Extracellular Vesicles That Herald the Scarcity of Oxygen

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Abstract
The natural, membrane-bound nanoscale particles, called extracellular vesicles (EVs) have emerged as an effective, versatile vehicle to transport desired drugs specifically to injury sites. Heralding the presence of the scarcity of oxygen, EVs produced from the cells upregulating the expression of the critical transcriptional regulator of hypoxia, HIF-1, can induce a response in ischemia-reperfusion-damaged cells to ameliorate renal tubular injury and inflammation.

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Impaired kidney functions ensuing from injury, infection, and other damaging incidences lead to a large share of mortality and morbidity worldwide [1]. Acute kidney injury (AKI), an abrupt reduction of the normal kidney functions, principally due to excessive oxidation, occurs widely among hospitalized patients and tends to progress to chronic kidney disease, end-stage renal disease, and other complications [2]. While a great deal of effort has been made to treat AKI, a definitive therapy for established AKI remains still lacking. Importantly, systemic administration of drugs to improve AKI is prone to delivering an inadequate amount to the injury site and often instigates unintended side effects in off-target tissues. A delivery means specifically targeting the injured kidney cells, therefore, needs to be established. In this regard, extracellular vesicles (EVs) have emerged as a vehicle to achieve such renal injury-specific drug targeting, for these natural, membrane-bound nanoparticles can be engineered to transport desired drugs to the injury site with great precision and accuracy [3, 4].

While damages ensuing from ischemia and reperfusion can cause AKI to develop on one hand, paucity of oxygen in the kidney can also induce a highly conserved functional adaptation on the other hand. That is, exposure to a sublethal level of hypoxia is known to confer upon the kidney cells the ability to tolerate subsequent ischemic injury [5, 6]. Notably, this so-called ischemic preconditioning (IPC) state can be transferred from a donor to a recipient, inducing remote IPC [5, 6]. Present in various body fluids such as blood, saliva, and urine, EVs are known to carry a specific set of cargo molecules that mirror the physiological and pathological status of the producing cells, eventually discharging the contents in EV-receiving cells [3]. Indeed, EVs derived from a donor
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of IPC are known to mount remote IPC in recipients, presumably through delivering a select set of cargos that reflect the IPC state, to assist injured kidney cells to recover [7]. In this regard, the ability of EVs in transferring an IPC state is actively being exploited to find a better way of treating AKI and other related kidney diseases [8, 9].

EVs have several favorable attributes as a drug delivery vehicle to target an injured tissue. Above all, EVs appear to be more biocompatible, less immunogenic, and more barrier-permeable, compared to other nanoscale particles [3]. A wide range of cargo molecules of potential therapeutic value have thus been examined whether they can be stably delivered via EVs [3]. In this vein, Ding et al. [10] in this issue of Kidney Diseases report a potential utility of the EVs collected from the cells that upregulate the expression of the fundamental transcriptional regulator of hypoxia, HIF-1. This new study shows that the stabilization of the α subunit of HIF-1, HIF-1α, by using the prolyl hydroxylase inhibitor FG-4592, constitutes a form of IPC and further demonstrates that EVs produced from the FG-4592-treated cells can induce a reno-protective response in recipient cells, ameliorating the renal tubular injury and inflammation caused by ischemia-reperfusion in both cultured cell and mouse models of AKI.

The basic helix-loop-helix PAS domain-containing transcriptional effector, HIF-1, orchestrates a gene expression program that helps adapt cells to hypoxia [11]. Expression of the genes involved in erythropoiesis, angiogenesis, glycolysis, and iron metabolism is either directly or proximally induced by HIF-1 [12, 13]. This concerted modulation of gene expression leads cells to gaining the ability of not only surviving the scarcity of oxygen but also evading programmed cell death, which would otherwise occur unless cells properly manage hypoxia. HIF-1 further coordinates gene expression toward inhibiting expression of proinflammatory cytokines, thereby reducing the risk of tissue damage caused by unrestrained immune response in hypoxia [14]. These fundamental properties of HIF-1-mediated gene expression appear to play a crucial role in protecting renal cells from ischemia-reperfusion-induced injury in AKI.

