Uremic Toxins Affect the Endothelium

Uremic toxins can induce endothelial dysfunction in patients with chronic kidney disease (CKD). Indeed, the structure of the endothelial monolayer is damaged in CKD, and studies have shown that the uremic toxins contribute to the loss of cell–cell junctions, increasing permeability. Membrane proteins, such as transporters and receptors, can mediate the interaction between uremic toxins and endothelial cells. In these cells, uremic toxins induce oxidative stress and activation of signaling pathways, including the aryl hydrocarbon receptor (AhR), nuclear factor kappa B (NF-κB), and mitogen-activated protein kinase (MAPK) pathways. The activation of these pathways leads to overexpression of proinflammatory (e.g., monocyte chemoattractant protein-1, E-selectin) and prothrombotic (e.g., tissue factor) proteins. Uremic toxins also induce the formation of endothelial microparticles (EMPs), which can lead to the activation and dysfunction of other cells, and modulate the expression of microRNAs that have an important role in the regulation of cellular processes.

1. Introduction

Chronic kidney disease (CKD) is caused by progressive loss of kidney function, leading to the accumulation of uremic toxins in the bloodstream; the endothelium, which is directly exposed to these toxins, is negatively affected. The endothelium plays an important role in the maintenance of vascular homeostasis and its dysfunction may contribute to the pathogenesis of cardiovascular diseases (CVD) [1][2]. Clinical studies have shown that CKD patients are at increased risk of morbidity and mortality from CVD, such as atherosclerosis and thrombotic events [1][3][4][5][6][7]. In fact, multiple studies indicate that uremic toxins mediate endothelial dysfunction and vascular inflammation, both main contributors to the development of CVD [1][2].

Endothelial cells are exposed to a variety of uremic toxins, which can be divided into three major groups: (I) small water-soluble compounds; (II) middle molecules; and (III) protein-bound uremic toxins [8][9]. Small water-soluble compounds have a molecular weight of less than 500 Da, such as urea, uric acid, and guanidines [8][10]. The middle molecules have a molecular weight greater than 500 Da, among which are leptin, adiponectin, and β2-microglobulin [8][10][11][12]. Finally, protein-bound uremic toxins generally have low molecular weight and are difficult to remove by dialysis therapies. Prototypes of this group are *p*-cresyl sulfate (PCS), indoxyl sulfate (IS), and indole-3 acetic acid (IAA) [3][8][10].

Recently, in vivo and in vitro studies have investigated the effects of isolated uremic toxins or uremic serum from CKD patients on endothelial cells to better understand the molecular and cellular mechanisms involved in endothelial dysfunction mediated by uremic toxins [13][14]. This review aims to discuss the main findings of the impact of uremic toxins on the endothelium, including effects on cell structure, expression of receptors and transporters of uremic toxins, activation of signaling pathways, microRNA (miRNA) regulation, inflammation and thrombosis processes, and formation of endothelial microparticles (EMPs) (**Figure 1**).
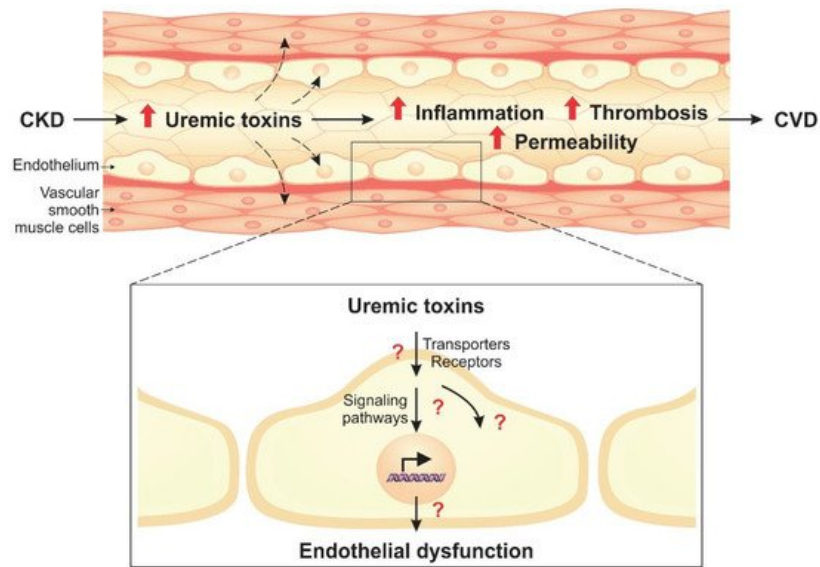
Figure 1. The progression of chronic kidney disease (CKD) results in the accumulation of uremic toxins in the bloodstream, which leads to endothelial dysfunction. Vascular endothelial cells exposed to uremic toxins have a proinflammatory and a prothrombotic phenotype, and the monolayer structure is damaged, increasing permeability. This impairment of endothelial function can contribute to cardiovascular diseases (CVD) pathogenesis.

2. Cell Signaling Pathways Altered by Uremic Toxins

Uremic toxins lead to phenotypic endothelial changes through the activation of cellular signaling pathways. Given the complexity of these pathways, important proteins that modulate the cellular response to uremic toxins stand out, such as AhR, NF-κB, and MAPKs. These molecules, therefore, have potential to be used as therapeutic targets in CKD.

