Make Precision Medicine Work for Chronic Kidney Disease

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Significance of the Study

- With increasing knowledge of omics and the development of computational models, precision medicine is coming of age in nephrology in order to identify specific biomarkers and develop targeted therapy. The breakthroughs of omics in chronic kidney disease (CKD) and the application of systems biology in the potential role of transforming growth factor- β_1 in the targeted intervention of renal fibrosis exemplify the use of precision medicine in CKD.

Keywords

Precision medicine · Chronic kidney disease · Omics ·
Transforming growth factor- β_1 · Renal fibrosis

Abstract

Precision medicine is based on accurate diagnosis and tailored intervention through the use of omics and clinical data together with epidemiology and environmental exposures. Precision medicine should be achieved with minimum adverse events and maximum efficacy in patients with chronic kidney disease (CKD). In this review, the breakthroughs of omics in CKD and the application of systems biology are reviewed. The potential role of transforming growth factor- β_1 in the targeted intervention of renal fibrosis is discussed as an example of how to make precision medicine work for CKD.

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Introduction

With increasing knowledge of human omics and the development of computational tools for biomedical analysis, precision medicine is unveiled as an accurate diagnostic modality and targeted treatment (using the right dose and time), with minimum adverse events and maximum efficacy [1]. Precision medicine requires the following critical elements: clinical informatics and bioinformatics, computational science, mathematics, and systems biology [2]. In recent years, precision medicine has made great progress; for example, the novel classifications of cancers are based on molecular testing of genetic markers. This has led to targeted drugs such as imatinib for patients with chronic myeloid leukemia who have a BCR-ABL mutation and crizotinib for those with lung cancer who carry the EML4-ALK fusion gene [1]. In kidney transplant patients, urinary-cell messenger ribo-

nucleic acid (mRNA) profile relevant to acute cellular rejection has been identified as a noninvasive diagnostic biomarker, leading to early antirejection therapy and monitoring response of drug treatment [3].

Chronic kidney disease (CKD) is a major health and socioeconomic burden [4]. Renal fibrosis is the common pathway for CKD of various origins, resulting in end-stage renal disease [4, 5]. The injury results in local inflammation and the production of proinflammatory cytokines, which contribute to the recruitment of inflammatory cells that synthesize and release profibrotic cytokines. This induces the activation and recruitment of matrix-producing cells and epithelial-to-mesenchymal transition (EMT), which ultimately leads to renal fibrosis in CKD pathogenesis [6]. Transforming growth factor (TGF)- β_1 is both a well-known EMT inducer and profibrotic molecule that participates in the pathogenesis of renal fibrosis [6].

In this report, the knowledge and understanding of omics (emerging technologies for analyzing big data) as well as novel diagnostic and therapeutic strategies for CKD are reviewed. The molecular pathways associated with TGF- β_1 are discussed as potential therapeutic targets for progressive CKD in order to shed light on the role of precision medicine in CKD management.

Genomic, Epigenetic, and Transcriptional Studies of CKD

Series of studies have reported that specific genetic polymorphisms, epigenetic and transcriptional variations, could increase the risk of CKD [3, 5, 7]. There are two basic approaches for identifying candidate genes of CKD. First, the genotype-to-phenotype approach: perform unbiased screening to identify the genes which are associated with CKD, and then unveil the mechanistic relevance through experimental studies. Second, the phenotype-to-genotype approach: screen the specific genes which have been confirmed in renal pathogenesis to analyze the polymorphisms, variations on transcription or expression, in the susceptibility of CKD [3].

Genome-wide association studies (GWASs) use genomic variations, termed single nucleotide polymorphisms (SNPs), to identify regions of the genome which are associated with the disease status or a clinical phenotype [5, 8]. Multiple GWASs have been conducted to expand the knowledge of genetic variants of CKD which reveal the pathways and mechanisms, and potentially represent the targets for therapeutic interventions [7, 9].

For example, GWASs identified the variants in the promoter of the UMOD gene, SNP rs4293393, which increased UMOD expression. Uromodulin overexpression led to salt-sensitive hypertension by upregulating Na-K-Cl transporter (NKCC2) phosphorylation, and contributed to renal damage. This mechanism indicated that pharmacological inhibition of NKCC2 would be more effective in lowering blood pressure in hypertensive patients who were homozygous for UMOD promoter risk variants than in other hypertensive patients, and the uromodulin might be a therapeutic target for lowering blood pressure and preserving renal function [10].

The epigenetic state determines the expression and transcription of genes by DNA methylation of CpG dinucleotides, nucleosomal histone modifications, and other mechanisms [11, 12]. Different from primary DNA sequences, epigenetic modifications are reversible and susceptible, depending on different time, locations, nutritional status, and environmental exposure; therefore these factors can be modified to suppress disease progression in clinical practice. The DNA methylation changes in CKD patients have shown both DNA hypomethylation and hypermethylation [13]. The hypermethylation downregulated the expression of the renoprotective gene KLOTHO and was associated with CKD progression; thus, KLOTHO might be a potential epigenetic drug target to suppress CKD progression [14].

