Role of Mammalian Sterile20-like Kinase 1 and 2 in Oxidative Stress

Sonali J. Rawat, Ph.D.
Fox Chase Cancer Center, 333 Cottman Avenue, Philadelphia, PA, USA
Email: Sonali.jalan@fccc.edu

Abstract
Mammalian sterile 20 like kinase (Mst) 1 and Mst2 are stress-activated kinases, activated by various stress stimuli including oxidative stress. Mst1/2 act as tumor suppressor proteins and their deregulation is associated with the development of cancers, such as liver cancer and prostate cancer. Cellular oxidative stress is known to upregulate Mst1/2 activity; activation of Mst1/2 induces apoptosis in oxidative stress conditions. Until recently, the exact mechanism/s of Mst1/2 activation and Mst1/2-induced apoptosis under oxidative stress conditions was not clear. But some recent reports have begun to elucidate the mechanisms of Mst1/2 activation by oxidative stress. Not only Mst1/2 activity is regulated by oxidative stress, but Mst1/2 are also involved in the regulation of redox levels in cells. In this short report we will discuss various mechanisms of Mst1/2 activation and Mst1/2-dependent apoptosis in response to oxidative stress.

Keywords: apoptosis, c-abl, FOXO1/3, Mst1, Mst2, oxidative stress

Introduction

Oxidative stress in cells is caused by overproduction of reactive oxygen species (ROS) and reactive nitrogen species (RNS)(Martinez and Andriantsitohaina, 2009; Mikkelsen and Wardman, 2003; Sasaki, 2006). RNS species in cells are mainly produced by the enzyme nitric oxide synthase; high levels of RNS can result in cell injury or cell death ((Martinez and Andriantsitohaina, 2009). ROS are mainly generated in cells by the electron transport chain. Antioxidant enzymes neutralize low levels of ROS produced in cells, while intermediate ROS levels act as secondary messengers in many signaling pathways ((Finkel, 2011). Accumulation of high levels of ROS in cells results in damage of intracellular molecules such as DNA, proteins, and lipids. Therefore, oxidative stress is implicated in a variety of diseases like Alzheimer's disease, pulmonary fibrosis and diabetes (Sasaki, 2006). Oxidative stress also plays an important role in the process of carcinogenesis (Reuter et al., 2010). Oxidative stress can cause oxidative DNA damage, which leads to genetic mutations, genomic instability and chromosomal abnormality. Oxidative stress can alter expression of genes involved in growth regulatory pathways and can also activate signaling pathways involved in cell survival and growth (Sasaki, 2006; Tien Kuo and Savaraj, 2006; Valko et al., 2006). Oxidative stress has been shown to activate Mst1 and Mst2, which are important tumor suppressor proteins (Kakeya et al., 1998; Lehtinen et al., 2006; Taylor et al., 1996). Mst1 and Mst2 are important mediators oxidative stress induced apoptosis. Until recently, the mechanisms of Mst1/2 activation by oxidative stress and the mechanisms by which Mst1/2 induces apoptosis in response to oxidative stress were not known. In this report we will briefly summarize some recent findings that describe these mechanisms. Mst1/2 are key components of the Hippo signaling pathway. This pathway regulates organ size and tissue homeostasis by regulating apoptosis and cell proliferation (Zeng and Hong, 2008; Zhao et al., 2010). The Mammalian sterile 20-like kinases are homologous to yeast sterile 20-like kinases; in yeast the sterile 20-like kinases play an important role in the mating pathway. In mammals sterile 20 family consists of two subfamilies, the p21-activated kinase

family and the germinal center kinase family. Mst1 and Mst2 belong to the germinal center kinase family (Ling et al., 2008). Mst1 protein consists 487 amino acids and Mst2 protein is made up of 491 amino acids. The structure of Mst1 and Mst2 proteins is remarkably similar. Further, there are many of similarities in the ways Mst1 and Mst2 are activated and also in the functions of Mst1 and Mst2. We will not discuss the structure and functions of Mst1/2 in this short report and the reader are advised to read the following comprehensive articles for further details (Avruch et al., 2012; Ling et al., 2008; Radu and Chernoff, 2009).

