UROLOGIC ONCOLOGY

Urologic Oncology: Seminars and Original Investigations 25 (2007) 120–127

Original article

Gene expression profile in squamous cell carcinoma of the urinary bladder using complementary deoxyribonucleic acid microarray

Ashraf A. Ewis, M.D., Ph.D.[a,b,*], Essam El-Samman, Ph.D.[c,d], Nermin Ali, M.D., Ph.D.[e], Kazuaki Kajimoto, Ph.D.[f], Yasuo Shinohara, Ph.D.[f], Mitsuru Ishikawa, Ph.D.[a], Hiro-Omi Kanayama, M.D., Ph.D.[c], Yoshinobu Baba, Ph.D.[a,g]

[a] *Health Technology Research Center, National Institute of Advanced Industrial Science and Technology (AIST-Shikoku), Takamatsu, Japan*
[b] *Department of Public Health and Occupational Medicine, Faculty of Medicine, El-Minia University, El-Minia, Egypt*
[c] *Department of Urology, The University of Tokushima School of Medicine, Tokushima, Japan*
[d] *Department of Urology, Egyptian National Institute of Oncology, Sohag, Egypt*
[e] *Department of Pharmacology, The University of Tokushima School of Medicine, Tokushima, Japan*
[f] *Division of Gene Expression, Institute for Genome Research, The University of Tokushima School of Medicine, Tokushima, Japan*
[g] *Department of Applied Chemistry, Graduate School of Engineering, Nagoya University, Nagoya, Japan*

Received 21 November 2005; received in revised form 20 March 2006; accepted 21 March 2006

Abstract

To date, molecular evidence studies for bladder cancer, using the microarray technology, are focusing on the transitional cell carcinoma, however, similar fingerprinting studies have rarely been performed on the other molecular phenotypes of bladder cancer, squamous cell carcinoma (SCC). This study was conducted to monitor the gene expression profiles for bilharzial-related SCC of the bladder to be able to compare its data with transitional cell carcinoma microarray data. A total of 17 paired bilharzial urinary bladder SCC specimens and their corresponding normal urothelium were analyzed using the complementary deoxyribonucleic acid microarray hybridization approach to study the molecular basis of the development of SCC of the urinary bladder. Validation of the microarray results was performed using the Northern blotting technique. After supervised analysis of the microarray data, there was at least a 3-fold difference in the expression between SCC of the bladder and normal urothelium in 82 genes. A total of 38 genes were up-regulated in SCC of the bladder, including matrix degradation-related genes, growth factors, different oncogenes, and immunology related genes. Conversely, 44 genes were down-regulated in SCC of the bladder, including integrins, laminins, cadherins, nonmetastatic cell 1 (NM23) and apoptosis-related genes. Our findings can explain the aggressive behavior of SCC of the bladder. Such gene profiling studies will add to our understanding of the mechanisms of carcinogenesis, and may also improve our ability to diagnose and treat bladder cancer.

Keywords: Bladder cancer; Bilharziasis; Squamous cell carcinoma; Complementary deoxyribonucleic acid microarray; Gene expression

1. Introduction

The urothelial lining, extending from the renal pelvis to the urethra, is susceptible to malignancy, which can be 1 of the major pathologic types, either transitional cell carcinoma (TCC) in Western countries and Japan, or squamous cell carcinoma (SCC), which is prevalent in tropical areas, Egypt, Middle East, and East African countries [1,2]. In Egypt, SCC of the urinary bladder accounts for as many as 75% of bladder cancers, with about 80% of SCC associated with chronic infections with Schistosoma hematobium (bilharziasis), and is said to behave much differently than other urothelial carcinoma (e.g., TCC). Generally, SCC of the urinary bladder is a rapidly growing, aggressive, and infiltrative tumor, and most patients present with advanced muscle invasive disease [3]. Bladder SCC caused by Schistosoma infection is usually exophytic, nodular, fungating and well-differentiated lesions [4]. SCC of the bladder can develop as a result of non-bilharzial causes such as chronic irritation of the bladder from urinary calculi, catheters, infections, or diverticula [5].

In the pre-genomic era, these molecular events have been

* Corresponding author. Tel.: +81-87-869-4104; fax: +81-87-869-4113.

E-mail address: ashraf-ewis@aist.go.jp (A.A. Ewis).

1078-1439/07/$ – see front matter
doi:10.1016/j.urolonc.2006.03.006

examined with traditional methods at a single gene level. However, molecular diagnostics based on assays for a single protein may be inadequate to account for the inherent complexity of cancer, and for the variability of both healthy and affected populations. Now, in the post-genomic era, such events can be monitored using complementary deoxyribonucleic acid (cDNA) or oligonucleotide microarrays, which are powerful tools for analyzing relationships among tumors, disease classifications, and assigning new classes of diseases. Microarrays help to study the up or down-regulated genes, and correlate them with disease stage, response to treatments, and clinical outcomes.

