Cell. Mol. Life Sci. (2013) 70:3773–3789
DOI 10.1007/s00018-013-1275-7

Cellular and Molecular Life Sciences

REVIEW

Deciphering microRNA code in pain and inflammation: lessons from bladder pain syndrome

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Received: 21 November 2012 / Revised: 22 January 2013 / Accepted: 28 January 2013 / Published online: 5 March 2013

Abstract MicroRNAs (miRNAs), a novel class of molecules regulating gene expression, have been hailed as modulators of many biological processes and disease states. Recent studies demonstrated an important role of miRNAs in the processes of inflammation and cancer, however, there are little data implicating miRNAs in peripheral pain. Bladder pain syndrome/interstitial cystitis (BPS/IC) is a clinical syndrome of pelvic pain and urinary urgency/frequency in the absence of a specific cause. BPS is a chronic inflammatory condition that might share some of the pathogenetic mechanisms with its common co-morbidities inflammatory bowel disease (IBD), asthma and autoimmune diseases. Using miRNA profiling in BPS and the information about validated miRNA targets, we delineated the signaling pathways activated in this and other inflammatory pain disorders. This review projects the miRNA profiling and functional data originating from the research in bladder cancer and immune-mediated diseases on the BPS-specific miRNAs with the aim to gain new insight into the pathogenesis of this enigmatic disorder, and highlighting the common regulatory mechanisms of pain and inflammation.

Keywords MicroRNA · Bladder · Pain · Inflammation · Gene expression · Bladder cancer · Inflammatory bowel disease

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Introduction

MicroRNAs (miRNAs) are quickly gaining recognition for their role in many biological processes and disease states [1, 2]. MiRNAs are endogenous non-coding single-stranded RNAs of approximately 22 nucleotides that regulate gene expression by post-transcriptional mechanisms upon sequence-specific binding to the 3′ untranslated regions (3′ UTRs) [1, 3] or, occasionally, the 5′UTRs [4, 5] or coding regions [6–8] of their mRNA targets. MiRNAs exhibit imperfect complementarity with mRNAs, allowing them to regulate multiple genes and thus complicating efforts to predict and functionally validate their targets [9]. Since their discovery [10], miRNAs have been acknowledged as important modulators of gene expression, and deficiency in their synthesis or function contributes to many human diseases [11, 12]. MiRNA expression profiles characteristic of a particular disorder may serve as a useful diagnostic tool, but more importantly, it is the first step towards unraveling miRNA functions. Follow-up studies in experimental models are carried out to identify the protein targets of differentially expressed miRNAs and delineate the signaling pathways activated in a diseased state.

Although a wealth of information has been gathered on miRNA expression in bladder cancer (BCa), which in this respect remains the best-studied disorder, only a few studies have been carried out in bladder dysfunction. The first miRNA profiling in bladder pain syndrome/interstitial cystitis (BPS), which was performed in our laboratory, has identified several miRNAs, regulating the expression of signaling and adhesion molecules [13, 14]. Pain and inflammation are characteristic of BPS, which has been suggested to share the pathogenetic mechanisms with other inflammatory diseases such as asthma, inflammatory bowel disease, and autoimmune diseases [15]. These are common co-morbidities

of BPS/IC, and miRNA expression and function in these immune-mediated diseases has been addressed in a number of studies. This review projects the miRNA profiling and functional data originating from the research in bladder cancer and immune-mediated diseases on the BPS-specific miRNAs aiming to gain new insight into the pathogenesis of this enigmatic disorder, and highlighting the common regulatory mechanisms of pain and inflammation.

MicroRNAs—general information

The first miRNAs lin-4 and let-7 were discovered as important regulators of the normal temporal control of diverse postembryonic developmental events in *C. elegans* [10, 16]. Most miRNA reduce protein synthesis, whereas some, such as miR369-3 and miR-373, enhance translation [17, 18]. The first link between miRNAs and cancer was made after uncovering the deletion of miR-15 and miR-16 in leukemias [19], and the potential for the miRNA profiling in cancer diagnosis became apparent [20]. This was followed by studies of the role of miRNAs in cardiovascular [21], neurodegenerative [22–24], and autoimmune disease [25, 26]. Therapeutic use of miRNAs was pioneered by administration of miRNAs [27], or their antagonists called antagomirs [28], to the affected cells restoring the normal levels of their protein targets. The locked nucleic acid (LNA)-antagomirs have been successfully introduced into the clinical practice: LNA-anti-miR-122 suppresses hepatitis C virus (HCV) replication in chronically infected animals [29].

The details of miRNA synthesis are well characterized and extensively reviewed [30–32]. Most canonical mammalian miRNA genes have been identified in introns of the protein-coding or noncoding RNAs and around one-third of them are located in the introns of target genes [33]. The miRNA gene transcripts are the primary precursor RNAs (pri-miRNA), which mature in two steps (Fig. 1). Pri-miRNAs are processed in the nucleus by Drosha–DGCR8 complex into a ~70-nucleotide RNA molecules [34]. These precursor hairpin miRNAs (pre-miRNAs) are then exported to the cytoplasm by Exportin-5 (Exp5) [35] and cleaved by Dicer-TRBP complex into a ~20-bp miRNA/miRNA* duplex. The guide strand of the miRNA/miRNA* duplex is usually incorporated into miRNA-induced silencing complex (miRISC) and the passenger strand or (miRNA*) is released and degraded [36]. The final product is RISC-loaded mature miRNA, which is guided to its target mRNA by means of base pairing (Fig. 1).

Some microRNA, such as miR-877 and miR-1226, derive from short intronic hairpins named "mirtrons" [37, 38]. Mirtrons are spliced from the host gene by the spliceosome, become linearized by debranching enzyme and then fold into hairpins, which enter the miRNA-processing pathway without Drosha-mediated cleavage [37, 39]. Recently, a third pathway of microRNA biogenesis has been described: some microRNAs (miR-1225 and miR-1228) do not need DGCR8, Dicer, Exportin-5, or Argonaute 2 for their biogenesis, which nevertheless involves Drosha. This class of microRNAs has been named "simtrons" [40]. The miRNA registry in humans includes 18,226 entries for hairpin precursor miRNAs, expressing 21,643 mature miRNA products [41, 42].

