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REVIEW

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Phosphorylation mechanisms in intensive care medicine

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Abstract *Introduction:* The phosphorylation states of proteins, lipids, carbohydrates, amino acids, and nucleotides control the mechanisms behind nearly all cellular functions. Therefore, not surprisingly, recent findings have shown that alterations in these phosphorylation pathways play a central role in the development and progression of many disease states. This review provides a brief summary of the function and activity of various phosphorylation mechanisms, outlines some of the major phosphorylation signaling cascades, and describes the role of these phosphorylation mechanisms in intensive care medicine. *Methods:* This article will comprise a comprehensive review of the literature in the context of intensive care medicine. Specifically, we will discuss the involvement of phosphorylation in the pathogenesis, diagnosis, and treatment of heart failure, myocardial infarction, stroke, respiratory failure, ventilation-induced lung injury, traumatic brain injury, acute organ failure, systemic sepsis, and shock. *Conclusion:* Phosphorylation mechanisms clearly play an important role in many pathologies and treatment strategies of intensive care and therefore further understanding of these mechanisms may lead to the development of novel therapies and improved patient care.

Keywords Mitogen-activated protein kinase · Phosphoinositide 3-kinase · JAK-STAT · GSK · NFκB

Introduction

Phosphorylation is one of the driving mechanisms behind nearly all cellular functions; however, a true understanding of the significance of this process has only begun within the past half century, and its role in the pathogenesis of critical illnesses remains largely unexplored.

Mechanism of phosphorylation

Phosphorylation and dephosphorylation are reversible modifications involving addition or removal of a high-energy phosphate group. This process plays a central role in intracellular signaling and cellular communication, thereby inducing a large array of functional consequences dependent on the specific phosphorylation target [36]. Phosphorylation is regulated by kinase and phosphatase enzymes. Kinases, also known as phosphotransferases, transfer phosphate groups from high-energy donor molecules, such as adenosine triphosphate (ATP), to specific substrates, including proteins, lipids, carbohydrates, amino acids, and nucleotides. In eukaryotic proteins, protein phosphorylation occurs on serine, threonine or tyrosine amino acid residues, of which the majority of events occur on serine (90%) or threonine (10%) [27]. While tyrosine phosphorylation encompasses only 0.1%

of protein phosphorylation events [27], it has profound importance in many basic cellular processes [36]. Lipid kinases often target sphingosine, ceramide, and phosphatidylinositol and play a central role in cell signaling, growth, motility, and inflammation [36]. Uniquely, lipid kinases can freely diffuse through plasma membranes, allowing the transfer of signals from the outside-in or from the inside-out of the cell [36]. Carbohydrate phosphorylation has particular importance in metabolism, where it regulates glucose/glycogen balance and thereby controls the energy supply within the body [36].

The effects of phosphorylation can be divided into three basic categories based on their speed of action: immediate, rapid, and slow (Fig. 1). Immediate effects result from phosphorylations that induce an instantaneous biological effect, such as glycogen phosphorylase, which upon phosphorylation becomes activated to produce glucose from glycogen, or connexin gap-junctional channels, which undergo a conformational change resulting in decreased permeability [36]. The second category is rapid, occurring within a few seconds to few hours. This slight delay normally occurs due to the necessity to recruit secondary molecules or induce molecular relocation before biological effects can be observed. Typical examples of rapid phosphorylation mechanisms are: (1) phosphoinositide 3-kinase (PI3K) phosphorylation induction of beta-adrenergic receptor endocytosis, (2) phosphorylation of transcription factors that trigger their proteasomic degradation, and (3) protein kinase A (PKA) activity that can potentiate insulin granule exocytosis and secretion [27, 36, 48]. Lastly, phosphorylation mechanisms can be slow acting, requiring several minutes to several hours to produce their physiological effects. This delay normally occurs due to long phosphorylation cascades, the requirement for nuclear translocation, and the subsequent alteration of transcription and translational mechanisms, ultimately altering protein synthesis. Many effects produced by the mitogen-activated protein kinases (MAPK) and nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB), which upregulate inflammation, fall under this category [26, 45].