2.1. AhR Pathway

AhR is a ligand-inducible transcription factor that belongs to the basic helix–loop–helix transcription factor family. Inactivated AhR is found in the cytoplasm bound to chaperones, which dissociate when AhR is activated by binding to a ligand [15]. Studies have identified several exogenous and endogenous ligands for AhR, such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), dioxin-like planar polychlorinated biphenyls (PCBs), and uremic toxins, including IS, IAA, indoxyl glucuronide, kynurenic acid, and other tryptophan derivatives [16][15][17][18][19][20][21]. Activated AhR can induce genomic signaling, known as the canonical pathway, as well as the non-genomic pathway.

In the genomic signaling pathway, activated AhR is translocated to the nucleus, where it forms a dimer with aryl hydrocarbon receptor nuclear translocator (ARNT) [15]. The AhR/ARNT complex binds to the xenobiotic responsive element (XRE; 5′-GCGTG-3′) in the promoter region of several target genes, inducing their expression [15][22]. Studies have shown that IS and IAA induce AhR translocation to the nucleus in endothelial cells [23][16][17]. In addition, overexpression of *CYP1A1* and *CYP1B1*, genes directly regulated by AhR/ARNT, was found in endothelial cells exposed to IS and IAA, an effect that was reversed with AhR inhibitors [23][16][24][17]. Furthermore, it has been demonstrated in HUVECs that IS and IAA, through AhR, induce AhRR expression, an AhR repressor that competes for ARNT binding, which ultimately leads to a negative regulatory loop for AhR [25][24][22].

In the non-genomic pathway, activated AhR interacts with various other signaling molecules, such as NF-κB and Src, independently of ARNT [16]. Ito et al. [23] demonstrated that IS increased the expression of E-selectin in an AhR-dependent manner in HUVECs. Despite that, the authors verified that AhR did not directly bind to the E-selectin gene promoter [23]. However, it was found that E-selectin overexpression was associated with the activity of the transcription factor activator protein-1 (AP-1), which is induced by AhR through the non-genomic pathway [23]. Similarly, Addi et al. [16] demonstrated that HUVECs exposed to IAA had an increase in TF gene expression, but AhR was not linked to the gene promoter despite the effect being reversed with AhR knockout and inhibition. The authors then found that NF-κB was essential for increasing TF expression, but its activity was decreased by the AhR inhibitor, suggesting a regulation between them [16].

Studies have shown that the activation of AhR by uremic toxins is involved in vascular inflammation, permeability, and the development of CVD [23][26][17]. In HUVECs, IS induced MCP-1 and E-selectin expression, proinflammatory molecules involved in leukocyte recruitment and adhesion to the endothelium, in an AhR-dependent manner [23][17]. In vivo, Ito et al. [23] also found that IS increased interaction between leukocytes and the endothelium of the femoral artery in wild-type mice, an effect that was not seen in endothelial cell-specific AhR knockout mice treated with IS. Furthermore, the

activation of AhR by IS also enhanced Src phosphorylation and, consequently, VE-cadherin phosphorylation, inducing an increase in endothelial permeability that was reversed with AhR inhibitors [26]. Koizumi et al. [27] demonstrated that IS-induced senescence of HUVECs is AhR-dependent and may contribute to CVD. In HUVECs, IAA and IS increased the expression of TF through AhR, which is associated with the pathogenesis of atherosclerosis and thrombosis [25][16]. Therefore, these studies suggest that AhR activation induced by uremic toxins has an important role in endothelial dysfunction and vascular injury. Interestingly, IS through the AhR pathway led to upregulation of OAT1 in renal proximal tubule cells as well as P-glycoprotein, an efflux pump that is part of the ABC transporter superfamily, in human hepatoma cells [28][29]. These data suggest that the AhR pathway may be involved in the regulation of the expression of cellular transporters.

AhR activating potential (AhR-AP) corresponds to the combination of all AhR agonists present in uremic serum, such as IS, IAA, and indoxyl glucuronide. Dou et al. [30] demonstrated that uremic serum from stage 3 to 5 and stage 5D CKD patients had higher AhR-AP than serum from healthy controls by an AhR-responsive bioassay. In addition, the authors reported that AHR-AP is associated with cardiovascular events in CKD patients [30]. In vivo, Dou et al. [30] detected higher serum levels of AhR agonists as well as overexpression of *Cyp1a1*, a gene regulated by AhR, in the aorta and heart of nephrectomized mice compared to AhR$^{-/-}$ nephrectomized or wild-type mice. In a cohort of patients with ESRD, Shivanna et al. [31] noted greater AhR activity in uremic serum compared to that of healthy controls. Kolachalama et al. [21] also found increased AhR activity and TF levels in serum from CKD patients who had thrombotic events compared to their counterparts without thrombosis. Taken together, these data indicate a relationship between uremic toxins, AhR activation, and the development of CVD [21][30][32].

