Activation of common signaling pathways during remodeling of the heart and the bladder

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ABSTRACT

The heart and the urinary bladder are hollow muscular organs, which can be afflicted by pressure overload injury due to pathological conditions such as hypertension and bladder outlet obstruction. This increased outflow resistance induces hypertrophy, marked by dramatic changes in the organs' phenotype and function. The end result in both the heart and the bladder can be acute organ failure due to advanced fibrosis and the subsequent loss of contractility. There is emerging evidence that microRNAs (miRNAs) play an important role in the pathogenesis of heart failure and bladder dysfunction. MiRNAs are endogenous non-coding single-stranded RNAs, which regulate gene expression and control adaptive and maladaptive organ remodeling processes. This Review summarizes the current knowledge of molecular alterations in the heart and the bladder and highlights common signaling pathways and regulatory events. The miRNA expression analysis and experimental target validation done in the heart provide a valuable source of information for investigators working on the bladder and other organs undergoing the process of fibrotic remodeling. Aberrantly expressed miRNA are amendable to pharmacological manipulation, offering an opportunity for development of new therapies for cardiac and bladder hypertrophy and failure.

KEYWORDS

Fibrosis, bladder, heart, miRNA, pressure overload, obstruction, signaling, gene expression

1. Introduction

Although the heart and the urinary bladder serve distinct functions in the body, both are designed to undergo repeated cycles of filling and emptying, albeit with remarkably different kinetics. The heart ensures a constant supply of blood to all other organs, contracting and relaxing continuously from early embryogenesis until death. The bladder on the other hand stores urine as it is being produced by the kidneys and empties when an appropriate opportunity arises, usually once every few hours. Contraction and relaxation of the heart and the bladder are governed by different mechanisms. However when there is a resistance interfering with liquid outflow, their respective contractile units, cardiomyocytes and bladder smooth muscle cells (SMCs), can be subjected to pressure and volume overload.

Notwithstanding the structural and functional differences between the bladder and the heart, both organs respond to the pathological pressure overload by hypertrophy and fibrotic remodeling leading to the loss of contractility [1, 2]. Due to the immediate life-threatening nature of these processes in the heart, the wealth of experimental and clinical data far surpasses that of the bladder. Although not all observations are immediately transferrable from one organ to the other, there are striking parallels in the cellular and molecular mechanisms, which we will discuss in this Review. We will regard the processes, occurring during pressure overload-induced organ remodeling in the heart and the bladder in the context of the role of miRNAs, important epigenetic regulators of stress signaling pathways.

2. Dysfunction of the heart and the bladder caused by sustained pressure overload

Normally, the heart responds to an increase in blood flow by adjusting the stroke volume and rate. Increased filling pressure and volume intensify the stretching force on the myocardium leading to a pressure overload. Because the adult mammalian myocardium has negligible regenerative capacity, it responds to this mechanical stress by producing additional sarcomeres leading to an increase in myocyte size. In the heart volume overload occurring due to mitral regurgitation or arteriovenous fistulas causes increased diastolic stress and results in eccentric hypertrophy. The heart is enlarged, but the wall is thin with big cavities; cardiomyocytes are elongated due to the serial insertion of new sarcomeres [3]. In contrast, hypertension, aortic stenosis and other stimuli, resulting in pressure overload, induce parallel insertion of sarcomeres and an increase in myocyte width, characteristic of concentric hypertrophy [4]. Both pressure overload and stretching of the cardiomyocytes activate growth signaling pathways, which lead to organ remodeling [5]. Cardiac hypertrophy can be physiological, when it is induced by prolonged exercise or during pregnancy, or pathological, when it develops in response to hypertension, aortic stenosis, heart valve insufficiency or myocardial infarction [6]. While both types of hypertrophy provoke increased myocardial mass, pathological hypertrophy is accompanied by extensive fibrosis, cardiac dysfunction and ultimately heart failure. Physiological hypertrophy, in contrast, is characterized by normal cardiac structure and function, and is reversible [7]. Not surprisingly, in each type of cardiac hypertrophy involve different signaling pathways [8]. Pressure overload-induced cardiac hypertrophy initially is an adaptive

response aiming to maintain output. However, when the underlying cause is not eliminated, an extensive organ remodeling program is initiated, resulting in increased myocardial stiffness due to fibrosis, diastolic dysfunction and eventually ventricular dilation and combined diastolic and systolic heart failure [9]. The pathological mechanisms of an isolated volume overload marked by eccentric left ventricular remodeling and dysfunction are less clearly defined, although many parallels are emerging [10]. It has been shown that chronic volume overload is a stimulus for myocardial fibrosis in human patients [11] and animal models [12], with similar signaling pathways activated in both in concentric and eccentric hypertrophy [12].

The lower urinary tract (LUT) comprises the bladder and urethra. The bladder is a musculo-membranous hollow organ, which stores and expulses urine. Prerequisite for normal storage and voiding is a coordinated interaction between the muscle components of the bladder wall (detrusor) and its outlet (urethra and sphincter). During the filling phase the bladder wall maintains a certain degree of tension, which is caused by myogenic contractile activity, while detrusor pressure remains low without detectable muscle contractions [13]. Activation of the sphincter in the absence of detrusor contractions during the filling phase preserves continence [14].

When this process is impaired, lower urinary tract dysfunction (LUTD) with the symptoms of urgency, frequency and incomplete emptying can occur. LUTD is caused by various pathologies including neurologic disorders and bladder outlet obstruction (BOO) [15]. BOO caused by benign prostatic hyperplasia (BPH), which affects the majority of men as they age [16], or urethral strictures, leads to an increased resistance during voiding and is more commonly observed in men than in women [17]. BOO necessitates an increased intravesical pressure to void completely and results in profound structural and functional changes in the bladder wall, bladder hypertrophy, which resemble those of concentric myocardial hypertrophy. The increase in wall thickness corresponds to the severity of obstruction [18], with a significant correlation between the two observed in men with BPH [19]. In contrast, volume overload injury in the bladder of human patients is a rare occurrence, and is usually prevented by catheterization [20]. Bladder hypertrophy is characterized by changes in the expression profile of SM contractile and signaling proteins, modification of extracellular matrix (ECM) proteins, and an increase in bladder innervation [21, 22]. Although relief of obstruction is a traditional form of therapy for this disorder, many storage and voiding symptoms, including urgency, frequency and incomplete emptying, persist after surgery, indicating that timing of the surgical intervention may be critical for complete recovery of bladder function. As bladder outlet obstruction progresses from inflammation to hypertrophy to fibrosis [23], early identification of structural changes can guide therapeutic decisions that might prevent further damage to the bladder and optimize the timing of treatment. Reliable markers of bladder function are urgently needed in order not to surpass the “point of no return”, leading to bladder decompensation/failure.