Oxygen tension of a tissue stringently controls the quantity of effective HIF-1 molecules, which function as a heterodimer [15]. While the level of the β subunit, HIF-1β, does not fluctuate considerably, that of the α subunit, HIF-1α, markedly increases posttranslationally as cells face the scarcity of oxygen. Under the normal range of oxygen tension, HIF-1α is hydroxylated at the two proline residues in the ODD domain, P402 and P564, by the coordinated activity of the prolyl hydroxylase domain-containing proteins [16]. The resultant hydroxylated HIF-1α is bound and ubiquitinated by the von Hippel-Lindau complex of E3 ubiquitin ligase, subsequently undergoing degradation by the proteasome [16]. As a result of this active removal of the α subunit, the functioning HIF-1 heterodimer, composed of HIF-1α and HIF-1β, does not form when oxygen is sufficiently available. A decline of oxygen tension below a threshold, however, triggers inhibition of the catalytic activity of the prolyl hydroxylase domain-containing proteins, thereby blunting the proteasomal degradation of HIF-1α, increasing the level of the HIF-1 heterodimer, and ultimately leading to transcription of HIF-1 target genes [17, 18].

Since HIF-1α becomes stabilized in such a hypoxic condition as ischemia, Ding et al. [10] postulated that emergence of the functioning HIF-1 heterodimer can establish an IPC state. An increase in HIF-1 activity induced by hypoxia is thought to promote kidney tubular cells to secrete EVs, laden with reno-protecting molecules in their interior [19]. Thus, Ding et al. [20] further hy-
pothesized that EVs released from HIF-1-expressing cells would induce a reno-protecting response when transferred to ischemia-reperfusion-injured cells of AKI. Of note, hypoxia was known to stimulate release of proinflammatory substances into EVs [20], precluding simple hypoxic cell culturing from preparing for the transferring EVs. As mentioned above, however, HIF-1 itself is known to suppress the expression of proinflammatory genes [14]. Therefore, Ding et al. [20] attempted to increase the expression of HIF-1 per se without exposing the EV-producing cells to hypoxia.

To this end, Ding et al. [20] utilized a specific chemical inhibitor of HIF-1 prolyl hydroxylases, called FG-4592, to stabilize HIF-1α in EV-donor cell lines such as HEK293 and HK2 with culturing them in normoxia (Fig. 1). Ding et al. [20] observed that such FG-4592-induced EVs could indeed ameliorate the injury phenotypes of ischemia-reperfusion when transferred to both cultured-cell and mouse models of AKI. Specifically, FG-4592-induced EVs were shown to alleviate engagement of apoptosis as well as to inhibit activation of the NF-κB pathway, which could account for another chief observation of the study; reduction of the release of proinflammatory cytokines, such as IL-6, from the injured kidney tubular cells upon receiving the FG-4592-induced EVs.

Ding et al. [20] further noted that the recipient injured kidney tubular cells lowered the expression of endogenous HIF-1 in response to the transferred FG-4592-induced EVs. Importantly, high doses of HIF-1 prolyl hydroxylase inhibitors are known to promote tubulointerstitial fibrosis via activating the HIF-1-KLF-5-TGF-β signaling cascade [21]. In this regard, this observation suggests an intriguing utility of the approach using FG-4592-induced EVs in minimizing the level of HIF-1 expression in the injured cells during the response, thereby avoiding the unintended fibrosis.

Overall, the study by Ding et al. [20] presents a straightforward means of utilizing fundamental properties of HIF-1 in EV-mediated amelioration of ischemia-reperfusion-induced kidney injury. In a figurative perspective, the EVs produced from HIF-1-upregulated cells might be viewed as though they heralded the presence of the scarcity of oxygen to the recipient injured cells. The cargo molecules loaded in the FG-4592-induced EVs were not defined by the present study by Ding et al. [20] but remain to be determined by future studies. However, one can speculate that the cargos of FG-4592-induced EVs likely include such molecules as can alter the phenotypes of the injured, recipient cells toward enduring the dearth of oxygen and repairing damaged parts appropriately.

In addition to defining the cargo contents of FG-4592-induced EVs, the current findings of the study by Ding et al. [20] may need more elaboration by further studies. This includes a consideration of how to increase the accuracy and precision of delivery to the injury site. Notably, the authors’ research group has recently published a successful engineering of the surface of EVs with a kidney injury site-specific homing signal, REV\textsubscript{LTH}, which was demonstrated to bind selectively to kidney injury molecule-1 (Kim-1) present on the injured kidney cells [22]. Exploring the knowledge on such “surface functionalization” will lead us to better utilizing FG-4592-induced EVs in ameliorating AKI and other related kidney injuries.

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### Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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### Author Contributions

Sekyung Oh and Sang-Ho Kwon drafted, reviewed, and edited the manuscript.

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