End-stage renal disease is different from chronic kidney disease in upregulating ROS-modulated proinflammatory secretome in PBMCs - A novel multiple-hit model for disease progression

Ruijing Zhang\textsuperscript{a,b,c}, Jason Saredy\textsuperscript{d}, Ying Shao\textsuperscript{a}, Tian Yao\textsuperscript{j}, Lu Liu\textsuperscript{a}, Fatma Saoud\textsuperscript{a}, William Y. Yang\textsuperscript{d}, Yu Sun\textsuperscript{a}, Candice Johnson\textsuperscript{a}, Charles Drummer IV\textsuperscript{a}, Hangfei Fu\textsuperscript{a}, Yifan Lu\textsuperscript{a}, Keman Xu\textsuperscript{a}, Ming Liu\textsuperscript{h}, Jirong Wang\textsuperscript{a}, Elizabeth Cutler\textsuperscript{a,i}, Daohai Yu\textsuperscript{e}, Xiaohua Jiang\textsuperscript{a}, Yafeng Li\textsuperscript{c}, Rongshan Li\textsuperscript{c}, Lihua Wang\textsuperscript{g}, Eric T. Choi\textsuperscript{f,g,h}, Hong Wang\textsuperscript{g,h}, Xiaofeng Yang\textsuperscript{a,g,h,**}

\textsuperscript{a} Center for Inflammation, Translational & Clinical Lung Research, Lewis Katz School of Medicine at Temple University, Philadelphia, PA, 19140, USA
\textsuperscript{b} Department of Nephrology, The Second Hospital of Shanxi Medical University, Taiyuan, Shanxi, 030013, China
\textsuperscript{c} Department of Nephrology, The Affiliated People’s Hospital of Shanxi Medical University, Taiyuan, Shanxi, 030012, China
\textsuperscript{d} Rutgers University, New Brunswick, NJ, 08901, USA
\textsuperscript{e} Department of Clinical Sciences, Lewis Katz School of Medicine at Temple University, Philadelphia, PA, 19140, USA
\textsuperscript{f} Division of Vascular and Endovascular Surgery, Department of Surgery, Lewis Katz School of Medicine at Temple University, Philadelphia, PA, 19140, USA
\textsuperscript{g} Centers for Metabolic Disease Research, Cardiovascular Research, & Thrombosis Research, Lewis Katz School of Medicine at Temple University, Philadelphia, PA, 19140, USA
\textsuperscript{h} Departments of Pharmacology, Microbiology and Immunology, Lewis Katz School of Medicine at Temple University, Philadelphia, PA, 19140, USA
\textsuperscript{i} School of Science and Engineering, Tulane University, New Orleans, LA, 70118, USA
\textsuperscript{j} Shanxi Medical University, Taiyuan, Shanxi Province, 030001, China

ARTICLE INFO

Keywords:
- Chronic kidney disease (CKD)
- End-stage renal disease (ESRD)
- PBMC secretome
- Reactive oxygen species (ROS)
- Trained immunity

ABSTRACT

Background: The molecular mechanisms underlying chronic kidney disease (CKD) transition to end-stage renal disease (ESRD) and CKD acceleration of cardiovascular and other tissue inflammations remain poorly determined.

Methods: We conducted a comprehensive data analyses on 7 microarray datasets in peripheral blood mononuclear cells (PBMCs) from patients with CKD and ESRD from NCBI-GEO databases, where we examined the expressions of 2641 secretome genes (SG).

Results: 1) 86.7% middle class (molecular weight >500 Daltons) uremic toxins (UTs) were encoded by SGs; 2) Upregulation of SGs in PBMCs in patients with ESRD (121 SGs) were significantly higher than that of CKD (44 SGs); 3) Transcriptomic analyses of PBMC secretome had advantages to identify more comprehensive secretome than conventional secretomic analyses; 4) ESRD-induced SGs had strong proinflammatory pathways; 5) Proinflammatory cytokines-based UTs such as IL-1β and IL-18 promoted ESRD modulation of SGs; 6) ESRD- and CKD-upregulated UTs such as IGFBP7 and downregulating anti-inflammatory TGF-β1 and telomere stabilizer SERPINE1/PAI-1; 7) ROS pathways played bigger roles in mediating ESRD-upregulated SGs (11.6%) than that in CKD-upregulated SGs (6.8%), and half of ESRD-upregulated SGs were ROS-independent.

Conclusions: Our analysis suggests novel secretome upregulation in PBMCs of patients with CKD and ESRD, act synergistically with uremic toxins, to promote inflammation and potential disease progression. Our findings have provided novel insights on PBMC secretome upregulation to promote disease progression and may lead to the identification of new therapeutic targets for novel regimens for CKD, ESRD and their accelerated cardiovascular disease, other inflammations and cancers. (Total words: 279).

https://doi.org/10.1016/j.redox.2020.101460

Received 11 December 2019; Received in revised form 28 January 2020; Accepted 7 February 2020
Available online 20 February 2020

© 2020 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
1. Introduction

The incidence of chronic kidney disease (CKD) is increasing worldwide [1]. A major cause of mortality in patients with CKD has been found to be atherosclerosis-related cardiovascular disease (CVD) [2]. Our and others’ recent reports showed that CVD stressors and risk factors such as hyperlipidemia [3,4], hyperglycemia [5], hyperhomocysteinemia [6,7], and chronic kidney disease [8–10], promote atherosclerosis and vascular inflammation via several mechanisms. These mechanisms include endothelial cell (EC) activation [3,11–14] and injury [15]; caspase-1/infammasome activation [8,10], mitochondrial reactive oxygen species (ROS) [4]; Ly6Chigh mouse monocyte and CD40+ human monocyte differentiation [7,16–18]; decreased/trans-differentiated regulatory T cells [19–22] (Treg); impaired vascular reparability of bone marrow-derived progenitor cells [23,24]; down-regulated histone modification enzymes [25] and increased expressions of trained immunity pathway enzymes [26].

CKD is classified into five stages [27] based on glomerular filtration rate (GFR, mL/min. per 1.73 m²); ≥90 mL/min (stage 1), 60–89 mL/min (stage 2), 30–59 mL/min (stage 3), 15–29 mL/min (stage 4) and <15 mL/min (stage 5). At stage 5, the patient develops end-stage renal disease (ESRD) and requires life-long renal replacement therapy (RRT). Clinical evaluations for kidney function include creatinine level, blood urea nitrogen (BUN) assessment and cystatin C level (MedlinePlus, NIH https://medlineplus.gov/kidneytests.html). CVD risk increases significantly according to the stages of CKD, ranging from 1.5-fold in stage 2, to between 20 and 1000-folds with ESRD [28]. Indeed, CVD accounts for 50% of deaths in patients receiving dialysis [29], demonstrating that CKD accelerates atherosclerotic pathology [28], which along with its complications such as myocardial infarction, stroke and peripheral artery disease, are the leading cause of morbidity and mortality in this country, and account for 75% of all CKD deaths from CVD [30]. The molecular and cellular mechanisms underlying CKD-accelerated atherosclerotic pathology, remain unknown.

It has been suggested that CKD-derived uremic toxins (UTs) [31], in combination with other risk factors, cause oxidative stress including mitochondrial ROS [4], low-grade inflammation with increased circulating cytokines and endothelial dysfunction [28,32]. Recently, in novel UT metabolomics/gene databases, we analyzed the expression changes of UT receptors and UT synthases in CKD and CVD. We made the following observations: 1) UTs represent only 1/80th of the human serum small-molecule metabolome; 2) Increased in CKD and CVD, some UTs induce or suppress the expression of inflammatory molecules; 3) The expression of UT genes are significantly modulated in CKD patients, and coronary artery disease (CAD) patients; and 4) The expressions of UT genes are upregulated by pathogen/danger associated molecular pattern receptors (PAMPs/DAMPs)/inflammasome-caspase-1 as we reported [8] and tumor necrosis factor-α (TNF-α) pathways but are inhibited in CD4+Foxp3+ regulatory T cells (Treg). These results demonstrate that UTs are selectively increased, and serve as DAMPs and homeostasis-associated molecular patterns (HAMPs) that modulate inflammation [33]; and that some UT genes are upregulated in CKD and CAD rather than by pure passive accumulation [10]. One well-characterized UT example is carbamylated low-density lipoprotein (cLDL) [34]. Protein carbamylation has been found in atherosclerotic plaque; and serum level of cLDL is increased significantly in patients with ESRD, which has been shown to have all of the major biological effects relevant to atherosclerosis, including EC injury and dysfunction [35] by binding to oxidized low-density lipoprotein (oxLDL) receptor (LOX-1) [36], increased expression of cell adhesion molecules, monocyte adhesion, and vascular smooth muscle cell (VSMC) proliferation [34]. However, an important question remains whether additional secretory proteins participate in the pathogenesis and inflammatory acceleration of CKD and ESRD.

The secretome, defined as a portion of total proteins secreted by cells to the extracellular space, secures a proper micro-environmental niche, thus maintaining tissue homeostasis [37,38]. Secreted molecules are key mediators in cell-cell interactions and influence the cross-talk with the surrounding tissues in addition to their endocrine functions in long-distance as previously demonstrated by hormones, growth factors, cytokines, adipokines, myokines, cardiokines [39], and chemokines [40]. There is strong evidence supporting that crucial cellular functions such as proliferation, differentiation, communication and migration are strictly regulated from the cell secretome [41]. The major difference between our current study and previous reports on the roles of cytokines and chemokines in CKD pathology is that secretome analyses provide a panoramic view on all the secreted genes in the human genome modulated in CKD and ESRD, as opposed to focusing on only one or a few cytokines/chemokines. Recent reports showed that aberrant endothelial secretome in kidney diseases contribute to fibroblast reprogramming [40]. More importantly, peripheral blood mononuclear cells (PBMCs) are first tier of sensors to uremic toxins and other proinflammatory molecules in serum during kidney dysfunction [42,43]. Gene expression profile, metabolite profile, monocytic counts of PBMCs are identified to provide an access to evaluate and predict the settings of CVD and CKD [7,44–46]. The PMC morphology, Treg/Th17 disequilibrium and activation of TLRs on membrane of PBMCs promote vascular calcification and endothelial dysfunction, which are closely related to cardiovascular risk in CKD patients [47–49]. Meanwhile, glomerular inflammation is correlated with IL-6 and IL-1β secretion in the peripheral blood [50]. However, an important question remains whether CKD and ESRD upregulate the secretome gene expressions in innate immune cells such as PBMCs, by which chronic systemic and tissue inflammations get accelerated.

In order to broaden our understanding of CKD and ESRD-accelerated inflammation, we hypothesized that CKD and ESRD induce differential secretomic gene (SG) expression patterns in PBMCs [51], by which CKD and ESRD accelerate inflammation. We conducted a comprehensive data analyses on a microarray dataset (GEO ID:GSE15072) containing genomic screening of PBMCs from patients with CKD and ESRD from the NIH-NCBI-GEO databases (https://www.ncbi.nlm.nih.gov/gds/), where we examined expressions of 2641 secretome genes (SG). We made the following findings: 1) 86.7% middle class UTs were encoded by SGs; 2) Upregulations of SGs in PBMCs in patients with ESRD (121 SGs) were significantly higher than that of CKD (44 SGs); 3) ESRD-induced SGs had strong proinflammatory pathways; 4) Proinflammatory cytokines-based UTs such as IL-1β and IL-18 promote ESRD modulation of SGs; 5) ESRD-upregulated co-stimulation receptors CD48 and CD58 increase secretomic upregulation in the PBMCs, which are magnified enormously in tissues; 6) M1-, and M2-macrophage polarization signals contribute to ESRD- and CKD-upregulated SGs; 7) ESRD- and CKD-upregulated SGs contain senescence-promoting regulators by upregulating proinflammatory IGFFBP7 and downregulating anti-inflammatory TGF-β1 and telomere stabilizer SERPINE1/PAI-1; and 8) ROS pathways play bigger roles in mediating ESRD-upregulated SGs (11.6%) than that in CKD-upregulated SGs (6.8%), and half of ESRD-upregulated SGs are ROS-independent. Novel PBMC-secretome acts synergistically with uremic toxins, to promote inflammation and potential disease progression. Our findings provided novel insights on secretomic upregulation in PBMCs of patients with CKD and ESRD and identification of new therapeutic targets on CKD, various inflammations and cancers.

2. Materials and methods

2.1. Expression profile of secretomic genes (SGs) and innate immunomac genes (IGs) in PBMC from patients with CKD and with ESRD

Microarray datasets were collected from National Institutes of Health (NIH)-National Center for Biotechnology Information (NCBI)-Gene Expression Omnibus (GEO) databases (https://www.ncbi.nlm.nih.gov/gds/) and analyzed with an online software GEO2R (https://
www.ncbi.nlm.nih.gov/geo/geo2r/). The numbers of 7 GEO datasets were listed in Table 2A. The detailed information of these GEO datasets was shown in Table 2A and other tables.

2.2. Statistical analysis of microarray data

As we reported [26,52], we applied a statistical method similar to that meta-analysis and analyzed the expressions of 9 house-keeping genes including ACTB, GAPDH, PGK1, PPIA, B2M, YWHAZ, SDHA, HMBS, TBP (Supplement Table of Housekeeping Genes) in all GEO datasets regardless of species that were chosen for this study. The house-keeping gene list was extracted from the list provided by Eisenberg and Levanon [53]. Briefly, the mean log fold change (LogFC) of house-keeping genes between treatment and control groups vary from −1.27 to 1.28. As this variation was very narrow, we concluded that the datasets (Table 2A) are of high quality. The target genes with expression changes more than 2-folds in CKD and ESRD were defined as the upregulated genes, while genes with their expression decreased more than 2-fold in CKD and ESRD were defined as downregulated genes [logFC] > 1).

2.3. Ingenuity Pathway Analysis

We utilized Ingenuity Pathway Analysis (IPA, Qiagen, https://www.qiagenbioinformatics.com/products/ingenuity-pathway-analysis/) to characterize clinical relevance and molecular and cellular functions related to the identified genes in our microarray analysis. Differentially expressed genes were identified and uploaded into IPA for analysis. The core and pathways analysis was used to identify molecular and cellular pathways, as we have previously reported [52,54].

