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Effects of curcumin on bladder cancer cells and development of urothelial tumors in a rat bladder carcinogenesis model

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Abstract

Curcumin, a well-known dietary pigment derived from *Curcuma longa*, inhibited growth of several types of malignant cells both *in vivo* and *in vitro*. Its effects on cell proliferation and the induction of apoptosis in human bladder cancer cell lines and intravesical activity in a rat bladder tumor model were studied. Exposure of human bladder cancer cells to curcumin resulted in the induction of apoptotic cell death and caused cells to arrest in the G2/M phase. The anti-apoptotic Bcl-2 and Survivin protein was downregulated by the curcumin treatment together with enhancement of the Bax and p53 expression. The inhibitory activities of curcumin were stronger than those of cisplatin and could not be prevented by catalase pretreatment in T24 cells. Clonal assay indicated large-dose and short-term curcumin was lethal to bladder cancer cells. Moreover, the *in vivo* study revealed curcumin did induce apoptosis *in situ*, inhibit and slow the development of bladder cancer. These observations suggest that curcumin could prove an effective chemopreventive and chemotherapy agent for bladder cancer.

Keywords: Bladder; Carcinoma; Transitional cell; Curcumin; Inhibition; Apoptosis

1. Introduction

Transitional cell carcinoma of the bladder is a significant health problem worldwide. Many transitional cell carcinoma (TCC) cases are superficial and may be treated with endoscopic resection; However, the recurrence rate is high for tumors treated with resection alone [1,2], which has led to the use of adjuvant therapy with intravesical agents [3]. However, the conventional chemotherapeutic regimens are often intolerable because of the strong systemic toxicity and local irritation [4–6], and the efficacy of available intravesical agents is only 30–70% [7]. These factors highlight the urgent need to find a novel adjuvant agent to reduce the recurrence rate.

Interest in plant food-derived phytochemicals with the capability to decrease the incidence of a variety of tumors has been rising. Food derivatives have the advantage of being relatively nontoxic. However, limited scientific evidence regarding the effectiveness of these natural derivatives in conjunc-

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tion with a lack of mechanistic understanding of their actions has prevented their incorporation into the mainstream of medical care [8]. Curcumin, a popular Indian food spice derived from the rhizome of the plant *Curcuma longa* Linn. (Zingiberaceae), has been recognized as a promising anti-cancer drug due to its multiple properties including anti-inflammatory, anti-oxidant and anti-carcinogenic activities. Among the possibilities, regulation of an array of cellular biochemical processes such as inhibition of nitric oxide synthase, receptor tyrosine kinase and protein kinase C activities and the alteration of transcriptional factors c-jun/AP-1 and nuclear factor κB, p53 by curcumin have been suggested [9]. Various suggested pathways of curcumin activity include the inhibition of arachidonic acid metabolism, lipoxygenase and cycloxygenase activity [10,11]. Moreover, curcumin is known to modulate the activities of certain enzymes involved in the bioactivation and disposition of chemical carcinogens [12]. Based on these observations, curcumin may be a potential cancer chemopreventive agent.

Curcumin may be responsible for the lower incidence of urothelial malignancies [13,14] and the lower rate of colorectal cancer [15] in the regions where curcumin is a staple part of the diet. It was reported that curcumin induced apoptosis of MBT-2 cells [16] and G2/M arrest of T24 cells [17]. Moreover, curcumin effectively inhibited tumor implantation and growth in a mouse intravesical tumor implantation model [16,18] and prevented OH-BBN-induced bladder carcinogenesis in the rodent model carcinogenesis systems [19]. However, little is known about its effect at molecular level on human bladder cancer cells and intravesical activity in the rat bladder tumor model induced by intravesical instillation of *N*-methyl-*N*-nitrosourea (MNU). To gain a better understanding of the effects of curcumin on bladder cancer, we examined whether curcumin has anti-cancer effects on human bladder cancer cells by inducing apoptosis and investigated its mechanism of action and intravesical activity in a MNU-induced rat bladder tumor model.

2. Materials and methods

2.1. Materials

Curcumin, catalase, MNU and MTT was purchased from Sigma. The following antibodies were obtained from Santa Cruz: Bcl-2 mouse monoclonal C-2 antibody; Survivin rabbit polyclonal FL-142 antibody; p53 rabbit polyclonal FL-393 antibody; Bax rabbit polyclonal P-19 antibody; β-actin rabbit polyclonal I-19 antibody; Horseradish peroxidase-conjugated goat anti-mouse and goat anti-rabbit secondary antibody (IgG antibodies). RPMI (Roswell Park Memorial Institute) Medium 1640 was obtained from GIBCO (Invitrogen Corporation). Fetal bovine serum (FBS) was from Hangzhou Sijiqing Biological Engineering Materials Co., Ltd. (Hangzhou, China). BCA protein assay kit and Hoechst Staining Kit for Apoptosis were purchased from Beyotime Biotechnology, Haimen, China.

