Effect of curcumin on the viability of SKOV3 cells and its probable mechanism of action

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Abstract

Purpose: To study the influence of curcumin on the survival of SKOV3 cells and the potential molecular mechanism of action.

Methods: SKOV3 cell proliferation was measured using MTT assay. Gene expression levels were assayed using QRT-PCR assay, while protein expression was determined with western blotting.

Results: Cell viability was reduced by curcumin at doses of 20, 40 and 80 µM (p < 0.05) in a dose-based fashion. Protein expression levels of Bax, caspase-3 and caspase-9 were upregulated by curcumin, while Bcl-2 protein level was downregulated (p < 0.05).

Conclusion: These results demonstrate that curcumin inhibits cell proliferation by promoting the protein expressions of pro-apoptotic genes (Bax, caspase-3 and caspase-9) while suppressing Bcl-2 protein level. Therefore, curcumin might be a novel alternative therapy for ovarian cancer.

Keywords: Curcumin, SKOV3, Apoptosis, Ovarian cancer

INTRODUCTION

Cancer of the ovaries (CO) ranks among the major causes of cancer-related mortality in women globally [1,2]. It is associated with very poor treatment outcomes, with a low 5-year survival [3]. Most CO cases are aggressive, highly metastatic and chromosomally instable [4,5]. The insidious nature of CO and inadequate screening strategies invariably result in delayed diagnosis in patients. The treatment option for CO involves chemotherapy, but it is associated with undesirable adverse effects such alopecia, tiredness, blood loss and deleterious changes in bone marrow, all of which are depressing to the patient [6].

Mixtures of synthetic drugs and herbal extracts have been effectively used for treatment of cancers [7,9]. Apart from producing good therapeutic effects, herbal products have very low toxicity and minimal adverse effects, relative to orthodox anticancer drugs. Thus, herbal products may serve as effective and alternative remedies to conventional drugs used in cancer therapy.

Curcumin, a polyphenolic compound isolated from turmeric, possesses antioxidant and anti-
inflammatory properties. Studies have demonstrated that curcumin exerts anti-carcinogenic potential through induction of apoptotic changes and suppression of angiogenesis [10]. Moreover, evidence for in vivo anti-cancer effects of curcumin have been reported in several investigations which showed its anti-lymphoma properties [11,12]. However, not much is known about the effect of curcumin on DLBCL. This study was aimed at investigating the influence of curcumin on the proliferative potential of SKOV3 cell lines, and the molecular mechanism of action.

EXPERIMENTAL

Chemicals

Curcumin, MTT and N-acetylcysteine (NAC) were products of Sigma. All other reagents and media were of the highest grade.

Anti-tumor assays

The SKOV3 cell lines were cultured in MEM spiked with 5 % FBS at 37 °C in a 5% CO2 incubator. The viability of SKOV3 cancer cells was measured using MTT method. The cells were seeded in 96-well plates at a density of 3 × 10^3 cells/well for each cancer cell line. Curcumin was solubilized DMSO to levels of 20, 40 and 80 μM in each well. A well without curcumin served as control. After incubating the cells for 4 h, 20 μL of DMSO was added to every well, and incubation was continued for three days. Prior to addition of MTT (20 μL/well), the medium was replaced with DMSO to solubilize the resultant formazan crystals, and the absorbance of each well was read at 550 nm in a microplate reader. From the absorbance values, the ED50 was calculated with reference to cells unexposed to curcumin.

Western blotting assay

Harvested SKOV3 cells were subjected to lysis in RIPA buffer spiked with protease and phosphatase blockers. The protein level in the lysate was measured with bicinchoninic acid method. The proteins were subjected to SDS-PAGE and transferred to polyvinylidene difluoride membranes which were incubated with non-fat milk solution (5 %) to block non-specific binding of the blot. Then, the membranes were incubated for 12 h at 4 °C with appropriate 1° antibodies, followed by incubation with HRP-conjugated 2° antibody at laboratory temperature for 60 min. The protein bands were subjected to ECL.

qRT-PCR

TRIzol reagent was applied for extraction of total RNA from the cells. The RNA (1.0 μ) was reverse-transcribed to cDNA. Then, qRT-PCR was carried out in Bio-Rad iCycler iQ5 instrument (Bio-Rad Lab. Inc.). The qRT-PCR reaction mixture comprised 12.5 μL Maxima SYBR Green qPCR Master mix, sense and anti-sense primers (0.3 μL each), 5.9 μL H2O free from nuclease, and 6 μL cDNA template. Cycling conditions were: initial denaturation at 95 °C for 10 min, 45 cycles at 95 °C for 15 sec, 55 °C annealing for 20 sec, extension at 72 °C for 30 sec, in total of 61 cycles. β-Actin was used as internal control. The relative gene expressions were calculated from a standard calibration curve. The primer sequences used are presented in Table 1.