3. Regulatory miRNAs and their impact in disease

Since their discovery in the early 1990s [24], miRNAs have emerged as central regulators of many biological processes, including proliferation, differentiation and cell death [25]. MiRNAs are

endogenous non-coding single-stranded RNAs of approximately 22 nucleotides which regulate gene expression by post-transcriptional mechanisms upon sequence-specific binding to the 3' untranslated regions (3' UTRs) [25, 26] or, occasionally, the 5'UTRs [27, 28] or coding regions [29-31] of their mRNA targets. The short "seed" sequences (nucleotides 2-8) at the 5' ends of miRNAs are most critical and often sufficient for target selection. MiRNAs exhibit imperfect complementarity with mRNAs, allowing them to regulate multiple genes and thus complicating efforts to predict and functionally validate their targets [32]. Each miRNA target is regulated in the range of a 2-fold change, therefore the additive effect of simultaneous regulation of a large number of transcripts is believed to bring about strong phenotypic effects [33]. The details of miRNA synthesis are well characterized and extensively reviewed elsewhere [34-36].

MiRNAs are produced in a wide range of species, with more than 1000 encoded by the human genome [37]. Changes in their expression profiles, occurring concomitant with emerging pathologies, have led to the notion that in addition to their developmental function, they might influence the responses of already differentiated tissues to physiologic and pathophysiologic stress [38]. This suggests a role of miRNAs in disease states, and indeed dysregulation of their synthesis contributes to fibrotic diseases of the lung, liver and the heart [39-41]. The first miRNA profiling of a LUTD was performed for bladder pain syndrome (BPS) in our laboratory. We identified several miRNAs, which affect the expression of signaling and adhesion molecules known to be relevant for the pathogenesis of bladder pain [42], suggesting a regulatory role of miRNAs. Follow-up experiments established the role of miRNAs in the regulation of urothelial permeability [43] and bladder SMC proliferation and morphology [44]. More recent studies implicated miRNAs in the regulation of bladder contractility [45, 46].

4. The cellular effectors of cardiac and bladder fibrosis

Although cell types comprising the heart and the bladder are different, their response to pathologic pressure bears a lot of similarity. The contractile cells of both organs, cardiomyocytes and detrusor SMCs (dSMC), are capable of a considerable morphologic and proliferative plasticity. Initially, both cardiomyocytes and dSMCs undergo an increase in size as a result of increased synthesis and incorporation of contractile elements. In the heart, this is associated with structural remodeling including changes in fibrillar collagen network and angiogenesis [8]. Chronic pressure overload, unlike physiologic hypertrophy, may then activate pro-fibrotic pathways leading to cell death (apoptosis, necrosis) and the replacement of the lost myocytes with excessive collagen. Fibrosis and reduced capillary density leads to myocardial ischemia, further exacerbating the damage and contributing to the transition from hypertrophy to heart failure [47]. Several cell types are implicated in the heart fibrosis, either directly (collagen-producing fibroblasts and myofibroblasts) or by secreting fibrogenic mediators (macrophages, mast cells, lymphocytes, cardiomyocytes and vascular cells).

Myofibroblasts combine the features of SMCs (expression of SM α -actin) with excessive extracellular matrix protein production, and myofibroblast accumulation has been reported in the pressure overloaded myocardium. Activation of resident cardiac fibroblasts by fibrogenic mediators such as transforming growth factor beta (TGF- β) is probably the most important source of myofibroblasts in the fibrotic heart [48], although their trans-differentiation from pericytes, endothelial cells and circulating progenitors has been described [49]. Immune cells, most notably monocytes and macrophages are increasingly implicated in the regulation of the fibrotic response, both during its initiation (M1 macrophages induced by IFN- γ alone or in combination with TNF- α and GM-CSF) and its resolution and the subsequent reduction of inflammation (M2 macrophages, induced by IL-4 or IL-13) [50]. Similarly, mast cells are instrumental in the regulation of inflammatory and fibrotic processes in the heart. They release a vast array of fibrogenic mediators, including histamine, TNF- α , TGF- β , IL-4, PDGFs and FGFs, and increased density of mast cells has been observed in pressure overloaded hearts [51].

Unlike terminally differentiated cardiomyocytes, dSMCs retain the ability to undergo a switch from contractile to proliferative phenotypes. Both hypertrophy and hyperplasia of dSMCs has been reported during remodeling of obstructed bladders [52]. Contractile properties of dSMCs are gradually impaired during BOO-induced remodeling, accompanied by a shift in myosin heavy chain mRNA isoform expression towards the embryonic/proliferative phenotype [53]. Myofibroblasts, differentiating from resident mesenchymal cells of the bladder serosa, have also been postulated to play a role in the development of detrusor overactivity during experimental outflow obstruction [54]. Similar to their function in the diseased heart, myofibroblasts could be a source of extracellular matrix produced during BOO-induced fibrosis. Experimental partial BOO (pBOO) in animal models progresses through several stages from inflammation to fibrosis [23]. Acute inflammation is characterized by release of cytokines such as IL-1, TNF- α and IL-6 [55], prostaglandines, histamine secretion, vasodilation and increased vascular permeability [56]. These mediators cause urothelial irritation, which is responsible for the symptoms of urgency, dysuria and increased frequency. Cytokines and growth factors have been shown to cause inflammation and bladder remodeling after pBOO. A recent study demonstrated that the inflammatory cytokine IL-1 β is essential in inducing bladder remodeling and deterioration of bladder function in pBOO [57].

5. Role of miRNAs in the signaling pathways activated by sustained pressure overload in the heart and the bladder

The activation and phenotypic modification of contractile and interstitial cells described above is accompanied by changes in gene expression reflected in differential activation of intracellular signaling pathways. In the heart, comparative studies between physiological and pathological hypertrophy revealed activation of pathways specific for each condition. In reversible, physiological cardiac hypertrophy, major activated pathways are IGF1-phosphoinositide 3-kinase (PIK3, (p110 α)-Akt pathway [58], gp130/JAK/STAT pathway [59], thyroid hormone signaling [60] and heat shock transcription factor (HSF1) [61]. In contrast, pathological cardiac hypertrophy is induced by G α q

signaling activated by GPCRs of angiotensin II (Ang II) [62], endothelin ET1 [63] and catecholamines [64] and eventually converging with intracellular Ca^{2+} /calcineurin [65], protein kinase C (PKC) and PTEN-PIK3 γ pathways [66].

