"Remote" myokine protects from pulmonary ischemia/reperfusion injury by a surprising "proximal" control mechanism

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The concept of remote ischemic preconditioning (RIPC) was first conceived more than quarter of a century ago in the context of myocardial ischemia/reperfusion (I/R) injury. It was originally based on the principle of "intra"-myocardial protection, i.e., to reduce myocardial I/R damage by subjecting the heart to cycles of "sub-lethal" myocardial I/R prior to prolonged coronary artery occlusion, which however was clinically restricted to elective cardiac surgery settings (1). Twenty-five years ago, Przyklenk and colleagues then showed that "remote" yet cardio-local ischemic preconditioning protected the otherwise unharmed myocardium from subsequent prolonged coronary occlusion. Although preconditioning occurred within the heart, it represented cardioprotection between two "remote" vascular beds within the heart, thus representing the first example of tissue protection from I/R damage by a remote site (2). This form of "remote" intramyocardial protection was next extended to protective interactions between the heart and other organs, with episodes of brief I/R applied in remote organs such as the kidney or the small intestine before sustained coronary artery occlusion (3). The concept holds that during the brief episodes of I/R in the remote organ, a protective mediator or signal produced by this organ, the "donor organ", traffics to the heart to exert its protective function there, i.e., in the "recipient organ". In the past 10–15 years, the concept of RIPC has been extensively studied and extended to various organs including liver, lung, stomach, and brain. Because of its potential clinical applicability, the skeletal muscle, exemplified by the upper or lower limbs and I/R episodes created by blood pressure cuffs (termed "limb RIPC" or "LRIPC"), has been the favorite "donor organ". Of note, the concept has also been extended to "recipient organs" other than the heart, including the lung (4).

Several blood-borne factor(s) and neural pathways have been suggested to constitute the "remote signal" conveying protection. For the heart, it has been suggested that these factors or pathways elicit cardioprotective signaling pathways in cardiomyocytes including the so-called RISK and SAFE pathways (5-9). While the downstream signaling routes are complex, they generally induce mito-protective activity by mediating an inhibitory effect on mitochondrial permeability transition pore (MPTP) opening (4). However, while several investigators have studied the endocrine and/or neuronal pathways that underlie the cross-organ interactions in LRIPC organ protection, the full sequelae of events from the "remote" source to the protective effect in the target organ is mechanistically still poorly understood. This includes the humoral tracks mediating RIPC cross-talk between skeletal muscle and is chemically stressed tissues, including their beneficial effects on lung damage such as in acute lung injury (ALI).

Chen *et al*. now have unraveled such a mechanistic LRIPC axis in the context of lung injury, as recently reported in *Sci Transl Med* (10). They started out from the clinical observation that patients with neonatal

respiratory distress syndrome (NRDS) show reduced irisin serum concentrations, while irisin concentrations in the bronchoalveolar lavage (BAL) fluid were elevated, suggesting recruitment of irisin protein from circulation to the lung under such physiologic stress conditions. Irisin is a 112-amino acid protein. Functionally, it is a myokine derived from the ecto-moiety of fibronectin domain-containing 5 protein (FNDC5) in skeletal muscle cells that has previously been shown to regulate glucose homeostasis and increase energy turnover by stimulating the "browning" of white adipose tissue (WAT) (11-13). Applying various *in vivo* and *in vitro* models of lung I/R injury, including pharmacological irisin administration or inhibition and *Ucp2* gene-deleted mice, Chen *et al.* demonstrated that LRIPC releases irisin to protect against injury to the lung. In mice, application of episodes of RIPC to the limbs stimulated irisin secretion from skeletal muscle. Following I/R injury, this then resulted in transfer of this myokine to the pulmonary tissue. They also identified the mechanism of irisin-mediated protection in the stressed lung. Surprisingly, this is not a conventional receptor-mediated pathway as would have been predicted for an endocrine-like mediator such as irisin. In vitro data suggest that upon transfer to the lung, irisin enters alveolar epithelial cells through lipid raft-mediated endocytosis. It then somehow exits the endosomal compartment and targets mitochondria, where it interacts with mitochondrial uncoupling protein 2 (UCP2), a close homolog of UCP1 that is preferably found in lung tissue. The study further shows that irisin acts to stabilize UCP2 and counter-act its degradation, which in turn leads to protection from I/R-induced oxidative stress of pulmonary type I epithelial cells and preservation of mitochondrial metabolism. Finally using mouse models, they demonstrated that injection of recombinant irisin protects against IR-induced pulmonary injury. They further show that this prevents impairment of mitochondrial function, whereas the protective effect of recombinant irisin was compromised in *Ucp2*-gene-deleted mice or by a small molecule pharmacologic inhibitor of UCP2. All in all, this provided convincing *in vitro* and *in vivo* evidence that irisin is a lung-protective myokine. It also uncovers a previously unrecognized intracellular mechanism, which implicates novel mitochondrial target structures that could qualify as a potential translational avenue in pulmonary I/R injury.

