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Caspase-1 Plays a Critical Role in Accelerating Chronic Kidney Disease-Promoted Neointimal Hyperplasia in the Carotid Artery

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Abstract

To determine whether caspase-1 is critical in chronic kidney disease (CKD)-mediated arterial neointimal hyperplasia (NH), we utilized caspase^{-/-} mice and induced NH in carotid artery in a CKD environment, and uremic sera-stimulated human vascular smooth muscle cells (VSMC). We made the following findings: (1) Caspase-1 inhibition corrected uremic sera-mediated downregulation of VSMC contractile markers, (2) CKD-promoted NH was attenuated in caspase^{-/-} mice, (3) CKD-mediated downregulation of contractile markers was rescued in caspase null mice, and (4) expression of VSMC migration molecule $\alpha v\beta 3$ integrin was reduced in caspase^{-/-} tissues. Our results suggested that caspase-1 pathway senses CKD metabolic danger signals. Further, CKD-mediated increase of contractile markers in VSMC and increased expression of VSMC migration molecule $\alpha v\beta 3$ integrin in NH formation were caspase-1 dependent. Therefore, caspase-1 is a novel therapeutic target for the suppression of CKD-promoted NH.

Keywords

Caspase-1; Vascular inflammation; Chronic kidney disease (CKD); Neointimal hyperplasia (NH); Vascular smooth muscle cell (VSMC)

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no competing interests.

Ethics Statement This study was conducted in accordance to the Helsinki declaration and with the ethical standards of the responsible committee on human experimentation (institutional and national). All participants provided written informed consent. All institutional and national guidelines for the care and use of laboratory animals were followed and approved by the Institutional Animal Care and Use Committee of Temple University School of Medicine.

Introduction

Chronic kidney disease (CKD) affects over 15 % of the adult population [1, 2] and significantly contributes to morbidity and mortality in the general population. CKD results from progressive loss in renal function and is identified by pathologically elevated levels of plasma creatinine, resulting from lower glomerular filtration rate (<60 ml/min/1.73 m²) [3]. Thus, due to decreased kidney function, CKD results in an accumulation of metabolic wastes such as urea and other uremic toxins [4]. When the kidney function drops by 10–15 %, hemodialysis is required for the patient's survival [5].

CKD patients acquire fatal cardiovascular diseases (CVD) and are predisposed to death from CVD instead of renal failure [6]. Several CVD, such as atherosclerosis, arteriovenous fistula (AVF), and allograft vasculopathy are aggravated by CKD. Nevertheless, the gene expression scores that were established to predict the risk for obstructive coronary disease does not account CKD as a risk factor [7]. Also, utilization of imaging modalities for early detection of atherosclerosis has not been explored in individuals with CKD [8]. This indicates that CKD-induced CVD have not been explored extensively.

CKD-mediated CVD are associated with the development of neointimal hyperplasia (NH) in the arteries. NH is defined as the inward proliferation and migration of vascular smooth muscle cells (VSMC), resulting in the thickening of the arterial wall and loss of the arterial lumen. Moreover, NH was present prior the AVF formation in CKD patients [9], and also, uremia accelerated the development of NH associated with AVF in rodent models with CKD [10, 11]. Furthermore, humans and pigs fed with high-fat diet developed atherosclerosis at the site of preexisting NH [12–15]. Therefore, it is of paramount importance to identify the molecular components that promote the initiation and progression of NH in CKD, which will also potentiate identification of novel therapeutic targets.

VSMC demonstrates extensive phenotypic plasticity during vascular repair and remodeling under various pathological conditions. In a healthy kidney-controlled plasmic and artery environment, fully differentiated VSMC highly express several specific contractile proteins that include smooth muscle α -actin, smooth muscle myosin heavy chain, SM22 α , and calponin, which is known to have a contractile phenotype [16]. In contrast, VSMC undergo from Bcontractile[^] to Bsynthetic[^] phenotypic change by the downregulation of VSMC contractile gene expression in response to vascular injury induced by CKD, uremia, and mechanic stress as we recently reported [17]. This synthetic phenotype of VSMC is characterized by the loss of contractility, abnormal proliferation, migration, and matrix secretion which are important steps in the formation of NH. The VSMC synthetic phenotype can induce inward remodeling, significantly narrow the vessel lumen, and accelerate the development of various vascular pathologies such as atherosclerosis, hypertension, and post-angioplasty restenosis [18, 19]. However, the sensing molecular mechanism which bridges the elevated metabolic wastes and uremic toxins in plasma and the accelerated development of NH in CKD patients remains unknown.

