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Urologic Oncology: Seminars and Original Investigations 23 (2005) 413–418

UROLOGIC ONCOLOGY

Original article

Micro-array analysis of the effect of post-transurethral bladder tumor resection urine on transforming growth factor-β1 dependent gene expression in transitional cell carcinoma☆

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Received 28 June 2004; received in revised form 17 May 2005; accepted 18 May 2005

Abstract

Introduction and Objectives: Prior studies have shown that bladder trauma occurring during transurethral bladder tumor resection increases urinary levels of the cytokine transforming growth factor (TGF)-β1. This study used complementary deoxyribonucleic acid micro-array technology to identify additional genes in human transitional cell carcinoma (TCC), whose expression is altered as a consequence of increased urinary levels of TGF-β1.

Methods: The human TCC line 253J was cultured in standard media, or media spiked with either 10% post-transurethral bladder tumor resection urine (PTU), or PTU and anti-TGF-β1 neutralizing antibody. Messenger ribonucleic acid from these conditions, together with messenger ribonucleic acid from stably transfected 253J cells over-expressing TGF-β1, was hybridized with ATLAS® micro-array membranes (Clontech, Palo Alto, CA) containing 588 human genes. Hybridization signal intensity was quantified using phospho-imaging. An analytic strategy based on the variance in the signal intensity ratio of specific housekeeping genes in control and experimental comparisons was used to identify significant changes in gene expression. Reverse transcriptase polymerase chain reaction of target genes was used to confirm gene over-expression and TGF-β1 responsiveness.

Results: Seven genes were identified on micro-array: v-RAF-1, colony stimulating factor-1 receptor, v-FGR, insulin growth factor-1 receptor, epidermal growth factor receptor, α5 integrin, and interferon receptor-1. Reverse transcriptase polymerase chain reaction confirmed over-expression in the autocrine TGF-β1 producing cell line and increased expression in response to exogenous TGF-β1.

Conclusions: TGF-β1 in PTU alters the expression of multiple genes in human TCC in vitro. The impact of these changes on the biologic phenotype of the malignant cell and the efficacy of adjuvant therapies requires further evaluation. © 2005 Elsevier Inc. All rights reserved.

Keywords: Bladder cancer; Transforming growth factor-β1; Gene expression

1. Introduction

Transurethral resection of bladder tumors (TURBT) is the cornerstone of treatment of nonmuscle invasive urothelial carcinoma. This procedure serves multiple clinical needs because it provides histologic definition of tumor type, information regarding the stage and grade of disease, and, most importantly, it is therapeutic. In skilled hands, it can be performed safely, with minimal morbidity, and, in many cases, on an outpatient basis.

Although TURBT is an effective and proved method for diagnosing and controlling nonmuscle invasive bladder cancer, it is not without its shortcomings. Principal among these is the fact that the tumor is removed in a piecemeal fashion. Tumor fragmentation during the process of resection, coupled with the fact that fragmentation occurs in the presence of a fluid media, has the potential to leave residual tumor at the resection site and disburse tumor cells to remote sites within the urinary bladder. Multiple studies have shown the ability of urothelial tumor cells, generated during the process of TURBT, to adhere to sites of urothelial injury [1–4]. These injury sites may be direct as in the case of the resection bed, or indirect occurring as a consequence of

☆ This work was supported by a grant from the Department of Veterans Affairs and the Milwaukee Veterans Affairs Medical Center.

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1078-1439/05/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.urolonc.2005.05.029

mechanical or pressure trauma to the bladder during transurethral resection [4]. Adherent neoplastic cells in turn can serve as foci for recurrence. Data accumulated over the past decade suggest that a substantial percentage of bladder tumor recurrences are clonal in etiology [5–7]. Implantation at transurethral resection is a recognized contributor to this recurrence rate.

Surgical wounding occurring as a consequence of TURBT has the potential to affect tumor biology in ways beyond that of simply predisposing to tumor cell adherence. Tissue trauma initiates a complex cascade of events that culminate in the beneficial outcome of wound healing. Clotting cascade activation followed by cytokine release, cell migration, and local cellular proliferation represent facets of the wound healing process. As much as these events have the potential to affect the normal population of cells that facilitate wound healing, there is also the potential for these events to affect the biology of residual tumor cells within the urinary bladder. In the case of cells remaining at the actual injury site, this effect may be direct. However, the nature of the bladder is such that soluble constituents of the wound healing process have the potential to diffuse into the urine and, consequently, affect neoplastic cells at sites remote from the actual wound. Given the multifocality of nonmuscle invasive bladder cancer and the occurrence of tumor disbursement and/or seeding at TURBT, the potential for surgical injury to affect cells at both injury site and remotely within the bladder has important implications.

