計畫名稱：Effect of Curcumin suppressed metastasis in tongue cell line (SCC-4).

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Abstract

Curcumin from the rhizome of the *Curcuma longa* plant has been noted for its chemo-preventative and chemo-therapy activities, and it inhibits the growth of many types of human cancer cell lines. In this study, the mechanisms of cell death involved in curcumin-induced growth inhibition, including cell cycle arrest and induction of apoptosis in human tongue cancer SCC-4 cells were investigated. Herein, we observed that curcumin inhibited cell growth of SCC-4 cells and induced cell death in a dose-dependent manner. Treatment of SCC-4 cells with curcumin caused a moderate and promoted the G2/M phase arrest, which was accompanied with decreases in cyclin B/CDK1 and CDC25C protein levels. Moreover, curcumin significantly induced apoptosis of SCC-4 cells with a decrease of the Bcl-2 level, reduction of mitochondrial membrane potential (ΔΨm), and promoted the active forms of caspase-3. Curcumin also promoted the releases of AIF and Endo G from mitochondria in SCC-4 cells by using confocal laser microscope. Therefore, we suggest that curcumin induced apoptosis through a mitochondria-dependent pathway in SCC-4 cells. In addition, we also found that curcumin-induced apoptosis of SCC-4 cells was partly through endoplasmic reticulum (ER) stress. In conclusion, curcumin increased G2/M arrest and induced apoptosis through ER stress and mitochondria-dependent pathways in SCC-4 cells.
Figure 1. Curcumin decreased the percentage of viable SCC-4 cells. Cells were cultured in DMEM/F-12 + 10% FBS with 0, 1, 5, 10, 15 or 20 μM of curcumin for 24 h (A) and 48 h (B). The percentage of viable SCC-4 cells was determined as described in Materials and Methods. Each point is mean ± S.D. of three experiments. *p < 0.05, significantly different compared between DMSO-treated control and curcumin treatment.

Figure 2. Curcumin affected on cell cycle distribution and induction of apoptosis in SCC-4 cells. Cells were cultured in DMEM/F-12 + 10% FBS with 10 μM of curcumin for 0, 6, 12, 24 and 48 h. The cells were examined and analyzed for cell cycle distribution (A) and evaluated for the percentage of cell cycle (B) by flow cytometry as described in Materials and Methods.

Figure 3. Curcumin induced chromatin condensation and DNA fragmentation in SCC-4 cells. Cells were cultured in DMEM/F-12 + 10% FBS with various concentrations of curcumin at 5, 10, 20 or 30 μM. The cells were harvested and were examined for apoptosis by DAPI staining for 24 h (A) and 48 h (B) and DNA fragmentation is performed by DNA gel electrophoresis for 48 h (C) as described in Materials and Methods.

Figure 4. Curcumin altered the levels of mitochondria membrane potential (ΔΨₘ), reactive
oxygen species (ROS) and the production of cytosolic Ca^{2+} in SCC-4 cells. Cells were treated with 10 μM of curcumin for 0, 3, 6, 12 and 24 h before being collected, and stained with DiOC₆ (1 μmol/l) for the levels of ΔѰₑₘ (A), DCFH-DA (10 μM) for ROS (B) and Indo 1/AM (3 μg/ml) for cytosolic Ca^{2+} production (C) as described in Materials and Methods. Each experiment was done with triple sets (Mean ± S.D.): *p < 0.05, significantly different when compared with DMSO-treated control group.

**Figure 5.** Representative Western blotting showing changes in the levels of G₂/M phase arrest and apoptosis-associated protein levels in SCC-4 cells after exposure to curcumin. Cells were treated with 10 μM of curcumin for 0, 6, 12, 24 and 48 h before the total proteins were prepared and determined, as described in Materials and Methods. The levels of apoptotic relative protein expressions (A: cyclin B, CDK1 and CDC25C; B: Bcl-2, cytochrome c, AIF, pro-caspase-8, pro-caspase-9 and caspase-3, XIAP and PARP; C: GRP78, pro-ATF-6, PERK and IRE-1) were estimated by Western blotting analysis as described in Materials and Methods. β-actin is as an internal control.

**Figure 6.** The translocations of AIF, Endo G (A), ATF-4 and GADD153 (B) were examined by confocal laser microscopy as described in Materials and Methods. The nuclei were stained by PI (red fluorescence). Areas of colocalization between AIF, Endo G, ATF-4 and GADD153
expressions, cytoplasm and nuclei in the merged panels are yellow. Scale bar, 40 μm.

**Figure 7.** The proposed model of molecular signaling pathways from SCC-4 cells after exposure to curcumin. Curcumin promoted G₂/M phase arrest followed by provoking ER stress and mitochondria-dependent apoptotic death in SCC-4 human tongue cancer SCC-4 cells *in vitro.*
Figure 1

A

Viability (%) vs. Concentration of curcumin (uM)

B

Viability (%) vs. Concentration of curcumin (uM)
Figure 4

A

Mitochondria membrane potential (\%)

Time (hr)

B

Reactive oxygen species (\%)

Time (hrs)

C

Calcium concentration (\%)

Time (hr)
Figure 6

A

Control

48 h

AIF

Endo G

B

Control

48 h

ATF-4

GADD153

Figure 7