Title: miR-195 inhibits tumor progression by targeting RPS6KB1 in human prostate cancer

Supplementary File S1

Patients and Tissue Samples

The study was approved by the human study ethics committees at MGH, Boston, MA and the Ministry of Public Health of P.R. China. Written informed consent was obtained from all patients. All specimens were handled and made anonymous according to the ethical and legal standards.

For quantitative real-time reverse transcriptase PCR (qRT-PCR) and western blot analyses, 12 pairs of primary PCa and self-matched adjacent non-cancerous frozen samples were obtained from the tissue bank at Guangzhou First People's Hospital. None of the patients recruited in this study received chemotherapy or radiotherapy before the operation. The pathological diagnosis was performed preoperatively and confirmed postoperatively. All patients were reviewed and all specimens were reexamined in November, 2012.

The Taylor dataset is a publicly available dataset including 113 primary PCa patients with microarray expression data and follow-up clinicopathological information of patients (1).

For Human PCa tissue microarrays (TMA), 225 consecutive PCa patients who underwent radical prostatectomy at the Massachusetts General Hospital (MGH, Boston, MA) from September 1993 to March 1995 were included in our study. The

follow-up clinicopathological information of patients was shown in Supplementary Tab. S1. Patients who received adjuvant or neoadjuvant hormonal or radiation treatment prior to cancer recurrence were excluded. All hematoxyolin-eosin (H&E)-stained sections from each case were reviewed, and the Gleason score was reassigned according to the current grading recommendation provided by the International Society of Urological Pathology (2). The tissue blocks containing the index PCa (tumor focus with the highest Gleason score) were selected for inclusion in the tissue microarray (TMA). Relative clinicopathological data included age, preoperative PSA, Gleason score, American Joint Committee on Cancer (AJCC) T stage, surgical margin status, BCR and overall survival. In the Taylor dataset, Biochemical recurrence (BCR) was defined as PSA ≥ 0.2ng/ml on two consecutive measurements after radical prostatectomy, while in the cohort from MGH, BCR was defined as PSA > 0.2ng/ml on two consecutive measurements. The BCR-free survival and non-metastatic BCR-free survival were defined as the time interval between the initial surgery and the day of BCR. The distant metastasis-free survival was defined as the time interval between the initial surgery and the day of the detection of metastatic lesions. The overall survival was determined from the date of surgery to the time of the last follow-up or death.

Reference:

1. Taylor BS, Schultz N, Hieronymus H, Gopalan A, Xiao Y, Carver BS, Arora VK, Kaushik P, Cerami E, Reva B, Antipin Y, Mitsiades N, Landers T, Dolgalev I, Major JE, Wilson M, Socci ND, Lash AE, Heguy A, Eastham JA, Scher HI, Reuter VE, Scardino

PT, Sander C, Sawyers CL, Gerald WL. Integrative genomic profiling of human prostate cancer. Cancer Cell. 2010; 18: 11-22.

2. Bjartell A. Words of wisdom. The 2005 International Society of Urological Pathology (ISUP) Consensus Conference on Gleason Grading of Prostatic Carcinoma. Eur Urol. 2006; 49: 758-9.

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Supplementary File S2

Proteomics expression profiling by mass spectrometric analysis using an isobaric tagging reagent iTRAQ:

Sample Preparation

LNCaP Cells were harvested and washed three times with PBS. Cell pellets were lysed in 150 µl of RIPA lysis buffer containing 1% (V/V) TritonX-100, 1% (W/V) Sodium deoxycholate, 0.1%(W/V) SDS, 150mM NaCl and 50mM Tris-HCl (pH8.0). Then, the sample was sonicated on ice and centrifuged at 12,000×g for20 min at 4°C and the middle layer of liquid was collected. Protein concentration was estimated by BCA Protein Assay Kit (Sangon Biotech, PR China).

Trypsin Digestion and iTRAQ Labeling

Briefly, 200µg of each protein sample was reduced and alkylated. Then, Proteins were digested with trypsin (Promega, USA) at 37 °C at a ratio of 1:50 (enzyme-to-substrate) overnight. iTRAQ labeling was performed according to the manufacturer's protocol (Applied Biosystems, Sciex). Each sample was labeled separately with two of the eight available tags (premiR-195: 113 and 114 tags; premiR-000: 115 and 116 tags; blank: 119 and 121 tags). All labeled peptides were pooled together.

High-pH Reversed-Phase Chromatography.

The Ultimate 3000 HPLC system (Dionex, USA) equipped with a 2.00-mm-inner diameter *100-mm-long Gemini-NX 3u C18110A columns (Phenomenex, USA) was used for High-pH fractionation. Peptides were loaded onto the column and washed isocratically at 95% eluent A (20 mM HCOONH4, 2M NaOH) (pH10). Peptide fractionation was performed using a linear binary gradient from 15 to 50% B (20 mM HCOONH4, 2 M NaOH, 80% ACN) (pH 10) at 0.2ml/min over 45 min. Finally, the column was washed at 90% B for 10 min and returned to 95% A for 10 min. The UV detector was set at 214/280 nm, and fractions were collected every 1 min. In total, 10 fractions were pooled and dried by vacuum centrifuge for subsequent nano-reversed phase liquid chromatography (nano-LC) fractionation.

RPLC-MS/MS Analysis.

Each fraction was resuspended in loading buffer (0.1% FA,2% ACN) and separated using an Ultimate 3000 nano-LC system equipped with a C18 reverse phase column (100-μm inner diameter, 10-cm long, 3-μm resin from MichromBioresources, Auburn, CA). The peptides were separated using the following parameters: 1) mobile phase A: 0.1% FA, 5% ACN, dissolved in water; 2) mobile phase B: 0.1% FA, 95% ACN; 3) flow rate: 300nl/min; 4) gradient: B-phase increased from 5% to 40%, 70min.

Then，the LC eluent was subject to Triple TOF5600 MS/MS system (AB SCIEX, CA) in an information dependent acquisition mode. MS spectra were acquired across the mass range of 400–1,250 m/z in high resolution mode (> 30,000) using 250 ms

accumulation time per spectrum. A maximum of 20 precursors per cycle were chosen for fragmentation from each MS spectrum with100 ms minimum accumulation time for each precursor and dynamic exclusion for 20 s. Tandem mass spectra were recorded in high sensitivity mode (resolution>15,000) with rolling collision energy on and iTRAQ reagent collision energy adjustment on.

Data analysis

Student t-test with Benjamini-Hochberg correction (p value ≤ 0.05) was used to identify differentially expressed proteins between premiR-195 and premiR-000 LNCaP cells. For iTRAQ high throughput data functional analysis, protein quantification data with relative expression of >1.5 or <0.67 and P-value < 0.05 was chosen to upload into the Ingenuity Pathways Analysis (IPA) online system (Ingenuity Systems http://www.ingenuity.com) and GO annotation system. Canonical pathways, biological functions were performed according to the user guider (1).

Reference:

1. Chen JH, He HC, Jiang FN, Militar J et al. Ran PY. Analysis of the specific pathways and networks of prostate cancer for gene expression profiles in the Chinese population. Med Oncol. 2012 Sep;29(3):1972-84.