Review Article

Multi-Omics Approach: New Potential Key Mechanisms Implicated in Cardiorenal Syndromes

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Keywords

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Abstract

Cardiorenal syndromes (CRS) include a scenario of clinical interactions characterized by the heart and kidney dysfunction. The crosstalk between cardiac and renal systems is clearly evidenced but not completely understood. Multi-factorial mechanisms leading to CRS do not involve only hemodynamic parameters. In fact, in recent works on organ crosstalk endothelial injury, the alteration of normal immunologic balance, cell death, inflammatory cascades, cell adhesion molecules, cytokine and chemokine overexpression, neutrophil migration, leukocyte trafficking, caspase-mediated induction of apoptotic mechanisms and oxidative stress has been demonstrated to induce distant organ dysfunction. Furthermore, new alternative mechanisms using the multi-omics approach may be implicated in the pathogenesis of cardiorenal crosstalk. The study of “omics” modifications in the setting of cardiovascular and renal disease represents an emerging area of research. Over the last years, indeed, many studies have elucidated the exact mechanisms involved in gene expression and regulation, cellular communication and organ crosstalk. In this review, we analyze epigenetics, gene expression, small non-coding RNAs, extracellular vesicles, proteomics, and metabolomics in the setting of CRS.

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Background

The “organ crosstalk” is the complex biological communication between different body systems, mediated via cellular, subcellular, molecular, neural, endocrine and paracrine factors through numerous feedback. The physiological crosstalk is fundamental to preserve body regular homeostasis and normal functioning. However, in the disease state, the initiation of toxic cell signaling by the primary damaged organ can cause structural and functional impairment of distant organs [1]. Particularly, heart performance and kidney function are strictly interconnected and communication between these 2 systems involves different dynamic and bidirectional pathways [2]. Multi-factorial mechanisms implicated in the pathogenesis of cardiorenal syndrome (CRS) involve not only hemodynamic parameters, such as extracellular fluid volume, cardiac output and arterial pressure, but also endothelial injury, immunologic imbalance, cell death, inflammatory cascades, cell adhesion molecules, cytokine and chemokine overexpression, oxidative stress, neutrophil migration, leukocyte trafficking, caspase-mediated induction of apoptotic mechanisms, extracellular vesicles (EVs), small non-coding RNAs, epigenetics and oxidative stress [3–18].

The interactive system of molecular layers, such as genetics, epigenetics, messenger RNA (mRNA) transcripts, proteins and metabolites, is involved in the development of human disease and it is influenced by several environmental factors. Recent technological advances have provided high-throughput measurement of human genome, epigenome, metabolome, transcriptome and proteome at the population level [19]. The integrative approach of multi-omic data may help in the understanding of the molecular dynamics implicated in the pathophysiology of human disease, thus leading to novel strategies for their early detection, treatment and prevention. In particular, in the “omic” technologies, there is a complete vision of the molecules of cells and tissues. “Omic” technologies intent is principally the widespread detection of genes (genomics), mRNA (transcriptomics), proteins (proteomics) and metabolites (metabolomics) in a definite biological sample in a non-targeted and non-biased way (Table 1). According to these approaches, a multifaceted system can be investigated more comprehensively if considered as a whole. Given the huge quantity of records generated in these studies, bioinformatics and statistic are fundamental for their understanding. Precise validation is important to confirm that findings are not just random results [19–22].

In this review, we analyze epigenetics, gene expression, small non-coding RNAs, EVs, proteomics and metabolomics in the setting of CRS (Fig. 1).