2.2. NF-κB Pathway

NF-κB is a family of transcription factors that play a crucial role in endothelial inflammation. There are five forms of NF-κB proteins: p50 (NF-κB1), p52 (NF-κB2), p65 (RelA), RelB, and c-Rel. The p52 and p50 proteins are derived from the p100 and p105 forms, respectively. NF-κB family members form dimers, of which the p50/p65 dimer is the most common [33][34]. In the inactivated state, NF-κB dimers are found in the cytoplasm bound to inhibitory proteins, such as IκBα and IκBβ. In the presence of a stimulus, NF-κB dissociates from the inhibitory proteins, which are degraded. In the canonical pathway, the activation of IκB kinase (IKK) mediates the phosphorylation of IκBα, releasing NF-κB (mainly the p50, p65, and c-Rel forms) which is then translocated to the nucleus [33][34]. On the other hand, in the non-canonical pathway, also known as the alternative pathway, the activation of NF-κB-inducing kinase (NIK) leads to p100 phosphorylation and processing to the p52 form, which is translocated to the nucleus along with RelB [33][34].

Studies have shown that the NF-κB pathway participates in gene regulation in endothelial cells in the uremic conditions [35][24][36]. HUVECs exposed to uremic serum had greater IκB degradation and, consequently, higher levels of p50/p65 in the nucleus compared to those exposed to healthy serum [37]. In HUVECs, Tumur et al. [36] demonstrated that IS induced p65 phosphorylation as well as MCP-1 and ICAM-1 overexpression, which was reversed with NF-κB inhibitors. Furthermore, Masai et al. [35] reported an increase in the translocation of p65 to the nucleus and MCP-1 upregulation in HUVECs exposed to IS. Interestingly, the authors verified that this effect was suppressed by ERK1/2 and p38 MAPK inhibitors, indicating the participation of these proteins in the activation of the IS-induced NF-κB pathway [35]. In addition, studies have demonstrated an increase in the translocation of p50 and p65 to the nucleus in HUVECs exposed to IAA, which was decreased with AhR and p38 MAPK inhibitors [16][24]. Inhibition of NF-κB also reversed IAA-induced COX-2 and TF overexpression in HUVECs [16][24]. Based on these data, it is suggested that the NF-κB pathway is important for the upregulation of proinflammatory proteins in uremic conditions.

2.3. MAPK Pathway

MAPK are a family of serine/threonine kinases that are activated by phosphorylation [38]. There are three main groups of MAPK: Extracellular signal-regulated kinase (ERK1 and ERK2), C-Jun N-terminal kinase (JNK1, JNK2, and JNK3), and p38 MAPKs (α, β, γ, and δ) [39]. The MAPK signaling pathway is activated in the presence of a stimulus and, consequently, mediates the cellular response through activation of other proteins, such as transcription factors, by phosphorylation [40][41]. Therefore, MAPK has a significant role in signal transduction [39].

In uremic conditions, studies have shown that the MAPK pathway is activated in endothelial cells. Uremic serum enhanced ERK1/2 phosphorylation in HUVECs compared to normal serum [37]. IAA and IS also induced phosphorylation of p38 MAPK and ERK1/2 in HUVECs in the first 30 min of exposure [35][24]. In addition, MAPK pathway inhibition can reverse the effects of uremic toxins [35][16][24]. Inhibition of p38 MAPK reduced IAA-induced TF and COX-2 overexpression in HUVECs, the expression of which are possibly modulated by the AhR/p38MAPK/NF-κB pathway [16][24]. In another study, it was shown that inhibition of ERK1/2 and p38 MAPK suppressed IS-induced p65 phosphorylation (NF-κB) as well

as MCP-1 overexpression in HUVECs [35]. **Figure 2** shows the mechanisms modulated by the AhR, NF-κB, and MAPK signaling pathways in endothelial cells exposed to uremic toxins.
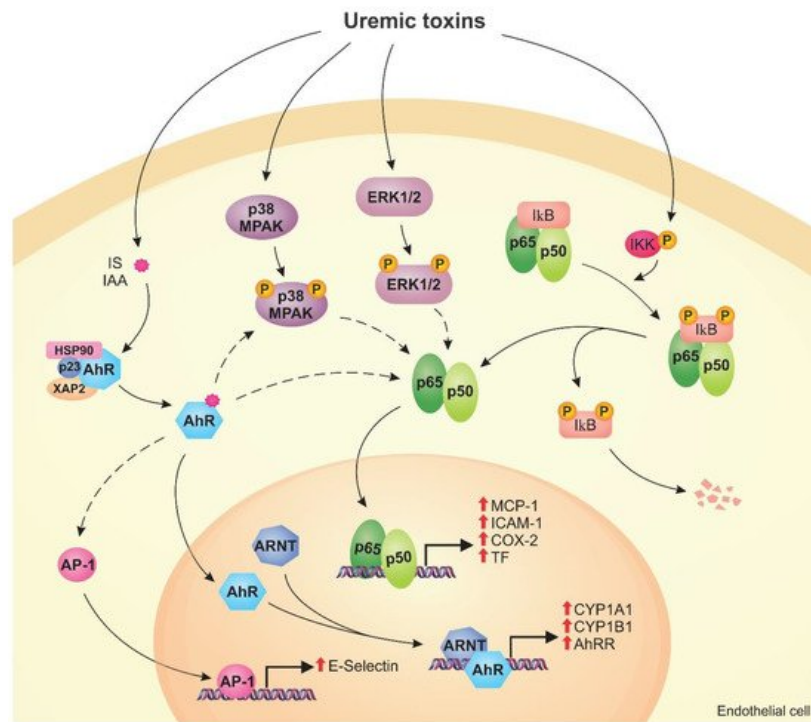

