ISOLATION AND CHARACTERIZATION OF A NOVEL HUMAN BLADDER CANCER CELL LINE: BK10

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SUMMARY

Molecular studies of bladder carcinomas have aided in determining causative genetic events and the prognosis of cancers endowed with certain abnormalities. In vitro bladder cancer characterization of key cytogenetic alterations is useful for study of molecular changes that may promote oncogenic events. In our laboratory, a novel human bladder cancer cell line, BK10, has been established in vitro and passaged for more than 20 mo. This new bladder cancer cell line (BK10) was derived from bladder tissue containing grade III-IV/IV transitional cell carcinoma. Bladder cancer tissue was obtained at the time of radical cystoprostatectomy extirpation. Cell cultures derived from this surgical sample exhibited an epithelial morphology and expressed epithelial cytokeratins. Immunostains of BK10 were negative for prostate specific antigen (PSA), fibronectin, smooth muscle actin alpha, and desmin. Karyotypic analysis revealed an aneuploid chromosomal content <4n> with many numerical and structural abnormalities previously linked to bladder oncogenesis. Translocations occurred in chromosomes 1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 13, 14, 15, 16, 17, 19, 20, 21, 22, X and Y. G-banding analysis revealed rearrangements involving chromosomes 9q and 17p, and the location of the abl1 oncogene and the p53 gene, respectively. The availability of this bladder cancer cell line will provide a useful tool for the further study of bladder carcinoma oncogenesis and gene therapy.

Key words: bladder; cancer cells; characterization; cytogenetics.

INTRODUCTION

Bladder cancer is the fifth most common form of cancer in the United States with more than 50 000 newly diagnosed cases per year (41). Transitional cell carcinoma (TCC) is the most common malignancy arising in the uroepithelium (41). The treatment of TCC is problematic when similar histologic appearances of tumors yield variable biologic behaviors (29,41). Molecular studies of bladder cancers endowed with certain abnormalities (41). To investigate this link, in vitro models have been employed to evaluate the differentiation, transformation, invasiveness, and oncogenesis of bladder cancer and to assess potential therapeutic modalities (10,29). Several TCC cell lines have been studied previously (2,10,19,23,30-32). Nevertheless, newly established, well-characterized TCC lines are needed to assist study of molecular changes that may be functionally important for bladder cancer invasion and metastasis.

As part of ongoing gene therapy initiatives in urogenital tissues, we routinely initiate primary bladder cultures for studies of liposomal-mediated transfection via cytokine-producing plasmids. Such gene-modified tumor vaccines (GMTV) are effective in a mouse MBT-2 bladder cancer model (4,40). Human prostate and bladder cancer cells may be so modified in vitro as well (20,47). Culture techniques for primary growth of prostate cells have been previously described by our laboratory (39). After initiation of a primary bladder culture in 1995, cells which possessed growth characteristics typical of long-term neoplastic cell lines were isolated. This cell culture exhibited persistent and rapid proliferation, lack of contact inhibition resulting in focus formation, and lack of senescence. In this report, we describe the isolation and characterization of a new TCC cell line, designated BK10.

MATERIALS AND METHODS

Case history. Tissue was harvested from a bladder specimen removed at the time of cystoprostatectomy for the treatment of muscle-invasive transitional cell carcinoma of the bladder in a 67-yr-old male patient. Pre-operative imaging studies revealed no evidence for bony metastasis or lymph node metastasis. Initial symptoms were those of urinary obstruction. Tumor was detected on routine cystoscopy with biopsy. Pathology returned positive for grade IV out of IV transitional cell carcinoma. The patient underwent pre-operative staging studies revealing no evidence for adenopathy. However, the left ureter was obstructed signifying muscle invasion and local invasion. At the time of surgery, pathology revealed metastatic transitional cell carcinoma to a left iliac node. In addition, a lung lesion was noted pre-operatively and...
Tissue was paraffin embedded and submitted to the Pathology Department at routine histologic evaluation by standard hematoxylin and eosin (H&E) staining and the patient was administered postoperative chemotherapy. It was felt to be likely a secondary malignancy. Radiotherapy was applied appropriately and the patient was administered postoperative chemotherapy.