Role of miRNAs in urinary bladder function and pathology

Pathogenesis of bladder pain syndrome (BPS)

Bladder pain syndrome (BPS) is a clinical syndrome of pelvic pain and urinary urgency-frequency in the absence of a specific cause. According to the European Society for the Study of Interstitial Cystitis (ESSIC) proposal and European Association of Urology (EAU) guidelines [43], BPS is diagnosed on the basis of symptoms of pain associated with the urinary bladder, accompanied by at least one other symptom, such as day-time and/or night-time urinary frequency after exclusion of confounding symptoms. Overall, this disease, which has a significant impact on social and psychological well-being, affects approximately 1 million patients in the USA alone [44], with at least 230 confirmed cases per 100,000 females [45]. The etiology of BPS is unknown, and its treatment largely empiric. A multitude of pathogenetic mechanisms have been postulated ranging from neuroinflammatory to autoimmune or possibly infectious or toxic agents, but an inflammatory component is commonly thought to be involved. Epithelial damage has often been invoked: the mucinous layer of the healthy bladder is often compromised in patients with BPS/IC, as well as in some animal models [46, 47].

The integrity of the urothelium is indispensable for the healthy bladder [48]. Mechanical or chemical damage, as well as a bacterial infection can lead to a compromised urothelium allowing urinary solutes to penetrate into the interstitium [49]. The loss of epithelial integrity is a predominant histopathologic finding in biopsies from BPS patients [50]. In human BPS patients, the molecular markers for bladder permeability and proteoglycan core proteins are often down-regulated [51, 52]. Recently, we have shown that the mRNA levels of the tight junction (TJ) proteins ZO-1, JAM-1, occludin, and tight claudins, normally present in water impermeable epithelia and abundant in normal urothelium, were significantly down-regulated in bladder biopsies from BPS patients, indicating a compromised tight junction structure and possibly increased permeability of the urothelium [13]. Therefore it seems possible that urothelial

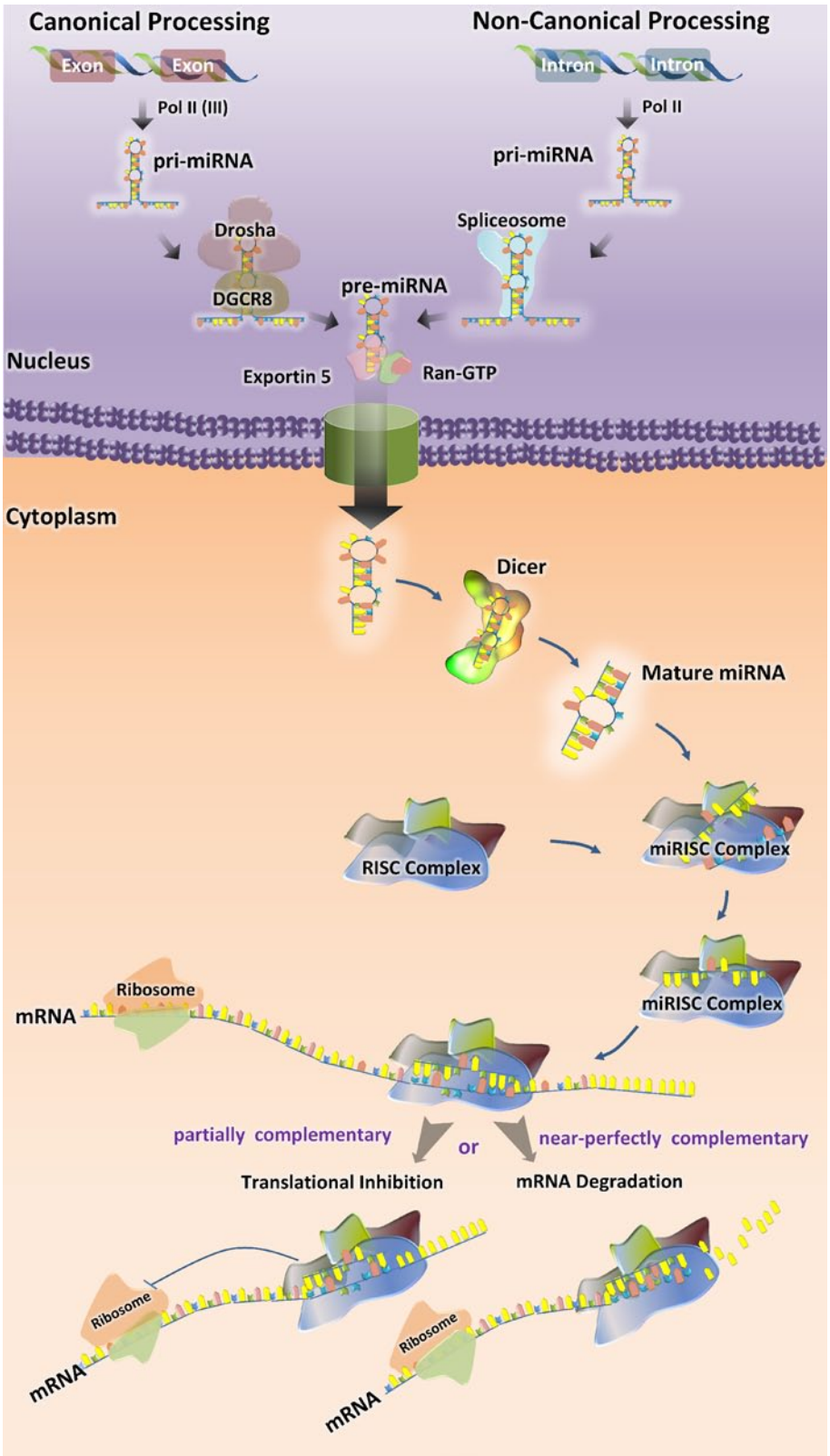

Fig. 1 Biogenesis of miRNAs. Mature microRNAs (miRNAs) are approximately 22 nucleotides long and generated by a two-step process. The first step takes place in the nucleus, where primary miRNAs (pri-miRNA) are converted into precursor miRNAs (pre-miRNAs) by the microprocessor complex containing Drosha and DGCR8 (in canonical process) or spliceosome, which excises the pri-microRNA transcribed from introns (in non-canonical process). Exportin-5 transports pre-miRNAs to the cytoplasm. In the second step, the hairpin structures of the pre-miRNAs are re-cleaved by the RNase III Dicer to generate mature miRNA. The functional strand of the mature miRNA gets incorporated into the RNA-induced Silencing Complex RISC (miRISC), where it guides miRISC to silence target mRNAs through translational repression or mRNA cleavage

damage is a preceding feature of this disorder and might be a causative factor in the pathogenesis of BPS. A decrease of E-cadherin mRNA levels, which we observed at mRNA level in BPS (Monastyrskaya, unpublished), was confirmed by recent findings of lower E-cadherin and ZO-1 expression in IC/BPS, but not in overactive (OAB) bladders, and

suggesting the urothelial barrier function was compromised in BPS but not affected in the OAB bladder [53].

