Role of prognostic biomarker decoy receptor 3 and immunomodulation in kidney diseases

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Abstract: Decoy receptor 3 (DcR3), also known as tumor necrosis factor receptor superfamily member 6b (TNFRSF6B), was recently identified as a novel biomarker for predicting progression of kidney diseases with potential immune modulation. The purpose of this review is to discuss the current evidence related to DcR3 in kidney diseases and to compare the differences between human and animal studies both in vivo and in vitro. High serum DcR3 predicts the occurrence of peritonitis in patients receiving chronic peritoneal dialysis and is positively correlated with inflammatory markers such as interleukin-6, high-sensitivity C-reactive protein, and adhesion molecules in patients on maintenance hemodialysis (HD). Higher serum DcR3 levels not only independently predict cardiovascular and all-cause mortality in HD patients but also identify older adults on HD at risk of protein-energy wasting in combination with a low geriatric nutritional risk index. Recently, renal tubular epithelial cells (RTECs) expressing DcR3 have also been used to predict progression of chronic kidney disease. Expression of DcR3 was correlated with a 2-fold increase in serum creatinine or failure of kidney allograft. DcR3 could protect renal myofibroblasts against Fas-induced apoptosis and subsequently lead to renal fibrosis. Locally expressed DcR3 in the RTECs may suppress the FasL-Fas-mediated apoptosis of T cells, resulting in an accumulation of allo-reactive T cells. In addition to traditional biological functions, recombinant DcR3, Fc and cytomegalovirus promoter-driven human DcR3 plasmid are able to modulate the activation and differentiation of dendritic cells and macrophages via “non-decoy” action. Both progressive IgA nephropathy and autoimmune crescentic glomerulonephritis in mice can be suppressed after hydrodynamics-based gene delivery of DcR3 plasmid. DcR3-mediated effects in vitro could be surveyed via over-expressing DcR3 or addition of recombinant DcR3.Fc, and CD68-driven DcR3 transgenic mice are suitable for investigating systemic effect in vivo. Inhibition of DcR3 expression in human may be a promising approach for pathomechanism.

Keywords: Adhesion molecules; Alloreactive T cells; Inflammatory markers; Renal tubular epithelial cells; Transgenic mice

1. INTRODUCTION

Chronic kidney disease (CKD) has emerged as an important public health burden in Taiwan, and cardiovascular disease (CVD) and malnutrition-inflammation-cachexia were the leading causes of both morbidity and mortality in patients with CKD, hemodialysis (HD), and peritoneal dialysis (PD).\textsuperscript{1} Decoy receptor 3 (DcR3) has emerged as a novel pleiotropic immunomodulator to modulate proinflammatory responses via “decoy” and “non-decoy” actions,\textsuperscript{2,3} and thus may serve as a biomarker of disease severity or a predictor of disease outcomes. DcR3 is not detectable in most normal human kidney tissues but serum and tissue DcR3 levels are higher in patients with late-stage CKD, especially in HD patients, when compared with those in cancer patients or normal individuals.\textsuperscript{4,5} Our previous study determined that DcR3 not only represents sustained low-grade systemic inflammation in CKD patients,\textsuperscript{4} but also indicates subsequent residual inflammation in localized kidney allograft and further allograft fibrosis.\textsuperscript{7} Systemic inflammation is especially high in PD and HD patients.\textsuperscript{6,7} Furthermore, DcR3 might serve as a marker for malnutrition-inflammation status. Serum DcR3 was inversely related to albumin and positively related to high-sensitivity C-reactive protein (hs-CRP) and low nutritional status.\textsuperscript{19}