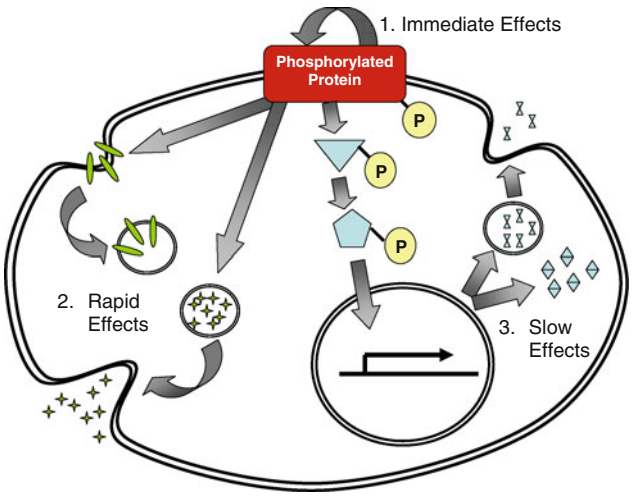

Fig. 1 Schematic representation of temporal classification of phosphorylation mechanisms. Immediate effects occur directly after the original phosphorylation event, rapid effects may require signal transduction, molecular recruitment, and/or molecular translocation within the cell, whereas slow effects often require complex phosphorylation of signal transduction cascades, nuclear translocation, and de novo synthesis and/or secretion of proteins

Functions of phosphorylation

One of the major functions of phosphorylation is the creation of energy in the form of adenosine triphosphate (ATP), guanosine-5′-triphosphate (GTP) or cytidine triphosphate (CTP), of which ATP is the most commonly used source of energy and phosphate donor used by kinase enzymes [36]. Furthermore, ATP itself is created by the kinase ATP synthase through the process of oxidative phosphorylation, which uses energy released by oxidation of nutrients [36].

Regulation of receptor and enzyme activity is also controlled through changes in phosphorylation, which often induces activation or inhibition through changes in tertiary structure, thereby allowing access or blocking their active sites and binding domains [27]. Examples of this regulation are glycogen phosphorylase, which becomes activated upon serine phosphorylation, and Src tyrosine kinase, which masks its kinase domain following phosphorylation [36]. Moreover, G-protein coupled receptors (GPCR) are regulated by GPCR regulatory kinases (GRKs), which phosphorylate the intracellular domains of GPCRs, allowing binding of arrestin proteins and thereby blocking G-proteins re-association and preventing reactivation of the signaling pathway [36]. Phosphorylation can also curtail receptor signaling as seen in the tumor necrosis factor receptor (TNFR), where phosphorylation inhibits its apoptotic activity while preserving its ability to activate NFκB [17].

In addition, phosphorylation can also regulate receptor/enzyme activity by altering their ability to recognize and bind to other proteins, resulting in tightly controlled specificity of protein–protein interactions. For example, tyrosine kinase receptors (RTKs) are phosphorylated within the activated receptor to create binding sites for proteins that contain specific domains (SH2 or PTB domains) [36].

Phosphorylation can also initiate protein degradation through ubiquitin/proteasome pathways, whereby E3 ubiquitin ligases bind to phosphorylated residues and trigger ubiquitination [48]. Classic examples include; (1) forkhead box class O (FOXO) transcription factors, phosphorylated by Akt [48], (2) β-caterin, whose degradation is initiated by glycogen synthase kinase-3 (GSK-3),

and (3) IκBα, an endogenous regulator of NFκB, ubiquitinated upon serine phosphorylation [48]. Additionally, proteasome activity itself is regulated through phosphorylation of its alpha subunits, thereby permitting the association of regulatory complexes required for proper proteasome function [69].

Major signaling networks

While many phosphorylation pathways certainly contribute to various pathologies or reparative mechanisms of acute illness and injury, the following five major signaling networks are believed to play a predominant role in critical care.

MAPK pathway

The five characterized MAPK pathways [MAP kinase kinase/Extracellular-signal-regulated kinase (MEK/ERK), c-Jun N-terminal kinase (JNK), p38, ERK3, and ERK5] can be activated by a variety of growth factors, cytokines, and environmental changes, following which they exert changes in proliferation, differentiation, cytoskeletal reorganization, and gene regulation (Fig. 2) [45]. Specificity of MAPK signaling is obtained through receptor-specific pathways, signaling kinetics, crosstalk between pathways, tissue-specific effectors, cellular compartmentalization, and/or coordination by scaffolding and adaptor proteins [45]. The most highly characterized pathways are the MEK/ERK, JNK, and p38 pathways [45]. Briefly, the MEK/ERK pathway, predominantly involved in proliferation and differentiation, is initiated through activation of RTKs which phosphorylate Ras-GDP to Ras-GTP. The activated Ras then recruits Raf, catalyzing the phosphorylation of MEK1/2 and subsequently ERK1/2 [45]. The JNK pathway responds to cytokines, GPCRs, and stress, such as DNA or protein synthesis inhibition [45]. Upon phosphorylation, JNK is transported to the nucleus, where it phosphorylates and upregulates transcription factors regulating apoptosis and immune cell maturation [45]. The p38 kinase family members are activated by cytokines, hormones, GPCRs, and stress, such as heat and osmotic shock, with their major effects being angiogenesis, cellular proliferation, and inflammation [45].