3. Results

3.1. 86.7% middle class (molecular weight > 500 Daltons) uremic toxins (UTs) were encoded by secretomic genes (SGs)

We recently reported that UTs, classified in three major groups including 1) small solutes, 2) protein-bound uremic toxins, 3) middle molecules, are significantly upregulated and modulated in patients with chronic kidney disease (CKD) [10,55–57]. To improve our understanding of how many uremic toxins are encoded by secretomic genes, we hypothesized that SGs encode the majority of middle class uremic toxins (UTs). To test this hypothesis, we collected a comprehensive list of human SGs containing 2641 secreted gene proteins as predicted by majority decision-based method for secreted proteins (MDSEC) used for protein classification within the Protein Atlas (https://www.proteinatlas.org/search/protein_class:Secreted + proteins + predicted + by + MDSEC, accessed December 4, 2019) [51]. As others reported, the expression levels of mRNAs are strongly correlated to that of proteins when comparing samples of the same cell type/tissue [58], justifying for us to estimate SG changes in the diseases using peripheral blood mononuclear cells (PBMC) transcriptomic changes for the SG changes. A list of 30 middle class UTs were collected from the European Uremic Toxins (EUTox) Database (www.uremic-toxins.org, assessed on December 4, 2019) [59–61]. As shown in Table 1A, 26 out of 30 middle molecular class UTs (86.7%) were well-characterized cytokines and encoded by SGs. In addition, as shown in Table 1B, the Ingenuity Pathway Analysis (IPA) showed that at least five out of top 10 pathways of middle class UTs were closely related with cytokine-associated signaling functions including apelin (adipokine) liver signaling [62], role of hypercortykemia and hyperchemokinemia, role of cytokines in mediating communication between immune cells, differential regulation of cytokine production in macrophages and T helper cells by IL-17A and IL-17F, and IL-10 signaling. If we associate the rest of five UTs top pathways with innate immune responses, and tissue inflammation including communication between innate and adaptive immune cells, glucocorticoid receptor signaling, graft-versus-host disease signaling [63], triggering receptor expressed on myeloid cells-1 (TREM1) signaling [64], and cardiac hypertrophy signaling [65], then we can classify all the top 10 pathways of UTs play significant roles in promoting inflammations related to ESRD. Therefore, these results suggest that secretomic changes in the PBMCs contributed to the generation of middle class UTs and therefore may play significant roles in the progression of CKD and end-stage renal disease (ESRD); and that secretomic changes detected in transcriptomic approaches reflected secretomic changes in protein levels, demonstrated by UTs, detected on protein levels, as examples, at least partially.

3.2. Uregulations of SGs in PBMCs in patients with ESRD (121 SGs) were significantly higher than that of CKD (44 SGs); and among ESRD specifically modulated 975 SGs, ESRD upregulated 116 SGs (11.9%) but downregulated 859 SGs (88.1%), respectively

To improve our understanding of how many secreted molecules are generated in the prototypic innate immune cell types, PBMCs, in patients with CKD and ESRD, we hypothesized that the expressions of secretome in PBMCs from patients with CKD and ESRD are modulated in comparing to that of healthy controls. As shown in Table 2B, we identified significant secretomic mRNA expression changes in PBMCs, from patients with CKD and patients with ESRD (see the references for the information regarding the classification of CKD and ESRD, patients and controls) [66–68]. Total 44 out of 2641 SGs (1.67%) were
upregulated in PBMCs from patients with CKD; in comparison, 121 out of total 2641 SGs (4.58%) were upregulated in the PBMCs from patients with ESRD. In addition, 55 out of 2641 SGs (2.08%) were downregulated in the PBMCs from patients with CKD and ESRD. These results suggest that 1) a panoramic view of secretome changes in PBMCs can be generated by analyzing microarray data from patients with CKD and ESRD; 2) secretome changes in PBMCs may contribute significantly to generation of UTs in patients with chronic kidney disease (CKD) and end-stage renal disease (ESRD) (also see Table 4A); and 3) UTs-based secretome in PBMCs in patients with CKD and ESRD may play significant roles in promoting CKD- and ESRD-associated accelerated systemic and tissue inflammations (also see Table 4D).

Secretome upregulation and downregulation in the PBMCs from patients with CKD and ESRD can be categorized into four groups by Venn Diagram analysis, Fig. 1. The results showed that: 1) 25 SGs upregulated in CKD (25/44, 56.8%) shared with that downregulated in ESRD, and shared four SGs with that upregulated in ESRD. The 25 SGs upregulated in CKD but downregulated in ESRD had potential in inhibiting the progression of ESRD. The four SGs downregulated in CKD but upregulated in ESRD suggest: a) their regulatory pathways switched from downregulation in CKD to upregulation in ESRD; and b) their potential roles in promoting ESRD progression and downregulates 88.1% SGs for disease progression, indicating for the first time that as high as 88.1% SGs in the PBMCs may play homeostatic functions which could potentially contribute to the inhibition of ESRD progression.

Therefore, we have demonstrated for the first time that ESRD upregulates 116 SGs that may promote ESRD progression and downregulates 859 SGs that may inhibit ESRD progression in the PBMCs in patients with ESRD. If ESRD-specific upregulated 116 SGs plus ESRD-specific downregulated 859 SGs as 100% (975 SGs), this might suggest that ESRD upregulates specifically only 11.9% SGs to promote disease progression and downregulates 88.1% SGs for disease progression, indicating for the first time that as high as 88.1% SGs in the PBMCs may play homeostatic functions which could potentially contribute to the inhibition of ESRD progression.

3.3. ESRD-upregulated SGs had 2 folds higher percentages of the cytoplasm and nucleus subcellular groups than the controls; and had the higher percentages of five out of 13 SG functional groups including enzyme, kinase, peptide, transcription regulator, and transmembrane in comparison to the controls.

We used IPA to map the subcellular locations for CKD- and ESRD-modulated SGs. As shown in Table 3A, CKD-upregulated SGs had higher potential roles in inhibiting the ESRD progression.
percentages of extracellular space SGs (56.82%) and plasma membrane SGs (20.45%) than that of total SGs controls, 49% and 16.19% respectively; but CKD-downregulated SGs had decreased percentages of extracellular space SGs (41.82%) in comparison to that total SGs control (49%). ESRD-upregulated SGs had higher percentages of cytoplasm SGs (47.11%) and nucleus SGs (8.26%) than that of total SGs controls, 22.08% and 4.46%, respectively. In contrast, ESRD-downregulated SGs had decreased percentages of cytoplasm SGs (15.95%) and other group SGs (3.23%) in comparison to that total SGs control (cytoplasm, 22.08%), and other group SGs (8.27%), respectively.

We then used IPA to map the 13 function groups for CKD- and ESRD-modulated SGs (Table 3A). In CKD-upregulated SGs, the percentages of five out of 13 functional groups including ion channel, kinase, peptide, transcription regulator, and transmembrane were increased. In ESRD-upregulated SGs, the percentages of five out of 13 functional groups including enzyme, kinase, peptide, transcription regulator, and transmembrane were increased in comparison to that of total SGs. These results demonstrated that CKD upregulated SGs have upregulated functional group of SGs similar to that of ESRD except for the functional groups of enzyme and ion channel. Of note, some PBMC secretome proteins identified with a transcriptomic approach localized in the subcellular locations other than the supernatants of cultured cells and plasma that conventional secretome analyses sampled and examined. Therefore, our data have also demonstrated that

Table 3a
All of the percentages of five subcellular location groups of SGs and 14 functional groups of SGs were significantly changed in CKD and ESRD compared with that of total SG controls according to IPA results. Gene list for all these up- and downregulated SGs in CKD and ESRD as well as all of the SGs are listed in Supplement Table 3. (# - number of SGs, % - percentage in each location).

| classification | Total SGs (control) | up in CKD* | down in CKD* | up in ESRD* | down in ESRD* |
|----------------|---------------------|------------|-------------|-------------|-------------|
| group          | #       | %         | #       | %         | #       | %         | #       | %         | #       | %         |
| Location       | cytoplasm           | 574       | 22.08%   | 8         | 18.18%    | 12       | 21.82%    | 57       | 47.11%    | 148      | 15.95%    |
|                | extracellular space | 1274      | 49.00%   | 25        | 56.82%    | 23       | 41.82%    | 31       | 25.62%    | 532       | 57.33%    |
|                | nucleus             | 116       | 4.46%    | 1         | 2.27%     | 4        | 7.27%     | 10       | 8.26%     | 31        | 3.34%     |
|                | other               | 215       | 8.27%    | 1         | 2.27%     | 4        | 7.27%     | 1        | 0.83%     | 30        | 3.23%     |
|                | plasma membrane     | 421       | 16.19%   | 9         | 20.45%    | 12       | 21.82%    | 22       | 18.18%    | 187       | 20.15%    |
| total          | 2600                | 100.00%   | 44       | 100.00%   | 55       | 100.00%   | 121      | 100.00%   | 928       | 100.00%   |
| Functional group | cytokine            | 156       | 6.00%    | 1         | 2.27%     | 4        | 7.27%     | 6        | 4.96%     | 68        | 7.33%     |
|                | enzyme              | 464       | 17.85%   | 5         | 11.36%    | 9        | 16.36%    | 32       | 26.45%    | 151       | 16.27%    |
|                | G-protein coupled receptor | 18 | 0.69% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | 11 | 1.19% |
|                | growth factor       | 120       | 4.62%    | 2         | 4.55%     | 3        | 5.45%     | 6        | 4.96%     | 69        | 7.44%     |
|                | ion channel         | 10        | 0.38%    | 1         | 2.27%     | 1        | 1.82%     | 0        | 0.00%     | 8         | 0.86%     |
|                | kinase              | 47        | 1.81%    | 2         | 4.55%     | 4        | 7.27%     | 4        | 3.31%     | 31        | 2.26%     |
|                | other               | 1279      | 49.19%   | 18        | 40.91%    | 22       | 40.00%    | 39       | 32.23%    | 389       | 41.92%    |
|                | peptide             | 232       | 8.92%    | 8         | 18.18%    | 2        | 3.64%     | 13       | 10.74%    | 198       | 11.64%    |
|                | phosphatase         | 25        | 0.96%    | 0         | 0.00%     | 0        | 0.00%     | 0        | 0.00%     | 11        | 1.19%     |
|                | transcription regulator | 46 | 1.77% | 2 | 4.55% | 3 | 5.45% | 5 | 4.13% | 12 | 1.29% |
|                | translation regulator | 2 | 0.08% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% |
|                | transmembrane receptor | 115 | 4.42% | 4 | 9.09% | 4 | 7.27% | 11 | 9.09% | 45 | 4.85% |
|                | transporter         | 86        | 3.31%    | 1         | 2.27%     | 3        | 5.45%     | 5        | 4.13%     | 35        | 3.77%     |
| total          | 2600                | 100.00%   | 44       | 100.00%   | 55       | 100.00%   | 121      | 100.00%   | 928       | 100.00%   |

*P < 0.05.
Transcription factor analysis using TRANSFAC database (http://genexplain.com/transfac/) through the access of the GATHER database (https://changlab.uth.tmc.edu/gather/) showed that top 10 transcription factors filtered by P value were involved in each of the four groups of SGs such as CKD upregulated SGs, CKD-downregulated SGs, ESRD-upregulated SGs and ESRD-downregulated SGs. Of note, five out of ten transcription factors that are involved in ESRD downregulated SGs are isoforms of E2F1.

| Annotation | -ln (p value)* | main function/effect | PMID     |
|------------|---------------|----------------------|----------|
| up SGs in  |               |                      |          |
| CKD        |               |                      |          |
| E2F1(V$E2F1_Q6_01) | 5.94       | control cell-cycle progression from G1 to S phase | 7969176  |
| fork head box J 2(V$FHK2_01) | 5.94     | suppression of migration and invasion | 25873280 |
| NF-kappaB (p65) (V$NFKBQ6_01) | 5.13     | proinflammation to disease progression | 30135182 |
| E2F1(V$E2F1_Q6) | 5.01     | control cell-cycle progression from G1 to S phase | 7969176  |
| KROX (V$KROX_Q6) | 5.01     | tooth development cell cycle | 12489153 |
| DP-1 heterodimer (V$E2FDP1_01: E2F-1) | 4.31     | pathogenesis of lymphocytic leukemia | 26301816 |
| E2A (V$E2A_Q2) | 4.31     | lipid metabolism and atherosclerosis cell growth, differentiation, and apoptosis | 15564374 |
| LEF1(V$LEF1TCF1_Q4) | 3.89     | cell proliferation | 31623618 |
| upstream stimulating factor (V$USF_Q6) | 3.84     | proinflammation and fibrosis cell growth, differentiation, and apoptosis | 31623618 |
| activator protein 1(V$AP1_Q4) | 3.84     | proinflammation and fibrosis | 31623618 |
| down SGs  |               |                      |          |
| in CKD     |               |                      |          |
| nuclear respiratory factor 2(V$NR2P01) | 5.56     | oxidative stress | 27646262 |
| SREBP(V$SREBP_Q3) | 5.51     | glucose metabolism | 28920951 |
| NF-κB (V$NFKBQ6_01) | 5.24     | proinflammatory response to cell differentiation | 31101940 |
| c-ETS-1 binding site (V$ETS1_B) | 4.9     | cell differentiation | 30566881 |
| AP-1 binding site (V$AP1_C) | 4.84     | cell growth, differentiation, and apoptosis | 15564374 |
| c-ets-2 binding sites (V$ETS2_B) | 4.84     | cell growth, differentiation, and apoptosis | 11175361 |
| ZTA (V$ZTA_Q2) | 4.78     | Epstein-Barr Virus Reactivation | 27708396 |
| c-Rel (V$CREL_Q1) | 4.61     | tumorogenesis | 26757421 |
| DEAF1(V$DEAF1Q6) | 4.61     | intellectual disability | 24726472 |
| TEL2(V$TEL2_Q6) | 4.49     | hematoipoiesis | 28693791 |
| up SGs in  |               |                      |          |
| ESRD       |               |                      |          |
| Egr-1(V$EGR1_Q6) | 8.42     | inflammation and fibrosis maintenance of peripheral nerve myelin | 21511034 |
| Egr-2(V$EGR2_Q6) | 7.4     | maintenance of peripheral nerve myelin | 15836632 |
| CEBPAGAMMA (V$CEBPAGAMMA_Q6) | 6.61     | antioxidant regulator | 26667036 |
| PIT1(V$PIT1_Q6) | 6.0     | obesity and insulin resistance | 27568561 |
| Ikaros 3(V$IK3_Q3) | 5.94     | T helper cell 2 transcription factor | 21469117 |
| Hepatic nuclear factor 1(V$HNF1_C) | 5.79     | proinflammatory molecules such as C-reactive protein, IL-6, NF1, and HNF4a in CKD | 29330688 |
| early growth response gene 3 product (V$EGR3_Q1) | 5.18     | IL-1β co-expressed inflammatory gene | 31612215 |
| POU1F1(V$POU1F1_Q6) | 5.13     | inflammation/immunity and hormone regulator | 27709872 |

(continued on next page)
transcriptomic analyses of PBMC secreteme have advantages to identify more comprehensive secreteme than conventional secretemic analyses [69].