2.2. Cell culture and treatment

Human bladder tumor cell lines T24, UMUC2 and EJ were cultured in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum, 100 U/ml penicillin and 100 mg/ml streptomycin (complete medium) in a humidified 95% air/5% CO_2 incubator at 37 °C. Cells were allowed to attach for 24 h before treatment. Curcumin (98% purity) was dissolved in dimethylsulfoxide (DMSO) as a 10 mg/ml stock solution and stored at −20 °C. Curcumin was diluted in complete medium and the final concentration of DMSO was not than 1% in clonal assay and *in vivo* study and not than 0.15% in other treatment protocols. Cisplatin (Yamanouchi Inc., Japan) was provided in the form of pure substance and dissolved in culture medium to a stock concentration of 100 mg/ml just before use.

2.3. MTT assay

Bladder cancer cell lines T24, UMUC2, and EJ were seeded at 2×10^3 per well in 96-well culture plates and incubated overnight with RPMI 1640 containing 10% FBS. The cells were then treated with 0–40 μmol/L concentrations of curcumin for 24 or 48 h. The fraction of cells surviving was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The effect of curcumin on growth inhibition was assessed as percent cell viability where vehicle-treated cells were taken as 100% viable. To evaluate the combined effect of cisplatin and curcumin, T24 cells were treated with different concentrations of cisplatin, curcumin or combination, and cell viability was determined as above. To assess possible effects of catalase, T24 cells were pretreated with 100–1000 U/ml catalase for 0.5 h, prior to exposing these different cell samples to curcumin (20 μM) for 24 h, and cell viability was determined as above.

2.4. Clonal assay

Approximately 200 cells of T24 or UMUC2 were plated respectively in 2.5 ml complete medium onto 30 mm tissue culture dishes. Cells were allowed to

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attach for 24 h before the addition of 2.5 ml complete medium containing 0–240 μM curcumin. Dishes were placed in the incubator for one hour, and then gently rinsed with RPMI 1640 three times to remove the curcumin. Complete culture medium was added to each dish. Dishes were returned to the incubator for up to 14 days and surviving tumor cells were allowed to form colonies. After 14 days colonies were fixed with 100% methanol, stained with Giemsa stain and the numbers of colonies per dish were counted. The assay was repeated three times using each cell line. Each experiment was repeated at least for three times to establish the association.

2.5. Detection of apoptosis by fluorescence microscopy

Curcumin induced apoptosis was determined by acridine orange/ethidium bromide (AO/EB) staining as described earlier [20]. Briefly, bladder cancer cells were grown to about 50% confluence and then treated with curcumin (0–20 μmol/l) or cisplatin (0–50 μmol/l) for 24 h.

2.6. Quantification of apoptosis and detection of cell cycle redistribution by flow cytometry

Upon reaching 70–80% confluency, the cells were exposed to 0–40 μM curcumin for 24 h. Cells (1×10^6 cells) were harvested by centrifugation and washed with PBS. The cells were fixed with ice-cold 80% ethanol for 30 min and stained with propidium iodide reagent (50 μg/mL propidium iodide in 0.1% sodium citrate containing 0.1% Triton X-100). Ten thousand events were acquired in a flow cytometer (Coulter EPICS XL, American) and the data were analyzed using multicycle software.

2.7. Western immunoblotting analysis of p53, Bcl-2, Bax and Survivin protein expression

Western blot analysis was done to detect Bcl-2 and Survivin protein expression. The cells were scraped, washed three times with ice-cold PBS, and then lysed in Triton buffer (50 mM Tris–HCl, pH 8.0, 150 mM NaCl, 100 μg/ml PMSF, 1% Triton X-100) for 30 min at ice. Lysates were then cleared by centrifugation at 14,000 rpm, aliquoted, and stored at −80 °C. Protein concentrations were determined with a BCA protein assay kit. 100 μg proteins of different groups boiled for 5 min in sample buffer and were separated in 10 or 15% SDS–PAGE and transferred onto nitrocellulose membrane. Blocking was performed for 1 h at room temperature in blocking buffer consisting of 5% skim milk powder in TBST (100 mM Tris–HCl, pH 7.5, 150 mM sodium chloride, 0.05% Tween-20). The membrane was incubated with Bcl-2, Survivin, p53 or Bax in blocking buffer (1:500) or β-actin (1:2000). After washing in TBST, the membrane was then incubated for 2 h at room temperature with goat anti-rabbit or goat anti-mouse IgG/HRP conjugate in blocking buffer (1:4000). Finally, after washing again in TBST, The blots were developed by use of a SuperEnhanced chemiluminescence detection kit (Applygen Technologies Inc., Beijing, China).

