The role of CCN1 in kidney-brain crosstalk

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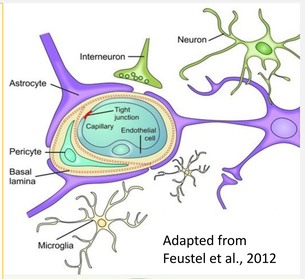
Abstract

Cognitive impairment and dementia are major global health problems of the aging human population. It is estimated that about 55 million people were living with dementia in 2019 and this number is expected to triple by 2050. Dementia can result from abnormal structure and function of brain blood vessels that may develop in a variety of conditions including chronic kidney disease (CKD). Recent studies identified new molecular mechanisms of kidney-brain crosstalk including newly identified angiogenic hormones such as the matricellular protein Cellular Communication Network factor 1 (CCN1) produced by the healthy kidney. My hypothesis is that CCN1 helps to maintain the normal structure and function of blood vessels throughout the body, including a healthy blood-brain barrier (BBB). In addition, I further hypothesize that reduced plasma CCN1 caused by diminished renal production of CCN1 due to kidney disease plays a major role in the development of brain vascular dysfunction and therefore in the pathogenesis and progression of dementia and cognitive impairment.

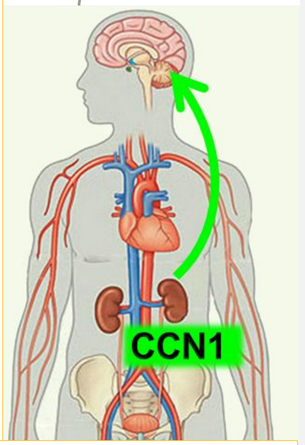
In this translational project, I measured and found reduced plasma CCN1 levels in patients at the USC's Alzheimer's Disease Research Center (ADRC) who exhibited mild cognitive impairment (MCI) compared to those who were cognitively normal. In addition, plasma CCN1 levels and the density of cell-to-cell junctions in brain endothelial cells were 20% reduced in a mouse model of CKD (5/6 nephrectomy) compared to control healthy mice. In brain endothelial cells cultured in vitro, CCN1 treatment had protective effects on cell proliferation, motility and angiogenesis as confirmed by scratch and tube formation assays. My study helped to improve the mechanistic understanding of the disease process of dementia and laid the foundation for future novel clinical diagnostic and therapeutic development in this area.

Introduction

- Cognitive impairment and dementia affects up to 50% and 70% of patients with moderate and severe CKD, respectively.
- The kidneys are well known to play major roles in maintaining whole body fluid and electrolyte balance, blood volume, blood cell number, and blood pressure via locally produced hormones released to the blood, such as renin and erythropoietin.
- The blood-brain barrier (BBB) is the specialized system of brain microvascular endothelial cells that shields the brain from toxic substances in the blood, supplies brain tissues with nutrients, and filters harmful compounds from the brain back to the bloodstream.



Adapted from Feustel et al., 2012



- CCN1 (Cyr61) is a matricellular protein and angiogenic inducer that acts through various integrin receptors and known to be a major positive regulator of endothelial cell function.
- Preliminary data show the majority of systemic CCN1, detected in the plasma, is produced by the kidney as evidenced by the drastic drop in plasma CCN1 24hrs after bilateral nephrectomy.

Aim

This study tested the hypothesis that CCN1 helps to maintain the normal structure and function of the blood-brain barrier (BBB), and that reduced plasma CCN1 caused by diminished renal production in kidney disease plays a major role in the development of brain vascular dysfunction.

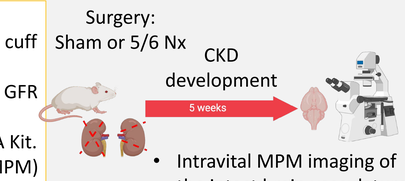
Methods

Mouse studies

- Systolic blood pressure (BP) was measured using the non-invasive tail cuff method.
- Glomerular filtration rate (GFR) was measured using transdermal GFR technology.
- Plasma CCN1 levels were measured using mouse Cyr61 SimpleStep ELISA Kit.
- BBB permeability was studied using intravital multiphoton (MPM) microscopy. Fluorescence intensity of the A680 conjugated albumin (70kDa) and the A488 conjugated Dextran (500kDa) was measured in brain interstitium and microvasculature.
- Anti-ZO1 antibody was used to visualize BBB tight junctions.

