Point-by-point responses to reviewers

Reviewer 1

The authors discuss the functional and molecular mechanisms in aging kidneys. They separately describe the glomerular, tubular, vascular and metabolic changes in detailed. However, many knowledge are already known to most researcher. Moreover, some issues need to be addressed.

(1) The authors discussed a lot about the measurement of aging kidneys. However, these contents may not be within the scope of IJMS. So I recommend this part should be removed.

We thought exact definition of kidney dysfunction using proper biomarkers for the elderly population was also helpful to understand the pathophysiologic mechanisms of aging kidney. However, we removed the part as reviewer's comments.

(2) More details should be added to provide complete information in Figure 1 as described in Section 3.2.

We would like to thank the reviewer's comment. According to the reviewer's comment, we tried to put more detailed information described in Section 3.2 in the Figure 1 (currently relocated Figure 2). We have now presented in the revised Figure 1 in revised manuscript at page 9 as below.

Page 9

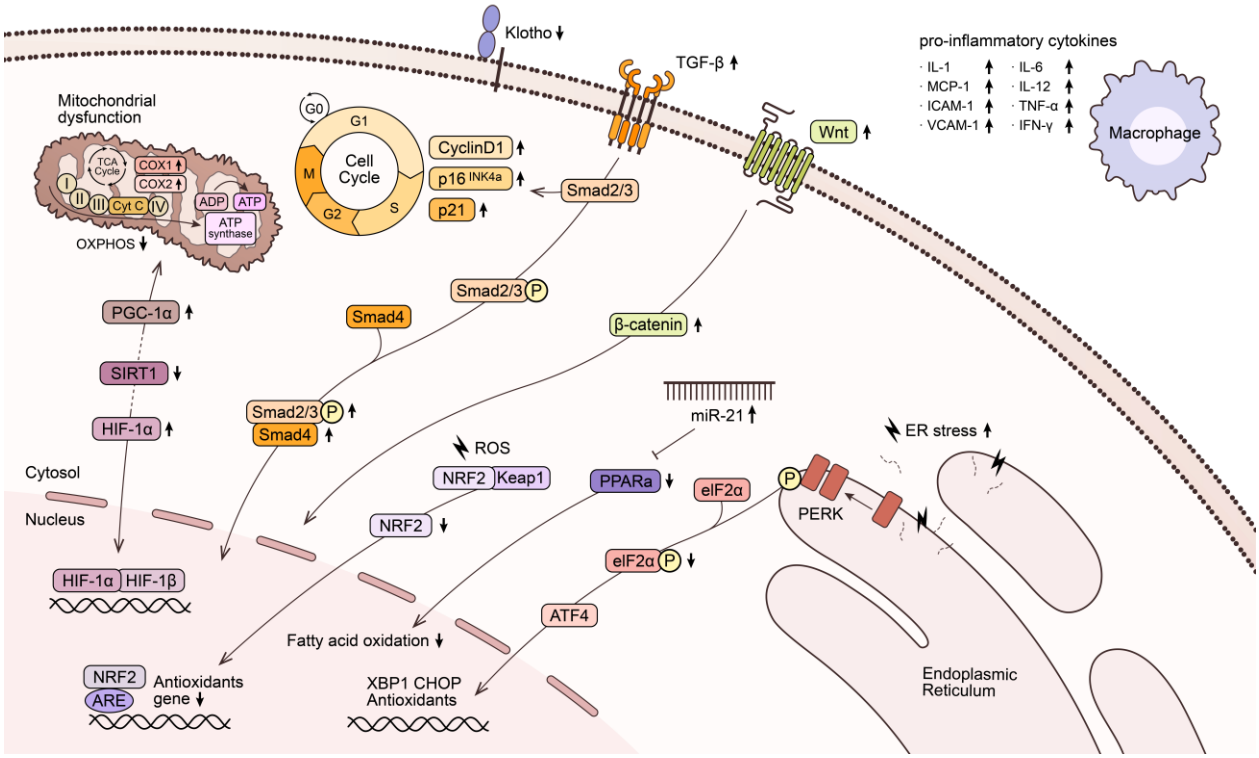


Figure 2. Molecular mechanisms in aged tubule.

