Functional genomic analyses of IC/BPS patient subgroups: a pilot study

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Introduction: *To further facilitate understanding of disease pathophysiology and patient stratification in interstitial cystitis/bladder pain syndrome (IC/BPS), we utilized molecular phenotyping to compare three clinically distinct IC/BPS patient subgroups.*

Materials and methods: *Total RNA (miRNA and mRNA) was isolated via standard protocols from IC/BPS patient bladder biopsies and assayed on whole genome and microRNA expression arrays. Data from three patient subgroups (n = 4 per group): (1) low bladder capacity (BC; ≤ 400 cc) without Hunner's lesion, (2) low BC with Hunner's lesion, and (3) non-low BC (> 400 cc) were used in comparative analyses to evaluate the influence of BC and HL on gene expression profiles in IC/BPS.*

Results: *The BC comparison (Group 1 v 3) identified 54 miRNAs and 744 mRNAs. Eleven miRNAs mapped to 40 genes. Hierarchical clustering of miRNA revealed two primary clusters: (1) 3/4 low BC patients; (2) 4/4 non-low and 1/4 low BC patients. Clustering of mRNA provided clear separation based on BC. The HL comparison (Group 1 v 2) identified 16 miRNAs and 917 mRNAs. 4 miRNAs mapped to 13 genes. Clustering of miRNA and mRNA revealed clear separation based on HL status.*

Conclusions: *Significant molecular differences in IC/BPS were found to be associated with the low BC phenotype (e.g., an upregulation of cell proliferation and inflammation marker genes), as well as additional molecular findings that further define the HL+ phenotype (e.g., upregulation of genes involved in bioenergetics reactions) and suggest oxidative stress may play a role.*

Key Words: gene expression, miRNA, interstitial cystitis/bladder pain syndrome

Introduction

Interstitial cystitis/bladder pain syndrome (IC/BPS) is a chronic pain condition that affects millions of women and men in the United States.[1,2] This disorder classically presents with irritative voiding symptoms (urgency, frequency, and dysuria) as well as pelvic pain that increases with passive bladder filling,[1,2] however the IC/BPS clinical presentation is highly heterogeneous, with variation in symptoms and severity across affected individuals.[1,2] Although the underlying pathophysiology of IC/BPS is an active area of research, there is not yet a clearly defined etiology. This presents a challenge not only for diagnosis of IC/BPS but also for symptom management in affected individuals.

The etiology of IC/BPS is thought to be multifactorial, whereby several pathophysiologic processes can present simultaneously to result in the clinical manifestation.[3] Abnormalities in the structure and function of urothelial cells,[4-6] mastocytosis with inflammatory cell infiltration,[7,8] and neurogenic inflammation[9] have all been suggested as factors that underlie the development of IC/BPS. Moreover, patients with a diagnosis of IC/BPS also report non-urologic symptom and syndromes such as migraines and depression, suggestive of a neurological etiology, and disorders such as irritable bowel syndrome, chronic fatigue syndrome, and fibromyalgia, suggestive of a systemic manifestation. To better understand the molecular characteristics that are associated with IC/BPS pathophysiology, we have been investigating gene

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expression profiles in bladder biopsies from patients with IC/BPS. In an earlier small pilot study, we found significant differences in samples from patients with low bladder capacity (BC) IC/BPS (≤ 400 cc) when compared to samples from patients with non-low (> 400 cc) BC IC/BPS, as well as between patients with low BC who were Hunner's lesion positive when compared to samples from patients with low BC who were found to be Hunner's lesion negative.[10] Differentially expressed genes identified in the comparison between these groups were found to be involved in inflammatory signaling and tight junction pathways.[10] The principal component analysis performed using molecular data from this pilot study revealed a clear separation, based on the patients' anesthetic BC, that allowed for a more detailed understanding of molecular variation between phenotypic subgroups within the IC/BPS patient population. Our group has also previously performed histologic evaluation of low BC IC/BPS patients and demonstrated that bladder samples from patients with low BC exhibited higher levels of acute inflammation, chronic inflammation, and erosion on microscopy when compared to non-low BC IC/BPS samples.[11]

