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Quercetin down-regulated *bcl*-2 gene expression in human leukemia HL-60 cells[1]

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KEY WORDS HL-60 cells; quercetin; gene expression; *bcl*-2 genes; DNA damage; immunohistochemistry; immunoblotting

AIM: To study the effect of quercetin (Que) on *bcl*-2 gene expression in human leukemia HL-60 cells. **METHODS:** Immunohistochemical analysis and RNA Dot blot hybridization were used to identify the expression of *bcl*-2 genes. **RESULTS:** The expression of *bcl*-2 protein was 94 % in control HL-60 cells, which became 45 % - 84 % when they were cultured with Que 15 - 60 $\mu mol \cdot L^{-1}$ for 48 h. The expression of *bcl*-2 mRNA in HL-60 cells was obviously decreased in treatment with Que 15 - 60 $\mu mol \cdot L^{-1}$. **CONCLUSION:** The apoptotic action of Que in HL-60 cells was associated with the down-regulation of the apoptosis-suppressing gene *bcl*-2.

Quercetin (Que) inhibited the growth of cancer cell lines[1-4]. The anti-proliferative activity of Que might be mediated by the induction of apoptosis[4-6]. The program of cell death by apoptosis was positively and negatively regulated by the products of different genes. The *bcl*-2 gene family is an apoptosis-regulating genes. The gene *bcl*-2 suppressed induction of apoptosis. The level of *bcl*-2 expression in different myeloid leukemias was associated with the degree of resistance to induction of apoptosis by cancer chemotherapy and some other agents[7]. In this study we investigated the effect of Que on *bcl*-2 gene expression in HL-60 cells.

MATERIALS AND METHODS

Materials Que produced by Shanghai Second Chemical Reagent Factory (lot No 900905) was dissolved in Me_2SO and diluted in medium immediately before each experiment with the Me_2SO < 0.1 % concentration. Diethyl pyrocarbonate (DEPC) was from Sigma. Trizol reagent (total RNA isolation reagent), and RPMI-1640 were from Gibco. Prime Gene Labeling System was from Promega. Monoclonal mouse anti-human *bcl*-2 oncoprotein (Clone 124) was from Dako. [α-^{32}P]dCTP was purchased from Yahui Biomedical Engineering Co (Beijing).

Cell culture HL-60, a human promyelocytic leukemia cell line, was cultured in RPMI-1640 medium supplemented with 10 % heat-inactivated fetal bovine serum in 5 % CO_2.

Immunohistochemical analysis To determine if Que decreased the intracellular levels of *bcl*-2, immunohistochemical assays[2] were performed. After washed with PBS buffer twice, the cells were transplanted onto cytospin slides, and fixed with 4 % paraformaldehyde in PBS at 24 ℃ for 10 min. Cells were then washed with PBS and incubated at 24 ℃ for 1 h with an anti-*bcl*-2 mouse monoclonal antibody. Hydrogen peroxide, normal horse blocking serum, biotinylated Igs, avidin-biotin complex, and 3-amino-9-ethylcarbazole substrate solutions were used according to the manufacturer's instruction (ABC ELITE detection system, Vector). The positivity of the reactions was independently assessed in blind fashion by two pathologists: 100 consecutive cells in three or more fields were counted.

Dot blot hybridization Total RNA was prepared from cell cultures[8]. Denaturing gel electrophoresis of RNA was done to confirm the purity of extracted RNA. The concentration of extracted RNA was determined by UV excitation with DU-50 UV spectrophotometer (λ = 260/280 nm). RNA samples were blotted onto nitrocellulose filter in the amount of 10 μg using a Dot blot apparatus. Filters were dried in vacuum oven at 80 ℃ for 2 h. Dried filters were prehybridized, hybridized with *bcl*-2 cDNA probe (850 bp) labeled by random primer method

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(Promega) with [α-^{32}P] dCTP 1.85 MBq, and washed, autoradiographed at −70 ℃ with a Cronex fluorescence enhance screen.

