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Angiogenic response following renal ischemia reperfusion injury: new players

Réponse angiogénique après lésion d'ischémie-reperfusion : nouveaux acteurs

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KEYWORDS

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Summary

Ischemia-reperfusion (IR) injury can negatively influence the short- and long-term outcomes of kidney transplantation because it promotes acute tubular necrosis and tissue scarring and activates innate alloimmunity. The adaptive responses to IR are centrally involved in reducing tissue damage but can also be deleterious when they activate programmed cell death and inflammation. The HIF-1 α -mediated angiogenic responses following IR at early and late stages are complex and poorly understood. The early stages of IR seem to be associated with an antiangiogenic response, whereas the hypoxia that follows IR at later stages may activate angiogenic factors such as vascular endothelial growth factor (VEGF) and may be beneficial by stabilizing the microvasculature and favoring local blood supply. In addition to HIF-1 α , new players in angiogenesis, including mTOR and the unfolded protein response, may lead to innovative therapeutic strategies for treating patients with ischemia- and reperfusion-associated tissue inflammation and organ dysfunction.

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Résumé

Les lésions d'ischémie-reperfusion peuvent altérer les résultats de la transplantation rénale à court et long terme par différents mécanismes liés à la survenue d'une nécrose tubulaire aigue, d'une fibrose tissulaire et à l'activation de l'immunité innée. La réponse immunitaire adaptative, qui vise essentiellement à restaurer l'intégrité tissulaire, peut en fait conduire à des effets délétères en activant la mort cellulaire programmée ou en déclenchant des cascades pro-inflammatoires.

La réponse angiogénique médie par le facteur HIF-1 α est un mécanisme complexe et méconnu. Lors la phase ischémique aigue, cette voie conduit à l'activation d'agents angiogéniques (comme le VEGF) associés à une stabilisation de la micro vascularisation rénale contribuant à lutter contre l'hypoxie tissulaire. Parallèlement, des acteurs nouvellement identifiés comme la protéine mTOR ou les protéines mal conformées pourraient permettre de définir de nouvelles stratégies thérapeutiques contre les lésions d'ischémie-reperfusion en transplantation rénale.

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Ischemia-Reperfusion Injury: definitions, controversies and approximations

The IR syndrome

Ischemia and reperfusion is a pathological condition characterized by an initial restriction of blood supply to an organ followed by the restoration of perfusion and concomitant reoxygenation. In its classic manifestation, an occlusion of the arterial blood supply results in a severe imbalance of metabolic supply and demand that causes tissue hypoxia. Perhaps surprisingly, the restoration of blood flow and reoxygenation is frequently associated with an exacerbation of tissue injury and a profound inflammatory response, which together are called “reperfusion injury” [1].

Ischemia-reperfusion (IR) is a cause of kidney structural deterioration that is omnipresent in kidney transplantation and contributes to acute and chronic kidney injury, loss of function, innate and adaptive immunity stimulation, and graft loss. Delayed Graft Function (DGF) is a clinical consequence of IR and is mostly often defined as the need for dialysis during the first week after transplantation [2]. Ischemic DGF is pathologically reflected by ischemic acute tubular necrosis, and its prevalence varies greatly depending on its definitions; however, recent comprehensive surveys indicate a prevalence of approximately 20% [3,4]. The limited oxygen availability (hypoxia) that occurs during the ischemic period is associated with impaired endothelial cell barrier function and a concomitant increase in vascular permeability and leakage. In addition, ischemia and reperfusion leads to the activation of cell death programs, including apoptosis, autophagy-associated cell death and necrosis [1]. Despite the restoration of its vascular supply, an ischemic organ may not immediately reperfuse (this is called the “no reflow phenomenon”). Moreover, reperfusion injury is characterized by immune responses that include natural antibody recognition of neoantigens and subsequent activation of the complement system. Innate and adaptive immune responses contribute to injury, including the activation of pattern recognition receptors such as Toll-like receptors (TLR) and inflammatory cell trafficking into the diseased organ (i.e., innate and adaptive immune activation).