BPS is characterized by several other gene expression changes: tachykinin receptors NK1R and NK2R were significantly down-regulated and bradykinin B1 receptor, cannabinoid receptor CB1, and muscarinic receptors M3–M5 were up-regulated in BPS patients' biopsies [13]. In addition, expression of acid-sensing channels, important for nociceptive pain, was altered in BPS: we detected an up-regulation of ASIC2a and ASIC3 mRNA, whereas ASIC1a remained unchanged [54]. These changes, in conjunction with deficiencies of urothelial barrier, might account for increased nociception in BPS patients.

MiRNAs in BPS and OAB

New information is emerging on the miRNA-mediated regulation of epithelial permeability, bladder contractility, and neurogenic inflammation in bladder dysfunction. While studying the gene expression changes characteristic of BPS, we observed a perplexing down-regulation of tachykinin receptors in the biopsies of BPS patients which prompted us to study its mechanisms [13]. Using cell-based models, we showed that prolonged exposure of NK1R to Substance P (SP) caused a decrease of NK1R mRNA levels and a concomitant increase of regulatory miRNAs miR-449b and miR-500. In the biopsies of BPS patients, the same miRNAs were significantly increased, suggesting that BPS promoted an attenuation of NK1R synthesis via activation of specific miRNAs. We confirmed this hypothesis by identifying 31 differentially expressed miRNAs in BPS patients and demonstrated a direct correlation between miR-449b, miR-500, miR-328, and miR-320 and a down-regulation of NK1R mRNA and/or protein levels. The results of the first miRNA profiling in biopsies of BPS patients are shown in Table 1.

Recently, using a mouse model with an induced deletion of Dicer, two groups examined the role of miRNAs in the regulation of bladder contractility. An induced smooth-muscle-specific Dicer knock-out resulted in significantly reduced levels of miRNAs, including miR-145, miR-143, miR-22, miR-125b-5p, and miR-27a, from detrusor preparations without mucosa. Deletion of Dicer resulted in a disturbed micturition pattern in vivo and reduced depolarization-induced pressure development in an isolated detrusor due to decreased levels of L-type Ca^{2+} channels [55]. In a similar study, the loss of Dicer exacerbated cyclophosphamide-induced bladder overactivity in mice, possibly because the miRNAs capable of targeting P2X mRNAs were impaired, leading to enhanced P2X receptor expression [56]. The authors describe an up-regulation of miR-34a and miR-25 in the mouse model of OAB. Interestingly, miR-25 is also elevated in BPS [13] (Table 1), pointing to the similarities of function of this miRNA in both disorders.

Our follow-up study of the miRNA function in BPS concerned the role of these molecules in the regulation of urothelial permeability [14]. We identified microRNA miR-199a-5p, which was increased in BPS patients' biopsies, as an important regulator of intercellular junctions. Upon overexpression in urothelial cells it impaired correct tight junction formation and caused a permeability increase. MiR-199a-5p directly targeted mRNAs encoding LIN7C, ARHGAP12, PALS1, RND1, and PVRL1 and attenuated their expression levels to a similar extent. Laser microdissection revealed that miR-199a-5p was predominantly expressed in the bladder smooth muscle, but also detected in the mature bladder urothelium and primary urothelial cultures. In the urothelium, its expression can be up-regulated following activation of cAMP signaling pathways. Our results point to a possible link between miR-199a-5p expression and the control of urothelial permeability in bladder pain syndrome. Up-regulation of miR-199a-5p and concomitant down-regulation of its multiple targets might be detrimental for the establishment of a tight urothelial barrier, leading to chronic pain.

miRNA profiling in bladder cancer (BCa)

Except the studies discussed above, most of the information on the role of miRNAs in the bladder comes from the work done in BCa. Here we summarize the published data in order to identify the organ-specific miRNA expression patterns and draw comparisons between BCa and bladder dysfunction.

BCa is one of the most common cancers in the world [57], the fifth most commonly diagnosed non-cutaneous solid malignancy, and, after prostate cancer, the second most frequently diagnosed genitourinary tumor [58]. In 2008, 386,300 new cases of bladder cancers were diagnosed globally [59]. The early miRNA screening attempts were aimed at defining the cancer-specific miRNAs, and differentiating between the tumor stages by means of miRNA profiling. The first study in human bladder compared the miRNA expression profiles of bladder and kidney cancers [60]. Both types of cancers were found to share many miRNAs. The most significant up-regulated miRNAs in BCa versus normal bladder (1.2-fold change cutoff; $p < 0.05$) were: miR-223, miR-26b, miR-221, miR-103-1, miR-185, miR-23b, miR-203, miR-17-5p, miR-23a, and miR-205. However, this study did not identify any significantly down-regulated miRNA in tumors and the data did not overlap with the later published results [61], possibly because of the limited number of reference samples (two normal mucosa).

Expression of 343 miRNA was studied in noninvasive and invasive bladder carcinoma cell lines in order to identify the miRNA signature in BCa suitable for discriminating the superficial from the invasive disease [62]. Nine miRNAs