Human Translation

- Urine and plasma samples were collected by USC's Alzheimer's Disease Research Center - ADRC.
- Dementia score was used to establish cognitive function.
- Plasma CCN1 and creatinine were measured by using human Cyr61 and creatinine ELISA kits.



- Intravital MPM imaging of the intact brain vasculature
- GFR measurement
- Urine collection
- Blood collection
- Tissue harvest

- Image analysis - Leica LAS X or Image J image analysis software
- Statistical analysis –GraphPad Prism
- Illustration: Biorender

Results

Plasma CCN1 levels are reduced in a mouse model of CKD

- No difference in systolic blood pressure.
- Significantly reduced GFR in mice with 5/6 Nx compared to control.
- Significantly reduced plasma CCN1 levels in mice with 5/6 Nx compared to control.

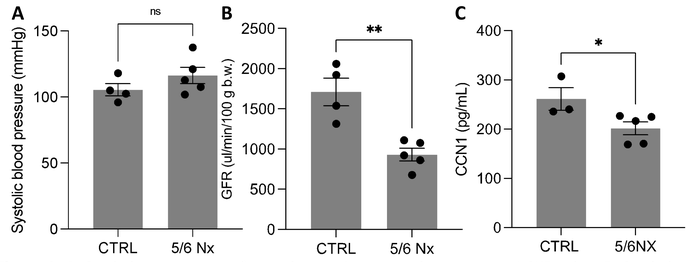


Figure 1: **A**: Statistical summary of systolic blood pressure in control and CKD mice. **B**: Statistical summary of glomerular filtration rate in control and CKD mice. **C**: Statistical summary of plasma CCN1 levels in control and CKD mice. (n=4 control, and n=5 CKD, ns: not significant, *p<0.05, ** p<0.01 using student T test).

Intravital imaging reveals increased BBB permeability in a mouse model of CKD

- Increased fluorescence intensity of mid-size plasma proteins (A680 conjugated Albumin 70 kDa) compared to high molecular weight markers (A488 conjugated Dextran 500 kDa) in brain interstitium in 5/6 Nx compared to control.

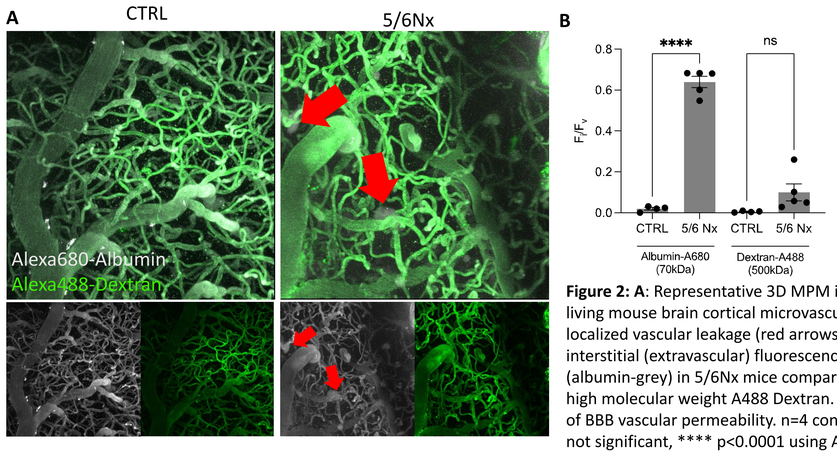
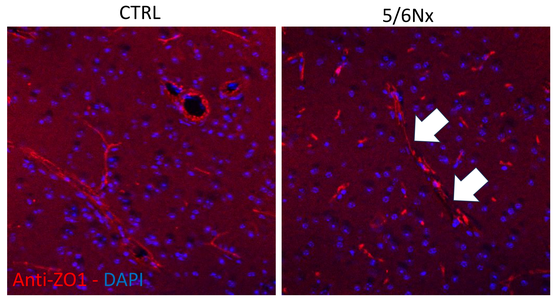