To date, the function of miRNA in stress-responsive pathways has predominantly been studied in the context of cardiovascular disease, and less well described in the bladder. Nevertheless, activation of the main intracellular signaling pathways, relevant for hypertrophy, fibrosis and decompensation such as Ca^{2+} /calcineurin signaling, TGF- β , Wnt/Frizzled/ β -catenin signaling and ECM remodeling have been reported in obstructed bladders, and some similarities in miRNA expression profiles are beginning to emerge. Here, we summarize the main processes involved in pressure overload-induced cardiac hypertrophy and decompensation, and define their role in BOO-induced bladder dysfunction.

5.1. Ca^{2+} /calcineurin/Nuclear Factor of Activated T cells (NFAT) pathway

Contractile function and cell growth of both cardiomyocytes and dSMCs are controlled by calcium. Calcium is an important second messenger for G protein-coupled receptors and biomechanical stress and its downstream effectors calmodulin and calcineurin transmit the information encoded in the fluctuations of intracellular Ca^{2+} concentration into cellular processes. The Ca^{2+} /calcineurin/NFAT pathway, activated by sustained elevation of intracellular Ca^{2+} levels, plays a central role in the development of cardiac hypertrophy and failure [67]. Phosphorylated NFAT proteins reside in the cytoplasm and are dephosphorylated by activated calcineurin. Their subsequent translocation to the nucleus initiates a muscle hypertrophy gene expression program [68]. Calcineurin activity is increased in hypertrophied and failing human hearts [69], animal models of aortic constriction [70] and transgenic animals, developing cardiac hypertrophy upon overexpression of an activated form of this enzyme [67].

There is evidence that muscle-specific miRNAs miR-1 and miR-133, which are clustered on the same chromosomal locus [71, 72], are involved in the regulation of cardiac hypertrophy. Expression levels of miR-1 increase during differentiation [73], making it the most abundant miRNA in the adult heart [74]. The data indicating that miRNAs affect the Ca^{2+} -induced gene expression are just emerging: in the heart, miR-1 inhibits the translation of calmodulin-encoding mRNAs and down-regulates calcium-calmodulin signaling through calcineurin to NFAT. MiR-1 also negatively regulates the expression of myocyte enhancer factor MEF2a and GATA4, key transcription factors mediating calcium-dependent changes in gene expression [75]. MiR-133a has been implicated in targeting NFATc4, reducing its levels and attenuating alpha-adrenergic receptor agonist-induced cardiomyocyte hypertrophy [76]. A suppression of miR-133 induces hypertrophy *in vivo* and *in vitro* by enhancing calcineurin expression [77], whereas its overexpression produces the opposite effect [72].

Studies in mice and humans showed that an overexpression of pre-miR-212 or pre-miR-132 leads to an enlargement of cardiomyocytes and development of cardiac hypertrophy. Mice without miR-

212/132 were protected from cardiac hypertrophy, and the pharmacological knock-down of miR-132 by antagomir injection effectively blocked cardiac hypertrophy [78]. Furthermore, this study demonstrated that the miR-212/132 family targets and negatively regulates the expression of FoxO3 transcription factor, which is an anti-hypertrophic and pro-autophagic factor in cardiomyocytes. MiR-212/132 overexpression and FoxO3 down-regulation result in a hyperactivation of the pro-hypertrophic calcineurin/NFAT signaling pathway [78]. Interestingly, it has also been shown that miR-1 expression in cardiomyocytes is regulated by the Akt- FoxO3 pathway [79] and miR-1 levels were down-regulated in miR-212/132 overexpressing transgenic hearts [78].

Both the α - and β -isoforms of the calcineurin catalytic subunit are direct targets of miRNA miR-499. MiR-499 inhibits cardiomyocyte apoptosis through the suppression of calcineurin-mediated dephosphorylation of dynamin-related protein-1 (Drp1), thereby decreasing Drp1 accumulation in mitochondria and Drp1-mediated activation of the mitochondrial fission program. It has been suggested that modulation of miR-499 levels may provide a therapeutic approach for treating myocardial infarction and heart fibrosis [80].

MiR-22 is essential for hypertrophic cardiac growth in response to stress. MiR-22-null hearts blunted cardiac hypertrophy and cardiac remodeling in response to 2 independent stressors: isoproterenol infusion and an activated calcineurin transgene. Loss of miR-22 sensitized mice to the development of dilated cardiomyopathy under stress conditions. Sirt1 and specific histone deacetylase HDAC4, which normally binds to and represses cardiac-specific transcription factors MEF2c and MEF2d were identified as miR-22 targets in the heart [81].

Similarly, miR-23a expression was up-regulated upon prolonged treatment with the isoproterenol and aldosterone. Knockdown of miR-23a could attenuate the ensuing hypertrophy, suggesting that miR-23a is able to convey the hypertrophic signal. The expression levels of miR-23a were controlled by Ca^{2+} /calcineurin via NFATc3 [82].

miR-199b is another direct calcineurin/NFAT target gene which increases expression in mouse and human heart failure, and targets the nuclear NFAT kinase dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1a (Dyrk1a), constituting a pathogenic feed-forward mechanism that affects calcineurin-responsive gene expression. Inhibition of miR-199b by a specific antagomir normalized Dyrk1a expression, reduced nuclear NFAT activity and caused marked inhibition and even reversal of hypertrophy and fibrosis [83].

The involvement of calcineurin / NFAT signaling in bladder hypertrophy is just beginning to emerge: mRNAs for all four known calcineurin-responsive NFAT isoforms were detected in urothelium and SMCs, and experimentally-induced pBOO in mice was shown to activate the calcineurin/NFAT pathway [84]. Treatment of pBOO mice with cyclosporine A (CsA), an inhibitor of calcineurin, significantly reduced hypertrophy secondary to obstruction and improved bladder performance [84]. In agreement with activation of calcineurin/NFAT signaling in obstructed bladders, a recent study has demonstrated an up-regulation of miR-212/132 cluster in pBOO animals [85]. The putative

binding sites in the miR-132/212 promoter were compared with transcription factors predicted to be active in the obstruction model, and Creb and Ahr were implicated in miR-212/132 induction. Additionally, serum stimulation and protein kinase C activation induced miR-212/132 expression in human detrusor cells. MeCP2, Pnkd and Ache were validated as direct targets of miR-132 and miR-212 in the detrusor, suggesting that this miRNA cluster influences gene expression and limits cell growth [85].

