Data in Brief

Gene expression profiling of WDR5 regulated genes in bladder cancer

Xu Chen a,b,1, Peng Gu a,b,1, Kuiqing Li a, Weibin Xie a, Changhao Chen a, Tianxin Lin a,b,⁎, Jian Huang a,⁎⁎

a Department of Urology, Sun Yat-sen Memorial Hospital, Sun Yat-sen University, Guangzhou, China
b Guangdong Provincial Key Laboratory of Malignant Tumor Epigenetics and Gene Regulation, Sun Yat-sen Memorial Hospital, Sun Yat-sen University, Guangzhou, China

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WD repeat domain 5 (WDR5) plays an important role in various biological functions through the epigenetic regulation of gene transcription (Wysocka et al., 2005[1]; Sandstrom et al., 2014[2]; Ang et al., 2011[3]). Recently, our study found that WDR5 was upregulated in bladder cancer tissues, promoted bladder cancer cell proliferation, self-renewal and chemoresistance to cisplatin in bladder cancer cells in vitro, and tumor growth in vivo (Chen et al., 2015). To gain a molecular understanding of the role of WDR5 in promoting bladder cancer, we performed a genome-wide analysis on WDR5 knockdown by microarray gene expression profiling. Here we provide detailed experimental methods and analysis for the microarray data, which have been deposited into Gene Expression Omnibus (GEO): GSE59132.

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1. Direct link to deposited data
The deposited data can be found at: http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE59132.

2. Experimental design, materials and methods

2.1. Cell culture

The human bladder cancer cell line UM-UC-3 was obtained from ATCC (CRL-1749, ATCC), and cultured in DMEM (Gibco, Shanghai, China). All media were supplemented with 10% FBS (Shanghai ExCell Biology, China) and 1% penicillin/streptomycin. Cells were grown in a humidified atmosphere of 5% CO2 at 37 °C.

2.2. RNA interference

WDR5 is a core component of the MLL/SET1 complexes and mediates H3K4 methylation, which is correlated with gene activation[1]. The present study reveals that WDR5 plays a critical role in undifferentiated progenitor cells of the baboon subventricular zone[2] and embryonic stem cell self-renewal[3]. We identified that WDR5 was upregulated in bladder cancer tissues, and elevated WDR5 protein levels positively correlated with advanced tumor stage and poor survival[4]. To study the role of WDR5 in bladder cancer, we knocked down WDR5 by siRNA. SiRNA oligos that targeted WDR5 (1-GCUGGGAAUAUCCGAUGUATT, 2-GCUCAGAGGAUAACCUUGUTT, 3-CCCAGUCCAACCUUAUUGUTT) were purchased from GenePharma (Shanghai, China). SiRNA transfections were performed with 75 nM siRNA and Lipofectamine RNAimax (Life Technologies) following the manufacturer’s instructions. WDR5 was remarkably downregulated in UM-UC-3 cells by the siRNA si-WDR5-2.
or si-WDR5-3, but not si-WDR5-1 [4]. To gain a molecular understanding of the mechanisms of WDR5 in bladder cancer cells, UM-UC-3 cells were transfected with a control siRNA, si-WDR5-2 or si-WDR5-3 siRNA respectively for 48 h in three independent experiments.

2.3. RNA isolation and qRT-PCR

We extracted total RNA from nine samples (three biological replicates) of UM-UC-3 cells that had been transfected with the control siRNA or with two siRNA targeting WDR5 expression using TRIzol reagent (Invitrogen) and further purified the RNA using Qiagen RNeasy Mini Kit according to the manufacturer’s protocol. The RNA quality was assessed by formaldehyde agarose gel electrophoresis and was quantitated by NanoDrop spectrophotometer. One microgram of total RNA of each sample was used for reverse transcription (PrimeScript RT-PCR kit, TaKaRa Biotechnology, Dalian, China) and was further performed qRT-PCR to detect the expression of WDR5. We confirmed that the expression of WDR5 was obvious downregulated in transfection with si-WDR5-2 or si-WDR5-3 siRNA compared with control siRNA in three biological replicates (Fig. 1). Therefore, we equally mixed the RNA of three biological replicates samples as a sample to further perform the microarray analysis.

2.4. Microarray and quality control

The Affymetrix PrimeView Human Gene Expression Array was used in this study and performed by CapitalBio Corporation (Beijing, China). 100 ng of total RNA was used to synthesize double-stranded cDNA. The bio-tagged cRNA was produced by using the MessageAmp™ Premier RNA Amplification Kit and were fragmented to strands of 35–200 bases in length according to the protocols from Affymetrix. The fragmented cRNA was hybridized to PrimeView Human Gene Expression Array containing about 36,000 transcripts. Hybridization was performed at 45 °C with rotation for 16 h (Affymetrix GeneChip Hybridization Oven 640). The GeneChip arrays were washed and then stained (streptavidin-phycoerythrin) on an Affymetrix Fluidics Station 450 followed by scanning on a GeneChip Scanner 3000.

The scanned images were assessed and analyzed to generate raw data files saved as CEL files using Affymetrix GeneChip Operating

Fig. 1. Efficiency of WDR5 knockdown in UM-UC-3 cells by siRNA was verified by qRT-PCR. The results are presented as the means ± SD of values obtained in three independent experiments. Statistical significance was calculated using the ANOVA analysis. "**p < 0.01.

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2.5. Data normalization and analysis

All samples were normalized through the Robust Multi-array Average (RMA) using DNA-chip analyzer (dChip). Differentially expressed genes were first identified with fold change of ≥ 2 or ≤ 0.5 in the group of control siRNA comprised with si-WDR5-2 or si-WDR5-3 siRNA. To further investigate the target genes of WDR5, differentially expressed genes were identified with both fold change of ≥ 1.5 and at least one group fold change of ≥ 2 in both siRNA compared with control siRNA. There were 136 upregulated genes and 42 downregulated genes in both siRNAs of WDR5 compared with control siRNA [4]. GO analysis and pathway analysis were performed by using Molecule Annotation System (MAS 3.0, CapitalBio Corporation, Beijing, China). The WDR5 regulated genes were mainly associated with regulation of transcription, cell cycle, anti-apoptosis and cell adhesion (Fig. 2).

2.6. Validation of microarray data by qRT-PCR

In order to validate the microarray data, qRT-PCR was performed to detect the differentially expressed genes in UM-UC-3. As shown in Fig. 3, these genes were well validated and the fold changes of them in qRT-PCR were more obvious than microarray.

3. Discussion

We described here a dataset composed of microarray gene expression profiling of WDR5 regulated genes in the human bladder cancer UM-UC-3 cells. With this experiment, we were able to demonstrate that WDR5 directly controls and coordinates the expression of a large number of genes involved in cell cycle and anti-apoptosis [4]. We believe that this dataset would be particularly valuable for investigating and underlying molecular mechanisms of WDR5 in bladder cancer.

Conflict of interest

The authors declare no conflict of interests.

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