Molecular and Genetic Mechanisms Involved in the Pathogenesis of Cardiorenal Cross Talk

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Key Words

Cardiorenal syndrome · Epigenetics · Extracellular vesicles · Small noncoding RNA

Abstract

The term ‘cardiorenal syndrome’ (CRS) encloses a scenario of clinical interactions in which cardiac and renal dysfunctions coexist. The cross talk between the heart and the kidney is clearly evidenced but not fully understood. Indeed, different factors have been shown to be involved in the pathogenesis of CRS, such as systemic inflammation, oxidative stress, apoptosis and immune dysregulation. Recently, considerable attention has been paid to the role of new alternative mechanisms which may be implicated in the pathogenesis of cardiorenal cross talk. In this review, we will focus on epigenetics, prenatal programming, small noncoding RNAs and extracellular vesicles, aiming to elucidate their possible role in heart and kidney diseases.

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Introduction

Heart performance and kidney function are closely interconnected. The physiological cross talk between these two organs is necessary to maintain the regular homeostasis and the normal functioning of the human body. However, during disease states, the damaged organ can induce structural and functional dysfunction in the other organ. The term cardiorenal syndrome (CRS) encloses a scenario of clinical interactions in which cardiac and renal dysfunctions coexist. Observational and clinical data showed that acute/chronic worsening of kidney function directly contributes to acute/chronic cardiac disease and vice versa, constituting the CRS [1–4]. The CRS classification includes a huge array of acute and chronic conditions of these two systems, where the primary failing organ can be either the heart or the kidneys. The current definition has been expanded into 5 subtypes whose etymology reflects the primary and secondary pathology, the time frame, as well as cardiac and renal co-dysfunction consequent to systemic disease (table 1) [1]. Moreover, epidemiological studies of CRS indicate that patients may move between different CRS subtypes [5–7]. Systemic in-

Table 1. CRS classification system

Acute cardiorenal syndrome	CRS type 1	Abrupt worsening of cardiac function leading to acute kidney injury, e.g. acute coronary syndrome, acute decompensated heart failure or cardiogenic shock causing acute heart failure and then renal dysfunction
Chronic cardiorenal syndrome	CRS type 2	Chronic abnormalities in cardiac function causing progressive CKD, e.g. congestive cardiac and chronic heart failure
Acute renocardiac syndrome	CRS type 3	Sudden worsening of renal function causing acute cardiac dysfunction, e.g. uremic cardiomyopathy secondary to acute renal failure, acute kidney ischemia or glomerulonephritis that leads to acute cardiac injury and/or dysfunction (such as acute myocardial infarction, ischemia, congestive heart failure, pulmonary edema and arrhythmia)
Chronic renocardiac syndrome	CRS type 4	Condition of primary CKD leading to an impaired cardiac function and/or increased risk of adverse cardiovascular events, e.g. left ventricular hypertrophy, diastolic heart failure secondary to renal failure and extreme burden of cardiovascular disease risk in patients with CKD, such as chronic glomerular disease and autosomal dominant polycystic kidney disease
Secondary cardiorenal syndrome	CRS type 5	Systemic disorders causing both cardiac and renal dysfunction, e.g. septic shock, vasculitis, diabetes mellitus, systemic lupus erythematosus, infections, drugs, toxins and connective tissue disorders

Table 2. Possible new mechanisms implicated in CRS cross talk

Epigenetic mechanisms	Changes in gene function that are mitotically and/or meiotically heritable and that do not entail a change in DNA sequence	Covalent modifications of DNA bases (e.g. DNA methylation) Histone modification RNA interference Chromatin remodeling
Prenatal programming	Environmental cues during fetal development can permanently affect the composition, homeostatic systems and functions of multiple organs and systems	Nutrition for example
Small noncoding RNA	RNA that does not encode a protein and regulates gene expression	miRNAs Small interfering RNAs Piwi-interacting RNAs
EVs	Cell-derived vesicles that are enclosed by a lipid bilayer, ranging from 30 to 2,000 nm	Apoptotic bodies Microvesicles Exosomes

flammation, oxidative stress, apoptosis and immune dysregulation have been demonstrated to play a pivotal role in the pathogenesis of CRS [1, 4, 8–16]. Furthermore, new alternative mechanisms, such as epigenetics, prenatal programming, small noncoding RNAs and extracellular vesicles (EVs), have recently been proposed to be implicated in CRS (table 2). In this review, we will focus on these mechanisms, aiming to elucidate their role in the pathophysiology of heart and kidney cross talk.