5.2. TGF- β in hypertrophy and fibrosis

TGF- β is a key player in wound healing, fibrosis and negative regulation of inflammation, and its levels are increased in the majority of fibrotic diseases [86, 87]. Its co-factors, the activating and inhibiting Smads, transmit the signal to the nucleus, resulting in activation of target genes, including contractile and ECM proteins. Non-canonical, Smad-independent signaling via TGF- β occurs via direct activation of TGF- β -activated kinase TAK1, and plays a pivotal role in pathological cardiac response to sustained pressure overload [88]. Importantly, bone morphogenetic protein BMP7, a negative regulator of TGF- β signaling during fibrosis, is selectively suppressed by activated TAK1 in TGF- β -stimulated myocytes. TAK1 can also be activated by inflammatory cytokines IL-1 and TNF- α , actively released during cardiac and bladder obstruction [89], and might represent an important cross-talk point between different fibrogenic pathways.

In addition to cardiomyocytes, the myocardium contains cardiac fibroblasts, endothelial and smooth muscle cells [90]. TGF- β not only activates cardiomyocytes, resulting in cell hypertrophy and production of fibrogenic mediators, but also has a profound and consistent effect on cardiac fibroblasts, which are probably its main effectors in the heart [91]. Cardiac fibroblasts, which derive from mesenchymal cells, are able to differentiate into myofibroblasts and produce vast quantities of ECM proteins [92]. Activated myofibroblasts are found in the myocardium during cardiac injury [93]. Accumulation of ECM proteins is the reason for stiffness of the myocardium and leads to impaired heart function. TGF- β 1 induces SM differentiation via activation of p38 MAPK, which is in turn required for activation of the SMC marker gene promoters. TGF- β also activates Rho A and its downstream target protein kinase N1 (PKN). Overexpression of active PKN increases the transcriptional activity of the promoters that control expression of SM α -actin, SM-myosin heavy chain, and SM22- α , and the activities of serum-response factor (SRF), GATA, and MEF2-dependent enhancer-reporters [94]. This enables TGF- β and its downstream target connective tissue growth factor (CTGF) to stimulate differentiation of myofibroblasts and enhance collagen deposition in the pressure-overloaded heart.

Similar to the heart, TGF- β 1 is increased in obstructed bladder undergoing hypertrophy [23] and collagen deposition [95]. Progressive accumulation of ECM, characteristic of the fibrotic bladder, leads to reduced contractility and impaired micturition during the final decompensated/fibrotic stage. CTGF increases as a response to stress and enhances the stretch-mediated collagen deposition in

the bladder, whereas TGF- β increases smooth muscle growth and differentiation, both resulting in the hypertrophy during the early stages of obstruction [96].

In addition to influencing mRNA expression, TGF- β induces expression of several miRNAs involved in fibrosis, including miR-192, and miR-491-5p. The role of miR-192, also up-regulated in BPS [97], in promoting fibrosis is best defined in the kidney [98], where it may be a critical downstream mediator of TGF- β /Smad3 signaling for the development of renal fibrosis [99]. CTGF also increases miR-192 expression [100], whereas vascular endothelial growth factor (VEGF), a key promoter of angiogenesis, suppresses epithelial-mesenchymal transition (EMT) by inhibiting Smad3 and miR-192, whose expression is Smad3-dependent [101]. In addition to miR-192, Smad3 mediates renal and cardiac fibrosis by up-regulating miR-21 and down-regulating the miR-29 and miR-200 families [87]. MiR-21 is especially important in the heart, where it is induced during hypertrophy [86], and has been postulated to target inhibitory Smad7, thus enhancing TGF- β signaling. MiR-21, which is increased in cardiac fibroblasts during stress, represses Sprouty homolog 1 (Spry1), a negative regulator of the extracellular signal-regulated kinase/mitogen-activated protein (ERK-MAP) kinase signaling pathway [102]. Spry1 is known to induce secretion of fibroblast growth factor and fibroblast survival, leading to increased interstitial fibrosis. Silencing miR-21 using an antagomir reduced cardiac ERK-MAP kinase activity and inhibited cardiac fibrosis in pressure-overload mouse models [102].

Overexpression of hepatocyte growth factor (HGF) reduces TGF- β 1 production and activity in cardiac fibroblast cultures and animal models of cardiomyopathy [103]. HGF is a multi-potent endogenous repair factor secreted primarily by mesenchymal cells, it enhances normal tissue regeneration and inhibits fibrotic remodeling by inhibiting myofibroblast proliferation. TGF- β 1 suppresses HGF expression via miR-199a-3p [104]. HGF also induces anti-fibrotic miR-29 expression, while TGF- β stimulation leads to a reduction in miR-29 expression and de-repression of collagen synthesis. Stimulation with HGF was associated with highly elevated miR-29 levels and markedly repressed synthesis of collagen-I and -IV [105].

MiRNAs 143/145 (miR-143/145) are highly expressed in adult SMC lineages and mediate, in part, the expression of several SMC contractile genes. They modulate cytoskeletal dynamics and promote SMC migration following an injury [106]. TGF- β 1 stimulates miR-143/145 expression in a dose- and time-dependent manner. The effect of TGF- β 1 on miR-143/145 is mediated by p38 MAPK and is dependent on the myocardin and serum response factor transcriptional switch as well as Smad4 [107]. Pre-miR-145 promotes, whereas an anti-miR-145 partially blocks, TGF- β 1-induced SMC differentiation [107].

MiR-199a-5p is another miRNA, strongly up-regulated by TGF- β [108]. It is particularly important in cells of mesenchymal origin. MiR-199a-5p is highly expressed in bladder smooth muscle [43], as well as cardiomyocytes. In cardiomyocytes it down-regulates hypoxia-inducible factor 1 α (HIF-1 α) and sirtuin 1 [109], and promotes cardiomyocyte hypertrophy [110]. MiR-199a-5p targets WNT2 [111], and regulates multiple cytoskeletal remodeling pathways [43, 44]. Its overexpression in the

dSMCs significantly increased cell size and up-regulated SM-specific contractile proteins. These changes were mediated by up-regulation of SM-specific transcriptional activator myocardin at mRNA and protein levels. Myocardin-related transcription factor (MRTF-A) downstream targets *Id3* and *MYL9* were also induced. Up-regulation of myocardin was accompanied by down-regulation of WNT-dependent inhibitory Krüppel-like transcription factor 4 (*KLF4*) in miR-199a-5p overexpressing cells. In contrast, *KLF4* was induced in anti-miR-expressing cells following the activation of WNT2 signaling, leading to repression of myocardin-dependent genes. MiR-199a-5p plays a critical role in the WNT2-mediated regulation of proliferative and differentiation processes in the smooth muscle and may act as a key modulator of smooth muscle hypertrophy, relevant for organ remodeling (Figure 1) [44].

