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Anti-proliferative benefit of curcumol on human bladder cancer cells via inactivating EZH2 effector

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ABSTRACT

We investigated the molecular mechanism of curcumol-induced apoptosis in bladder cancer cells. The mitochondrial membrane potential was measured using JC-1 staining. ROS generation of bladder cancer cells was determined using the DCFH staining method. The apoptosis of bladder cancer cells was examined using the Annexin V-FITC and PI double-staining method. Enforced expression of EZH2 in bladder cancer cells was accomplished by transfecting an EZH2 expression plasmidinto EJ and T24 cells. siRNAs targeting EZH2 were used to inhibit endogenous expression of EZH2. Curcumol dose-dependently inhibited proliferation and colony formation and induced apoptosis in EJ and T24 bladder cancer cells. These effects correlated with decreased accumulation of EZH2. In addition, suppression of EZH2 enhanced the inhibitory effects of curcumol on cell growth and colony formation and increased curcumol-induced apoptosis. Conversely, enforced expression of EZH2 ameliorated the inhibitory effects of curcumol on cell growth and colony formation and decreased curcumol-induced apoptosis in EJ and T24 cells. We also found that suppression of EZH2 induced ROS generation and MMP loss in both EJ and T24 cells. Conversely, up-regulation of EZH2 suppressed ROS generation and MMP loss. Our data indicate that curcumol inhibits proliferation and induces apoptosis by targeting EZH2 and modulating the mitochondrial apoptosis pathway.

1. Introduction

Bladder cancer is the most common malignant tumor of the urinary system and has the highest morbidity rate of all urinary system cancers. Its etiology is unclear. The most common treatments for bladder cancer are surgery, radiation and chemotherapy. Gene therapy is used to supplement the existing treatments. Chemotherapy often causes damage to normal tissues and produces a variety of side effects. The most urgent task is to find effective drugs that target tumor cells and do not harm normal cells.

Curcumol is the main active ingredient in Rhizoma Curcuma, a common traditional Chinese medicine (TCM). It is reported to possess antitumor [1–8] and anti-inflammatory properties [9], but little is known about the molecular mechanism underlying these effects. Recent studies have attempted to investigate the antitumor activity of curcumol in various types of cancers, including lung cancer [1], esophageal carcinoma [3], colorectal cancer [7], breast cancer [8]and nasopharyngeal carcinoma [10]. These studies indicate that the p38 MAPK [7] and NF-kappaB [8]signaling pathways are involved in the antitumor effects of curcumol.

Enhancer of zeste homolog 2 (EZH2) is a member of the Polycomb-group (PcG) family [11]. PcG family members form multimeric protein complexes that are involved in maintaining the transcriptional repression of genes over successive cell generations. EZH2 associates with the embryonic ectoderm development protein, the VAV1 oncoprotein, and the X-linked nuclear protein. EZH2 may play a role in the hematopoietic and central nervous systems. Recent research has demonstrated that EZH2 plays an important role in tumorigenesis, metastasis and invasion. Targeting EZH2 is now considered to be a therapeutic strategy in cancer [12], and EZH2 inhibitors are showing early signs of promise