The microRNAs (miRNAs) are short (20–23 nucleotides in length), noncoding, endogenous, single-stranded RNA molecules that regulate target mRNAs at the posttranscriptional level. miRNAs inhibit protein synthesis, either by inhibition of translation or mRNA degradation [15]. The miRNAs are more stable than mRNA and present in different body fluids, especially in urine, suggesting that miRNAs could serve as ideal biomarkers of CKD of various pathologies [12]. Previous studies have demonstrated that urinary miRNA abundance was variable in CKD (Table 1). miRNA-29 was reported to regulate the expression of collagen genes and extracellular matrix proteins which are involved in modulating renal fibrosis, negatively correlating with tubulointerstitial fibrosis in urine exosome [16]. Identifying the regulation and function of these miRNAs in renal pathogenesis might pinpoint miRNAs as new therapeutic targets for progressive CKD and renal fibrosis.

Table 1. Main findings of omics associated with renal fibrosis: positive and negative correlations

Field	Positive correlation	Negative correlation	Suppl. ref. No.
Genomics	IL-18 (+137 GG, -607CC); TGF- β_1 (-509 TT); AS (int 2 CC); UMOD (rs12917707; rs12446492); ELMO1 gene; Nox2 gene	TGF- β_1 (+869 TT); UMOD (rs13333226, rs12917707); Sirt1 gene	1–10
Epigenomics	DNA methylation (e.g., Klotho promoter); histone modifications (e.g., profibrotic and ER stress-related genes)	apelin-13, KLF4	11–16
Transcriptomics	miR-192, miR-29, miR-21, miR-150, mRNA (e.g., APE1, AT1R, CXCR4, THBS1, TRIB1)	miR-93, miR-217, miR-200a, miR-26a, mRNA (e.g., BMP7, CD2AP)	13, 17–25
Proteomics	TGF- β_1 , α -SMA, NGAL, KIM-1, CD147, CXCL1, annexin A1, HE4, NGAL, MBL, MMP-7, MMP-9, CTGF, uVDBP, periotin, CKD273 peptides	HO-1, E-cadherin	26–37
Metabolomics	cystatin C, lipids (e.g., ectopic, oxidized), glycolysis, acetoacetate, phosphorylcholine/choline, H-1 NMR-based metabolomics	pyruvate, glycine, L-carnitine	12, 38–43

References of this table are listed in online supplementary file 1, www.karger.com/doi/10.1159/000455101. TGF, transforming growth factor; AS, aldosterone synthase; UMOD, uromodulin; ELMO1, engulfment and cell motility protein 1; Nox2, NADPH oxidase 2; ER, reticulum stress; KLF4, Krüppel-like factor 4; APE1, apyrimidinic endonuclease 1; AT1R, angiotensin II-1 receptor; CXCR4, chemokine (C-X-C motif) receptor 4; THBS1, thrombospondin 1; TRIB1, tribbles homolog 1; BMP7, bone morphogenetic protein 7; α -SMA, α -smooth muscle actin; NGAL, neutrophil gelatinase-associated lipocalin; KIM-1, kidney injury molecule 1; HE4, human epididymis protein 4; MBL, mannose-binding lectin; MMP, matrix metalloproteinase; CTGF, connective tissue growth factor; uVDBP, urinary vitamin D binding protein excretion; HO1, heme oxygenase 1; H-1 NMR, H-1 nuclear magnetic resonance.

Proteomics and Metabolomics for CKD

The biggest challenge of studies in proteomics and metabolomics in CKD is the identification of potential biomarkers. The term “biomarker” is defined as a quantitative biological molecule or pathological process that is typically used for the diagnosis of a disease or the assessment of disease activity. Biomarkers of CKD mainly indicate the proteins and metabolites from blood, urine, and kidney biopsy specimens; recently, the molecules from DNA and miRNA analyses can also be biomarkers. The ideal biomarkers of CKD should accurately predict an individual’s risk of developing CKD, detect and classify the disease early based on the individual molecular mechanism, and then monitor the response to therapy for further adjustment.

Proteomics serve not only as a screening method to identify potential biomarkers but also as a diagnostic method itself [17]. The majority of urinary proteins are generated by the kidney and, hence, hold substantial information on renal pathogenesis. With ongoing studies on urinary proteome, the abundance of molecular information will replace kidney biopsy [18]. The CKD273 classifier, based on 273 urinary peptides, was well suited for the early detection of CKD and prognosis of progression

[19]. The PRIORITY study validated the use of CKD273 to detect diabetic nephropathy (DN) from type 2 diabetic patients, who had no sign of DN with normal estimated glomerular filtration rate and normoalbuminuria. These patients will be randomized to the groups of low-dose spironolactone plus standard treatment and placebo plus standard treatment, aiming at demonstrating the benefit of the proteomics-guided intervention [20].