Statistics Positively stained cells were expressed as $\bar{x} \pm s$ and assessed by ANOVA.

RESULTS

Immunolocalization of *bcl*-2 *bcl*-2 was localized to outer mitochondrial membranes and endoplasmic reticulum as well as nuclear membranes (Fig 1).

Expression of *bcl*-2 protein, as analyzed by immunohistochemistry, was 94 % in control HL-60 cells, which became 45 % − 84 % when they were cultured with Que 15-60 μmol·L^{-1} for 48 h (Tab 1).

Expression of *bcl*-2 mRNA HL-60 cells highly expressed *bcl*-2 mRNA. Compared with HL-60 cells in control, the expressions of *bcl*-2 mRNA in HL-60 cells with Que 15 and 60 μmol·L^{-1} were obviously decreased (Fig 2).

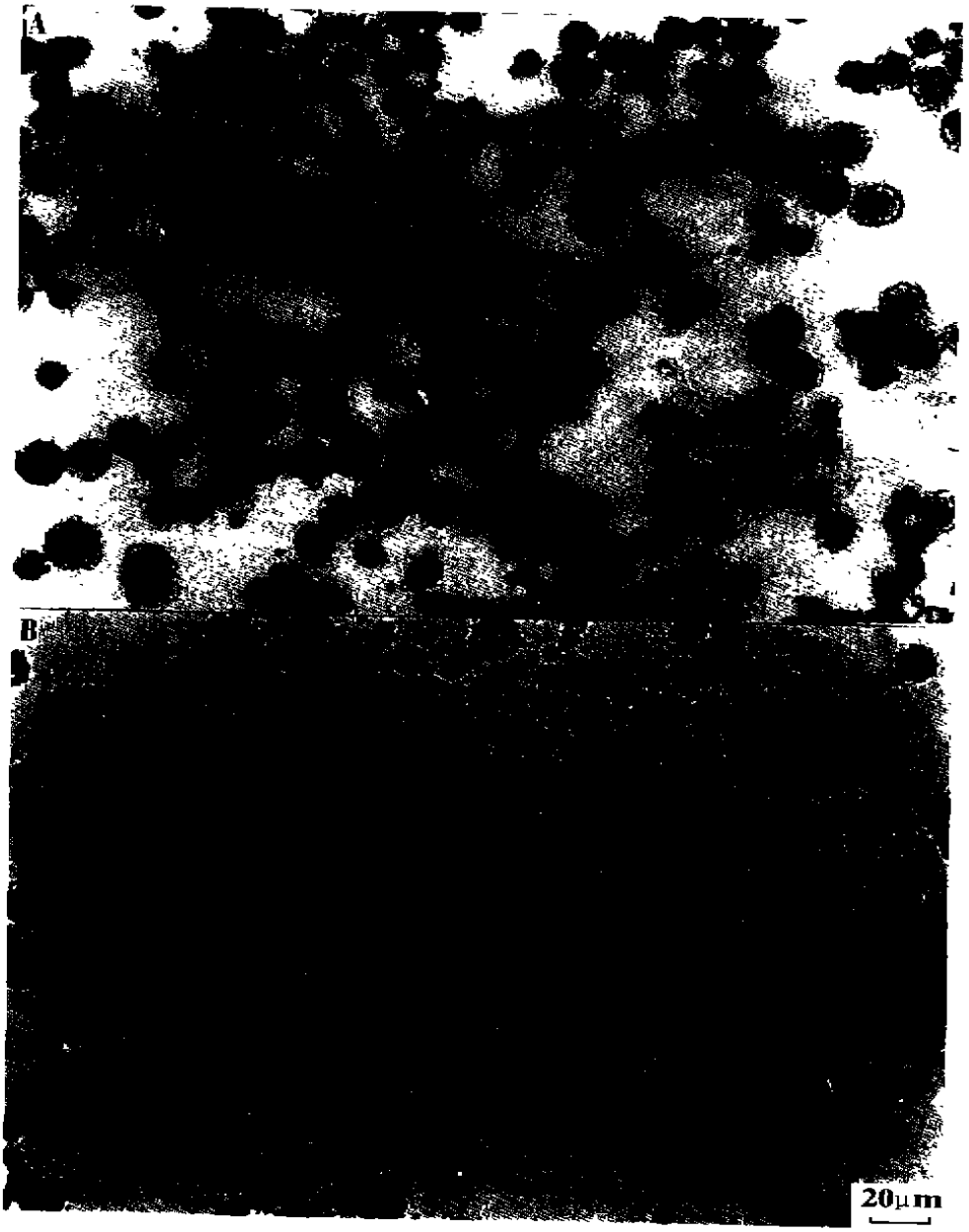

Fig 1. Immunolocalization of *bcl*-2 in HL-60 cells treated with Que for 48 h, × 400. A) Control; B) Que 60 μmol·L^{-1}.

Tab 1. Effect of Que on *bcl*-2 protein expression in HL-60 cells at 48 h. $n = 6$ experiments. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control.

Quercetin/μmol·L^{-1}	Positively stained cells/%
0	94 ± 5
7.5	88 ± 8^a
15	84 ± 9^b
30	54 ± 7^c
60	45 ± 7^c

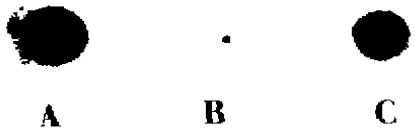

Fig 2. Expression of *bcl*-2 mRNA in HL-60 cells treated with Que for 48 h. (A) Control; (B) Que 60 μmol·L^{-1}; (C) Que 15 μmol·L^{-1}.

DISCUSSION