**3.4. Although CKD- and ESRD-upregulated SGs were highly diversified in signaling, ESRD-induced SGs had strong proinflammatory pathways**

To characterize the signaling pathways that CKD- and ESRD-modulated SGs are involved in, we adapted IPA to map SGs pathways. As shown in Fig. 2A, IPA indicated that upregulated and downregulated SGs in CKD were highly diversified in signaling pathways and were not classified into any signaling pathways in a statistically significant manner. The results suggest that there are diversified and multi-regulatory factor-based signals involved in controlling SGs modulations in CKD. In Fig. 2B, the IPA results showed that 22 out of total 121 (18.2%) upregulated SGs in the PBMCs from patients with ESRD were classified in five active pathways according to IPA core analysis including dermatan sulfate biosynthesis, neuroprotective role of Thimet oligopeptidase (THOP1) [70] in Alzheimer’s disease, IL-8 signaling, cardiac hypertrophy and neuroinflammation signaling pathway. The rest of 99 SGs (81.8%) upregulated SGs in the PBMCs from patients with ESRD were in a diversified manner similar to that observed in CKD. Of note, the chondroitin sulfate/dermatan sulfate (CS/DS)-containing proteoglycans (CS/DSPGs) are extracellular matrix (ECM) molecules, the chondroitin sulfate/dermatan sulfate (CS/DS)-containing proteoglycans (CS/DSPGs).

| down SGs in ESRD | Annotation | -ln (p value)* | main function/effect | PMID |
|------------------|------------|---------------|----------------------|------|
| E2F1(V$E2F1_Q6) | 12.62      | control cell-cycle progression from G1 to S phase | 7969176 |
| nuclear respiratory factor 2(V$NRF2_01) | 12.62 | oxidative stress | 27646262 |
| E2F1(V$E2F1_Q6) | 12.62 | control cell-cycle progression from G1 to S phase | 7969176 |
| E2F1(V$E2F1_Q3_01) | 12.62 | control cell-cycle progression from G1 to S phase | 7969176 |
| E2F(V$E2F_Q6) | 12.62 | control cell-cycle progression from G1 to S phase | 7969176 |
| E2F(V$E2F_Q3_01) | 12.62 | control cell-cycle progression from G1 to S phase | 7969176 |
| KROX (V$KROX_Q6) | 12.62 | tooth development | 12489153 |
| CREB(V$CREB_Q6) | 12.62 | formation of long-lasting memories | 20223527 |
| CREB(V$CREB_Q4_01) | 12.62 | formation of long-lasting memories | 20223527 |
| NRF1(V$NRF1_Q6) | 12.62 | Cholesterol Homeostasis | 29149604 |

*P value were calculated based on the probability of seeing a Bayes factor of a particular magnitude in a query.

A total of 113 active pathways were identified in ESRD downregulated SGs via IPA analysis. Six out of 113 pathways (5.31%) were positively activated by downregulated SGs in ESRD, including SPINK1 Pancreatic Cancer Pathway, Inhibition of Matrix Metalloproteases, PPAR Signaling, Apelin Cardiac Fibroblast Signaling Pathway, Antioxidant Action of Vitamin C, and PTEN Signaling. The rest of the pathways were downregulated, suggesting that a large number of SGs pathways in the PBMCs from patients with ESRD were downregulated for ESRD progression, which potentially drives physiological functions and homeostasis. Of note, the five active pathways induced by upregulated SGs in ESRD (Fig. 2B) were also included in 113 active pathways induced by downregulated SGs in ESRD according to Venn Diagram, which showed that some components in these ESRD-upregulated pathways can be fully functional in the absence of the other components in these pathways downregulated in ESRD.

In addition, to identify the upstream regulating transcription factors (TFs) for CKD- and ESRD-modulated SGs, we used the GATHER database (https://gather.genome.duke.edu/) [72] to map the TF binding in the promoters of the modulated SGs. As shown in Table 3B and Fig. 2F, the top 10 TFs bound to the promoters of CKD-upregulated SGs were E2F1, FoxJ2, NFkBP65, E2F1, ROX, DP-1 heterodimer, E2A, LEPITCF1, upstream stimulating factor and activator protein 1, which were different from that of ESRD-upregulated SGs. The top 10 TFs bound to the promoters of ESRD-upregulated SGs included Egr-1 (inflammation and fibrosis) [73], Egr-2 (maintenance of peripheral nerve myelin), CCAAT-enhancer-binding protein (CEBPgamma) (antioxidant regulator) [74], phosphate inorganic transporter 1 (PIT1) (obesity and insulin resistance) [75], Ikaros 3 (T helper cell 2 transcription factor) [76], hepatic nuclear factor 1 (HNF1) (transcription inducer for proinflammatory molecules such as C-reactive protein, IL-6, HNF1a and HNF4a in CKD) [77], early growth response gene 3 (IL-1B co-expressed inflammatory gene) [78], POU1F1 (inflammation/immunity and hormone regulator) [79], E4BP4 (obesity and insulin resistance) [80], and cell-division control protein 5. In addition, we noticed that nuclear respiratory factor 2 (different from the antioxidant transcription factor nuclear factor-erythroid-derived 2-like 2, Nrf2) [81] was
downregulated in ESRD-downregulated SGs (Table 3B and Fig. 2F). In summary, the seven TFs out of ten TFs identified with the Gather database indicated that ESRD-upregulated SGs promote inflammation, obesity and insulin resistance and fibrosis.

The Venn Diagram Analysis results (Fig. 3A) on the three secretory gene groups such as 35 UT genes (encoded for 30 UTs in Table 1A), 44 CKD upregulated SGs and 121 ESRD-upregulated SGs showed that: 1) UTs have no overlaps with CKD-upregulated SGs; 2) UTs have two toxins (CFD, and RETN) overlapped with ESRD-upregulated SGs; 3) ESRD-upregulated SGs have four SGs (ADAM Metallopeptidase Domain 9 (ADAM9), complement C3 (C3), Heat Shock Protein 90 Beta Family Member 1 (HSP90B1), and S100 Calcium Binding Protein A12

---

**Fig. 2a.** Top 10 pathways of upregulated and downregulated SGs in CKD from IPA. These pathways were highly diversified in signaling pathways and were not classified into any signaling pathways in a statistically significant manner (cutoff: P value < 0.05, |z-score| > 2). (Lists of all pathways associated with these up- and downregulated SGs in CKD via IPA are listed in Supplement Table 5.)
In summary (Table 4D), based on the four aspects above including 1) IPA analyses on proinflammatory signaling pathways of ESRD-upregulated SGs; 2) Neuroinflammation Signaling Pathway (Fig. 2A); 3) Gather/TRANSFAC-identified TFs involved in proinflammatory and profibrotic pathways (Table 3A); 4) ClueGo (Cytoscape)-identified ESRD-upregulated SGs shared with that of UTs (Fig. 2B), our results demonstrated that UT genes and ESRD-upregulated SGs share many signaling pathways, especially in some pro-inflammatory/profibrotic pathways. These results were correlated well with our recent report on our new model of inflammation-driven upregulation for uremic toxin generation rather than the traditional model of passive accumulation of metabolites fully due to kidney dysfunction to generate uremic toxins [10].

3.5. Proinflammatory cytokines-based middle class UTs such as interleukin-1β (IL-1β) and IL-18 promote ESRD modulation of SGs

To identify the molecular mechanisms underlying ESRD modulation of SGs, we selected proinflammatory cytokine-based UTs such as IL-1β and IL-18 stimuli to demonstrate proof of principle. As shown in Table 4A, IL-1β stimulation of PBMCs resulted in upregulation of five out of 121 ESRD-upregulated SGs such as C-X-C Motif Chemokine Ligand 2 (CXCL2), Interleukin 1 Receptor Associated Kinase 3 (IRAK3), Phospholipase A2 Group VII (PLA2G7), Sphingomyelin Phosphodiesterase Acid Like 3A (SMPDL3A) and Thrombospondin 1 (THBS1). Of note, the Geo dataset (microarray experiments) used as many as 17 innate immune stimuli, IL-1β-stimulated data were chosen for our analysis since the rest of innate immune stimuli were not the reported UTs. In addition, IL-1β stimulation downregulated 29 out of 928 ESRD-downregulated SGs (Table 2B) (3.13%). Moreover, IL-18 stimulation upregulated five out of 121 ESRD-upregulated SGs (4.13%) such as complement factor D (CFD), Interleukin-6 Receptor Subunit Beta (IL6st), IRAK3, Platelet-Derived Growth Factor C (PDGFC), and S100A12) overlapped with CKD-upregulated SGs. In addition, one signaling pathway “Role of Cytokines in Mediating Communication between Immune Cells” was shared by the top 10 pathways associated with UTs and the five active pathways upregulated by SGs in ESRD. These results suggest that the signaling pathway “Role of Cytokines in Mediating Communication between Immune Cells” may be significant in ESRD progression.

ClueGo (v2.5.4) from Cytoscape (v3.7.2) was also used to verify a close functional relationship between UT genes further and up-regulated SGs in ESRD (Fig. 2B) [82-84]. Of note, the small dots were the genes that connected UT gene group and upregulated SGs in ESRD. ClueGo identified four ESRD-upregulated SGs pathways (big balls in blue color), neuropeptide receptor finding, regulation of endocrine process, lung fibrosis and hyaluronan metabolic process. In addition, ClueGo identified five UT genes pathways, such as prostaglandin biosynthetic process (big balls in red color). Moreover, ClueGo found 20 shared (big balls in grey color, connected) regulatory pathways (Supplemental Table 8).

In summary (Table 4D), based on the four aspects above including 1) IPA analyses on proinflammatory signaling pathways of ESRD-upregulated SGs (Fig. 2B) and 2) IPA analyses on activating profibrotic pathways in ESRD-downregulated SGs (Fig. 2D); 3) Gather/TRANSFAC-identified TFs involved in proinflammatory and profibrotic pathways (Table 3B); 4) ClueGo (Cytoscape)-identified ESRD-upregulated SGs shared with that of UTs (Fig. 3B), our results demonstrated that UT genes and ESRD-upregulated SGs share many signaling pathways, especially in some pro-inflammatory/profibrotic pathways. These results were correlated well with our recent report on our new model of inflammation-driven upregulation for uremic toxin generation rather than the traditional model of passive accumulation of metabolites fully due to kidney dysfunction to generate uremic toxins [10].
upregulated in ESRD were cytokines including inflammation-modulating aminoacyl tRNA synthetase complex interacting multifunctional protein 1 (AIMP1), inflammatory C-X-C motif chemokine ligand 2 (CXCL2), inflammatory CXCL8, T cell and B cell promoting IL7, T cell and natural killer cell-activating IL-15 and inflammatory X–C motif chemokine ligand 1 (XCL1).

In Fig. 3C, the Venn Diagram Analysis results showed that four cytokines downregulated in CKD were shared with that downregulated in ESRD. The expanded list of 68 cytokines downregulated in ESRD can be split into two groups. First are the chemokine subset of cytokines. Chemokines are involved in cell migration, activation and tissue injury and thus key mediators of inflammation, especially in cardiovascular disease [85]. According to previous studies, the majority of these proteins were involved in the homeostatic function of immune cells due to their ligand promiscuity [86]. The profile of chemokines downregulated in ESRD were mostly “homeostatic” compartments rather than “inflammatory” ones. Typically, CCL2, 3, 4, 5, 11, CXCL1, 2, 8, and 10 played proinflammatory roles in kidney disease; and these chemokines were not included in this list [87–89]. Secondly, pro-inflammatory cytokine drivers were “common” in kidney disease including IL6, IL8, IL10, IL17 and IL18 and were not included in our downregulated cytokine list [88]. These cytokines may be contributed by cell types other than PBMCs during kidney diseases. Interestingly, both proinflammatory and anti-inflammatory members (IL-1Ra and IL-36G) were all downregulated, indicating that a compensatory balance weighed by PBMCs was associated with disease progression [90]. Of note, IL10 family members, including IL-19, IL-22, IL-24, IL-26 and IL-28 were downregulated [90]. Based on the overall proinflammatory phenotype associated with secretomic changes during ESRD, these results suggested that in addition to the modulation by cytokines and chemokines, additional secretomic changes modulated by other mechanisms may play significant roles in disease progression. One cytokine upregulated in CKD, DKK3, was shared with that downregulated in ESRD. The six cytokines upregulated in ESRD were ESRD-specific. The results suggest that first, the numbers of SGs in the cytokine groups upregulated in ESRD are significantly higher than that of CKD; and second, highly focused six cytokines and chemokines upregulated in ESRD may play significant roles in promoting ESRD progression and systemic inflammations. To integrate all the findings on proinflammatory cytokines from UTs and from SGs upregulated in
In ESRD, we proposed a new mechanism in Fig. 3D. Proinflammatory cytokines (primary, upstream) play significant roles in combination with other uremic toxins and other mechanisms in upregulating SGs (secondary, downstream), promoting the pathogenesis of ESRD and inflammations. Of note, the classification of primary/upstream cytokines and secondary/downstream cytokines is conceptual to demonstrate the cytokine interaction as the proof of principle. We used the proinflammatory cytokines IL-1β and IL-18 from UTs as prototypic secretomic proteins to demonstrate the mutual promotion and modulation among the secretomic proteins as the role-switching of "primary" or "secondary" cytokines during ESRD. Future time course experiments will be needed to characterize chronological upregulation of cytokines in upregulated UTs and SGs in ESRD.