IR, DGF and kidney allograft outcomes

Recent works have challenged the impact of ischemic DGF on long-term allograft outcomes [5,6]. Whereas the duration of cold ischemic time (CIT), i.e., the period of time from kidney removal until arterial declamping, is significantly associated with DGF, CIT does not influence long-term graft outcomes. This does not mean that DGF does not influence outcomes, but rather that the ischemic tubular necrosis that occurs following cold ischemia that contributes to DGF may be totally reversible without scar. One implication of one of these studies [5], which was performed with 9082 paired deceased-donor kidneys and 18164 recipients registered in the national Scientific Registry of Transplant Recipients and may explain why CIT is widely accepted as a factor that negatively influences graft outcomes, is that minimizing the deleterious impact of cold ischemia is dependent on the proper storage of kidneys, which, according to some authors [7], is not correctly performed in many centers. When placed directly into a cold bath and flushed with cold solution, vasoconstriction occurs, and blood remained trapped in vessels. Ideally, the kidney should be flushed at room temperature with warm solution.

These considerations of the clinical impact of IR are important because the medical and economic consequences of the therapeutic options that must be developed to avoid them need to be evaluated. Furthermore, the data regarding the effect of ischemic DGF on graft outcomes should be more than anecdotal. This observation also fuels discussions based on clinical and experimental evidence that acute (ischemic) tubular necrosis is not reliably followed by a regeneration ad integrum of the tubular epithelium and thus scarring may occur. Clinically, acute kidney injury is widely accepted as a factor that negatively influences long-term kidney function, especially if these episodes are severe, frequent and occur in already-altered kidney parenchyma [8]. The biological mechanisms that convert an acute kidney injury into a chronic injury are beyond the scope of this review; however, ischemia and hypoxia are centrally implicated in this process. Ischemic stress generates the conditions necessary for the establishment of a permanent state of tissue ischemia, and acute ischemia may thereby promote lesions (e.g., capillary

destruction, interstitial fibrosis, inflammation, and vasoconstriction) and activate profibrotic and inflammatory molecular signaling pathways (e.g., HIF-1 α and angiotensin 2) that initiate a vicious cycle that perpetuates tissue ischemia and structural deterioration [8]. The chronic structural changes associated with chronic allograft nephropathy contribute to tissue ischemia: chronic vasoconstriction, arteriosclerosis, capillary rarefaction and interstitial fibrosis (which reduces oxygen diffusion) are the main factors that contribute to the maintenance of a hypoxic state [9, Fine, 2008 #174]. Therefore, if we accept that ischemic acute kidney injury followed by reperfusion is not a self-limiting process and that it initiates pathological processes (e.g., programmed cell death, inflammation and alloimmunity) that promote chronic structural deterioration (e.g., interstitial fibrosis and tubular atrophy), it becomes necessary to decipher the adaptive molecular pathways activated by IR because advances in their understanding may lead to innovative therapeutic strategies for treating patients with IR.