An additional tool that is increasingly being used to assess molecular variation in disease, especially when the goal is to better understand regulation of gene expression, is microRNA (miRNA) expression analyses. MiRNAs are small, non-coding molecules that modify gene expression and play an important role in the regulation of cellular function. Studies that measure miRNA variation in urologic disease are increasingly present in the literature, with many reports focused on variation within urologic malignancies. One such study found that several miRNAs were significantly overexpressed in patients with upper urinary tract urothelial carcinoma (UUTUC) when compared to control patients without UUTUC.[12] Another study demonstrated that specific miRNAs were found to have a regulatory effect on pro-oncogenic genes involved in the development of urothelial adenocarcinoma.[13] These studies suggest that differences in miRNA expression may have significant implications for the development of urological disease.

The primary objective of the present study was to extend our previous findings of molecular variation between phenotypic subgroups of IC/BPS patients by including a co-expression analysis, using miRNA and mRNA derived from the same samples, to identify differential miRNA-regulated gene expression differences between specific IC/BPS patient subgroups. The identification of differential co-expression of miRNAs and their putative target genes (mRNAs), and the molecular pathways and processes they participate in, can provide new biological insights into IC/BPS subtypes as well as disease pathophysiology.

Materials and methods

Patient selection

Adult women between 18-80 years old with a diagnosis of IC/BPS were prospectively enrolled into this study at the time of a therapeutic hydrodistension procedure, Table 1. Patients with a history of any

TABLE 1. **Demographic characteristics of study participants**

	Bladder capacity (cc)	Hunner's lesion status	Comparison group	Age (years)	BMI	Ethnicity
IC08	400	No	low BC, HL-	30	30.0	Caucasian
IC10	400	No	low BC, HL-	49	30.2	Caucasian
IC12	350	No	low BC, HL-	50	24.9	African American
IC19	400	No	low BC, HL-	52	25.6	African American
IC09	300	Yes	low BC, HL+	41	24.1	Caucasian
IC16	300	Yes	low BC, HL+	69	23.2	African American
IC17	400	Yes	low BC, HL+	62	25.1	Caucasian
IC18	350	Yes	low BC, HL+	68	23.3	Caucasian
IC30	600	No	non-low BC HL-	26	25	Caucasian
IC34	675	No	non-low BC, HL-	29	40.8	Caucasian
IC42	900	No	non-low BC, HL-	58	25.3	Caucasian
IC48	1500	No	non-low BC HL-	41	41.2	African American

BC = bladder capacity; HL = Hunner's lesion

urogenital cancer, urethral diverticula, neurologic disease, cyclophosphamide use, radiation cystitis, or bladder tuberculosis were excluded. Patients who were catheterized at the time of surgery, patients with an active genital herpes episode, and patients with an active bladder infection were also excluded. Informed consent was obtained from all patients prior to study enrollment through Wake Forest University Health Sciences Institutional Review Board approval (IRB00018552).

Tissue collection and storage

Bladder tissue samples were obtained from IC/BPS patients at the time of a scheduled hydrodistension procedure. While under general anesthesia, the bladder was hydrodistended to 1 meter of water height and held for 5 minutes. The bladder was then drained, and anesthetic bladder capacity was measured. We subsequently performed a systematic cystourethroscopy to assess for Hunner's lesion presence. Bladder tissues were then obtained via cystoscopic cold cup biopsy technique from the posterior bladder wall. For Hunner's lesion positive patients, biopsies were obtained along the posterior bladder wall near, but not directly from, a Hunner's lesion. Following retrieval, bladder biopsies were immediately placed in RNALater solution, transported back to our laboratory facilities, and stored at -20°C until further processing.

RNA isolation and molecular assays

Total RNA (messenger RNA [mRNA] and microRNA [miRNA]) was isolated from the bladder tissue biopsies using the miRNeasy Kit (Qiagen) according to the manufacturer's instructions. RNA quality and purity were assessed using a NanoDrop spectrophotometer by measuring absorbance at 260/280 nm and RNA quality was measured using a bioanalyzer (Agilent Technologies). RNA was assayed on whole genome microarrays via standard protocols as previously described,[14] and on miRNA expression arrays (nCounter miRNA Expression Array; NanoString Technologies) using the nCounter SPRINT Profiler instrument (NanoString Technologies) following the manufacturer's instructions and protocols.

