行政院國家科學委員會專題研究計畫　成果報告

人類 Ste20 蛋白激酵素 3 於細胞凋亡中分子作用機轉之研究

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計畫主持人： 袁俊傑

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行政院國家科學委員會補助專題研究計畫成果報告

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□出席國際學術會議心得報告及發表之論文各一份

□國際合作研究計畫國外研究報告書一份

執行單位：國立交通大學生物科技系

中　華　民　國九十五年十月三十日

行政院國家科學委員會專題研究計畫成果報告

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Preparation of NSC Project Reports

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一、中文摘要

蛋白激酵素 Mst3 在細胞凋亡中扮演重要的角色。由共軛焦顯微鏡觀察我們發現 Mst3 會與粒線體螢光標記 Mitotracker、AIF 及 EndoG 之螢光訊號重疊。Mst3 是一粒線體蛋白這一發現已由免疫電子顯微鏡之觀察及細胞分液的西方墨點法等實驗進一步証實，而造成細胞凋亡。進一步實驗我們發現 Mst3 會同時與粒線體中的促凋亡蛋白質結合成促凋亡蛋白複合體，Mst3/AIF/endoG 的複合體亦會在抗癌藥 staurosporine 的刺激下而從粒線體中轉移到細胞核內。在 staurosporine 刺激下所造成的細胞凋亡會有效的受到 Mst3 失活突變株 $Mst3^{KR}$ 的抑制。有趣的是 AIF 及 EndoG 因刺激而有的活化現象亦顯著的受到 $Mst3^{KR}$ 的抑制。我們認為 Mst3 受到刺激後是藉由調控 AIF 及 EndoG 的活性而促成細胞凋亡作用的發生。這些研究結果已經集結成兩篇論文，並交由期刊審閱中。

關鍵詞：Mst3、細胞凋亡、AIF、內切酵素 G

Abstract

Mst3 (mammalian Ste20-like serine/threonine protein kinase 3) was shown to play an important role in the apoptosis. Using confocal microscopy, Mst3 was demonstrated to co-localize with mitochondrial specific marker, Mitotracker, and proteins, such as apoptosis-inducing factor and endonuclease G. The localization of Mst3 in mitochondria was further confirmed by immunoelectron microscopy and Western blotting. In mitochondria, Mst3 associates with AIF and endonuclease G forming a pro-apoptotic complex that may redistribute to the nucleus in response to treatment with staurosporine. The staurosporine-induced apoptosis of HeLa cells can be effectively suppressed by the $Mst3^{KR}$, a dominant negative form. Interestingly, the staurosporine-induced activation of AIF and endonuclease G is also suppressed by $Mst3^{KR}$, suggesting the essential role of Mst3 in regulating AIF and endonuclease G during apoptosis. We conclude that Mst3 may induce cell apoptosis in response to the treatment of apoptotic signal by regulating the activity of AIF and endonuclease G. Two manuscripts bout these studies have been submitted to the SCI journal for reviewing.

Keywords: Mst3, Apoptosis, AIF, Endonuclease G

二、緣由與目的

Apoptosis is a fundamental and indispensable process that eliminates excess cells during tissue homeostasis (1), embryo development (2) and immune system maturation (3). Although caspases are shown to play important roles in apoptosis (4), apoptosis can be induced independent of the activity of caspases. Mitochondria were proven to be essential in the pathway to caspase-independent apoptotic cell death (5). The permeability of outer mitochondrial membrane is altered during apoptosis and causes the release of cytochrome C (Cyto C) and/or other pro-apoptotic proteins, such as apoptotic-inducing factor (AIF) (6), endonuclease G (EndoG) (7), Smac/DIABLO

(8) and DNA fragmentation factor (DFF) endonuclease (9). Subsequently, pro-apoptotic proteins, may translocate to the nucleus and trigger chromosome condensation and DNA fragmentation.

EndoG is responsible for the oxidative stress-indiuced nuclear DNA fragmentation and apoptosis in rat primary hepatocytes (7). Moreover, the ischemia-induced nuclear DAN fragmentation of mouse cerebrum is induced by the nuclear translocation and activation of EndoG (10). AIF, a mitochondrial flavoprotein with NADH oxidase activity, has a dual role in controlling cellular life and death (11,12). In normal mitochondria, AIF may support the energy production by mediating the biogenesis and/or maintenance of complex I (12). However, with the stimulation of apoptotic signal, AIF may translocate from the mitochondria to nucleus to function as a pro-apoptotic factor to trigger the apoptosis (17,30,31).

Mst3 (mammalian sterile 20-like kinase 3), a 47.5 kDa human serine/threonine protein kinase, belongs to a growing family of mammalian Ste20-like protein kinases. Ste20-like family kinases are found to be important in regulating various cellular events in response to environmental cues (13). Although its physiological functions remain unclear, Mst3 has been shown to be essential in triggering apoptosis (14,15). The molecular mechanism that underlies Mst3-induced apoptosis, however, remains unclear. In this report evidences were presented to indicate that Mst3 was found to be present in both cytoplasm and mitochondria. Interestingly, in mitochondria, Mst3 associates with AIF and EndoG. Evidences were further provided that the activity of Mst3 may be required in the staurosporine-induced activation of AIF and EndoG during apoptosis.