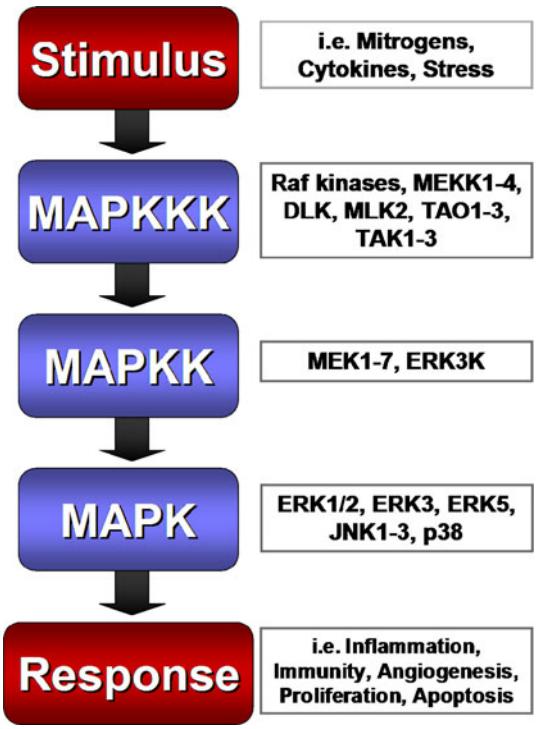

Fig. 2 A simplified cascade of the MAP kinase pathway and the various molecules involved

NFκB pathway

Nuclear factor kappa-light-chain-enhancer of activated B cells is ubiquitously expressed and activated by nearly all forms of stress, including physical stress, oxidative stress, chemical exposure, bacteria, and viruses [26]. The five identified members of the NFκB family, i.e., relA (p65), relB, c-rel, NFκB1 (p50 or its precursor p105), and NFκB2 (p52 or its precursor p100), form homo- or heterodimers, which when inactive are bound in the cytosol to IκB [26]. Upon stress stimulation, IκB kinases (IKK) phosphorylate IκB, resulting in its disassociation from NFκB and ubiquitination [26]. The unbound NFκB then translocates into the nucleus, where it binds target DNA, inducing transcription and protein synthesis [26]. This NFκB pathway augments the inflammatory process and induces antiapoptotic effects through upregulation of TRAF1/2 and blockade of caspase activity [26].

p53 pathway

The transcription factor p53 is an important regulator of the cell cycle and tumor suppression, therefore playing a central role in conserving genetic stability [28]. Under resting conditions, p53 is cytosolically bound to ubiquitin ligases (mdm2 or mdm4) that continuously mark p53 for degradation [28]. Cellular stress induces phosphorylation of p53 in its N-terminal domain by either MAP kinases (described above) or genome integrity proteins (i.e., ATR, ATM, CHK1/2, DNA-PK, and CAK) [28], which disrupts its binding to mdm2/4, allowing its nuclear translocation and binding to transcriptional coactivators [28]. Upon activation, p53 has three functions: (1) induction of cell cycle arrest, (2) activation of DNA repair proteins, and (3)

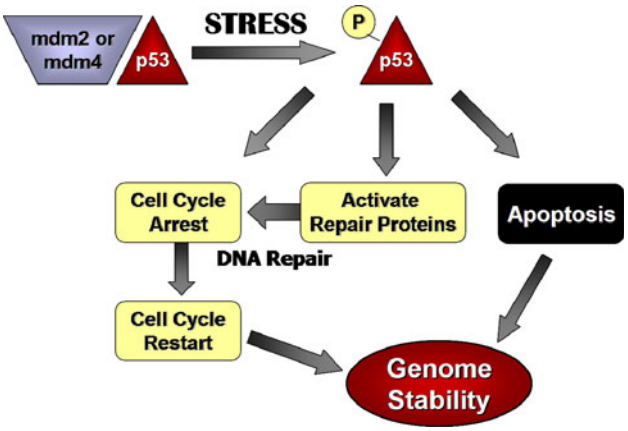

Fig. 3 Schematic flowchart of how p53 maintains genomic stability through either DNA repair or apoptotic mechanisms

induction of apoptosis in unrepairable cells (Fig. 3) [28]. Through these mechanisms, p53 prevents replication of damaged cells and ensures DNA quality control [28].