To date, almost all diagnostic and prognostic researches who study molecular evidence for bladder cancer are focusing on the TCC molecular fingerprinting, and many are using the microarray technology for that purpose [6–9]. However, similar fingerprinting studies have rarely been performed on the other molecular phenotypes of bladder cancer (i.e., SCC) [10,11]. In this study, we have applied the cDNA microarray hybridization approach to study the molecular basis of the development of SCC of the urinary bladder. Such microarray gene profiling data of SCC of the bladder are important to be compared with the existing gene profiles of TCC, and help in the proper understanding of the underlying mechanisms guiding the development of the 2 major histotypes of bladder cancer, with the likelihood of identifying relevant molecular markers for the bladder SCC that may assist in diagnosis, outcome prediction, and therapeutic interventions.

2. Materials and methods

2.1. Tissue samples

A total of 26 pairs of urinary bladder tumor biopsy samples and their corresponding normal urothelial tissue were obtained from the Egyptian National Institute of Oncology at El-Minia, Egypt. We selected only 17 paired specimens to be included in this study after getting the results of the routine pathology examination confirming that they are SCC related to Schistosoma infection (i.e., bilharzial bladder cancer), and they are all either stage T3a or T3b. All tissue samples were immediately submerged in Ribonucleic Acid Later (Takara Bio Inc., Shiga, Japan) and incubated at 4°C overnight before storage at −80°C until the ribonucleic acid (RNA) extraction process started. Each tumor sample was staged according to previously published criteria [12] and reevaluated by a pathologist in the department of second pathology, Tokushima University School of Medicine, Tokushima, Japan. Informed consent was obtained from all patients, and the corresponding medical ethical committee approved all protocols.

2.2. RNA Preparation

After collecting the tumor specimens and the pathologist confirmed their stage, grade, and histotype, the areas of the frozen tissues corresponding to the areas of slides that contain more than 80% of the malignant cells were macro-dissected and collected into the tubes for RNA extraction. We extracted total RNA from frozen tissue stored in ribonucleic Later using the RNeasy Mini kit (QIAGEN, Inc., Hilden, Germany) according to the manufacturer's instructions. Total RNA was quantified using an ultraviolet spectrophotometer (Ultrospec 3000 UV/Visible spectrophotometer; Pharmacia Biotech, Cambridge, UK). Electrophoresis on agarose gel stained with ethidium bromide and examined under ultraviolet light confirmed RNA quality and integrity.

We started our investigation by performing the array analysis on 2 samples (i.e., 2 individual SCC samples along with normal urothelium control ones) that were analyzed on a microarray chip because each chip of the commercially purchased microarray chips that we used contains 2 arrays. We then proceeded to the pooling process to reduce study expenses during the preliminary stages. The pooling process was performed at the messenger RNA level, with similar amounts of each RNA sample. We made 2 pools for the selected SCC tumor samples derived from bilharzial bladder cancer. All the 17 selected samples having stage T3a or T3b SCC were pooled into 2 groups, and 2 pools for their corresponding normal urothelial samples were prepared for comparison purposes. We repeated the microarray experiments using the 2 groups of pooled RNA to have a wide range of data for all the possible genetic alterations that accompany the occurrence of urinary bladder SCC. For further validation of the microarray results, we repeated the microarray experiment twice. From the comparison and alignment analysis of the microarray experiments of the duplicate pools analysis and that of the 2 individual samples, we found that the expression profiles of the pools were fairly representing the cohort.

2.3. Hybridization and microarray scanning

The reverse transcription labeling and hybridization were performed as described in the protocol recommended by Agilent Technologies Incorporated (Palo Alto, CA) intended for Agilent microarray analysis. Briefly, a 10-μg aliquot of total RNA samples of each group was reverse transcribed into a cDNA probe with an oligo (dT) primer and labeled nucleotides. The reaction was performed in a solution containing 50 μM dATP/dGTP/dTTP, 25 μM dCTP, 25 μM cyanine 3 (Cy3)-dCTP (for SCC samples) or cyanine 5 (Cy5)-dCTP (for normal urothelium samples) (Enzo Diagnostics, Inc., New York, NY), and 400 units of MMLV (Moloney Murine Leukemia Virus enzyme) reverse transcriptase at 42°C for 1 hour. Incubating the reaction mixture at 70°C for 10 minutes terminated the labeling reaction. Adding 0.05 μg RNaseIA, followed by incubation