Figure 2. Uremic toxins activate the aryl hydrocarbon receptor (AhR), nuclear factor kappa B (NF-κB), and mitogen-activated protein kinase (MAPK) pathways in endothelial cells. Toxins such as indoxyl sulfate (IS) and indole-3 acetic acid (IAA) activate AhR, which is translocated to the nucleus, forms a dimer with aryl hydrocarbon receptor nuclear translocator (ARNT), and induces the expression of CYP1A1, CYP1B1, and AhRR (genomic pathway). Activated AhR can stimulate other pathways (non-genomic pathway), including the activation of AP-1 that induces E-selectin expression. Uremic toxins also cause the activation of the MAPK pathway, such as p38MAPK and ERK1/2, by phosphorylation. MAPK and AhR can induce the NF-κB pathway. In uremic conditions, IκB is degraded and p50/p65 are translocated to the nucleus, inducing monocyte chemoattractant protein-1 (MCP-1), intercellular adhesion molecule-1 (ICAM-1), cyclooxygenase-2 (COX-2), and tissue factor (TF) expression. The activation of these pathways by uremic toxins can lead to endothelial dysfunction.

3. Modulation of MicroRNAs by Uremic Toxins in Endothelial Cells

MicroRNAs (miRNAs) play an important role in the regulation of endothelial cell function via the modulation of eNOS-derived nitric oxide bioavailability, angiogenesis, and innate immune response [42]. Recent studies have demonstrated the involvement of miRNAs in endothelial dysfunction since they act as regulators in endothelial cells [43][44][45]. miRNAs are part of a family of small endogenous noncoding RNA made up of about 21 nucleotides, and influence physiological/pathological processes, including cell growth, differentiation, and apoptosis [42][45]. Uremic toxins can upregulate miRNA-92a, as well as miR-142-3p, miRNA-92a-3p, and miRNA-489-3p, suppressing the expression of genes critical for endothelial homeostasis, thus contributing to their dysfunction [42][45][46]. Uremic toxins can also cause the downregulation of miRNAs, such as lower levels of miRNA-214, which promotes apoptosis [47]. In patients with CKD, lower levels of circulating miR-126 and miR-223 were associated with a lower estimated glomerular filtration rate and with higher mortality and cardiovascular events [48]. These mechanisms may lead to the discovery of a new perspective for the treatment of CKD [49][50]. All these data suggest possible new targets for the treatment of endothelial injury in CKD, demonstrating the importance of studies of the other effects of uremic toxins on endothelial dysfunction.

4. Uremic Toxins Induce the Formation of Endothelial Microparticles

Endothelial microparticles (EMPs) are vesicles derived from the cell membrane, between 0.1 μm and 1 μm in size, which carry content from the source cell, including proteins and miRNAs [51][52]. EMPs interact directly with target cells, such as VSMCs, monocytes, endothelial progenitor cells, and other endothelial cells [52][53][54]. This interaction leads to the internalization of EMPs and the transfer of their active biomolecules to the target cell, which may activate signaling pathways [53]. Therefore, EMPs are important tools of intercellular communication formed in physiological and pathological processes, including in CKD [51][55].

In uremic conditions, it was demonstrated that PCS, IS, and inorganic phosphate induced the formation of EMPs from endothelial cells [38][52][56][57][58]. Studies have also shown that EMPs are related to endothelial dysfunction in CKD patients [59][56][60][61]. In vitro, Carmona et al. [52] showed that EMPs from endothelial cells exposed to IS had increased levels of ICAM-1 and PECAM-1, and miRNAs (e.g., miR-181a-5p, miR-4454, and miR-150-5p). In the same study, the authors found that IS-induced EMPs had an anti-angiogenic effect on endothelial progenitor cells, which are important in the endothelium repair process [52]. Furthermore, IS- and IAA-induced EMPs had greater procoagulant activity, due to the production of factor Xa by TF, compared to EMPs from cells not exposed to these toxins [25]. Similarly, EMPs from cells exposed to hyperphosphatemia conditions caused an increase in thrombin formation, also indicating procoagulant activity [38]. Soriano et al. [54] isolated EMPs from CKD patients and found that they increase osteocalcin (OCN) expression in endothelial progenitor cells, VSMCs, and fibroblasts, which indicates cellular dysfunction and vascular calcification. Furthermore, IS-induced EMPs enhanced the proliferation of VSMCs in vitro and in an ex vivo model, effects that contribute to neointimal hyperplasia [53][62]. These findings suggest that EMPs formed in the uremic conditions can mediate the dysfunction of target cells and contribute to vascular damage and the development of CVD.

References

1. Jing, Y.J.; Ni, J.W.; Ding, F.H.; Fang, Y.H.; Wang, X.Q.; Wang, H.B.; Chen, X.N.; Chen, N.; Zhan, W.W.; Lu, L.; et al. p-Cresyl sulfate is associated with carotid arteriosclerosis in hemodialysis patients and promotes atherogenesis in apoE −/− mice. Kidney Int. 2016, 89, 439–449.

2. Wang, C.H.; Lai, Y.H.; Kuo, C.H.; Lin, Y.L.; Tsai, J.P.; Hsu, B.G. Association between serum indoxyl sulfate levels and endothelial function in non-dialysis chronic kidney disease. Toxins 2019, 11, 589.