Genomics and Transcriptomics

Genomics, the most mature of the omics fields, focuses on the systematic study of body genome, identifying genetic variants associated with human disease, treatment response and future prognosis. The genome is the whole DNA of a cell or organism. The human genome comprehends 3.2 billion bases and 30,000–40,000 protein-coding genes. DNA microarrays provide a measurement of DNA sequence differences among individuals, and the expression of thousands of genes can be analyzed simultaneously [22, 23]. Transcriptomics examines genome RNA levels, both qualitatively (transcripts types, new splice sites, RNA editing sites) and quantitatively (the expressed quantity of each transcript). According to the central dogma of biology, RNA is considered the single-strand mediator between DNA and proteins. RNA reads-out DNA. Other RNA functions comprehend structural function (e.g., ribosomal complexes) and regulatory function (e.g., small non-coding RNAs). Large transcriptomic researches have established that although only ~3% of the genome encodes proteins, up to 80% of the genome is transcribed [24]. Gene expression analysis is the determination of gene patterns expressed at

Table 1. New mechanisms implicated in CRS crosstalk

Gene expression	Translation of information encoded in a gene into protein or RNA structures; it is cell and timing specific	Up-regulation Down-regulation
Epigenetic mechanisms	Changes in gene function that are mitotically and/or meiotically heritable and that do not entail a change in DNA nucleotide sequence; it is responsible for phenotypic differences between cell types in multicellular organisms	Covalent modifications of DNA bases DNA methylation – Histone modifications – RNA interference Chromatin remodelling
Prenatal programming	Environmental signals during foetal development can affect composition, homeostatic systems and functions of multiple organs and systems; In utero environment and maternal and parental status may influence foetal and prenatal programming, thus predisposing the foetus to the disease state	Nutritional environment other factors
Small non-coding RNA	RNA that does not encode a protein and regulate gene expression and gene transcription through sequence-specific recognition	miRNAs siRNAs piRNAs
EVs	Cell-derived vesicles, enclosed in a lipid bilayer, ranging from 30 to 2,000 nm; EVs contain a specific subset of common proteins related to biogenesis and trafficking, as well as specific components derived from their origin cell or tissue	Apoptotic bodies MVs Exosomes
Proteomics	Protein profile produced by the genes of a particular cell, tissue or organism at a certain setting and timing	Modifications of structure, function, and interactions of different proteins
Metabolomics	Complete profile of metabolites derived by the ending and final products of cellular processes at specific times	Variations of molecules and metabolomics patterns

CRS, cardiorenal syndrome; EV, extracellular vesicles; miRNA, microRNAs; siRNA, small interfering RNA; piRNA, piwi-interacting RNAs; MVs, microvesicles.

the level of genetic transcription in a specific cellular type. Limited data exist on gene expression and activation in the setting of acute and chronic CRS. In the setting of CRS type 1, Virzi et al. [8] investigated gene expression of IL-6, IL-18, and NGAL by quantitative real-time PCR in renal tubular epithelial cells (TECs) incubated with plasma from healthy controls (CTR), and patients with CRS type 1 or heart failure (HF). In the CRS type 1 group, mRNA expression of these factors resulted significantly higher compared with patients with HF and CTR. The authors concluded that the in vitro exposure to plasma from CRS type 1 patients altered the gene-expression profile of TECs. In particular, the TEC profile is characterized by an increased gene-expression of pro-inflammatory mediators, tubular damage markers (NGAL), and pro-apoptotic factors [8]. Moreover, the same group investigated the gene activation of apoptotic pathway in the context CRS type 1. According to the authors, BAD and BAX gene expression was significantly higher in monocytes treated with plasma from CRS type 1 patients compared to AHF group. On the contrary, FASL expression was similar in monocytes treated with plasma from CRS type 1 patients and the AHF group [25]. In the setting of chronic CRS, Angelini et al. [26] studied the contribution of the prevalent congestion to the development of kidney injury in a rat model of CRS type 2. They confirmed that kidney injury is characterized by tubular damage and systemic inflammation. In particular, they reported double mRNA expression of NGAL in the kidney

Fig. 1. Multi-factorial mechanisms implicated in the pathogenesis of CRS.

tissue of chronic HF rats. On the basis of their histological and protein data, they suggested that the upregulated NGAL complexed with MMP9 perpetuates the vicious circle of kidney/heart damage by enhancing the enzymatic activity of MMP9 with extracellular matrix degradation, worsening heart remodelling [26].