2.8. Tumorigenecity studies in a rodent bladder cancer model

A cohort of 40 animals six to eight week old female Wistar rats (Lanzhou University experiment animal center) was used and divided equally into two treatment groups. The animals were anesthetized with intraperitoneal chloral hydrate. One gram of *N*-methyl-*N*-nitrosourea (MNU) was dissolved in 100 ml of normal saline. The all animals received 0.15 ml (1.5 mg) of this solution via a 22 gauge Teflon angiocath intravesically within 45 min after preparation of the MNU solution, every other week (week 0, 2, 4, 6 and 8) for a total of five doses after the bladder was drained. Whereas the 20 group 1 animals received 0.2 ml of curcumin (240 μM) in complete culture medium, the 20 group 2 received complete culture medium with the same concentration of DMSO alone and served as the control group, every other week (week 1, 3, 5, 7, 9, 11 and 13) for a total of seven doses. The animals remained anesthetized for approximately 2 h after catheterization without urination. Animals were housed at the animal facility at our institution under standard temperature and humidity conditions. The animals were sacrificed by CO_2 gas overdose after 15 weeks and necropsy was performed. The urinary bladder and urethra were excised in toto. The liver and lungs were inspected for metastases. The kidneys and ureters were dissected and inspected. The bladders were bivalved at the dome, fixed in 10% phosphate buffered formalin at least 24 h and embedded in paraffin and submitted for histopathology. Sections were stained with hematoxylin and eosin (H&E) or with Hoechst 33258. The tumors were categorized by histologic grade using conventional criteria. The incidence of tumor growth was scored while blinded to the treatment protocol.

2.9. Statistical analysis

Data are presented as means ± SE. All analysis was performed using SPSS 13.0 statistical software. All error bars shown represent the SE. Comparison of cell viabilities, apoptotic rate, clonal growth and cell cycle redistribution between treatment and control were conducted using Student's *t*-test. χ-square test was used to test the tumor incidence data with 2-tailed significance at $p < 0.05$. $p < 0.05$ was considered statistically significant.

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Comparisons lacking significance are specifically denoted in the legends and text.

3. Results

3.1. Effect of curcumin treatment on cell survival

We first examined the effect of curcumin on cell viability. The treatment of T24, UMUC2 and EJ cells with 0–40 μmol/L concentrations of curcumin significantly decreased cell viability in a dose-dependent and time-dependent manner (Fig. 1A). Catalase pretreatment had no effect on the cytotoxic activity of curcumin in T24 cell line (data not shown).

3.2. Effect of curcumin in combination with cisplatin on the growth of T24 cells

To investigate whether there is any synergism between curcumin and cisplatin, we performed a combinatorial study of curcumin and cisplatin. Whereas 25 μM cisplatin and 20 μM curcumin induced 9 and 52% cytotoxicity, respectively, a combination treatment of them produced only 54% cytotoxicity, which is almost the same as that by 20 μM curcumin (Fig. 1B). Moreover, the other combinations at higher concentration had the similar results. These observations suggest that there was not any synergistic effect between curcumin and cisplatin. Unexpectedly, the inhibitory effect of curcumin was stronger than those of cisplatin.

3.3. Clonal growth assay

To mimic clinical intravesical therapy, 1-h treatment was adopted. Fig. 1C shows the results of the clonal assay in the T24 and UMUC2 cell lines. At 240 μM visual inspection of the all dishes revealed absent cells or colonies, indicating that curcumin was completely lethal to each cell line.

3.4. Apoptosis induced by curcumin in T24 and UMUC2 and by cisplatin in T24 cells

We next determined whether curcumin-mediated inhibition of cell viability in bladder cancer cells is caused by the result of induction of apoptosis. The induction of apoptosis of T24 and UMUC2 by curcumin (10–20 μM) and T24 cells by cisplatin (50 μM) was evident from the morphology of cells as assessed by fluorescence microscopy (Fig. 2A). We next quantified the extent of apoptosis by flow cytometric analysis of curcumin-treated cells. Compared with control, curcumin treatment resulted in 18.2% and 17.2% apoptotic cells at 40 μM on T24 and UMUC2 cells, respectively, for 24 h (Fig. 2B). Consistent with the fluorescence microscopy data, flow cytometry revealed that treatment of T24 and UMUC2 cells with curcumin resulted in a dose-dependent induction of apoptosis. The percent of apoptosis was increased significantly (Fig. 3A).