Table 1 Comparative analysis of miRNA expression profiles

Comparison of miRNA profiles					
miRNA	BPS	BCa	IBD (CD, UC)	Asthma, RA, MS, SLE	Pain
let-7 g	Up				Up after opioid treatment [109] Altered in complex regional pain syndrome (CRPS) [113]
miR-23a	Up	Up [60]	Up [150]	Up in SMC remodeling/ asthma [88]	
miR-23b	Up	Up [60]	Up [151]	Up in SMC remodeling/ asthma [88]	Up in neuropathic pain [110]
miR-25	Up			Up in SMC remodeling/ asthma [88]	
miR-26a	Up			Up in SMC remodeling/ asthma [126]	
miR-27a	Up				
miR-30e-5p	Up				
miR-95	Up				
miR-130b	Up				Down in CRPS [113]
miR-133b	Up	Up [61], Down [66]	Up [103]		Down after morphine—role in pain [152]
miR-148a	Down			Up in SLE [79]	
miR-182	Down				
miR-186	Up				
miR-192	Up		Up [104]		Down in CRPS [113]
miR-199a(-5p)	Up	Down [66]	Up [153]		
miR-320(a)	Up	Up [61]	Down [151]		
miR-324-3p	Up		Up [103]		Down in CRPS [113]
miR-328	Up				
miR-342(-3p)	Up				
miR-379	Up				
miR-422a	Up			Up in MS [94]	Down in CRPS [113]
miR-422b(378)	Up				
miR-449b	Up				
miR-485-5p	Up				
miR-493	Down				
miR-500(*)	Up				
miR-502(-5p)	Up				
miR-511	Up				
miR-572	Up			Up in MS [94]	
miR-594	Up		Up [151]		
miR-597	Up				

Occurrence and regulation of the miRNAs, altered in BPS, *BCa* bladder cancer, *IBD* inflammatory bowel disease *CD* Crohn's disease, *UC* ulcerative colitis, asthma, *RA* rheumatoid arthritis, *SLE* systemic lupus erythematosus, *MS* multiple sclerosis and pain

with significant differential expression included mir-21, mir-31, mir-200a, mir-200c, mir-205, mir-373*, mir-487b, mir-498, and mir-503. MiR-21 expression was up-regulated and miR-205 down-regulated in the invasive compared with the noninvasive bladder cell lines. In 2009, Dyrskjot et al. investigated the expression profile of 290 microRNAs in immortalized urothelial cell lines, tumorigenic cell lines, 106 bladder tumors and 11 normal samples and identified some differentially expressed miRNAs between cancer and normal urothelium. In agreement with the previous study [62], miR-21 was the most up-regulated and miR-145 was the most down-regulated in cancer compared to the normal samples. Several differentially expressed miRNAs were found comparing different tumor stages. Most significant miRNAs up-regulated in progressing tumors were miR-129-5p, miR-518c*, miR-185, miR-133b, miR-373*,

miR-320a, miR-145; and miRNAs, which were mostly down-regulated in progressing tumors were miR-29c, miR-29b, miR-29a, miR-361-5p, miR-203, and miR-205 [61].

Investigation of 322 miRNAs expressed in normal urothelium from patients with high-grade urothelial cell carcinomas (UCC) and disease-free controls revealed that 11 % of miRNAs were differentially expressed [63]. Down-regulation of miRNAs is a common phenomenon in low-grade tumors, and aberrant promoter hyper-methylation influences miRNA down-regulation in low-grade UCC. Many miRNAs down-regulated in low-grade tumors are predicted to target FGFR3 (miRs-99a/100/214/145/30a/125b/507). In contrast, in high-grade UCC up-regulation of many miRNAs including miRNA-21 can suppress p53 function [64].

Lin et al. performed miRNA profiling in BCa and matched normal urothelial epithelium controls and identified 37 up-regulated and 38 down-regulated miRNAs. Among them, miRNA-143 was most down-regulated, 13.7 times lower in tumor than in the matched control [65]. miRNA-143 was not expressed in the human BCa cell lines EJ and T24 and its transfection inhibited cell proliferation and reduced RAS protein levels [65]. Ichimi et al. investigated tumor-suppressive miRNAs in BCa [66]. Upon screening of 156 miRNAs in 14 BCas, five normal bladder epithelium (NBE) samples and three BCa cell lines, miR-145, miR-30a-3p, miR-133a, miR-133b, miR-195, miR-125b, and miR-199a* were shown to be significantly down-regulated in BCas [66]. Keratin 7 (KRT7) mRNA was a common predicted target for the down-regulated miRNAs. Compared to normal bladder epithelium, BCa samples had significantly higher mRNA levels of KRT 7 [66].

Using microarray technology, Song et al. reported that the expression profile of miRNAs was significantly altered in bladder urothelial carcinoma tissue compared to adjacent normal bladder tissue. Consistent with previous observations, most differentially expressed miRNAs were down-regulated. The top ten dis-regulated miRNAs including miR-1, miR-145, miR-143, miR-100, miR-200b, miR-708, miR-133a, miR-133b, and mir-125b were validated by real-time RT-PCR [67].

The first report of genome-wide miRNA expression profiling in human bladder urothelial carcinoma by deep sequencing was published in 2011 by Han et al. [68]. A total of 656 differentially expressed miRNAs were detected comprising known human miRNAs and miRNA antisense sequences (miRNA*s). MiR-490-5p was the most significantly down-regulated and miR-96 was the most significantly up-regulated miRNA [68].

Notwithstanding a long list of miRNAs de-regulated in BCa [69, 70], there is very little overlap in the patterns of miRNA expression between BCa and BPS (Table 1), which might be indicative of differential organ responses to specific diseases.

Inflammation in BPS and the role of miRNA in immune-mediated diseases

BPS is an inflammatory disorder

Inflammation occurs frequently in the lower urinary tract, and most often is a result of a urinary tract infection. One of the intriguing features of BPS is the presence of inflammation, often confirmed by biopsy, in the absence of an inflammatory agent (toxin or microorganism) [71]. Many causative factors have been suggested for BPS, including chronic or subclinical infection, autoimmunity and genetic susceptibility, which could be responsible for initiating the inflammatory response. However, a central role of inflammation has been confirmed in the pathogenesis of interstitial cystitis [72]. Epithelial dysfunction, often observed during BPS, might cause nerve sensitization, which in turn could lead to up-regulation of neurotransmitter release (tachykinins, glutamate, calcitonin gene-related peptide) [73]. Secretion of inflammatory mediators such as SP from sensory nerves has been implicated in the pathophysiology of pain triggering mast cell secretion/neurogenic inflammation [74]. Indeed, the obligatory requirement of tachykinin receptor NK1R in cystitis and its role in mast cell degranulation and inflammation have been confirmed in experiments with NK1R knockout mice [75]. Recently, increased urinary NGF levels were described in BPS/IC patients suggesting that chronic inflammation is involved in this bladder disorder [76].

