Technology in Cancer Research & Treatment
Volume 19: 1-9

DOI: 10.1177/1533033820926591
journals.sagepub.com/home/tct
SAGE

Desloratadine, a Novel Antigrowth Reagent for Bladder Cancer

Jianfeng Ma, MM[1], Jinchun Qi, MM, PhD[2], Shoubin Li, MM[3], Chaohua Zhang, MM[1], Haijiang Wang, MM[1], Lijun Shao, MM[1], Xiaofei Yuan, BM[1], and Quan Sha, MM[1]

Abstract

Desloratadine, a potent antagonist for human histamine H1 receptor, has been revealed to exhibit antihistaminic activity and anti-inflammatory activity. However, it is not yet known whether desloratadine has any effect on the biological behaviors of tumor cells. In this study, we aimed to investigate the effects of desloratadine on cell growth and invasion in bladder cancer EJ and SW780 cells *in vitro*. We observed that desloratadine inhibited cell viability of EJ and SW780 cells in a dose- and time-dependent manner. Desloratadine treatment was also revealed to suppress colony-formation ability and induce cell cycle arrest at G1 phase in EJ cells. Desloratadine promoted cell apoptosis via modulating the expression of Bcl-2, Bax, cleaved caspase 3, and cleaved caspase 9 in EJ and SW780 cells. Western blot resulted showed that desloratadine also impaired the expression of autophagy-related proteins, such as Beclin 1, P62, and LC3I/II in EJ and SW780 cells; while autophagy inhibitor LY294002 reversed the effects of desloratadine on these proteins. Moreover, desloratadine remarkably attenuated cell migration and invasion. Furthermore, we illustrated that desloratadine downregulated the expression of N-cadherin, Vimentin, Snail1, and Snail2, while upregulated the expression of E-cadherin in EJ and SW780 cells *in vitro*. The level of interleukin 6 was reduced in desloratadine-treated cells, while upregulation of interleukin 6 significantly abolished the anticancer activity of desloratadine in cell invasion and Bcl-2, Bax, Beclin1, LC3-I/II, N-cadherin, and E-cadherin expression in EJ cells. Taken together, our data suggest a potential anticancer activity of desloratadine on cell growth and invasion for bladder cancer, which may be mediated by diminishing the epithelial-to-mesenchymal transition and interleukin 6.

Keywords

desloratadine, bladder cancer, cell cycle, apoptosis, EMT

Abbreviations

CC8, Cell Counting Kit 8; DMSO, dimethyl sulfoxide; EMT, epithelial-to-mesenchymal transition; IL, interleukin; NC, negative control; OD, optical density; PI, propidium iodide; RC, radical cystectomy

Received: August 29, 2019; Revised: March 9, 2020; Accepted: March 30, 2020.

Introduction

Bladder cancer is the most prevalent urologic tumor worldwide, with approximately 350 000 new cases in the world annually.[1,2] Despite advances in the treatment of bladder cancer, including radical cystectomy (RC), radiotherapy and postoperative chemotherapy, or immunotherapy, the prognosis of patients with bladder cancer is still unsatisfied due to its high frequency of recurrence and metastasis.[3-5] Hence, discovering and identifying novel antitumor reagent is urgently needed. Currently, increasing studies are now focusing on reassessing known drugs for other diseases in order to discover their potential antitumor effects.[6]

Desloratadine, a potent antagonist for human histamine H1 receptor, is originally used for the treatment of allergies and allergic rhinitis.[7,8] Desloratadine has been reported to exhibit

[1] Department of Urology Surgery, Baoding No. 1 Hospital, Baoding, China
[2] Department of Urology Surgery, Second Affiliated Hospital of Hebei Medical University, Shijiazhuang, China
[3] Hebei General Hospital, Shijiazhuang, China

Corresponding Author:
Jianfeng Ma, Department of Urology Surgery, Baoding No. 1 Hospital, No. 966 Baihua East Road, Baoding 071000, China.
Email: majianfeng01@aliyun.com

antihistaminic activity and anti-inflammatory activity.[7] Desloratadine inhibits the production and release of proinflammatory cytokines, such as the interleukin (IL) 6 and IL-8. It is demonstrated that desloratadine has no adverse effects on the central nervous system, cardiovascular system, or gastrointestinal system, and no significant drug interactions.[7] However, whether desloratadine affects the biological behavior of cancers is not well understood.