Transcribed Regulatory Elements: Small Non-Coding RNAs and EVs

Small non-coding RNAs contain about 18–30 endogenous nucleotides and represent an alternative intrinsic resource of gene regulation. Understanding the role of these non-coding molecules both in health and disease states is fundamental since they perform critical biological function [27, 28]. Small non-coding RNAs regulate the expression of protein-coding genes through sequence-specific recognition, binding to 3' or 5'-untranslated region of target mRNA or promoter sequences, thus controlling mRNA levels by post-transcriptional mechanisms [29, 30]. Moreover, the role of these molecules in specific cellular or physiological processes can be investigated through the deletion or inhibition of microRNA (miRNA) processing machinery [6, 31]. In addition, RNAs are secreted from cells in the bloodstream and directing themselves to the targeted cells. This fact denotes a novel communication approach in cell-cell or cell-organ signal transduction. Non-coding RNAs have been classified into 3 main categories: miRNAs, small interfering RNA and piwi-interacting RNAs according to their features related to the origin, structure, associated effector proteins and biological functions [32, 33]. As notable discovery, miRNAs have been found in the extracellular space and in biological fluids, in a relatively stable condition in spite of the existence of RNase [34].

The inclusion in EVs, such as microvesicles, exosomes and apoptotic bodies, as well as the formation of protein-miRNA complexes, have been reported as possible mechanisms against RNase degradation [35]. EVs are cell-derived vesicles walled in a lipid bilayer, with a diameter ranging from 30 to 2,000 nm depending on the origin [36, 37]. They contain widespread proteins linked to biogenesis and trafficking, as well as precise cellular and tissue factors, for example, specific proteins and DNA and RNA [38–42]. Thus, the learning of the content of EVs in term of proteome and nucleic acid may offer information on the cell or tissue of origin and, importantly, their physiological condition [43]. Exosomes and small non-coding RNAs might participate in the pathophysiology of kidney and heart disease, mediating intercellular communication and signaling mechanisms in the target cell, transferring genetic materials and proteins [44–47].

Indeed, according to recent evidence, these vesicles may be investigated as molecular indicators of renal dysfunction and structural injury, both in acute and chronic kidney damage and in graft rejection [47–54]. In cardiovascular disease (CVD), platelets have been reported to release exosomes, which may have a role in the intricate cross-talk between different cells in atherosclerosis plaques [55]. Moreover, cardiomyocytes as well have been revealed to secrete exosomes able to transfer DNA and RNA to many cellular types [56]. Furthermore, Rana et al. [14] have recently investigated the function of cardiac miR21 and miR29b and the inhibition of myocardium fibrosis in myocardial infarction after lowering uremic toxins levels in a rat model of CRS. Higher miR21 expression and a reduction of miR29b were demonstrated in the heart after the exposure to augmented levels of indoxyl sulphate (IS). Additionally, a significant positive correlation between cardiac miR21 and serum concentration of IS and a significant inverse correlation between cardiac miR29b and serum levels of IS were described [14]. Recently, the same author investigated whether the cardiac changes caused by kidney injury may be induced by the dysregulation of cardiac miRNAs that are involved in the regulation of gene expression implicated in fibrosis, apoptosis and hypertrophy processes. For the first time, they reported increased cardiac expression of miRNA-212 and -132 after subtotal nephrectomy in a rat model of AKI. Furthermore, they described that treatment with an angiotensin-converting enzyme inhibitor lead to a reduction of the expression of these 2 miRNAs [57]. Moreover, Chuppa et al. [58] longitudinally studied left ventricle pathology in a 5/6 nephrectomy rat model of CRS type 4, thus recognizing new molecular mediators. Next-generation sequencing of left ventricle mRNA and miRNA was applied at physiologically distinct points in disease progression. They described gene alterations in the immune system, as well as, alterations in lipid metabolism and inflammatory pathways. They reported alterations in miRNAs patterns and identifying several specific miRNAs. In particular, these authors analyzed miR-21-5p-mediated targeting of peroxisome proliferator-activated receptor α , thus showing the therapeutic benefit of peroxisome proliferator-activated receptor α agonists on cardiac remodelling in a rat model of chronic kidney disease (CKD) [58].

Not many specific and appropriate studies have been performed on the role of non-coding RNA and EVs in the pathogenesis of CRS, but recent evidence suggests their possible implication in the cross-talk between distant organs in the setting of CRS.

