HSP70-Mediated NLRP3 Inflammasome Suppression

Subjects: Immunology
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Acute kidney injury (AKI) is the sudden loss of renal function, usually due to ischemia, nephrotoxic agents, or urinary tract obstructions. Although AKI is a relatively common condition, especially in hospitalized and chronically ill patients, treatments remain largely supportive, despite mortality associated with this condition being as high as 20%. Hence, there is growing interest in developing regenerative therapies for AKI that can repair renal injury as well as prevent its progression to chronic kidney disease. AKI is associated with both systemic and intrarenal inflammation, which is believed to be a key component underlying its pathophysiology. Although inflammation in the acute phase can facilitate tissue repair following injury, disruption of this process can lead to persistent inflammation, causing tissue damage and fibrosis. Many molecular mediators of inflammation have been identified in AKI, which include the NLRP3 inflammasome, toll-like receptors (TLRs), and various secreted cytokines that promote neutrophil- and monocyte-mediated inflammatory responses. Indeed, blockade of innate immune receptors seems to confer protection against AKI in several preclinical studies.

In a recent study conducted by Ullah et al., the authors demonstrated the effect of combination therapy with pulsed focused ultrasound (pFUS) and mesenchymal stem cell derived extracellular vesicles (MSC-derived EVs) in a mouse model of cisplatin-induced AKI. Here we evaluated their ability to suppress AKI-related inflammation by downregulation of HSP70, which in turn reduced the formation of the NLRP3 inflammasome, resulting in the attenuation of the pro-inflammatory environment characteristic of AKI. The authors validated this effect using in vitro knockdown studies which also suggested that HSP70 is a positive regulator of the NLRP3 inflammasome.

HSP70, Inflammasome, NLRP3, Kidney Injury

Introduction

Acute kidney injury (AKI) is the sudden loss of renal function, usually due to ischemia, nephrotoxic agents, or urinary tract obstructions [1]. Although AKI is a relatively common condition, especially in hospitalized and chronically ill patients, treatments remain largely supportive, despite mortality associated with this condition being as high as 20% [2]. Hence, there is growing interest in developing regenerative therapies for AKI that can repair renal injury as well as prevent its progression to chronic kidney disease.

AKI is associated with both systemic and intrarenal inflammation, which are believed to be key components underlying its pathophysiology [3]. Although inflammation in the acute phase can facilitate tissue repair following

injury, disruption of this process can lead to persistent inflammation, causing tissue damage and fibrosis [4]. Many molecular mediators of inflammation have been identified in AKI [5], which include the NLRP3 inflammasome [6], toll-like receptors (TLRs) [7], and various secreted cytokines that promote neutrophil- and monocyte-mediated inflammatory responses [5,8]. Indeed, blockade of innate immune receptors seems to confer protection against AKI in several preclinical studies [9,10,11,12].

Another therapeutic strategy for immune modulation lies in mesenchymal stromal cell (MSC)-based therapies [13]. MSCs are multipotent cells that have been investigated as a cell therapy for regenerative medicine applications, including AKI [14,15]. Their therapeutic effect arises from their ability to home to damaged tissue and secrete extracellular vesicles (EVs) and other factors that act in a paracrine manner to exert proliferative, pro-survival, and anti-inflammatory effects [16,17,18,19]. More recent studies have begun exploring purified MSC-derived EVs as a cell-free alternative to MSC therapy [20,21,22]; the advantages of using EVs compared to MSCs include their higher safety profile, ability to cross barriers with minimal sequestration in the pulmonary microvasculature following intravenous infusion, lower immunogenicity, and avoidance of complications related to stem cell-induced tumor formation [23,24,25,26,27,28].

While EVs can achieve a therapeutic effect comparable to their parent MSCs in the context of AKI, there remains great interest in optimizing their efficacy. Pulsed focused ultrasound (pFUS), where target organs are selectively treated with focused sound waves, has recently emerged as a method to improve MSC-based therapies [29]. Pre-treatment of target organs with pFUS has been shown to locally upregulate cytokines and trophic factors, improve MSC homing, and subsequently their therapeutic efficacy [30,31,32]. However, the full range of mechanisms underlying pFUS has yet to be elucidated [29,33], and its effect on EV therapy is particularly lacking. Here, we assess the effect of combination therapy with pFUS and MSC-derived EVs in a mouse model of cisplatin-induced AKI, evaluating in particular their ability to suppress AKI-related inflammation.