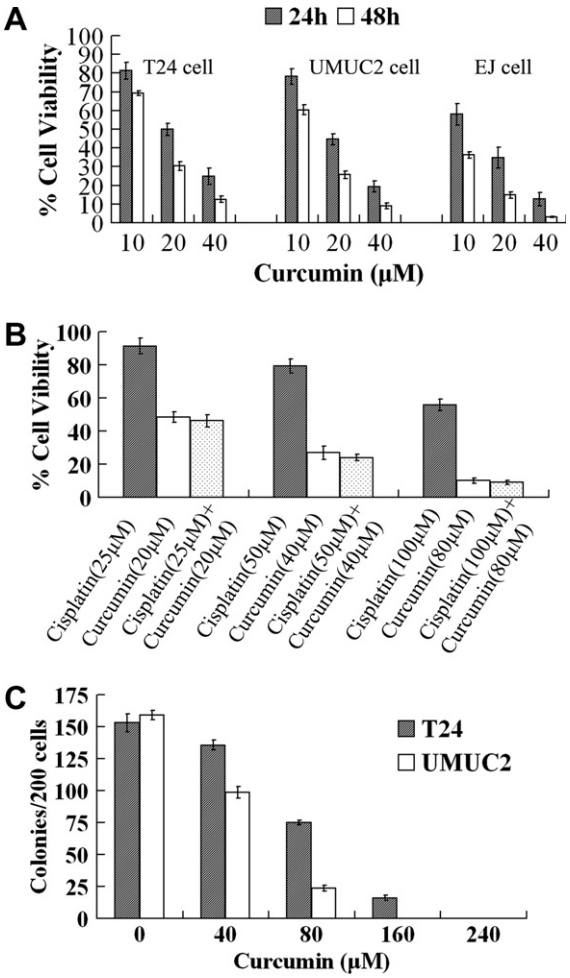

Fig. 1. Effect of curcumin treatment on bladder cancer cell viability, in combination with or without cisplatin and Clonal assay. (A) T24, UMUC2, and EJ cells were treated with 0, 10, 20, and 40 μmol/l curcumin for 24 or 48 h, and the surviving fraction was assayed for by MTT. Each concentration was repeated in 10 wells. Curcumin treatment affects cell viability in a dose-dependent fashion. For each cell line, there are significant differences between groups and within the same duration (24 and 48 h) of treatment (Student's t-test, $p < 0.05$). (B) T24 cells were treated with specified concentrations of curcumin alone, curcumin, or with combination (curcumin + cisplatin) for 24 h, and cell viability was determined by MTT assay. There are significant differences between cisplatin and curcumin or combination treatment (Student's t-test, $p < 0.05$), but no statistically significant difference between curcumin and the combination treatment ($p > 0.05$). (C) Clonal assay for T24 and UMUC2 cells lines At 160 and 240 μM doses curcumin was lethal to UMUC2 line. At 240 μM dose curcumin was lethal to T24 cell line. The data are from three separate experiments. Statistical analysis used student's t-test ($p < 0.05$). Details are described in Section 2.

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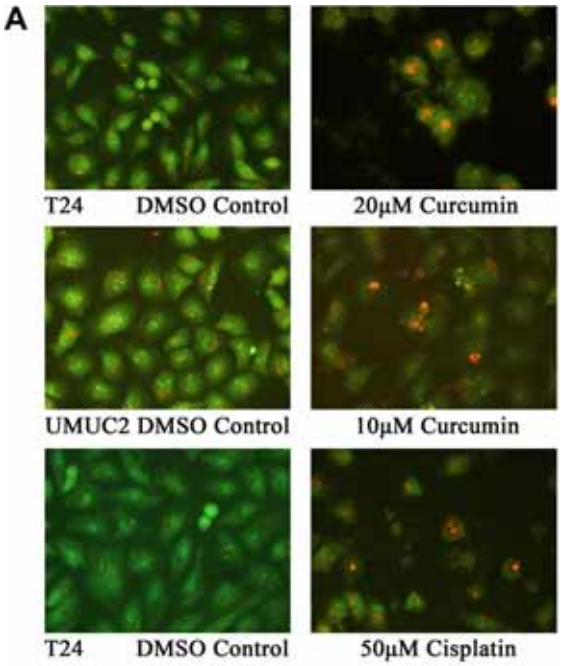