Abbreviations: COX-1, cyclooxygenase 1; COX-2, cyclooxygenase2; ADP, adenosine diphosphateta, adenosine triphosphate; TCA, tricarboxylic acid; Cyt C, Cytochrome C , PGC1α, PPAR-α coactivador-1; OXPHOS, oxidative phosphorylation; SIRT1, Sirtuin 1; HIF-1α, hypoxia-inducible factor 1 subunit α; HIF-1β, hypoxia-inducible factor 1 subunit β; PPARα, peroxisome proliferator activated receptor α; TGF-β, transforming growth factor-β; Smad2/3, SMAD family member 2/3; Smad4, SMAD family member 4; NRF2, Nuclear factor erythroid-2-related factor 2; ARE, antioxidant responsive element; PERK, protein kinase RNA-like endoplasmic reticulum kinase; eIF2α, eukaryotic initiation factor 2α; ATF4, Activating Transcription Factor 4; XBP1, X-binding protein 1, CHOP, CCAAT-enhancer-binding protein homologous protein; ER, endoplasmic reticulum; IL, interleukin; TNF- α, tumor necrosis factor-α; IFN-γ, interferon γ.

(3) Figure 3 provides an overview of aging kidneys from four different points. The figure should be provided in advance, rather than put at the end.

We appreciate the reviewer's comments. We prepared two figures about the changes happened in aged kidneys according to various parts. Figure 3 (currently relocated Figure 1) has been relocated from the end to the front part, page 4 in response to the reviewer's comment.

Page 4

Complex changes associated with multiple molecular alterations in various parts of the kidney are implicated in the decline of kidney dysfunction with age. Figure 1 presents a schematic summary of changes in the molecular mechanisms of aged kidneys. Each change can occur as an interaction among different kidney parts. The detailed mechanisms of those molecular alterations are described below.

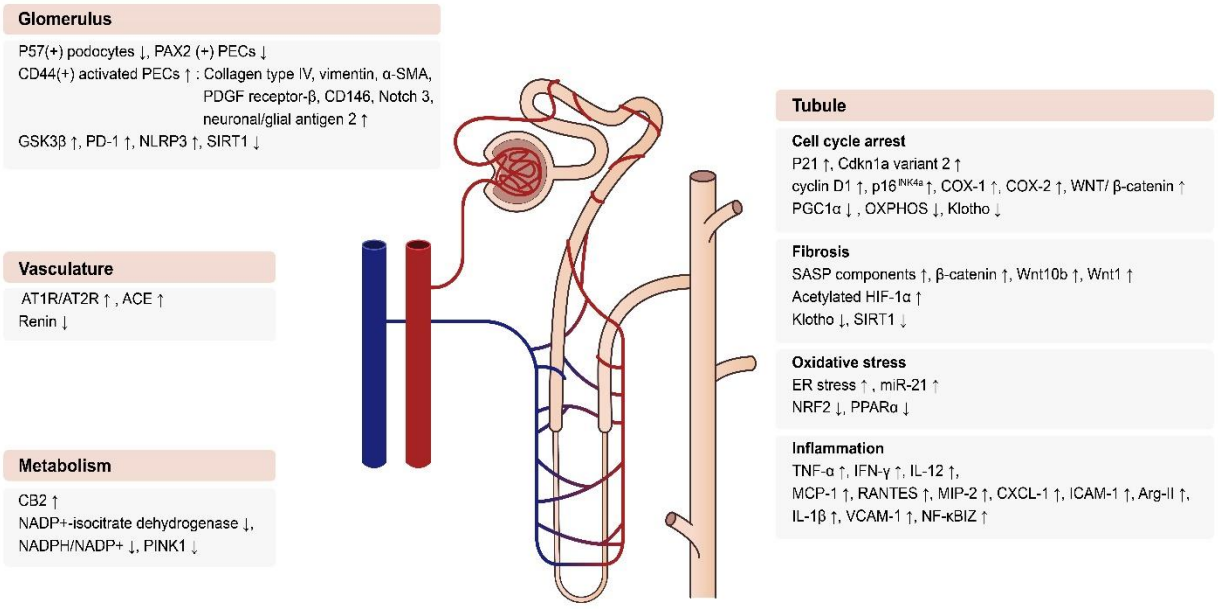


Figure 1. Changes in molecular mechanisms of aged kidney.

