Integrated microRNA-mRNA profiling identifies molecular biomarkers in bladder outlet obstruction-induced lower urinary tract dysfunction

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1. Introduction
Bladder outlet obstruction (BOO) induces significant organ remodeling accompanied by changes in bladder function leading to lower urinary tract symptoms (LUTS) and dysfunction (LUTD). Early diagnosis of BOO-induced LUTS is complicated by the lack of methods to assess specific molecular changes in the bladder wall. MicroRNAs (miRNAs) are a class of small non-coding regulatory RNAs altered in patients with LUTD and animal models of partial BOO. Previously using next generation sequencing (NGS) method, we identified alterations in mRNA and miRNA expression profiles associated with urodynamically-defined states of BOO-induced LUTD in human patients and studied the potential regulatory role of miRNAs. Here, using RT-qPCR we validated our sequencing results in a bigger cohort of patients and examined mRNA and miRNAs for distinguishing the BOO states at the molecular level.

2. Experimental setup
Bladder dome biopsies were collected from human patients with BOO selected based on a complete urological evaluation, uroflowmetry, post void residual and urodynamic studies. The subjects were divided in 4 groups. NGS was performed on total isolated RNA of 6 patients per group and RT-qPCR validation of miRNA and mRNA expression profile was done on n=212 patients for each group.

3. NGS results – clustering of mRNAs and miRNAs
Using Illumina NGS methodology, we investigated the differentially expressed mRNA and miRNAs characteristic of the defined states of BOO-induced LUTD in human patients. MicroRNA and mRNA expression patterns detected in the samples of BOO with detrusor overactivity (DO) were similar to the normal bladder samples, whereas BOO without overactivity (BO) were clustered with underactive bladder (UA) samples and clustering of patients based on mRNA and miRNA datasets was highly correlated.

4. Validation of NGS results by RT-qPCR
To validate mRNA and miRNA sequencing results, we carried out qPCR using mRNA and miRNA expression assays on n ≥ 12 samples per group of BOO-induced LUTD patient biopsies. The clustering of patients based on mRNA (top) and miRNA (bottom) qPCR results was similar to the clustering of patients based on mRNA or miRNA sequencing data.

5. ROC analysis of NGS data: mRNA and miRNA biomarkers
Receiver operating characteristic curve analysis (ROC) was performed to predict the best mRNA and miRNA candidates suitable for further validation of NGS results. The ROC curves were calculated for individual miRNAs using NGS data. The area under the ROC curve is calculated with 95% confidence interval. The data were validated by QPCR and boxplots for the response variables in the mRNA PCR dataset classified by groups are shown.

6. RT-qPCR of group-specific markers
Three-dimensional scatterplots and point identification for 3 mRNA and 3 miRNA biomarkers. Log2 fold change values ≥ 12 samples per group. *p<0.05 and **p<0.001

7. 3D scatterplots of three-mRNA and three-miRNA signatures for BOO states
Taking into account that individual ROC-predicted miRNAs failed to offer acceptable specificity and sensitivity, we performed further analysis of the data using a combination of validated differentially expressed miRNAs/mRNAs. A combination of 3 miRNAs/mRNAs was sufficient to discriminate each patient group.

8. Conclusions
Testing NGS-based ROC analysis in a bigger sample cohort rejects the potential of using single miRNAs as biomarker determinants for specific BOO states. Our data supports the power of using a group of 3 miRNAs or mRNAs instead of individual miRNAs/miRNAs. It remains to be determined whether they can also predict the treatment outcome.

Disclosure:

Significantly regulated miRNAs. Log2 fold change of 2634 significantly regulated miRNA (Y-axis) and 24 samples (X-axis).

Significantly regulated mRNAs. Log2 fold change of 342 significantly regulated mRNA (Y-axis) and 24 samples (X-axis).

miRNA-mRNA tanglegram. Entanglement is a measure between full entanglement (1) and 0 (no entanglement).

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Three-dimensional scatterplots and point identification for 3 mRNA and 3 miRNA biomarkers. Log2 fold change values of 49 patients with different BOO states are plotted for NRXN3, BMP7, UFK1A mRNAs and miR-103a-3p, hsa-miR-199a-3p and hsa-miR-199a-5p miRNAs suggested by ROC analysis. Concentration ellipsoids are drawn to show the DO patients in green, BO patients in blue and UA patients in orange color.

Regulation of 6 significantly changed mRNAs in 28 patients. qPCR results in 3 patients' groups are shown as log2 fold changes compared to controls. *p<0.05 and **p<0.001

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