

Mst1: Function and Mechanism in Brain and Myocardial Ischemia Reperfusion Injury

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Abstract: Mammalian STE20-like kinase-1 (Mst1) is a generally expressed apoptosis-promoting kinase and a key bridgebuilder of apoptotic signaling in the etiology of tissue injury. Despite the fact that the biological function of Mst1 and its role in the cell's signalling network have yet to be determined, however, there is a lot of evidence that Mst1 plays an important role in cell death which results from tissue injury. Previous studies have shown that Mst1 is not only a target for some apoptosis-related molecules such as caspase 3 and P53, but also act as an activator of these proteinases to magnify apoptosis signal pathways. This article reviews the role of Mst1 in the signaling pathways which is related with the neuronal cell apoptosis or microglia activation following myocardial and brain injury. Therefore, this work contributes to better understanding of the pathological process of myocardial and brain injury.

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1. INTRODUCTION

Hypoxic-ischemic cardio-cerebrovascular diseases has become the highest morbidity, disability and mortality of the disease [1-3]. The best approach to rescue myocardial and brain ischemia patients is to restore myocardial and brain tissue blood supply timely. But at the same time bringing new damage to myocardial and brain, that is ischemia-reperfusion (I-R) injury [4]. Although significant advances in the technological and medical treatments have been made, many patients still suffer from varying degrees of dysfunction, which directly weakens their quality of life [5]. Therefore, it is extremely important to find effective strategies to improve the functional recovery of stroke patients.

The mechanism of myocardial and cerebral IR injury refers to oxidative stress, mitochondrial dysfunction, cell inflammation and apoptosis, a series of cell and molecular biology events [6, 7]. In the previous studies, Oxidative stress is still regarded as a major contributor to cell death during I-R injury, although different mechanisms of cell death are likely simultaneously activated [8, 9]. Oxidative stress leads to the gene expression not only in innate immunity but programmed cell death and has an effect on cell survival and homeostasis [10, 11]. Although many research results of oxidative stress injury have been reported and it is

well established that a number of related regulators *in vivo*, but remarkably little is known about the mechanisms underlying the biological effects of oxidative stress.

In 1996, Lu *et al.* indicated that apoptosis of some cell lines like NIH-3T3, HL-60, and human prostatic tumour (LnCap), induced by an array of apoptotic stimuli was connected with the activation of a phosphorylated myelin basic protein kinase which weight 36 kDa [12]. Although they postulated that the 36 kDa myelin basic protein kinase is a common component in some signalling pathways of cell apoptosis and that maybe it is a cleavage product of a higher molecular weight enzyme, until a long time later, the protein was identified as a cleavage fragment of Mst1. Actually, this enzyme had only been described by Creasy and Chernoff a year earlier [13, 14]. Mst1 is widely known as a major component of the Hippo signaling pathway to mediate organs size and homeostasis by regulating cell proliferation and differentiation [15-17]. Recently, there are emerging studies that have shown that Mst1 plays an important role in regulating inflammation, stress response and apoptosis in myocardial injury, spinal cord injury and brain injury [18, 19]. Mst1 exerts apoptotic effect by being activated by its activator, autophosphorylation and in turn phosphorylation of downstream targets such as FOXO3 (Forkhead box O3) and Bcl-xL [20, 21]. Recently, Mst1 has been proven to play a crucial role in neuronal cell death which is induced by oxidative stress [8, 22, 23]. In conclusion, there is growing evidence indicating that Mst1 plays an important role in cell death caused by I-R injury.

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2. MST1 AND CEREBRAL ISCHEMIA-REPERFUSION INJURY

During the past decade, Cerebral ischemic stroke has become a major public health issue characterized by high rates of morbidity, mortality and disability [24]. In cerebral I-R-induced injury, even though the neuronal cell death has always been the focus of investigation [25, 26], a growing number of evidences demonstrate that microglial act as the critical regulator of extracellular environment of neurons, its activation plays a paramount role in neuronal cell death caused by I-R injury [8, 11]. In the resting state, microglia are the common immune cells of the central nervous system and have an action of immunologic surveillance [27]. When I-R injury occurs, oxidative stress leads to neuronal cell death and releases damage-associated molecular pattern molecules (DAMPs) and purines (ATP) that activate scavenger receptors and Toll-like receptors (TLRs) in microglia, leading to the accumulation of activated microglia around the injury region [28]. In addition, another similar study demonstrated that in response to infection or brain injury, microglial migrate to the injury region of the central nervous system, where they accumulate and become activated, the accumulation of microglial cells subsequently clear up the dead cells and cell debris [29]. Nevertheless, the inundatory microglial activation may have a detrimental effect on neurons [8, 30], which can be indicative of, besides the decrease of neuronal cell death, a potential therapeutic strategy that may inhibit microglial activation in the therapy of the stroke.

