Molecular Signaling Pathways in Ischemia/Reperfusion

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Ischemia and reperfusion (I/R) is an important pathologic phenomenon that has not been completely defined from the perspective of the molecular signaling pathways developed immediately at its inception to minutes and hours thereafter. From the practical point of view, we have divided I/R into 3 phases: *phase I*, which occurs seconds to minutes after the injury and is associated with changes dependent on the activation of phospholipases, intracellular calcium, eicosanoids, other lipid molecules, protein kinases, inducible nitric oxide synthase, and the expression of preformed adhesion molecules like P-selectin; *phase II*, which occurs minutes to hours after I/R injury and is associated with the active transcription of protein synthesis of molecules like inflammatory cytokines (mainly tumor necrosis factor-α and interleukin 1) starting their signaling downstream from the membrane into the cytoplasm where kinases will be activated and send signals to the nucleus for the activation of transcription factors and further continuing with the inflammatory event; and *phase III*, which occurs several hours to days after I/R and is associated with the appearance of molecular chronic mechanisms of protection like the presence of anti-inflammatory cytokines of the IL-10 type, late adhesion molecules, and other growth factors such as TGF-β. This completes the whole molecular event related to I/R injury.

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Experimental and Clinical Transplantation (2004) 2: 174-177

Keywords: *Molecular signaling, Ischemia, Reperfusion, Update*

Ischemia and reperfusion (I/R) is a complex phenomenon directly associated with the inflammatory changes following lack of blood supply with subsequent hypoxia/anoxia (ischemia) and resumption of circulation (reperfusion). Physical, chemical, and molecular responses are part of this vastly abnormal environment. Because of the overall pathologic process being part of a severe inflammatory condition, I/R has been managed with various antiinflammatory strategies. This review focuses on the molecular signaling pathways occurring immediately at the beginning of reperfusion (phase I), minutes to hours thereafter (phase II), and those events developing after a few hours and days following the I/R injury (phase III).

Immediate Response to Reperfusion (Phase I)

Immediately upon reperfusion (within seconds to minutes), a series of molecular cascades, particularly those dependent on the activation of phospholipases and intracellular calcium, are readily stimulated. Changes in the cytoskeleton—as well as in the response of eicosanoids (and other lipid molecules such as prostaglandins, thromboxane, and leukotrienes), protein kinases and iNOS (inducible nitric oxide synthase)—are facilitated at the beginning of reperfusion. This phase is represented mainly by lipids and preformed proteins that appear as soon as the reperfused blood enters the circulation and establishes contact with the endothelium. Also expressed in this phase are certain preformed adhesion molecules like P-selectin, which is already existent on the surface of the endothelium and platelets. Its expression in I/R is secondary to cytoskeleton changes and calcium release, which permits P-selectin transport from the palate globules to the surface to begin the series of

events recognized as leukocyte-endothelial cell interactions.

Early Response to Reperfusion (Phase II)

Early in the molecular response to I/R (within minutes to hours), active transcription of protein synthesis begins, especially for those molecules associated with inflammation. In this regard, tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1) are the most important components. These inflammatory cytokines, and probably most significantly TNF-α, are at center stage regulating the I/R molecular pathway. The initial molecular signaling starts at this point and continues into the cytoplasm where kinases (e.g MAPKs) will be activated, sending a signal to the nucleus that activates transcription factors and further perpetuates the inflammatory event.

Activation of TNF

TNF is a homotrimer of 157 amino acid subunits mediating the inflammatory response. Today, the TNF signaling pathway is fairly well characterized [1]. In essence, TNF signals through cell surface receptors known as tumor necrosis factor-receptor 1 (TNF-R1) and tumor necrosis factor-receptor 2 (TNF-R2). It appears that TNFR-1 initiates the majority of TNF activities. The binding of TNF to TNFR-1 triggers a series of intracytoplasmic events that result in nuclear activation of nuclear factor kB (NF-κB) and oncogene c-jun (c-Jun) [1]. These nuclear factors are the basis for the induction of inflammatory genes. Downstream, TNF-R-associated death domain (TRADD) recruits other factors such as TNF-R-associated factor 2 (TRAF2) which is thought to activate MAPK kinase kinase (MAPKKK) of the MEK kinase-1 (MEKK1) or JNK (c-Jun N-terminal kinase) types. JNK will phosphorylate c-Jun producing subsequent transcriptional activity. Through another part of the TNF signaling cascade, the protein kinase RIP (receptor interacting protein) will activate NF-κB, even though it is not always required for the activation of this transcriptional factor [1] (Figure 1).