A recent study investigated whether bladder inflammation could directly modulate the apoptotic signaling pathway in urothelial cells in interstitial cystitis/painful bladder syndrome (IC/PBS, equivalent definition of BPS) [77]. The levels of pro-apoptotic proteins, including phospho-p53 (Ser 15), Bad, Bax, and cleaved caspase-3 were significantly increased in the IC/PBS bladders. These results suggested that the tissue damage and abnormal urothelium in the IC/PBS bladders might be regulated concurrently by inflammatory signals, such as p38 mitogen-activated protein kinase and TNF-alpha. The contents of phospho-p38α and TNF-α in IC/PBS samples were significantly greater than in the control. The in vitro analysis showed that the apoptotic process could be induced by TNF-alpha treatment and anisomycin stimulation in normal urothelial cells [77].

miRNAs as regulators of immune response

During the recent years, plentiful evidence has been amassed pointing to the critical role of miRNAs both in the development of immune system and its function in innate and adaptive responses [78]. Several miRNAs, including miR-155, miR-181a, miR-146a, miR-150, miR-223 and miR-17-92 have been shown to regulate development, differentiation, and function of immune cells (reviewed by [79, 80]).

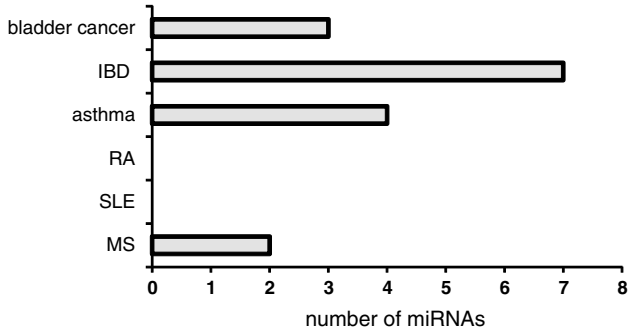

Fig. 2 Similarly regulated miRNAs. Number of miRNAs either up- or down-regulated both in BPS and BCa, IBD, RA, SLE, or MS

The innate immune response is a cellular response, comprising macrophages, monocytes, granulocytes, and dendritic cells, which is started by activation of Toll-like receptors (TLR). TLR signaling induces numerous miRNAs including miR-155, miR-146a, and miR-21 [81]. Additionally, miR-125b and let-7 are important in controlling innate immune responses: miR-125b targets TNF-α, while let-7 targets IL-6 mRNA [82, 83]. Interestingly, although in our study we detected an increased neutrophil infiltration in the BPS patients [13], none of the classical immune cell-specific miRNAs were significantly up-regulated. However, we detected an increase in miR-500* and miR-511, which are important for the dendritic cell, monocyte and macrophage function [84, 85]. These results indicate that the elevated miRNAs levels might originate from the bladder tissue remodeling during BPS, rather than the immune cell infiltration.

Taking into account the role of inflammation in BPS and the lack of data on miRNA regulation in the similar inflammatory disorders of the bladder, we compared the miRNAs identified in other immune-mediated diseases, to the BPS profile. These data are summarized in Table 1 and Fig. 2.

Asthma

Asthma is a common chronic airway disease characterized by airway remodeling (epithelial alteration, fibrosis, smooth muscle hypertrophy) and associated with Th2 response that stimulates eosinophile and mast cell infiltration. Asthma is a common co-morbidity of BPS [86], therefore we correlated the available information on miRNA profiling in this disorder with our data in BPS [13]. In the mouse models of asthma, miR-1 was down- and miR-21 up-regulated, along with 21 other differentially expressed miRNAs, and in another model, miR-16, -21, and -126 were up-regulated. MiR-133, -25, 146a- and -26a regulate human airway smooth muscle cells in asthma models (reviewed by [87]). MiR-23a, -23b, and -25 play an important role in regulating the phenotype of airway smooth muscle via modulating the expression of inflammatory mediators like RANTES, eotaxin, and TNF-α. These genes are responsible for extracellular matrix turnover and expression of contractile proteins (myosin heavy chain) [88]. Interestingly, some of the miRNAs described in this study appear to be important in BPS—we found miR-23a, -23b, -25, and -320 up-regulated in BPS, arguing for an increased smooth muscle phenotype, which is consistent with low bladder volumes and thick bladder walls observed in patients [13].

Autoimmune diseases

The role of miRNAs in autoimmune diseases including rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and multiple sclerosis (MS) has been postulated and confirmed by altered levels of several important miRNAs (reviewed in [79]). Although no data support a direct causal role of autoimmune reactivity in the pathogenesis of BPS/IC, there is ample indirect evidence, such as the strong female preponderance and the clinical association between BPS and other known autoimmune diseases within patients and families [15]. BPS is associated with the diagnosis of RA [89], SLE, and Sjögren's syndrome [90]. In RA, miRNAs miR-146a, miR-223, and miR-155, characteristic of immune cell activation, were significantly up-regulated in synovial fluid samples [91]. In MS, miRNAs miR-34a, -155 and -326 were elevated in active lesions [92]. In SLE, miR-146a, a negative regulator of TLR signaling was profoundly decreased, whereas miR-148a and miR-21 were increased in T cells from patients with lupus [93]. Both miR-422a and miR-22 have previously been implicated in MS [94]. Taken together, miRNA profiling in autoimmune diseases revealed a strong prevalence of immune cell-specific miRNAs and some overlap with BPS profile established in our study [13].

Inflammatory bowel disease (IBD)

IBD, comprising Crohn's disease (CD) and ulcerative colitis (UC), is a gastrointestinal chronic inflammatory disorder and a frequent co-morbidity of BPS [95]. Earlier work has demonstrated that both the number of SP-positive nerve endings and mast cell count were increased in patients with BPS and IBD [96], implicating a similar pathogenic mechanism. There are numerous reports indicating that patients with CD and UC have altered miRNA profiles [97–105]. Analysis of miRNA expression in patients with active UC, inactive UC, CD, irritable bowel syndrome, infectious colitis, and microscopic colitis revealed eight miRNAs (miR-16, miR-21, miR-23a, miR-24, miR-29a, miR-126, miR-195, and Let-7f) which were significantly increased in active UC tissues and three miRNAs (miR-192, miR-375, and miR-422b) were significantly decreased in the UC tissues. Up-regulation of

