

Appendix 1

Extracting miRNAs in urine using the nanowire device

We extracted miRNAs from 500 μ L urine sample using a nanowire-microfluidic device (3,4). We fabricated the nanowire device by assembling ZnO nanowire scaffolds, COP resin microfluidic substrate, COP resin substrate, two stainless steel holders, and PEEK tubes. After assembling the device, the inlet PEEK tube was connected to a syringe pump (KDS-200, KD Scientific Inc.) to introduce urine and lysis buffer. On the other hand, the outlet PEEK tube was put in an RNase-free microfuge tube (Eppendorf AG.) to collect the flow-through urine and miRNA-containing solution. The miRNA-containing solution extracted with lysis buffer was purified using Wako microRNA Extractor SP Kit (FUJIFILM Wako Chemical Corporation) according to the manufacturer's instructions. We profiled miRNA expression using microarray.

Microarray analysis of miRNA expression

Comprehensive miRNA expression analysis was performed using the 3D Gene miRNA Labeling Kit and the 3D-GeneHumanmiRNA Oligo Chip (Toray Industries, Inc.), which was designed to detect 2,632 miRNAs registered in miRBase release 22 (<http://www.mirbase.org/>; ref. 22). Fluorescent signals for each spot on the microarray were obtained using the 3D-Gene Microarray Scanner (Toray Industries, Inc.) and digitized using the accessory digitizing application "Extraction" (Toray Industries, Inc.) Background (BG) signals were subtracted from the raw signals by statistically inferring the true signal based upon the assumption of normal distribution for the BG signal and exponential distribution for the true signal (7). miRNAs with (BG-subtracted signal) $>2^6$ with at least 50% of the samples were selected, and the other miRNAs were removed from the down-stream analyses. Quantile normalization was applied after BG subtraction and low-expression miRNAs removal. Log₂ transformation was applied after quantile normalization.

Data accessibility

The microarray data have been deposited in the GEO database under accession codes GSE196156.

Statistical analysis

The urinary samples were analyzed to identify IC/BPS in the following way: the top 20 miRNAs by accuracy to correctly classify HIC/BPS and controls in ROC curve analysis were identified by leave-one-out cross-validation; miRNAs with AUC value ≥ 0.85 and cross-validated accuracy ≥ 0.85 were selected. Categorical and continuous variables of patients' characteristics were compared using chi-square test and Mann-Whitney U test, respectively. Statistical analysis were performed using R version 3.4.3 (R Foundation for Statistical Computing, <http://www.R-project.org/>), compute.es package version 0.2-4, hash package version 2.2.6.1, MASS package version 7.3-51.3, mutoss package version 0.1-12, and pROC package version 1.14.0. Heat maps were created using the online tool Morpheus (<https://software.broadinstitute.org/morpheus>). Other statistical analyses were performed using STATA version 14 (StataCorp). For all analyses, a two-sided P value less than 0.05 was considered statistically significant.