Experimental comparison groups

Three phenotypically distinct IC/BPS patient subgroups were selected for this pilot study. Patients in Group 1 (n = 4) demonstrated low anesthetic bladder capacity, defined here as ≤ 400 cc on cystoscopic hydrodistension, and were negative for Hunner's lesions (HL-). Patients in Group 2 (n = 4) also demonstrated low anesthetic bladder capacity and were Hunner's lesion positive (HL+). Group 3 (n = 4) patients had non-low anesthetic bladder capacity, defined here as BC > 400 cc on hydrodistension, and were HL-.

To evaluate differential expression, two group comparisons were performed: comparison 1 - between groups 1 vs. 3 to evaluate low vs. non-low anesthetic bladder capacity IC/BPS patients and, comparison 2 - between groups 1 and 2 to evaluate Hunner's lesion positive vs Hunner's lesion negative IC/BPS patients. Of note, all patients with Hunner's lesion positive disease demonstrated low anesthetic bladder capacity at the time of hydrodistension in this analysis.

Data analysis

Differential gene expression analysis for mRNA was performed using Qlucore Omics Explorer software (Qlucore, Lund Sweden). A p value of < 0.01 was used to determine statistical significance for all mRNA analyses. For differential miRNA expression analyses, the nCounter Analysis System (NanoString Technologies) was used. A p value of < 0.05 was used to determine statistical significance for all miRNA analyses. Principal component analysis (PCA), as well as unsupervised hierarchical clustering, were used to determine differences in miRNA and mRNA expression between subgroups for both comparisons. Ingenuity Pathway Analysis (IPA) software was used to perform pairwise mapping of differentially expressed miRNA to differentially expressed mRNA. IPA was also used to identify experimentally demonstrated functional gene pathways involved for each comparison.

Results

Demographic data

The mean age for all patients enrolled was 47.92 ± 14.85 years. Patients with low BC were, on average, older than those with non-low BC (52.6 ± 12.5 vs. 38.5 ± 12.6) however this difference did not reach statistical significance. All patients (12/12, 100%) were female. Mean BMI for all patients enrolled was 28.04 ± 7.34. ANOVA of mean demographic variables across groups revealed no significant differences in demographic characteristics between the three IC/BPS patient subgroups.

Comparison 1: low vs. non-low bladder capacity IC/BPS

A total of 54 differentially expressed miRNAs ($p < 0.05$) and 744 differentially expressed mRNAs ($p < 0.01$)

were identified in the comparison between low and non-low bladder capacity IC/BPS subgroups, Figure 1. Hierarchical clustering of miRNA revealed two primary clusters: one consisting of 3 low BC IC/BPS patient bladder samples, and the other consisting of all 4 non-low BC IC/BPS patient bladder samples and 1 low bladder capacity sample, Figure 1a. Hierarchical clustering of mRNA revealed 2 primary clusters: one consisting entirely of low BC IC/BPS patient bladder samples, and the other consisting entirely of non-low BC IC/BPS patient samples, Figure 1b.

Using the microRNA Target Filter feature in IPA to identify both predicted and experimentally verified functional relationships between genes and miRNAs, 11 of the 54 differentially expressed miRNAs mapped to 40 of the 744 differentially expressed mRNAs identified in this comparison between low and non-low BC patient samples, Table 2.