In the tumor necrosis factor receptor (TNFR) family, DcR3 is the only member capable of neutralizing three ligands: Fas ligand (Fasl), LIGHT (homologous to lymphotoxin, exhibits inducible expression and competes with HSV glycoprotein D for binding to herpesvirus entry mediator, a receptor expressed on T lymphocytes), and tumor necrosis factor-like ligand 1A (TL1A). From this perspective, DcR3.Fc-mediated anti-inflammation involves TNFRSF6B/DcR3-induced T-cell costimulation, which possibly operates through FasL-mediated reverse signaling.\textsuperscript{11} However, inflammation is also down-regulated due to DcR3-induced M2/H2-like phenotype via activation of heparin sulfate proteoglycans, such as syndecan-2 and CD44v3.\textsuperscript{12} In basic studies, in addition to “decoy” function, DcR3 can suppress the Th1 response and attenuate cell-mediated immunity in vitro.\textsuperscript{11,12} The recombinant DcR3.Fc fusion protein is able to induce CD14\textsuperscript{low} monococyte differentiation into CD14\textsuperscript{low}/CD40\textsuperscript{low}/CD54\textsuperscript{low}/CD80\textsuperscript{low}/CD86\textsuperscript{high}
dendritic cells (DCs), which then skew T cell differentiation into the Th2 phenotype.1,2,13 Hsieh et al also found DcR3 modulates macrophage activation toward an M2-like phenotype in vitro and that DcR3 downregulates MHC class II expression in tumor-associated macrophages (TAMs) via epigenetic control.12,13 Compared with wild-type CT26 colon cancer cells, enhanced migration and invasion have been observed in CT26-DcR3 stable transfectants. In an animal model, compared with wild-type mice, significantly enhanced tumor growth and spreading were observed in CD68 promoter-driven DcR3 transgenic (Tg) mice.14 Therefore, DcR3-Fc-treated DCs skew T cell differentiation into the Th2 phenotype, while DcR3-Fc-treated macrophages behave in the same manner as the M2 phenotype. Based on the current available evidence, the precise roles of DcR3 in kidney diseases remain unclear and thus further study is needed to elucidate the underlying mechanisms.

2. PURPOSE OF THE REVIEW

With respect to candidate biomarkers in the progression of CKD itself and outcomes of CKD patients, there are currently no identical and representative biomarkers, which carry both immunological and nonimmunological factors. Accordingly, traditional methods, such as baseline estimated glomerular filtration rate (eGFR) by category, absolute change in eGFR value, annual eGFR decline or annual percentage change of eGFR, velocity of eGFR slopes, and eGFR variability, have been widely used for predicting cognitive deterioration, cardiovascular risk, renal outcome, and patient mortality.17–21 The other established risk factors to predict progression of CKD, survival of kidney allograft, and outcomes of patients are diabetes mellitus, hypertension, hyperlipidemia, hyperuricemia, proteinuria, and Charlson Comorbidity Index score.24,26 Specific risk factors for survival of kidney allograft and renal transplant recipients (RTRs) are posttransplantation diabetes mellitus (PTDM),27 metabolic syndrome,28 posttransplantation glomerulonephritis (PTGN),29 sarcopenia,30 and recurrent kidney allograft rejection.20

Several candidate biomarkers such as longitudinal measurements of cystatin C,14 urine neutrophil gelatinase-associated lipocalin (NGAL),31 serum cytokines, chemokines, and microRNA–RNA32 are now available. However, there is some controversy in the literature. Serum or tissue DcR3 is not inferior to the diagnostic performance of several available tests, and serves as an adequate biomarker in terms of accuracy of risk prediction.2,10 However, DcR3 is not found in mouse and rat genomes.2,3 The purpose of the current review includes the comparison of the difference between human and animal studies. Overexpression of DcR3 in Tg mice model or hydrodynamics-based gene delivery of DcR3 plasmid could mimic pathophysiologic expression in human after acute or chronic insults. The real pathomechanistic roles for upregulation of DcR3 during inflammation inducing positive or negative-feedback reaction in patients with CKD, HD, PD, or kidney transplantation may be performed in the inhibition of DcR3 expression in human primary cells or transformed cells.

3. SCIENTIFIC EVIDENCE

3.1. Serum DcR3 as a biomarker for inflammation and patient outcomes

We found that high serum DcR3 levels were associated with occurrence of PD-related peritonitis in patients receiving chronic PD. Baseline serum DcR3 in PD patients was 1.94 ± 1.23 ng/mL.39 However, relatively high serum DcR3 concentrations ranged from 0.05 to 17.78 ng/mL in maintenance HD patients.9 Baseline serum DcR3 showed a strong positive correlation with inflammatory markers (interleukin-6 [IL-6] and hs-CRP) and adhesion molecules, including intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1).8 Low serum albumin and history of CVD, the leading two causes of morbidity in patients on HD, were significantly and negatively associated with DcR3 levels.9 More importantly, serum DcR3 levels were elevated in HD patients and were closely related to inflammation, cardiovascular, and all-cause mortality.9 The potential mechanism involves DcR3 modulating the proinflammatory response via “non-decoy” activities through NF-κB-mediated expression of ICAM-1, VCAM-1, and IL-8 by monocytes, whose binding capacity to endothelium was shown to be enhanced in circulation.9

