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◎ 第 35 屆年會暨學術研討會

論 文 集

Imatinib mesylate-induced apoptosis in melanoma cells B16 via reactive oxygen species-dependent mitochondria caspases activation

Imatinib mesylate 藉由活性氧物質刺激線粒體 caspases 活化誘導黑色素瘤細胞 B16 凋亡

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BACKGROUND: Imatinib mesylate (IM) is approved by the FDA for chronic myelogenous leukemia and gastrointestinal stromal tumor treatment and has been shown to regulate the cell growth through targeting the ATP-binding site of BCR-ABL tyrosine kinases, and other receptors tyrosine kinases including platelet derived growth factor receptors α/β and c-kit. IM had been tried on other malignant tumors which over-express those indicated tyrosine kinase receptors. Expression of c-kit and PDGFR in melanoma had been identified, and induction of apoptosis in melanoma cells elicited by IM had been reported *in vitro* and *in vivo*. Except classical caspases-dependent apoptosis pathway, many studies indicated there were other alternative pathways of IM on tumor cell apoptosis such as enhancing human melanoma cell susceptibility to TRAIL-induced cell death or down-regulating telomerase activity to inhibit cell proliferation. Reactive oxygen species (ROS) had been proved to play important roles in initiating cell apoptosis. ROS can be a regulator in cell growth and is abundant in both normal melanocytes and melanoma. ROS is important in regulation of Akt and RAS-MAPK signaling pathways in melanoma cells.

OBJECTIVES: We investigate IM inducing apoptosis in melanoma through ROS generation and which downstream pathways are involved.

METHODS: Melanoma B16F0 was used and cell viability and cytotoxicity were measured by MTT and LDH assay. We studied JNK, p38, Akt, ERK activation by Western blotting with antibodies respectively. ROS generation was studied with flow cytometry and dichlorofluorescein diacetate (DCFH-DA) assay.

RESULTS: Activation of caspase-3 cascade was identified by inducing cleaved caspase-3 protein and its downstream substrate D4-GDL. An increase in intracellular peroxide level is detected and IM-induced apoptosis is significantly inhibited by adding a ROS scavenger N-acetyl cysteine (NAC) in accordance with reducing ROS production. JNK and p38 phosphorylation were activated after IM treatment. Inhibition of JNK or p38 activation by SP-600125 or SB-203580 could enhance IM inducing apoptosis.

CONCLUSIONS: IM induces ROS generation which leads to apoptosis of melanoma cell B16F0. ROS generation activates JNK and p38 pathways and inhibition of JNK and p38 enhances the IM induction of apoptosis in melanoma B16F0.

背景: Imatinib mesylate (IM) 為 FDA 核准於慢性髓性白血病與腸胃道間質瘤之標靶治療藥物，能調控具有 BCR-ABL、 α/β 型血小板生長因子與 c-KIT 酪胺酸激酶接受器的細胞生長。許多研究嘗試使用 IM 來治療過度表現前述酪胺酸激酶接受器的其他惡性細胞；黑色素瘤會表現 α/β 型血小板生長因子與 c-KIT 接受器；研究證實發生體內、外，IM 有抑制黑色素瘤之效果。除了藉由活化 caspases 而誘導的傳統細胞凋亡路徑外，IM 也被證實具有其他誘導細胞凋亡的路徑，例如引起 TRAIL 誘導之細胞死亡或減少終端酶活性，進而抑制細胞增生。

活性氧物質在啟動細胞凋亡生長扮演重要角色。特別是黑色素細胞與黑色素瘤細胞中，存在有豐富的活性氧物質，且已知會調控黑色素瘤細胞的 Akt 與 RAS-MAPK 訊息傳導路徑。我們希望研究活性氧物質在 IM 對於黑色素瘤細胞 B16F0 凋亡表現的角色，與其活化之相關訊息路徑關連性。

方法: 我們利用 MTT 與 LDH 分析方法評估 IM 對於黑色素瘤細胞株 B16F0 的存活與細胞毒性之影響。以不同抗體利用西方墨點實驗觀察 JNK、p38、Akt、ERK 的表現量增加或減少。使用流式細胞儀與 DCFH-DA 測量法評估活性氧物質的表現量。

結果: 經由 caspase 3 的斷裂表現，與其下游蛋白質受體 D4-GDL 的表現增加，證實 IM 經由黑色素瘤細胞 caspase 3 啟動細胞凋亡之路徑；而活性氧物質的產生量與細胞凋亡表現，可以經由添加抗氧化劑 NAC 來阻斷。加入 IM 可以增加 JNK 與 p38 的磷酸化表現，並藉由 JNK 與 p38 抑制劑 SP-600125 與 SB-203580 可以分別增進 IM 誘導黑色素瘤細胞 B16F0 細胞株之凋亡。

結論: IM 可以藉由活性氧物質的產生誘導黑色素瘤細胞 B16F0 細胞株凋亡，且活性氧物質會活化 JNK 與 p38 路徑；而抑制該訊息路徑可以增強 IM 誘導黑色素瘤細胞 B16F0 之表現。