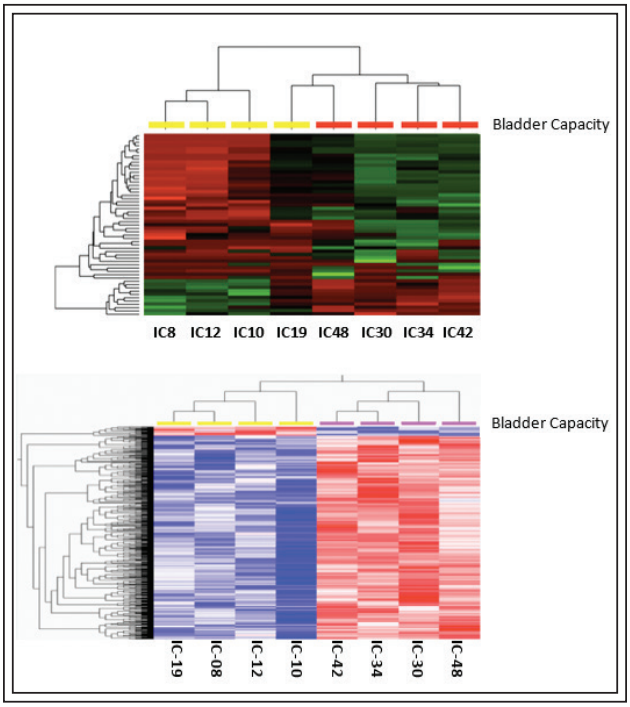

Figure 1. Hierarchical clustering of RNA expression profiles in IC/BPS patient bladder biopsy samples for the low vs. non-low bladder capacity comparison. **Top:** Heatmap representation of miRNA expression profiles. Yellow columns indicate low bladder capacity IC/BPS patients. Red columns indicate non-low bladder capacity IC/BPS patients. Within the heat map: Red indicates higher gene expression and Green indicates lower gene expression. **Bottom:** Heatmap representation of mRNA expression profiles. Yellow columns indicate low bladder capacity IC/BPS patients. Magenta columns indicate non-low bladder capacity IC/BPS patients. Within the heat map: Blue indicates higher gene expression and Red indicates lower gene expression.

Comparison 2: Hunner's lesion positive vs. HL negative IC/BPS

A total of 16 differentially expressed miRNAs ($p < 0.05$) and 917 differentially expressed transcripts ($p < 0.01$) were identified when comparing the Hunner's lesion positive and negative IC/BPS subgroups, Figure 2. Hierarchical clustering of miRNA revealed two

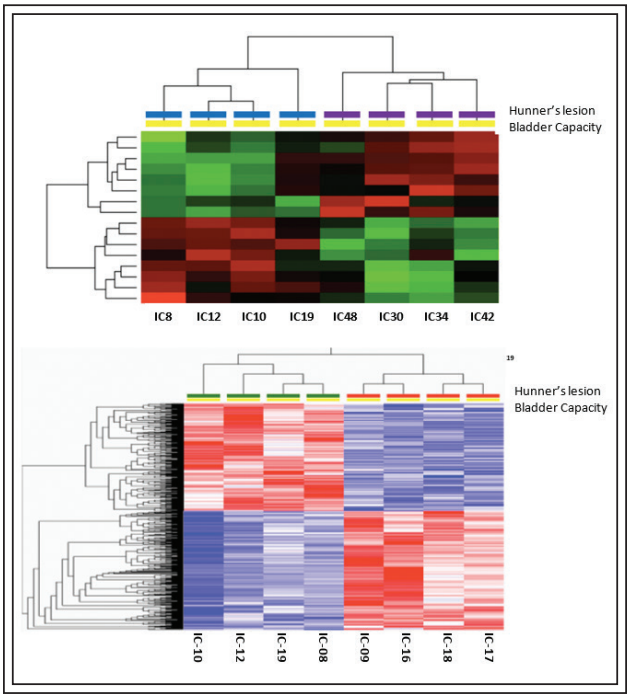

Figure 2. Hierarchical clustering of mRNA expression profiles in IC/BPS patient bladder biopsy samples for the Hunner's lesion positive vs. HL negative comparison. **Top:** Heatmap representation of miRNA expression profiles. Yellow columns indicate low bladder capacity IC/BPS patients. Blue columns indicate HL positive. Purple columns indicate HL negative IC/BPS patients. Within the heat map: Red indicates higher gene expression and Green indicates lower gene expression. **Bottom:** Heatmap representation of mRNA expression profiles. Yellow columns indicate low bladder capacity IC/BPS patients. Green columns indicate HL positive IC/BPS patients. Red columns indicate HL negative IC/BPS patients. Within the heat map: Blue indicates higher gene expression and Red indicates lower gene expression.