3. Barreto, F.C.; Barreto, D.V.; Liabeuf, S.; Meert, N.; Glorieux, G.; Temmar, M.; Choukroun, G.; Vanholder, R.; Massy, Z.A. Serum Indoxyl Sulfate Is Associated with Vascular Disease and Mortality in Chronic Kidney Disease Patients. Clin. J. Am. Soc. Nephrol. 2009, 4, 1551–1558.

4. Wu, I.W.; Hsu, K.H.; Hsu, H.J.; Lee, C.C.; Sun, C.Y.; Tsai, C.J.; Wu, M.S. Serum free p-cresyl sulfate levels predict cardiovascular and all-cause mortality in elderly hemodialysis patients—A prospective cohort study. Nephrol. Dial. Transplant. 2012, 27, 1169–1175.

5. Liabeuf, S.; Barreto, D.V.; Barreto, F.C.; Meert, N.; Glorieux, G.; Schepers, E.; Temmar, M.; Choukroun, G.; Vanholder, R.; Massy, Z. A free p-cresylsulphate is a predictor of mortality in patients at different stages of chronic kidney disease. Nephrol. Dial. Transplant. 2010, 25, 1183–1191.

6. Rattazzi, M.; Villalta, S.; De Lucchi, L.; Sponchiado, A.; Galliazzo, S.; Faggin, E.; Pagliara, V.; Zilli, C.; Callegari, E.; Carberlotto, L.; et al. Chronic kidney disease is associated with increased risk of venous thromboembolism recurrence. Thromb. Res. 2017, 160, 32–37.

7. Betriu, A.; Martinez-Alonso, M.; Arcidiacono, M.V.; Cannata-Andia, J.; Pascual, J.; Valdivielso, J.M.; Fernandez, E. Prevalence of subclinical atheromatosis and associated risk factors in chronic kidney disease: The NEFRONA study. Nephrol. Dial. Transplant. 2014, 29, 1415–1422.

8. Vanholder, R.; Pletinck, A.; Schepers, E.; Glorieux, G. Biochemical and Clinical Impact of Organic Uremic Retention Solutes: A Comprehensive Update. Toxins 2018, 10, 33.

9. Boelaert, J.; Lynen, F.; Glorieux, G.; Schepers, E.; Neirynck, N.; Vanholder, R. Metabolic profiling of human plasma and urine in chronic kidney disease by hydrophilic interaction liquid chromatography coupled with time-of-flight mass spectrometry: A pilot study. Anal. Bioanal. Chem. 2017, 409, 2201–2211.

10. Duranton, F.; Cohen, G.; De Smet, R.; Rodriguez, M.; Jankowski, J.; Vanholder, R.; Argiles, A. Normal and pathologic concentrations of uremic toxins. J. Am. Soc. Nephrol. 2012, 23, 1258–1270.

11. Okuno, S.; Ishimura, E.; Kohno, K.; Fujino-Katoh, Y.; Maeno, Y.; Yamakawa, T.; Inaba, M.; Nishizawa, Y. Serum β2-microglobulin level is a significant predictor of mortality in maintenance haemodialysis patients. Nephrol. Dial. Transplant. 2009, 24, 571–577.

12. De Oliveira, R.B.; Liabeuf, S.; Okazaki, H.; Lenglet, A.; Desjardins, L.; Lemke, H.-D.; Vanholder, R.; Choukroun, G.; Massy, Z.A. The clinical impact of plasma leptin levels in a cohort of chronic kidney disease patients. Clin. Kidney J. 2013, 6, 63–70.

13. Maciel, R.; Cunha, R.; Busato, V.; Franco, C.; Gregório, P.; Dolenga, C.; Nakao, L.; Massy, Z.; Boullier, A.; Pecoits-Filho, R.; et al. Uremia Impacts VE-Cadherin and ZO-1 Expression in Human Endothelial Cell-to-Cell Junctions. Toxins 2018, 10, 404.

14. Favretto, G.; Souza, L.M.; Gregório, P.C.; Cunha, R.S.; Maciel, R.A.P.; Sassaki, G.L.; Toledo, M.G.; Pecoits-Filho, R.; Souza, W.M.; Stinghen, A.E.M. Role of Organic Anion Transporters in the Uptake of Protein-Bound Uremic Toxins by Hu

man Endothelial Cells and Monocyte Chemoattractant Protein-1 Expression. J. Vasc. Res. 2017, 54, 170–179.

15. Soshilov, A.A.; Motta, S.; Bonati, L.; Denison, M.S. Transitional States in Ligand-Dependent Transformation of the Aryl Hydrocarbon Receptor into Its DNA-Binding Form. Int. J. Mol. Sci. 2020, 21, 2474.

16. Addi, T.; Poitevin, S.; McKay, N.; El Mecherfi, K.E.; Kheroua, O.; Jourde-Chiche, N.; de Macedo, A.; Gondouin, B.; Cerini, C.; Brunet, P.; et al. Mechanisms of tissue factor induction by the uremic toxin indole-3 acetic acid through aryl hydrocarbon receptor/nuclear factor-kappa B signaling pathway in human endothelial cells. Arch. Toxicol. 2019, 93, 121–136.