Serum DcR3 level in RTRs was relatively low when compared with the levels in mice treated with hDCR3 or Tg overexpression (150 to 850 ng/mL),1,2,13,14 RTRs in the high DcR3 expression (HDE) in RTECs had high serum DcR3 levels (1.52 ± 0.36 ng/mL) compared with the levels (0.71 ± 0.27 ng/mL) of the low DcR3 expression (LDE) in RTECs.9 The human serum DcR3 concentration or endogenous DcR3 expression may not be high enough to cope with the modulation of the T cell response in acute T cell-mediated rejection (TCMR). We proposed a two-hit theory in human DcR3 expression in kidney allograft associated with allograft survival after kidney transplant rejection. In the acute stage of the cellular immunologic storm, DcR3 could directly induce NF-κB-mediated expression of adhesion molecules and inflammatory cytokines.12,15,16 Furthermore, locally expressed DcR3 in the RTECs of kidney allograft may suppress the FasL-Fas-mediated apoptosis of T cells, leading to an accumulation of activated allo-responding T cells.37 The second-hit could be caused by subsequent residual inflammation, indicating that HDE in the damaged tubuli could facilitate peripheral myofibroblast escaping from Fas and FasL-induced apoptosis.3

3.2. Tissue DcR3 as a biomarker for sporadic IgA nephropathy, CKD progression, and kidney allograft failure

In CKD patients, we found that the higher the expression of DcR3 in RTECs was, the greater the expression of α-smooth muscle actin (α-SMA) and fibrosis in the interstitium.4 CKD patients with HDE had a higher risk of poor composite disease outcomes, including doubling of serum creatinine and/or end-stage renal failure. The effect of this novel tissue biomarker on outcomes was more pronounced compared with conventional risk factors, such as proteinuria, diabetes mellitus, hypertension, hyperlipidemia, and eGFR.4 A potential mechanism may involve DcR3 acting as a death decoy receptor to neutralize the proapoptotic effects of FasL, LIGHT, and LT1A. Human renal myofibroblasts with inducible DcR3 by TNF-α survived FasL-induced apoptosis due to constitutive expression of Fas receptor (FasR) in human renal interstitial fibroblasts.6

The TFNFRSF6B (DcR3) gene variants using high-throughput single nucleotide polymorphism (SNP) test over biopsy tissues are associated with sporadic IgA nephropathy (IgAN), with the exception of familial clustering of IgAN.8 The proposed biologic relevance of tag SNPs of the DcR3 gene, which has a positive association with IgAN, is that they serve as an antagonist to downregulate LIGHT-LTBR signaling.8 The main cause is related to an overexpression of LIGHT in Tg mice, leading to an IgAN phenotype characterized by T cell-mediated intestinal inflammation, dysregulation of immunoglobulin A production and clearance, mesangial IgA deposition, hematuria, and proteinuria.19

In RTRs, HDE in RTECs can independently predict poor graft outcome (2-fold increase in serum creatinine and/or graft failure) and significantly increase the predictability of kidney
3.3. Immunological phenotype of cell-mediated rejection in renal histopathology

In RTRs, the greater expression of DcR3 immunoreactivity in RTECs was correlated with acute TCMR manifesting as tubular atrophy and interstitial inflammation. The DcR3 expression was more specifically related to the severity of acute TCMR (grade 1B and grade 2A versus grade 1A) in the subgroup analysis.7

In situ hybridization and immunohistochemical (IHC) staining were concurrently used for analyzing different severity of acute TCMR in kidney allograft. We not only found that intense DcR3 mRNA expression in RTECs infiltrated with mononuclear cells corresponded to the areas of active inflammation and severe rejection-related architecture, but also that the pattern seemed to show endogenous DcR3 was locally produced by mononuclear cells and RTECs. There was no significant difference between HDE and LDE in severity levels of acute antibody-mediated rejection. There was a positive correlation between HDE and tubulitis. A positive correlation between HDE and interstitial fibrosis was found, but there were no direct relationships among HDE, peritubular capillaritis, and glomerulitis.7