Abbreviations: PAX2, paired box gene 2; PEC, parietal epithelial cell; SMA, α-smooth muscle actin; PDGF, platelet-derived growth factor; GSK3, glycogen synthase kinase 3; PD-1, programmed cell death protein-1; NLRP3, nod-like receptor protein 3; SIRT1, sirtuin 1; AT1R, angiotensin II type 1 receptor; AT2R, angiotensin II type 2 receptor; ACE, angiotensin-converting enzyme; CB2, Cannabinoid receptor 2; NADP, nicotinamide adenine dinucleotide phosphate; PINK1, Phosphatase and tensin homolog-induced kinase 1; COX-1, cyclooxygenase 1; COX-2, cyclooxygenase2; PGC1α, PPAR-α coactivador-1; OXPHOS, oxidative phosphorylation; SASP, senescence-associated secretory phenotype; HIF-1α, hypoxia-inducible factor 1 subunit α; ER, endoplasmic reticulum; NRF2, nuclear erythroid-related factor 2; PPARα, peroxisome proliferator activated receptor α, TNF- α, tumor necrosis factor-α; IFN-γ, interferon γ; MCP-1, Monocyte chemoattractant protein 1; RANTES, Regulated upon Activation, Normal T cell Expressed and presumably Secreted; MIP-2, Macrophage inflammatory protein-2; CXCL-1, C-X-C motif chemokine ligand 1; ICAM-1, Intracellular Adhesion Molecule-1; Arg-II, arginase-II; IL, interleukin; VCAM-1, Vascular cell Adhesion Molecule-1; NF-κBIZ, NF-κB inhibitor zeta.

(4) The Future Perspectives section is too broad and only convey limited knowledge.

As reviewer's mentioned, we tried to convey more specific information about the directions for future researches. The following has been added to the future perspectives section at page 11 as below.

Page 11

For example, drugs newly developed to treat other diseases, such as cancer, have gained attention for

their potential to prevent renal damage. PD-1/PD-L1 inhibitors, known as immune checkpoint inhibitors, have shown therapeutic promise for the treatment of various cancers and might also offer renal protection, as we reviewed above. PD-1 signaling is being explored for its therapeutic potential in clinical trials focused on renal aging [48, 103].

In particular, single-cell RNA sequencing with a spatial consideration could elucidate target molecules in specific parts of aged kidneys. For example, a single-cell RNA sequencing analysis revealed tubular cells changing into an inflammatory phenotype and associated target molecules in aged murine and human kidney tissues [104]. A single-cell RNA sequencing analysis of aged mouse kidneys revealed seven subtypes of renal endothelial cells (ECs), with glomerular ECs and angiogenic ECs being the most aged. Furthermore, a pro-coagulant and pro-inflammatory microenvironment manifested in aged glomerular ECs [105].

To alleviate the deterioration of renal function in aging kidneys, it is necessary to detect the decline in renal function at an early stage, and factors that affect those early changes must be found. To date, creatinine has been used as the standard test to monitor kidney dysfunction, but because muscle mass is low in elderly people, the ability of creatinine to reflect early decline in renal function is limited. Therefore, efforts have been made to discover a more sensitive biomarker. The most fully verified substance to date is cystatin C, but confirmative results have yet to be published. Efforts to detect kidney damage in the early stages using substances related to aging should continue.

(5) The manuscript should have a conclusion Section.

We appreciate reviewer's precise comments. As reviewer mentioned, we added a conclusion section in the revised manuscript at page 12 as below.

Page 12

6. Conclusions

Complex changes in various parts of the kidney are implicated in kidney dysfunction in elderly people. Changes that occurs sequentially with aging are connected organically to molecular alterations in adjacent tissues. An individual's sensitivity and vulnerability to those molecular alterations might be linked to the degree of decline in kidney function with age. A schematic description of changes that happen in the various tissues of aged kidneys is presented in Figure 3. Because aging kidneys are clinically associated with increased vulnerability to various renal diseases, studies to prevent and treat kidney dysfunction in aging kidneys should continue. Research on the molecular underpinnings of aging in kidneys is also incomplete, and distinctive biomarkers for aging kidneys are needed. The most recent research provides an overview of the functional and molecular mechanisms of aging kidneys and might

ultimately be used to develop kidney disease diagnostic, therapeutic, and prognostic prediction technologies.

(6) As mentioned in Section 5, some single cell and spatial omics data have been reported, like PMID: 37675124, PMID: 37827693, etc. How do these single cell studies contribute to our improvement in investigating aging kidney?

We thank the reviewer for pointing this out. Single cell studies may be helpful to verify key molecules in the changes shown in the aged kidney of the specified region. Verifying target molecules could be developed to elucidate new therapeutic strategies. The reference as reviewer mentioned was cooperative in the manuscript. We have added reference recommended by reviewers and additional reference to the discussion section of the revised manuscript at page 11 as below (reference number : [104], [105]).

Page 11

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Comments on the Quality of English Language

: Minor editing of English language required

We thank the reviewer for pointing this out. The English in this review has undergone English language editing by MDPI (Manuscript ID: English-72157) as following ”The text has been checked for correct use of grammar and common technical terms, and edited to a level suitable for reporting research in a scholarly journal.” Based on the reviewer’s comment, we received English editing service once more from the site what our institute (Korea University) suggested (eWorldEditing Inc.). The confirmation letter from the site was attached as a supplement document.