Recently, Mst1/2 have become a subject of intense investigation due to their critical role as tumor suppressor proteins in a variety of cancers (Avruch et al., 2011; Liu et al., 2010; Lu et al., 2010; Song et al., 2010; Zhou et al., 2009; Zhou et al., 2011). Mst1 and Mst2 can be activated by various apoptotic and stress stimuli, such as staurosporine, UV radiation, hydrogen peroxide, TNF-α, retinoic acid, okadoic acid, and anti-cancer drugs (Graves et al., 1998; Kakeya et al., 1998; Lee et al., 1998; Lehtinen et al., 2006; Lu et al., 1996; Watabe et al., 2000). Mechanism/s of Mst1/2 activation by most of these signals is largely unknown, but recently some mechanisms of Mst1/2 activation by oxidative stress have been proposed.

Mechanisms of Mst1/2 activation by oxidative stress

While it is well established that Mst1/2 are activated by oxidative stress stimuli, the mechanisms of this activation are just starting to uncover. Three different mechanisms of Mst1/2 activation by oxidative stress have been proposed (Fig.1). The first mechanism of Mst1/2 activation, under oxidative stress conditions was shown in neuronal cells. In these cells, oxidative stress induced c-abl-dependent tyrosine phosphorylation and activation of Mst1 and Mst2 (Liu et al., 2012; Xiao et al., 2011). c-abl induced Mst1 phosphorylation at Y433 increased Mst1 interaction with the transcription factor FOXO (Forkhead Box O) 3. Mst1 phosphorylated FOXO3 leading to the activation of FOXO3 and resulting in neuronal cell death (Xiao et al., 2011). Mst2 was phosphorylated at Y81 by c-abl, resulting in Mst2 activation and neuronal cell death (Liu et al., 2012). Interestingly, Mst1 was also shown to activate c-abl in the presence of oxidative stress in astrocytes, suggesting reciprocal activation of c-abl and Mst1 under oxidative stress conditions in astrocytes (Lee et al., 2014). The second mechanism of Mst1 activation under oxidative stress conditions involved H_2O_2 and thioredoxin, an antioxidant enzyme. It is well established that Mst1 and Mst2 can homodimerize followed by autophosphorylation that leads to Mst1 and Mst2 activation (Creasy et al., 1996; Radu and Chernoff, 2009) Thioredoxin was shown to interact with Mst1 and this interaction inactivated Mst1 by preventing homodimerization and autophosphorylation of Mst1. On the other hand, H_2O_2 disrupted the interaction between Mst1 and thioredoxin, resulting in Mst1 activation. Therefore, thioredoxin acts as a molecular switch that regulates oxidative stress-induced Mst1 activation (Chae et al., 2012). The third mechanism of regulation of Mst1/2 activation under oxidative stress involves another antioxidant enzyme, Peroxiredoxin 1. Peroxiredoxin 1 is thought to be a critical regulator of Mst1 activity under oxidative stress conditions and knockdown of Peroxirdoxin 1 was shown to prevent Mst1 activation by H_2O_2 (Morinaka et al., 2011). H_2O_2 was shown to induce Peroxiredoxin 1 oxidation to form Peroxiredoxin 1 homo-oligomers. The homo-oligomers interacted with Mst 1 resulting in autophosphorylation of Mst1 and increase in its kinase activity. Therefore, the effect of Peroxiredoxin 1 on Mst1 activity is opposite to that of thioredoxin, even though both are anti-