TABLE 2. **Differentially expressed miRNAs and pair-wised mapped differentially expressed mRNAs with corresponding fold changes in patients with low bladder capacity IC/BPS compared to non-low bladder capacity IC/BPS**

miRNA	Fold change (miRNA)	mRNA	Fold change (mRNA)	Gene pathway
let-7b-5p	4.03 (↑)	DUSP12	1.354 (↑)	Cell proliferation and differentiation
		PGRMC1	1.303 (↑)	Cell proliferation and differentiation
		POLR2C	1.19 (↑)	Transcription regulation
		RRP8	1.254 (↑)	Cell cycle arrest, Apoptosis
miR-613	3.99 (↑)	BLCAP	1.279 (↑)	Apoptosis
		CHST11	1.059 (↑)	Apoptosis
		CPOX	1.363 (↑)	Heme biosynthesis
		KCNJ2	1.139 (↑)	Cell excitability and development
		LARP4	1.152 (↑)	Cell organization
		PPIB	1.167 (↑)	Cell proliferation
		SAC3D1	1.161 (↑)	Cell morphology
		TRAPPC3	1.218 (↑)	Protein binding
		UHMK1	1.489 (↑)	Cell cycle progression
		XPO6	1.183 (↑)	Protein binding
miR-146b-5p	2.04 (↑)	BLMH	1.278 (↑)	Cell death and fibrosis
		TLR1	1.188 (↑)	Cell activation, signaling, differentiation
miR-155-5p	3.35 (↑)	CYP51A1	1.302 (↑)	Cell maturation and adipogenesis
		LCLAT1	1.198 (↑)	Cell fragmentation and depletion
		MPZL1	1.238 (↑)	Cell migration
		POLE3	1.415 (↑)	Cell cycle progression
		TXMDC12	1.233 (↑)	Glutathione redox reactions
miR-200c-3p	1.69 (↑)	ELMO2	1.11 (↑)	Chemotaxis, cell activation and migration
miR-520e	4.48 (↑)	TFAP4	1.104 (↑)	Apoptosis
		ZNF226	1.183 (↑)	Transcription regulation
miR-367-3p	3.41 (↑)	PCGF1	1.191 (↑)	Cell proliferation, ubiquitination, autophagy
miR-1271-5p	3.62 (↑)	ODF2	1.071 (↑)	Cell organization
miR-30d-5p	1.64 (↓)	NUCB1	1.167 (↑)	Signal transduction regulation
		PEX11B	1.271 (↑)	Cell proliferation
		TMCO1	1.403 (↑)	Calcium channel activity
		TMEM59	1.346 (↑)	Cell function
		UAP1	1.411 (↑)	Cell growth
		WNT5A	1.466 (↑)	Cell proliferation and differentiation
miR-222-3p	1.64 (↓)	CDKN1B	1.454 (↑)	Progression through the cell cycle
miR-15a-5p	2.43 (↓)	CDK5RAP1	1.213 (↑)	Cell differentiation
		CREBL2	1.208 (↑)	Cell differentiation, Apoptosis
		GRB2	1.138 (↑)	Cell proliferation and differentiation
		GTF2H	1.195 (↑)	Progression through cell cycle
		OSGEPL1	1.115 (↑)	Transferase activity
		SRPRB	1.391 (↑)	Protein binding
		YIF1B	1.149 (↑)	Protein binding

TABLE 3. **Differentially expressed miRNAs and pair-wised mapped differentially expressed mRNAs with corresponding fold changes in patients with Hunner's lesion positive IC/BPS when compared to patients with Hunner's lesion negative IC/BPS**