The (mal)adaptive responses to hypoxia

Cells subjected to hypoxic stress engage in adaptive responses aimed at maintaining basal metabolic functions (e.g., anaerobic glycolysis), increasing oxygen and nutrient supplies (e.g., neoangiogenesis and EPO synthesis) and activating antiapoptotic molecular programs. Notably, the HIFs (hypoxia inducible factors) are central regulators of cellular adaptation to hypoxia [10]. The best-known adaptive response to ischemia is the stabilization of the transcription factor Hypoxia Inducible Factor-1 α (HIF-1 α), which escapes from proteasomal degradation during hypoxia. Under hypoxic conditions, HIF α induces the transcription of hundreds of genes that encode proteins responsible for regulating glucose uptake and metabolism, angiogenesis, inflammatory cell chemotaxis, cell proliferation and survival, and extracellular matrix formation and turnover [10]. If adaptation fails, the cell will die by apoptosis or necrosis. The permeabilization of the plasma membrane that occurs during necrosis and late apoptosis allows the release of intracellular components that prime antigen-presenting cells through the activation of pattern recognition receptors. Moreover, HIF α activates nuclear factor- κ B (NF- κ B) signaling and enhances the immune functions of myeloid cells [11]. In addition to inflammation, adaptive responses to hypoxia induce tissue remodeling, leading to interstitial fibrosis and tubular atrophy. HIF α regulates the expression of profibrotic cytokines including TGF α , platelet derived growth factor (PDGF), connective tissue growth factor (CTGF) and VEGF [10], and it activates the epithelial-to-mesenchymal transition. The stability of HIF is regulated by the oxygen-sensing prolyl hydroxylase domain (PHD) enzymes, of which there are three isoforms, PHD1 - PHD3. Treatment with pharmacological PHD inhibitors results in an increased ischemia tolerance by the kidneys [12,13]. To date, PHD inhibitors seem to be well tolerated in humans, suggesting that they could be readily tested in larger clinical trials. Other adaptive processes implicated in the response to nutrient and oxygen deprivation involve the mammalian target of rapamycin (mTOR) kinase and the unfolded protein response (UPR) [14] (see below).

The secretion of angiogenic factors by epithelial and endothelial cells during ischemia contributes to the maintenance of an intact tubulo-interstitial compartment and slows the progression of kidney disease, although the situation is complex, as a dysregulated angiogenic response can result in deleterious effects and amplify an injury [15-18]. Therefore, a better understanding on the intimate regulators of an angiogenic response in the setting of IR injury would be of great importance to protect kidneys against structural deterioration and to prevent DGF.

The endothelium and angiogenic responses during IR injury

Endothelial consequences of IR

Ischemic endothelial cell injury participates in the extent and maintenance of acute kidney injury. Early alterations in peritubular capillary blood flow during reperfusion have been documented and are associated with the loss of normal endothelial cell function. Distorted peritubular capillary morphology is associated with a loss of barrier function that may contribute to early alterations in vascular stasis. In addition, ischemia induces alterations in endothelial cells that may promote inflammation and procoagulant activity, thus contributing to vascular congestion. Reductions in microvasculature density may play a critical part in the progression of chronic kidney disease following initial recovery from IR injury [19]. The exact nature of how capillary loss alters renal function and predisposes individuals to renal disease is thought to be due at least in part to hypoxia. Finally, the loss of endothelial cell function may represent an important therapeutic target in which vascular trophic support mediated by angiogenic factors may be important in ameliorating the acute and/or chronic effects of ischemic acute kidney injury.

Vascular Endothelial Growth Factor in the angiogenic response to IR

As previously stated, HIF1 α signaling is the master regulator of angiogenic responses during ischemia. HIF-1 α upregulates a number of factors implicated in cytoprotection, including angiogenic growth factors - such as vascular endothelial growth factor (VEGF), endothelial progenitor cell recruitment via the endothelial expression of SDF-1, heme-oxygenase 1 (HO-1), and erythropoietin (EPO) - and vasomotor regulation. VEGF is the most well-studied HIF-1 α -activated growth factors; it is known to act on the microcirculation through various mechanisms and can stimulate endothelial cell proliferation and differentiation, increase vascular permeability, and mediate endothelium-dependent vasodilation. Furthermore, VEGF promotes monocyte chemotaxis and the expression of adhesion molecules.