Epigenome and Epigenetics

Epigenomics focuses on genome-wide description of mutable modifications of DNA or DNA-associated proteins, such as DNA methylation or histone acetylation. Covalent changes of DNA and histones are main regulators of gene transcription, and consequently of cellular natural life. These modifications may be predisposed by both genetic and environmental

contributors, can be long lasting and are sometimes heritable [59–61]. Epigenetics is responsible for phenotypic differences among cell types in multicellular organisms, and it is considered a dynamic process regulating gene expression patterns [62, 63]. Signals coming from intracellular environment, neighbouring cells, and extracellular factors, such as diet, drugs and nutrition may induce epigenome changes [4, 62]. In this context, numerous lines of evidence have emphasized the role of epigenetic changes in the pathogenesis of CKD and CVD: smoking, mitochondrial alterations, hypertension and nephron quantity are significantly influenced by the in utero environment programming [64–66]. Innovative and fascinating evidence suggested the involvement of epigenetic mechanisms in the development of acute and chronic subtypes of CRS. Unfortunately, it is still uncertain how CRS risk factors may be influenced by histone changes, methylation and RNA interference. In the setting of kidney and heart cross-talk, renal failure has been documented to increase cardiac histone H3 epigenetics; these findings directly link renal failure with the induction of cardiomyopathy-related genes [67]. Furthermore, uraemia can also affect DNA methylation, thus suggesting that epigenetic alterations are involved in the pathogenesis of CRS [62]. Lately, emerging and promising data is increasing about overnutrition and CRS. Nistala et al. [68] described that maternal and paternal malnutrition (both under- and overnutrition) may affect foetal and prenatal programming, thus prompting the foetus to CRS. Recently, several evidence revealed that in utero undernutrition and nutrient restriction promote hypertension, CVD and CKD in the offspring; furthermore, high birth weight is related with enlarged predisposition to CRS [69–75]. In the setting of cigarette smoking, Drummond et al. [76] investigate renal and cardiac injury progression and functional changes in the Sprague-Dawley rat model of CKD exposed to 4 weeks of daily combustible cigarette smoke. They concluded that smoking, tobacco and nicotine induced significant decreases in important epigenetic antifibrotic mediators, such as miRNA-29b-3p, with the consequent higher expression of fibrosis markers (collagen-1A1, -3a1, -4a1, integrin, elastin, fibrillin, matrix metalloprotease-2) and extracellular matrix remodelling. This finding confirmed the negative effect of smoking on epigenetic regulation of fibrosis development in renal and cardiac tissues [76].

Proteomics and Metabolomics

Proteome is a dynamic reflection of both genome and environment and it is thought to hold special promise for biomarker discovery, since proteins are most likely to be affected in the disease state [77]. Proteomics is a branch of biotechnology that uses the techniques of molecular biology, biochemistry, and genetics to analyze proteins' structure, function, and interactions. It is the set of expressed proteins in a given type of cell or organism, at a given time, under defined conditions. Proteomics is performed to measure peptide abundance, to investigate peptide modifications and to study protein interactions. Furthermore, the functions of a great fraction of proteins are mediated by post-translational changes such as proteolysis, glycosylation, phosphorylation, nitrosylation and ubiquitination. These modifications play a key role in intracellular signalling, enzyme activity control, protein turnover and transport, and overall cell structure maintenance [78, 79]. Proteins analysis and quantification have been completely transformed and modified by mass spectrometry (MS)-based methods. MS is a revolutionary method adapted for high-throughput analyses of thousands of proteins in cells or body fluids [80–82]. Recent technological advances in proteomics have allowed a wide-scope analysis of protein patterns in bodily fluids, allowing the identification of numerous protein markers in various conditions. For instance, urine and saliva are ideal biological fluids for proteomic analysis; they are easily collected in a non-invasive manner.

Proteomic biomarkers may improve the management of patients with cardiac, renal and cardiorenal disease by providing more accurate and earlier detection of the pathology. In addition, proteomic biomarkers may also be useful to define the most suitable therapeutic targets in a given patient or disease setting [83–86]. For example, urinary proteomics has been used in several studies in order to identify and validate biomarkers associated with different acute and CKDs [87–91]. Research to determine prognostic and diagnostic value as well as clinical utility in renal, cardiac and cardiorenal disease states is ongoing [92].