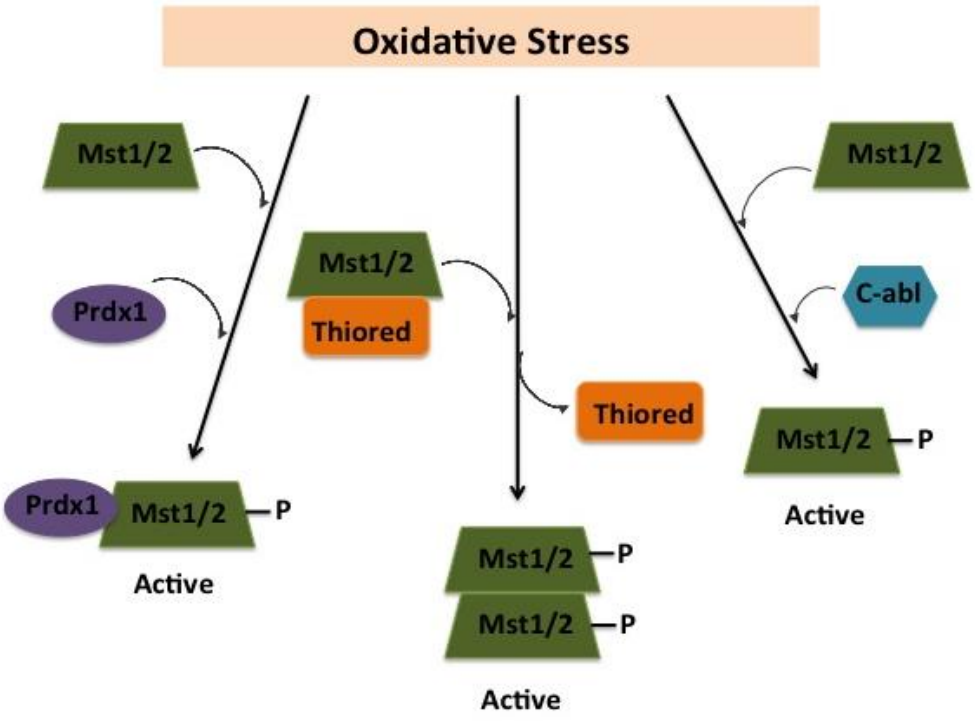

Figure 1. Different mechanisms of Mst1/2 activation by oxidative stress. See text for details.

oxidant enzymes. While thioredoxin inactivates Mst1/2, Peroxiredoxin 1 activates the kinases.
The different mechanisms of activation of Mst1/2 under oxidative conditions could be due to the use of different cell-types. In neuronal cells, c-abl was shown to be an important mediator of oxidative stress induced Mst1/2 activation. Thioredoxin was shown to negatively regulate Mst1 activation in human embryonic kidney cells (HEK293), while Peroxiredoxin 1 was shown to be essential for Mst1 activation in Cos-7 cells.

Mechanisms of Mst-induced apoptosis in response to oxidative stress

Activation of Mst1 under oxidative stress conditions results in cellular apoptosis. Mst1 was shown to phosphorylate FOXO transcription factors under oxidative stress conditions. FOXO is a family of transcription factors that mediates apoptosis by regulating the transcription of numerous apoptotic genes (Brunet et al., 2004; Kops et al., 2002). Under normal conditions, 14-3-3 proteins sequester FOXO transcription factors in the cytoplasm, inhibiting FOXO-dependent transcription and cell death (Van Der Heide et al., 2004). Under oxidative stress conditions, Mst1 was shown to phosphorylate FOXO1 at Ser212 and FOXO3 at Ser207 in the forkhead domain. Mst1-induced phosphorylation of FOXO1/3 disrupted FOXO1/3 interaction with 14-3-3 proteins, resulting in the nuclear translocation of FOXO1/3 and induction of neuronal cell death (Lehtinen et al., 2006; Yuan et al., 2009). While in neuronal cells, Mst1 activation, induced apoptosis by FOXO-mediated transcriptional upregulation of pro-apoptotic genes, a different mechanism was proposed in cardiomyocytes. In cardiomyocytes, activation of Mst1 by oxidative stress resulted in Bcl-xL phosphorylation. Bcl-xL is a member of Bcl-2 family of anti-apoptotic proteins; Bcl-xL prevents apoptosis by binding and inhibiting the function of pro-apoptotic protein Bax. A pro-apoptotic stimulus to cells results in activation of Bax,

which gets inserted into the mitochondrial membrane resulting in the release of cytochrome c, causing apoptosis (Pawlowski and Kraft, 2000). In cardiomyocytes phosphorylation of Bcl-xL by Mst1 prevented Bcl-xl association with Bax, resulting in Bax activation and subsequently, in mitochondria-mediated apoptosis (Del Re et al., 2014). This is the first study that describes a role of Mst1 in inducing apoptosis by regulating the intrinsic pathway of apoptosis, under oxidative stress conditions. This also suggests that Mst-FOXO signaling pathway is not universal for Mst-induced apoptosis and perhaps the exact mechanism/s is context dependent. As discussed in the following section, activation of Mst1 and subsequently FOXO, in fact, results in a decrease in apoptosis in immune cells.