Recently, the innate immune system was identified to play a role in the development of NH in response to vascular injury. Toll-like receptors (TLR), which play a vital role in innate

immunity, are located in the plasma membrane and recognize a variety of conserved microbial pathogen-associated molecular patterns (PAMP) and endogenous metabolite danger signal-associated molecular patterns (DAMP) that promote pro-inflammatory gene transcription. As we described previously [20], for tissues in which receptors for DAMP are not constitutively expressed, TLR also work in synergy with cytosolic sensing receptor families which include NLR (NOD (nucleotide binding and oligomerization domain)-like receptors), to recognize endogenous DAMP and mediate the activation of a wide range of inflammatory genes [21]. Caspase-1 is a member of the cysteine protease family of caspases that requires assembly of a NLR family member-containing protein complex called Inflammasome[^] for activation. Caspase-1 is present in the cytosol as an inactive zymogen pro-caspase-1, and undergoes post-translational cleavage to be activated. Activated caspase-1 is required for cleaving and processing of pro-interleukin-1 β (pro-IL-1 β) and pro-interleukin-18 (pro-IL-18) into functional mature pro-inflammatory cytokines IL-1 β and IL-18, respectively, as well as the activation of other inflammatory pathways. Recently, we reported that caspase-1 promotes aortic endothelial cell activation, facilitates early atherosclerosis [22, 23], inhibits endothelial cell angiogenesis [24], and weakens progenitor cell vascular repair [25]. Previously, we also reported that CKD alters vascular smooth muscle cell phenotype [26]. Although caspase-1 inflammasome activation has been reported in the pathogenesis of kidney disease [27], an important issue of whether caspase-1 plays any role in CKD-mediated NH and VSMC phenotypic change from contractile to a synthetic phenotype remains unknown.

In this study, we examined a novel hypothesis that caspase-1 promotes the CKD-induced VSMC switch from contractile to synthetic phenotype and the NH development. We applied a carotid artery ligation mouse model reported previously [28] on the CKD model [11] for the development of NH in wild-type (WT) and caspase-1 gene-deficient (caspase-1^{-/-}) mice [29]. Our data has demonstrated that caspase-1 plays a critical role in the downregulation of VSMC contractile marker gene expression and promotes NH formation in CKD mouse models.

Materials and Methods

Human Vascular Smooth Muscle Cell Culture and Uremic Sera Collection

This study was approved by Temple University School of Medicine Institutional Review Board (IRB). After informed consent, blood samples were collected from 6 healthy donors with normal kidney function and 20 patients with end-stage renal disease who were on hemodialysis (HD). Blood was collected prior to the routine HD session [30]. Blood samples were centrifuged at 3000 rpm, and the sera were aliquoted and stored at -80C.

Human aortic vascular smooth muscle cells (HAVSMC) were cultured as we previously described [17]. HAVSMC were serum starved for 24 h and then treated with the serum collected from CKD/healthy donors (growth medium supplemented with 10 % v/v serum) for another 24 h. We used pooled serum from (3–5) maintenance HD patients in each experiment and caspase-1 inhibitor (Z-YVAD-FMK, BioVision, Inc., Milpitas, CA) at 10 μ m concentration. Untreated serum-starved cells were used as a control.

Mice

Proposed experiments were approved by Institutional Animal Care and Use Committee of Temple University School of Medicine. Wild-type (WT, C57BL/6) mice were purchased from Jackson Laboratory (Bar Harbor, Maine). Genetically modified caspase-1 gene-deficient mice in C57BL/6 back-ground (caspase-1^{-/-}) were generously provided by Dr. Richard Flavell from Yale University [29].