Epigenetics and Prenatal Programming in CRS

Epigenetics is a relatively recent science, defined as ‘the structural adaptation of chromosomal regions so as to register, signal or perpetuate altered activity states’, and referring to heritable changes in gene expression patterns not caused by alterations in the nucleotide sequence itself [17, 18]. Epigenetics plays a pivotal role both in mammalian development and in several pathological conditions, such as cancer and immune dysfunction. The epigenome

Fig. 1. Epigenetic biochemical mechanisms. Epigenomic changes in response to specific signals coming from the intracellular environment, neighboring cells and extracellular factors, such as diet, drugs and nutrition.

is crucial for the transcriptional outcome, allowing certain genes to be expressed while others cannot access the transcriptional machinery. Understanding the epigenome is critical for the comprehension of cell-type-specific gene regulation both in baseline and disease conditions [19]. The epigenome changes in response to specific signals coming from the intracellular environment, neighboring cells and extracellular factors, such as diet, drugs and nutrition. Epigenetic biochemical mechanisms include DNA methylation, cytosine modifications, covalent histone tail changes, higher-order chromatin organization and short noncoding RNA molecules, which are associated with chromatin remodeling and gene expression regulation (fig. 1). Recently, considerable attention has been paid to the role of epigenetic mechanisms and factors implied in cellular and sub-cellular responses associated with acute kidney injury, chronic kidney disease (CKD), heart failure and cardiovascular disease [20–31]. It is known that DNA methylation and histone modifications closely interact to control

gene expression, and they may have a role in the organ cross talk [32, 33]. In particular, these mechanisms seem to be involved in the determination of gene accessibility to RNA polymerase II and to relevant transcription factors [34].

Epigenetic inheritance is responsible for a greater amount of phenotypic cellular differences in multicellular organisms [35]. This concept may explain why subjects with both similar genetic background and environmental risk factors for cardiovascular disease and/or CKD could have different clinical outcomes and manifestation of the disease [33]. In particular, several lines of evidence are pointing to the fact that epigenetic modifications might play a specific role in CKD development: smoking, mitochondrial dysfunction, hypertension and nephron number are significantly influenced by the in utero environment programming [36–38]. Furthermore, multiple studies have been performed to analyze methylation changes in peripheral blood samples obtained from healthy controls, and diabetic and CKD patients. These studies indicated modified methylation profiles in blood samples obtained from CKD patients [25–27, 39–41]. Initial analyses on global DNA methylation changes have demonstrated both DNA hypo- and hypermethylation; later genome-wide analyses have identified several different loci associated with DNA methylation changes in CKD [25–27, 39–41]. Unfortunately, the interpretation of the results is difficult because of the specific cell type nature of the epigenome. Indeed, general consensus exists on the toxic effects of the uremic milieu on epigenetic gene regulation, thus perpetuating CKD-associated accelerated arteriosclerosis and cardiovascular disease [42–46].

Limited data exist regarding epigenetic mechanisms involved in the setting of acute and chronic subtypes of CRS. Unfortunately, it is still unclear how CRS risk factors are affected by histone modification, methylation and RNA interference. Recently, fascinating and promising evidence has emerged about overnutrition and CRS. Nistala et al. [47] reported that maternal and paternal malnutrition (both under- and overnutrition) may affect fetal and prenatal programming, thus predisposing the fetus to CRS development. While in utero nutrient restriction has been shown to promote hypertension, cardiovascular disease and CKD in offspring, a high birth weight is associated with an increased propensity for the development of CRS [48–54]. Collectively, it is still unclear how CRS risk factors are affected by histone modification, methylation and RNA interference. A better elucidation of the epigenetic paradigm in CRS context

could revolutionize and change both diagnosis and treatment of this syndrome: drugs and therapies modulating epigenetic modifications could prevent and/or attenuate CRS.