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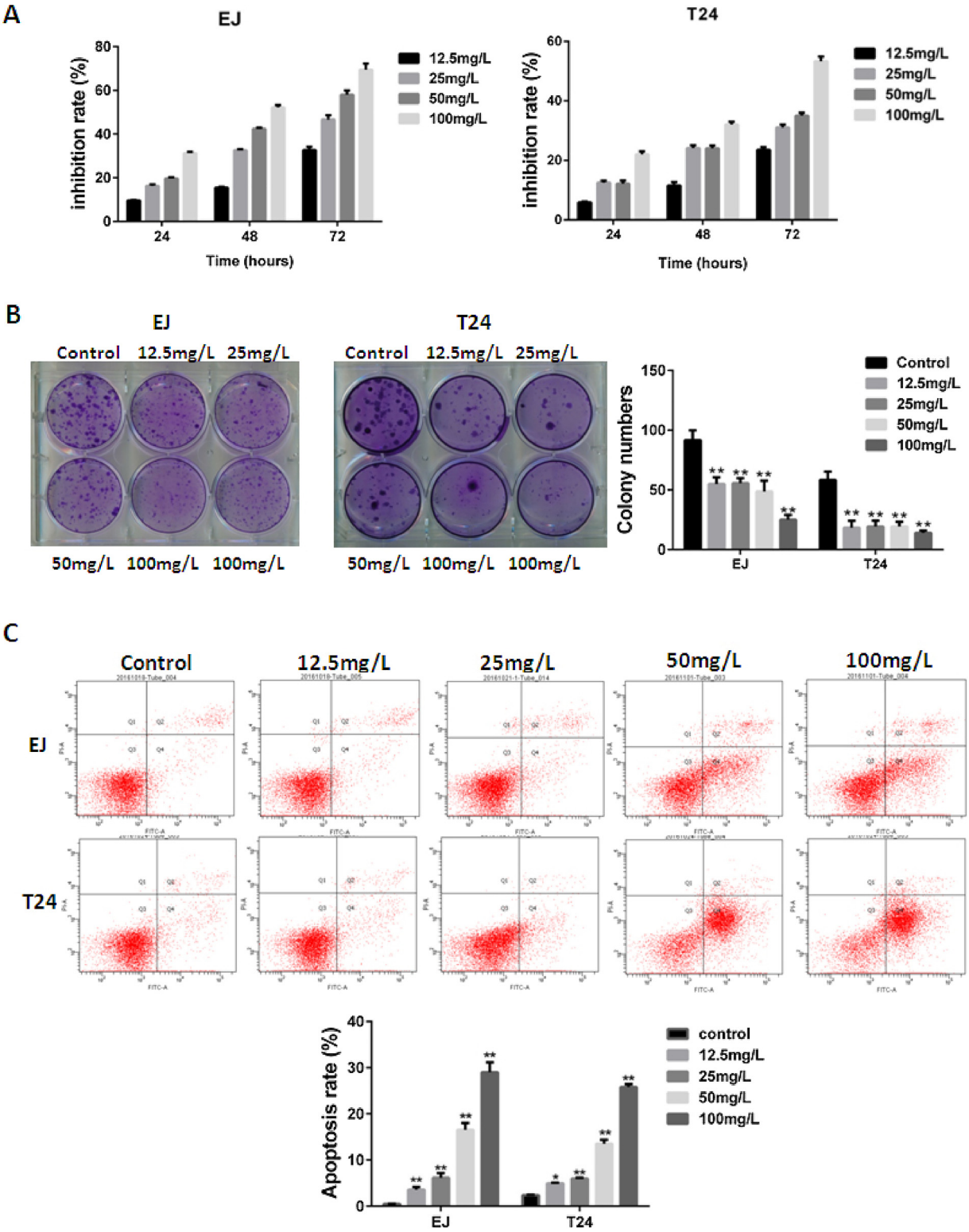

Fig. 1. Curcumol induces growth inhibition and apoptosis in bladder cancer cells. EJ and T24 cells were exposed to different concentrations of curcumol (12.5 mg/L, 25 mg/L, 50 mg/L and 100 mg/L) for the indicated times. CCK-8 assays (A), colony formation assays (B) and apoptosis assays (C) were performed to examine cell growth, colony formation and apoptosis. Data represent the means ± SD of at least three independent experiments. **, $P < 0.01$.

in clinical trials.

In the current study, we investigated the mechanism underlying the inhibitory effects of curcumol on bladder cancer cells. Two bladder cancer cell lines, EJ and T24, were used. We investigated the effects of EZH2 on curcumol-induced growth inhibition, apoptosis, loss of mitochondrial membrane potential (MMP) and ROS generation. We also investigated the effects of EZH2 on key factors in the mitochondrial apoptosis pathway, including Bcl-2, BAX, BAK1 and cytochrome. Our work provides a better understanding of the molecular mechanism of curcumol-induced growth inhibition.