17. Watanabe, I.; Tatebe, J.; Namba, S.; Koizumi, M.; Yamazaki, J.; Morita, T. Activation of Aryl Hydrocarbon Receptor Mediates Indoxyl Sulfate-Induced Monocyte Chemoattractant Protein-1 Expression in Human Umbilical Vein Endothelial Cells. Circ. J. 2013, 77, 224–230.

18. Ambolet-Camoit, A.; Ottolenghi, C.; Leblanc, A.; Kim, M.J.; Letourneur, F.; Jacques, S.; Cagnard, N.; Guguen-Guillouzo, C.; Barouki, R.; Aggerbeck, M. Two persistent organic pollutants which act through different xenosensors (alpha-endosulfan and 2,3,7,8 tetrachlorodibenzo-p-dioxin) interact in a mixture and downregulate multiple genes involved in human hepatocyte lipid and glucose metabolism. Biochimie 2015, 116, 79–91.

19. Han, S.G.; Han, S.S.; Toborek, M.; Hennig, B. EGCG protects endothelial cells against PCB 126-induced inflammation through inhibition of AhR and induction of Nrf2-regulated genes. Toxicol. Appl. Pharmacol. 2012, 261, 181–188.

20. Asai, H.; Hirata, J.; Watanabe-Akanuma, M. Indoxyl glucuronide, a protein-bound uremic toxin, inhibits hypoxia-inducible factor−dependent erythropoietin expression through activation of aryl hydrocarbon receptor. Biochem. Biophys. Res. Commun. 2018, 504, 538–544.

21. Kolachalama, V.B.; Shashar, M.; Alousi, F.; Shivanna, S.; Rijal, K.; Belghasem, M.E.; Walker, J.; Matsuura, S.; Chang, G.H.; Gibson, C.M.; et al. Uremic Solute-Aryl Hydrocarbon Receptor-Tissue Factor Axis Associates with Thrombosis after Vascular Injury in Humans. J. Am. Soc. Nephrol. 2018, 29, 1063–1072.

22. Larigot, L.; Juricek, L.; Dairou, J.; Coumoul, X. AhR signaling pathways and regulatory functions. Biochim. Open 2018, 7, 1–9.

23. Ito, S.; Osaka, M.; Edamatsu, T.; Itoh, Y.; Yoshida, M. Crucial Role of the Aryl Hydrocarbon Receptor (AhR) in Indoxyl Sulfate-Induced Vascular Inflammation. J. Atheroscler. Thromb. 2016, 23, 960–975.

24. Dou, L.; Sallée, M.; Cerini, C.; Poitevin, S.; Gondouin, B.; Jourde-Chiche, N.; Fallague, K.; Brunet, P.; Calaf, R.; Dussol, B.; et al. The cardiovascular effect of the uremic solute indole-3 acetic acid. J. Am. Soc. Nephrol. 2015, 26, 876–887.

25. Gondouin, B.; Cerini, C.; Dou, L.; Sallée, M.; Duval-Sabatier, A.; Pletinck, A.; Calaf, R.; Lacroix, R.; Jourde-Chiche, N.; Poitevin, S.; et al. Indolic uremic solutes increase tissue factor production in endothelial cells by the aryl hydrocarbon receptor pathway. Kidney Int. 2013, 84, 733–744.

26. Assefa, E.G.; Yan, Q.; Gezahegn, S.B.; Salissou, M.T.M.; He, S.; Wu, N.; Zuo, X.; Ying, C. Role of resveratrol on indoxyl sulfate-induced endothelial hyperpermeability via aryl hydrocarbon receptor (AHR)/Src-dependent pathway. Oxid. Med. Cell. Longev. 2019, 2019.

27. Koizumi, M.; Tatebe, J.; Watanabe, I.; Yamazaki, J.; Ikeda, T.; Morita, T. Aryl Hydrocarbon Receptor Mediates Indoxyl Sulfate-Induced Cellular Senescence in Human Umbilical Vein Endothelial Cells. J. Atheroscler. Thromb. 2014, 21, 904–916.

28. Jansen, J.; Jansen, K.; Neven, E.; Poesen, R.; Othman, A.; van Mil, A.; Sluijter, J.; Sastre Torano, J.; Zaal, E.A.; Berkers, C.R.; et al. Remote sensing and signaling in kidney proximal tubules stimulates gut microbiome-derived organic anion secretion. Proc. Natl. Acad. Sci. USA 2019, 116, 16105–16110.

29. Machado, T.S.; Poitevin, S.; Paul, P.; McKay, N.; Jourde-Chiche, N.; Legris, T.; Mouly-Bandini, A.; Dignat-George, F.; Brunet, P.; Masereeuw, R.; et al. Indoxyl sulfate upregulates liver P-glycoprotein expression and activity through aryl hydrocarbon receptor signaling. J. Am. Soc. Nephrol. 2018, 29, 906–918.

30. Dou, L.; Poitevin, S.; Sallée, M.; Addi, T.; Gondouin, B.; McKay, N.; Denison, M.S.; Jourde-Chiche, N.; Duval-Sabatier, A.; Cerini, C.; et al. Aryl hydrocarbon receptor is activated in patients and mice with chronic kidney disease. Kidney Int. 2018, 93, 986–999.