3.6. ESRD-upregulated co-stimulation receptors CD48 and CD58 increase secretomic upregulation in the PBMCs; may not be even limited in the PBMCs, CD48/CD58-CD2 signaling can be actually magnified enormously in tissues

We hypothesized that CKD- and ESRD-upregulated T cell activation co-stimulation receptors (CSRs) and co-inhibition receptors (immune checkpoint receptors, ICRs) [91], as prototypic cell membrane receptors in mediating cell-cell interactions, upregulate SGs in PBMCs (antigen-presenting cells, APC) via reverse signaling as we reported [22,92]. As shown in Table 5A, the expression of 14 CSRs, 4 dual-function receptors, and 10 ICRs were examined in the microarrays of the PBMCs from patients with ESRD and CKD (Table 2A) as we reported [92]. The results show that 1) ESRD upregulates CSRs CD48 and CD58 but downregulates seven out of 14 CSRs including Inducible T Cell Costimulator Ligand (ICOSLG), CD70, TNF Superfamily Member 14 (TNFSF14), CD40, TNFSF15, TNFSF18, and Signaling Lymphocytic Activation Molecule Family Member 1 (SLAMF1); 2) ESRD downregulated one out of four dual function receptors poliovirus receptor (PVR) (con-stimulation at naïve T cells but co-inhibitory at activated T cells); 3) ESRD downregulated four out of 10 immune checkpoint receptors (co-inhibition receptors) such as nectin cell adhesion molecule 3 (NECTIN3), programmed cell death 1 ligand 2 (PDCD1LG2), human endogenous retrovirus-H long terminal repeat-associating protein 2 (HHLA2) and butyrophilin like 2 (BTNLI2); and 4) CKD upregulated one immune checkpoint receptor HHLA2 but downregulated one co-stimulation receptor TNFSF8.

To determine whether ESRD upregulated co-stimulation receptors [92], CD48 and CD58, play any causative roles in regulating ESRD-modulated SG expressions, we tried to find available microarray or RNA-sequencing datasets associated with overexpression or deficiency of CD48 and CD58. As no such datasets are available at the time of this writing, we used the GEO datasets (GSE15215) related to CD2, a membrane protein acting as the ligand for both CD48 and CD58 on dendritic cells (DCs, CD2+ DCs versus CD2− DCs), to determine whether the forward signaling of CD48 and CD58 (from antigen-presenting cells toward T cells) can modulate the expression of SGs modulated in ESRD. These results showed that CD2 upregulates 14 out of 121 SGs (11.6%) upregulated in ESRD; and CD2 downregulates 25 out of 928 SGs downregulated in ESRD.

Fig. 2f. The Venn Diagram of transcript factors analysis in Table 3B. E2F1 was shared by CKD-upregulated SGs and ESRD-downregulated SGs in ESRD. In addition, nuclear respiratory factor 2 (Nrf2), a key transcription factor in Redox Oxygen Species (ROS), was shared in two groups of SGs, CKD-downregulated SGs and ESRD-downregulated SGs, indicating these two transcription factors may serve as an important inhibitor of disease progression.

Fig. 3a. The Venn Diagram results on the three groups such as 35 UT genes (encode total 30 UTs), 44 CKD upregulated SGs and 121 ESRD-upregulated SGs showed that 1) UTs have no overlaps with CKD-upregulated SGs; 2) UTs have two toxins (CFD, and RETN) overlapped with ESRD-upregulated SGs; 3) ESRD-upregulated SGs have four SGs (ADAM9, C3, HSP90B1, and S100A12) overlapped with CKD-upregulated SGs. In addition, one signaling pathway “Role of Cytokines in Mediating Communication between Immune Cells” was shared by the top 10 pathways associated with UTs and the five active pathways upregulated by SGs in ESRD.
SGs (2.7%) downregulated in ESRD (see Table 5b). We found that CD48/CD58-CD2 signaling may amplify the SGs alteration in ESRD. These results suggest that CD48/CD58-CD2 signaling promotes SGs upregulation in ESRD. Of note, the justifications for this analysis are:

1) DCs can be the parts of PBMCs in patients with ESRD [93]; 2) CD2 protein is also expressed in monocytes, B lymphocytes, CD4+ T cells, CD8+ T cells NK cells, platelets, bone marrow stromal cells, which were the CD2 expression data collected from the GeneCards database (https://www.genecards.org/cgi-bin/carddisp.pl?gene=CD2#protein_expression); 3) new RNA-sequencing data from Human Protein Atlas Database (https://www.proteinatlas.org) indicated that CD48 and CD58 are expressed in every one of 27 tissue examined; and that their ligand (CD2) is also expressed in every one of 27 tissues examined, which are correlated with the CD2 protein expression data collected in the GeneCards database shown above (see Fig. 4a); and 4) as shown in Fig. 4b, the protein expression data from the Proteomics Database (https://www.proteomicsdb.org/) showed that CD2 protein can be highly enriched in cytotoxic T-lymphocyte, natural killer cell, bone marrow stromal cell, helper T-lymphocyte, B lymphocyte and monocyte, which indicated the possibility of the signal amplification induced by co-stimulation of CD48/CD58-CD2 pathway. Of note, we reported previously that CD40+ proinflammatory monocytes accelerate inflammation in CKD [7]. Taken together, these data suggest that first, ESRD-upregulated CD48 and CD58 increase secretomic upregulation in the PBMCs, whose signals may not be even limited in the PBMCs from patients with ESRD examined in this study; second, CD48/CD58-CD2 pathway can be actually magnified enormously in tissue levels so that the CD48/CD58-CD2 pathway-activated PBMCs in blood circulation accelerate vascular and other inflammations; and third, reverse signaling from CD2+ T cells to CD48+/CD58+ PBMCs play significant roles in modulating PBMC secretomic changes in ESRD (see Fig. 4c).

3.7. Classically activated macrophages (M1)-, and alternatively activated macrophages (M2)- macrophage polarization signals contribute to ESRD- and CKD-upregulated SGs

We recently identified 20 new disease group-specific and 12 new shared pathways in macrophages in eight groups of 34 diseases including 24 inflammatory organ diseases and 10 types of cancers [94,95]. It has also been reported that M1 proinflammatory macrophages contribute to infection clearance, inflammation and renal injury, and M2 anti-inflammatory macrophages can contribute to the resolution phase of the response to injury [96,97]. We hypothesized...
that M1 and M2 macrophage polarization signals contribute to CKD- and ESRD-upregulated PBMC SG expressions. As shown in Table 6, eight out of 44 (18.18%) CKD-upregulated SGs were found in M1 macrophage polarization dataset; six out of 44 (13.64%), including three out of 44 (6.8%, M2a), four out of 44 (9.1%, M2b), one out of 44 (2.3%, M2c), CKD-upregulated SGs, were found in M2a, M2b, and M2c macrophage subset polarization, respectively. In addition, 15 out of 121 (12.4%) ESRD-upregulated SGs were found in M1 macrophage polarization dataset; 16 out of 121 (13.2%), including one out of 121 (0.83%, M2a), 11 out of 121 (9.1%, M2b), and 9 out of 121 (7.4%) ESRD-upregulated SGs were found in M2a, M2b, and M2c macrophage polarization, respectively. When examining those modulated SGs with IPA, no significant pathways were found. As shown in Fig. 5, these results suggest that:

1) macrophage polarization pathways participate CKD- and ESRD-upregulated secretomic changes in PBMCs in patients with CKD and ESRD;
2) M1 proinflammatory macrophage polarization signal may play more important roles in facilitating PBMC secretomic upregulations in CKD and ESRD than the signals mediating three M2 macrophage subset polarizations and
3) M1- and M2-polarization signaling pathways involving in upregulating SGs are diversified.

3.8. ESRD- and CKD-upregulated SGs in PBMCs contain senescence-promoting regulators by upregulating proinflammatory growth factor IGFBP7 and downregulating anti-inflammatory cytokine TGF-β1 and leukocyte telomere length stabilizer SERPINE1/PAI-1

The senescence program is implicated in diverse biological processes, including embryogenesis, tissue regeneration and repair, tumorigenesis, aging and inflammation. Two main classes of senescent cells have been identified: acute and chronic senescent cells. Acute senescent cells are generated during coordinated, beneficial biological processes characterized by a defined senescence trigger, transient senescent-cell signaling functions, and eventual senescent-cell clearance. In contrast, chronic senescent cells arise more slowly from cumulative, diverse stresses and are inefficiently eliminated, leading to their accumulation and deleterious effects through a secretory phenotype [98]. Senescent cells secrete a variety of proteins collectively known as the senescence-associated secretory phenotype (SASP) [99]. Recent murine studies have shown that depletion of chronically senescent cells extends healthy lifespan and delays age-associated disease, implicating senescence and the senescence-associated secretory phenotype as drivers of organ (kidney) dysfunction [100]. Previous reports suggest that secretomic changes in CKD and ESRD modulate cellular senescence and disease-modulated aging process, renal fibrosis and cancers. In addition to senescence in the kidney, senescent vascular cells, both endothelial and smooth muscle cells, participate in atherosclerosis; senescent pre-adipocytes and adipocytes have been shown to lead to insulin resistance [101]. Thus, we hypothesize that CKD- and ESRD-related senescence signaling contributes to upregulated SGs in PBMCs. To examine this hypothesis, we compared 71 senescence regulators [102] with CKD-regulated SGs and ESRD-upregulated SGs, respectively. As shown in Table 7, first, no senescence regulators matched with CKD-upregulated SGs; second, two out of 71 senescence regulatory genes were decreased in CKD downregulated SGs [102], specifically ID1 (a member of ID family of helix-loop-helix transcriptional regulatory proteins, a kidney damage inhibitor and target of bone morphogenetic proteins [103]) and secreted protein acidic and rich in cysteine (SPARC). It has been reported that SPARC accelerates disease progression in experimental crescentic glomerulonephritis [104]; and SPARC leads to a progressive reduction in podocyte number, thus fueling the future development of glomerulosclerosis [105]. In addition, two out of 71 senescence regulatory genes including cellular repressor of E1A stimulated genes 1 (CREG1) and insulin-like growth factor-binding protein 7 (IGFBP7) were increased in ESRD-upregulated SGs. Of note, CREG1 haploinsufficiency confers increased susceptibility of adipose tissue to inflammation, leading to aggravated obesity and insulin resistance when challenged with a high fat diet [106]. IGFBP7 is one of the growth factors upregulated in patients with inflammatory breast cancer [107]. Moreover, five out of 71 senescence regulators such as serpin family E member 1, (SERPINE1, plasminogen activator inhibitor 1, PAI-1),
Table 4a
As a novel mechanism, cytokine-based m.w. UTs can amplify the ESRD signals
in inducing secretomic changes in the PBMCs from patients with ESRD. The first
example is that 4.13% of upregulated SGs in ESRD were upregulated in
Interleukine-1 beta (IL-1B)-treated human blood leukocytes (GO ID: GSE103500)
and 3.13% were downregulated.

| Uremic Toxin | Primary Change | Gene        | P.Value    | logFC     |
|-------------|----------------|-------------|-----------|-----------|
| IL-1B       | upregulated ESRD SGs | CXCL2       | 6.53E-05  | 4.959039  |
|             |                 | IRAK3       | 0.002333  | 1.785057  |
|             |                 | PLA2G7      | 0.033483  | 1.751108  |
|             |                 | SMPD3A      | 0.038285  | 1.081000  |
|             |                 | THBS1       | 0.027777  | 1.307772  |

| downregulated ESRD SGs | 29 (3.13%) |
|------------------------|------------|
| IL-1B                  |            |
| ADAM12                 | 0.003899   | −2.62067 |
| B3GNT3                 | 0.02258    | −3.46046 |
| BRINP2                 | 0.044496   | −5.65328 |
| CAMP                   | 0.024428   | −1.31643 |
| CLPS                   | 0.049925   | −6.62095 |
| EGFL7                  | 0.042206   | −2.06849 |
| FG6                    | 0.015567   | −4.70715 |
| FN1                    | 0.018602   | −2.11926 |
| GREM1                  | 0.031547   | −1.73607 |
| INSL6                  | 0.012816   | −3.0913  |
| IZUMO4                 | 0.029209   | −5.18373 |
| KLK10                  | 0.017674   | −2.11833 |
| KLK11                  | 0.037963   | −2.50713 |
| LIFP                   | 0.028216   | −1.38852 |
| LLRCL7                 | 0.015359   | −7.35399 |
| MIA                    | 0.013939   | −1.3311  |
| MMP11                  | 0.021633   | −1.8703  |
| MMP28                  | 0.004459   | −3.31363 |
| MUC2                   | 0.034662   | −2.09434 |
| NRG1                   | 0.038783   | −1.98528 |
| OPRIN                  | 0.049001   | −3.26088 |
| PGC                    | 0.034397   | −1.69163 |
| POFTU1                 | 0.011287   | −1.93524 |
| PON1                   | 0.036631   | −4.57456 |
| PRL                    | 0.015419   | −4.23738 |
| PPR1                   | 0.04216    | −1.79944 |
| PVR                    | 0.034772   | −4.70854 |
| SERPINE1               | 0.012631   | −5.17299 |
| SPARCL1                | 0.023002   | −2.49064 |

Table 4b
As a novel mechanism, cytokine-based m.w. UTs can amplify secretomic
changes in ESRD. The second example is that 4.13% upregulated SGs in ESRD
were upregulated in IL-18-treated human blood leukocytes (GO ID: GSE103500)
and 3.13% were downregulated (the full gene list is attached in
supplement Table 9).

| Uremic Toxin | upregulated ESRD SGs | Gene       | P.Value    | logFC     |
|-------------|-----------------------|------------|-----------|-----------|
| IL-1B       | 5 (4.13%)             | CFD        | 0.007418  | 1.755116 |
|             |                       | IL6ST      | 0.007849  | 3.379721 |
|             |                       | IRAK3      | 0.005272  | 1.357006 |
|             |                       | PDGF5C     | 0.01745   | 1.366625 |
|             |                       | S1L1       | 0.024563  | 1.612347 |

| downregulated ESRD SGs | 105 (11.31%) |
|------------------------|-------------|
| IL-1B                  |            |
| ADAM12                 | 0.047778   | −3.15133 |
| ALPLP2                 | 0.036972   | −1.34452 |
| AMBN                   | 0.048098   | −1.73612 |
| AMELX                  | 0.010632   | −2.25732 |
| ANTRXR1                | 0.032292   | −2.91483 |
| ARHGAP6               | 0.017996   | −3.93008 |

SPARC, transforming growth factor β1 (TGFB1), insulin-like growth factor 1 (IGF1), and insulin-like growth factor-binding protein 5 (IGFBP5) were downregulated in ESRD-downregulated SGs. Of note, TGFB1 is a key reactive oxygen species (ROS) promoting cytokine in renal fibrosis [108]. TGF-β1 promotes the cell cycle G2/M arrest based senescence-associated secretory phenotype (SASP) rather than DNA-damage based G1/S arrest [109]. TGFβ1-Smads form an anti-proliferation pathway [110]. Anti-aging gene Klotho deficiency exacerbates early diabetic nephropathy via enhancing TGFβ1 signaling in kidneys, which is a strong inducer of cellular senescence in a mouse model of chronic kidney injury [111]. Insulin-like growth factor 1 (IGF1) were increased in secretomic genes upregulated in ESRD. Circulating IGF-1 forms a complex with two other proteins – the IGF binding protein (IGFBP) and the acid labile subunit (ALS). High level concentrations of circulating IGF-1 are related to a higher risk of prostate, colorectal and breast cancers [112]. The results suggest that secretomic genes modulated in PBMCs from patients with ESRD modulate senescence via the following mechanisms: first, increasing expressions of proinflammatory growth factor IGFBP7; second, promoting inflammation and inhibiting fibrosis by decreasing TGF-β1 [113]; third, on the other hand, as potential negative feedback mechanisms, inhibiting inflammation and kidney injury by upregulating anti-inflammatory CREG1 and downregulating proinflammatory SPARC, respectively; fourth, inhibiting higher risk of prostate, colorectal and breast cancers by decreasing IGFBP1 and IGFBP5; and fifth, promoting senescence by decreasing leukocyte telomere length (LTL) [114] via inhibiting SERPINE1/PAI-1 expression [115]. Therefore, our data suggest that modulating secretomic gene expressions in PBMCs may have beneficial therapeutic effects in the treatment of ESRD-related cancer and aging-related diseases [116] (see Fig. 6).