JAK-STAT pathway

Signal transducers and activator of transcription (STAT) proteins regulate cell growth, survival, differentiation, apoptosis, hematopoiesis, and the immune system [65]. When unphosphorylated, the seven STAT family members (STAT1-4, 5A/B, 6) shuttle between the cytosolic and nuclear compartments [65]. STAT signaling is predominantly initiated by cytokines, which bind to various receptors, resulting in intracellularly activation of Janus kinase (JAK) [65]. JAK activation causes phosphorylation of STAT protein tyrosine residues, which promotes dimerization [65] and induces their active transport into the nucleus, where they bind consensus DNA-recognition motifs located on cytokine inducible genes, leading to upregulation of proinflammatory mediators [65]. This pathway is then negatively controlled by nuclear phosphatases which dephosphorylate the STAT proteins [65].

PI3K pathway

Phosphoinositide 3-kinases are a family of lipid kinases that phosphorylate phosphatidylinositol, important for intracellular signaling [29]. The PI3K family has four classes (I–IV) based on their structure and function [29]. Class I, which are the most highly characterized, are further subdivided into class IA (PI3Kα, β, δ), and class IB (PI3Kγ) [29]. Classical PI3K signaling is initiated by stimulation of RTKs or GPCRs, which with adaptor and scaffolding proteins, phosphorylate phosphatidylinositol 4,5-bisphosphate (PIP2) to phosphatidylinositol (3,4,5)-trisphosphate (PIP3) in the plasma membrane [29]. PIP3 then phosphorylates several downstream target molecules, including Akt/protein kinase B (PKB) [29]. Akt, in turn, phosphorylates an array of molecules, including MAPKs, NFκB, and p53 (described above), leading to a vast number of physiological effects including cell growth, proliferation, differentiation, motility, survival, inflammation, and intracellular trafficking [29]. The phosphorylation of PI3K is counterbalanced by the phosphatase actions of phosphatase and tensin homolog (PTEN) and src-homology 2-containing inositol 5′ phosphatase (SHIP) [29].

The role of phosphorylation in pathologies of intensive care medicine

Heart failure and myocardial infarction

Heart disease is the leading cause of death in the USA, culminating in myocardial infarction (MI) in 40% of cases. Recent studies have shown that phosphorylation mechanisms play a large role in the pathogenesis, diagnosis, and treatment of MI and cardiovascular failure (Table 1). Examples include creatine kinase, commonly used as a diagnostic biomarker of MI, and aspirin, β-blockers, and angiotensin-converting enzyme (ACE) inhibitors, each used as standard therapies and known to alter phosphorylation. Specifically, aspirin, a cyclooxygenase (COX)1/2 inhibitor, alters STAT signaling, nitric oxide synthase (NOS) phosphorylation, and NFκB inhibition [13, 64]. Beta-adrenoceptors, inhibited by β-blockers, are coupled to Gs-proteins that form cyclic adenosine monophosphate (cAMP) to stimulate PKA [52], and ACE inhibitors increase Akt and protein kinase C (PKC) phosphorylation, while inhibiting phosphorylation of MAPKs, IKKs, and mammalian target of rapamycin (mTOR) (a PI3K family member) [6].

Activation of the PI3Kγ and delta isoforms plays a central role in MI, and a combined PI3K$\gamma\delta$ inhibitor (TG100-115) is currently in phase I/II trials (Fig. 4) [21]. Additionally, a separate phase II trial targeting the activity of PKCδ, a downstream target of PI3K, is currently being conducted with a primary end point aimed at reducing infarct size [7]. Phosphorylation of glycogen synthase kinase 3β (GSK3β), another downstream target of PI3K/Akt signaling, during reperfusion correlates with myocardial infarct size in situ [59], and pharmacological inhibition reduces infarct size [59], possibly representing another novel therapeutic target.

The JAK-STAT pathway largely contributes to heart failure, with STAT1 and STAT3 playing a predominant role [10]. Specifically, STAT3 conditional knockout mice develop early age-related heart failure, while mice overexpressing STAT3 displayed a significant reduction in myocardial infarct size, indicating the cardioprotective