References

7. Irizarry RA, Hobbs B, Collin F, et al. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics* 2003;4:249-64.

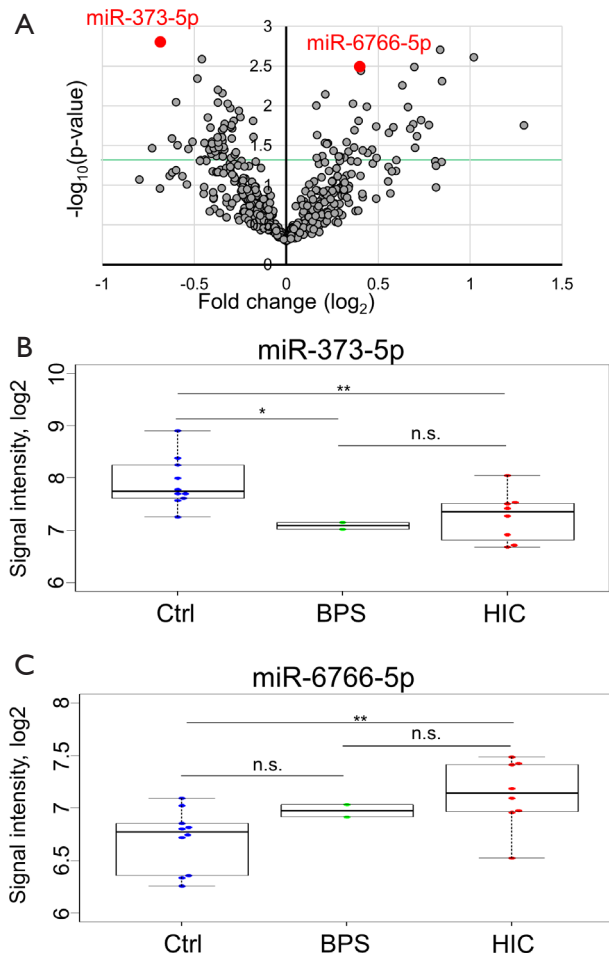


Figure S1 Evaluation of miRNA expression profiles in urine. (A) Volcano plot of the fold change of miRNAs. Green line indicates P=0.05. (B) Expression level of miR-373-5p. (C) Expression level of miR-6766-5p. Ctrl, control; BPS, bladder pain syndrome; HIC, Hunner type interstitial cystitis; miRNA, microRNA.

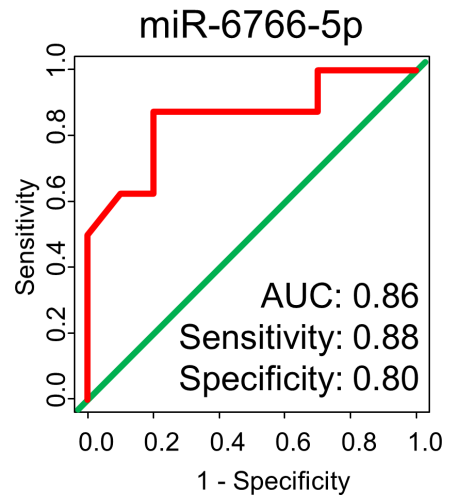
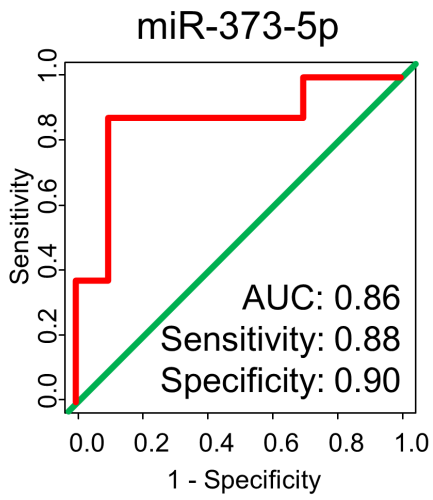


Figure S2 ROC curve analysis of miR-373-5p and miR-6766-5p in HIC patients and controls. AUC, area under the curve; ROC, receiver operating characteristic; HIC, Hunner type interstitial cystitis.