However, several questions and challenges remain to be addressed. First of all, this refers to the "donor organ" of the "remote" signal axis, i.e. the skeletal muscle. The concept of the skeletal muscle functioning as an endocrine organ secreting myokines upon exercise, which participate in tissue crosstalk, is still in its infancy and several questions remain regarding the validation of myokines (14). This also holds true for irisin. There has been some controversy about the validity of the antibodies used to detect circulating irisin and the molecular weight and amino acid sequence of irisin also has been debated (11). Moreover, the mechanism of irisin secretion is still incompletely understood. The transcription cofactor peroxisome proliferator-activated receptor-γ co-activator 1α (PGC1α) that regulates energy metabolism (12), induces fibronectin III domain-containing protein-5 (FNDC5), a 212-residue transmembrane protein, located in the plasma membrane. Post-translational processing by as yet poorly understood cleavage mechanism, followed by glycosylation and presumably dimerization eventually leads to the generation of a 112-residue moiety called "irisin". In order to eventually capitalize on a skeletal muscle exercise/health paradigm, it will be important to fully elucidate the mechanism of irisin generation (14). Next, the methodology and diagnostics to quantitate circulating irisin levels will need to be optimized to reliably predict the serum levels of this myokine before and after exercise, but also to possibly establish a personalized medicine basis depending on to-be-discovered polymorphisms. Knowledge about the concentration of irisin in the circulation as well as its half-life are important parameters to fully characterize the "inter-organ axis" between "donor" and "recipient" organ.

In this context, it will also be interesting to learn which tissues other than lung may be irisin-target sites. Are there uptake mechanisms for irisin in WAT, brown adipose tissue (BAT), or liver as well? Given the presumed critical role of irisin in the regulation of glucose homeostasis (11,13), this might be predicted. If yes, are the uptake rates and mechanisms similar to those in lung or is the lung a preferred target site? What about irisin effects in the preconditioned ischemic heart? Interestingly, the classical CXC-type chemokine CXCL12/SDF-1α has been suggested to contribute to RIPC in myocardial ischemia, although it is unlikely to be "the unidentified humoral protein" of approximately 10 kDa that was suggested to be produced by limb conditioning remote from the heart to stimulate cardioprotection (15). The non-classical, atypical, chemokine macrophage migration-inhibitory factor (MIF) that is abundantly produced by hypoxic endothelium but also skeletal muscle (16,17), has endocrine-like properties, and shares receptor pathways and functional similarities with CXCL12 (18-22), also is not the sought-for cardioprotective LRIPC factor (23). Myokines have not

been implicated in cardioprotective RIPC so far.

Similarly, several interesting questions need to be asked and resolved on the "recipient organ" side. Mechanistically, the most challenging issue is to clarify how irisin molecules that have entered alveolar epithelial cells through lipid raft-mediated endocytosis exit from the endosomal compartment. Does irisin stay intact or are only fragments released? What is the actual translocation mechanism that leads to the transfer of irisin from the endosomal lumen to the cytosolic compartment? Translocation of endocytosed inflammatory mediators from the endosomal lumen to the cytosol is not unprecedented. The atypical chemokine MIF was shown to be rapidly taken up by monocytes/macrophages and to at least partially translocate into the cytosolic compartment where it regulates the cell cycle by interacting with the COP9 signalosome subunit CSN5/JAB1 (24). Fibroblast growth factor-2 (FGF-2) was reported to traffic into the nucleus (25). Regarding the study by Chen *et al.*, it also needs to be clarified how irisin is translocated into the mitochondrial inter-membrane space and if UCP2 is its only interaction partner? Although UCP2 appears to be an UCP isoform that is enriched in lung tissue (26), it is possible that irisin interacts with yet additional proteins. This could be both mitochondrial proteins related to redox regulation and mitochondrial function but also cytosolic proteins. On the other hand, UCP2 appears to be particularly suited as irisin-regulated target protein. It is rapidly turned-over with a half-life of only 30 min compared to >1 day for the homolog UCP1. To this end, heat production by UCP1 in adipocytes of the BAT type is an adaptive, enduring process, whereas the control of reactive oxygen-species (ROS) production by UCP2 in mitochondria is prone to be subtle and acute (26). Interestingly, UCP2 has been suggested to be post-translationally modified by redox-dependent glutathionylation, raising the question whether irisin binds to glutathionylated or unmodified UCP2.

Lastly, these mechanistic considerations entail important translational questions related to the therapeutic targeting of irisin/UCP2 for treating ALI or other human diseases. For example, pharmacologically administered irisin might need to be optimized to improve membrane permeability. Depending on the mechanism to be unraveled for endosome/cytosol transfer of irisin, optional nanoparticle-based strategies may have to be tailored in a pH-regulated fashion. Finally, although the lung is especially vulnerable to I/R injury because of its remarkable vascular structure and the permanent demand for oxygen replenishment, the path to clinical translational may be difficult and bumpy. Initial euphoria regarding the application of LRIPC for cardiac protection in cardiac surgery patients was recently dampened when two large-scale multi-center clinical studies, the ERICCA and RIPHEART study, did not improve clinical outcomes in patients undergoing complex on-pump coronary artery-bypass grafting (CABG), despite numerous promising experimental pre-clinical studies and proof-of-concept clinical studies (27-29).

Nevertheless, the current study suggests several intriguing novel mechanistic angles that could qualify for translational strategies in lung injury based on the uncovered irisin/UCP2 axis.

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Footnote

Conflicts of Interest: The author has no conflicts of interest to declare.

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