Statistics

Data are presented as mean ± standard deviation. Analysis of variance and Tukey test were used for comparing means amongst the groups. All data analyses were done with GraphPad Prism 5.0 software. Values of p < 0.05 were assumed as indicative of statistical significance.

RESULTS

Effect of curcumin on viability of SKOV3 cells

To illustrate the effect of curcumin on the viability of SKOV3 cells, the potency of curcumin-treated SKOV3 cells was measured with MTT method. At doses below 20 μM, curcumin had no effect on cell viability. However, dose-based suppression of cell viability occurred at curcumin doses >20 μM (Figure 1).

Table 1: Primers used

| Gene   | Sense             | Anti-sense               |
|--------|-------------------|--------------------------|
| Bax    | 5'-TGCTTCAAGGTTCATCCAGG-3' | 5'-TGCGAAAGGCTAGAAAAGGGCCA-3' |
| Bcl-2  | 5'-GGATTGTGGCCCTTCTTGGAG-3' | 5'-CCAAAAGCAAGAGCTGCTTC-3' |
| Caspase 3 | 5'-AATCTGAGCTGAGAGGCAG-3' | 5'-ACAAAGGACTGGAAGACC-3' |
| Caspase 9 | 5'-GGAAGAGGGGACAGATGAG-3' | 5'-TGTTTGGGACAGACCTCAG-3' |
| β-actin | 5'-GTGGGCGGCGCCCAGGCAACCA-3' | 5'-CTCCTTTAATGTGACGACACAGTTTCC-3' |
Figure 1: Curcumin affected SKOV3 cell growth. Group 1 = untreated cells; groups 2, 3 and 4 are 20, 40 and 80 μM curcumin, respectively; **<p < 0.01, vs group 1

Influence of curcumin on apoptotic protein levels in SKOV3 cells

To determine the influence of curcumin on the apoptosis proteins of SKOV3 cells, the apoptosis protein expression levels in cells exposed to curcumin at doses of 20, 40, and 80 μM were assayed using Western blotting. At doses >20 μM, curcumin produced dose-dependent downregulation of Bcl-2 and upregulation of caspase-3, Bax and caspase-9 (Figure 2).

Figure 2: Curcumin affects apoptotic protein levels in SKOV3 cells. Group 1 = untreated; groups 2, 3 and 4 are 20, 40 and 80 μM curcumin, respectively

Effect of curcumin on apoptosis gene expression levels in SKOV3 cells

Curcumin, at levels >20 μM, downregulated the mRNA expressions of the anti-apoptotic gene Bcl-2 and enhanced mRNAs of Bax, caspase-3 and caspase-9 in SKOV3 cells in a dose-based manner (Figure 3).

DISCUSSION

The anticancer effect of curcumin has been demonstrated in several types of cancers [13,14]. Studies have shown that curcumin affects several metabolic processes through many molecular targets [15]. It has also been reported that curcumin does not exert toxic effects in humans even if administered at a dose as high as 10 g daily [16]. The present study has shown that curcumin suppressed the growth of SKOV3 cancer cells.

Figure 3: Effect of curcumin on expression levels of apoptotic genes in SKOV3 cells. Group 1 = untreated; groups 2, 3 and 4 are 20, 40 and 80 μM curcumin, respectively. *<p < 0.05; **<p < 0.01, vs group 1

It belongs to the Bcl-2 family of proteins that regulate cell death (apoptosis) [17]. The ratio of Bax to Bcl-2 is a standard indicator of apoptosis [18] and pro-apoptotic potential [19]. Caspase levels (caspases-3, 8 and 9) are associated with apoptotic potential [20,21]. In this study, curcumin exposure induced changes in concentrations of Bax and Bcl-2. Of particular relevance is the curcumin-induced decrease in Bax level, which point to cell death via the intrinsic route.

CONCLUSION

The findings of this study show that curcumin lowers SKOV3 cell viability, increases pro-apoptotic proteins, and decreases Bcl-2 level, thereby inhibiting SKOV3 tumor growth. These findings may be helpful in the development of a new therapeutic strategy for cancer management.

DECLARATIONS

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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