Early cardiac marker genes, such as cardiac-specific homeobox (*Csx/Nkx2.5*), myocardin, homeodomain only protein, *GATA4*, and *MEF2C*, are thought to participate in cardiomyocyte differentiation and contribute to cardiac hypertrophy in animal models. Myocardin, a potent transcriptional coactivator of SM and cardiac genes, is up-regulated in the myocardium in animal models and human end-stage heart failure. Up-regulation of myocardin expression in the heart has been linked to hypertrophic cardiomyopathy in human patients [112]. Silencing *MYOCD* gene by a short hairpin RNA in a mouse model of heart failure normalized the levels of myocardin and several fetal genes, including SMC genes and attenuated cardiac muscle dysfunction [113]. Interestingly, hypoxia has been shown to induce both *MYOCD* gene expression and cardiomyocyte hypertrophy [114], although the exact nature of this link is not clear. Taking into account that miR-199a-5p is also up-regulated by hypoxia [115], and in view of its ability to indirectly induce the *MYOCD* gene which we observed in SMCs [44], it might act as an important factor promoting increased, pathological myocardin synthesis in cardiovascular diseases. A recent study has demonstrated that *KLF4* attenuates isoproterenol-induced cardiac hypertrophy by inhibiting myocardin expression and activity [116], therefore a down-regulation of WNT-dependent *KLF4* protein by miR-199a-5p [44] could have an opposite effect, ultimately up-regulating myocardin and causing heart hypertrophy.

In contrast to the examples above, TGF- β is also capable of repressing the synthesis of several miRNAs, such as miR-29, miR-30 and miR-133 which have anti-fibrotic properties [117]. In the heart, the miR-29 family targets mRNAs that encode proteins involved in fibrosis, including multiple collagens, fibrillins, and elastin. Down-regulation of miR-29 de-represses the expression of these mRNAs and enhances fibrotic response [118]. MiR-133a1/a2, expressed in cardiomyocytes, are down-regulated during cardiac injury. MiR-133a-1 and miR-133a-2 are identical, muscle-specific miRNAs that are regulated during muscle development by the SRF transcription factor. Their deletion enhanced ectopic expression of smooth muscle genes in the heart and aberrant cardiomyocyte proliferation by up-regulating SRF (negative feedback loop) and cyclin D2 [119].

In the bladder, a recent study addressed the role of miR-133 during BOO-induced TGF- β -mediated hypertrophic and fibrotic response. Similar to the diseased heart, miR-133 was down-regulated during bladder remodeling in the rat and stimulation of the bladder SMCs with exogenous TGF- β 1 resulted in a dose-dependent decrease of miR-133a/b levels. Transfection of miR-133 mimics

attenuated TGF- β 1-induced SM α -actin, ECM proteins and fibrotic growth factor expression, and upregulated high molecular weight caldesmon expression in the bladder SMCs. Downregulation of p-Smad3, not p-Smad2 by miR-133 was also detected. Additionally, miR-133 overexpression suppressed TGF- β 1-induced SMC hypertrophy and proliferation by directly targeting CTGF. In the obstructed bladder, miR-133 modulates TGF- β 1-induced SMC phenotypic changes by down-regulating CTGF through the TGF- β -Smad3 signaling pathway [120]. Muscle-specific miR-133 clusters together with miR-1, which has been implicated in regulation of bladder overactivity by reducing the expression levels of connexin 43 (GJA1), a major gap junction protein in the detrusor, in a myocardin-dependent manner [121]. Myocardin decreased GJA1 expression through miR-1 up-regulation, thereby contributing to the maintenance of normal sensitivity and increase in bladder capacity.

In the bladder miR-29 is negatively regulated by TGF- β and plays a role in remodeling of the extracellular matrix during BOO. MiR-29 was repressed in an animal model of pBOO leading to matrix remodeling and increased bladder stiffness [46]. c-Myc, NF-kappaB and Smad3 repressed miR-29, causing increased levels of miR-29 target mRNAs tropoelastin, the matricellular protein Sparc and collagen IV. Thus, TGF- β signaling via its downstream effectors reduced miR-29 in BOO, and the resulting decline in miR-29 contributed to matrix remodeling and altered passive mechanical properties of the detrusor [46].

5.3. Renin-angiotensin system

The renin-angiotensin system (RAS) is emerging as one of the key players in hypertrophy and fibrosis, in both the heart and the bladder [122, 123]. Angiotensin II (Ang II) is a peptide that regulates blood pressure, vascular tone and sodium homeostasis. Most of its effects, including contractile increase, are mediated via AT1 and less well described AT2 G α q protein-coupled receptors and Ca²⁺ release from the sarcoplasmic reticulum. The heart contains a local RAS system, which is activated by pressure overload [124], and blocking Ang II formation with angiotensin converting enzyme inhibitors attenuates pressure overload-mediated heart hypertrophy in animals and humans [125]. Ang II stimulates AT1 receptors which are expressed in the bladder in both smooth muscle and urothelial cells, and Ang II-mediated detrusor contractility has been reported [126]. Application of AT1 receptor antagonists in animal models of pBOO significantly reduced organ hypertrophy and collagen deposition, implicating activation of RAS in bladder fibrosis [127]. It is possible that the pro-fibrotic activity of Ang II is mediated by downstream activation of TGF- β signaling and subsequent collagen accumulation [128]. These observations were further confirmed by the fact that inhibitory Smad7 prevented Ang II-induced cardiac fibrosis via mechanisms involving the Sp1-TGF- β /Smad-NF- κ B-miR-29 regulatory network [128].

Many miRNA that affect activated Ang II-AT1 signaling act on TGF- β or Ca²⁺ pathways. A global miRNA array analysis of Ang II-mediated miRNA regulation in cells overexpressing the AT1 receptor focused on separating the role of G α q/11-dependent and -independent pathways. Five

miRNAs (miR-29b, -129-3p, -132, -132* and -212) were induced by Ang II, and their up-regulation was blocked following Gαq/11 and MEK1 inhibition [129]. Chronic Ang II infusion induced an up-regulation of the hypertrophic miR-132 and miR-212 [130].