Small Noncoding RNAs: A Possible New Entity Implicated in Heart-Kidney Cross Talk

Small noncoding RNAs, which are constituted by about 18–30 endogenous nucleotides, represent an alternative intrinsic resource of gene regulation. Almost 2,500 types of these molecules have been isolated in many life forms. They have been found in all human cells and they are evolutionarily well conserved. Understanding the role of these noncoding molecules both in healthy and disease conditions is crucial due to their possible association with many critical biological functions [55, 56]. Well over half of the human transcriptome is, indeed, predicted to be under small noncoding RNA regulation [57]. Small noncoding RNAs regulate the expression of protein-coding genes through sequence-specific recognition, binding to the 3'- or 5'-untranslated region (3'-UTR) of target messenger RNA (mRNA) or promoter sequences, thus regulating mRNA levels by posttranscriptional mechanisms [58, 59]. Partial sequence complementarity between a small noncoding RNA and its target site in a specific mRNA is often sufficient for binding [57]. Several algorithms are usually used to discover these interactions, with computational predictions suggesting that a single small noncoding RNA can potentially regulate multiple RNAs [57, 60]. However, *in vitro* experimentation is always necessary to validate the predicted targets as well as the role of small noncoding RNAs in the regulation of the cell signaling pathway, cell communication and cellular phenotype [57, 60]. Moreover, the general role of these molecules in specific cellular or physiological processes can be examined by deleting or inhibiting the microRNA (miRNA)-processing machinery. There is an increasing number of reports on the regulatory roles of these RNAs, including transcriptional gene silencing/activation and posttranscriptional gene silencing events [61, 62]. In animals, partial pairing between small noncoding RNA and mRNA target site usually results in diminished protein expression through a variety of mechanisms, such as mRNA destabilization, degradation, translational repression and even activation of gene expression [57, 63]. Additionally, these small RNAs are secreted from cells and enter the bloodstream directed toward targeted cells, thus denoting a new communication approach in cell-cell or cell-organ signal transduction. Different tech-

niques have been established to isolate small RNAs from cell-free bodily fluids, such as serum, plasma and urine [64]. Thus, small RNAs are reportedly present in the circulation and found to be stable [65]. Consistent with tissue-specific functions, many small RNAs have different biological effects depending on the cell nature. It is not unexpected, therefore, that small RNAs play an essential role as transcriptional regulators in a wide range of biological processes such as cellular differentiation, growth/proliferation, apoptosis/death, migration, stress responses, metabolism and defense [58, 66–69]. Given these diverse roles, small RNAs could be pivotal regulators in the development of disease states [66, 70]. Different noncoding RNAs exist and they have been classified into three main categories: miRNAs, small interfering RNAs and piwi-interacting RNAs on the basis of their features related to the origin, structure, associated effector proteins and biological functions [71, 72]. miRNAs have been studied more extensively. In particular, increasing evidence indicates that miRNAs might be associated with several pathological conditions, such as cardiovascular and renal diseases [68–70, 73–79]. Circulating miRNAs have been demonstrated to possess different profiles in patients with heart failure, acute kidney injury and CKD when compared to controls, suggesting their possible use as innovative biomarkers for these conditions.

In the heart, miRNAs seem to be involved in the regulation of different mechanisms, including excitation-contraction, cardiac remodeling and regeneration, myocyte hypertrophy, ventricular dilatation, apoptosis and myocardial fibrosis [80, 81]. In this regard, Tijssen et al. [77] reported that miR423-5p was particularly high in the blood of patients with heart failure, and its level was related to the severity of the disease. Increased levels of miR423-5p, miR320a, miR22 and miR92b have also been found in heart failure patients [82]. Likewise, Corsten et al. [83] observed higher circulating levels of miR499 and miR122 in the setting of acute heart failure. Moreover, miR21 and miR29 are abundantly expressed in the heart and seem to regulate myocardial fibrosis by acting on mRNA of extracellular matrix proteins and TGF- β_1 [84, 85]. Rana et al. [13] have recently reported the role of cardiac miR21 and miR29b together with the inhibition of myocardial fibrosis in myocardial infarction after lowering uremic toxin levels in a rat model of CRS. Exposure to elevated serum concentrations of indoxyl sulfate (IS) was associated with an increase in miR21 expression and a reduction in miR29b in the heart. Furthermore, a significant correlation between cardiac miR21 and serum levels of IS, and a significant inverse association between