Mst1 is well known as a Pro-apoptotic molecule and is commonly expressed in mammalian cells. In cerebral IR injury, Tyrosine kinase c-Abl phosphorylates Mst1 at Y-433 and activates Mst1, in turn Mst1 phosphorylates forkhead box O1/3 (FoxO1/3) transcription factors at serine 207 [8, 31], thereby promoting nuclear translocation of FoxO1/3 and activates the Mst1-FoxO1/3 signaling pathway and may directly activate the neuronal cell death program by promoting BIM expression [11]. Recently, studies confirmed that post-translational modification of FoxO₃ distinctly and differentially regulate microglial functions according to a time-dependent manner of oxygen-glucose deprivation (OGD), an *in vitro* model of ischemia [11, 32]. FoxO₃a stimulates the activation and proliferation of microglia at the early stages of OGD, however, at later phase after OGD, induces apoptosis of microglial [11] (Fig. 1). In conclusion, these findings demonstrate that c-Abl/Mst1/FoxO₃ signaling pathway plays a considerable role in regulating different microglial functions.

Furthermore, in OGD-BV-2 cells model, microglia were activated and divided into two phenotypes, which are termed as M1 microglia and M2 microglia [33, 34]. Although M1 microglia plays an important role in clearing injured tissue fragments and pathogens, meanwhile, these microglia could also evoke new injury to surrounding neurons because M1 microglia can release some kinds of inflammatory cytokines. On the contrary, M2 microglia are essential in reducing inflammatory response and tissue protection because of its

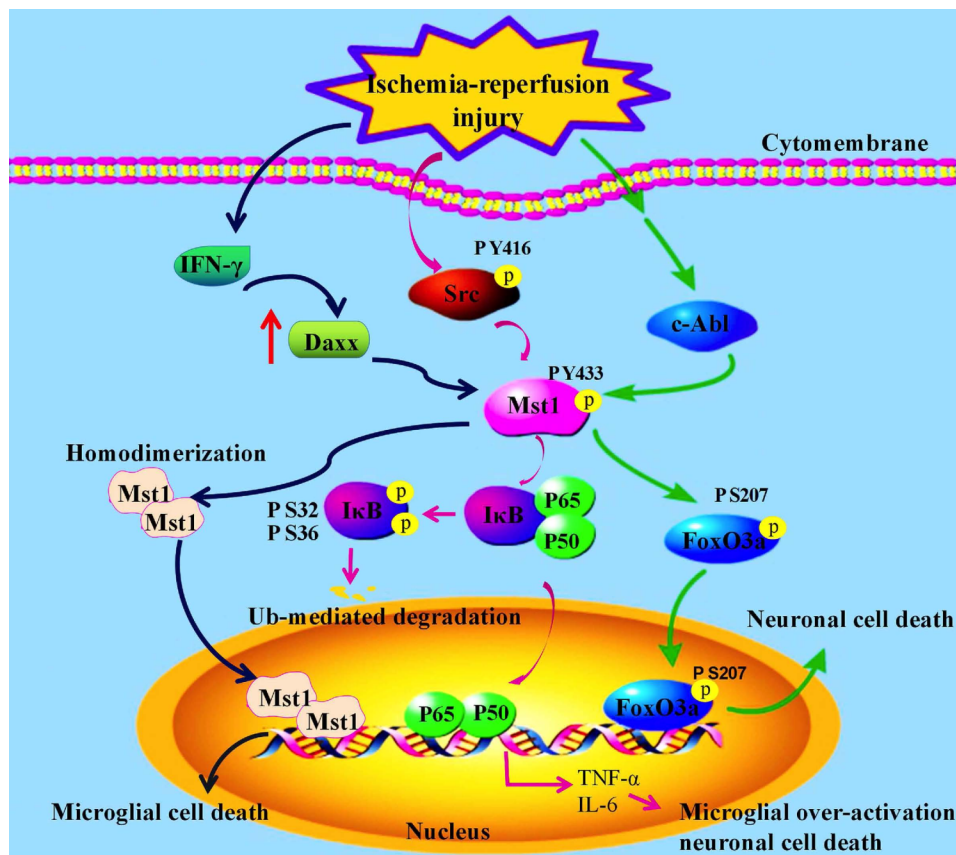


Fig. (1). The roles of Mst1 in cerebral ischemia-reperfusion injury.

function of release neuroprotective factors. Therefore, inducing activated microglia toward M2 state will contribute to reduced neuronal damage, evoked by cerebral ischemic injury. Recently, there is evidence showing that Mst1 participates in the activation of microglia and its transition to M2 state in OGD-BV-2 cells model [35]. In this model, the expression level of p-MST1-Y433 was significantly increased following OGD. Besides, Malibatol A could promote the expression of M2 microglia makers instead of M1 state by inhibiting the expression of p-MST1(Y433) induced by OGD in BV-2 cells, thereby reducing BV-2 cells apoptosis.