Model of CKD

CKD was established in mice as previously described [31]. Blood urea nitrogen (BUN) was measured after 1 week of post nephrectomy using a commercial kit (STANBIO Laboratory, Issaquah, WA).

Left Common Carotid Artery Ligation

WT CKD, caspase-1^{-/-} CKD, and WT sham-surviving mice underwent LCCA ligation as previously described [11].

RNA Extraction and Quantitative Real-Time PCR

Total RNA from cultured cells and mouse carotid arteries were extracted as previously described [17]. CDNA was amplified with inventoried gene assay products containing two human gene-specific primers (ACTA2 (SMA), Hs00909449_m1; CNN1 (calponin), Hs00923894_m1; Applied Biosystems, Grand Island, NY), four mouse gene-specific primers (Acta2, Mm725412_s1; Cnn1, Mm00487032_m1; Sm22 (Tagln), Mm00441661_g1; and Smtn, Mm00449973_m1; Applied Biosystems), and one FAM dye labeled Taq Man MGB probe using 7500 Real Time PCR System (Applied Biosystems). Relative gene expression was calculated by 2^{-C_t} method after normalization to eukaryotic 18S.

Histomorphometry

LCCA were harvested and treated as previously described [17]. Volumetric measurements for NH lesion and thrombus were performed using ImageJ software. Measurements were made of the vessel lumen area, neointimal area (enclosed by the black arrows), medial area, and adventitia (Fig. 1).

Statistics

Results were shown as the mean $\pm$ 2 standard deviations (SD) and analyzed for statistical significance using an unpaired t test.

Results

Caspase-1 Inhibition Corrected CKD Patients' Sera-Induced Downregulation of VSMC Contractile Gene Markers

Recently, caspase-1/inflammasome pathway was identified as a major sensor for endogenous metabolic waste-related DAMP [21]. Therefore, we formulated the hypothesis that caspase-1 pathway may play a role in sensing uremic toxins in CKD patients' plasma and promote VSMC to undergo from contractile to synthetic phenotypic change. To examine this novel

hypothesis in vitro, we treated HAVSMC with sera collected from CKD patients and studied the gene expression of VSMC contractile markers, α -actin and calponin (Fig. 1b). Treatment of HAVSMC with uremic sera significantly decreased the expression of α -actin and calponin by a fold change of 0.329 and 0.394, respectively (Fig. 1c and d). This data suggested that our in vitro model was valid. Interestingly, co-administration of caspase-1 inhibitor with uremic sera markedly ameliorated the suppression of α -actin and calponin in HAVSMC (Fig. 1c and d). These results suggested that the caspase-1 pathway in VSMC play a role in sensing elevated uremic metabolic wastes in CKD patients' sera and make VSMC undergo the contractile to synthetic phenotypic change.

BUN Level was not Significantly Altered in Wild-Type CKD Mice Versus Caspase-1^{-/-} CKD Mice

To determine the role of caspase-1 in the development of NH in the carotid artery, murine CKD model was established in 10 WT mice and 10 caspase-1^{-/-} mice (Fig. 2a). In addition, 10 WT mice were used as sham controls. After the surgeries were conducted to establish CKD, mice were subjected to BUN analysis. Although the BUN levels in WT CKD mice were significantly higher than that reported for the WT mice (24.60 ± 2.62) [32], BUN levels were not significantly different between WT CKD mice and caspase-1^{-/-} CKD mice (65.9 ± 11.64 versus 71.43 ± 35.5 mg/dL; $p < 0.18$) (Fig. 2b). Also, the BUN levels in WT CKD mice were similar to that of what we reported previously [11], suggesting that the CKD model was established successfully. Moreover, there were no significant differences in body weight between the groups at the time of LCCA ligation, and the body weights were in the reported range [11]. Of note, a previous report showed that neither inflammasome components NLRP3 and ASC nor caspase-1 deficiency had any significant effect on renal histopathology nor the proteinuria of serum nephritis [32], which were well correlated with our observation that no significant change was found in the BUN levels of WT CKD mice and caspase-1^{-/-} CKD mice. Taken together, these analyses suggested that the caspase-1 inflammasome pathway may not play a significant role in the kidney pathogenesis caused by the trauma in the CKD model nor does it have a direct adaptive immune response in the serum/antibody-triggered nephritis model [32].