In the context of “omics” sciences, metabolomics is the investigation of the complete set of metabolites – in a cell, tissue, organ or organism – which are the final products of cellular processes in health and disease condition. It could also be defined as a field linking genomics, transcriptomics and proteomics, modifying totally the way to explore and interpret the above stated cellular procedures [93]. It is not only the product of genes and RNA expression modification but also that of protein activity and environmental elements, including nutrition and pharmacological therapies [94]. In a clinical circumstance, the quick characterization of small-molecule metabolites in numerous biologic fluids (such as urine, blood, saliva) permit the examination of the interaction genotype-phenotype and genotype-environment, thus generating the chance to explore a snapshot of the metabolic status whether normal or pathological [95, 96]. Metabolomics has been shown to have a substantial impact on the investigation of various CVD and renal disease [94, 96]. For instance, MS-based profiling of plasma metabolites was performed in over 400 HF patients by Cheng et al. [97] in order to assess the diagnostic and prognostic value of metabolomics in HF. Their results demonstrated that metabolomics is able to provide significant prognostic value, independent of brain natriuretic peptide and other traditional risk factors [97]. In a recent work, Bassareo et al. [93] investigated the differences in the urinary metabolic profile and in the hematic asymmetric dimethylarginine levels in young grown-up subjects who were born preterm with an extremely low birth weight and a control group of subjects who were born at term with a weight appropriate for their gestational age. Interestingly, the authors reported a correlation between the urinary metabolic profile in extremely low birth weight and their blood asymmetric dimethylarginine levels, suggesting the presence of a subclinical CRS in these subjects [93].

New Potential Therapeutic Approaches and Precision Medicine

A key concept of modern biology is that genes and their products participate in complex, interconnected networks, rather than linear pathway. Given the molecular diversity of biomarkers available, the high-throughput multi-omics technologies offer an amazing opportunity for holistic investigations. The approach of multi-omics in the setting of cardiac, renal and cardiorenal diseases (i.e., inhibition of miRNAs and the study of EVs) may improve disease management by identifying molecular abnormalities that could guide disease detection and diagnosis, suggest a link between genotype and phenotype and new therapeutic strategies. Furthermore, it could predict personal response to therapy with the reduction of the cost. In this context, the multi-omics approach may represent the basis for precision medicine, providing personalized health care, especially through the extrapolation of disease risk and the early intervention for a potentially better outcome with lower cost. Precision medicine also holds the promise of individualized treatment, and challenges the clinician to translate diagnosis into better personal management for each patient. The multi-omics approach can gain a comprehensive and global picture of biological processes such as interactions in and between signal transduction pathways leading to a better understanding of diseases, in particular in the context of cardiac, renal and cardiorenal disease. Omic strategies lend themselves to biomarker discovery, as they investigate multiple molecules simultaneously, and the creation of multi-biomarker panel for the diagnosis of the disease. The multi-omics technologies allow us to discover new molecular contexts, generate hypotheses and find approaches

to medication and therapy. Applications of these innovative technologies in the field of oncology are now revealing the spectra of genetic modifications implicated in the pathogenesis of thousands of human cancers and can offer the possibility to address the correct genotype-phenotype approach. Further studies are necessary for other fields to apply the multi-omics approach. Furthermore, education and dissemination of knowledge on the real-life deliverables and challenges of omics technologies for health professionals are essential for this revolution.

Conclusion

In conclusion, while “omic” studies in the area of cardiorenal medicine are relatively small in number and remained at early stage, with advancing technology, it is a rapidly expanding field of research. “Omic” strategies still provide many challenges: the technology and the software are still evolving and mapping the human coding and non-coding genome and transcriptome, proteome and metabolome is still ongoing. Carefully designed experiments, accompanied by appropriate analytical techniques and statistical analyses, will assist in tackling many of these challenges. Results from “omic” research in the setting of CRS will undoubtedly improve future therapeutic approaches for patients with this multifaceted syndrome.

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