In addition, recent studies have shown that in microglia, specific absence of Mst1 reduces stroke-induced brain injury [15]. Mst1 regulates the stroke-induced activation of microglia by direct phosphorylation of I κ B α at serine residues S32 and S36. Further study reveal that Src kinase directly phosphorylates Mst1 at Y433 and activates Mst1, so Src kinase plays a key role in the upstream of the Mst1-I κ B signal during microglia activation [36] (Fig. 1). The phosphorylation and degradation of I κ B is a pointer of NF- κ B activation and an inhibitor of NF- κ B during ischemic stroke [37]. In particular, the activation of NF- κ B has an effect on delaying inflammation and neurotoxicity [38]. Consequently, Src-Mst1-I κ B signaling pathway plays a significant role in stroke-induced microglial activation.

The above studies show that phosphorylated Mst1 promotes the activation of microglia. Interestingly, homologous dimerization of Mst1 inhibits microglial activation in inflammatory stimuli or tissue injury. As an example, Hee Jae Yun *et al.* [30] have shown that in answer to tissue injury or inflammatory stimulation, microglia is activated and releases both neurotrophic and neurotoxic substances subsequently [39]. Consistent activation of microglia for a long time may trigger non-reversible damage to neurons and lead to neurological diseases [40-43]. Daxx and Mst1 regulate apoptosis which is caused by interferon- γ (IFN- γ) in microglia [30]. The expression level of Daxx is upregulated by IFN- γ [44], then the high level of Daxx can lead to the homodimerization of Mst1 and activates Mst1 [30]. Subsequently, activated Mst1 dimer nuclear translocation and promote induced microglia death. Knockdown Daxx also weakens IFN- γ causing cell death in rat microglia. Additionally, deficiency of Mst1, the death of microglia induced by IFN- γ was meaningfully reduced compared with wild-type mice [30] (Fig. 1). Consequently, dimerized Mst1 is a pivotal mediator of microglia death caused by the inflammatory factor.

In summary, phosphorylated Mst1 promotes the activation of microglia, leading to neuronal cell death in cerebral I-R injury or stroke, whereas dimerization of Mst1 inhibits the activation of microglia caused by tissue injury or inflammatory reaction.

4. MST1 AND MYOCARDIAL ISCHEMIA REPERFUSION INJURY

In recent several years, there has been an increasing awareness of Ischemic heart disease which is a significant

component of cardiovascular disease and a primary cause of death worldwide [45, 46]. Although many approaches in understanding the causes of ischemic heart disease have been discovered, the mechanism of myocardial I-R injury has not yet been enough elucidated. Consequently, the treatment method of myocardial I-R injury is immature at present.

In response to a variety of stresses, including myocardial I-R, volume overload and pressure, Mst1 is activated in myocardial cells, through many different kinds of molecular mechanisms involving mechanical stress, cytokines and oxidative stress [47, 48]. Mst1 can be translocated to the nucleus, the ER and mitochondria, although is localized primarily in the cytoplasm and activated by scaffolding proteins, like NF2 and Rassf1A, by different mechanisms [49, 50]. Particularly, Under the stimulation of myocardial I-R injury Mst1 moved to the mitochondria in cardiomyocytes, and activated by Rassf1A and K-Ras-dependent regulatory mechanisms. In turn, the activated Mst1 phosphorylates Bcl-xL at Ser14 [47, 51]. On account of the phosphorylation at the Ser14 of Bcl-xL which resulted in the dissociation of Bcl-xL from B cell leukemia/lymphoma 2-associated (Bcl-2-associated) protein x (Bax), accordingly activating Bax and inducing mitochondrial apoptotic pathways [51, 52]. In addition, in Mst1 knockout mice, deficiency of Ser14 phosphorylation of Bcl-xL obviously weakens I-R injury and the development of cardiomyopathy, because the phosphorylation of Bcl-xL at Ser14 has completely disappeared due to the lack of Mst1 [45]. Therefore, the function of Mst1 in response to myocardial I-R is particularly mediated by Ser14 phosphorylation of Bcl-xL. In knockin (KI) mice, Ser14 of Bcl-xL is replaced by Ala, so Mst1 cannot phosphorylate Bcl-xL and unsuccessfully activates mitochondrial apoptosis pathway [47]. The *in vivo* data further indicate that significant protection represents that the phosphorylation of Bcl-xL at Ser14 is the master mechanism mediating the proapoptotic function of Mst1 during myocardial I-R injury (Fig. 2). Consequently, inhibition of Mst1 is able to reduce the I-R injury and forestalls cardiac remodeling/dysfunction after chronic myocardial infarction, suggesting that Mst1 is a promising target of cardiac therapy for ischemic heart injury.