31. Shivanna, S.; Kolandaivelu, K.; Shashar, M.; Belghasim, M.; Al-Rabadi, L.; Balcells, M.; Zhang, A.; Weinberg, J.; Francis, J.; Pollastri, M.P.; et al. The Aryl Hydrocarbon Receptor is a Critical Regulator of Tissue Factor Stability and an Antithrombotic Target in Uremia. J. Am. Soc. Nephrol. 2016, 27, 189–201.

32. Lano, G.; Laforêt, M.; Von Kotze, C.; Perrin, J.; Addi, T.; Brunet, P.; Poitevin, S.; Burtey, S.; Dou, L. Aryl Hydrocarbon Receptor Activation and Tissue Factor Induction by Fluid Shear Stress and Indoxyl Sulfate in Endothelial Cells. Int. J. Mol. Sci. 2020, 21, 2392.

33. Mussbacher, M.; Salzmann, M.; Brostjan, C.; Hoesel, B.; Schoergenhofer, C.; Datler, H.; Hohensinner, P.; Basílio, J.; Petzelbauer, P.; Assinger, A.; et al. Cell Type-Specific Roles of NF-κB Linking Inflammation and Thrombosis. Front. Immu

nol. 2019, 10, 85.

34. Sun, S.-C. The non-canonical NF-κB pathway in immunity and inflammation. Nat. Rev. Immunol. 2017, 17, 545–558.

35. Masai, N.; Tatebe, J.; Yoshino, G.; Morita, T. Indoxyl sulfate stimulates monocyte chemoattractant protein-1 expression in human umbilical vein endothelial cells by inducing oxidative stress through activation of the NADPH oxidase-nuclear factor-kappaB pathway. Circ. J. 2010, 74, 2216–2224.

36. Tumur, Z.; Shimizu, H.; Enomoto, A.; Miyazaki, H.; Niwa, T. Indoxyl Sulfate Upregulates Expression of ICAM-1 and MCP-1 by Oxidative Stress-Induced NF-κB Activation. Am. J. Nephrol. 2010, 31, 435–441.

37. Buendía, P.; Carracedo, J.; Soriano, S.; Madueño, J.A.; Ortiz, A.; Martín-Malo, A.; Aljama, P.; Ramírez, R. Klotho Prevents NFκB Translocation and Protects Endothelial Cell From Senescence Induced by Uremia. J. Gerontol. Ser. A Biol. Sci. Med. Sci. 2015, 70, 1198–1209.

38. Abbasian, N.; Burton, J.O.; Herbert, K.E.; Tregunna, B.-E.; Brown, J.R.; Ghaderi-Najafabadi, M.; Brunskill, N.J.; Goodall, A.H.; Bevington, A. Hyperphosphatemia, Phosphoprotein Phosphatases, and Microparticle Release in Vascular Endothelial Cells. J. Am. Soc. Nephrol. 2015, 26, 2152–2162.

39. Coulthard, L.R.; White, D.E.; Jones, D.L.; McDermott, M.F.; Burchill, S.A. p38MAPK: Stress responses from molecular mechanisms to therapeutics. Trends Mol. Med. 2009, 15, 369–379.

40. Morrison, D.K. MAP Kinase Pathways. Cold Spring Harb. Perspect. Biol. 2012, 4, a011254.

41. Cargnello, M.; Roux, P.P. Activation and Function of the MAPKs and Their Substrates, the MAPK-Activated Protein Kinases. Microbiol. Mol. Biol. Rev. 2011, 75, 50–83.

42. Shang, F.; Wang, S.-C.; Hsu, C.-Y.; Miao, Y.; Martin, M.; Yin, Y.; Wu, C.-C.; Wang, Y.-T.; Wu, G.; Chien, S.; et al. MicroRNA-92a Mediates Endothelial Dysfunction in CKD. J. Am. Soc. Nephrol. 2017, 28, 3251–3261.

43. Kumar, S.; Kim, C.W.; Simmons, R.D.; Jo, H. Role of Flow-Sensitive microRNAs in Endothelial Dysfunction and Atherosclerosis. Arterioscler. Thromb. Vasc. Biol. 2014, 34, 2206–2216.

44. Schober, A.; Weber, C. Mechanisms of MicroRNAs in Atherosclerosis. Annu. Rev. Pathol. Mech. Dis. 2016, 11, 583–616.

45. Wiese, C.B.; Zhong, J.; Xu, Z.-Q.; Zhang, Y.; Ramirez Solano, M.A.; Zhu, W.; Linton, M.F.; Sheng, Q.; Kon, V.; Vickers, K.C. Dual inhibition of endothelial miR-92a-3p and miR-489-3p reduces renal injury-associated atherosclerosis. Atherosclerosis 2019, 282, 121–131.

46. Kétszeri, M.; Kirsch, A.; Frauscher, B.; Moschovaki-Filippidou, F.; Mooslechner, A.A.; Kirsch, A.H.; Schabhuettl, C.; Aringer, I.; Artinger, K.; Pregartner, G.; et al. MicroRNA-142-3p improves vascular relaxation in uremia. Atherosclerosis 2019, 280, 28–36.

