Semaphorin3A-Inhibitor Ameliorates Doxorubicin-Induced Podocyte Injury

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Podocyte injury is an independent risk factor for the progression of renal diseases. Semaphorin3A (SEMA3A), expressed in podocytes and tubular cells in the mammalian adult kidneys, has been reported to regulate diverse biological function and be associated with renal diseases. Here, we investigated pathological roles of SEMA3A signaling on podocyte injury using doxorubicin (Dox)-induced mouse model and examined the therapeutic effect of SEMA3A-inhibitor (SEMA3A-I). We demonstrated that Dox caused massive albuminuria and podocyte apoptosis as well as increase of SEMA3A expression in podocytes, all of which were ameliorated with SEMA3A-I treatment. In addition, c-Jun N-terminal kinase (JNK), known as a downstream of SEMA3A signaling, was activated in Dox-injected mouse podocytes while SEMA3A-I treatment partially blocked the activation. *In vitro*, SEMA3A-I protected against Dox-induced podocyte apoptosis and recombinant SEMA3A caused podocyte apoptosis with activation of JNK signaling. JNK inhibitor, SP600125, attenuated SEMA3A-induced podocyte apoptosis, indicating that JNK pathway would be involved in SEMA3A-induced podocyte apoptosis. Furthermore, the analysis of human data revealed a positive correlation between urinary SEMA3A level and proteinuria, suggesting that SEMA3A is associated with podocyte injury. In conclusion, SEMA3A has essential roles on podocyte injury and it would be the therapeutic target for protecting from podocyte injury.

semaphorin3A podocyte proteinuria apoptosis c-Jun N-terminal kinase

SEMA3A has been reported to play important roles in multiple aspects of renal diseases [7]. For example, urinary SEMA3A has been shown to be an early, predictive biomarker of acute kidney injury (AKI) as well as later-onset AKI and progression of AKI [20,21]. Previously, we reported that urinary SEMA3A levels in MCNS, IgA-N, and MN groups were higher than in the control group, and that urinary SEMA3A might be an indicator for remission of MCNS patients [12]. Consistent with these reports, Aggarwal et al. reported that excess SEMA3A might promote diabetic nodular glomerulosclerosis, massive proteinuria, and renal failure in diabetic nephropathy mice [22]. Likewise, Mohamed et al. reported increased levels of urinary SEMA3A in diabetic mice and human diabetic patients with nephropathy [23]. In addition, it has also been reported that SEMA3A can be a predictive biomarker for ankylosing spondylitis [24] and systemic lupus erythematosus [25], suggesting the effect of SEMA3A signaling on the regulation of the immune system. These findings suggest that SEMA3A has common effects on the podocyte injury and subsequent progression of renal injury in renal diseases. Our results indicated the increased expression of podocyte SEMA3A in Dox-induced mouse kidneys, and SEMA3A-I protected from Dox-induced renal injury by lowering albuminuria and podocyte injury, demonstrating that SEMA3A signaling does have the association with podocyte injury and targeting the SEMA3A–NRP1 axis with SEMA3A-I would be a therapeutic option to treat renal injury. Interestingly, SEMA3A-I treatment also inhibited Dox-induced SEMA3A expression in podocytes, which seemed somehow strange considering the effect of SEMA3A-I to block the binding of SEMA3A–

NRP1. We assumed that SEMA3A-I attenuated Dox-induced podocytopathy, which resulted in the reduction of SEMA3A from injured podocytes. In addition, there was the positive correlation between urinary SEMA3A level and proteinuria in a human study, reinforcing our conclusion.

On the point of SEMA3A inhibition, several chemicals have been reported. For example, Tian et al. have applied (-)-Epigallocatechin-3-gallate (EGCG) as a SEMA3A inhibitor, which is the major polyphenol constituent from green tea, in LPS-induced AKI [17,26,27]. They indicated the increase in tubular SEMA3A expression with LPS treatment, and EGCG suppressed LPS-induced cell apoptosis and inflammation through the regulation of JNK and Rac1/NF-κB p65 signaling [17]. In addition, Kumagai et al. identified a novel, highly selective SEMA3A inhibitor (SM-345431, vinaxanthone) [14]. SM-345431 was isolated from the cultured broth of a fungus *Penicillium sp.* and interacts with SEMA3A directly and inhibits the binding of SEMA3A to NRP1 with the same physicochemical properties as SM-216289 (xanthofulvin), but develops a higher pharmaceutical quality [14,15,16]. Indeed, SM-345431 has been shown to enhance regenerative response and functional recovery of the injured spinal cord [28]. It is also reported that SM-345431 accelerated peripheral nerve regeneration and sensitivity in a murine corneal transplantation model [29]. In this study, we demonstrated that SM-345431 protected from Dox-induced podocyte injury through an anti-apoptosis mechanism.