Prior work by our group has shown that TURBT increases the levels of cytokines present in the urine [8,9]. Moreover, we have shown that PTU, in a manner that is contingent on the presence of transforming growth factor (TGF)-β1, alters fibronectin expression in urothelial cell lines [10]. The purpose of this study was to take a broader look at gene expression by urothelial carcinoma cells in response to TURBT. Specifically, this study used an analytic strategy intended to identify genes whose expression was altered in a human urothelial carcinoma cell line in response to trauma (i.e., TURBT) induced increases in urinary levels of TGF-β1. Using a complementary deoxyribonucleic acid (cDNA) array analysis of the 253J cell line, we identified 7 genes whose expression was altered by post-TURBT urine (PTU) in a TGF-β1 dependent manner.

2. Materials and methods

2.1. Cell culture

The human transitional carcinoma cell line 253J was obtained from American Type Cell Culture (Rockville, MD). Cells were maintained at 37°C, 5% carbon dioxide in RPMI 1640 (Invitrogen Life Technologies, Carlsbad, CA), supplemented with 10% fetal bovine serum, penicillin, and streptomycin (complete media). The 253J TGF-β1-8 cell line, which constitutively expresses TGF-β1, was used to assess the effect of autocrine TGF-β1 production on gene expression. This cell line has been previously described [10]. Briefly, pcDNA3/h TGF-β1 and pcDNA3/CAT vectors were transfected into 253J cells using the LipofectAMINE reagent (Invitrogen Life Technologies) according to the manufacturer's instructions. The G418 resistant clones were expanded, and TGF-β1 expression determined by Northern analysis and TGF-β1 bioassay. The cell line was recloned before these experiments. A Northern blot for TGF-β1 messenger ribonucleic acid (mRNA), comparing the parental 253J line to the recloned 253J TGF-β1-8 line, is shown in Fig. 1.

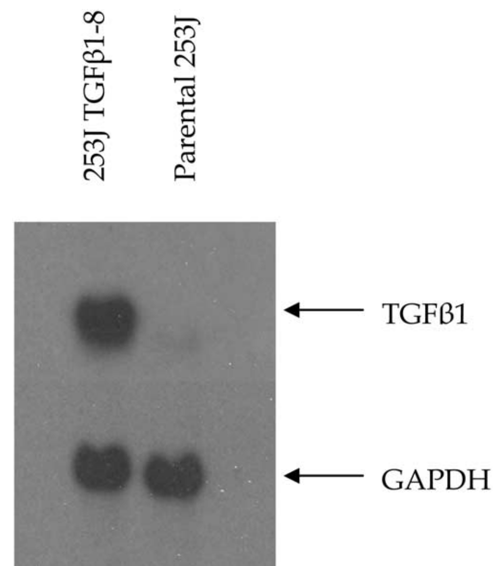

Fig. 1. Northern blot analysis of TGF-β1 expression in parental 253J cells and the constitutive TGF-β1 over-expressing cell line 253J TGF-β1-8. glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is shown as a lane loading control.

2.2. Northern analysis

Northern analysis for TGF-β1 expression was performed as previously described [10]. Briefly, 20 μg total RNA from both parental 253J and 253J TGF-β1-8 lines was subjected to 1% denaturing gel electrophoresis. The RNA was transferred to a nylon membrane and immobilized by ultraviolet cross-linking. Pre-hybridization was performed at 42°C in a solution of 5× saline sodium citrate (SSC), 50% formamide, 1× Denhardt, 100 μg/ml denatured ss DNA, and 1% sodium dodecyl sulfate (SDS) for 4 hours. The membrane was then hybridized overnight in an identical solution containing a P32-labeled human TGF-β1 cDNA probe. After hybridization, the membrane was washed in 1× SSC and 0.1× SDS at room temperature for 2 × 20 minutes, in 0.1× SSC and 0.1× SDS at 50°C for 2 × 15 minutes. Finally, the membrane was exposed to Kodak XAR-5 film (Sigma-Aldrich, St. Louis, MO) at −75°C with an intensifying screen. The membrane was stripped, and reprobed with a

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