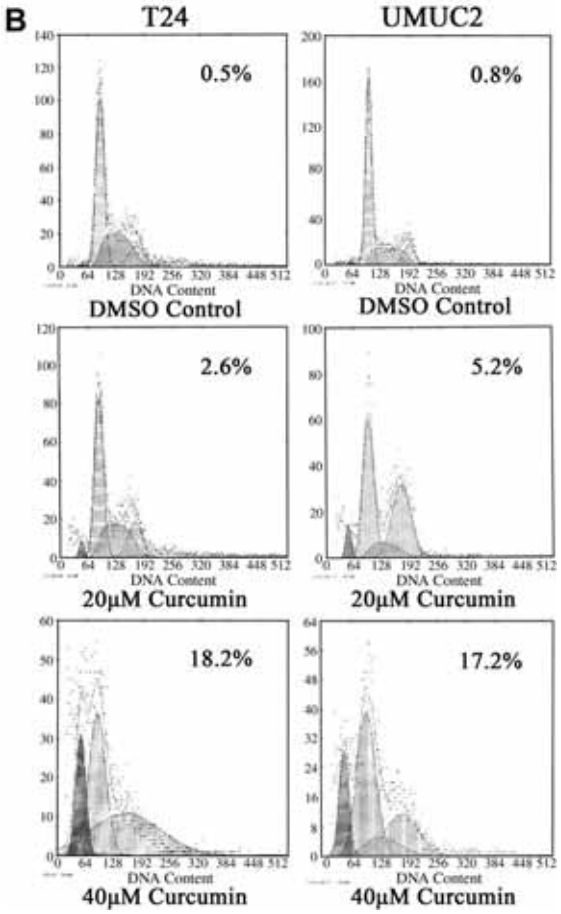

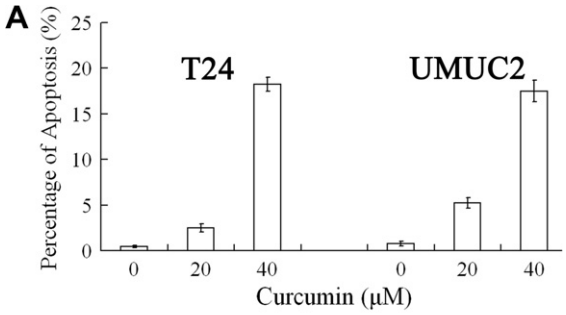

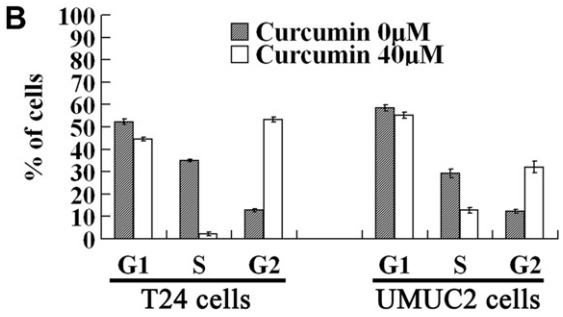

Fig. 3. Quantitative representation of curcumin caused apoptosis (A) and cell cycle redistribution (B) in T24 and UMUC2 cells was assessed by flow cytometry. Cells were exposed to various curcumin concentrations for 24 h. Percent cell cycle was evaluated after propidium iodide staining and flow cytometry of 20,000 acquired events. Details are described in Section 2. Data represent means ± SE of three experiments. Curcumin increased the percent of apoptosis significantly (Student's *t*-test, $p < 0.05$). The flow cytometric analysis revealed that 40 μM curcumin arrested the cell cycle at the G2/M phase and decreased the S fraction in T24 and UMUC2 cells significantly (Student's *t*-test, $p < 0.05$).

3.5. Curcumin induced cell cycle arrest of T24 and UMUC2

The flow cytometric analysis for apoptosis also revealed that 40 μM curcumin arrested the cell cycle at the G2/M phase and decreased the S fraction in T24 and UMUC2 cells significantly (Fig. 3B).

Fig. 2. Curcumin induces apoptosis in T24 and UMUC2 cells and cisplatin in T24 as assessed by fluorescence microscopy (A) and by flow cytometry (B). (A) Representative micrographs of T24 and UMUC2 cells undergoing apoptosis induced by curcumin or cisplatin as assessed by fluorescence microscopy. Cells were treated with vehicle alone or specified concentration of curcumin or cisplatin for 24 h. Apoptotic cells were identified by the condensation and fragmentation of their nuclei. (B) T24 and UMUC2 cells were treated with curcumin (0–40 μM/l for 24 h). Percent apoptosis was evaluated after propidium iodide staining and flow cytometry of 20,000 acquired events. Representative of three independent experiments with similar results. Details are described in Section 2.