miRNA	Fold change (miRNA)	mRNA	Fold change (mRNA)	Gene pathway
miR-155-5p	5.06 (↑)	MARC1	1.433 (↑)	Oxidation-reduction processes
		TXNDC12	1.184 (↑)	Oxidation-reduction processes, cell death
miR-15b-5p	2.64 (↑)	CA12	2.178 (↑)	Cell growth, ion transport, homeostasis
		CCNF	1.33 (↑)	Progression through cell cycle, degradation
		CDC14B	1.215 (↑)	Degradation and senescence
		GRB10	1.17 (↑)	Cell growth, Apoptosis
		HSDL2	1.361 (↑)	Oxidation-reduction processes
		HSPA1A/HSPA1B	1.961 (↑)	Apoptosis, cell death, ubiquinatio
		PSAT1	1.343 (↑)	Catalytic, transaminase, transferase activity
		SLC35A1	1.284 (↑)	Transcription regulation
miR-29c-3p	2.25 (↓)	DNMT3B	1.127 (↑)	Cell proliferation and growth
		SHROOM2	1.396 (↑)	Cell organization
miR-200a-3p	3.26 (↓)	DLX5	1.692 (↑)	Apoptosis

primary clusters: one consisting entirely of Hunner's lesion positive IC/BPS patient samples and the other consisting entirely of Hunner's lesion negative IC/BPS patient samples, Figure 2a. Hierarchical clustering of mRNA revealed the same two primary clusters, Figure 2b.

As in comparison 1, using the microRNA Target Filter feature in IPA to identify functional relationships between genes and miRNAs, we found that 4 of the differentially expressed miRNAs mapped to 13 of the differentially expressed mRNAs in this comparison between samples form HL+ and HL- IC/BPS patients, Table 3. Each of these miRNA-mRNA pairs corresponded to either a predicted or an experimentally derived functional relationship.

Discussion

The goal of this pilot study was to identify functional genomic differences between phenotypic subgroups of IC/BPS patients through the measurement of gene and regulatory microRNA expression levels in bladder biopsy tissue taken at the time of therapeutic bladder hydrodistention. Significant differences were found in the expression of several miRNAs and mRNAs in the low vs. non-low bladder capacity IC/BPS subgroup comparison. The finding of gene expression differences here is consistent with a previously published report by our group in which significant mRNA differences were shown, for the first time, to segregate patients into distinct low and non-low bladder capacity IC/BPS subgroups.[10] In the current study we also identified miRNA-mRNA pairs that showed significantly different co-expression between the subgroups and that suggest functional relevance in IC/BPS.

One of the miRNAs down-regulated in low BC samples, miR-222-3p, corresponded to the upregulation of the gene for cyclin dependent kinase inhibitor 1B (CDKN1B), a member of the cyclin dependent kinase inhibitor 1B gene family known to play a functional role in cell proliferation, cell cycle progression, and cell growth. Another miRNA that was upregulated in low BC samples, miR-613, corresponded to an upregulation of the mRNA for BLCAP apoptosis inducing factor (BLCAP). The associated biological pathway is reported to play a functional role in the stimulation of apoptosis.

Overall, in the comparison between low BC and non-low BC samples, several miRNAs (4/11) demonstrated the classic relationship in which decreased expression of a microRNA is associated with increased expression of the paired mRNA, however the majority (7/11) of miRNA-mRNA pairs in this comparison had an increased expression of a miRNA associated with an increased expression of the paired mRNA, Table 2. These seemingly oppositional findings may not be surprising given that previous studies have found a similar dual role relationship between miRNA-mRNA pairs. For example, Vasudevan et al reported that two specific miRNAs, Let-7 and the synthetic

miRNA miRcxcr4, induce translation upregulation of target mRNAs on cell cycle arrest, yet they repress translation in proliferating cells.[15] Another study by Lin et al reported that miR-206 either inhibited or promoted the expression of KLF4 depending on the context of the cellular conditions in which they were placed, such that miR-206 demonstrated a negative regulatory effect of KLF4 expression in normal epithelial cell lines and a positive regulatory effect of KLF4 expression in cancerous epithelial cell lines.[16] These studies illustrate that miRNAs may have a positive or negative regulatory function on the mRNAs they act upon depending on cell type and the specific cellular environment they inhabit.

We also identified canonical pathways that were predicted to be impacted by the differentially regulated miRNA-mRNA pairs in this low vs. non-low BC comparison. Functional over-representation of pathways including cell proliferation, cell differentiation, progression through the cell cycle, and apoptosis was demonstrated. Taken together, these findings suggest that abnormalities in urothelial cell structure and function may underlie the low bladder capacity IC/BPS phenotype.