Mst1/2 regulate cellular oxidant levels

Recent reports have suggested that Mst1/2 are not only activated by oxidative stress, but they also regulate oxidant levels in cells. Choi et al demonstrated that peripheral T-cells from Mst1/2 (-/-) mice have decreased activity of FOXO1/3, resulting in reduced expression of the proteins SOD2 (superoxide dismutase 2) and catalase. SOD2 and catalase are antioxidant proteins and thus, a decrease in their expression led to an increase in the cellular ROS levels followed by induction of apoptosis (Choi et al., 2009; Qin et al., 2013). The study further demonstrated that crossing Mst1 transgenic mice with Mst1 (-/-) mice restored Mst1 expression in the progeny. The progeny had lower cellular ROS levels as compared to the Mst (-/-) mice and the numbers of peripheral naïve T cells were also restored. This study, in contrast with the studies mentioned in the previous section, demonstrated that activation of FOXO1/3 by Mst1 reduces ROS levels and cellular apoptosis in T-cells.

In another report, Mst1/2 deletion in liver cells elevated the expression of antioxidant enzymes, thereby, reducing cellular ROS levels (Qin et al., 2013; Wu et al., 2013). The reduction in cellular ROS levels in hepatocytes isolated from Mst1/2 knockout mice was attributed to increased Yap activity. In the canonical hippo-signaling pathway, Mst1/2 phosphorylates the protein kinase Lats, which in turn phosphorylates the transcription coactivator Yap to inhibit Yap's activity (Chan et al., 2005; Hao et al., 2008). Yap promotes the expression of a number of antioxidants including Peroxiredoxin1 and thus in liver cells loss of Mst1/2 upregulated Yap activity to increase the levels of anti-oxidant enzymes (Wu et al., 2013). Overall, unlike in T-cells, the study in liver cells demonstrated that Mst1/2 activation increases ROS levels. These studies also suggest that not only the mechanism of activation of Mst1/2 under oxidative stress condition is context dependent, downstream functions of Mst1/2 are also dependent upon cellular context. It is possible that Mst-Yap pathway is predominantly activated in liver cells leading to increased ROS production, while Mst-FOXO signaling pathway is activated in immune cells resulting in reduced ROS production and protection of peripheral T cells from oxidative stress induced apoptosis.

Mst1 activation was also shown to elevate ROS levels and cause DNA damage in HEK293 cells, under oxidative stress conditions (Rawat et al., 2013). Upon stimulation by H_2O_2, Mst1 directly interacted with Peroxiredoxin1, which resulted in phosphorylation and inactivation of peroxiredoxin1 by Mst1. This study suggested that by inhibiting Peroxiredoxin 1, Mst1 maintains high oxidant level in cells exposed to H_2O_2, which might induce apoptosis and promote tumor suppressor function of Mst1/2 (Rawat et al., 2013).

Conclusions

Although Mst1/2 activation by oxidative stress was known for more than a decade now, the mechanism of Mst1/2 activation by oxidative stress as well as the downstream signaling pathways that lead to apoptosis was a mystery for a long time. Recent findings have partly solved this mystery and we now have answer to some of these questions.

While several different mechanisms have been proposed for oxidative stress- induced Mst1/2 activation and induction of apoptosis in different cell types, there is no doubt that activation of c-abl-Mst1/2-FOXO1/3 pathway is critical for oxidative stress-induced neuronal cell death. Since oxidative stress induced neuronal cell death is a hallmark of a number of neurodegenerative diseases, understanding the precise mechanism by which oxidative stress activates the c-abl-Mst1/2-FOXO1/3 pathway in neuronal cells is crucial to target this pathway to treat neurodegenerative diseases.

As discussed above, Mst1/2 also plays crucial role in regulating oxidant level in cells. It is evident from these studies that depending on the cell type, Mst1/2 can either elevate or reduce cellular ROS levels. These findings suggest that regulation of ROS level by Mst11/2 is complex and more work needs to be done to understand the differential behavior of Mst1/2 in regulating oxidative stress in different cell types.

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