Discussion

We have shown that the pretreatment of kidneys suffering from AKI with pFUS enhances the therapeutic effect of MSC-derived EVs. This synergistic effect is at least in part due to downregulation of HSP70, which in turn reduces the formation of the NLRP3 inflammasome, resulting in the attenuation of the pro-inflammatory environment characteristic of AKI (Figure 4).

pFUS is a non-invasive procedure with an excellent safety profile that can be precisely targeted to deep body tissues, and is already FDA-approved for several clinical applications [41]. pFUS has previously been shown to enhance MSC therapy for AKI. However, the mechanism by which this occurs has yet to be fully understood, likely due to differences in ultrasound parameters used between various groups [29]. Some studies have reported that pFUS upregulates local cytokines which serve as a homing signal for MSCs, thereby increasing their accumulation in sonicated tissue and increasing their therapeutic effect [31]. On the other hand, we have previously found that pFUS may have an independent therapeutic effect in AKI, and can enhance MSC therapy independent of increased homing [33]. Consistent with our previous study, we have found here that pFUS is independently able to

attenuate NLRP3-mediated inflammation, with subsequent improvements in physiological kidney function. Additionally, we demonstrate that pFUS acts synergistically with EV therapy to reverse AKI.

The NLRP3 inflammasome is an intracellular protein complex consisting of NLRP3, ASC, and pro-caspase-1, which upon activation releases active caspase-1 that proceeds to convert pro-inflammatory cytokines IL-1β and IL-18 into their mature form [42]. The inflammasome has been shown to be upregulated in both mouse models of AKI and human renal biopsies from different pathologies [6]. NLRP3 also has inflammasome-independent effects in tubular epithelial cells [9], including participating in SMAD2 and SMAD3 phosphorylation in response to TGFβ signaling, triggering renal fibrosis [43]. Though there have been previous reports on the suppression of the NLRP3 inflammasome by MSCs [44,45,46] and MSC-derived EVs [47,48], our study is the first to show that pFUS has both an independent and synergistic role in its regulation.

Heat shock proteins (HSPs) are molecular chaperones known to broadly regulate inflammation, including the formation of the NLRP3 inflammasome [34]. However, the exact direction of their regulation appears to be context dependent. HSP90 has been shown to be a positive regulator of the NLRP3 inflammasome in various studies [35,36,37]. HSP70 has also been shown to be a positive regulator of airway inflammation, with HSP70 knockout mice showing significant reductions in airway inflammation compared to wild type mice following intratracheal antigen challenge [39]. Extracellular HSP70 has been shown to act as a cytokine, binding to monocytes through CD14 and activating NF-κB signaling to increase the production of IL-1β, IL-6, and TNF-α [38]. On the contrary, intracellular HSP70 has also been shown to inhibit NLRP3 inflammasome activation in a mouse model of peritonitis [40], where HSP70 deficiency caused worsened NLRP3-dependent peritonitis and enhanced caspase-1 activation and IL-1β production by macrophages, while genetic or heat shock-induced HSP70 overexpression had the opposite effect. The highly context-dependent effects of HSP70 on NLRP3 inflammasome regulation highlights the need for careful studies investigating their molecular links and the involved cell types, details that become crucial should HSP inhibitors be considered for preclinical investigation [49].

Our study has found that in cisplatin-induced AKI, HSP70, HSP90, and NLRP3 are all highly upregulated, and can be suppressed with pFUS and EV therapy. We found that HSP70 knockdown in vitro leads to significant suppression of NLRP3 expression, suggesting HSP70 to be a positive regulator of the NLRP3 inflammasome. We thus propose a mechanism by which pFUS and EVs, likely through intermediate effectors, converge to suppress HSP70, which reduces NLRP3 inflammasome formation and subsequent release of proinflammatory cytokines (Figure 4). Alternative mechanisms must also be considered, including the possibility of another protein targeting both HSP70 and NLRP3. It would be necessary to repeat our experiments in an HSP70 knockout mouse before we can conclude whether the observed therapeutic effect is in fact dependent on HSP70.

In summary, our study demonstrates that pFUS has both independent and synergistic therapeutic effects when used in combination with MSC-derived EVs to treat cisplatin-induced AKI. Both pFUS and EV converge to suppress HSP70/90, which leads to decreased expression of the NLRP3 inflammasome and downstream pro-inflammatory cytokines, ultimately improving kidney function. The growing worldwide prevalence and morbidity of AKI prompts the development of regenerative therapies to restore kidney function and avoid progression to chronic

kidney disease. Though EVs have seen substantial preclinical success for AKI, improving their therapeutic efficacy may be necessary for clinical translation. The safety profile and non-invasive nature of pFUS make it an attractive tool, though much remains to be understood about its physiological and molecular effects. Careful characterization of these mechanisms will serve to further its development and optimization as a clinical tool.

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