There is evidence of a cross-talk between interleukin-6 (IL-6), which plays a role in cardiac hypertrophy and fibrosis, and Ang II. Binding of Ang II to AT-1 receptors stimulates IL-6 secretion [131]. Expression of both IL-6 and its signal transducer gp130 has been reported in cardiomyocytes and cardiac fibroblasts [132] and IL-6 concentration in cultured cardiomyocytes was decreased following application of the AT-1 receptor antagonist, strengthening the link between the two signaling systems [132]. These results suggest that IL-6 contributes significantly to cardiomyocyte hypertrophy by an autocrine pathway and to fibroblast proliferation by a paracrine pathway and that these effects could be mediated by Ang II.

AT1 receptor is a target for miR-155 [133], a miRNA important both in regulation of immune response and cardiac hypertrophy. MiR-155 is expressed in cardiomyocytes, where it represses the expression and function of MEF2A and Jarid 2. In mouse models of cardiac hypertrophy, loss of miR-155 suppressed cardiac hypertrophy and cardiac remodeling in response to two independent pathological stressors, transverse aortic restriction and an activated calcineurin transgene and prevented the progress of heart failure thus substantially extending the survival of calcineurin transgenic mice [134].

5.4. Hypoxia

As obstruction- or pressure overload-induced bladder and heart remodeling progresses from activated contractility to compensatory hypertrophy, the intrinsic demands for oxygen steadily grow, and cause up-regulation of HIF-1 α (hypoxia inducible factor-1 α) [135, 136]. HIF-1 α functions as a master regulator of O₂ homeostasis by controlling O₂ delivery and utilization. Heart tissue hypoxia or ischemia resulting from arterial stenosis induces HIF-1 α activity, which is required for the production of angiogenic growth factors that stimulate vascular remodeling to increase blood flow through collateral vessels [135]. Interestingly, volume overload, which is less pro-fibrotic than pressure overload in the heart [137] is characterized by reduced propensity for ischemia and linked to a reduced expression of a novel cytokine resistin [138], which interacts with the profibrotic factors.

As obstruction progresses in the bladder, hypoxia develops in the detrusor smooth muscle, marked by an elevation of inducible nitric oxide synthase and VEGF mRNA levels and a decrease in the intercontraction interval [139]. Similarly, hypoxia-induced endogenous up-regulation of VEGF was shown to accelerate growth of the bladder by detrusor and urothelium hypertrophy and hyperplasia [140]. HIF-1 α activation in bladder outlet obstruction involves mechanisms beyond the accumulation of HIF-1 α protein and results in a switch of the energetic support of contraction to anaerobic glycolysis characterized by increased expression of glucose transporters and glycolytic enzymes combined with mitochondrial remodeling. Together, these changes uphold contractility when mitochondrial respiration is limited [141].

Hypoxia has been shown to influence the expression of many miRNAs via a plethora of signaling pathways. In vascular smooth cells, hypoxia induces SMC proliferation by increasing the expression levels of miRNA-21, thus regulating the smooth muscle phenotype. Hypoxia-induced platelet-derived growth factor (PDGF) has been shown to induce the transcription of miRNA-221 and miRNA-222, leading to the phenotypic switching in vascular smooth muscle cells [142]. Overexpression of these miRNAs causes down-regulation of myocardin, instrumental in maintaining the contractile phenotype of smooth muscle. The induction of miRNA-210 and -373 is dependent on hypoxia-induced factor HIF-1 α . [143]. On the other hand, HIF-1 α is itself a target of miR-199a-5p, predominantly expressed in cardiomyocytes, and playing a role in the regulation of cardiac hypertrophy [110]. Because miR-199-5p is increased by cardiac hypoxia following activation of DNM3os, a noncoding transcript that harbors the miRNA cluster miR-199a~214 [115], it forms a negative feed-back loop controlling the levels of HIF-1 α . A spectrum of miRNAs, including miR-23, -24, -26, -27, -103, -107, -181, -210, and -213 is induced in response to low oxygen, at least some via a HIF-dependent mechanism [144]. Among these, hypoxia-induced miR-210 is a master-regulator and marker of hypoxic response [145], and has an anti-apoptotic effect on SMCs [146].

5.5. Phosphoinositide 3-kinase (PI3K) signaling in pathologic hypertrophy

Phosphoinositide 3-kinase (PI3K), Akt and mammalian target of rapamycin (mTOR) pathway is important for regulation of cardiomyocyte size, survival, angiogenesis and inflammation in cardiac hypertrophy [147]. In contrast to the receptor tyrosine kinase-coupled p110 α isoform of PI3K, involved in signal transduction during physiological heart growth, GPCR-coupled p110 γ PI3K is prominent in heart pathology. p110 γ PI3K does not affect cardiomyocyte size, but is a negative regulator of heart muscle contractility [66]. Activated PI3K creates phospholipid binding sites for a serine/threonine kinase Akt on the cell membrane. Akt mediates growth signals through inactivation and phosphorylation of pro-apoptotic proteins [148]. Phosphatase and tensin homologue on chromosome ten (PTEN) is a negative regulator of Akt. PTEN is targeted by miR-21, a pro-fibrotic miRNA discussed earlier, and modulation of miR-21 regulated expression of matrix metalloprotease-2 (MMP-2) via a PTEN pathway [149]. Similarly, reduction of miR-22 levels attenuated an Ang II-induced hypertrophic reaction by directly targeting PTEN mRNA. During cardiomyocyte hypertrophy, attenuation of miR-22 in rat cardiomyocytes efficiently protected them from hypertrophic effects through de-repressing PTEN [150]. PTEN dephosphorylates the phospholipids, which are necessary for membrane targeting and activation of Akt [151]. Interestingly, moderately activated Akt showed cardioprotection, whereas its prolonged chronic activation was detrimental for heart function [152]. Similarly, cardiac mTOR overexpression inhibited necrosis, reduced expression of proinflammatory cytokines, including IL-6 and TNF- α and protected hearts against ischemia-reperfusion injury [153].

In BOO, the role of PI3K signaling has not been investigated in great detail however, there is evidence of its importance: activation of this pathway has been reported during bladder hypertrophy in acute and chronic cystitis [154]. In SMC culture models of BOO, hydrostatic pressure stimulated

cell proliferation via the PI3K/SGK1 signaling pathway [155], emphasizing its importance in mechanotransduction. The growth factor PDGF promotes the division of fibroblasts and members of the PDGF family are involved in myocardial fibrosis [156]. PDGF receptor tyrosine kinase transactivates PI3K-Akt pathway, causing fibrosis. PDGF is released from bladder fibroblasts in response to hydrostatic pressure [157]. In vitro studies in cultured bladder SMCs showed that in response to stretch or mechanical overload PDGF-BB is released and activates PI3K-Akt signaling [158], potentially linking stretch-activated signaling and cell proliferation.