Activation of NF-κB

The activation of NF-κB is induced by TNF in the following manner: first, phosphorylation-dependent ubiquitination and degradation of inhibitor kB (IκB) occurs in the cytoplasm [2]. The phosphorylation is dependent on MAPKs and the ubiquitination and proteolytic degradation on the ubiquitin-proteome system. Second, the IKK (IκB kinase) is activated together with the TNF-R1. This activation is dependent on RIP. Adaptor proteins also participate in the activation of TNF-R1 which leads to NF-κB and JNK activation. Subsequently, a significant amount of cross talk occurs among NF-κB, JNK, and TNF-R1 to maintain the whole signaling pathway fully engaged and continuously active [1,2] until it reaches the nucleus where other gene expression of transcription factors will occur (Figure 1).

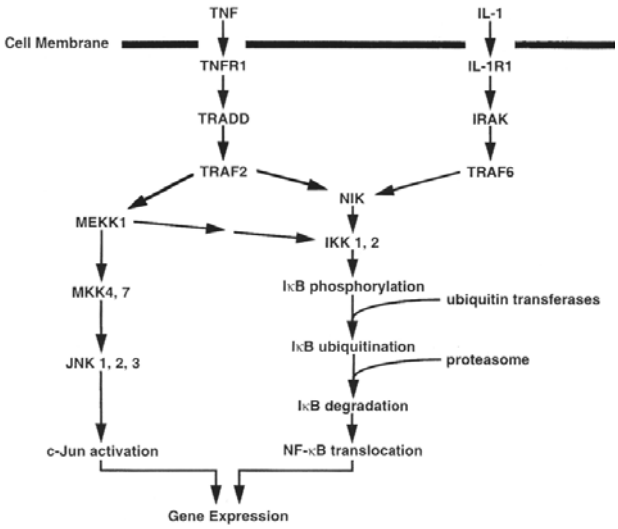

Figure 1. TNF and IL-1 signaling pathway (obtained from Manning AM and Rao A. Agents Targeting Transcription Factors in Inflammation, Gallin JI et al (eds.), Lippincott, Williams, and Wilkins, Philadelphia, 1999)

Activation of MAPKs

As has been indicated, MAPKs are activated as a consequence of the early events following I/R, namely, activation of TNF and related proteins, which are conducive to stimulation of MAPKs and nuclear transcription factors such as NF-κB. Several members of the MAPK superfamily are associated with I/R, including p38 MAPK, ERK (extracellular signal-regulated kinase), JNK, several MAPKs of p44/42 type, and others [3] (Figure 2).

p38 MAPK

Of all stress-induced kinases, p38 MAPK is best known and characterized. It is recognized as the most-important inflammatory kinase [3]. It is activated in response to inflammatory signals such as those seen after I/R. There are 5 isomers—α,β, β2,δ,γ—each with different characteristics. p38α is primarily expressed in leukocytes and bone

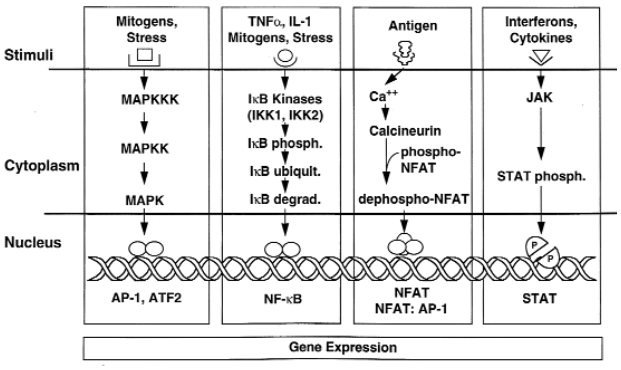

Figure 2. Gene regulation pathways critical to the inflammatory response (partially modified from Manning and Rao, see Figure 1)

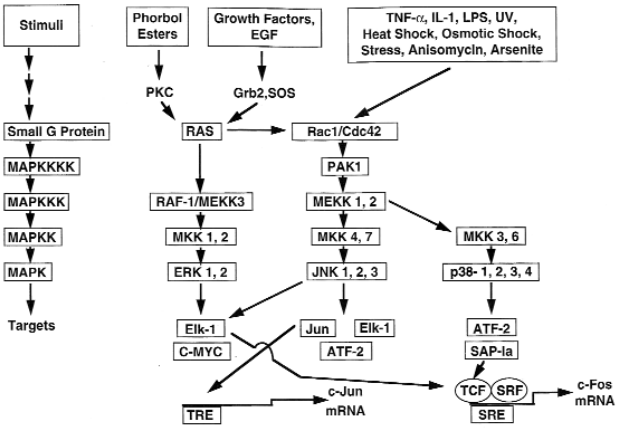

Figure 3. Complex MAPKs pathways from stimuli to target (obtained from Manning and Rao, see Figure 1)