Caspase-1 Deficiency Significantly Decreased CKD-Promoted NH of the Carotid Artery

Based on our observation that inhibition of caspase-1 activity can partially rescue the attenuation of contractile markers in vitro, we hypothesized that caspase-1 activation in VSMC may suppress the VSMC Bcontractile[^] phenotype, promote VSMC migration, and accelerate the development of NH in the artery. In order to test this hypothesis, we established a mouse LCCA ligation model as previously reported [11]. Six WT CKD mice, eight caspase-1^{-/-} CKD mice, and seven WT sham mice underwent LCCA ligation procedure and were kept 3 weeks for histophotometric analysis. After preparing and staining the histological samples with the VVG staining (elastic fibers), the level of NH in each of these sections was analyzed. Results showed that NH volumes were increased significantly in WT CKD mice from $239,775.07$ to $1,440,023.70 \mu\text{m}^3$ relative to WT shams ($p = 0.035$) (Fig. 3a and b). In addition, we observed that the NH volumes were significantly reduced by five folds in caspase-1^{-/-} CKD mice to $71,69.97$ from $1,440,023.70 \mu\text{m}^3$ in WT CKD mice ($p = 0.0196$) (Fig. 3a and b). Moreover, no differences were found in the NH volumes

between WT sham mice and CKD caspase-1^{-/-} mice. In order to consider the potential variations caused by the process of histological slides, we also calculated the NH (NI) to media (M) (NI/M) ratio for each group of mice (Fig. 3c). We observed that NI/M ratio was markedly elevated in WT CKD mice relative to WT shams. The NI/M volume ratio of the caspase-1^{-/-} CKD mice was comparable to WT shams and significantly lower relative to WT CKD mice. In addition, the percentages of stenosis in carotid arteries were also examined (Fig. 3d). The results showed that WT sham mice had lumen stenosis percentages of 19.24 %, which was relatively low compared to that of the WT CKD mice which was at 68.25 %. Most interestingly, caspase-1^{-/-} mice had significantly low percentage of lumen stenosis compared to WT CKD mice. Moreover, no significant differences were found between the media volumes of WT CKD and that of caspase-1^{-/-} CKD groups (Fig. 3e). These results demonstrated that caspase-1 plays a critical role in accelerating CKD-promoted NH in the carotid artery and that caspase-1 deficiency significantly ameliorates this effect.

Caspase-1 Deficiency Rescued CKD-Mediated Reduction of Contractile VSMC Marker Expression in NH Lesion

Our results clearly indicated that caspase-1 deficiency significantly decreases CKD-promoted NH in the carotid artery (Fig. 3), and also that caspase-1 inhibition partially corrected the uremic sera-induced attenuation of VSMC contractile gene markers (Fig. 1). Therefore, we hypothesized that caspase-1 deficiency inhibits NH by preserving the expression of contractile smooth muscle marker genes in the carotid artery. To examine this hypothesis, we analyzed the gene expression of contractile smooth muscle markers that include α -actin, calponin, SM22, and smoothelin (Fig. 4). The results showed that the expression of α -actin in caspase-1^{-/-} CKD mice was increased by 1.4-fold relative to WT CKD mice. The gene expression of calponin (1.28 folds), SM22 (1.22 folds), and smoothelin (1.41 folds) were moderately elevated in caspase-1^{-/-} CKD mice compared to that of WT CKD mice. These results suggested that caspase-1 deficiency attenuate the phenotypic switch of VSMC from contractile to synthetic, thus subsequently suppress NH development.