3.9. Reactive oxygen species (ROS) pathways play much bigger roles in ESRD-upregulated SGs (11.6%) than that in CKD-upregulated SGs (6.8%), and half of ESRD-upregulated SGs are ROS-independent

It has been well documented that reactive oxygen species (ROS) plays a key role in regulating pathophysiological signaling in endothelial cell activation [117], cardiovascular diseases [118] and chronic kidney disease/end-stage renal disease [119]. We also reported that mitochondrial ROS plays a significant role in mediating EC activation [4,120,121]. Overproduction of ROS by impaired mitochondria can lead to positive feedback to enhance the cellular damage and generate uremic toxins, especially those produced by oxidation or peroxidation, such as creatine, urea and Melatonin [122,123]. This process aggravated by the accumulation of uremic toxins was hallmark by mitochondria dysfunction defined as increased proton leaks as we reported [4,120,121,124,125], impaired mitochondria dynamics, alteration of mitochondria morphology and remodeling, which lead to dysfunction of podocytes and endothelial cells in the kidney. Mitochondria is both a source and a target for uremic toxins. In addition, it has been reported that uremia is associated with a reduction in the numbers and functions of lymphoid cells, whereas numbers of myeloid cells in uremic patients are either normal or increased with increased production of inflammatory cytokines and ROS [119]. Moreover, to find the evidence that ROS pathway genes are modulated by CKD and ESRD, the 84 oxidative and anti-oxidative regulatory genes [126] were examined. As shown in Table 8A, ESRD upregulated an antioxidant enzyme peroxiredoxin 4 (PRDX4) and a potential neuron development regulator prion protein (PRNP), and downregulated eight oxidative/anti-oxidative genes including antioxidant glutathione peroxidase 3 (GPX3), anti-oxidant glutathione peroxidase 5 (GPX5), antimicrobial lactoperoxidase (LPO), microbical myeloperoxidase (MPO), anti-oxidant superoxide dismutase 3 (SOD3), anti-inflammatory cytokine IL19, proinflammatory cytokine IL-22, anti-inflammatory apolipoprotein E (APOE) in PBMCs, respectively. These results suggest that ESRD downregulates more antioxidant enzymes/proteins than upregulate them, whereby promoting ROS generation. In addition, CKD downregulated one anti-oxidative gene SOD3. These results suggest that ESRD and CKD modulate ROS regulome (oxidative and anti-oxidative regulatory genes).

However, an important question remains whether ROS signaling and antioxidant signaling mediate CKD and ESRD-modulation of SGs. Thus, we examined a novel hypothesis that ROS signaling and anti-oxidant signaling mediate CKD-, and ESRD-, modulation of SGs. By
The IPA classified the parts of the SGs modulated in the PBMCs from patients with CKD and patients with ESRD as the cytokine group. The results showed that 4 out of 55 SGs (7.2%) downregulated in CKD (also shown in Table 2B) were cytokines; one out of 44 SGs (2.27%) upregulated in CKD was cytokine; 68 out of 928 SGs (7.3%) downregulated in ESRD were cytokines; and 6 out of 121 SGs (4.96%) upregulated in ESRD were cytokines.

| Symbol | Entrez Gene Name | Expr p-value | Expr Log Ratio | Location |
|--------|-----------------|--------------|----------------|----------|
| down in CKD |                |              |                |          |
| [4] IL24 interleukin 24 | 0.0327 | −1.174 | Extracellular Space |
| IL36RN interleukin 36 receptor antagonist | 0.0319 | −1.2 | Extracellular Space |
| FF4 platelet factor 4 | 0.0149 | −1.11 | Extracellular Space |
| EDA ectodysplasin A | 0.00949 | −1.069 | Extracellular Space |

| Symbol | Entrez Gene Name | Expr p-value | Expr Log Ratio | Location |
|--------|-----------------|--------------|----------------|----------|
| up in CKD (1) |                |              |                |          |
| DKK3 dickkopf WNT signaling pathway inhibitor 3 | 0.0094 | 1.827 | Extracellular Space |

| Symbol | Entrez Gene Name | Expr p-value | Expr Log Ratio | Location |
|--------|-----------------|--------------|----------------|----------|
| down in ESRD |                |              |                |          |
| [68] BMP8A bone morphogenetic protein 8a | 0.000409 | −2.355 | Extracellular Space |
| CCL1 C-C motif chemokine ligand 1 | 0.00184 | −1.28 | Extracellular Space |
| CCL8 C-C motif chemokine ligand 8 | 0.0221 | −1.682 | Extracellular Space |
| CCL13 C-C motif chemokine ligand 13 | 5.57E-05 | −1.797 | Extracellular Space |
| CCL17 C-C motif chemokine ligand 17 | 0.0196 | −1.465 | Extracellular Space |
| CCL18 C-C motif chemokine ligand 18 | 0.00866 | −1.367 | Extracellular Space |
| CCL19 C-C motif chemokine ligand 19 | 0.0044 | −2.113 | Extracellular Space |
| CCL21 C-C motif chemokine ligand 21 | 0.00242 | −2.011 | Extracellular Space |
| CCL22 C-C motif chemokine ligand 22 | 3.47E-05 | −1.656 | Extracellular Space |
| CCL23 C-C motif chemokine ligand 23 | 0.00434 | −2.54 | Extracellular Space |
| CCL24 C-C motif chemokine ligand 24 | 0.00354 | −1.942 | Extracellular Space |
| CCL25 C-C motif chemokine ligand 25 | 0.0037 | −1.042 | Extracellular Space |
| CRH corticotropin releasing hormone | 0.00196 | −1.518 | Extracellular Space |
| CSF1 colony stimulating factor 1 | 7.44E-05 | −1.84 | Extracellular Space |
| CSF2 colony stimulating factor 2 | 0.00115 | −1.158 | Extracellular Space |
| CXCL1 C-X3-C motif chemokine ligand 1 | 0.000352 | −1.231 | Extracellular Space |
| CXCL5 C-X3-C motif chemokine ligand 5 | 0.0264 | −1.939 | Extracellular Space |
| CXCL9 C-X3-C motif chemokine ligand 9 | 0.014 | −1.063 | Extracellular Space |
| CXCL11 C-X3-C motif chemokine ligand 11 | 0.0439 | −1.101 | Extracellular Space |
| CXCL12 C-X3-C motif chemokine ligand 12 | 0.000564 | −1.422 | Extracellular Space |
| CXCL14 C-X3-C motif chemokine ligand 14 | 0.000645 | −1.355 | Extracellular Space |
| DKK3 dickkopf WNT signaling pathway inhibitor 3 | 0.00168 | −1.867 | Extracellular Space |
| EDN1 endothelin 1 | 0.0271 | −1.424 | Extracellular Space |
| EPO erythropoietin | 0.00154 | −2.113 | Extracellular Space |
| FASLG Fas ligand | 0.000484 | −1.074 | Extracellular Space |
| IFNA5 interferon alpha 5 | 0.0351 | −1.309 | Extracellular Space |
| IFNA7 interferon alpha 7 | 0.0259 | −1.167 | Extracellular Space |
| IFNA16 interferon alpha 16 | 0.0243 | −1.327 | Extracellular Space |
| IFNB1 interferon beta 1 | 0.0248 | −1.679 | Extracellular Space |
| IFNW1 interferon omega 1 | 0.000648 | −1.794 | Extracellular Space |
| IL2 interleukin 2 | 0.00227 | −1.245 | Extracellular Space |
| IL3 interleukin 3 | 0.00158 | −1.82 | Extracellular Space |
| IL4 interleukin 4 | 0.000549 | −2.658 | Extracellular Space |
| IL5 interleukin 5 | 0.00255 | −1.791 | Extracellular Space |
| IL9 interleukin 9 | 0.00207 | −1.852 | Extracellular Space |
| IL11 interleukin 11 | 0.00046 | −1.87 | Extracellular Space |
| IL16 interleukin 16 | 0.000374 | −1.476 | Extracellular Space |
| IL19 interleukin 19 | 0.00667 | −1.564 | Extracellular Space |
| IL21 interleukin 21 | 0.000431 | −2.258 | Extracellular Space |
| IL22 interleukin 22 | 0.0124 | −1.315 | Extracellular Space |
| IL24 interleukin 24 | 0.000213 | −2.524 | Extracellular Space |
| IL25 interleukin 25 | 0.00486 | −1.597 | Extracellular Space |
| IL26 interleukin 26 | 0.00285 | −1.789 | Extracellular Space |
| IL37 interleukin 37 | 0.031 | −1.296 | Extracellular Space |
| IL17A interleukin 17A | 0.0004 | −1.944 | Extracellular Space |
| IL18 interleukin 1 alpha | 1.52E-07 | −2.584 | Extracellular Space |
| IL1RN interleukin 1 receptor antagonist | 4.04E-05 | −1.628 | Extracellular Space |
| IL36A interleukin 36 alpha | 0.00185 | −1.492 | Extracellular Space |
| IL36G interleukin 36 gamma | 0.0143 | −1.615 | Extracellular Space |
| IL36RN interleukin 36 receptor antagonist | 0.0281 | −1.175 | Extracellular Space |
| LIF LIF interleukin 6 family cytokine | 0.0323 | −1.402 | Extracellular Space |
| LTA lymphotixin alpha | 2.73E-05 | −2.497 | Extracellular Space |
| OSM oncostatin M | 0.00294 | −2.002 | Extracellular Space |
| PF4 platelet factor 4 | 0.0432 | −1.106 | Extracellular Space |
| PRL prolactin | 0.00443 | −1.693 | Extracellular Space |
| PRHL prolactin releasing hormone | 0.00346 | −1.191 | Extracellular Space |
| SCG2 secretogranin II | 0.00633 | −2.162 | Extracellular Space |
| SCGB1A1 secretoglobin family 1A member 1 | 0.0166 | −1.372 | Extracellular Space |
| SLURP1 secreted LY6/PLAUR domain containing 1 | 0.000616 | −1.435 | Extracellular Space |
| SPP1 secreted phosphoprotein 1 | 0.000139 | −2.907 | Extracellular Space |
| THPO thrombopoietin | 0.00506 | −1.895 | Extracellular Space |
| TNFSF11 TNF superfamily member 11 | 0.000298 | −1.936 | Extracellular Space |
| TNFSF14 TNF superfamily member 14 | 1.85E-05 | −3.778 | Extracellular Space |
| WNT1 Wnt family member 1 | 0.00424 | −1.889 | Extracellular Space |
| WNT2 Wnt family member 2 | 0.0082 | −1.681 | Extracellular Space |
mining the microarray datasets in the NIH-NCBI-GeoDataset database, we found several microarray datasets with the inhibition of nicotinamide adenine dinucleotide phosphate oxidase 2 (NOX2) [118] and the deficiency of antioxidant transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2) [118]. Nrf2 was chosen since Nrf2 was considered as a novel therapeutic target for mitochondria dysfunction in chronic kidney disease by animal experimental researches [121,127–132]. In Fig. 7A, we classified all the SGs into four groups: first, the SGs increased in NOX2 inhibited cells and decreased in Nrf2 deficient cells are ROS-suppressed genes; second, the SG expressions decreased in NOX2 inhibited cells and increased in Nrf2 deficient cells are ROS-promoted genes; third, the SGs either promoted by ROS pathways in one group of microarray datasets or suppressed by ROS pathways in another group of microarray datasets are ROS-independent genes; and fourth, the SGs that were not significantly modulated in NOX2 inhibited and Nrf2 deficient are ROS-independent genes (Fig. 7A). Of note, only 1030 out of 2641 SGs were found in the NOX2-, Nrf2-deficient datasets and were focused in our analyses. As shown in Table 8B, 144 out of 1030 SGs (14.0%) were ROS-suppressed genes; 191 out of 1030 SGs (18.5%) were ROS-promoted SGs; 3 out of 1030 SGs (0.29%) were ROS-unsettled SGs; and 692 out of 1030 SGs (67.2%) were ROS-independent. In addition, 17 out of CKD-upregulated 44 SGs (38.6%) were found to be modulated in ROS-related manners including 5 as ROS-suppressed SGs (29.41%), 3 as ROS-promoted SGs (17.65%), 0 as ROS-unsettled SGs, and 9 as ROS-independent SGs (52.94%); moreover, 26 out of CKD-downregulated 55 SGs (47.3%) were found to be modulated in ROS-related manners including 6 as ROS-suppressed SGs (7.14%), 8 as ROS-promoted SGs (30.77%), 0 as ROS-unsettled SGs, and 16 as ROS-independent SGs (61.54%); furthermore, 84 out of ESRD-upregulated 121 SGs (69.4%) were found to be modulated in ROS-related manners including 6 as ROS-suppressed SGs (7.69%), 8 as ROS-promoted SGs (30.77%), 0 as ROS-unsettled SGs, and 64 as ROS-independent SGs (76.19%); and finally, 341 out of ESRD-downregulated 928 SGs (36.7%) were found to be modulated in ROS related manners including 59 as ROS-suppressed SGs (17.30%), 74 as ROS-promoted SGs (21.70%), 2 as ROS-unsettled SGs (0.59%), and 206 as ROS-independent SGs (60.41%). These results suggest that first, ROS play much bigger roles in ESRD-upregulated SGs (69.4%) than that in CKD-upregulated SGs (38.6%); second, ROS-independent ESRD-upregulated SGs (76.19%) are much more than ROS-independent CKD-upregulated SGs (52.94%), indicating that ESRD uses more ROS-independent mechanisms than CKD in upregulating SGs in the PBMCs; and third, ROS-suppressed CKD-upregulated SGs (29.41%) are much higher than that of ESRD-upregulated SGs (7.14%) (see Fig. 7b).