Table 2 Validated BPS miRNA targets

miRNA	Regulation in BPS	Target; function	Reference
let-7 g	Up	1. Lectin-like oxidized low-density lipoprotein receptor-1; inhibition of SMC apoptosis 2. Type I collagen alpha2 (COL1A2); inhibition of migration 3. μ opioid receptor; role in pain	1. [115] 2. [116] 3. [109]
miR-23a	Up	1. Transcription factor Foxo3a; role in cardiac hypertrophy 2. Muscle-specific ubiquitin ligases, MAFbx/atrogin-1 and muscle RING-finger 1 (MuRF1); inhibition of muscle atrophy 3. Sprouty2 and Sema6A proteins; promote angiogenic activity. 4. E-cadherin	1. [117] 2. [118] 3. [154] 4. [119]
miR-23b	Up	1. Smads 3, 4 5; regulation of TGF-β signaling 2. FZD7 or MAP3k1; metastasis control 3. TAB 2, TAB 3, and IKK-α; suppresses IL-17-induced inflammation	1. [121] 2. [120] 3. [122]
miR-25	Up	1. Bim, pro-apoptotic protein; increase in proliferation, reduction of apoptosis 2. TRAIL Death Receptor-4; reduction of apoptosis	1. [124] 2. [125]
miR-26a	Up	1. SMAD1, 4; increase SMC proliferation, delay differentiation 2. Glycogen synthase kinase GSK-3β; smooth muscle hypertrophy 3. Cyclin D2, EZH2; inhibition of cancer cell proliferation	1. [126] 2. [127] 3. [155]
miR-27a	Up	1. Sprouty2 and Sema6A; enhance angiogenesis 2. Myostatin; role in skeletal muscle proliferation 3. Sprouty 2; enhances proliferation	1. [154] 2. [123] 3 [156].
miR-30e-5p	Up	No validated targets	
miR-95	Up	Sorting nexin 1 (SNX1); increases proliferation in cancer cells	[129]
miR-130b	Up	ZEB1; represses EMT in cancer cells 2. RUNX3 apoptotic protein; increase cell proliferation	1. [130] 2 [157].
miR-133b	Up	1. Pitx3 transcription factor; role in neuron differentiation and pain 2. FAIM, GSTP; promotes apoptosis	1. [152] 2. [158]
miR-148a	Down	1. ROCK1; increase motility 2. WNT1 and WNT10B; activation of WNT/β-catenin pathway	1. [131] 2. [133]
miR-182	Down	1. FOXO1; proliferation 2. CREB1; inhibition of proliferation	1. [159] 2. [160]
miR-186	Up	P2X7; Ca homeostasis	[134]
miR-192	Up	1. SIP1, Ebox repressor; increase of Col1a2 and fibrosis 2. ZEB1/2; increase in fibrosis	1. [135] 2. [136]

Table 2 continued

miRNA	Regulation in BPS	Target; function	Reference
miR-199a(-5p)	Up	1. Cell junction proteins LIN7C, ARHGAP12, PALS1, RND1 and PVRL1; causes epithelial dysfunction 2. HIF1alpha, Sirtuin; hypertrophy in cardiomyocytes 3. Discoidin domain receptor 1; decrease cancer cell proliferation	1. [14] 2. [161] 3. [162]
miR-320(a)	Up	1. β-Catenin; suppresses cancer cell proliferation 2. Neuropilin 1; suppression of cell proliferation 3. Aquaporins 1 and 4; increase of edema 4. NK1R; role in neurogenic inflammation	1. [139] 2. [140] 3. [141] 4. [13]
miR-324-3p	Up	Prolyl endopeptidase; promotes fibrosis	[142]
miR-328	Up	1. NK1R; role in neurogenic inflammation 2. L-type calcium channel a1C, IGFR1; smooth muscle remodeling	1. [13] 2. [143]
miR-342(-3p)	Up	No functional data	
miR-379	Up	ABCC2 transporter; inhibition of efflux for various endogenous and exogenous compounds	[163]
miR-422a	Up	Tumor suppressor	[164]
miR-422b(378)	Up	1. IGFR1; cardiac remodeling and decreased survival 2. Sufu (Suppressor of fused) and Fus-1; increased cell survival and proliferation	1. [144] 2. [165]
miR-449b	Up	1. NK1R; role in neurogenic inflammation 2. CDK6 and CDC25A; cell cycle arrest	1. [13] 2. [145]
miR-485-5p	Up	No functional data	
miR-493	Down	1. FZD4 and RhoC; decrease bladder cancer cell growth and migration 2. IGF1R; inhibition of cell growth and metastasis	1. [146] 2. [147]
miR-500(*)	Up	1. NK1R; role in neurogenic inflammation 2. Marker of dendritic cells and monocytes, regulation of immune response	1 [13]. 2. [85]
miR-502(-5p)	Up	No functional data	
miR-511	Up	TLR4 and CD80; regulation of immune response	[84]
miR-572	Up	No functional data	
miR-594	Up	No functional data	
miR-597	Up	No functional data	

miR-192 was observed in IBD-associated dysplasia [104]. UC patients show an up-regulation of immune cell-specific miR-21 and miR-155 in inflamed tissue [106].

Comparing active to quiescent UC, it was reported that four miRNAs (miR-188-5p, miR-25, miR-320a, miR-346) were down-regulated and five miRNA (miR-29a, miR-29b, miR-126*, miR-127-3p, miR-324-3p) were up-regulated [103]. We found a strong overlap in miRNA expression profiles between BPS and IBD (Table 1).

Inflammatory pain disorders

Pain is one of the hallmarks of inflammation, and chronic inflammatory conditions are often accompanied by chronic pain. Inflammatory pain serves as a warning that hinders the normal bodily function until the stimulus abates or the tissue repairs. Noxious stimuli activate numerous receptors and ion channels, and signals propagate to the central nervous system, where pain is perceived [107]. Epigenetic modifications influence inflammatory cytokine metabolism, steroid responsiveness, and opioid sensitivity thus contributing to the development of chronic pain [108], however, there are little data implicating miRNA in peripheral pain. It was shown that miRNAs might regulate the action of opioid drugs: let-7 miRNA family, including let-7 g, which is up-regulated in BPS, was found to be critical for μ opioid receptor function. Chronic exposure to morphine caused the reduction of MOR levels and concomitant increase in let-7 miRNA synthesis [109]. It is possible that miRNA induction might serve as an adaptive response, aimed at reducing pain and inflammation: the animals with neuropathic pain showed significant improvements after infusion of miR-23b.