Figure 2: A: Representative 3D MPM images of the intact living mouse brain cortical microvasculature. Note the localized vascular leakage (red arrows) indicated by the high interstitial (extravascular) fluorescence intensity of A680 (albumin-grey) in 5/6Nx mice compared to control and the high molecular weight A488 Dextran. B: Statistical summary of BBB vascular permeability. n=4 control, n=5 5/6Nx, ns: not significant, **** p<0.0001 using ANOVA.

Results

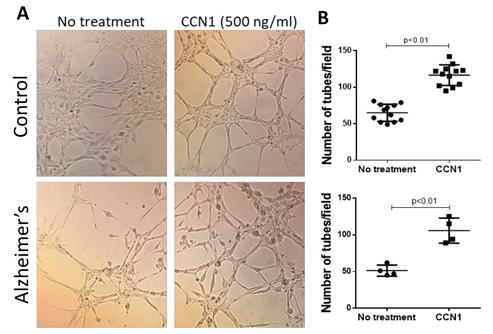
Reduced expression of ZO1 in brain endothelial cells in a mouse model of CKD



- Reduced ZO1 expression in brain endothelial cells in a mouse model of CKD compared to control.
- This suggests the disruption of BBB tight junctions, which is a known hallmark of the vascular changes in dementia.

Figure 3: **A**: Representative immunofluorescence images of ZO1 (red) expression in mouse brain vasculature. Note the reduced or patchy expression of ZO1 in 5/6 Nx mice compared to control (white arrows) indicating the disruption of tight junctions of BBB.

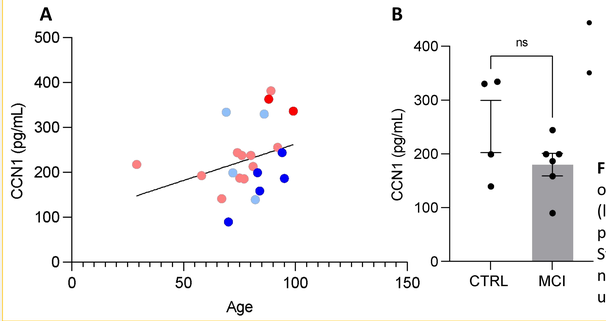
CCN1 improves endothelial cell function in health and disease



- CCN1 had strong angiogenic effects in both non-AD and AD patient-derived endothelial cells.

Figure 4 A: Representative images of in vitro angiogenesis (tube formation) assay in cultured endothelial cells derived from human control and Alzheimer's patients. **B**: Statistical summary of the in vitro angiogenesis assay. N=4-12, using student T test.

Human translation



- Plasma CCN1 levels showed positive correlation with aging.
- MCI was associated with reduced plasma CCN1 levels compared to control.

Figure 5 A: Plasma CCN1 levels as a function of age in male (blue), female (red), control (light), and mild cognitive impairment patients (MCI) (dark) in the ADRC cohort. **B:** Statistical summary of plasma CCN1 levels. n=4 control, and n=6 MCI, ns: not significant, using student T test).

Summary and Conclusion

- Plasma CCN1 levels were reduced in patients at the USC's Alzheimer's Disease Research Center (ADRC) who exhibited mild cognitive impairment (MCI) compared to those who were cognitively normal.
- Plasma CCN1 levels and the density of cell-to-cell junctions in brain endothelial cells were 20% reduced in a mouse model of CKD (5/6 nephrectomy) compared to control healthy mice.
- In brain endothelial cells cultured in vitro, CCN1 treatment had protective effects on cell proliferation, motility and angiogenesis as confirmed by tube formation assays.
- In conclusion, our study helped to improve the mechanistic understanding of the disease process of dementia in CKD and laid the foundation for future novel clinical diagnostic and therapeutic development in this area.

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