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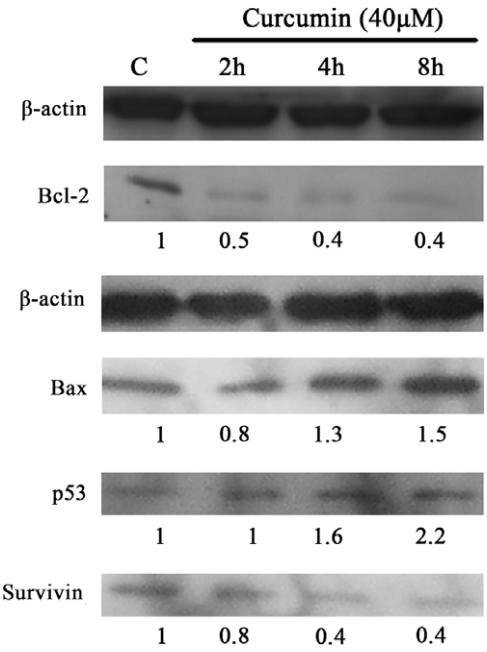

Fig. 4. Effect of curcumin treatment on protein expression of p53, Bcl-2, Bax and Survivin in T24 cells. T24 cells were plated (2×10^6 cells) in 100-mm dishes. At approximately 80% confluence, cells were treated with 40 μmol/L curcumin for 0, 2, 4 and 8 h. Then, whole cell lysate was prepared and resolved on a 12 or 15% gel, blotted against anti-p53, Bcl-2, Bax or Survivin, respectively, and detected by enhanced chemiluminescence. Details are described in Section 2. C, control. Representative immunoblots of three independent experiments with similar results.

3.6. Effect of curcumin on the expression of p53, Bcl-2, Bax and Survivin

To determine the pathway adopted by curcumin to cause apoptosis of T24 cells, we measured the expression of those pro-apoptotic proteins and anti-apoptotic proteins by Western blot analysis. The results indicate that the levels of Bcl-2 and Survivin were decreased, and p53 and Bax were increased, which may have led to apoptosis (Fig. 4). The all protein levels were measured by quantitative Western blot analysis after normalizing with β-actin content.

3.7. Effect of curcumin on tumorigenecity in a rats bladder model induced by intravesical instillation of MNU

Because curcumin was observed to be effective in inhibiting the growth of bladder cancer cells *in vitro*, we next investigated whether these results could be translated into an autochthonous animal model of bladder cancer induced by intravesical instillation of *N*-methyl-*N*-nitrosourea (MNU). The model appears appropriate for screening and developing new intravesical treatments for superficial bladder cancer [21]. Approximately 18% (7 of 40) of all animals did not survived all 5 doses. The caused of death were usually related to complete ulceration of the urinary bladder or urosepsis secondary to urethral stricture formation and urinary obstruction. At week 15, when the bladders were bivalved tumors were grossly visible in bladders with areas of necrosis and hemorrhage. These large tumors weigh as one to two grams (the normal rat bladder weighs approximately 50 mg). Of the 16 group 1 curcumin-treated rats 4 had tumor for an incidence rate of 25%. In the 17 group 2 controls that received culture medium containing dimethyl sulfoxide tumor was evident in the bladder of 12 for an incidence rate of 70.6%. Results were statistically significant for curcumin-treated rats compared with control rats (Table 1). The bladders treated with MNU developed neoplastic changes included hyperplasia, atypia, carcinoma in-situ and papillary TCC (Fig. 5A–C). There were significant differences for histologic stage in curcumin-treated group versus the control group (Table 2). Hoechst 33258 Staining for apoptosis *in situ* revealed that the induction of apoptosis of bladder cancer cells by curcumin treatment was evident from the morphology of cells as assessed by fluorescence microscopy (Fig. 5D–E). From these data, we conclude that curcumin is an effective intravesical agent to inhibit and slow the tumorigenicity of bladder cancer. Moreover, curcumin treatment did not cause any loss in the body weight, food intake, or exhibited apparent signs of toxicity in animals.

Table 1
Effect of curcumin on bladder tumor development in female Wistar rats

	Control	Curcumin
No. subjects	17	16
No. tumor (%)	12 (70.6)	4 (25.0)

The observed differences for tumor development in curcumin-treated rats compared with control rats were statistically significant with $p < 0.05$ (Fisher's exact test).

4. Discussion

Current treatment for superficial disease includes endoscopic tumor ablation. The most effective and widely used agent for intravesical instillation is bacillus Calmette–Guerin. However, side effects of BCG therapy are common, and approximately one third of patients fail to respond [22]. Mitomycin, thiotepa and epirubicin have been used as agents to prevent recurrence but they are not without side effects. Oosterlinck et al. reported that administering epirubicin immediately after bladder tumor resection was beneficial for preventing implantation but no long-term survival benefit or freedom from