cardiac miR29b and serum levels of IS were observed. These results collectively suggest a clear role of IS in altering miRNA21 and miRNA29b in the heart, which leads to cardiac fibrosis [13]. Moreover, miR1, miR133, miR208, miR23a and miR199b have been demonstrated to play a major role in the development of cardiac hypertrophy, whereas miR21, miR199a, miR210 and miR494 are fundamental for myocyte survival during ischemia [86].

The kidney mostly expresses miR192, miR194, miR204, miR215 and miR216, which have been implicated in the migration and proliferation of renal cells [87, 88]. The deletion of the miRNA30 family is responsible for the decrease in renal cell numbers, vascular damage and extensive fibrosis [74, 89]. In addition, miRNA192 has been identified as a key regulator of collagen formation in diabetic kidney disease in a mouse model, whereas in renal biopsies from patients with diabetic kidney disease, TGF- β up-regulated miRNA192 expression in proximal tubule cells, correlating with fibrosis and a reduction in estimated glomerular filtration rate [90, 91]. Furthermore, miR21 expression was found to be increased in proliferating tubular epithelial cells, whereas knockdown of miR21 resulted in enhanced apoptosis in these cells [92]. In an animal model, targeted deletion of the gene *Dicer* from the proximal tubular epithelium protects from ischemia/reperfusion-induced renal injury and is associated with changes in the expression of several miRNAs, such as miR132, miR362 and miR379 [93]. The role of miRNA in the pathogenesis of acute kidney injury is still not well understood. Lee et al. [94] have recently evaluated an integrative network of miRNAs and mRNA data by microarray analyses to discover a possible master regulator of acute kidney injury. miR122 was found to be down-regulated by cisplatin, whereas miR34a was up-regulated by the drug. In addition, miR192 and miR377 seem to be implicated in matrix deposition and fibrosis [91, 95], while miR200 and miR205 seem to be related to the epithelial-to-mesenchymal transition [95].

The Role of EVs in the Cardiorenal Cross Talk

As remarkable discovery, miRNAs have been found in the extracellular space and in biological fluids in a relatively stable state despite the existence of RNase [96]. These extracellular miRNAs, excreted through various and incompletely understood pathways, may be protected from degradation by several mechanisms. The inclusion in EVs, such as microvesicles, exosomes and apo-

ptotic bodies, as well as the formation of protein-miRNA complexes have been reported as possible mechanisms against RNase degradation [69]. EVs are all cell-derived vesicles enclosed in a lipid bilayer, ranging from 30 to 2,000 nm in diameter depending on their origin. In fact, three main populations of EVs have been identified, according to their intracellular origin and dimension [97, 98]. EVs contain a specific subset of common proteins related to biogenesis and trafficking, as well as specific components derived from their cell or tissue of origin [99, 100], such as proteins and nucleic acids [101–103]. Therefore, the study of the proteome and the nucleic acid content of EVs may provide information about the cell or tissue of origin and, importantly, their physiological state. Three public online databases including all gathered information about EV content are available: EVpedia, ExoCarta and Vesiclepedia [103–106]. Exosomes are vesicles 30–150 nm in diameter derived from inward budding of endosomal membranes, resulting in the progressive accumulation of intraluminal vesicles within large multivesicular compartments. They are released extracellularly when these multivesicular compartments fuse with the plasma membrane [99, 107]. Microvesicles are bigger than exosomes (100–1,000 nm) and they are produced by direct budding of the plasma membrane [108]. The first evidence of exosome-mediated transfer of mRNAs and miRNAs has recently been shown by Valadi et al. [109], who observed substantial amounts of RNA in the exosomes of mouse mast cells. Apoptotic bodies appear as a heterogeneous group of vesicles, ranging from 50 nm to 5 μ m in size and 1.16–1.28 g/ml in buoyant density [110–112]. They contain DNA, RNA and histones, and display ‘eat-me’ signaling molecules, causing their phagocytosis by macrophages [113, 114]. Due to their specific cellular content and high density, they may be distinguished from two other major vesicle populations, which show considerably more overlap [97].