Similarly, another work has demonstrated that in response to oxidative stress caused by myocardial I-R injury, Mst1 is activated both in mouse myocardium *in vivo* and cultured cardiomyocytes [49]. In order to simulate oxidative stress during I-R, treatment of neonatal rat ventricular myocytes (NRVMs) was conducted with Hydrogen Peroxide and induced activation of MYPT-1, showing a lack of Ser696 phosphorylation of MYPT-1 indicative of its activation [53]. Previous work identified that PP1-MYPT-1 was identified as NF2 activator by dephosphorylation of Ser518 in *Drosophila* and in mammalian cells [54]. So, MYPT-1 is activated by oxidative stress, mediated NF2 dephosphorylation and activation in cardiomyocytes. In NRVMs, overexpression of NF2 promoted Mst1 activation [50]. Mst1 is known to promote cell death [20, 55], therefore, through evaluated cardiomyocyte apoptosis in the case of increased NF2 expression, it was found that increased NF2 expression prompted a significant increase in TUNEL-positive cardiomyocytes, and this reaction is greatly weakened through inhibition of Mst1. Similarly, NF2 activates caspase-3, which was meaningfully

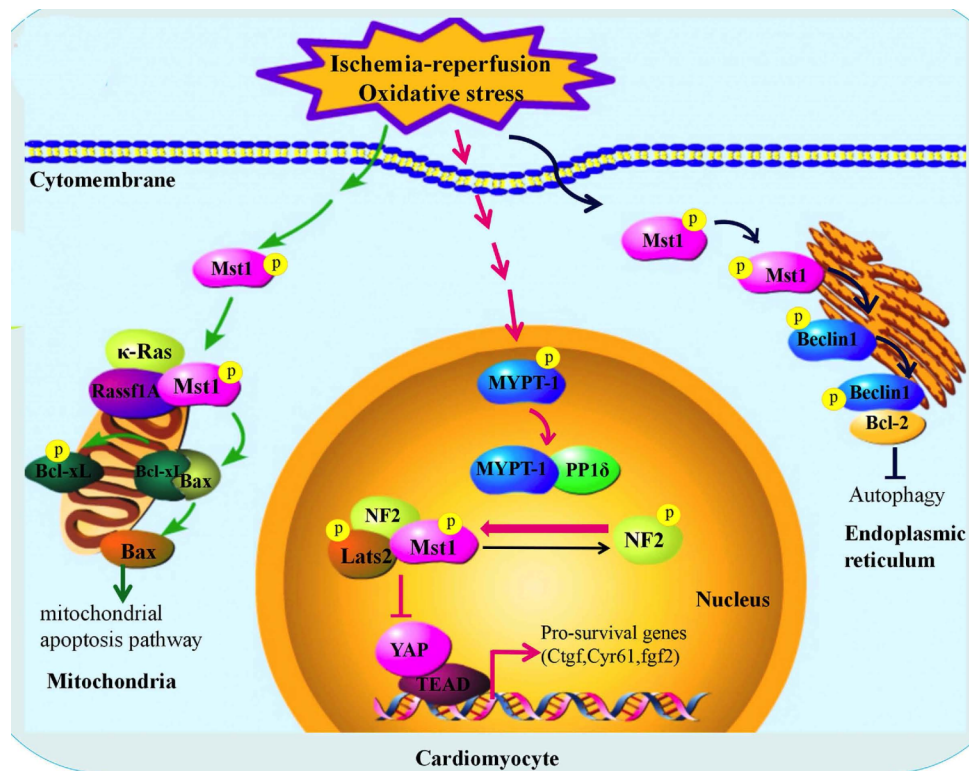


Fig. (2). The roles of Mst1 in myocardial ischemia-reperfusion injury.

attenuated by inhibition of Mst1 [56]. On the other hand, reducing the level of endogenous NF2 using siRNA weakens both the activation of Mst1 and cardiomyocyte death induced by myocardial I-R injury [10, 49]. Mst1 is known as an important part of the Hippo pathway, and NF2 can promote cardiomyocyte death through engaging Hippo signaling at the level of Mst1. Furthermore, NF2 forms a complex with Mst1 and Lats2 which are the main components of Hippo pathway, then the complex inhibits nuclear translocation of YAP which is another important component of Hippo pathway [57, 58] (Fig. 2), therefore, YAP cannot initiate the transcription of the pro-survival genes such as *ctgf*, *cyr61*, finally, loss of cytoprotective effect.