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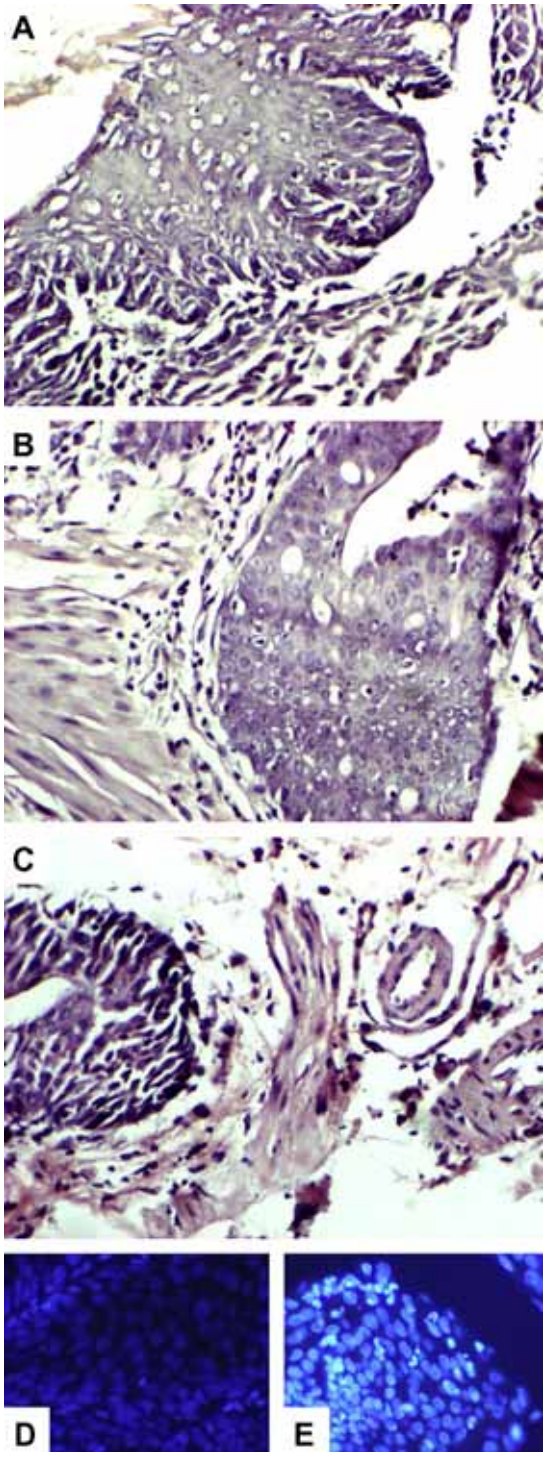
disease progression was evident [23]. The toxicity and incomplete efficacy of the commonly used intravesical agents has promoted a research for other treatments for superficial TCC of the bladder.



Curcumin has been consumed for centuries in Asia. It has the advantage of being a nontoxic natural product [24]. The pharmacological safety of curcumin is shown by the nontoxic consumption of up to 8000 mg/day when taken by mouth for 3 months in humans [25] and up to 5 g/kg in rats [24,26]. In addition, curcumin is nonmutagenic [24,27]. Interestingly, curcumin, up to 50 μM, had no effect on normal cells [28,29] and did not prevent cell growth of normal peripheral blood mononuclear cells [30]. However, the bioavailability of curcumin *in vivo* is low after oral ingestion [26,31], but can be dramatically elevated by coingestion of piperine in both rats and humans [32]. The average peak serum concentrations after taking 8000 mg of curcumin in patient were no more than two microM and urinary excretion of curcumin was undetectable [25], but the concentrations used in many *in vitro* study for obviously anti-tumor effect usually were ten or more microM [29] which could not be achieved *in vivo* in humans following oral administration, making their results less relevant for *in vivo* use. Of course, if the concentration was achieved in human serum through intravenous injection, the safety must be evaluated again. We supposed that the high lipophilia of curcumin with poor water solubility lead to poor absorption, the later may be the reason of its safety after taken orally. So we hypothesized that there would no system toxicity if curcumin was instilled intravesically because of poor absorption just like oral administration, moreover, a large doses could be used.

The anti-neoplastic activity of curcumin has been studied in various cell lines [33–36]. Various mechanisms have been proposed to explain the anti-tumor activity of curcumin. Curcumin has been shown to induce apoptosis in many tumor cell lines [37,38], including those of bladder origin [16]. In the present study, evidence of apoptosis was found in T24 and UMUC2 cells exposed to curcumin. More specifically, the flow cytometry analysis, AO/EB staining positively identified markers of apoptosis. The pres-

Fig. 5. Sections staining with hematoxylin and eosin (H&E) for histopathology (A–C) or with Hoechst 33258 for apoptosis (D–E). Wistar female rat bladder treated with five doses of MNU with hyperplasia (A, from curcumin treatment), with areas of marked atypia and dysplasia involving full mucosal thickness (B, from curcumin treatment), with muscle invasive TCC (C, from control)). (D–E) Apoptotic cells were identified by the condensation and fragmentation of their nuclei. (D) From control. (E) From curcumin treatment.