5.6. ECM and contractile protein remodeling

Remodeling of ECM takes place in pressure-overloaded hearts and obstructed bladders following activation of the TGF- β pathway and leads to increased deposition of collagen and activated synthesis of fibronectin and tropoelastin. In the heart, ECM deposition contributes to organ stiffness and causes a progressive loss of contractility, further exacerbated by the shift in the expression of contractile proteins, including the myosin heavy chain or the alpha-subunit of Na⁺,K⁽⁺⁾-ATPase towards the fetal type [159]. Similarly, obstruction-induced hypertrophy of the bladder leads to the development of a de-differentiated smooth muscle phenotype, as evidenced in SMCs by a reversal to fetal (non-muscle) gene expression patterns [160]. In addition to their function in regulation of ECM components, several muscle-enriched miRNAs directly affect the expression levels of contractile proteins. MiR-208a is one of the most important heart-enriched miRNAs playing a crucial role in heart health and disease [161]. It is a member of a miRNA family that also includes miR-208b and is encoded by an intronic region of the *Myh6* gene. Within the heart, miR-208a and miR-208b are involved in the regulation of the myosin heavy chain isoform switch during development and in pathophysiological conditions. Transgenic overexpression of miR-208a was sufficient to induce hypertrophic cardiac growth in mice, which resulted in pronounced repression of the miR-208 regulatory targets thyroid hormone-associated protein 1 and myostatin, two negative regulators of muscle growth and hypertrophy [162]. MiR-208a was required for proper cardiac conduction and expression of the cardiac transcription factors homeodomain-only protein and GATA4 and the gap junction protein connexin 40 [162]. Down-regulation of this miRNA together with a TNF- α increase activates the complex mediator of transcription 13/nuclear receptor corepressor 1 axis, which in turn promotes MEF2 inhibition. This closes a self-limiting feedback loop, driving the transition from compensation toward decompensation in pulmonary hypertension-induced right ventricular failure [163].

MiR-378, which is expressed in cardiac myocytes [164], is also known to control cardiac hypertrophy by repression of the mitogen-activated protein kinase (MAPK) pathway. Four components of the MAPK pathway, MAPK1, insulin-like growth factor receptor 1 (IGF1R), growth factor receptor bound protein 2 (GRB2) and kinase repressor of ras (KSR1) are among miR-378 targets [165]. Studies in mouse models and humans with heart failure showed that the expression level of miR-378 was significantly decreased compared to non-failing hearts. Thus, a decrease of miR-378 expression is related with the development of cardiac hypertrophy [164]. The roles of

several TGF- β -regulated miRNAs, including miR-29, miR-143/145, miR-133a and miR-199a-5p in regulation of ECM and contractile protein expression have been described above.

In the bladder, mechanical strain and traction forces from SMCs are transmitted to the surrounding ECM by coupling molecules including the adhesion molecules integrins, leading to reorganization of ECM during wound repair and pathological fibrosis. In pBOO animal models, a down-regulation of decorin and up-regulation of biglycan, which are markers for fibrotic changes, have been reported [166]. Knock-down of integrin- β 1 in the urothelium of transgenic mice resulted in bladder overfilling, overactivity and incontinence due to abnormal mechano-transduction [167]. Integrin- α 1 β 1 is the main collagen receptor involved in reorganization of ECM. Its interaction with collagen is mediated by decorin, which helps ECM fibril packaging and compression [168]. Integrin- α 1 β 1 plays a role in angiotensin-mediated SM hypertrophy, which is one of the important mechanisms in BOO [169]. 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 SM-specific Dicer knock-out resulted in significantly reduced levels of miRNAs, including miR-145, miR-143, miR-22, miR-125b-5p and miR-27a, disturbed the micturition pattern *in vivo* and reduced depolarization-induced pressure development in an isolated detrusor due to decreased levels of L-type Ca²⁺ channels [170]. In a similar study, the loss of Dicer exacerbated cyclophosphamide-induced bladder overactivity in mice [45].

6. Therapeutic options for targeting miRNA dysregulation in disease

The pressure overload- and obstruction-induced hypertrophy and fibrosis of the heart and the bladder share many signaling pathways (Figure 2). Important hubs of signaling activated by pathologic stress are targeted by miRNAs, whose altered expression contributes to the progression of the disease. Restoration and inhibition of miRNA function are the two possible approaches to modulate miRNA activity for therapeutic purposes. Many mature miRNA sequences are conserved among species, allowing direct knowledge transfer from animal models to humans which is a big advantage for drug development [171]. On the other hand, due to the fact that miRNAs regulate a large number of transcripts and each gene can also be regulated by several different miRNAs, the potential off-target effects have to be considered, since changing miRNA expression levels might perturb multiple cellular functions [172]. Delivery of miRNAs and organ- or tissue-specific targeting is a challenge and therefore might require an addition of cell surface receptor ligands to increase specificity [173]. Half-life and/or chemical modification of miRNAs might affect their function and cause toxicity.

MiRNAs are readily inhibited by antisense nucleotides, allowing a “loss-of-function” approach. Antisense oligonucleotides with locked nucleic acid (LNA) modification can be delivered systematically via an injection with little or no toxicity and a half-life sufficient to achieve therapeutic efficacy: LNA-anti-miR-17-5p markedly decreased the area of infarction and apoptosis induced by ischemia following reperfusion in mice [174]. The efficacy of a locked nucleic acid (LNA)-modified

inhibitor of miR-192, designated LNA-anti-miR-192, was tested in the kidney, where LNA-anti-miR-192 significantly reduced levels of miR-192 and decreased renal fibrosis [175]. MiRNA sponges contain multiple tandem binding sites for a miRNA seed family of interest. High expression levels of miRNA sponges inhibit the activity of miRNA families, which share a common seed. The main advantage of miRNA sponges is that functional miRNA classes can be better inhibited than when using anti-miR antisense oligonucleotides [176]. In order to induce oligonucleotide uptake, viral vectors can be used. They can also deliver transgenes from miRNA sponges [177].