三、結果與討論

1) **In this study, we first demonstrated that Mst3 may trigger cell apoptosis via a caspase-independent pathway:** In this study we found that Mst3-induced cell death could not be suppressed by caspase inhibitor, Z-VAD-fmk, as indicated by exogenous β-galactosidase assay.

2) **A mitochondrial localization of Mst3 was also demonstrated:** Mitochondria have been proven to be essential in the pathway to caspase-independent apoptosis. Mst3 may have a role in mediating either the integrity of mitochondria or the activity of mitochondrial pro-apoptotic proteins. Therefore, the subcellular distribution of Mst3 in HeLa cells was investigated. Interestingly, the endogenous Mst3 was found to co-localize with Mitotracker, AIF and EndoG by Immunofluorescent staining, suggesting that Mst3 is probably present in the mitochondria. The mitochondrial localization of Mst3 was also demonstrated by immunogold labeling with specific antibody against Mst3 and the Western blot analysis of the subcellular fractionations. Above results reveal that, in resting cells, Mst3 may be present in both cytoplasm and mitochondria.

3) **Mst3 forms a complex with AIF and EndoG in the IMS of mitochondria:** Mst3 may play a role in regulating the activity of AIF and EndoG. Interestingly, both AIF and EndoG were found to be co-immunoprecipitated with Mst3 using anti-Mst3 antibody. Similarly, Mst3 could be co-immunoprecipitated with AIF and EndoG by anti-AIF and anti-EndoG antibodies, respectively. These results indicate that Mst3, AIF and EndoG form a pro-apoptotic complex in the IMS of the mitochondria.

4) **Staurosporine induces apoptosis of HeLa cells in a Mst3-dependent manner:** Staurosporine has been established to induce the apoptosis of HeLa cells, which may be a Mst3-dependent process (14). As expected, staurosporine induced a significant apoptosis in HeLa and HeLa(pcDNA) cells; while it was attenuated in HeLa($Mst3^{KR}$) cells. These results reveal that Mst3 is required to induce the apoptosis of HeLa cells by staurosporine. Further study shows that the

expression of Mst3 in HeLa cells was unaffected by staurosporine until after 18 h of treatment. These results indicate that the staurosporine-induced apoptosis of HeLa cells is not caused by the increase in the protein level of Mst3, AIF or EndoG.

5) **Staurosporine-induces a nuclear translocation of Mst3, AIF and EndoG:** Mst3 was previously suggested to translocate to the nucleus during apoptosis (14,15). Thus, the subcellular redistribution of Mst3 in HeLa cells in response to staurosporine treatment was investigated. Interestingly, upon the treatment with staurosporine, Mst3 was found to translocate to the nucleus. This result shows that staurosporine can induce the nuclear translocation of Mst3. However, the mechanism by which staurosporine triggers the nuclear translocation of full length Mst3 is unknown.

Further studies show that AIF and EndoG can also translocate to nucleus in response to staurosporine. In nucleus, Mst3 still forms a complex with AIF and EndoG as demonstrated by co-immunoprecipitation with Mst3 antibody.

6) **Mst3 is required for the activation of AIF and EndoG:** We postulate that the activity of AIF and EndoG may be tightly regulated by the associated Mst3 in the pro-apoptotic complex. Hence, the immunoprecipitated endogenous AIF and EndoG from cell extracts of HeLa, HeLa(pcDNA) and HeLa($Mst3^{KR}$) cells before and after staurosporine treatment were subjected to activity assay. The result show that the activity of AIF and EndoG could be greatly induced by staurosporine in both HeLa and HeLa(pcDNA) cells.However, the staurosporine-induced DNA binding capacity of AIF and DNA degradation ability of EndoG was greatly suppressed in the stable clone HeLa($Mst3^{KR}$). These results indicate that the staurosporine-induced activation of AIF and EndoG depends on the activity of Mst3. However, the mechanism by which Mst3 mediates the activation of AIF and EndoG remains to be elucidated.

四、計畫成果自評

The main objective of this project is to elucidate the molecular mechanisms of mammalian Ste20-like protein kinase 3 (Mst3). Specifically, we aim to study following issues: i) What is the molecular mechanism underlying Mst3-meidated apoptosis of HeLa cells? ii) What are the roles of Mst3 in mitochondria as well as in nucleus? iii) Searching for the intracellular proteins that may regulate the subcellular redistribution of Mst3. The results obtained in this study at least fulfill most of the goals proposed in this project. These data have been submitted to top-ranked journal for reviewing (16). In addition, the role of Mst3 in inducing trophoblast apoptosis and facilitating placenta delivery was also studied and submitted to top-ranked journal for reviewing (17).

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