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Table 2
Histopathological findings as evaluated by TNM system in female Wistar rat bladders treated with five doses of intravesical MUN and sacrificed at 15 weeks after initiation of the carcinogen

Treatment group	Percent with hyperplasia, flat or papillary atypia/dysplasia	Percent with superficial TCC	Percent with muscle invasion
Control $N = 17$	5 (29.4)	9 (52.9)	3 (17.6)
Curcumin $N = 16$	12 (75.0)	4 (25.0)	0 (0)

The observed differences for histologic stage in curcumin-treated rats versus the control group were statistically significant with $p < 0.05$ (Pearson Chi-square test).

ent findings corroborate and expand similar findings by Sindhwani and coworkers [16], who earlier reported that curcumin induced features characteristic of apoptosis in bladder cancer cells. Moreover, There is evidence that curcumin promotes formation of ROS [39,40], and in a previous study of leukemia cell lines curcumin induced apoptosis was prevented by anti-oxidants [41], Hence, an experiment was performed to determine if anti-oxidant could prevent the cytotoxic effect caused by curcumin. Unexpectedly, in our experiment potent anti-oxidant catalase did not prevent the cytotoxic effect caused by curcumin in the T24 cell lines. The apoptosis may be not mediated through the generation of reactive oxygen species. Previous studies have shown that curcumin potentiated the apoptotic effects of the chemotherapeutic agents (gemcitabine and paclitaxel) [29,42]. But in our experiment, there was not any synergistic effect between curcumin and cisplatin, although both curcumin and cisplatin induced T24 cells apoptosis. Unexpectedly, the inhibitory activities of curcumin were stronger than those of cisplatin.

Tumor cells often evade apoptosis by over-expressing anti-apoptotic proteins such as Bcl-2, NF-κB, Survivin, etc., which give them a survival advantage. Curcumin is a known inhibitor of nuclear factor-κB activation, which is essential for the induction of bcl-2 protein [43]. Bax, the pro-apoptotic member of the Bcl-2 family, has also been shown to be a p53 target and is up-regulated in a number of systems during p53-mediated apoptosis. On the other hand, Bcl-2 and Bax are the key factors for apoptosis or survival and cells are into apoptosis or survival depending on the ratio of Bax to Bcl-2. We found that there was reduced expression of Bcl-2 and Survivin together with enhancement of the Bax and p53 expression. Our observation leads to the possibility that curcumin induces T24 cell apoptosis in a p53-dependent pathway. Survivin is main factors for apoptosis resistance and are overexpressed in bladder tumors [44], and its expression correlates with tumor grade, recurrence risk, and survival in bladder cancer [45]. Therefore, curcumin's inhibition of Survivin expression is a good reason to make further research. Several studies have reported the role of curcumin in the growth arrest of cancer cells in culture and in animal models [46]. These studies have implicated curcumin-induced growth arrest, mostly at the G2/M stage. In line with their report, we found curcumin treatment caused cell cycle arrest at G2/M phase.

Many studies suggested curcumin has potential of chemopreventive and chemotherapy effect *in vivo* [25,34]. Curcumin inhibits tumor promotion against skin, oral, intestinal, and colon carcinogenesis [47–49]. For example, ingestion of curcumin by mice with familial adenomatous polyposis resulted in a reduction of intestinal tumors in the animals, in association with increased apoptosis in enterocytes of the intestinal tissue examined [50]. *In vitro* studies indicated that curcumin was lethal to bladder cancer cells at higher concentration within short-term through apoptosis, and it effectively inhibits tumor implantation and growth in murine bladder as an intravesical agent [13]. Our *in vitro* study also revealed that 240 μM curcumin was completely lethal to the 2 cell lines after a 1-h incubation period. Since triggering the 'molecular switch' of apoptosis may not take long [51], it would be possible to induce apoptosis of TCC cells by transient chemotherapy. Based on these results the same dose of curcumin and 2-h dwell time to mimic clinical instillation was selected for our *in vitro* experiments. Curcumin did inhibit and slow the tumorigenicity of bladder cancer. In line with *in vitro*, apoptosis was induced *in situ* by curcumin treatment. At the same time, curcumin treatment did not cause any side-effect. Further studies are in progress in our laboratory to determine whether or not administration of curcumin to animals by intraperitoneal or intravenous injection leads to better effects on preventing the development and/or the

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growth of tumors in the rat bladder tumor model, and the safety would be evaluated.

5. Conclusions

Taken together, our present findings showed the anti-cancer efficacy of curcumin against human bladder cancer cells *in vitro* and *in vivo*, especially, as an intravesical agent, it did inhibit tumor development and growth in murine bladder.

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