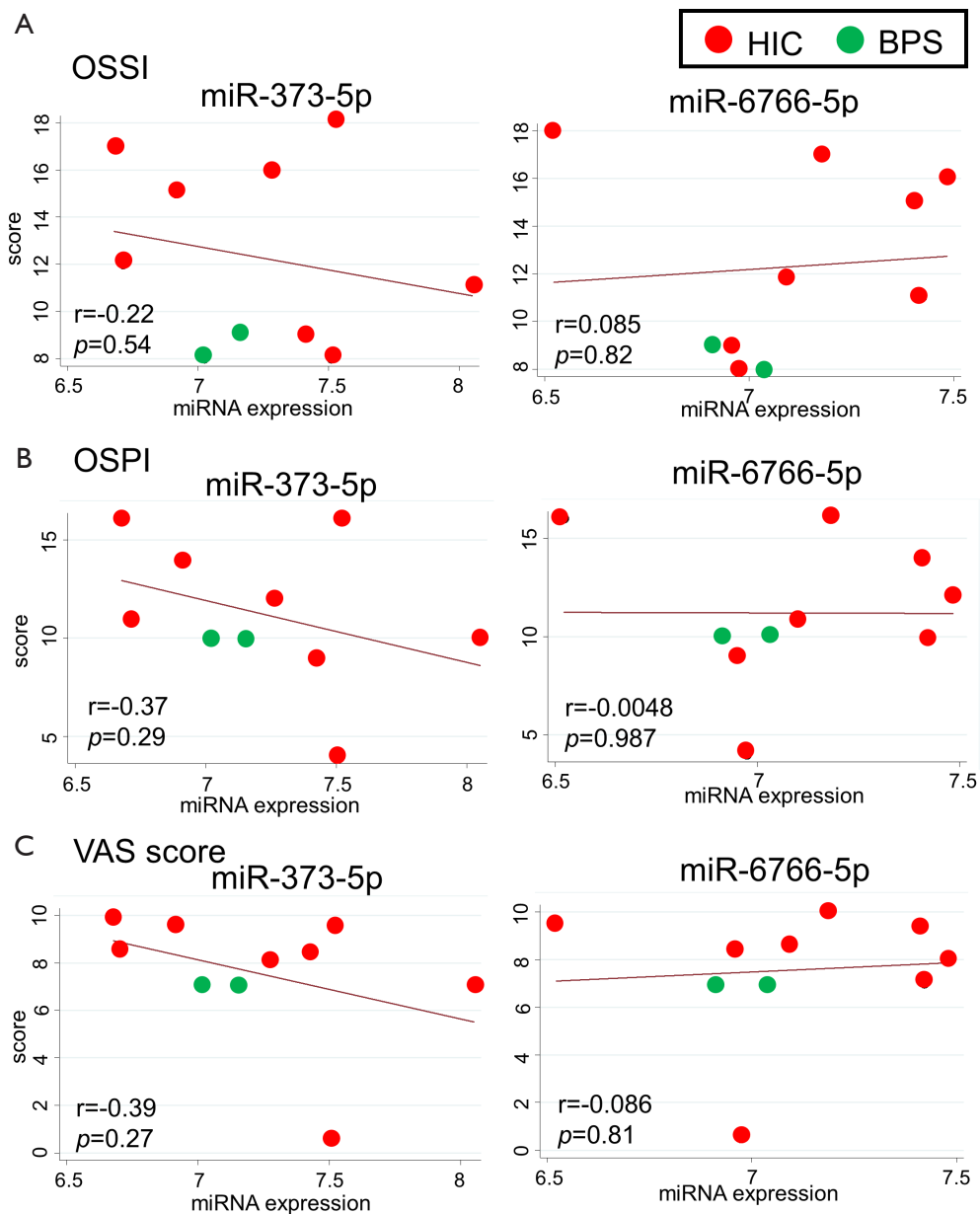


Figure S3 Scatter plots show the relationship between each miRNA expression and (A) OSSI, (B) OSPI, and (C) VAS score. HIC, Hunner type interstitial cystitis; BPS, bladder pain syndrome; OSSI, O’Leary-Sant score symptom indexes; OSPI, O’Leary-Sant score problem indexes; VAS, visual analog scale; miRNA, microRNA.

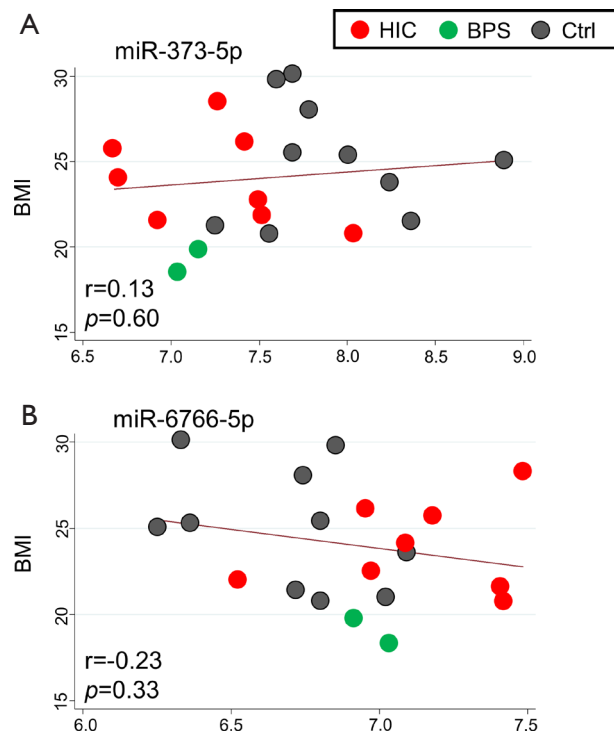


Figure S4 Scatter plots show the relationship between each miRNA expression and BMI. HIC, Hunner type interstitial cystitis; BPS, bladder pain syndrome; Ctrl, control; BMI, body mass index; miRNA, microRNA.