4. Discussion

Since UTs have been first identified via low throughput mass spectrometry, 130 UTs are documented in the European Uremic Solutes (EUTox) Database (http://www.uremic-toxins.org/DataBase.html). It remains unknown whether secreted proteins generated in innate immune cells in response to UTs stimulation can contribute to the pathogenesis of CKD and ESRD. We recently reported that uremic toxins (UTs) are selectively increased and serve as danger signal-associated molecular patterns (DAMPs) and homeostasis-associated molecular patterns (HAMPs) that modulate inflammation. These results also show that some UT genes are upregulated in CKD and CAD via caspase-1/ inflammatory cytokine pathways, rather than by purely passive accumulation. Our findings raised the possibility that UTs-stimulated innate immune cells and other types of cells increase their secretome, which contributes to the CKD/ESRD progression. As reported, the secretory proteins are important for maintaining cell-cell communication and proliferation. Examples of secretory proteins include hormones, digestive enzymes, cytokines, chemokines, interferons (IFNs), colony-stimulating factors (CSFs), growth factors, and tumor necrosis factors (TNFs) [133]. However, secretomic studies in CKD and other metabolic diseases have been at a low pace due to the low throughput technologies [134]. Our previous reports demonstrated that innate immune cells, PBMCs containing such as Ly6C<sup>hi</sup> (mice) [7,17,18,135–137] and CD40<sup>+</sup> (human) [7] monocytes, contribute significantly to the pathogenesis of metabolic vascular diseases including CKD [7]. However, three important questions remained, first, whether UTs are the only soluble molecular drivers for the progression of CKD to ESRD; second, whether secretome changes in innate immune cells, PBMCs, upregulated in CKD and ESRD contribute to the pathogenesis and progression

### Table 4c (continued)

| Symbol | Entrez Gene Name | Expr p-value | Expr Log Ratio | Location |
|--------|-----------------|--------------|----------------|----------|
| WNT4   | Wnt family member 4 | 0.00799 | −1.371 | Extracellular Space |
| WNT5A  | Wnt family member 5A | 0.00932 | −2.049 | Extracellular Space |
| EDA    | ectodysplasin A | 3.23E-06 | −1.747 | Plasma Membrane |

### Table 4d

Proinflammatory and profibrotic molecules, pathways and transcription factors played important roles in pathophysiological process of ESRD.

| Method | Table/Figure | Result |
|--------|--------------|--------|
| IPA    | Fig. 2B | upregulated SGs in ESRD can active proinflammatory pathways such as IL8 signaling, neuroinflammation signaling pathway. |
| IPA    | Fig. 2D | downregulated SGs in ESRD can active profibrotic pathways such as inhibition of matrix metalloproteases |
| TRAN- SF- AC | Table 3C | upregulated SGs in ESRD may be modulated by transcription factors involved in inflammation and fibrosis (Egr-1, POU1F1) |
| ClueGo | Fig. 3B | UT-encoded genes and up-regulated SGs in ESRD are shared in some proinflammatory pathways |
| GEO2R  | Tables 4A and 4B | Proinflammatory uremic cytokines IL1b and IL18 in UTs can amplify the upregulation of SGs in ESRD |
| IPA    | Table4C | Cytokines can be modulated during CKD progression to induce imbalance of anti-inflammatory and proinflammatory function. |
Most of 28 co-stimulation receptors and immune checkpoint (co-inhibition) receptors (expressed in the antigen presenting cell (APC) surface) are modulated in the PBMCs from patients with CKD and ESRD. The results showed that: 1) seven out of 14 co-stimulation receptors, one out of four dual receptors (functional as co-stimulation for naïve T cells and co-inhibition receptors for activated T cells), and four out of 10 immune checkpoint receptors were downregulated in ESRD, respectively; 2) two co-stimulation receptors CD48 and CD58 were upregulated in ESRD; 3) one immune checkpoint receptor HHLA2 was upregulated in CKD; and 4) one co-stimulation receptor TNFSF8 was downregulated in CKD.

| Gene symbol | logFC up in CKD | logFC down in CKD | logFC up in ESRD | logFC down in ESRD |
|-------------|-----------------|-------------------|------------------|--------------------|
| ICOSLG      | 1.9552          | -2.93849          | -3.77804         | -2.12532           |
| CD70        | -1.5467         | -2.93849          | -3.77804         | -2.12532           |
| TNFSF14     | -3.77804        | -3.77804          | -2.93849         | -2.93849           |
| CD40        | -2.93849        | -2.93849          | -3.77804         | -3.77804           |
| TNFSF9      | -2.93849        | -2.93849          | -3.77804         | -3.77804           |
| TNFSF4      | -2.93849        | -2.93849          | -3.77804         | -3.77804           |
| TNFSF15     | -1.11762        | -1.11762          | -1.11762         | -1.11762           |
| TNFSF18     | -1.25532        | -1.25532          | -1.25532         | -1.25532           |
| TNFSF8      | -1.36894        | -1.36894          | -1.36894         | -1.36894           |
| TIMD4       |                |                   | -1.36894         |                    |
| SLAMF1      |                |                   | -1.51147         |                    |
| CD48        |                |                   | 1.089174         |                    |
| SEMA4A      |                |                   | 1.68807          |                    |
| CD58        |                |                   | 1.68807          |                    |

Since there are no datasets of CD48 and CD58 deficient/overexpressed microarray datasets available in the NIH-NCBI-GEO database, we use the GEO datasets (GSE15215) related to CD2, a membrane protein acting as CD48/CD58 ligand on dendritic cells (DCs), CD2 + DCs versus CD2 − DCs to determine whether the forward signaling of CD48 and CD58 can modulate the expression of SGs modulated in ESRD. These results showed that CD2 upregulates 14 out of 121 SGs (11.6%) upregulated in ESRD; and CD2 downregulates 25 out of 928 SGs (2.7%) downregulated in ESRD.

| Gene symbol | log FC up in ESRD | log FC down in ESRD |
|-------------|-------------------|---------------------|
| ANXA1       | 2.18431           | 0.018331            |
| ANXA2       | 1.797703          | 0.006552            |
| ASGR1       | 2.235951          | 0.022441            |
| ASGR16      | 1.910232          | 0.004221            |
| EREG        | 1.079933          | 0.020802            |
| HS2ST1      | 2.614528          | 0.001885            |
| IGFBP7      | 2.731112          | 0.001049            |
| IRAK3       | 2.871895          | 0.006719            |
| MTHFD2      | 1.340201          | 0.01361             |
| NLRP3       | 2.662663          | 0.011059            |
| PINK1       | 1.876757          | 0.041052            |
| RNASE4      | 2.112123          | 0.007783            |
| TTP         | 1.607275          | 0.033834            |
| XCL1        | 1.997939          | 0.003066            |
| ANXA1       | 2.18431           | 0.018331            |
| ANXA2       | 1.797703          | 0.006552            |
| ASGR1       | 2.235951          | 0.022441            |
| ASGR16      | 1.910232          | 0.004221            |
| EREG        | 1.079933          | 0.020802            |
| HS2ST1      | 2.614528          | 0.001885            |
| IGFBP7      | 2.731112          | 0.001049            |
| IRAK3       | 2.871895          | 0.006719            |
| MTHFD2      | 1.340201          | 0.01361             |
| NLRP3       | 2.662663          | 0.011059            |
| PINK1       | 1.876757          | 0.041052            |
| RNASE4      | 2.112123          | 0.007783            |
| TTP         | 1.607275          | 0.033834            |
| XCL1        | 1.997939          | 0.003066            |
| ANXA1       | 2.18431           | 0.018331            |
| ANXA2       | 1.797703          | 0.006552            |
| ASGR1       | 2.235951          | 0.022441            |
| ASGR16      | 1.910232          | 0.004221            |
| EREG        | 1.079933          | 0.020802            |
| HS2ST1      | 2.614528          | 0.001885            |
| IGFBP7      | 2.731112          | 0.001049            |
| IRAK3       | 2.871895          | 0.006719            |
| MTHFD2      | 1.340201          | 0.01361             |
| NLRP3       | 2.662663          | 0.011059            |
| PINK1       | 1.876757          | 0.041052            |
| RNASE4      | 2.112123          | 0.007783            |
| TTP         | 1.607275          | 0.033834            |
| XCL1        | 1.997939          | 0.003066            |
of CKD and ESRD; and third, whether CKD and ESRD differentially modulate the secretomic changes via disease stage-specific pathways. To fill in these important knowledge gaps, in this study, we used cutting-edge molecular database mining approaches that we pioneered in 2004 [25,113,138,139] and analyzed PBMC secretomic (all the signal peptide sequence-containing secreted protein genes) changes in induced by CKD and ESRD. Our data analyses have made for the first time the following significant findings: 1) 86.7% middle class (molecular weight >500 Daltons) uremic toxins (UTs) were encoded by secretomic genes (SGs); 2) Upregulations of SGs in PBMCs in patients with ESRD (121 SGs) were significantly higher than that of CKD (44 SGs); and among ESRD specifically modulated 975 SGs, ESRD upregulated 116 SGs (11.9%) but downregulates 859 SGs (88.1%), respectively; 3) ESRD-upregulated SGs had 2 fold higher percentages of the cytoplasm and nucleus subcellular groups than the controls; and had the higher percentages of five out of 13 SG functional groups including enzyme, kinase, peptide, transcription regulator, and transmembrane in comparison to the controls. Transcriptomic analyses of PBMC secretome have advantages to identify more comprehensive secretome than conventional secretomic analyses; 4) Although CKD-, and ESRD-upregulated SGs were highly diversified in signaling, ESRD-induced SGs had strong proinflammatory pathways; 5) Proinflammatory cytokines-based middle class UTs such as interleukin-1β (IL-1β) and IL-18 promote ESRD modulation of SGs; 6) ESRD-upregulated co-stimulation receptors CD48 and CD58 increase secretomic upregulation in the PBMCs; may not be even limited in the PBMCs, CD48/CD58-CD2 signaling can be actually magnified enormously in tissues; 7) Classically activated macrophages (M1), and alternatively activated macrophages (M2)- macrophage polarization signals contribute to ESRD- and CKD-upregulated SGs; 8) ESRD- and CKD-upregulated SGs in PBMCs contain senescence-promoting regulators by upregulating proinflammatory growth factor IGFBP7 and downregulating anti-inflammatory cytokine TGF-β1 and leukocyte telomere length stabilizer SERPINE1/PAI-1; and 9) Reactive oxygen species (ROS) pathways play much bigger roles in ESRD-upregulated SGs (11.6%) than that in CKD-upregulated SGs (6.8%); and half of ESRD-upregulated SGs are ROS-independent.

Clinical and epidemiological studies have identified more than 10 risk factors in accelerating CKD progression and potential transition to ESRD as shown in Table 9A. However, the molecular pathways driving the pathogenesis of ESRD remained poorly characterized. Based on our
findings, we propose a new working model (Fig. 8), under the stimulation of uremic toxins, more than 121 novel secreted proteins are significantly upregulated in innate immune cells, PBMCs, in patients with ESRD, which makes PBMCs the major cell types in upregulating secretomes. This is the first time for us to understand that in addition to uremic toxins identified, significant secretomic changes may play highly significant roles in driving ESRD pathogenesis. In addition, since some PBMC secretome proteins identified with transcriptomic approach are localized in the subcellular locations other than the supernatants of cultured cells and plasma that conventional secretomic analyses sampled and examined, therefore, our data have also demonstrated that transcriptomic analyses of PBMC secretome have advantages to identify more comprehensive secretome than conventional secretomic analyses [69]. To determine the mechanisms underlying the SGs, we identified several novel molecular mechanisms: first, UTs play significant roles in upregulating PBMC SGs in patients with CKD and ESRD; and ESRD-induced SGs have strong proinflammatory pathways. In addition, our IPA results indicate that SGs in PBMCs upregulated in ESRD have a novel proinflammatory signaling pathway overlapped with that of UTs, role of cytokines in mediating communication between immune cells (Fig. 3A). We also used the Cytoscape and found that UTs pathways and ESRD-upregulated pathways share 20 regulatory or regulators. These results have demonstrated for the first time that ESRD-upregulated PBMC SGs have synergistic effects with that of UTs, which contribute to the disease progression significantly; second, we found strong functional evidence that proinflammatory cytokines-based middle class UTs such as interleukin-1β (IL-1β) and IL-18 promote ESRD modulation of SGs, which also serve as a new working model for UTs and PBMC secretome interactions in patients with ESRD and CKD; third, as a novel membrane Toll-like receptor-mediated signaling and interferon-g receptor signaling mechanisms, we found that classically activated macrophages (M1)-, and alternatively activated macrophages (M2)- macrophage

![Diagrams](image-url)

**Fig. 4c. Novel mechanism.** Co-stimulation receptors CD48 and CD58 can initiate signaling cascades via their interactions with their ligand CD2 to amplify the expression changes of SGs upregulated in the PBMCs in patients with ESRD.
polarization signals contribute to ESRD- and CKD-upregulated SGs; fifth, ESRD- and CKD-upregulated SGs in PBMCs contain senescence-promoting regulators by upregulating proinflammatory growth factor IGFBP7 and downregulating anti-inflammatory cytokine TGF-β1 and leukocyte telomere length stabilizer SERPINE1/PAI-1. Therefore, our data have demonstrated for the first time that controlling senescence-associated inflammation cell by targeting specific inflammatory mediators may have a beneficial therapeutic effect in treatment of ESRD-related cancers, aging and inflammatory diseases [116]; and sixth, Reactive oxygen species (ROS) pathways play much bigger roles in ESRD-upregulated SGs (11.6%) than that in CKD-upregulated SGs (6.8%); and half of ESRD-upregulated SGs are ROS-independent.