Table 1 Clinical studies targeting phosphorylation in critical care

Targeted condition	Compound	Action	Clinical phase	Study design	Status	Results/outcomes
Myocardial infarction	TG100-115	PI3K$\gamma\delta$ inhibitor	Phase I/II	Multicenter, double-blind RCT	Completed	Outcomes include: safety, pharmacokinetics, infarct size. No current published results
Myocardial infarction	KAI-9803	δPKC inhibitor	Phase II	Multicenter, double-blind RCT	Recruiting	Primary outcome: effect on infarct size
Myocardial infarction	Streptokinase	Plasminogen activator	Phase IV	Single-center, double-blind RCT	Completed	Streptokinase significantly limits long-term infarct size and preserves left ventricular volumes and functions [72]
Stroke	Cilostazol	Phosphodiesterase inhibitor	Phase III	Multicenter, double-blind RCT	Completed	No significant difference in the overall rate of recurrence of stroke but lower rates of ischemic and hemorrhagic stroke in the cilostazol group versus aspirin control [35]
Stroke prevention	Dabigatran	Thrombin inhibitor	Phase III	Multicenter, blind RCT	Completed	Dabigatran induced lower rates of stroke and systemic embolism than warfarin [16]
Acute lung injury	SB-681323	p38α inhibitor	Phase II	Multicenter, double-blind RCT	Recruiting	Outcomes include: safety, tolerability, pharmacokinetics, and anti-inflammatory effect on the lung
COPD	SB-681323	p38α inhibitor	Phase II	Multicenter, double-blind RCT	Recruiting	Outcomes include: safety, tolerability, lung inflammation, function, and dyspnea
Renal failure Liver failure	Sarafenib	Tyrosine protein kinase inhibitor	Phase I	Nonrandom, open-label, uncontrolled	Completed	Confirmed safety in using these compounds in patients with either acute renal or liver failure
Renal transplant	Everolimus	mTOR inhibitor	Phase III	Multicenter, open-label RCT	Completed	Everolimus obtained low acute rejection rate and good graft survival in renal transplantation [70]
Liver transplant			Phase III	Multicenter, open-label RCT	Recruiting	Outcomes include: renal function assessment and liver graft failure

Table 1 continued

Targeted condition	Compound	Action	Clinical phase	Study design	Status	Results/outcomes
Liver cirrhosis	INT747	Reduces alkaline phosphatase levels	Phase II	Multicenter, double-blind RCT	Recruiting	Outcomes include: alkaline phosphatase levels, safety, liver function, inflammation, fibrosis, and health symptoms
Renal function in sepsis	Alkaline phosphatase	Dephosphorylates many nucleotides, proteins, and alkaloids	Phase II	Single-center, double-blind RCT	Completed	Alkaline phosphatase treatment improves renal function in severe sepsis or septic shock patients [30]
Sepsis and MOF	Alkaline phosphatase	Dephosphorylates many nucleotides, proteins, and alkaloids	Phase II	Multicenter, double-blind RCT	Completed	Outcomes include: adverse events, blood pressure, coagulation, ECG parameters, mortality, ICU stay, and MVF days No current published results

PI3K phosphoinositide-3 kinase, *RCT* randomized controlled trial, *PKC* protein kinase C, *COPD* chronic obstructive pulmonary disease, *ECG* electrocardiogram, *ICU* intensive care unit, *MVF* mechanical ventilation free

role of STAT3, thought to occur through inhibition of apoptosis [10]. While clinical trials targeting JAK-STAT signaling have not been preformed to date, patients with end-stage heart failure display severely reduced STAT3 expression and activation [67], suggesting that such trials may be warranted.

Phosphorylation mechanisms have also been used to improve current therapies, as seen by the use of the vasodilator-stimulated phosphoprotein phosphorylation index, an indication of platelet reactivity, used to adjust clopidogrel dosing and reducing the amount of cardiac events [11].

Stroke

Stroke, defined as loss of brain function due to disturbance in blood flow, is the second leading cause of death in developed countries. Therapies, such as aspirin and clopidogrel, overlap considerably with those for MI; therefore, this section will not rediscuss these phosphorylation mechanisms but refers the reader to the above section.

The pathogenesis of acute ischemic stroke often involves thrombin activity, since 80% are thrombotic in nature. In addition to inducing clotting and ischemia, thrombin also induces inflammation, edema, and neuronal death, through various phosphorylation mechanisms. For example, thrombin can increase inflammation through phosphorylation of ERK1/2 and p38 [39], and upregulate COX2 through phosphorylation of the ERK and NFκB pathways [74]. Furthermore, PI3Kγ phosphorylation has been shown to contribute to thrombin-induced platelet aggregation and increased mortality associated with acute thromboembolism (Fig. 4) [31]. Furthermore, a recent clinical trial has shown that inhibition of thrombin activity by dabigatran significantly reduced incidence of stroke compared with the gold-standard therapy (Table 1) [16].