Although the expression of VEGF has been shown to increase in renal diseases and during hypoxia, experimental works did not find any increase in VEGF expression in a rat model of renal I/R. The loss of the renal vasculature appears in stark contrast to the regenerative potential of the renal tubular system following IR. As a possible explanation for the

lack of vascular repair, it is hypothesized that renal IR results in a net shift of expressed factors in favor of anti-angiogenesis vs. angiogenesis/vascular stabilization. For example, potential inhibitors of angiogenesis that are stimulated in the setting of I/R, such as endostatin, PAI-1, MMPs, ADAMTS1 and angiostatin, have been identified [20,21]. Nevertheless, this hypothesis is counterintuitive because hypoxia following I/R may be expected to stimulate the expression of proangiogenic molecules, particularly VEGF, in a HIF1 α -dependent fashion [1]. Because renal I/R results in persistent hypoxia and damage to the vascular system, the VEGF system should be investigated in both the early injury and later phases of this model as a possible explanation for the lack of vascular repair. Although VEGF is a promising therapeutic modality, a potential pitfall of the induction of vessels by the overexpression of a single gene such as VEGF is that the resulting vessels may be leaky, immature, or irregular. This is because the formation of a functionally intact microvasculature requires the coordinated activation of various genes.

Other angiogenic mediators

Fibroblasts Growth Factors (FGF) are non-classically secreted growth factors that regulate angiogenesis, tissue repair, inflammation, and carcinogenesis and are expressed in an HIF1 α -independent manner during ischemia. FGFs stimulate endothelial cells proliferation and migration. The role of FGF1 in the outcomes of IR is controversial because FGF-1 administration attenuates renal damage following IR, whereas the transgenic expression of nonclassically released FGF is compatible with normal development and morphology of kidneys but suppresses postischemic kidney repair [22]. Rather, FGF2 seems to be protective as the knockdown of FGFR2 exacerbated and delivery of recombinant FGF2 attenuated postischemic kidney damage [23,24].

Beside VEGF and FGF, the specific expressions of other components of an angiogenic response that usually occurs during kidney ischemia such as angiopoietin, angiogenin, platelet Derived Growth Factor (PDGF) or Connective Tissue Growth Factor (CTGF), have not been specifically analyzed. Moreover, array-based differential gene expression analysis targeting reperfusion injury in animal models and humans post-transplant failed to identify any potential angiogenic candidate expressed after IR [25,26].

New players in the angiogenic response to ischemia-reperfusion

Most of the research on the angiogenic response following IR has focused on HIF-1 α -mediated signaling pathways and the genes regulated by them, including VEGF. This focus was, at least in part, because HIF-1 α plays a central role in the response to hypoxia in cancer and some genetic diseases and is thus a promising therapeutic target. However, there is no doubt that other pathways activated during IR may also promote an angiogenic response and, like HIF-1 α , may constitute potential therapeutic targets. Investigating these other pathways can be performed using unbiased approaches, such as the analysis of gene expression profiles in tissues related

to a particular condition, such as acute kidney injury. A more analytical method is to investigate the IR process at the biological level. Following similar cases in cancer research, ischemia should not be seen only as oxygen blood shortage (hypoxia) but also as a deprivation of nutrients, aminoacids and glucose. Given these considerations, we identified two important transduction pathways that are activated by nutrient deprivation rather than oxygen deprivation and that are potential regulators of angiogenesis: the Unfolded Protein response and the mechanistic target of Rapamycin (mTOR) signaling.