In the current study, the anticancer property of desloratadine on bladder cancer was investigated *in vitro*. For the first time, our data revealed that desloratadine suppressed cell viability, growth, migration, and invasion of bladder cancer, suggesting a potential anticancer activity of desloratadine for bladder cancer therapy. Furthermore, we also found that desloratadine promoted cell apoptosis and autophagy and inhibited epithelial-to-mesenchymal transition (EMT) process in bladder cancer, which might be the mechanism underlying the anticancer activity of desloratadine.

Materials and Methods

Cell Culture

The human bladder cancer cell lines EJ and SW780 were obtained from the Cell Bank of Chinese Academy of Sciences (Shanghai, China). Cells were cultured in Dulbecco modified Eagle medium (Hyclone) that contained 10% fetal bovine serum (Gibco) and antibiotics (100 U/mL, Sigma Aldrich) at 37 °C with 5% CO_2. LY294002 was obtained from MedChemExpress.

Dose-Dependent Assay

EJ and SW780 cells were seeded into 96-well plates and then treated with different concentrations of desloratadine (0, 0.5, 1, 2, 4, 8, 16, 24, 32, and 64 μM, MedChemExpress) for 24 hours. After treatment, Cell Counting Kit 8 (CCK8) reagent (10 μL/well, Beijing Solarbio Science & Technology) was added into each well, followed by incubation at 37 °C for 90 minutes. The optical density (OD) was measured at 450 nm.

CCK8 Assay

Cell Counting Kit 8 was used to assess the effect of desloratadine on cell viability. Briefly, EJ and SW780 cells were seeded in 96-well plates at 1×10^3 per well and cultured for 24 hours; 32 or 12 μM of desloratadine was added into medium to treat EJ or SW780 cells for 24, 48, 72, and 96 hours, respectively; Dimethyl sulfoxide (DMSO) was used as negative control (NC). With an addition of CCK8 reagent, cells were incubated at 37 °C for 90 minutes, following by the measurement of OD value.

Colony Forming Assay

Cells were seeded in 6-cm culture dish at a density of 500 per well, followed by the treatment with desloratadine. After incubation for 1 week at 37 °C with 5% CO_2, visible colonies were fixed in 5 mL of 4% paraformaldehyde for 30 minutes followed by staining with 0.1% crystal violet for 30 minutes. The colonies were counted and imaged.

Cell Cycle Analysis

Flow cytometry was used for assessing the effect of desloratadine on cell cycle distribution. After treated with desloratadine or DMSO for 24 hours at 37 °C, cells were harvested and fixed in 70% precooling ethanol at −20 °C overnight. Then, cells were stained with propidium iodide (PI), and cell cycle analysis was performed by a flow cytometer (BD FACSC anto II, BD Biosciences). The obtained data were analyzed using BD FACSDiva software (BD Bioscience).

Cell Apoptosis Analysis

Annexin V-fluorescein isothiocyanate (FITC)/PI kit (BD Bioscience) was used for detection of cell apoptosis. After treated with desloratadine or DMSO at 37 °C for 24 hours, cells were collected and resuspended in 1× binding buffer at a density of 1 to 5×10^6 cells/mL; 100 μL of cell suspensions was incubated with 5 μL of Annexin V-FITC for 5 minutes in the dark prior to staining with 10 μL of PI. The samples were analyzed using flow cytometer and calculated by BD FACSDiva software.

Wound-Healing Assay

Wound-healing assay was performed for assessment of cell migration ability. Cells were seeded in 6-well plates at a density of 5×10^5 cells/well for 12 hours. After that, wounds were generated using pipette tips followed by the treatment with desloratadine in serum-free medium for 24 hours. The wound closure was imaged and analyzed using ImageJ software (National Institutes of Health).

Cell Invasion Assay

Transwell chamber (Millipore) coated with Matrigel (BD Bioscience) was carried out for cell invasion assay. Cells treated with desloratadine or DMSO at 37 °C for 24 hours were trypsinized and resuspended in serum-free medium at 1×10^6 cells/mL; 100 μL of cell suspensions was added into the upper compartment of Transwell chambers, meanwhile the complete medium was added to the lower chamber as the chemo attractant. Following the incubation at 37 °C for 24 hours, the non-invaded cells were wiped off with a cotton swab. The invaded cells were fixed with 4% paraformaldehyde for 30 minutes followed by staining with 0.1% crystal violet for 20 minutes. The invaded cells were imaged (magnification, ×40) and counted under the microscope.