47. Li, S.; Xie, Y.; Yang, B.; Huang, S.; Zhang, Y.; Jia, Z.; Ding, G.; Zhang, A. MicroRNA-214 targets COX-2 to antagonize indoxyl sulfate (IS)-induced endothelial cell apoptosis. Apoptosis 2020, 25, 92–104.

48. Fourdinier, O.; Schepers, E.; Metzinger-Le Meuth, V.; Glorieux, G.; Liabeuf, S.; Verbeke, F.; Vanholder, R.; Brigant, B.; Pletinck, A.; Diouf, M.; et al. Serum levels of miR-126 and miR-223 and outcomes in chronic kidney disease patients. Sci. Rep. 2019, 9, 4477.

49. M'baya-Moutoula, E.; Marchand, A.; Six, I.; Bahrar, N.; Celic, T.; Mougenot, N.; Maitrias, P.; Massy, Z.A.; Lompreh, A.-M.; Metzinger, L.; et al. Inhibition of miR-223 expression using a sponge strategy decreases restenosis in rat injured carotids. Curr. Vasc. Pharmacol. 2019, 17.

50. Metzinger-Le Meuth, V.; Metzinger, L. miR-223 and other miRNA's evaluation in chronic kidney disease: Innovative biomarkers and therapeutic tools. Non-Coding RNA Res. 2019, 4, 30–35.

51. Favretto, G.; Cunha, R.S.d.; Dalboni, M.A.; Oliveira, R.B.d.; Barreto, F.d.C.; Massy, Z.A.; Stinghen, A.E.M. Endothelial Microparticles in Uremia: Biomarkers and Potential Therapeutic Targets. Toxins 2019, 11, 267.

52. Carmona, A.; Guerrero, F.; Buendia, P.; Obrero, T.; Aljama, P.; Carracedo, J. Microvesicles Derived from Indoxyl Sulfate Treated Endothelial Cells Induce Endothelial Progenitor Cells Dysfunction. Front. Physiol. 2017, 8, 666.

53. Ryu, J.H.; Jeon, E.Y.; Kim, S.J. Indoxyl sulfate-induced extracellular vesicles released from endothelial cells stimulate vascular smooth muscle cell proliferation by inducing transforming growth factor-beta production. J. Vasc. Res. 2019, 56, 129–138.

54. Soriano, S.; Carmona, A.; Triviño, F.; Rodriguez, M.; Alvarez-Benito, M.; Martín-Malo, A.; Alvarez-Lara, M.-A.; Ramírez, R.; Aljama, P.; Carracedo, J. Endothelial damage and vascular calcification in patients with chronic kidney disease. Am. J. Physiol. Physiol. 2014, 307, F1302–F1311.

55. Gao, C.; Xie, R.; Yu, C.; Ma, R.; Dong, W.; Meng, H.; Zhang, Y.; Si, Y.; Zhang, Z.; Novakovic, V.; et al. Thrombotic role of blood and endothelial cells in uremia through phosphatidylserine exposure and microparticle release. PLoS ONE 20

15, 10, 1–16.

56. Meijers, B.K.I.; Van kerckhoven, S.; Verbeke, K.; Dehaen, W.; Vanrenterghem, Y.; Hoylaerts, M.F.; Evenepoel, P. The U remic Retention Solute p-Cresyl Sulfate and Markers of Endothelial Damage. Am. J. Kidney Dis. 2009, 54, 891–901.

57. Faure, V.; Dou, L.; Sabatier, F.; Cerini, C.; Sampol, J.; Berland, Y.; Brunet, P.; Dignat-George, F. Elevation of circulating endothelial microparticles in patients with chronic renal failure. J. Thromb. Haemost. 2006, 4, 566–573.

58. Di Marco, G.S.; König, M.; Stock, C.; Wiesinger, A.; Hillebrand, U.; Reiermann, S.; Reuter, S.; Amler, S.; Köhler, G.; Buc k, F.; et al. High phosphate directly affects endothelial function by downregulating annexin II. Kidney Int. 2013, 83, 213– 222.

59. Amabile, N.; Guerin, A.P.; Leroyer, A.P.; Mallat, Z.; Nguyen, C.; Boddaert, J.; London, G.M.; Tedgui, A.; Boulanger, C.M. Circulating Endothelial Microparticles Are Associated with Vascular Dysfunction in Patients with End-Stage Renal Failur e. J. Am. Soc. Nephrol. 2005, 16, 3381–3388.

60. Mörtberg, J.; Lundwall, K.; Mobarrez, F.; Wallén, H.; Jacobson, S.H.; Spaak, J. Increased concentrations of platelet- an d endothelial-derived microparticles in patients with myocardial infarction and reduced renal function- a descriptive stud y. BMC Nephrol. 2019, 20, 71.

61. Jalal, D.; Renner, B.; Laskowski, J.; Stites, E.; Cooper, J.; Valente, K.; You, Z.; Perrenoud, L.; Le Quintrec, M.; Muhame d, I.; et al. Endothelial Microparticles and Systemic Complement Activation in Patients With Chronic Kidney Disease. J. Am. Heart Assoc. 2018, 7, e007818.

62. Ryu, J.-H.; Park, H.; Kim, S.-J. The effects of indoxyl sulfate-induced endothelial microparticles on neointimal hyperplas ia formation in an ex vivo model. Ann. Surg. Treat. Res. 2017, 93, 11.