Podocyte phospholipase A2 receptor (PLA2R) is an antigenic target in autoimmune membranous nephropathy (MN). Anti-PLA2R antibodies in serum and glomerular deposits could serve as diagnostic biomarkers of idiopathic MN [21] and could also differentiate idiopathic MN from secondary MN [22], with high specificity and sensitivity. Moreover, in anti-PLA2R-associated idiopathic MN, serum titers of anti-PLA2R antibodies were positively correlated with proteinuria and disease activity and could predict the remission and relapse of idiopathic MN. Thus, anti-PLA2R antibody titers could have an important role in the selection of patients for immunosuppressive therapy and in monitoring the response to treatment [23].

The human metabolome is the product of multiple physiological and pathophysiological processes and might provide a novel insight into CKD etiology and pro-

Fig. 1. Schema of precision medicine for CKD. The figure visualizes the order and complex interconnections of the numerous interacting components which give rise to precision medicine.

gression. In contrast to traditional biochemical approaches that focus on a single metabolite, metabolomics involves a series of metabolites to gain an overall understanding of metabolism [24]. Compared with other omics technologies, there are fewer metabolites (3×10^3) than there are genes (2×10^4), transcripts ($>10^6$), proteins ($>10^6$), and posttranslational modified proteins ($>10^7$) [5]. Metabolomic biomarkers of diabetes were reported as sugar metabolites, ketone bodies, free fatty acids, and branched chain amino acids [25]. Distinct phospholipids, sphingolipids, and sphingomyelins in serum could identify DN from type 1 and type 2 diabetic patients. Urinary levels of acylcarnitines and hippuric acid were associated with early kidney damage and reflected alterations in β -oxidation and uremic toxin elimination, respectively [26]. Furthermore, increases in γ -butyrobetaine, citrulline, symmetric dimethylarginine, and kynurenine as well as a decrease in azelaic acid (β -oxidation) indicated the progression from micro-DN to macro-DN [27].

Systems Biology Brings Forward Precision Medicine for CKD

Systems biology is the computational and mathematical modeling of complex biological systems that can integrate the big data in omics as well as clinical phenotypes

to gain a mechanistic understanding of disease [28]. Systems biology will help physicians to understand the function of the whole organism in order to expand the scope of precision medicine by establishing the predictive models and achieve the tailored intervention. For the nephrologist, each patient's disease should be analyzed according to data cloud and systems biology to establish diagnosis or classification at the molecular level and select appropriate treatments. Then, the patient's response should be monitored, and clinical trials should be conducted to adjust the predictive models and personalized treatments (Fig. 1) [29]. RNA microarrays have been used to analyze gene expression profiles in renal glomerular and tubular samples from diabetic kidney disease (DKD) patients. Then, weighted gene co-expression network analysis identified 10 modules of genes in tubuli and 12 modules in glomeruli, which indicated that dysregulation of cell proliferation might contribute to the development of DKD, and those genes could be therapeutic targets for DKD [30].

The Role of TGF- β_1 in Renal Fibrosis

Renal fibrosis is the hallmark of CKD, leading to irreversible loss of tissue and impaired kidney function. Understanding the mechanisms of renal fibrosis would bring new taxonomy of CKD and targeted therapy in order to

TGF- β antagonists which reduce or block the activation of TGF- β_1 , including anti-TGF- β antibodies, the TGF- β receptor antagonist, and the targets of inhibiting TGF- β synthesis, might ameliorate renal fibrosis. Fresolimumab, a human monoclonal antibody that neutralizes all 3 isoforms of TGF- β , is being studied in a phase 2 clinical trial in patients with steroid-resistant primary focal segmental glomerulosclerosis. LY2382770, an antibody that neutralizes the bioactivity of TGF- β_1 , is also being investigated in a phase 2 clinical trial in DN patients. GW788388, a TGF- β receptor antagonist and small interfering RNA for the TGF- β receptor, could also inhibit TGF- β signaling activity. Pirfenidone, with antifibrotic and anti-inflammatory activities via the inhibition of TGF- β_1 synthesis, has been demonstrated to be safe and effective in many disorders such as idiopathic pulmonary fibrosis, multiple sclerosis, and liver fibrosis. Clinical trials of pirfenidone in CKD have been performed in patients with focal segmental glomerulosclerosis and DN. The results suggested that pirfenidone might be an effective agent to slow renal function decline in CKD [40]. In addition, the inhibition of TGF- β signaling by Smad7, bone morphogenetic protein-7 agonists, connective tis-

sue growth factor inhibitors, in vivo delivery of small interfering RNA, as well as all other molecules and pathways in Figure 2 could be potential therapeutic targets to inhibit or slow the progressive loss of kidney function in patients with CKD.

Conclusion

Precision medicine promises to combine omics data with clinical phenotypes to develop new clinical implementation that can predict CKD risks and therapeutic responses in a very precise way. For instance, by understanding the mechanisms of TGF- β_1 -related renal fibrosis, CKD would be refined by different pathogeneses of renal fibrosis depending on its involved pathway individually, and thus more precise biomarkers and therapeutic strategies would be achieved.

Disclosure Statement

The authors have no conflicts of interest to declare.

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