Que induced apoptosis in HL-60 cells[4] as well as in other cancer cells[5-6]. The program of cell death by apoptosis was positively and negatively regulated by the products of different genes[7]. Que down-regulated the mutant p53 protein levels in the human breast cancer cell line MDA-MB468[5]. *bcl*-2 protected a wide variety of cell types from undergoing apoptosis in response to such diverse stimuli as ionizing radiation, viral infection, or chemotherapeutic agents[9,10]. In our immunohischemical analysis, Que induced a decrease in the level of *bcl*-2 protein in HL-60 cells over 48-h treatment. The effect was dose-dependent and took place within a range of concentrations similar to that to inhibit the proliferation and to induce apoptosis of HL-60 cells[4]. To gain some insight into the mechanism by which Que reduced the level of *bcl*-2 protein, we analyzed its effects on the levels of *bcl*-2 mRNA using Dot blot hybridization. Que treatment decreased *bcl*-2 mRNA levels. This paper demonstrated for the first time that Que specifically inhibited the expression of *bcl*-2 in cellular transformation and transcription in HL-60 cells. This effect was directly related to the growth inhibitory properties and the apoptotic action of Que in this cell line.

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槲皮素下调人白血病 HL-60 细胞 *bcl*-2 基因表达[1]

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关键词 HL-60 细胞; 槲皮素; 基因表达; *bcl*-2 基因; DNA 损伤; 免疫组织化学; 免疫印迹

目的: 研究槲皮素对人白血病 HL-60 细胞 *bcl*-2 基因表达的影响. **方法**: 采用免疫组织化学技术和 RNA 点杂交观察基因表达. **结果**: Que 15 - 60 μmol·L^{-1}处理 HL-60 细胞 48 h 使 *bcl*-2 蛋白由对照组的94 %表达下调到 45 % - 84 %, 并能明显下调 HL-60 细胞 *bcl*-2 mRNA 表达. **结论**: Que 诱导 HL-60 细胞凋亡, 其诱导凋亡的分子机制与下调 *bcl*-2 基因表达有关.