利用小鼠模式探討 Mcl-1 與 Tom70 間交互作用在生理上的重要性

Characterization of the physiological significance of Mcl-1-Tom70 interaction by mouse models

中文摘要

Mcl-1 是一個半衰期很短的 Bcl-2 家族蛋白成員，在抗細胞凋亡中扮演上游重要的角色。在先前實驗中發現 Mcl-1 會與 Tom70 交互作用，且幫助 Mcl-1 定位於粒線體。在這篇論文中我們要利用小鼠模式研究 Mcl-1 與 Tom70 間的交互作用在生理上的重要性。我們建立一個會表現但不會與 Tom70 交互作用的突變 hMcl-1(DM mutant)，接著產生 Wt 與 DM hMcl-1 基因轉殖小鼠去 rescue mcl-1 基因剔除小鼠。我們的數據指出 hMcl-1 蛋白質的表現量對小鼠胚胎的發育十分的重要，只有表現量最高的 line (Wt hMcl-1 Tg#30) 可以得到出生後的 rescued 小鼠。而中等表現相當量的兩個 line (Wt hMcl-1 Tg#20 與 DM hMcl-1 Tg#27)卻無法得到出生之後的 rescued 小鼠，而有趣的是 DM hMcl-1(Tg#27)與 Wt hMcl-l(Tg#20)相比，可以 rescue Mcl-1 基因剃除小鼠胚胎至更進階的程度。我們也利用 rescued 小鼠纖維母細胞(MEF)作爲研究模式，觀察到 DM hMcl-1 在 DNA damage 傷害下與 Wt hMcl-1 相比具有更好的抗細胞凋亡能力，在 ER stress 下兩者就沒有明顯的差異。免疫螢光染色分析中我們無法分辨 Wt 與 DM hMcl-1 定位至粒腺體能力的差異。然而在 cytochrome C 釋出分析中，DM hMcl-1 保護 cytochrome C 由粒線體釋放的能力是比 Wt hMcl-1 差的。此外由共同免疫沉澱實驗中發現 DM hMcl-1 與 Puma 和 Noxa 間的親和力是比 Wt hMcl-1 好的。結合所有實驗指出，在胚胎發育時期，Mcl-1 與 Tom70 間的交互作用扮演著很複雜的角色，而此交互作用生理上的意義需要更深入的研究才可得知。

英文摘要

Mcl-1 is a labile Bcl-2 family member that plays an apical role in many cell survival and death programs. In our previous study, Tom70, is found to be able to facilitate Mcl-1 targeting to the mitochondrial compartment. The purpose of this project is to characterize the physiological significance of Mcl-1's interaction with Tom70 inside a mouse model. To address this issue, transgenic mice over-expressing wild-type or mutant Mcl-1defective in binding to Tom70 (the DM mutant) have been generated and used to rescue Mcl-1 KO mice. Our data show that Mcl-1 protein expression level is important for the development of mouse embryo. Only highly expression line (Wt hMcl-1 Tg#30) but not moderately expression line (Wt hMcl-1 Tg#20) can rescue Mcl-1 KO mouse to post natal stage. Interestingly, with a similar protein expression line, transgenic mice expressing DM hMcl-1(Tg#27) can rescue Mcl-1 KO embryos to a stage that is more advanced than that rescued by the Wt hMcl-1(Tg#20) level.

Furthermore, using mouse embryonic fibroblast (MEF) isolated from Wt or DM hMcl-1 rescued-embryos as model systems, we demonstrate that DM hMcl-1 has a better activity than the Wt protein in protecting DNA damage, but not ER-stress induced apoptosis. Confocal microscopy analysis did not reveal any significant difference in the ability of Wt or DM hMcl-1 to be localized to mitochondria. However, in vitro cytochrome C releasing assay demonstrated that the DM mutant manifests a weaker activity than the Wt protein in protecting cytochrome C releasing from mitochondria. On the other hand, co-immunoprecipitation assay revealed that the DM mutant seems to have a higher affinity than the Wt protein for both Puma and Noxa. Taken together, these results suggest that the Mcl-1 and Tom70 interaction may have a very complicated role during embryo development. The exact functional significance of such interaction needs further investigation.