4. DCR3 AS A POTENTIAL THERAPEUTIC AGENT IN KIDNEY DISEASE IN VIVO

The possible utility of hydrodynamics-based gene delivery (CMV promoter-driven human DcR3 plasmid, pCMV-DcR3) and recombinant DcR3 (DcR3.Fc) can prevent the development of autoimmune crescentic glomerulonephritis in (C57BL/6 crossing DBA/2J) hybrid mice and progressive IgAN in B-cell-deficient mice by daily injection of purified IgA antiphosphorylcholine antibodies and pneumococcal C-polysaccharide (Pnc). The beneficial effect might be due to modulation of T cell activation/proliferation or B cell activation. The other protective effect of DcR3 is against apoptosis of spleen and kidney parenchyma. Suppression of mononuclear leukocyte infiltration was evidenced by histopathology with hematoxylin and eosin stain, flow cytometry of splenic T cells, IHC staining for CD3, F4/80, or CD11b, real-time PCR assay for monocyte chemotactic protein-1 (MCP-1) and IL-6, and renal tissue NF-xB expression.40

5. DCR3 TG MICE

To understand the systemic effects of DcR3 in vivo and pathophysiologic expression in human after acute or chronic insults, Tg mice were studied using rat-insulin promoter (RIP)41 and CD68 promoter:27.37,40 IHC stain for DcR3 and insulin from Tg islet graft (overexpression of human DcR3 in islet β-cells by RIP promoter)—bearing kidneys in nonobese diabetic mice showed that DcR3 was potentially effective in prolonging islet graft survival, but did not provide permanent protection from diabetes recurrence.41 In CD68 promoter-driven DcR3 Tg (CD68-DcR3 Tg) mice, DcR3 was found to be a potent tumor-secreted factor that skews macrophage polarization toward TAM or macrophage phenotype 2. When BALB/c mice were infected with Listeria monocytogenes, DcR3 Tg mice showed up-regulation of anti-inflammatory cytokines (IL-4 and IL-10) and down-regulation of proinflammatory cytokines (IFN-γ, IL-12, and TNF-α).41 However, further study is needed to comprehensively demonstrate molecular evidence of systemic DcR3-mediated anti-inflammation in kidney disease.

<p>| Table 4. DCR3 as a prognostic biomarker in kidney diseases in a literature review |</p>
<table>
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<th>Population</th>
<th>Type of study</th>
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<td>Chronic HD patients</td>
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<td>RTRs with acute rejection</td>
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<td>NF-xB-mediated expression of adhesion molecules and inflammatory cytokines; suppress the Fas-L-Fas-mediated apoptosis of T cells in acute stage; peripheral myofibroblast escaping from Fas and Fas-L-induced apoptosis</td>
<td>Doubling of serum creatinine and/or graft failure</td>
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</table>

AGGN = autoimmune crescentic glomerulonephritis; BCD = B-cell-deficient; CKD = chronic kidney disease; DcR3, decay receptor 3; GN = glomerulonephritis; HD = hemodialysis; ICAM-1 = intercellular adhesion molecule-1; IgAN = IgA nephropathy; MIC = malnutrition-inflammation cachexia; PD = peritoneal dialysis; PEW = protein-energy wasting; RTRs = renal transplant recipients; VCAM-1 = vascular cell adhesion molecule-1.
6. KNOCKDOWN OF DCR3 IN HUMAN

There is evidence that knockdown of DcR3 by lentivirus-delivered short hairpin RNA inhibited ectopic adhesion of endometrium and abrogated endometriosis progression. Tsai et al. found that DcR3 is upregulated in human endometrial cells with high serum estrogen levels, and DcR3 expression level correlates positively with adhesion molecules (ICAM-1 and homing cell adhesion molecule) via Akt-NFκB signaling pathways. Knockdown of DcR3 not only suppresses endometriosis tissue growth and adhesion in an orthotopic xenograft endometriosis mouse model, but also reduces adhesion molecule expression and cell migration of the HEC1B cell line. There is no available data in DcR3 knockout in human renal cells, which could be more representative for the function of DcR3 in human.

In conclusion, in RTRs, DcR3 expression seemed to be associated with nonspecific expression in damaged tubuli, renal interstitium, peritubular capillaries, and infiltrating leukocyte subsets. Upregulation of DcR3 during acute inflammatory reactions induces negative-feedback to suppress inflammation. In CKD patients, DcR3 affects immunomodulation by serving as a traditional decoy receptor to antagonize Fasl-Fas-mediated apoptosis of myofibroblasts. The cell-specific expression of DcR3 on RTECs seemed to be derived from TNFα stimulation. In HD, PD, and older adults with malnourishment on HD, DcR3 functions as an effector molecule that modulates proinflammatory responses via “non-decoy” activities through NFκB-mediated expression of ICAM-1, VCAM-1, and IL-8 by monocytes. Our sequential studies (Table) demonstrated that DcR3 may be an appropriate prognostic biomarker for patients with CKD, HD, PD, malnourishment on HD, and RTRs.

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