This miRNA down-regulated NADPH oxidase 4 (NOX4), a reactive oxygen species (ROS) family member overexpressed in neuropathic pain [110].

MiR-124a is expressed in the spinal cord and has been implicated in pain. Knock-down of miRNA-124a increased the nociceptive behavior via an up-regulation of its pain-relevant target MeCP2 and proinflammatory marker genes [111]. Another recent study identified miR-181a as a modulator of GABA(Aα-1) receptor subunit down-regulation in spinal cord following neonatal cystitis-induced chronic visceral pain in rats [112].

Several miRNAs were profiled from whole blood samples of patients with complex regional pain syndrome (CRPS), a chronic pain condition resulting from dysfunction of central or peripheral nervous systems [113]. Like BPS, CRPS bears many signs of neurogenic inflammation. MiRNA profiling identified differential expression of 18 miRNAs in CRPS patients. Most miRNAs were down-regulated, however there is a significant overlap between the miRNAs, altered in CRPS and BPS (Table 1).

Functional information on miRNAs, altered in BPS

At the time of miRNA profiling in BPS, very few miRNA targets were validated, making it difficult to determine their function and potential importance for the disease pathogenesis. Since then, there has been a surge of publications on the miRNA-regulated proteins in cell lines and disease states. Sometimes multiple targets have been attributed to the same miRNA, implicating it in several, often contradictory, cellular functions. This divergence reflects the pleiotropic activity of miRNAs, which are known to regulate multiple mRNAs [1, 9]. Nevertheless, out of 31 miRNAs from our original study, no targets have been defined for miR-30e-5p, miR-342-3p, miR-485-5p, miR-502-5p, miR-572, miR-594, and miR-597. In contrast, numerous proteins have been identified as effectors of miRNAs belonging to the well-characterized families like let-7. The information on the validated targets of miRNAs altered in BPS is summarized in Table 2 and Fig. 3. Below, we refer in more detail to the functional data, relevant for BPS pathogenesis.

Let-7g belongs to the Let-7 family of miRNAs. Let-7 miRNAs regulate cell differentiation, proliferation, and neuromuscular development [16, 114]. The specific functions of let-7g include the inhibition of smooth muscle cell (SMC) apoptosis by targeting lectin-like oxidized low-density lipoprotein receptor-1 [115]. Additionally, it targets type I collagen alpha2 (COL1A2) [116], as well as μ opioid receptor, and is predicted to have a role in pain perception [109].

miR-23a, -23b, and -27 are members of miR-23 ~ 27 ~ 24 gene cluster. miR-23a is a versatile regulator of muscle development and promotes cardiac hypertrophy

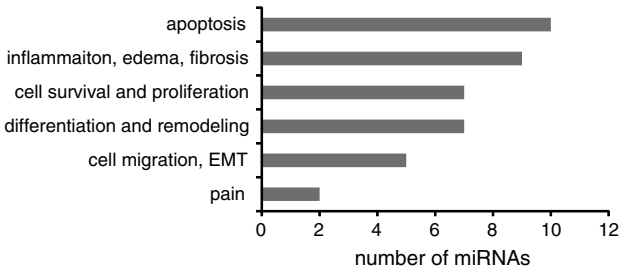

Fig. 3 miRNA functions. Number of miRNAs, altered in BPS and associated with a specific function

[117]. Ectopic expression of miR-23a was sufficient to protect muscles from atrophy in vitro and in vivo [118]. In cancer cell lines, the expression of miR-23a resulted in inhibition of E-cadherin expression [119]. Interestingly, we and the others have shown a decrease of E-cadherin levels in BPS, concomitant with an up-regulation of miR-23a. MiR-23b has pleiotropic functions in cell proliferation, and has been altered in many cancers [120]. It targets and down-regulates Smads and consequently TGF-beta signaling [121]. Recently, an important function of this miRNAs in regulation of autoimmune responses has been highlighted: miR-23b suppresses IL-17-associated autoimmune inflammation by targeting TGF-β-activated kinase 1/MAP3K7 binding protein 2 (TAB 2), TAB 3, and inhibitor of nuclear factor κ-B kinase subunit α (IKK-α) causing a decrease of IL-17-, tumor necrosis factor α (TNF-α)- or IL-1β-induced NF-κB activation and inflammatory cytokine expression and repression of autoimmune inflammation[122]. miR-27 plays a role in cell survival, and its overexpression in C2C12 cells resulted in myoblast proliferation by reducing the expression of myostatin, a critical inhibitor of skeletal myogenesis [123].

miR-25 was found up-regulated in BPS and in a mouse model of OAB, where it has been suggested to down-regulate P2X receptors, although the authors did not proceed beyond the in silico analysis [56]. miR-25 stimulates cell proliferation in ovarian and other cancers [124]. It was shown to protect cells against TNF-related apoptosis-inducing ligand (TRAIL)-induced apoptosis [125].

miR-26a is an important regulator of smooth muscle proliferation and function. miR-26a promotes vascular SMC proliferation while inhibiting cellular differentiation and apoptosis, and alters TGF-β pathway signaling [126]. Overexpression of miRNA-26a blunted SMC differentiation. miRNA-26a influences TGF-β-pathway signaling by targeting Smad-1 and Smad-4. Mechanical stretch up-regulates miR-26a expression and consequently induces SMC proliferation leading to hypertrophy [127]. miR-26a is a regulator of skeletal muscle: it was induced during skeletal muscle regeneration and its inhibition de-repressed Smad activity and inhibited differentiation [128].

miR-95 expression is up-regulated in many tumors. Mechanistic studies revealed that miR-95 repressed the expression of sorting nexin 1 (SNX1), whereas miR-95 silencing up-regulated SNX1 expression [129].

miR-130b targets and inhibits transcription factor ZEB1, suppressing EMT transition. Its transcription depends on p53, and repressed expression of miR-130b triggered ZEB1-dependent EMT [130].

miR-148a is down-regulated in BPS. Rho-associated coiled-coil containing protein kinase 1 (ROCK1) is one of its main targets. In skeletal muscle, increase of miR-148a helps myogenic differentiation through inhibition of ROCK1 [131], and in cancer cells it leads to suppressed tumor cell invasion and metastasis [132]. Similarly, silencing miR-148a in cancerous fibroblasts stimulated cell motility by de-repressing its targets WNT1 and WNT10B and activation of WNT/β-catenin pathway [133].

miR-186 was shown to target P2X7 Ca channel, implicated in cell survival and apoptosis [134].

miR-192 is highly expressed in the kidney epithelial cells, where it controls TGF-β-induced collagen 1 a2 expression via down-regulating Smad-interacting protein SIP1, a E-box repressor [135]. In a separate study, it was shown to increase collagen expression by targeting the E-box repressors Zeb1/2 [136]. Both studies allow concluding that miR-192 promotes fibrosis.

miR-199a-5p is an important regulator of intercellular junctions. Upon overexpression in urothelial cells, it impairs correct tight junction formation and leads to increased permeability. MiR-199a-5p directly targets mRNAs encoding LIN7C, ARHGAP12, PALS1, RND1 and PVRL1 and attenuates their expression levels to a similar extent. It is predominantly expressed in the bladder smooth muscle, but also detected in the mature bladder urothelium and primary urothelial cultures [14].