Western Blot Analysis

Cells were collected after 24 hours of treatment with desloratadine or DMSO prior to protein extraction using radioimmunoprecipitation assay lysis buffer (CWBIO). Equal amounts of proteins were electrophoresed by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis gel followed by transferring onto a polyvinylidene fluoride membrane (Millipore). Thereafter, 5% nonfat milk was used to block the membrane prior to the incubation with primary antibodies (1:1000, Proteintech Group) at 4 °C overnight. Subsequent to that, the membrane was incubated with the horseradish peroxidase-conjugated secondary antibodies (1:3000, Proteintech Group) for 1 hour. Finally, an enhanced chemiluminescence kit (CWBIO) was performed for signal development. Glyceraldehyde 3-phosphate dehydrogenase was used as an internal control.

ELISA Assay

After the cells were treated with desloratadine for 24 hours, the supernatant was collected and centrifuged at 300*g* for 10 minutes. Enzyme-linked immunosorbent assay kit (R&D Systems) was performed to detect IL-6 level according to the manufacturer's instructions.

Statistical Analysis

Each assay was conducted at least 3 times independently, and the data were expressed as mean ± standard deviation. GraphPad Prism 7.0 was employed for statistical analysis and comparisons between 2 groups were estimated with student *t* test or 1-way analysis of variance. $P < .05$ was considered statistically significant.

Results

Desloratadine Inhibits the Viability and Growth of Bladder Cancer Cells

In order to assess whether desloratadine affects the biological function of bladder cancer, bladder cancer EJ cells were treated with different concentrations of desloratadine (0, 0.5, 1, 2, 4, 8, 16, 24, 32, and 64 μM). As indicated in Figure 1A, after treatment for 24 hours, cells treated with 24, 32, and 64 μM of desloratadine presented significantly decreased viability by CCK8 assay ($P < .05$). The half-inhibitory concentration (IC_{50}) of desloratadine for EJ cells was 47.32 μM, and 32 μM of desloratadine was used for EJ cells in all the rest experiments for the appropriate effect, DMSO was used as NC. While desloratadine with a concentration of 8 μM or more significantly inhibited SW780 cell viability (Figure 1B), the IC_{50} of desloratadine for SW780 cells was 18.21 μM, and 12 μM of desloratadine was used for SW780 cells in all the rest experiments. To further determine the effect of desloratadine on cell proliferation and viability *in vitro*, CCK8 assay was performed. It was suggested that 32 μM of desloratadine apparently blocked the proliferation rate of EJ cells after treatment for 72 or 96 hours ($P < .05$, Figure 1C). The proliferation of SW780 cells was also inhibited by 12 μM of desloratadine (Figure 1D). Moreover, the colony formation assay also revealed a significant decrease in the colony numbers in the desloratadine-treated cells, compared to the NC group ($P < .05$, Figure 1E and F). Besides, flow cytometry was employed for assessing the effect of desloratadine on cell cycle distribution. Our data highlighted that compared with the NC group, the proportion of EJ cells in the G1 phase was increased after treatment with desloratadine, but the proportion of cells in the S phase decreased accordingly ($P < .05$, Figure 1G and H), suggesting that desloratadine treatment could induce cell cycle arrest at G1 phase in EJ cells. Furthermore, Western blot results further indicated that desloratadine reduced the expression of cyclin D1 and P70S6K in EJ cells ($P < .05$, Figure 1I and J). Altogether, these data indicated that desloratadine may inhibit cell growth capability of bladder cancer through regulating the cell cycle.

Desloratadine Promotes Bladder Cancer Cell Death by Inducing Apoptosis and Autophagy

Aimed at investigating the effect of desloratadine on bladder cancer cell death, cell apoptosis was analyzed using flow cytometry assay. The results suggested that desloratadine significantly enhanced apoptotic cell rate of EJ and SW780 cells compared with the NC cells ($P < .05$, Figure 2A). Next, apoptosis-related proteins were detected using Western blot to further figure out the mechanism involved in the increasing apoptosis by desloratadine. We observed that desloratadine enhanced the expression of cleaved caspase 3 and cleaved caspase 9 in both EJ and SW780 cells ($P < .05$, Figure 2B). In addition, the expression of Bcl-2, a pivotal antiapoptotic protein, was significantly blocked in desloratadine-treated cells, whereas the proapoptosis protein Bax was upregulated by desloratadine treatment ($P < .05$, Figure 2B). Besides, the expression levels of autophagy-related proteins were also changed for the desloratadine treatment. As shown in Figure 2C, compared to the NC cells, the expression of Beclin 1 was significantly upregulated in desloratadine-treated cells, and the ratio of LC3II/LC3I was also remarkably increased, at the meantime, the expression of P62 was downregulated (Figure 2C), indicating a significant autophagy was induced by desloratadine in bladder cancer cells. Further, treatment with LY294002, an autophagy inhibitor,[9] could reverse the promoting effect of desloratadine on Beclin 1 and LC3II/LC3I and the inhibiting effect on P62 expression (Figure 2D).