Glycoprotein VI (GPVI) is a membrane platelet glycoprotein that not only coordinates collagen interactions involved in thrombus formation, but also acts as a signaling receptor, leading to PI3Ks, SHIP1, and PKC phosphorylation [12]. Moreover, inhibition studies have demonstrated that PI3Kβ plays an essential role in GPVI-mediated platelet aggregation (Fig. 4) [42]. Additionally, GPVI inhibitors can reduce collagen-induced platelet aggregation both in vivo in rats and ex vivo in nonhuman primates, without prolonging bleeding time [66]. While no clinical trials targeting GPVI have been attempted, there is enhancement of platelet expression of GPVI in patients with transient ischemic attacks or stroke versus patients with nonischemic events [9], indicating GPVI as a possible therapeutic target.

Stroke-induced apoptosis is mediated through PKC pathways, in that PKCδ activity induces cell death, while PKCε promotes neuronal survival. In a rat model of

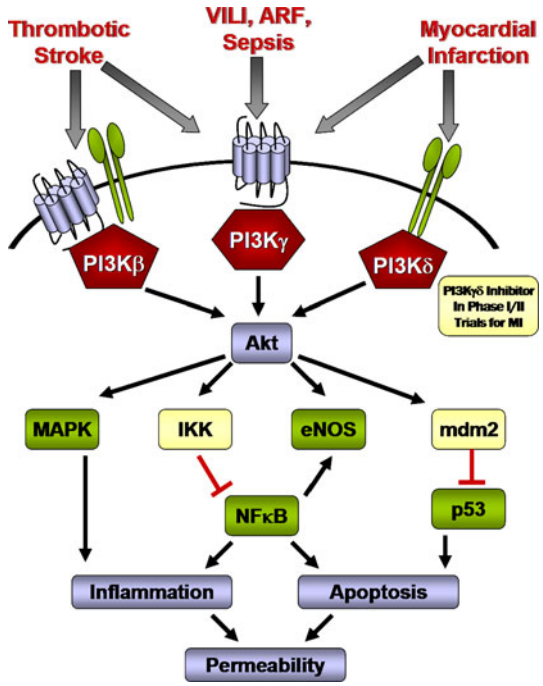

Fig. 4 The role of phosphoinositide-3 kinase isoforms in various aspects of critical illness, highlighting the major role of some dominant downstream intracellular signaling molecules

ischemic stroke, two studies have shown that bryostatin-1, a PKC activator, can rescue ischemia-induced deficits in synaptogenesis, neurotrophic activity, and long-term memory [78]. While bryostatin-1 is currently in phase II trials for other indications, it has not yet be tested for treatment of stroke.

Respiratory failure and mechanical ventilation

Respiratory failure results from various etiologies, including infection, fibrosis, chronic obstructive pulmonary disease, trauma, acute lung injury (ALI) or acute respiratory distress syndrome (ARDS), each resulting in inadequate gas exchange and hypoxemia, causing cellular stress, activation of intracellular signaling pathways, and cell death. A key molecule upregulated in response to low oxygen is hypoxia-inducible factor-1 (HIF1), which in turn regulates transcription factors and enzymes attempting to maintain homeostasis [2]. Some kinases and phosphatases effected by HIF1 include adenylate kinase, hexokinase, phosphoglycerate kinase, and MAPK phosphatase-1 [2]. Additionally, HIF1 has proapoptotic properties due to its ability to activate p53 [2]. Independently of HIF1, hypoxia can also induce ζPKC-mediated phosphorylation of Na,K-ATPase, inducing its endocytosis and contributing to pulmonary edema [18].

Oxygen supplementation is the primary supportive therapy for hypoxic patients; however, exposure to high oxygen concentrations can cause PI3K/Akt activation, leading to Bax phosphorylation, MAPK phosphorylation (JNK, ERK), and cell death [44].

If oxygen supplementation remains insufficient, patients are further supported by mechanical ventilation; however, this therapy induces ventilator-induced lung injury (VILI), also involving alteration of various phosphorylation pathways [55]. Best characterized in VILI is the involvement of the MAP kinases (Table 1), which through the activation of both p38 and ERK lead to pulmonary edema [22]. Furthermore, p38 inhibition is shown to block ventilation-induced caspase activation and alveolar apoptosis [47]. Moreover, ventilator-induced stretch can activate GPCRs and RTKs, leading to PI3K activation and increased lung vascular permeability, of which the PI3Kγ isoform has been shown to contribute to VILI through activation of Akt and ERK1/2 phosphorylation [51]. Moreover, rapamycin, an inhibitor of the Akt target mTOR, significantly reduces cellular injury induced by cyclic stretch [68]. While no clinical studies have tested the effect of targeting phosphorylation in respiratory failure or VILI, these studies suggest the need for further research in this area.

