluorouracil/mg protein/h, respectively, showing a statistically significant difference. Among the cancer tissues, differences in activity were seen according to the stage and grade of tumors. Low-grade (grade 1) tumors had significantly lower activities than high-grade tumors, and high-grade invasive tumors showed significantly higher activities than low-grade superficial tumors. These results demonstrate that a high TdR-Pase activity in bladder cancer is associated with the tumor characteristics of invasion and malignant potential. Our conclusions support the hypothesis that angiogenesis that is mediated by this molecule may be involved in the development of invasive human bladder cancer, as suggested by T. O'Brien et al. (Cancer Res., 55: 510–513, 1995), and also suggest that TdR-Pase is a potential therapeutic target in human bladder cancer.

INTRODUCTION

Pyrimidine nucleoside phosphorylase, TdR-Pase2 in humans, is the enzyme involved in the salvage pathway of pyrimidine nucleotide synthesis. High levels of activity of this enzyme have been shown in several malignant tumors (2, 3), and the biological significance of this enzyme in tumors has gradually become clear in recent years.

Cytokines such as tumor necrosis factor, interleukin 1α, and IFN-γ have been shown to induce TdR-Pase activity in tumor cells (4). 5'-dFUr, a pro-drug of 5-FUra, is converted to 5-FUra in vivo by this enzyme (5–7). Interestingly, TdR-Pase has been shown to be identical to a potent angiogenic factor, PDECGF (8, 9).

Bladder cancer, a major urogenital cancer, consists mainly of transitional cell carcinoma histologically and is classified into two distinct types: (a) superficial (pTa or pT1), usually low-grade (grade 1) tumors; and (b) invasive (pT2 or greater), mostly high-grade (grade 3) tumors. Although there is a significant distinction between the clinical behaviors of these two types of bladder cancer, the molecular and biochemical bases of these two types of bladder cancer remain unclear.

Because angiogenesis is generally known to commence at a relatively early stage of carcinogenesis and might be involved in the invasion and metastasis of cancer, the study of angiogenesis in human bladder cancer may be important for understanding the progression steps of human bladder cancer. O'Brien et al. (10) showed that PDECGF mRNA expression levels in invasive bladder cancer were markedly elevated and suggested that this angiogenic factor may facilitate the progression of superficial tumors to invasive disease.

We investigated TdR-Pase activity, which is the direct function of PDECGF, in bladder cancer tissues. TdR-Pase activity in human bladder cancer was higher than that in normal bladder mucosa, and high-grade invasive tumors showed significantly higher TdR-Pase activity than low-grade superficial tumors.

MATERIALS AND METHODS

Tumors and Normal Tissues. Tissue samples of human bladder cancer were obtained from 37 patients by either transurethral cold-knife resection or total cystectomy. Eight normal bladder epithelial tissue samples were obtained from the same patients either at total cystectomy of bladder cancer or at the time of operations for other cancers. All of the tumors were histologically diagnosed as transitional cell carcinoma and staged and graded based on the 1987 tumor-node-metastasis criteria of the International Union Against Cancer. The eight normal tissue samples were histologically proven to be normal epithelium. To obtain normal tissue from the cystectomy samples, several macroscopically normal portions of the bladder were dissected and frozen in liquid nitrogen, and part of each was examined histologically. Only microscopically normal bladder epithelial tissue without atypia, dysplasia, or carcinoma in situ was used for the study. All of the specimens were rapidly

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2 The abbreviations used are: TdR-Pase, thymidine phosphorylase; PDECGF, platelet-derived endothelial cell growth factor; 5'-dFUr, 5'-deoxy-5-fluorouridine; 5-FUra, 5-fluorouracil.
frozen in liquid nitrogen and stored at \(-80^\circ\text{C}\) until the enzyme assay was performed.

**Tdr-Pase Assay.** Tissues were homogenized in 10 mM Tris-HCl buffer (pH 7.4) containing 15 mM NaCl, 1.5 mM MgCl\(_2\), and 50 mM potassium phosphate. This mixture was then centrifuged at 105,000 \(\times g\) for 90 min. The supernatant was dialyzed overnight against 20 mM potassium phosphate buffer (pH 7.4), and 1 mM \(\beta\)-mercaptoethanol and used as a source of crude Tdr-Pase. The protein concentration was determined by the method of Lowry et al. (11). All procedures were carried out at 4°C. The reaction mixture (120 \(\mu\)l) for the assay of enzyme activity contained 183 mM potassium phosphate (pH 7.4), 10 mM 5'-dFUrd, and crude enzyme from human tissues. The reaction was carried out at 37°C for 60 min and then terminated by the addition of 360 \(\mu\)l of methanol. After removal of the precipitate by centrifugation, an aliquot of the reaction mixture (100 \(\mu\)l) was added to 400 \(\mu\)l of 20 mM 5-FUra as the internal standard in 50 mM phosphate buffer (pH 6.8) and then applied to a high-performance liquid chromatography column (ERC-ODS-1171). The solvent system used was 50 mM sodium phosphate (pH 6.8) containing 5 mM 1-decane sulfonic acid:methanol (v/v, 85:15). The amount of 5-FUra produced was measured with a UV monitor (280 nm).