Of note, the interactions of the pathways in our findings have been reported by other researches. These interactions may collectively lead to disease progression: First, cytokines exerted as key initiators, mediators and effectors for other pathway entries. Uregulated co-stimulating secretomic member CD48 can be induced under proinflammatory environment; secretion of IL2 was decreased in T cells isolated from CD48-deficient mice [140]. Second, co-signaling molecule CD48 and CD58 are widely reported to function as scavengers to clear the interstitial environment of extraneous cellular materials and also as antigen-presenting cells to stimulate adaptive immune response. Based on functionality, resident and infiltrating macrophages can produce a set of pro-inflammatory cytokines and polarization signals contribute to ESRD- and CKD-upregulated SGs; fifth, ESRD- and CKD-upregulated SGs in PBMCs contain senescence-promoting regulators by upregulating proinflammatory growth factor IGFBP7 and downregulating anti-inflammatory cytokine TGF-β1 and leukocyte telomere length stabilizer SERPINE1/PAI-1. Therefore, our data have demonstrated for the first time that controlling senescence-associated inflammation cell by targeting specific inflammatory mediators may have a beneficial therapeutic effect in treatment of ESRD-related cancers, aging and inflammatory diseases [116]; and sixth, Reactive oxygen species (ROS) pathways play much bigger roles in ESRD-upregulated SGs (11.6%) than that in CKD-upregulated SGs (6.8%); and half of ESRD-upregulated SGs are ROS-independent.

Of note, the interactions of the pathways in our findings have been reported by other researches. These interactions may collectively lead to disease progression: First, cytokines exerted as key initiators, mediators and effectors for other pathway entries. Uregulated co-stimulating secretomic member CD48 can be induced under proinflammatory environment; secretion of IL2 was decreased in T cells isolated from CD48-deficient mice [140]. Second, co-signaling molecule CD48 and CD58 are widely reported to function as scavengers to clear the interstitial environment of extraneous cellular materials and also as antigen-presenting cells to stimulate adaptive immune response. Based on functionality, resident and infiltrating macrophages can produce a set of pro-inflammatory cytokines and...
Among all SGs we investigated, no senescence regulators out of 71 were upregulated in CKD; two out of 71 senescence regulator genes such as Inhibitor Of DNA Binding 1 (ID1) and secreted protein acidic and rich in cysteine (SPARC) were downregulated in CKD; two out of 71 senescence regulator genes such as Cellular Repressor Of E1A Stimulated Genes 1 (CREG1) and Insulin Like Growth Factor Binding Protein 7 (IGFBP7) were upregulated in ESRD while five were downregulated in ESRD such as Serpin Family E Member 1 (SERPINE1), SPARC, transforming growth factor b1 (TGFB1), insulin-like growth factor (IGF1) and Insulin Like Growth Factor Binding Protein 5 (IGFBP5).

### Table 7

| Group                  | Gene Symbol | p value  | log FC     | Function                                      |
|------------------------|-------------|----------|------------|-----------------------------------------------|
| Upregulated in CKD     | N/A         |          |            |                                               |
| Downregulated in CKD   | ID1         | 0.042591 | -1.10654   | p53/pRb signaling & cell cycle                |
|                        | SPARC       | 0.028006 | -1.00435   | p53/pRb signaling & cell cycle                |
| Upregulated in ESRD    | CREG1       | 7.63E-05 | 1.463896   | p53/pRb signaling & cell cycle                |
|                        | IGFBP7      | 5.81E-05 | 2.059804   | p53/pRb signaling & cell cycle                |
| Downregulated in ESRD  | SERPINE1    | 0.0234   | -1.17965   | Other Senescence Response Gene                |
|                        | SPARC       | 0.00428  | -1.93975   | p53/pRb signaling & cell cycle                |
|                        | TGFB1       | 3.62E-06 | -2.48915   | p53/pRb signaling & cell cycle; Cell Adhesion Molecules |
|                        | IGF1        | 0.000375 | -2.37204   | insulin growth factor related                 |
|                        | IGFBP5      | 0.000673 | -1.7563    | insulin growth factor related                 |

![Fig. 6. Novel mechanism. Uremic toxins-promoted secretome accelerated renal disease and inflammation by inducing cellular senescence and senescence-associated secretory phenotype (SASP) according to p53 signaling and insulin growth factor (IGF) related pathways (PI3K/Akt) in ESRD.](image)

### Table 8a

Oxidative stress-related gene expressions (reactive oxygen species, ROS, regulatome) contribute to the progression of kidney dysfunction. We analyzed the gene expression of total 84 ROS regulatome genes (shown in supplement table 11) in CKD and ESRD. We found that 2 out of total 121 (1.65%) secretomic genes in ESRD while none of SGs in CKD are upregulated. Meanwhile, 8 out of 928 (0.8%) in ESRD and 1 out of 55 (1.82%) in CKD are downregulated.

| Group                  | Gene Symbol | p value  | log FC     |
|------------------------|-------------|----------|------------|
| Upregulated in CKD     | N/A         |          |            |
| Downregulated in CKD   | SOD3        | 0.031602 | -1.04179   |
| Upregulated in ESRD    | PRDX4       | 3.25E-05 | 1.308793   |
|                        | PRNP        | 8.80E-08 | 1.915437   |
| Downregulated in ESRD  | GPX3        | 4.56E-04 | -1.13831   |
|                        | GPX5        | 0.0101   | -1.48694   |
|                        | LPO         | 0.00185  | -1.8362    |
|                        | MPO         | 4.01E-03 | -1.85159   |
|                        | SOD3        | 0.0214   | -1.23203   |
|                        | IL19        | 0.00647  | -1.56396   |
|                        | IL22        | 0.0124   | -1.3153    |
|                        | APOE        | 4.61E-06 | -1.78856   |

other metabolites under the stimuli such as uremic toxins in ESRD and their phenotype can be reversely reprogrammed by the different subsets of cytokines and polarized into different subsets [142–144]. Fourth, senescence has been induced by signaling through a bevy of critical cytokines such as TNF-a, IFN-g as an important extrinsic pathway of senescence and those cytokines initiated an inflammatory network acted both cause and consequences during senescence [145–147]. Fifth, redox oxygen species has been widely reported to be a marker and an inducer to deteriorate diseases which could establish the inflammatory network [148–152]. This process could be carried out directly or indirectly. Direct pathways were according to the enhancing secretion of pro-inflammatory cytokines such as IFN-g, TNF-a and IL-1 by activating TCR and mTOR signaling while indirect pathways was activated by imbalance of M1-and M2-macrophages, SASP, and T cell signaling [153–162]. In conclusion, these pathways cross talk directly or
indirectly, and make ROS pathway as potential therapeutic targets to suppress disease progression.

One limitation of the current study is that due to the low throughput nature of verification techniques so that we could not verify every result we identified with the analyses of high throughput data (Table 10). We acknowledge that carefully designed in-vitro and in-vivo experimental models will be needed to verify the CKD-, and ESRD-upregulated PBMC secretomes further and underlying mechanisms we report here. Nevertheless, our findings provide novel insights on the roles of PBMC secretomes in the pathogenesis of ESRD and CKD, novel pathways under-lying the multi-hit models as well as new targets for the future therapeutic interventions for CKD, ESRD and their related diseases, aging and cancers.

Authors' contributions

RJZ carried out the data gathering, data analysis and prepared tables and figures. JS, YS, TY, LL, FS, WYY, YS, CJ, CDIV, HF, YL, KX, ML,
JW, EC, DY, XJ, YL, RL, LW, ETC, HW aided with analysis of the data. XFY supervised the experimental design, data analysis, and manuscript writing. All authors read and approved the final manuscript.

**Funding**

This work was supported by the hospital fellowship to RJ Zhang.

**Consent for publication**

Not applicable.

**Ethics approval and consent to participate**

Not applicable.

**Declaration of competing interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

---

**Fig. 7b. Novel Mechanism:** Reactive oxygen species (ROS)-related mechanisms, regulated by ROS generating enzyme NADPH oxidase 2 (NOX2) and antioxidant transcription factor Nrf2 pathways, modulated the secretomic changes during kidney dysfunction. The modulation was closely related to the balance of ROS and antioxidants and the imbalance contributes to the alteration of ROS-dependent SGs which could promote disease progression.

---

**Table 9a**

| clinical risk factor for CKD progression | scientific mechanism | PMID |
|----------------------------------------|----------------------|------|
| ethnicity                              | APOI mutant in African | 27312436 |
|                                        | Epigenetically heritable | 25993323 |
| gender                                | direct effects of sex steroids on kidney | 29355169 |
|                                        | sex differences in NO metabolism and oxidative stress | 29355169 |
|                                        | gender-differential impact of comorbidities and lifestyle risk factors | 29355169 |
| diabetes                              | Renal hemodynamic changes | 29486908 |
|                                        | ischemia and inflammation | 29486908 |
|                                        | Overactive renin-angiotensin-aldosterone system | 29486908 |
| hypertension                          | Global RAS activation | 29144825 |
|                                        | renal inflammation | 29144825 |
| obesity                               | RAAS activation | 31015582 |
|                                        | renal compression | 31015582 |
|                                        | Metabolic abnormalities | 31015582 |
| gut microbiota                        | production of uremic toxins | 30464044 |
|                                        | promoting translocation of bacterial component | 31243394 |
|                                        | secreting metabolites favoring insulin resistance favoring insulin resistance and endothelial dysfunction | 31243394 |
| nephrotoxicity                        | inducing apoptosis, autophagy and necrosis | 29341864 |
|                                        | Oxidant-Induced Renal Injury | 15519281 |
|                                        | mitochondria dysfunction in the proximal tubules | 29939355 |
| Hepatitis B and C infections          | glomerular immune complex deposition | 28149647 |
|                                        | direct viral invasion of the renal parenchyma | 31155101 |
| dyslipidemia                          | Direct lipid-induced cellular injury | 22290079 |
|                                        | inflammatory cytokines | 29176657 |
| proteinuria                           | Increased intraglomerular hydraulic pressure damage to glomerular filtration barrier | 22137726 |
| mineral bone disorder                 | VSMC dedifferentiation | 28119179 |
|                                        | neointimal atherosclerotic calcification | 28119179 |
Our new findings suggested a new model that not only passive accumulation of uremic toxins, but also other active upregulation of secretome during ESRD contributes to disease progression through proinflammatory and profibrotic pathways and molecules. This active accumulation modulated by both ROS-dependent and –independent pathways could promote systemic inflammation and fibrosis to accelerate disease progression. Uremic toxin-related cytokine switching, macrophage polarization and co-signaling by the interaction of CD48 and CD58 with CD2 were important pathways associated with ROS-independent SGs. Of note, there has been researches identified those pathways could modulate and interact with ROS-dependent pathway. The active accumulation of secretomic changes are the key mediators when combined with and modulated by other risk factors as multiple hits in the transition from CKD to ESRD. Ranking of all the mechanisms in our research by the numbers of SGs upregulated in each entry suggested that ROS serves as an important complementary role for prior knowledge of CKD progression, multiple-hit model of CKD progression.
Table 10
A novel research publication type with big-omics experimental database mining analyses leads to original new findings and generate anew hypotheses. A few aspects of comparisons were made within this study using big-omics experimental database mining approaches, the traditional literature reviews and the meta-analysis.

| category | Big-omics Database mining | Traditional literature review | meta analysis |
|----------|----------------------------|------------------------------|--------------|
| Analysis of experimental Data (NIH-Geo-DataSets with microarray experimental data, etc.) | yes | no | yes |
| Original new Findings | no | no | yes |
| Association research (gene co-expression patterns at the same pathology or stimuli) | yes | no | no |
| Causative research (upstream regulator gene deficient microarrays, ...) | yes | no | yes |
| Panoramic view at multiple mechanisms and pathways | yes | yes | yes |
| Improvement of our understanding | yes | yes | yes |
| Searchable Database requirements and tools | yes | no | yes |
| New publication types after –omics and high throughput experimental data generation | yes | no | yes |
| Different focuses from original papers | yes | no | no |
| Use of Ingenuity Pathway Analysis (IPA) to analyze experimental data | yes | no | no |
| Bioinformatic prediction | no | no | no |
| Future experimental verification | yes | yes | yes |
| Summary of previous reports | no | yes | yes |
| Example | PMID: 22438968 [our datamining paper focusing on IL-35 (highly cited by 173 papers)] | PMID: 24060958 [a Nature Review paper of management of hyperglycemia in diabetes] | PMID: 23083786 [a meta-analysis paper focusing on Effects of fibrates in kidney disease] |
| Experimental papers verifying the findings originated from example paper | PMID: 26085994; 29371247 | N/A | PMID: 25419705 |
| Use of multiple NIH databases including PubMed database (https://www.ncbi.nlm.nih.gov/books/NBK143764/) | yes | no | no |

Acknowledgements
This work was supported by NIH RO1 HL131460.

Appendix A. Supplementary data
Supplementary data to this article can be found online at https://doi.org/10.1016/j.redox.2020.101460.