Many miRNAs play a beneficial role in disease, and their function needs to be restored (“gain-of-function”) using synthetic miRNA mimics, which contain chemical modifications to improve stability and cellular uptake [178]. MiRNA mimics need to be delivered as RNA duplexes of guide (i.e. identical to the mature miRNA sequence) and passenger (complementary) strands without the latter inhibiting the function of the former. Supplementation of endogenous miRNA using miRNA mimics (pre-miRs) has been explored in cystic fibrosis using polymeric nanoparticles for delivery of miR-126 mimic into airway epithelial cells. Polymeric nanoparticles can deliver pre-miRs effectively in order to modulate gene expression but must be tailored specifically for miRNA delivery [179]. This approach might be interesting for the bladder, which is easily accessible for instillations with solutions containing nanoparticle-conjugated miRNA mimics. Alternatively, in order to optimize the oligonucleotide uptake, viral vectors can be employed. In an animal model of liver fibrosis, a single systemic injection of a miR-29a expressing adeno-associated virus (AAV) prevented and even reversed histologic and biochemical evidence of fibrosis. Given the consistent fibrosis-associated downregulation of miR-29 described in the heart and bladder, AAV-miR-29- based therapies may be an effective treatment option [180].

7. Future directions

Despite clear differences in the morphology and function, when faced with cellular stress induced by pressure overload, both the heart and the bladder undergo similar adaptive changes, manifested in activation of the same signaling pathways (Figure 2). Recent high-throughput genomic and transcriptomic studies have been used to identify new pathways and molecular signatures of fibrotic response. Integrative mRNA-miRNA expression profiling studies offer an exciting opportunity to gain insight into the regulatory role of miRNAs in bladder and heart pathologies and identify important hubs of signaling activity among their targets [181]. These studies are well underway in the field of cardiology, and are beginning to emerge in the field of bladder dysfunction.

This Review highlighted the converging roles of miRNAs in the regulation of key signaling processes in the heart and the bladder (summarized in Table 1). Further research in BOO-induced LUTD is needed to add to these data. Molecular leads (both mRNAs and miRNAs) identified during studies of pressure overload in the heart could be tested in animal models of pBOO and human patients’ screened to uncover further similarities.

Circulating miRNAs have been detected in the extracellular space and body fluids and are hailed as biomarkers for cardiovascular and hematological diseases, cancer and CNS disorders. Packaged within exosomes and microvesicles, they are present in a stable form in many body fluids, and might act as key molecules in cell-to-cell communication and signaling. Urine is a useful source of circulating RNAs as it is easy to collect non-invasively in large amounts. Urinary circulating miRNAs could be explored in the context of LUTD and used as diagnostic and/or prognostic markers.

Selective pharmacological interference with pathways activated during the progression from hypertrophy to decompensation/acontractility will allow prevention of organ failure in the heart and the bladder. Identification of the key mRNA targets of miRNAs needs to be followed up by functional validation, and the regulatory role of selected miRNAs in particular signaling pathways should be tested using pharmacologic tools and animal models.

Development and evaluation of miRNA inhibitors of fibrosis will provide a novel therapeutic tool. As discussed above there are several issues which need to be addressed, including safe and precise delivery of miRNAs, possible off-target effects, stability and reversibility of miRNA changes. Despite these challenges, the use of miRNAs as therapeutics holds great promise. Profound understanding of biosynthesis and regulation in different disease processes and validation of target mRNAs is required to fully exploit their potential. Development of precise methods to manipulate miRNA levels with good efficacy over extended periods of time is required. By altering the expression levels of miRNAs, which affect the key signalling pathways activated in fibrosis, it would be possible to prevent acontractility, and assist full restoration of organ function following relief of obstruction, which will be beneficial for ailing hearts and bladders alike.

ACKNOWLEDGMENTS

We thank Dr. Ali Hashemi Gheinani for his support and assistance during the preparation of this manuscript and Dr. Stéphanie Lecaude for critical suggestions. The financial support of the Swiss National Science Foundation (SNF Grant 320030_156161/1) and the Velux Foundation (Grant 895) is gratefully acknowledged. We apologize to all authors, whose excellent work could not be mentioned here due to publication size limits.

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FIGURE LEGENDS

Figure 1: MicroRNA miR-199a-5p in smooth muscle differentiation and hypertrophy.

Increased expression of miR-199a-5p in smooth muscle cells affects WNT signaling by down-regulating WNT2 and influences cytoskeleton remodeling.

1) Inhibition of WNT signaling pathway leads to down-regulation of KLF4, a WNT-dependent repressor of the myocardin synthesis and the transcription of SRF/myocardin-stimulated genes. KLF4 decrease leads to elevation of myocardin levels. Together with SRF myocardin then induces genes encoding SM-specific and contractile proteins, including SM22, SM α -actin (ACTA2), SM myosin heavy chain (MYH11), transforming growth factor-beta 1-induced transcript 1 (TGFB1I1).

2) MiR-199a-5p-regulated cytoskeleton remodeling activates actin-dependent MRTF-A, which translocates to the nucleus, associates with SRF and stimulates expression of MYL9 and Id3. Id3 is a part of regulatory network, promoting smooth muscle differentiation. The resulting increase in synthesis of contractile and structural proteins leads to SMC hypertrophy. Based on [44].

Figure 2: Common signaling pathways in the bladder and the heart.

Bladder and heart follow the same regulatory mechanisms leading to hypertrophy, fibrosis and contractility defects. Ca²⁺-dependent phosphatase calcineurin – NFAT, TGF- β , Renin-angiotensin system (RAS), Hypoxia, Phosphoinositide 3-kinase (PI3K) signaling, and CaMk/MEF2 signaling cause hypertrophy in both organs. TGF- β , Renin-angiotensin system (RAS), Hypoxia and ECM remodeling result in fibrosis in the bladder and the heart tissue. Contractility defects can arise in both organs from the alterations of CaMk/MEF2 signaling.

Table 1. Common miRNAs altered in bladder and heart pathologies.

MicroRNA	Bladder		Heart	
	Regulation	Function	Regulation	Function
miR-199a-5p	Up	Increases urothelium permeability [43] and SMC differentiation, reduces proliferation [44]	Up	Promotes cardiac hypertrophy [110]
miR-212/132	Up	Increases cell size and proliferation [85]	Up	Induces cardiac hypertrophy and represses autophagy [78]
miR-133	Down	Induces contractile protein synthesis, reduces bladder fibrosis [120]	Down	Reduces cardiac hypertrophy [76]
miR-1	Down	Reduces connexin-mediated bladder overactivity [121]	Down	Down-regulates MEF2a and GATA4 [75]
miR-29c	Down	Reduces collagen expression and bladder stiffness [46]	Down	Reduces fibrosis [118]