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p53 標的基因探討

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一、中文摘要

非固醇類抗炎性藥物(NSAIDs)如阿斯匹靈(aspirin, acetylsalicylic acid, ASA)已知為許多腫瘤之化學治療藥物。然而其所牽涉之訊息傳導物質則仍未明確。本實驗目的乃在探討 ASA 所誘發細胞凋亡之可能路徑-p53 路徑。以不同濃度之 ASA(0, 0.5, 1, 2, and 4 mM)處理，可使細胞外形顯著改變、降低細胞存活率及 DNA 片段鏈解，顯示 ASA 能誘發細胞凋亡。同時，不論是在時間或劑量的實驗中 p53 蛋白質表現皆伴隨 ASA 劑量之增加而增加。Cox-2 則降低。Caspase-3 於 ASA 處理後 3 小時既顯著誘發。當以 ERK 抑制劑 PD98059 抑制 ERK 活性時 p53 蛋白質顯著增加，此顯示 ERK 扮演相對之角色。ASA 能藉由 p53 路徑誘發細胞之凋亡且與 ERK 之活性表現有關。

關鍵詞：阿斯匹靈、p53、ERK、cox-2、PD98059、細胞凋亡

Abstract

Nonsteroidal anti-inflammatory drugs (NSAIDs) such as acetylsalicylic acid (ASA, also called aspirin) are well known chemotherapeutic agents of cancers; however, the signaling molecules involved remain unclear. The aim of this study was to investigate the possible existence of a putative p53-dependent pathway underlying the acetylsalicylic acid-induced apoptosis in OC-2 cells, a human oral cancer cell line. By increasing concentrations of ASA (0, 0.5, 1, 2, and 4 mM), changes in morphology leading to cell death took place. The MTT assay was employed to quantify differences in cell activity and viability. DNA ladder formation on agarose electrophoresis was also performed. The expression levels of several master regulatory molecules controlling various signal pathways were monitored using the immunoblotting techniques. Patterns of changes in expression were scanned and analyzed using the NIH image 1.56 software. Drastic morphological changes, reduced cell viability, and presence of internucleosomal DNA fragmentation all indicated that ASA is capable of inducing apoptosis in OC-2 cells. In the meanwhile, accumulation of wild type p53 protein significantly increased in time- and dose-dependent manners upon treatment with ASA. The expression of Cox-2 significantly decreased in response to ASA. The caspase-3 induced early at 3 hours after treated with ASA. The p53 protein induction by ASA was markedly enchanced when ERK activation was inhibited by ERK-specific inhibitor PD098059, thus indicating a negative role for ERK. ASA can induce apoptosis via p53-dependent pathway, at least, in cells from this particular oral cancer cell line. Furthermore, the expression of p53 was involved of ERK 1/2 blocking in response to treatment with ASA.

Keywords: acetylsalicylic acid, aspirin, p53,ERK, cox-2, PD098059, apoptosis

二、緣由與目的

Nonsteroidal anti-inflammatory drugs (NSAIDs) such as acetylsalicylic acid (ASA), also called aspirin, have been used as chemotherapeutic agents of cancers to induce apoptosis or reduce the incidence of various cancers, to name a few, like colon[1], lung[2], stomach[3] and colorectum[4]. It has been reported that a 40-50% reduction in mortality

from colorectal cancer patients who took aspirin or other NSAIDs on a regular basis[4]. Apoptosis, also known as programmed cell death, is a major model of cell death induced by medicine used as chemotherapeutic agents. The important function of apoptosis is the removal of those damaged cells containing mutated DNA. This prevents the proliferation of malignant clones or the propagation of cells infected with viral DNA[5].

ASA and other NSAIDs are known to act by directly suppressing cyclooxygenase 1 and 2 (Cox-1 and Cox-2, also known as prostaglandin H synthase), the key enzyme catalyzing the biosynthesis of prostaglandins, thereby blocking the production of proinflammatory prostaglandins[6-7]. Extensive studies of the mechanism of ASA-induced apoptosis have been focused on physio-pathological changes of cyclooxygenases and their regulatory pathways for decades. The current prevailing theory is that the mechanism for the suppressor effect of NSAIDs on carcinogenesis is attributed mainly to the inhibition of Cox-2. However, Zhu and coworkers (1999) reported that addition of PGE_2, the primary intermediate of the action of Cox-2, did not inhibit ASA-induced apoptosis in gastric cancer cells, suggesting that apoptosis was probably mediated by an alternative non-Cox-2 related pathway[8]. Compared to studies of the cyclooxygenase-centered mechanism, our understanding of p53-dependent or non-Cox-related apoptotic pathway induced by NSAIDs is far behind.

p53 is the most frequently mutated gene in human cancers and its functions have been described, including induction of G1 arrest or apoptosis following DNA damage or other cellular insults, moreover, in the maintenance of genomic stability and inhibition of angiogenesis[9-10]. In response to most stressors, the p53 protein level increases rapidly within 1 to 12 hours after treatment with UV or ionizing radiation[11]. The MAP kinase pathway including extracellular signal-regulated kinase (ERK), c-JUN amino-termianl protein kinase (JNN) and p38 MAPK. The activation of JNK and p38 MAP kinase is generally associated with promotion of apoptosis, while p42/44 ERK activity inhibits apoptosis. The caspase-3 is required for DNA fragmentation and some of the typical morphological changes of cells undergoing apoptosis, it initiates apoptotic DNA fragmentation by proteolytically inactivating DFF45 (DNA fragmentation factor-45)/ICAD (inhibitor of caspase-avtivated DNase)[12-13].

The present study was aimed to explore the possible existence of a p53-dependent apoptotic cascade in oral cancer cell line upon ASA treatment. Our data show that ASA is capable of inducing a p53-dependent apoptotic cascade which is under complicated controls of various regulatory molecules. Taken together, a temporary operational model of an ASA-induced COX-independent, and p53-related mechanism underlying apoptosis was proposed.

五、參考文獻

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