MiR-199a-5p is up-regulated in cardiac hypertrophy and its overexpression in cardiomyocytes leads to cell-size increase [137]. A separate study claims that in cultured cell lines, the expression of miR-199a and miR-199a* (miR-199a/a*) is confined to fibroblasts [138].

miR-320a has anti-proliferative effect, which it exerts by targeting β-catenin [139], neuropilin 1 (NRP-1), which is a co-receptor of vascular epithelial growth factor [140], and aquaporins 1 and 4 [141]. We have shown that miR-320a down-regulates tachykinin NK1 receptor [13].

miR-324-3p is implicated in the pathogenesis of renal fibrosis. A predicted target of miR-324-3p is prolyl endopeptidase. In cultured tubular cells, transient transfection with a miR-324-3p mimic increased deposition of collagen [142].

miR-328 is regulating the NK1R expression levels [13], and also has a role in smooth muscle remodeling by targeting L-type calcium channel-alpha1C and the insulin growth factor 1 receptor expression, ultimately leading to apoptosis of pulmonary arterial smooth muscle cells [143].

miR-378 is a cardioabundant microRNA that targets IGF1R. In tissues such as fibroblasts and fetal hearts, where IGF1 levels are high, there were either absent or significantly low miR-378 levels, suggesting an inverse relationship between these two factors [144]. miR-449b, in addition to influencing NK1R expression, targets and inhibits oncogenic CDK6 and CDC25A, resulting in cell-cycle arrest [145].

miR-493 is down-regulated in BPS. Interestingly, it has been implicated in regulation of bladder cancer tumorigenesis: expression of miR-493 in bladder cancer (T24, J82, and TCCSUP) cells and tissues was down-regulated. miR-493 decreased cell growth and migration by reducing the protein expression of FZD4 and RhoC [146]. IGF1R was identified as a direct target of miR-493, and its inhibition partially mimicked the anti-metastatic effects [147].

miR-550* was identified by us as a regulator of NK1R expression. Recently, it was shown to be specific for plasmacytoid dendritic cells (pDC) and monocytes [85]. miR-511 is an important regulator of immune cell function in dendritic cells and macrophages: inhibition of the two most highly up-regulated miRNAs, miR-511 and miR-99b, resulted in reduced lower DC-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN) level. Prediction of miRNA-511 targets revealed a number of genes with known immune functions, of which TLR4 and CD80 were validated. Interestingly, under the cell-cycle arrest conditions, miR-511 seems to function as a positive regulator of TLR4 [84].

Conclusions and future directions

Here we conducted a comparative analysis of the miRNA expression profile in BPS, BCa, and several inflammatory disorders, and summarized the validated functional information on the differentially expressed miRNAs. Despite the existence of several miRNA profiling studies in BCa, we found strikingly little overlap between the similarly regulated miRNA species in BCa and BPS.

On the other hand, seven out of 31 miRNA, altered in BPS showed the same regulatory pattern in IBD (Table 1, Fig. 2). IBD shares many features with BPS, including inflammation, pain, smooth muscle remodeling, and changes in epithelial permeability [97]. Disruption of epithelial barrier function was identified as one of the pathologic mechanisms in IBD, and miR-199a-5p, which we have recently characterized as a major regulator of urothelial tight and adherens junctions [14], is also up-regulated in IBD patients (Table 1). Similarly, fibrosis is a common feature of an advanced inflammatory disease and has been

described in both IBD [148] and BPS [149]. Interestingly, miR-192, up-regulated in both disorders (Table 1), has validated targets whose repression leads to increased fibrosis (Table 2). Based on this information, it would be interesting to take a closer look at these miRNAs in order to determine their therapeutic potential.

We found less overlap between BPS miRNAs and miRNAs de-regulated in the other inflammatory and autoimmune diseases, with the exception of asthma, which shared four up-regulated miRNAs. All of them (miR-23a, -23b, -25, and -26a) have been implicated in smooth muscle remodeling, and some have validated targets among the proteins involved in the regulation of muscle growth and differentiation (Table 2). The majority of BPS patients enrolled in our study had low-volume thick-walled bladders [13], and it would be tempting to speculate on the role of these four elevated miRNA in SMC proliferation during BPS.

Some of the functions of the miRNAs altered in BPS could be deduced based on the information about their validated protein targets identified in other cell systems (Fig. 3). Due to the pleiotropic nature of miRNAs, the same miRNA is often implicated in the regulation of opposing processes, i.e., promoting both cell proliferation and apoptosis. Based on the data in Table 2, we grouped the miRNAs according to their functions (Fig. 3). Most of miRNAs, altered in BPS (ten out of 31) have validated targets whose down-regulation results in increased apoptosis. These findings fit well with the emerging data demonstrating increased apoptosis in BPS [77]. The second prominent group was miRNAs influencing inflammation, edema, and fibrosis (nine out of 31). We also identified several miRNAs whose targets include regulators of cell proliferation and differentiation. It would be interesting to determine which bladder layers harbors the miRNAs belonging to these functionally opposing groups, in order to evaluate the differential effects of inflammation on the bladder urothelium and smooth muscle.

MicroRNA research is an exciting and challenging field, and we are witness to its burgeoning. Recent years have brought about a flood of publications containing both the expression profiling and target validation data. Bringing these results together yields helpful insights into the miRNA function in human diseases and their potential therapeutic applications.

Acknowledgments We gratefully acknowledge the financial support of the Swiss National Science Foundation (SNF Grant 320030_135783/1 to K. Monastyrskaya).

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