Endoplasmic reticulum stress and the unfolded protein response

Ischemia and reperfusion induce stress in the endoplasmic reticulum (ER). Hypoxia and glucose starvation promote ATP shortage, decrease the intra-reticular calcium concentration, impair the activity of chaperone molecules, interfere with disulfide bridge formation and impair the maturation of native proteins, a process that promotes endoplasmic reticulum (ER) stress and activates the UPR [27,28]. The UPR involves three major mediators: the kinase PKR-like ER Kinase (PERK), the protein kinase/endoribonuclease Inositol Requiring Enzyme 1 α (IRE-1 α) and the transcription factor Activating Transcription Factor 6 (ATF6). The role of the UPR is to modify cellular functions in response to ER stress and to re-establish normal ER functioning both at the translational and transcriptional levels. Specifically, the UPR is responsible for the attenuation of general mRNA translation by the phosphorylated form of elongation Initiation Factor 2 α (eIF2 α) and for the transcriptional regulation of the UPR genes that encode chaperones, folding and proteasomal degradation enzymes, and the activation of macroautophagy [29,30]. As an adaptive response to nutrient stress, the UPR is involved in the regulation of an angiogenic response, which aims to increase blood supply and, consequently, reduce ischemic stress. The secretion of VEGF-A is regulated by the UPR, and the mode of regulation of VEGFA expression by UPR mediators seems to depend on the cell type and the identity of ER stress factor. In mouse embryonic fibroblasts, VEGFA is transcriptionally regulated by IRE1 α and PERK through their respective transcription factors, sXBP1 and ATF4 [31], but in the HepG2 hepatoma cell line, VEGFA is regulated by ATF6 [32]. A more complex regulation of VEGFA was observed in a medulloblastoma cell line involving regulatory action at both the transcriptional and post-transcriptional levels [33]. We have demonstrated, both in vitro and in vivo models of ischemic stress as it occurs during cold ischemia, that kidney epithelial cells subjected to glucose deprivation secrete VEGFA, bFGF and angiogenin independently of HIF-1 α , and we identified the PERK pathway as a central regulator of this angiogenic response [34]. Whether the angiogenic response mediated by the UPR following ischemia and reperfusion is beneficial remains to be established, but this signaling pathway may have therapeutic potential because the UPR can be pharmacologically targeted at multiple levels to increase its adaptive properties and reduce programmed cell death.

Mechanistic Target of Rapamycin (mTOR) signaling

As part of mTOR complex 1 (mTORC1), mTOR activation under nutrient- and energy-replete conditions stimulates protein synthesis and cell growth through the phosphorylation of ribosomal protein S6 kinase (p70S6K, also known as RPS6KB1), eukaryotic initiation factor 4E binding protein 1 (4E-BP1) and eukaryotic elongation factor 2 kinase (EEF2K)3 (Wouters, *Nat Rev Cancer*, 2008). mTOR signaling pathways activate global protein translation, which promotes cell growth and proliferation. In kidney tissues, mTORC1 increases the cellular levels of HIF-1 α (by increasing its translation and transcription), which in turn stimulates the production of proangiogenic factors such as VEGF, PDGF- α , and TNF- α [35]. Therefore, under ischemic conditions, when nutrients and oxygen are scarce, mTOR signaling is inhibited, and the angiogenic response mediated by mTOR is reduced, as is the case with the mTOR inhibiting immunosuppressant drug Rapamycin. However, the picture is more complicated because other data suggest that the inhibition of mTOR may paradoxically increases VEGF translation and synthesis. Although 4E-BP1 phosphorylation inhibits the translation of most transcripts, a small subset of genes remain preferentially translated owing to various elements in their 5' untranslated regions. In particular, a number of genes, including the hypoxia-inducible genes encoding HIF1 α and vascular endothelial growth factor A (VEGFA), contain regulatory elements that support preferential translation under conditions of 4E-BP1 hypophosphorylation [14]. Clearly, the mechanisms of mTOR-dependent regulation of HIF1 α and VEGF expression and activity during normoxia and hypoxia and their functional consequences are in need of further investigation.

Conclusion

Research into ischemia and reperfusion injuries in kidney transplantation constitute a growing and ever-changing field, both in terms of their clinical consequences and understanding of the adaptive processes activated. The impact of the angiogenic responses to IR injury remains to be established because experimental evidence suggests that IR promotes an anti-angiogenic rather than a proangiogenic response. Taking into account that IR generates a hypoxic state within the kidney that will contributes to late structural deterioration, the modulation of mediators of angiogenesis, such as HIF-1 α and VEGF, may be useful. Beside the well-known functions of HIF-1 α in mediating responses to ischemia, other pathways, including the UPR and mTOR signaling, warrant further investigation into their role as new players of an angiogenic response to IR and as potential biomarkers and therapeutic targets.

Disclosure of interest

The authors have no conflicts of interest to declare in relation to this review.

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