Surgery and trauma

Surgical patients, especially those with severe trauma, hemorrhage or traumatic brain injury (TBI), are known to experience intense suppression of many immune functions, which renders these patients prone to nosocomial infections [4]. Many studies convincingly show that NFκB, Akt, MAPK, and apoptosis signaling pathways are strongly involved in this dysregulation of the inflammatory response induced by acute stress [15, 33, 34, 37, 86]. These pathways have been extensively studied in animal models of hemorrhage and/or trauma, showing a role for several phosphorylation mechanisms, such as Akt in liver function [33], NFκB and p38 in cardiac function [37, 86], and ERK in lung function [34]. Furthermore, high-mobility group box nuclear protein 1 (HMGB1), which interacts with many phosphorylation pathways included PI3Ks, MAPKs, and NFκB, is known to play an essential role in development of systemic inflammation and organ dysfunction following trauma [49]. In addition, clinical studies have correlated the state of posttraumatic immunosuppression in severe trauma patients with some critical phosphorylation signaling events, such as NFκB [3] and HMGB1 [15].

TBI is specifically linked to many phosphorylation pathways; however, MAPKs, PI3K/Akt, and GSK seem to play a dominant role in this pathogenesis [58]. In terms of MAPKs, in vivo models of TBI show that ERK phosphorylation is consistently activated, while p38 and JNK vary depending on the injury [33]. The temporal profile of ERK activation may be important, since phosphorylation

occurred in the cortex and hippocampus within 10 min [19], while activation in the parietal and occipital cortexes was seen after 7 days [24]. Moreover, TBI-induced ERK phosphorylation occurs early in neurons, but later in astrocytes and the hippocampus [58]. Furthermore, while most studies agree that TBI increases ERK phosphorylation, many debate whether this has detrimental or beneficial affects, since ERK inhibition can decrease cortical lesion size and atrophy [56], but its inhibition impairs memory and motor skills following TBI [19]. The temporal nature may, in fact, determine its positive or negative role, where early activation is detrimental as it induces premature neurite outgrowth, while late activation can increase plasticity and synaptic formation [58].

Following cortical impact in mice, Akt phosphorylation is increased adjacent to the lesion [62], collocalizing in neurons with increased Bcl-2-associated death promoter (BAD) phosphorylation and the absence of apoptosis, indicating that Akt plays a prosurvival role in TBI. Nerve transactional studies further demonstrated that Akt overexpression leads to faster axonal regeneration, while dominant negative Akt correlated with increased cell death [57]. Moreover, simvastatin treatment in rats increased Akt phosphorylation and restoration of cognitive function following cortical impact [81].

Phosphorylation of GSK-3β is also believed to contribute to TBI [58], since unilateral hemisection of the spinal cord and lithium inhibition of GSK-3β enhanced regeneration of neurons and significantly improved recovery of forelimb function [85]. No clinical trials have attempted to target phosphorylation during TBI, partly due to the reservations that TBI lacks an appropriate therapeutic window; however, in vivo studies show that injury may be worsened by sustained kinase activation, while other phosphorylation events may assist in neuronal repair [58].

Acute organ failure

Acute organ failure can manifest from a variety of etiologies, affecting the cardiovascular, respiratory, renal, hepatic, and/or neurological systems; however, to reduce overlap with other sections, we will focus on phosphorylation mechanisms contributing to acute renal and hepatic injury and failure (Table 1).

Renal dysfunction

Causes of acute renal failure (ARF) can be categorized as (1) prerenal, (2) intrinsic, or (3) postrenal, each of which can alter phosphorylation cascades. For example, in rats, JAK/STAT blockade decreases the severity of renal ischemia–reperfusion injury [83], while in mice, tyrosine kinase inhibitors significantly reduce zymosan-induced creatinine induction [20]. Additionally, renal failure from medication toxicity, such as due to cisplatin, can induce ARF through PI3Kγ-Akt, p38, and p53 phosphorylation [46], and ureteric obstruction-induced renal injury is ameliorated through blockade of p38 [77].

ARF treatment therapies also act through phosphorylation mechanisms. For example, in rat myoglobinuric kidney injury, glutamine decreases tubular apoptosis and reduces renal damage by inhibiting JNK phosphorylation of the protein 14-3-3 [43]. Moreover, estrogen therapy can induce protection through upregulating PI3K/Akt and phosphorylation of endothelial nitric oxide synthase (eNOS) [71].