Desloratadine Inhibits cell Migration, Invasion, and Epithelial-to-Mesenchymal Transition in Bladder Cancer Cells

To further investigate the effect of desloratadine on the metastatic potential of bladder cancer cells, a wound-healing assay was carried out to detect cell migration ability. As evident from Figure 3A, EJ cells treated with desloratadine (32 μM) showed a remarkably delay in the ability to migrate into the blank space compared with the NC cells ($P < .05$); SW780 cell migration ability was also decreased by desloratadine ($P < .05$).

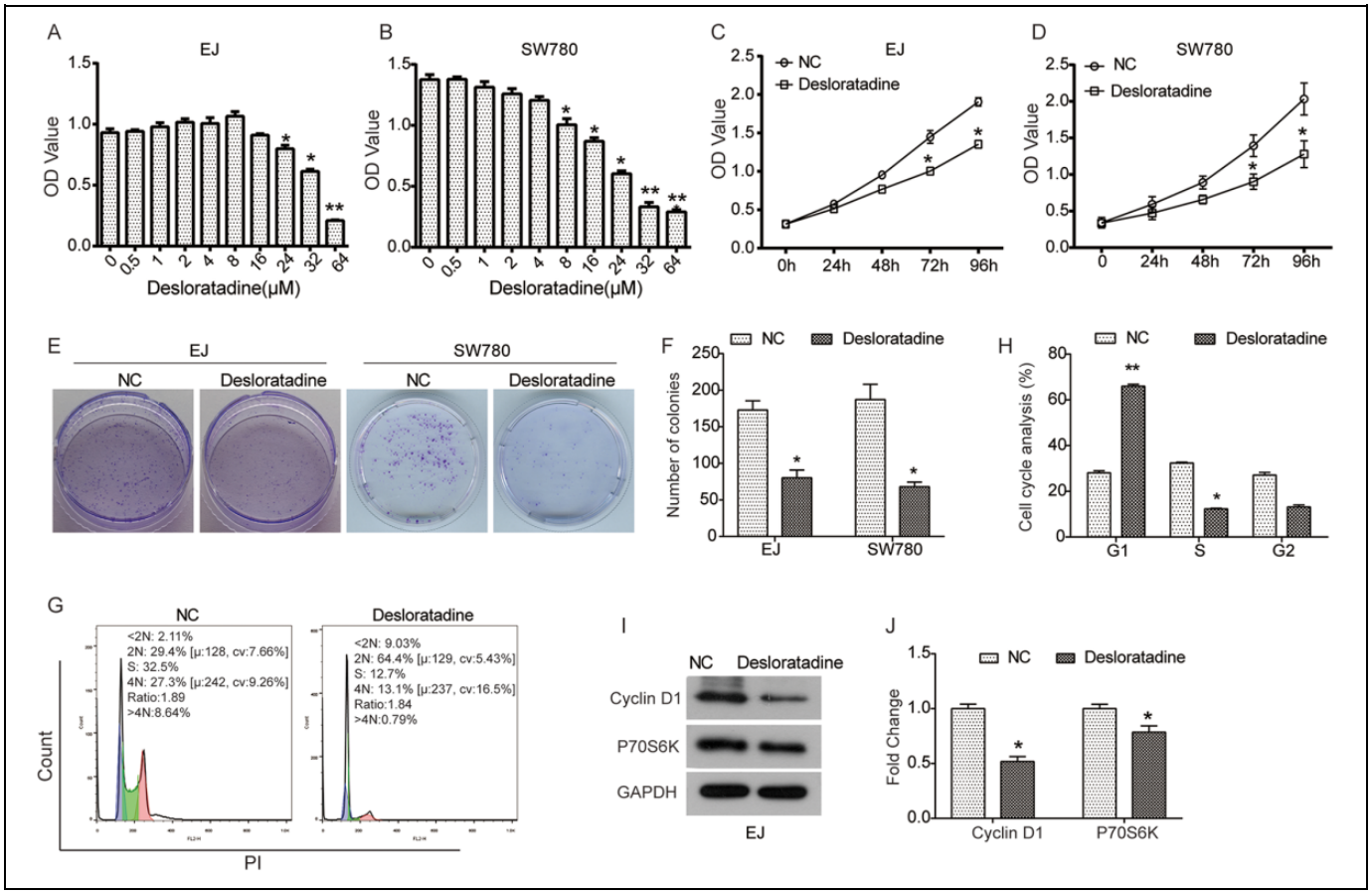

Figure 1. Desloratadine inhibits cell viability and growth and induces cell cycle arrest in bladder cancer cells. EJ (A) and SW780 (B) cells were treated with different concentrations of desloratadine (0, 0.5, 1, 2, 4, 8, 16, 24, 32, and 64 μM) for 24 hours, and cell viability was assessed using CCK8 assay. CCK8 assay was carried out to examine the effect of desloratadine on cell proliferation rate in EJ (C) and SW780 (D) cells, and DMSO was used as negative control (NC). E, EJ and SW780 cells were treated with desloratadine and allowed to form colonies in fresh medium for 1 week, DMSO was used as NC. F, Quantitative analysis of colony formation results. G, EJ cells were treated with desloratadine (32 μM) for 24 hours, and the cell cycle distribution was analyzed using flow cytometry. H, Quantitative analysis of cell cycle distribution. I, The relative expression of cyclin D1 and P70S6K in EJ cells treated with 32 μM of desloratadine for 24 hours. J, Quantitative analysis of Western blot results. GAPDH was used as a loading control. Data are expressed as the mean ± SD from 3 independent experiments. $^{*}P < .05$, $^{**}P < .01$ versus the control group. CCK8 indicates Cell Counting Kit 8; DMSO, dimethyl sulfoxide; GAPDH, Glyceraldehyde 3-phosphate dehydrogenase; SD, standard deviation.