In the comparison between the HL positive and HL negative IC/BPS subgroups, there were significant differences in the expression of 16 miRNAs and 917 mRNAs. These finding are consistent with previous studies that have identified molecular differences in the ulcerative (i.e. HL+) IC/BPS subtype.[17-19] In the present study, four of the differentially expressed miRNAs in this comparison mapped to 13 mRNAs, with half of the miRNA-mRNA pairs showing concordant upregulation and the other half showing a down-regulation of miRNA associated with an upregulation of the predicted target genes, Table 3.

One of down-regulated miRNAs, miR-29c-3p, is predicted to regulate expression of the DNA methyltransferase 3 beta (DNMT3B) gene. The DNA methyltransferase pathway is known to play a functional role in cell proliferation, cell growth, and methylation. One of the upregulated miRNAs, miR-15b-5p, is known to impact the expression of the growth factor receptor bound protein 10 (GRB10), a member of a family of genes that play a functional role in VEGF signaling, insulin receptor signaling, growth factor receptor signaling, cell growth, and apoptosis. An upregulation of miR-15b-5p also corresponded to an upregulation of the gene for heat shock protein family A/B (HSPA1A/HSPA1B), members of a group of proteins that play a functional role in bioenergetics (oxidative-reduction processes), DNA repair in response to cellular stress, cell death, apoptosis, and ubiquination.

As in the first comparison (between patients with low v.s non-low BC), this comparison between IC/BPS patients with and without HL identified cellular pathways, predicted by the differential expression of miRNA-mRNA pairs, to include cell proliferation, progression through the cell cycle, and apoptosis. However, the involvement of pathways that include inflammatory signaling and bioenergetics (reduction-oxidation) reactions were also predicted by these findings. This suggests that in addition to abnormalities in urothelial cell structure and function, inflammation and oxidative stress may play a potential role in the Hunner's lesion positive phenotype as well. These findings are consistent with previous reports in the literature regarding molecular features associated with the HL positive IC/BPS phenotype. In a 2019 study, Yoshiyuki et al demonstrated significantly different genomic and histological findings associated with the Hunner's lesion positive patients, with most notable differences present in genes that have a functional role in immune system and inflammatory pathways.[17] Additional studies have also reported upregulation of inflammatory signals in ulcerative IC/BPS. A 2012 study by Shie et al found significant molecular differences in patients with ulcerative IC/BPS with regards to both inflammatory signals as well as genes having a functional role in apoptosis.[18] Another study by Ogawa et al in 2010 reported the upregulation of specific pro-inflammatory cytokines and chemokines in patients with ulcerative IC/BPS compared to non-IC/BPS controls.[19]

One important limitation of this study is the small number of patients in each of the three IC/BPS patient subgroups. Because the study was designed to generate pilot data, we reasoned that if we found statistically significant differences in mRNA and miRNA co-expression levels with a modest n = 4 in each group, then it would provide evidence for the generalizability of these findings and warrant further investigation. A second limitation, that the study evaluated only female patient samples, is justified in the same way, i.e. since an n = 4 patient samples per group does not yield sufficient statistical power to account for gender differences, that would not be considered here. In a disorder like IC/BPS, where a vast majority of patients are female, this rationale can be justified in an exploratory pilot study of this size. Finally, the results presented here must be viewed as preliminary and more hypothesis generating than hypothesis testing, since the functional relevance of the mRNA-miRNA pairs that have been identified here have not been verified experimentally in the context of IC/BPS.

Conclusions

We have demonstrated unique molecular differences between three phenotypically distinct IC/BPS subgroups. In the low vs. non-low bladder capacity groups comparison, upregulated genes were over-represented in cell proliferation pathways, suggesting potential biological themes for the low BC phenotype. In addition to over-representation of these same pathways in the Hunner's lesion positive vs. negative comparison, upregulated genes were additionally over-represented in inflammation and oxidation-reduction pathways. This suggests that in addition to abnormal cell proliferation, inflammation and oxidative stress may underlie the HL positive phenotype. These findings create a framework for additional investigation and, if functionally validated, could be significant for diagnosis and management of IC/BPS as well as providing additional insights regarding IC/BPS subgroup specific biology and pathophysiology. □

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