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Original article

Micro-RNA profiling in kidney and bladder cancers☆

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Abstract

Objectives: Micro-RNAs are a group of small noncoding RNAs with modulator activity of gene expression. Recently, micro-RNA genes were found abnormally expressed in several types of cancers. To study the role of the micro-RNAs in human kidney and bladder cancer, we analyzed the expression profile of 245 micro-RNAs in kidney and bladder primary tumors.

Methods and materials: A total of 27 kidney specimens (20 carcinomas, 4 benign renal tumors, and 3 normal parenchyma) and 27 bladder specimens (25 urothelial carcinomas and 2 normal mucosa) were included in the study. Total RNA was used for hybridization on an oligonucleotide microchip for micro-RNA profiling developed in our laboratories. This microchip contains 368 probes in triplicate, corresponding to 245 human and mouse micro-RNA genes.

Results: A set of 4 human micro-RNAs (*miR-28, miR-185, miR-27*, and *let-7f-2*) were found significantly up-regulated in renal cell carcinoma ($P < 0.05$) compared to normal kidney. Human micro-RNAs *miR-223*, *miR-26b*, *miR-221*, *miR-103-1*, *miR-185*, *miR-23b*, *miR-203*, *miR-17-5p*, *miR-23a*, and *miR-205* were significantly up-regulated in bladder cancers ($P < 0.05$) compared to normal bladder mucosa. Of the kidney cancers studied, there was no differential micro-RNA expression across various stages, whereas with increasing tumor-nodes-metastasis staging in bladder cancer, *miR-26b* showed a moderate decreasing trend ($P = 0.082$).

Conclusions: Our results show that different micro-RNAs are deregulated in kidney and bladder cancer, suggesting the involvement of these genes in the development and progression of these malignancies. Further studies are needed to clarify the role of micro-RNAs in neoplastic transformation and to test the potential clinical usefulness of micro-RNAs microarrays as diagnostic and prognostic tool.

Keywords: Micro-RNA; Microarray; Tumor marker; Bladder cancer; Renal cancer

1. Introduction

Kidney cancer accounts for 3% of adult malignancies [1]. It represents a nonhomogeneous entity, although between 80% and 95% of the malignancies of the adult kidney can be classified as clear-cell or papillary renal cell carcinomas (RCCs). The histopathology of these tumors has been correlated with distinctively different genetic changes, indicating that unrelated molecular mechanisms underlie the development of each type of tumor [2].

Urothelial carcinoma, previously designated as transitional cell carcinoma (TCC), is the second most common

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malignancy of the genitourinary tract [1]. No specific and exclusive cytogenetic aberration has been identified for urothelial carcinoma, but various nonrandom deletions, gain of chromosomes, polyploidization, and formation of isochromosomes have been observed [3].

Micro-RNAs are an abundant class of small noncoding RNAs of about 22 nucleotides in length, which function as negative regulators (cleavage or translational repression) of gene expression by antisense complimentarily to specific messenger RNAs [4,5]. Increasing evidence shows that expression of micro-RNA genes are deregulated in human cancer [5,6]. Micro-RNA genes show high-frequency genomic alterations in tumors [7], and a high proportion of known micro-RNAs are located at fragile sites or in cancer-associated genomic regions, including minimal regions of loss of heterozygosity, minimal amplicons, or breakpoint cluster regions [6].

Specific overexpression or underexpression has been correlated with particular tumor types [8–11]. Micro-RNA overexpression could also result in down-regulation of tumor suppressor genes, whereas their underexpression could lead to oncogene up-regulation [5,6]. This permits micro-RNAs to act both as tumor suppressors and oncogenes [12,13]. Most importantly, micro-RNA expression signatures have been described to predict the outcome in several tumors, including lung cancer and chronic lymphocytic leukemia [14–16], and it may also predict response to chemotherapy [17]. Micro-RNAs might prove to be new therapeutic targets for a wide range of diseases, including cancer [13].

In this study, we analyzed the micro-RNA expression profiles in human bladder and kidney cancer, and detected that different micro-RNAs are involved in these 2 common malignancies that appear so different in their molecular biology and clinical behavior. We further evaluated micro-RNA expression pattern across different tumor stages to determine if different micro-RNAs could be implicated in tumor progression. Our results confirm that micro-RNA expression profiles could be used in the future together with other biomolecular markers to classify, diagnose, and predict the behavior of human bladder and renal cancers.

2. Materials and methods

2.1. Patients

Normal and pathologically diagnosed biopsy specimens were obtained from patients with kidney masses who underwent partial or radical nephrectomy and patients with bladder tumors who underwent radical cystectomy or endoscopic resection for primary or recurrent TCC. Tissue samples from bladder and kidney specimens were collected between 1998 and 2000 at Department of Urology at Thomas Jefferson University, with informed consent and approval by the internal review board.

2.2. Pathologic studies

A total of 27 kidney (RCC = 20, oncocytomas 2, angiomyolipomas 2, and normal renal parenchyma = 3) and 27 bladder specimens (urothelial carcinoma = 25 and normal mucosa = 2) were included in this study. Tumors were classified histologically according to the World Health Organization classification of tumors of the Urinary System and Male Genital Organs (2004) and staged according to the tumor-nodes-metastasis (TNM) classification [18].

2.3. Micro-RNA microarray hybridization

Samples were snap frozen immediately after resection. Total RNA isolation was performed with the Trizol protocol according to the manufacturer's instructions (GIBCO, Rockville, MD). RNA labeling and hybridization on micro-RNA microarray chips [19] was performed as previously described [8]. Micro-RNA oligonucleotide microchips were developed in our laboratories, and contained 368 probes in triplicate corresponding to 248 micro-RNA clusters (161 human, 84 mouse, and 3 *Arabidopsis*) and 15 t-RNA (8 human and 7 mouse). For 76 micro-RNAs, 2 different oligo-probes were designed, 1 containing the active sequence and the other specific for the precursor. Using these distinct sequences, we were able to analyze separately the expression of micro-RNA and pre-micro-RNA transcripts for the same gene. A PerkinElmer ScanArray XL5K Scanner (PerkinElmer Inc., Wellesley, MA) was used to scan processed slides, and QuantArray software (PerkinElmer, Inc.) quantified images. Signal intensity for each spot was calculated by subtracting local background (based on the median intensity of the area surrounding each spot) from total intensity.

2.4. Data analysis

Raw data were normalized and analyzed using the GeneSpring software (version 7.1; Silicon Genetics, Redwood City, CA). GeneSpring generates an average value of the 3 spot replicates of each micro-RNA. After data transformation (to convert any negative value to 0.01), normalization was performed using a per chip 50th percentile method that normalizes each chip on its median, allowing comparison among chips. Statistical analysis was performed using both a filter on fold change and a Welch *t*-test, for 2 groups' comparison, or Welch analysis of variance (ANOVA), for more than 2 groups' comparison. The Benjamini and Hochberg False Discovery Rate correction was used to reduce the number of false-positives. Micro-RNAs significantly deregulated were further analyzed according to predicted targets found in TargetScan [20,21], the database of conserved 3′ untranslated region micro-RNA targets.