Upregulation of autophagy during myocardial I-R injury [2, 59]. Autophagy within the normal physiological range is usually beneficial to clear protein aggregates and damaged mitochondria. However, Mst1 suppresses autophagy and promotes apoptosis by phosphorylating Beclin1 and inducing homodimerization of Beclin1 [60]. The conclusion reiterates that Mst1 is a promising target of myocardial I-R injury.

In summary, the above research proves that Ser14 sites of endogenous Bcl-xL is a critical target of Mst1 and that it plays an essential role in mediating the effect of Mst1 in I-R injury. Oxidative stress stimulates dephosphorylation of NF2 via MYPT-1-PP1 thereby promoting an active conformation of NF2. Activated NF2 forms a complex with Mst1 and Lats2 in the cardiomyocyte nucleus, promotes Mst1 activation, and negatively regulates YAP target gene expression, leading to YAP which cannot initiate the transcription of the pro-survival genes such as *ctgf* and *cyr61*, eventually leading

to cell death. In addition, Mst1 also inhibits autophagy during myocardial I-R injury.

CONCLUSION AND PROSPECTIVE

Research in recent years has discovered several interacting proteins and downstream signal pathways of Mst1 in IR injury or stroke, cerebral infarction and myocardial infarction following reperfusion, which are both typical I-R injury. This review summarizes a novel role of Mst1 in microglia activation, apoptosis and neuronal cell death during I-R injury or stroke. Mst1 activation appears to be linked to different molecules activation such as Src, c-Abl and Daxx under the conditions of oxidative stress induced by I-R injury or stroke. The main contents of this review can be summarized as follows: (1) Phosphorylated Mst1 at Y433 has an activating effect on microglia and promotes neuron death, whereas dimerization of Mst1 from the cytoplasm into the nucleus, inhibits microglia activation and promotes its death during brain I-R injury. (2) Under the stimulation of oxidative stress, phosphorylated Mst1 enters mitochondria and interacts with molecules such as Bax and Rassf1A to induce mitochondrial apoptosis, and phosphorylated Mst1 can also enter the nucleus, and form a complex with Lats2 and NF2 in the regulation of MYPT-1, thereby blocking the regulation of YAP on pro-survival genes transcription, leading to apoptosis. In addition, Mst1 also promotes cell death by inhibiting autophagy during myocardial I-R injury. Hence, these studies prove that Mst1 plays a central role in microglial activation, apoptosis induced by ischemic stroke or I-R injury, indicating that Mst1 is an effective target for the treatment of brain and myocardial I-R injury.

Although the studies of the mechanism of IR injury is deep and developed, the pivot point of the mechanism is not yet clear and needs further study. In cerebral I-R injury, Mst1 not only participates in the activation of microglia through the Src-Mst1-I κ B signaling pathway, but is also involve in the death of primary rat microglia through the IFN- γ -Daxx-Mst1 signaling pathway. It is well known that M1 microglial can release toxic factors what would be detrimental for the survival of the surrounding neurons. Therefore, blocking Src-Mst1-I κ B signaling pathway and meanwhile promoting IFN- γ -Daxx-Mst1 signaling pathway may be an effective way to treat activated microglia-induced neuronal apoptosis after I-R brain injury. In myocardial I-R injury, Mst1 not only participates in the regulation of transcription of some apoptosis-related molecules, but also involves in cell death through activation of mitochondrial apoptotic pathway and autophagy pathway. Therefore, affecting targeted silencing Mst1 or inhibiting its phosphorylation and blocking the related pro-apoptotic pathway may be a new direction for the effective treatment of I-R injury.

In addition, the drugs of heart and cerebral I-R injury which are obtained from animal experiment are not able to achieve the desired effect in clinical trials, this may be related to complicated mechanism of I-R injury, single-drug approach is not sufficient to combat the pathophysiological changes of cardiovascular and cerebrovascular disease caused by reperfusion. So the physiological, pharmacological and thrombolytic therapy should be combined with the the clinical treatment trends of heart and cerebral I-R injury. How to fully and effectively use the known mechanism of I-R injury to prevent and treat ischemic cardio-cerebrovascular diseases requires further clinical practice.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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