References
[1] Centers for Disease Control and Prevention, Chronic Kidney Disease in the United States, 2019, US Department of Health and Human Services, Centers for Disease Control and Prevention, Atlanta, GA, 2019.[https://www.cdc.gov/kidneydisease/publications-resources/2019-rational-fact.html]
[2] S.T. Reikes, Trends in end-stage renal disease. Epidemiology, morbidity, and mortality, PGM (Postgrad. Med.) 108 (1) (2000) 124–126 9-31, 35-6 passim.
[3] Y. Yin, X. Li, X. Sha, H. Xi, Y.F. Li, Y. Shao, et al., Early hyperlipidemia promotes endothelial activation via a caspase-1-sirtuin 1 pathway, Arterioscler. Thromb. Vasc. Biol. 35 (4) (2015) 804-816.
[4] X. Li, P. Fang, Y. Li, Y.M. Kuo, A.J. Andrews, G. Nanayakkara, et al., Mitochondrial reactive oxygen species mediate lysophosphatidylcholine-induced endothelial cell activation, Arterioscler. Thromb. Vasc. Biol. 36 (6) (2016) 1090-1100.
[5] P. Fang, D. Zhang, Z. Zheng, C. Yan, X. Jiao, W.D. Kruger, et al., Hyperhomocysteinemia potentiates hyperglycemia-induced monocyte differentiation and atherosclerosis, Diabetes 63 (12) (2014) 4275-4290.
[6] J. Xu, Y. Zhang, K. Xu, W.Y. Yang, X. Jiang, X. Sha, et al., Caspase-1 inflammasome activation mediates homocysteine-induced pyrop-apoptosis in endothelial cells, Circ. Res. 118 (10) (2016) 1525–1539.
[7] J. Yang, P. Fang, D. Yu, L. Zhang, D. Zhang, X. Jiang, et al., Chronic kidney disease induces inflammatory CD40+ monocyte differentiation via homocysteine elevation and DNA hypomethylation, Circ. Res. 119 (11) (2016) 1226–1241.
[8] L.M. Ferrer, A.M. Monroy, J. Lopez-Pastrana, G. Nanayakkara, R. Cueto, Y.F. Li, et al., Caspase-1 plays a critical role in accelerating chronic kidney disease-progression in LDLr/CBS-deficient mice, Circ. Res. 113 (12) (2018) 37–49.
[9] X. Li, X. Song, X. Li, Y. Huan, C. Wang, et al., Inhibition of caspase-1 activation in endothelial cells improves angiogenesis: a novel therapeutic potential for ischemia, J. Biol. Chem. 290 (28) (2015) 17485–17494.
[10] X. Yang, Y. Wei, Y.H. Liu, J.H. Peng, Q. Tian, G.P. Liu, et al., Hyperhomocysteinemia increases beta-amyloid by enhancing expression of gamma-secretase and phosphorylation of amyloid precursor protein in rat brain, Am. J. Pathol. 174 (4) (2009) 1481-1491.
[11] D. Zhang, P. Fang, X. Jiang, J. Nelson, J.K. Moore, W.D. Kruger, et al., Severe hyperhomocysteinemia promotes bone marrow-derived and resident inflammatory monocyte differentiation and atherosclerosis in LDLr/CBS-deficient mice, Circ. Res. 111 (1) (2012) 37–49.
[12] Y. Shao, Z. Cheng, X. Li, V. Chernyaya, H. Wang, X.F. Yang, Immunosuppressive/anti-inflammatory cytokines directly and indirectly inhibit endothelial dysfunction—a novel mechanism for maintaining vascular function, J. Hematol. Oncol. 7 (2014) 80.
[13] X. Li, L. Wang, P. Fang, Y. Sun, Y. Jiang, et al., Lysophospholipids induce innate immune transdifferentiation of endothelial cells, resulting in prolonged endothelial activation, J. Biol. Chem. (2018) 002752jbc.RA118.
[14] A. Li, Y. Sun, C. Drummer, Y. Li, D. Yu, Y. Zhou, et al., Increasing upstream chromatin long-range interactions may favor induction of circular RNAs in LysoPC-activated human aortic endothelial cells, Front. Physiol. 10 (2019) 433.
[15] J. Lopez-Pastrana, L.M. Ferrer, Y.F. Li, X. Xiong, H. Xi, R. Cueto, et al., Inhibition of caspase-1 activation in endothelial cells improves angiogenesis: a novel therapeutic potential for ischemia, J. Biol. Chem. 290 (28) (2015) 17485-17494.
[16] C.E. Zhang, W. Wei, Y.H. Liu, J.H. Peng, Q. Tian, G.P. Liu, et al., Hyperhomocysteinemia increases beta-amyloid by enhancing expression of gamma-secretase and phosphorylation of amyloid precursor protein in rat brain, Am. J. Pathol. 174 (4) (2009) 1481-1491.
[17] D. Zhang, P. Fang, X. Jiang, J. Nelson, J.K. Moore, W.D. Kruger, et al., Severe hyperhomocysteinemia promotes bone marrow-derived and resident inflammatory monocyte differentiation and atherosclerosis in LDLr/CBS-deficient mice, Circ. Res. 111 (1) (2012) 37–49.
[18] P. Fang, X. Li, H. Shan, J.J. Saredy, R. Cueto, J. Xia, et al., Ly6C+ inflammatory monocyte differentiation partially mediates hyperhomocysteinemia-induced vascular dysfunction in type 2 diabetic db/db mice, Arterioscler. Thromb. Vasc. Biol. 39(10) (2019) 2097–2119, ATVBHAA11931338.
[19] Z. Xiaoqiong, J. Song, Y. Yan, Y. Huang, A. Cowan, H. Wang, et al., Higher expression of Bax in regulatory T cells increases vascular inflammation, Front. Biosci. 13 (2008) 7143–7155.
[20] Z. Xiaoqiong, Y. Yan, J. Song, P. Fang, Y. Yin, Y. Yang, et al., Expression of TCTP antisense in CD25(high) regulatory T cells aggravates cuff-induced vascular inflammation, Atherosclerosis 203 (2) (2009) 401–408.
[21] W.Y. Yang, Y. Shao, J. Lopez-Pastrana, J. Mai, H. Wang, X.F. Yang, Pathological conditions re-shape physiological Tregs into pathological Tregs, Burns Trauma 3 (1) (2015).
[22] K. Xu, W.Y. Yang, G.K. Nanayakkara, Y. Shao, F. Yang, W. Hu, et al., glaTa3, g12αc, and Bcl6 regulate FOXP3+ Treg Plasticity and Determine Treg conversion into either novel antigen-Presenting-cell-like Treg or Tbi-Treg, Front. Immunol. 9 (2018) 45.
[23] J. Nelson, Y. Wu, X. Jiang, R. Berretta, S. Houser, E. Choi, et al., Hyperhomocysteinemia suppresses bone marrow CD34+/VEGFR-2 receptor 2 + cells and inhibits progenitor cell mobilization and homing to injured vasculature—a role of beta1-integrin in progenitor cell migration and adhesion, Faseb. J. : official publication of the Federation of American Societies for Experimental Biology 29 (7) (2015) 3085–3094.
[24] Y.F. Li, X. Huang, X. Li, R. Gong, Y. Yin, J. Nelson, et al., Caspase-1 mediates hyperlipidemia-weakened progenitor cell vessel repair, Front. Biosci (Landmark Ed) 23 (2018) 348-387.
[25] Y. Shao, V. Chernyaya, C. Johnson, W.Y. Yang, R. Cueto, X. Sha, et al., Metabolic
diseases downregulate the majority of histone modification enzymes, making a few upregulated enzymes novel therapeutic Targets—‘Sand out and gold stays, diseases downregulate the majority of histone modification enzymes, novel qualification markers for chronic disease risk factors and conditional DAMPs, Redox Biol 24 (2021) 101211.

[46] M. Kim, M. Kim, J.Y. Han, S.H. Lee, S.H. Jee, J.H. Lee, The metabolites in peripheral blood mononuclear cell showed greater differences between patients with chronic kidney disease, Am. J. Nephrol. 38 (2) (2017) 124–126.

[49] T. Wang, Y.Cui, J.Jin, J.Guo, G.Wang, X.Yin, et al., Translating mRNAs strongly correlated to protein mRNAs, in patients with chronic kidney disease, Am. J. Nephrol. 38 (2) (2017) 230–240.

[54] E. Kaczmarski, A. Frank, E. Weglie, L. Szczepanik, L. Blasiak, G. Muller, et al., The induction of endothelial dysfunction and atherosclerosis, J. Hematol. Oncol. 7 (2014) 205.

[56] T. Wang, Y. Li, G. Nanayakkara, Y. Shao, B. Liang, L. Cole, et al., Lysophospholipid receptors, as novel conditional danger receptors and homeostatic receptors modulate inflammation novel paradigm and therapeutic potential, J Cardiovasc Transl Res 9 (4) (2016) 343–359.

[59] C. J. Pelham, D.K. Agrawal, Emerging roles for triggering receptor expressed on myeloid cells receptor family signaling in inflammatory diseases, Expet Rev. Clin. Immunol. 10 (2014) 243–256.

[61] H. Mehta, A.G. Bamadda, Activation of cardiomyldi LDI in the induction of endothelial dysfunction and atherosclerosis, Eur. J. Heart 35 (2014) 3021–3032.

[62] H. Farhan, C. Rabouille, Signalling to and from the secretory pathway, J. Cell Sci. 124 (Pt 2) (2011) 171–180.

[65] C. Rabouille, Pathways of unconventional protein secretion, Trends Cell Biol. 27 (3) (2017) 230–240.

[66] G.Bindea, B.Mlecnik, H.Hackl, P.Charoentong, M.Tosolini, A.Kirilovsky, et al., Resistance to immunosuppressive therapy: novel markers for chronic disease risk factors and conditional DAMPs, Eur. J. Immunol. 41 (6) (2011) 1573–1582.

[67] K. Serre, E. Mohr, C. Benezech, R. Bird, M. Khan, J.H. Caamano, et al., Selective endothelial dysfunction-a novel mechanism for maintaining vascular function, J. Hematol. Oncol. 7 (2014) 205.
H.Peng, F.Yeh, J.Lin, L.G.Best, S.A.Cole, E.T.Lee, et al., Plasminogen activator

A. Tasanarong, S. Kongkham, S. Khositseth, Dual inhibiting senescence and epithelial-to-mesenchymal transition by erythropoietin preserve tubular epithelial cells: implications for glomerulosclerosis, Am. J. Physiol. Ren. Physiol. 289 (3) (2009) 1827–1836.

X.Tian, C.Yan, M.Liu, Q.Zhang, D.Liu, Y.Liu, et al., CREG1 heterozygous mice are susceptible to high fat diet-induced obesity and insulin resistance, PloSOne 12 (2005) F577–F584.

M. Nishikawa, N. Ishimori, S. Takada, A. Saito, T. Kadoguchi, T. Furihata, et al., Canonical BMP signaling in tubular cells mediates recovery after acute kidney injury, Kidney Int. 95 (1) (2019) 108–122.

R.Curi, R.deSiqueiraMendes, L.A.deCamposCrispin, G.D.Norata, S.C.Sampaio, et al., Inflammasomes are involved in microvascular dysfunction in sepsis: findings from the Strong Heart Family Study, J. Thromb. Haemostasis 15 (6) (2017) 27–35.

R. Zhang, et al.
functional outcomes, Clin Sci (Lond). 131 (12) (2017) 1329–1342.

[145] P. Li, Y. Gan, Y. Xu, L. Song, L. Wang, B. Ouyang, et al., The inflammatory cytokine TNF-alpha promotes the premature senescence of rat nucleus pulposus cells via the PI3K/Akt signaling pathway, Sci. Rep. 7 (2017) 42938.

[146] T. Wieder, E. Brenner, H. Braunmueller, O. Bischof, M. Rocken, Cytokine-induced senescence for cancer surveillance, Canc. Metastasis Rev. 36 (2) (2017) 357–365.

[147] A. Freund, A.V. Orjalo, P.Y. Desprez, J. Campisi, Inflammatory networks during cellular senescence: causes and consequences, Trends Mol. Med. 16 (5) (2010) 238–246.

[148] R. Lavieri, A. Rubartelli, S. Carta, Redox stress unbalances the inflammatory cytokine network: role in autoimmune patients and healthy subjects, J. Leukoc. Biol. 99 (1) (2016) 79–86.

[149] R. Voltan, P. Secchiero, F. Cesciano, D. Milani, G. Zauli, V. Tisato, Redox signaling and oxidative stress: cross talk with TNF-related apoptosis inducing ligand activity, Int. J. Biochem. 81 (Pt B) (2016) 364–374.

[150] G. Varga, M. Gattorno, D. Foell, A. Rubartelli, Redox distress and genetic defects conspire in systemic autoinflammatory diseases, Nat. Rev. Rheumatol. 11 (11) (2015) 670–680.

[151] M. Sengupta, R. Pal, A. Nath, B. Chakraborty, L.M. Singh, B. Das, et al., Anticancer efficacy of noble metal nanoparticles relies on reprogramming tumor-associated macrophages through redox pathways and pro-inflammatory cytokine cascades, Cell. Mol. Immunol. 15 (12) (2018) 1088–1090.

[152] R.M. Gorkzynski, C. Alexander, K. Brandenburg, Z. Chen, A. Heini, D. Neumann, et al., An altered REDOX environment, assisted by over-expression of fetal hemoglobin, protects from inflammatory colitis and reduces inflammatory cytokine expression, Int. Immunopharm. 50 (2017) 69–76.

[153] L.E. Padgett, K.A. Broniowska, P.A. Hansen, J.A. Corbett, H.M. Tse, The role of reactive oxygen species and proinflammatory cytokines in type 1 diabetes pathogenesis, Ann. N. Y. Acad. Sci. 1281 (2013) 16–35.

[154] L. Diebold, N.S. Chandel, Mitochondrial ROS regulation of proliferating cells, Free Radic. Biol. Med. 100 (2016) 86–93.

[155] A.V. Belikov, B. Schraven, L. Simon, T cells and reactive oxygen species, J. Biomed. Sci. 22 (2015) 85.

[156] H. Tang, J. Li, X. Liu, G. Wang, M. Luo, H. Deng, Down-regulation of HSP60 suppresses the proliferation of glioblastoma cells via the ROS/AMPK/mTOR pathway, Sci. Rep. 6 (2016) 28386.

[157] J.H. Kim, S.G. Chu, J.L. Gramlich, Y.B. Pride, E. Babendreier, D. Chauhan, et al., Activation of the PI3K/mTOR pathway by BCR-ABL contributes to increased production of reactive oxygen species, Blood 105 (4) (2005) 1717–1723.

[158] N. Malaquin, A. Martinez, F. Rotier, Keeping the senescence secretome under control: molecular reins on the senescence-associated secretory phenotype, Exp. Gerontol. 82 (2016) 39–49.

[159] Y. Zhu, J.L. Armstrong, T. Tchkonia, J.L. Kirkland, Cellular senescence and the senescent secretory phenotype in age-related chronic diseases, Curr. Opin. Clin. Nutr. Metab. Care 17 (4) (2014) 324–328.

[160] M. Shan, J. Qin, F. Jin, X. Han, H. Guan, X. Li, et al., Autophagy suppresses isoprenaline-induced M2 macrophage polarization via the ROS/ERK and mTOR signaling pathway, Free Radic. Biol. Med. 110 (2017) 432–443.

[161] H.R. Griffiths, D. Gao, C. Pararasa, Redox regulation in metabolic programming and inflammation, Redox Biol 12 (2017) 50–57.

[162] H.Y. Tan, N. Wang, S. Li, M. Hong, X. Wang, Y. Feng, The reactive oxygen species in macrophage polarization: reflecting its dual role in progression and treatment of human diseases, Oxid Med Cell Longev 2016 (2016) 2795090.