Caspase-1 Deficiency Decreases α v β 3 Integrin Expression

As we discussed in our previous review, α v β 3 integrin blockade led to a significant reduction in NH formation [33], which suggested that α v β 3 integrin and its ligand vitronectin mediate VSMC migration in NH formation. Importantly, α v β 3 integrin is a heterodimer of integrin α v and integrin β 3 subunits [34]. Therefore, we hypothesized that caspase-1 may promote the expression of α v β 3 integrin subunits, and its ligand vitronectin, that leads to VSMC migration into the neointima. To test this hypothesis, we utilized microarray data conducted on caspase-1-deficient tissue, which were deposited in the NIH-GEO Profile database. Our analysis revealed that that caspase-1 expression in WT mouse tissues was 22 folds higher than that of caspase-1 gene knock-out tissues ($p < 0.01$) (Fig. 5a and b). Furthermore, the expression of the three house-keeping genes (Gapdh, Aldoa, and Nono) in the microarrays of caspase-1^{-/-} mice versus WT control mice was comparable with a confidence interval ($\chi \pm 2$ standard deviations) of 1.07 ± 0.08 . This suggested that the tissue RNA samples utilized in this array were of good quality. Interestingly, the expression

of integrin α_v in WT mouse tissue was 1.53 folds higher than that in caspase-1^{-/-} mouse tissues ($p < 0.0247$) whereas the expression of the $\alpha_v\beta_3$ integrin ligand vitronectin in WT tissue was suppressed by 1.19 folds than that in caspase-1^{-/-} mouse tissues ($p < 0.1910$). We did not find significant changes of integrin β_3 expression with caspase-1 depletion. However, when we analyzed the DNA sequence data in the NIH/NCBI-UniGene database, we found that integrin β_3 RNA transcripts were expressed at a relative high level in human vessels (not shown). This finding suggested the possibility that highly expressed integrin β_3 can associate with caspase-1-promoted integrin α_v in order to upregulate the functional $\alpha_v\beta_3$ integrin to mediate VSMC migration. In addition, integrin α_v is a ubiquitously expressed protein in most tissues (see the RNA transcript expression profile at the NIH-NCBI-UniGene database Hs.436873). Thus, integrin α_v expression may not be significantly regulated by tissue differentiation signals but may possibly be regulated by pathological inflammatory signals. Since there are no microarray data sets obtained from caspase-1^{-/-} vessels available, our database mining results of $\alpha_v\beta_3$ integrin was from the microarray data set of GSE25205 that had used epididymal white adipose tissues were justified based on these considerations and manipulations. These results implicated that caspase-1 promoted the expression of functional $\alpha_v\beta_3$ integrin, but did not significantly affect the expression of the $\alpha_v\beta_3$ integrin ligand vitronectin. These results implied that caspase-1 potentially promotes NH by enhancing the expression of the VSMC migration driving molecule $\alpha_v\beta_3$ integrin.

Discussion

Patients who undergo HD-utilizing AVF often need to be surgically re-intervened in order to correct stenotic lesions [35]. These surgically repeated interventions increase the morbidity and mortality in CKD patients who receive dialysis [36]. The major cause of vascular access failure in AVF is due to NH [35], which is accelerated by the uremic state in CKD [10, 11]. However, no effective therapies are available to inhibit the initiation and progression of NH associated with CKD. For the first time, our results have demonstrated that the caspase-1 pathway plays a critical role in NH formation. Therefore, caspase-1 has the potential to serve as a novel therapeutic target for the suppression of CKD-promoted NH in various vascular pathologies including atherosclerosis, hypertension, and post-angioplasty restenosis [18, 19].

Previously, apoptosis-associated speck-like protein containing caspase recruitment domain (ASC) was found to be critical for neointima formation after vascular injury in mice [37]. ASC is an adaptor protein required for caspase-1-mediated inflammatory IL-1 β and IL-18 generation. However, this publication only reported the role of bone marrow-derived ASC and has not looked into ASC expressed in vascular residential cell (for example, VSMC) in NH formation. Also, caspase-1 pathway plays an important role in pulmonary vascular remodeling [38], atherosclerosis [39], and the pathogenesis of kidney disease [27]. Nevertheless, whether caspase-1 is a critical player in CKD-induced VSMC contractile to synthetic phenotypic switch that leads to development of NH was not explored before.