marrow, p38β in heart and brain, and p38γ in skeletal muscle [4]. There are also differences in substrate specificity and inhibitors. For example, pyrindinyl imidazoles are potent inhibitors of p38 α,β,β2 but not of p38δ [4,5]. Even though the upstream kinases are different for each isoform, the major substrates are the MAP kinase-activated protein kinase-2 and the MAP kinase-activated protein kinase-3 [3]. Multiple stimuli including I/R induce p38 activation with its subsequent cascade.

JNK

It is a 54-kDa protein, activated in response to several conditions, including stress, LPS (lipopolysaccharide), TNF, IL-1, and others [6]. JNK plays an important role in AP-1 (activated protein-1) activation, TNF expression, T-cell proliferation, and IL-1 production [3]. JNK does not appear to have the dominant role that p38 and other MAPKs have in inflammation, particularly I/R [7-20].

ERK

ERK is not a stress-induced MAPK, instead it is associated with proliferation, transformation, and differentiation. It has two isoforms, ERK1 (p44) and ERK2 (p42). It can participate in cytokine production in response to the extracellular matrix [3]. When used with MEK inhibitors, ERK plays a role in the production of inflammatory cytokines [21]. The ERK downstream cascade includes activated Raf, MEK1/2, and ERK1/2. The Raf family is a serine/threonine kinase and Ras is G-protein (Figure 3).

MAPK's Overlapping Pathways

The MAPK's pathways are not independent from each other but contain a series of overlapping signaling mechanisms. p38 and ERK, JNK and p38, p38, JNK, and ERK all have interrelated functions. It is not practical and feasible to completely isolate each one of the different pathways. Thus, we need to plan treatment based on these highly dependent networks [3] (Figure 3).

Role of MAPKs in Ischemia/Reperfusion

The current medical literature indicates that MAPKs are important targets for study as the management of I/R syndromes emerges. Experimental work [8-20] clearly delineates the importance of MAPKs in enhancing the damage associated with I/R. Inflammatory signaling is generally intensified by MAPKs and downregulated by inhibitors under most circumstances [7-20,22,23]. Thus, it appears that the appropriate inhibition of MAPKs would control and ameliorate the ischemic response (Figure 4).

Late Response to Reperfusion (Phase III)

Phase III is associated with the last event of the I/R response, the chronic molecular mechanisms of protection (days to weeks) that include the pathways associated with satisfactory cell healing. Antiinflammatory cytokines (of the IL-10 type), late adhesion molecules, and growth factors (of the TGF-β type) will be active in the process of recovery. At the cellular level, macrophages and fibroblasts are evident. When this phase is reached, the pathways for complete tissue recovery are in progress.

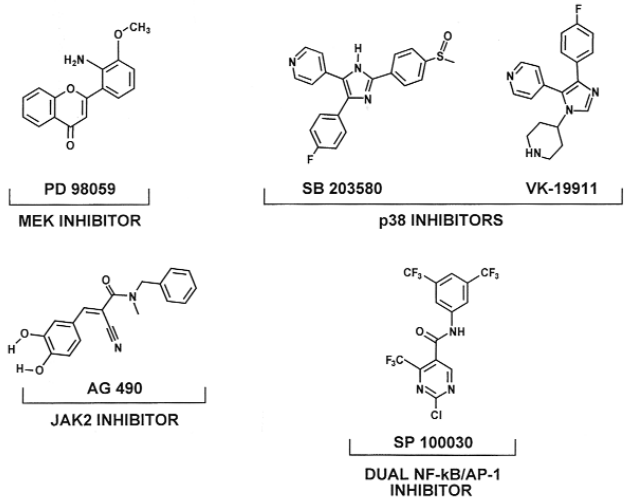

Figure 4. Selected therapeutic compounds modifying directly or indirectly the MAPKs pathways (partially modified from Manning and Rao, see Figure 1)

Conclusions

I/R is a complex phenomenon associated with a multitude of molecular networks. Three phases are described in regard to the chronology of the pathologic response. Well-known mediators (eicosanoids, TNF-α, IL-1, adhesion molecules, NO, and iCa++) begin a series of cascades associated with the damage characteristic of I/R. MAPKs are central to this pathologic event, with their signaling and gene response dependent on the degree of the severity of I/R [24-29]. Manipulation of these molecular inflammatory cascades with MAPK inhibitors offers a new possibility for overcoming the I/R lesion.

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