Interestingly, the transwell invasion assay also illuminated that exposed to desloratadine, EJ and SW780 cells displayed a notable decrease in cell invasion ability compared with the NC cells ($P < .05$, Figure 3B). It is well known that EMT plays a pivotal role in the metastasis of cancers.[10] Herein, the expression of EMT marker proteins was examined after EJ and SW780 cells were treated with desloratadine. As indicated by Western blot assay, the expression of N-cadherin, Vimentin, Snail1, and Snail2 was apparently downregulated in desloratadine-treated cells, in contrast, the expression of E-cadherin was upregulated ($P < .05$, Figure 3C), suggesting that desloratadine diminishes EMT process in bladder cancer cells *in vitro*.

Upregulation of IL-6 Partially Restored the Anticancer Activity of Desloratadine

Desloratadine has been reported to inhibit the release of IL-6 from basophils and mast cells.[7] As a multifunctional cytokine, IL-6 is involved in the progression of bladder cancer.[11,12] As shown in Figure 4A, desloratadine treatment significantly reduced the IL-6 level in EJ cells ($P < .05$). Moreover, upregulation of IL-6 could partially restore the inhibitory effect of desloratadine on EJ cell invasion compared with desloratadine-treated cells (Figure 4B). Further, upregulation of IL-6 also abolished the regulation of desloratadine in Bcl-2, Bax, Beclin1, LC3-II/LC3-I, N-cadherin, and E-cadherin expression in EJ cells (Figure 4C).

Discussion

Bladder cancer is characterized by high recurrence rate, rapid progression, and high mortality. Finding new agents that inhibit the growth and metastasis of bladder cancer is a new perspective for developing new therapeutic strategies for bladder cancer. In this study, for the first time, we identified that desloratadine inhibited cell viability in a dose- and time-