**Statistical Analysis.** Statistical analysis was performed using Wilcoxon’s test or the Kruskal-Wallis test to evaluate the significance of differences. \(P < 0.05\) was considered to be statistically significant.

**RESULTS**

Tdr-Pase activities determined by measuring the conversion of 5'-dFUr to 5-FUra in all of the cancer tissues and normal tissues are shown in Table 1. The mean activity in eight normal tissue samples was 19.20 \(\mu\)g FUra/mg protein/h. The mean activity in all 37 cancers was 108.5, showing a statistically significant difference (\(P = 0.0002\)). The activities in all of the superficial cancers (stages Ta and T1) and invasive cancers (stages T2–T4) were 90.9 and 141.1, respectively. Tdr-Pase activity in both superficial and invasive bladder cancers was obviously higher than that in normal tissues (Fig. 1). In five cases in which both tumor samples and adjacent normal tissue were taken, a difference in activity between tumor and normal tissue was seen as shown in Table 1.

In the cancers, several factors associated with tumor growth were analyzed for a difference in Tdr-Pase activity. A statistically significant difference in activity was shown for factors associated with superficial (stages Ta and T1) versus invasive (stages T2–T4) tumors (\(P = 0.04\)), grade 1 versus grade 2 and 3 tumors (\(P = 0.0008\)), and also for macroscopically papillary tumors versus nonpapillary tumors (\(P = 0.046\)). Fig. 2 shows Tdr-Pase activities in tumors with different grades. Grade 1 tumors had significantly lower Tdr-Pase activity than grade 3 tumors, and a statistically significant difference was also seen between grade 1 tumors and higher-grade tumors (\(P = 0.0008\)).

The association between Tdr-Pase activity and the stage and grade of tumors is shown in Fig. 3. Tdr-Pase activity seems to increase according to stage (T category), especially among grade 3 tumors.

**DISCUSSION**

Several studies have shown that Tdr-Pase activity in cancers was higher than that in normal tissue (2, 3). The biological significance of a high level of Tdr-Pase activity, however, is not fully understood. Our study demonstrated that high TdR-
Pase activity in bladder cancer is associated with the tumor characteristics of invasion and high grade.

TdR-Pase is identical to PDECGF, a potent angiogenic factor (8, 9, 12), and PDECGF mRNA expression was shown to be involved in invasive bladder cancer by O'Brien et al. (10). Our study confirms their suggestion that angiogenesis mediated by PDECGF may be involved in the development of invasion of human bladder cancer. O'Brien et al. demonstrated differential angiogenesis factor expression in human bladder cancer samples (10). They observed that PDECGF expression was higher in invasive tumors than in superficial tumors, but the reverse was true for the expression of another angiogenic factor, vascular endothelial growth factor. They suggested that the angiogenic pathway associated with PDECGF expression and the molecular genetic changes of chromosome 17p might be deeply involved in the development of invasive cancer.

Our results also clearly showed that TdR-Pase activity was
up-regulated in bladder cancer and that the activity in invasive bladder cancers was higher than that in superficial tumors, as shown in Fig. 1.

In invasive bladder cancer (stages T2–T4), tumors penetrate the detrusor muscle and surrounding tissues, usually show solid bladder cancers was higher than that in superficial tumors, as up-regulated in bladder cancer and that the activity in invasive poorly differentiated, with high-grade (grade 3) histological features. Among the invasive grade 3 tumors, the mean activity of TdR-Pase seems to increase with the depth of invasion, as shown in Fig. 3. Thus, TdR-Pase (PDECGF) might be involved in facilitating the progression of high-grade bladder cancer to invasive disease. The question of whether or not TdR-Pase activity would predict a bad prognosis as an independent prognostic factor might be an important clinical point. We are now carefully following the patients in this study.

TdR-Pase hydrolysis of thymidine gives rise to 2-deoxyribose 1-phosphate, which is readily dephosphorylated to 2-deoxyribose, which is reported to be angiogenic (12). Our study, in combination with the results of O’Brien et al. (10), gives strong support to the hypothesis that PDECGF/TdR-Pase-mediated angiogenesis is a very important factor in the progression (invasion) of these cancers.

Cytokines are also suggested to induce TdR-Pase activity (4). Interestingly, urothelial cells have also been shown to produce several cytokines (13). These cytokines are suggested to induce PDECGF/TdR-Pase-initiated angiogenesis and tumor invasion of human bladder cancer.

Our data again suggest therapeutic targets in bladder cancer. Invasive bladder cancer patients carry a poor prognosis, although several radical treatments are available. The obvious up-regulation of PDECGF/TdR-Pase is important as a therapeutic target in these tumors. 5′-dFURd is catalyzed to 5-FUr a in vivo by this TdR-Pase (5, 6). Thus, it would be interesting to study 5′-dFURd in relation to targeted chemotherapy for the treatment of invasive bladder cancer as well as for the prophylaxis of progression from superficial to invasive bladder cancer.

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