Among broad biological functions of SEMA3A, several reports indicated important roles of SEMA3A signaling on the regulation of cell apoptosis through the SEMA3A–NRP1/JNK axis [17,30]. The JNK/c-Jun pathway belongs to MAPK signaling, which can be activated by diverse stimulus, including reactive oxygen stress, inflammatory cytokines, and mechanical stress [31,32]. The JNK/c-Jun pathway has been shown to promote apoptosis in a variety of cell types [32]. In addition to the JNK pathway, various pathways, including Janus kinase/signal transducers and activators of transcription (JAK/STAT), protein kinase B (Akt) and other MAPK pathways of extracellular signal-regulated kinase (ERK) and p38, play critical roles in the cell apoptosis/survival paradigm. Wen et al. reported that macrophages, inhibiting SEMA3A signaling by knockout of plexinA4, reduced JNK phosphorylation, but no change was observed in phosphorylation of ERK1/2, p38, STAT1, and Akt under the stimulation [33], suggesting that SEMA3A signaling might specifically regulate JNK signaling. Therefore, we focused on the SEMA3A–JNK axis in the present study. On the other hand, it is also reported that SEMA3A signaling regulates dendritic development through the activation of the Akt pathway [34]. In addition, Guan et al. reported that SEMA3A might decrease Akt phosphorylation and induce apoptosis in cultured podocytes [35], indicating the possibility that SEMA3A might also regulate pathways other than JNK signaling. While SEMA3A-induced podocyte apoptosis was induced through the regulation of JNK signaling in the present study, which demonstrated the involvement of the JNK pathway in SEMA3A signaling, at least to some extent, further analysis is required to elucidate the detailed mechanisms by which SEMA3A signaling regulates the cell apoptosis/survival paradigm.

On the point of the therapeutic target for the MAPK pathway, there are several candidates reported to protect against podocyte injury. For example, Liu et al. reported that activation of ERK signaling as well as the JNK pathway was observed in a rat puromycin aminonucleoside (PAN) nephropathy model, and that treatment with U0126, an inhibitor of ERK, suppressed podocyte apoptosis caused by PAN [36]. Yu et al. reported that

transforming growth factor beta 1 (TGF-β1)-induced podocyte injury was ameliorated with U0126 treatment through the inhibition of the increment of transient receptor potential cation channel 6 (TRPC6) protein [37]. Lei et al. reported that mammalian target of rapamycin (mTOR) activation is associated with endoplasmic reticulum (ER) stress and apoptosis in high-glucose-treated podocyte, which was ameliorated with U0126 treatment [38]. Taken together, targeting ERK pathway might be a potential target for podocyte injury. In addition to the ERK pathway, involvement of the p38 pathway under podocyte injury is also reported. Koshikawa et al. reported the activation of the p38 MAPK and ERK pathways in rodent PAN and Dox nephropathy models, and that the treatment with FR167653, an inhibitor of p38 MAPK, completely blocked the increase of proteinuria caused by PAN or Dox [39]. Pengal et al. applied another inhibitor of p38 MAPK, SB203580, which reduced PAN-induced podocytopathy and actin cytoskeletal disruption [40], indicating the therapeutic potential of p38 inhibition under podocytopathy. Furthermore, the blockade of the JNK/c-Jun pathway has been shown to suppress renal injury in several disease models and has therapeutic value [18,41,42]. However, clinical trials evaluating JNK inhibitors in human fibrotic disorders showed side effects of liver toxicity [43]. Thus, other therapeutic strategies are needed to reduce the renal injury via the JNK/c-Jun pathway. On this point, apoptosis signal-regulating kinase 1 (ASK1), the member of the mitogen-activated protein kinase kinase kinase (MAPKKK) family, is another candidate to regulate the JNK/c-Jun pathway [44]. Accumulating evidence revealed that ASK1 activation accelerates renal injury through the activation of p38 and JNK cascades in rodent models of kidney injury, including ischemia/reperfusion-induced AKI, unilateral ureteric obstruction, and diabetic nephropathy [45,46,47], and that treatment with GS-444217, an inhibitor of ASK1, limited the loss of podocytes most likely through the anti-apoptosis pathway in a diabetic kidney disease mouse model, indicating ASK1 as an important target for podocyte injury [48]. In the present study, we demonstrated that SEMA3A-I may decrease podocyte apoptosis through the suppression of the JNK/c-Jun pathway, indicating that SEMA3A might be another candidate to target JNK pathway for podocyte protection.

Another important point to discuss is the timing of the treatment with SEMA3A-I or JNK-I. In our in vitro experiment, we added these inhibitors and/or Dox or SEMA3A at the same time. To examine whether these inhibitors might revert the podocyte apoptosis caused by Dox or SEMA3A, we might make the time lag to add these inhibitors after Dox or SEMA3A treatment. We assume that these inhibitors might not revert the podocyte apoptosis after the apoptosis signaling cascade proceeds over the SEMA3A or JNK signaling. In such situation, we would propose to use these inhibitors before or under the podocyte injury, not after the podocytopathy has been established. Further study is still required to understand the timing at which these potential inhibitors work and how SEMA3A-I functions on the JNK/c-Jun pathway in a Dox-induced podocytopathy mouse model.

In conclusion, our results demonstrated that SEMA3A-I treatment protected from Dox-induced podocytopathy by inhibiting podocyte apoptosis through the regulation of the JNK pathway. It would be the therapeutic target for preventing podocyte injury.

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