Hepatic dysfunction

Common etiologies of acute liver failure are paracetamol (acetaminophen) overdose, medication toxicity, alcoholic or viral hepatitis, and idiopathic origin, many of which effect phosphorylation pathways. Acetaminophen-induced hepatic injury results, in part, due to decreased Akt-NFκB activation and increased STAT1 phosphorylation resulting in necrosis [63]. Alcoholic hepatitis has been linked to eNOS phosphorylation [38], and increased STAT3 phosphorylation in hepatocytes and bile ducts [32]. Viral hepatitis, particularly B and C, are also linked to phosphorylation mechanisms, since hepatitis C increases IκBα phosphorylation and NFκB activation [50], while hepatitis B alters both the c-Src and GSK3β pathways [84]. Treatment therapies also depend on phosphorylation, since clevudine, a hepatitis B inhibitor currently in phase III clinical trials, requires phosphorylation in hepatocytes to reach its active form [60].

Sepsis and septic shock

Sepsis is the second-leading cause of noncoronary death in intensive care units, progressing to multiple organ failure (MOF). Both sepsis and MOF are highly associated with activation of various phosphorylation pathways (Table 1).

The two receptors of tumor necrosis factor (TNFRI and TNFRII) are strongly involved in the pathogenesis of systemic sepsis, through alteration of apoptosis, immunity, and inflammation, and are regulated via changes in phosphorylation [79]. In neutrophils, TNFRI induces activation of PI3K-PKCδ, which phosphorylates and regulates the formation of the TNF receptor associated factor-receptor interacting protein-TNF receptor type I-associated death domain (TRAF2–RIP–TRADD) complex, resulting in antiapoptotic affects [41]. Furthermore, ERK2 phosphorylation of TNFRI induces its relocation from the Golgi complex to the endoplasmic reticulum [17], and spleen tyrosine kinases (Syk) can be activated by TNFRI or TNFRII, leading to JNK, p38, ERK, and IκBα phosphorylation, thereby decreasing apoptosis [79].

A significant contributor to sepsis is lipopolysaccharide (LPS) signaling through toll-like receptors (TLR), which in turn activate kinases and transcription factors including Src, MAPKs, PI3K, and NFκB [1]. Furthermore, losartan, used for ALI/ARDS, can prevent sepsis-induced lung injury by blocking phosphorylation of NFκB, p38, ERK, and JNK [73]. Moreover, in neutrophils from patients with septic-induced ALI, NFκB activation correlates with prolonged mechanical ventilation [82].

While PI3K activation is clearly involved in sepsis, its specific role remains unclear. Broad-spectrum inhibitor studies, using wortmannin or LY294002, found that general PI3K inhibition increases septic mortality, inflammation, and apoptosis [80]. To determine isoform-specific contributions, our recent study demonstrates that PI3Kγ kinase activity significantly contributes to septic-induced mortality, lung, liver, and cardiovascular damage, as well as coagulation derangements in polymicrobial sepsis [53]. Moreover, we show that pharmaceutical inhibition of PI3Kγ, even following initiation of sepsis, reduces mortality, indicating that PI3Kγ may serve as a novel therapeutic target.

Despite advances in the understanding of septic pathology, few clinical trials have successfully reduced overall mortality or altered daily practice, with the exception of activated protein C (APC) (drotrecogin alfa activated), which based on the phase III PROWESS study, has been approved for treatment of patients with severe sepsis [8]. The mechanisms leading to APC-induced protection continue to be investigated and have been linked to changes in protein phosphorylation. Specifically, APC increases ERK1/2 phosphorylation, while decreasing NFκB [54]. Additionally, APC decreases p38 phosphorylation and regulates p53 in a piglet model of severe sepsis [61]. Furthermore, APC can phosphorylate sphingosine 1-phosphate in a PI3K-dependent manner, leading to improved endothelial barrier protection [23].

Despite some controversial clinical studies, recent recommendations for treatment of sepsis advocate use of long-term low-dose hydrocortisone in septic shock patients [5, 75]. While short-term high-dose corticosteroids display powerful anti-inflammatory effects that may favor nosocomial infections, long-term, low-dose hydrocortisone may restore adequate immunity through mechanisms involving protein phosphorylation [76]. Specifically, hydrocortisone is believed to restore immunity through the phosphorylation of MAPK [25] and interactions with the NFκB pathway [14]. Through alteration of these phosphorylation mechanisms, hydrocortisone has been shown to stimulate immunoglobulin synthesis, enhance opsonization and diapedesis of neutrophils, and increase dendritic cell homing without altering monocyte function or diminishing anti-inflammatory cytokine levels [40].

Overall conclusions

This review outlines some of the many phosphorylation mechanisms involved in various aspects of critical care medicine. Furthermore, the central role of phosphorylation signaling and the reversible nature of this system highlight the possible advantages of targeting phosphorylation in novel therapeutic treatments. While some clinical trials have already started to take advantage of these mechanisms, this field remains in its infancy and provides vast possibilities for future development and exploitation.

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