Glutamine, and 2 pM insulin (Sigma Chemical Co., St. Louis, MO) and transported to the research laboratory. Bladder tissue was immediately placed in a 100-mm tissue culture dish with 5 ml TTM, and minced with opposing tissue euhure dishes containing 5 ml of PEC media each (39). Dishes were passaged with 0.25% trypsin-7 nM ethylene-diaminetetraacetic acid (EDTA) (FBS) (Table 1) were passaged similarly but without the addition of trypsin inhibitors, lOB broth with inhibitors, and A8 selective agar (Remel Co., Lenexa, KS). Standard methods for isolation for Mycoplasma pneumonia, M. hominis, and Ureaplasma urealyticum were used (1).

Doubling time determination. Cells were plated at 2 × 10^4 in a 100-mm dish and were allowed to incubate for 96 h in PEC medium or medium F. Doubling time calculations were performed by dividing 96 h by the log base 2 of the cell count at time 96 h minus the log base 2 of the cell count at time 0 (16).

Immunohistochemical characterization. BK10 cells were grown to log phase, growth medium was removed, and cells were rinsed with phosphate buffered saline (PBS) (GIBCO). Cells were harvested by scraping in PBS. BK10 cells were cytospan on clean glass slides, 5 × 10^6 cells/slide, air-dried overnight and stored at −20°C until use. Slides were immunostained by the protocol of Hsu (13) and Kerna (17) with few modifications. Briefly, the slides were fixed in acetone for 10 min, air-dried for 20 min, and incubated 15 min with 5% normal horse serum (GIBCO) to block nonspecific Fc receptor sites. The appropriate primary antibody was applied to the slides and slides were incubated overnight at 4°C in a humidified chamber. The monoclonal antibody, AEI/AE2 (Boehringer Mannheim, Indianapolis, IN), diluted 1:500 in antibody-diluting buffer (ADB) (Bio-Meda Corporation, Foster City, CA) was used to stain for acidic and basic molecular weight epithelial cytokeratins. The monoclonal antibody, PSA (prediluted from Biogenex, San Ramon, CA), was used to stain for prostate-specific antigen (PSA). Monoclonal antibodies to cytokeratin 7, vimentin, fibronectin, desmin, and smooth muscle actin alpha were also obtained from Biogenex in a prediluted form. Monoclonal antibody to Uro-9 was obtained from Signet (Bedford, MA) and was diluted 1:500 in ADB. Monoclonal antibody to bcl-2 (Oncogene Science, Uniondale, NY) was diluted 1:100 in ADB. Monoclonal antibodies to p53 (clones 1801, 1620, and 240, Oncogene Science) (diluted 1:200 in ADB), and Rh (Oncogene Science) (diluted 1:100 in ADB) were used to stain for tumor suppressor gene products. IgG1 (Coulter Immunology, Hialeah, FL), used as the negative control, was diluted 1:200 in ADB. Following the primary antibody incubation, slides were washed three times in PBS, pH 7.4, for 8 min each wash (standard wash protocol). Biotinylated secondary antibody horse anti-mouse IgG (Vector Laboratories, Burlingame, CA) was diluted 1:100 in ADB and applied for 30 min, followed by washing in PBS. Slides were subsequently incubated with premixed Elite reagent avidin-biotin complex (Vector) for 30 min. Slides were again washed in PBS and developed in 0.05 M Tris pH 7.6, 2 ml 0.6% H2O2 for 8 min. Finally, the slides were washed in tap water, counterstained in 1.5% methyl green, dehydrated in increasing ethanol, cleared in xylene, and coverslipped in permanent mounting media (Proteox, Baxter, Charlotte, NC). Slides were observed by light microscopy and compared to the negative control (IgG1).

Doubling layer soft agar assay. Cells were harvested by the modified trypsinization protocol and diluted in 0.3% agar in PEC media plus 5% filter-sterilized FBS. Five dilutions were prepared: 1 × 10^2, 3, 4, 5, 6. Cellular suspensions were then layered over a pregelled 60-mm dish containing 0.5% agar. Cells were harvested from T150 flasks by mitotic shake-off with a mitotic inhibitor, colcemid (GIBCO, 0.07 µg/ml), 1 h before harvest (28). Cell pellets were resuspended in 0.075 M KCl for 20 min at room temperature and then fixed with methanol-acetic acid (3:1). Air-dried chromosome spreads were banded by the Giemsa-trypsin method (44). The two karyotypes present are composites of several comparably extended metaphases to ensure that representative examples of each chromosome were present.

RESULTS

Pathological studies of surgically resected bladder tumor. Histopathology of the resected bladder tumor revealed poorly differenti-