We utilized HAVSMC treated with uremic serum in the presence of caspase-1 inhibitor as our in vitro model, and LCCA ligation-induced NH in surgically induced CKD in caspase-1 null mice as our in vivo model to test whether caspase-1 plays any role in NH formation. In

our experimental models, we made the following observations: (1) Caspase-1 inhibition partially corrected uremic sera-induced attenuation of VSMC contractile markers; (2) BUN levels were not significantly different in WT CKD mice versus caspase-1^{-/-} CKD mice; (3) caspase-1 deficiency significantly decreased CKD-promoted NH of the carotid artery; (4) mechanistically, caspase-1 deficiency rescued CKD-induced attenuation of VSMC contractile markers in NH lesion; and (5) caspase-1 deficiency decreased the expression of the VSMC migration molecule $\alpha v\beta 3$ integrin. Our results implicated that the caspase-1 pathway senses CKD-elevated metabolic waste-related danger signals and promotes the switch from the VSMC contractile phenotype to synthetic phenotype both in vitro and in vivo. CKD promoted caspase-1-dependent NH formation in the LCCA by decreasing the expression of VSMC contractile phenotypic markers and enhancing the expression of VSMC migration molecule $\alpha v\beta 3$ integrin. This is in line with the previous finding that caspase-1 depletion in ApoE mice significantly reduced the VSMC content in the atherosclerotic plaque [40]. Our results suggested that caspase-1 depletion significantly decreases CKD-promoted NH but does not cause changes in the media volumes of the NH lesion arteries. Also, we observed that CKD-promoted NH is an inward remodeling process involved in the migration and proliferation of VSMC primarily in the tunica intima, that are adjacent to the intima but not distal to the intima, which results in the thickening of arterial walls and a decreased arterial lumen space.

In summary, the mechanisms underlying the initiation of VSMC migration and the switch from contractile to synthetic phenotype in response to CKD still remain unknown. Recent reports demonstrated that TLR work in synergy with cytosolic sensing receptor families such as NLR in recognizing endogenous DAMP and in mediating the upregulation and activation of a range of inflammatory genes [21]. Our results have clearly demonstrated that the caspase-1 pathway serves as the mechanisms underlying the initiation of VSMC migration and the switch from contractile to synthetic VSMC phenotypes in response to CKD-mediated elevation of metabolic danger signals. We further consolidated this by demonstrating that caspase-1 deficiency completely inhibits CKD-mediated NH formation. Our data mining studies had provided a possible underlying mechanism that caspase-1 can enhance CKD-mediated expression of VSMC migration molecule $\alpha v\beta 3$ integrin. The proposed novel molecular mechanism of caspase-1 is presented in Fig. 6. Our study concludes that caspase-1 can be a novel therapeutic target for the suppression of CKD-promoted NH.

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Abbreviations

CKD	Chronic kidney disease
CVD	Cardiovascular diseases

AVF	Arteriovenous fistulas
NH	Neointimal hyperplasia
VSMC	Vascular smooth muscle cells
TLR	Toll-like receptors
PAMP	Pathogen-associated molecular patterns
DAMP	Danger signal associated molecular patterns
NOD	Nucleotide binding and oligomerization domain
NLR	NOD-like receptors
HD	Hemodialysis
HAVSMC	Human aortic vascular smooth muscle cells
BUN	Blood urea nitrogen
WT	Wild type
VVG	Verhoeff elastic-van Gieson stain
H & E	Hematoxylin and eosin stain
ASC	Apoptosis-associated speck-like protein containing caspase recruitment domain

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Fig. 1.