Exosomes might play a pivotal role in the pathophysiology of kidney and heart diseases due to their action as mediators of intercellular communication and signaling mechanisms in the target cell, transfer of mRNAs, miRNAs and proteins, or the establishment of a way of cellular content disposal [115, 116]. Circumstantial evidence has demonstrated, indeed, that these vesicles may be considered as molecular markers of renal dysfunction and structural injury, both in acute and chronic kidneys (i.e. diabetic nephropathy, focal segmental glomerulosclerosis and glomerulonephritis) and in graft rejection [117–124]. Exosomes are released by podocytes, pass through the renal tubule and they can be taken up by re-

Fig. 2. EVs and the cardiorenal cross talk. The role of EVs acquires special significance in the context of CRS in which the bidirectional cross talk and interaction between heart and kidneys is known.

cipient epithelial cells of the collecting duct, thus influencing their function through the secretion of their content. Finally, they appear in the urine where they reflect the state of the urinary system, from podocytes to renal-tubular cells, thus making them an excellent source of samples for the study of kidney physiology and pathology [125, 126]. In the setting of cardiovascular diseases, platelets have been found to secrete exosomes, which may be involved in the complex cross talk between diverse cell types in atherosclerosis plaques [127]. Cardiomyocytes have also been shown to release exosomes able to transfer DNA and RNA to different cells [128]. However, the involvement of exosomes either in vascular diseases or in cardiovascular protection mechanisms has not been deeply investigated and fully unraveled. The role of EVs acquires special significance in the context of CRS in which the cross talk between heart and kidneys is known but not gone through. In fact, apart from being a source of potential new biomarkers, they participate in intercellular communication, in terms of protein and miRNA transfer from the origin cell to the target cell or act as functionalized messengers to ensure specific drug delivery to the desired point of action (fig. 2).

Future Research

Research on EVs is not only focused on their potential role as source of biomarkers but also as a new therapeutic tool. Taking into account the properties and functions of EVs, different clinical studies have been developed aiming to use them in therapy [112]. Unfortunately, a gold standard technique for the isolation of EVs in clinical practice is still missing. Different approaches exist, such as ultracentrifugation, filtration, immune-affinity method, aggregating agents and size exclusion chromatography. Once methodological difficulties are overcome, this research in the context of cardiorenal pathology is, therefore, more than justified. EVs are currently attracting increased attention and they constitute a promising field in this and other pathologies [14].

In the setting of acute kidney diseases, few studies have tested different sources of EVs for their therapeutic potential. Cantaluppi et al. [129] tested the effect of EVs from endothelial progenitor cells in a rat model for ischemia and reperfusion injury. The miRNA content of these vesicles seems to have a positive effect on renal tubular epithelial cells, reducing apoptosis and promoting cell

proliferation [130]. Chen et al. [131] probed, in a mouse model, that injection of exosomal transcriptional repressor activating transcription factor 3 (ATF3), which has an anti-apoptotic effect and inhibits inflammatory responses, is able to reduce ischemia-reperfusion kidney injury. Similarly, the effect of liver stem cell EVs in the regeneration of renal-tubule injury has been suggested in the study of Herrera Sánchez et al. [132], who demonstrated the inhibitory effect of these vesicles on renal-tubular cell apoptosis in a murine model of acute kidney injury.

Conclusion

Elucidation of the cardiorenal puzzle demands for expanded knowledge of many still missing pieces. Heart-kidney cross talk has significant clinical rele-

vance and the management of CRS is challenging due to the multitude and complexity of the pathophysiological interactions between these two organs. Alternative factors, such as epigenetics, prenatal programming, small noncoding RNA and EVs, might represent a new way of communication between cells and organs in the setting of cardiorenal cross talk. Nevertheless, this field of research still remains largely unexplored. Further studies are needed, because genetic mechanisms may represent very attractive therapeutic targets, thus improving clinical outcomes of patients with heart and kidney disease.

Disclosure Statement

The authors declare no conflict of interest.

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