The exposure of HAVSMCs to uremic sera decreased the gene expression of contractile markers, which was reversed by caspase-1 inhibition. **a** Schematic representation of the NH development. The *black arrows* show the direction of inward remodeling. **b** Schematic expression of the work flow. HAVSMCs were serum starved for 24 h and exposed to 10 % pooled uremic sera from CKD patients with/without caspase-1 inhibitor for another 24 h. The gene expressions of VSMC contractile markers (α -actin and calponin) were measured by qRT-PCR using Taqman primers and probes. **c** The uremic sera-mediated attenuation of α -actin expression was ameliorated in the presence of caspase-1 inhibitor. **d** Uremic sera-mediated suppression of calponin gene expression was rescued with caspase-1 inhibitor treatment

Fig. 2.

The CKD model was established in WT and caspase-1^{-/-} mice, followed by ligation of LCCA to induce NH. **a** The experimental design to establish CKD and NH in mice. The right kidney was ablated in 9-week-old mice. After 1 week, left nephrectomy was performed to establish CKD state. To verify the CKD state in mice, the BUN levels were measured. At week 4 after right kidney ablation, LCCA ligation was performed to induce NH. Three weeks after LCCA ligation, the artery was perfused and fixed with formalin and harvested for immunohistochemistry analysis. The VVG staining was used to determine the development of NH. **b** Mouse weight and BUN levels between WT and caspase^{-/-} CKD mice were not significant

Fig. 3.

Caspase-1 deficiency reduced CKD-induced carotid NH development in mice. **a** Representative cross-sections of VVG-stained LCCA from WT sham, WT CKD, and caspase-1^{-/-} CKD mice after 3 weeks of LCCA ligation. The carotid artery in caspase-1^{-/-} CKD mice had decreased NH development in comparison to WT CKD mice. **b** The neointimal (NI) volume was significantly increased in WT CKD mice relative to WT sham; NI was diminished in caspase-1^{-/-} CKD mice compared to WT CKD mice. **c** The NI/media (NI/M) ratio (%) was significantly increased in WT CKD mice relative to WT sham; the NI/M ratio was diminished in caspase-1^{-/-} CKD mice compared to WT CKD mice. **d** Vessel stenosis was increased in WT CKD mice compared to WT sham and was decreased in caspase-1^{-/-} CKD mice compared to WT CKD mice. **e** The media volume in WT CKD mice was not significantly different to caspase-1^{-/-} CKD mice. **p* value < 0.05

Fig. 4.

CKD decreased the gene expressions of VSMC contractile markers in the carotid artery in vivo, which was reversed with caspase-1 depletion. RNA extracted from carotid arteries of WT CKD and caspase-1^{-/-} CKD mice were converted to cDNA and analyzed by qPCR using Taqman probes. The gene expression of VSMC contractile markers α -actin, calponin, SM22, and smoothelin was measured. The gene expression of VSMC contractile markers were increased in the carotid arteries of caspase-1^{-/-} CKD mice relative to WT CKD mice

Fig. 5.

Caspase-1 depletion significantly suppressed the expression of integrin α v subunit of α v β 3 integrin. **a** Microarray data analysis revealed that the expression of integrin α v RNA transcripts in WT tissue was significantly higher than that in caspase-1^{-/-} mice. **b** The mean $\pm$ 2SD of the ratio of three housekeeping genes was used at 95 % confidence interval. * p < 0.05

Fig. 6.

The proposed novel mechanism of caspase-1 in development of CKD-promoted NH. 1 The NLR/inflammasome caspase-1 pathway in the contractile VSMCs senses the uremic toxins as DAMPs in a CKD-state, which consequently activates caspase-1. The activation of caspase-1 promotes the maturation and secretion of pro-inflammatory cytokines IL-1b and IL-18, decreases the expression of VSMC contractile markers and presumably makes VSMCs switch to synthetic phenotype and induce proliferation. 2 Caspase-1 activation in the contractile weakened VSMCs increases the expression of integrin $\alpha\text{V}\beta 3$, which mediates the formation of NH induced by inward remodeling due to the migration of VSMCs, leading to stenotic arterial lesion, occlusion of arterial lumen, and causes obstruction to the blood flow