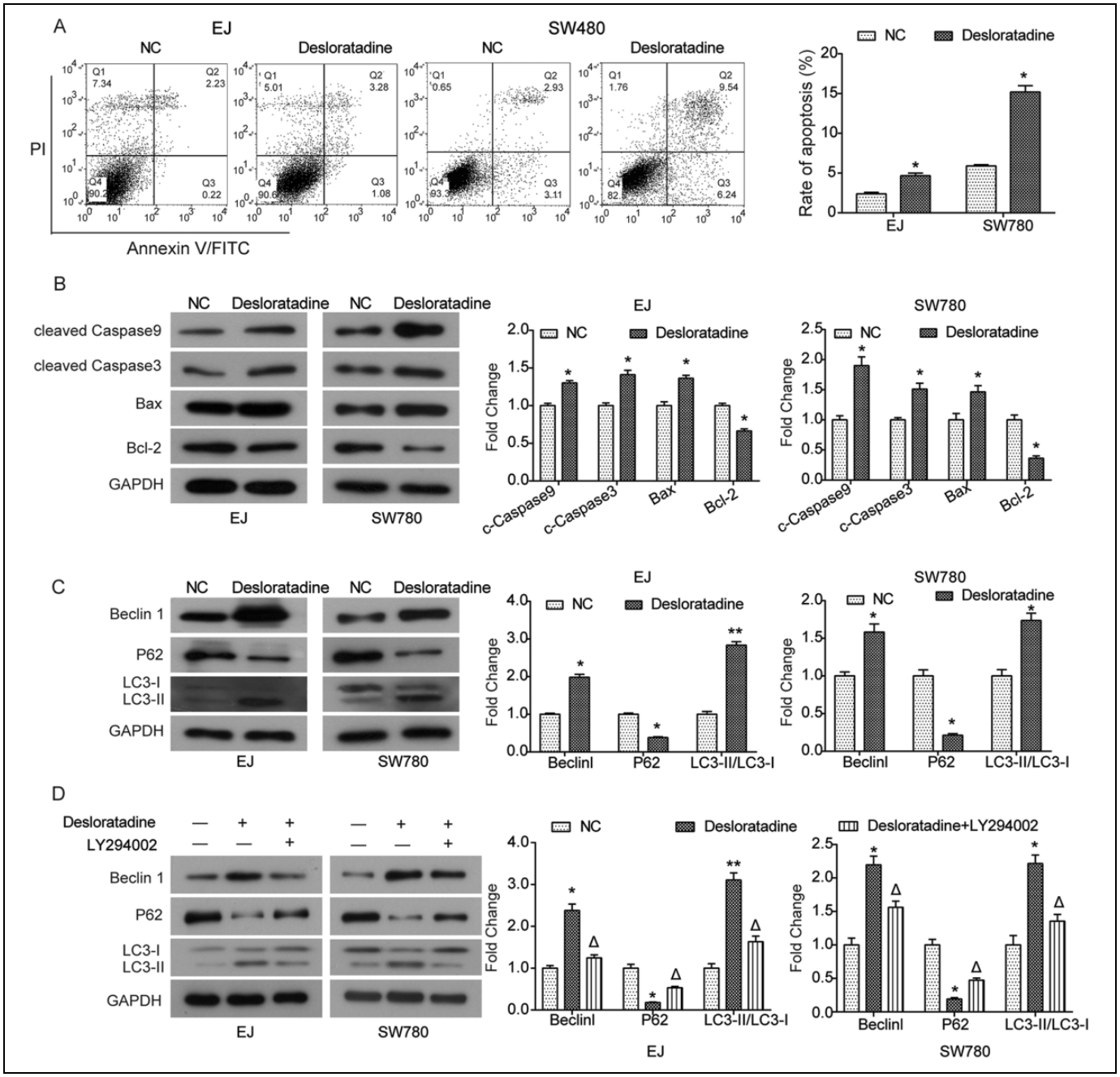

Figure 2. Desloratadine promotes apoptosis and autophagy in bladder cancer cells. A, EJ and SW780 cells were treated with desloratadine for 24 hours, and flow cytometry was carried out to assess apoptosis rate in EJ cells. B, Western blot assay was performed to detect the expression of cleaved caspase 3, cleaved caspase 9, Bax, and Bcl-2 in EJ and SW780 cells treated with desloratadine for 24 hours. C, The expression of autophagy-related proteins, Beclin 1, P62, and LC3I/II, were detected in EJ and SW780 cells treated with desloratadine for 24 hours using Western blot assay. D, The expression of autophagy-related proteins was detected after bladder cancer cells treated with desloratadine or desloratadine + LY294002 (15 μM) for 24 hours using Western blot assay. Data are expressed as the mean ± SD from 3 independent experiments. $*P < .05$, $**P < .01$ versus the control group; $\Delta P < .05$ versus desloratadine-treated group. SD indicates standard deviation.

dependent manner using CCK8 assay, and clonogenic ability of both EJ and SW780 cells was also significantly decreased by desloratadine, suggesting an antigrowth activity of desloratadine in bladder cancer. As well known, p53 is frequently mutated or inactivated in bladder cancer. Our data showed that desloratadine could inhibit the proliferation of p53 mutated EJ cells and p53 wild-type SW780 cells, indicating that the antitumor proliferation activity of desloratadine is independent of p53.

Normal cell cycle progression is the important mechanism for cell proliferation, and aberrant cell cycle activity is frequently occurred during tumorigenesis.[13,14] Blocking cell cycle

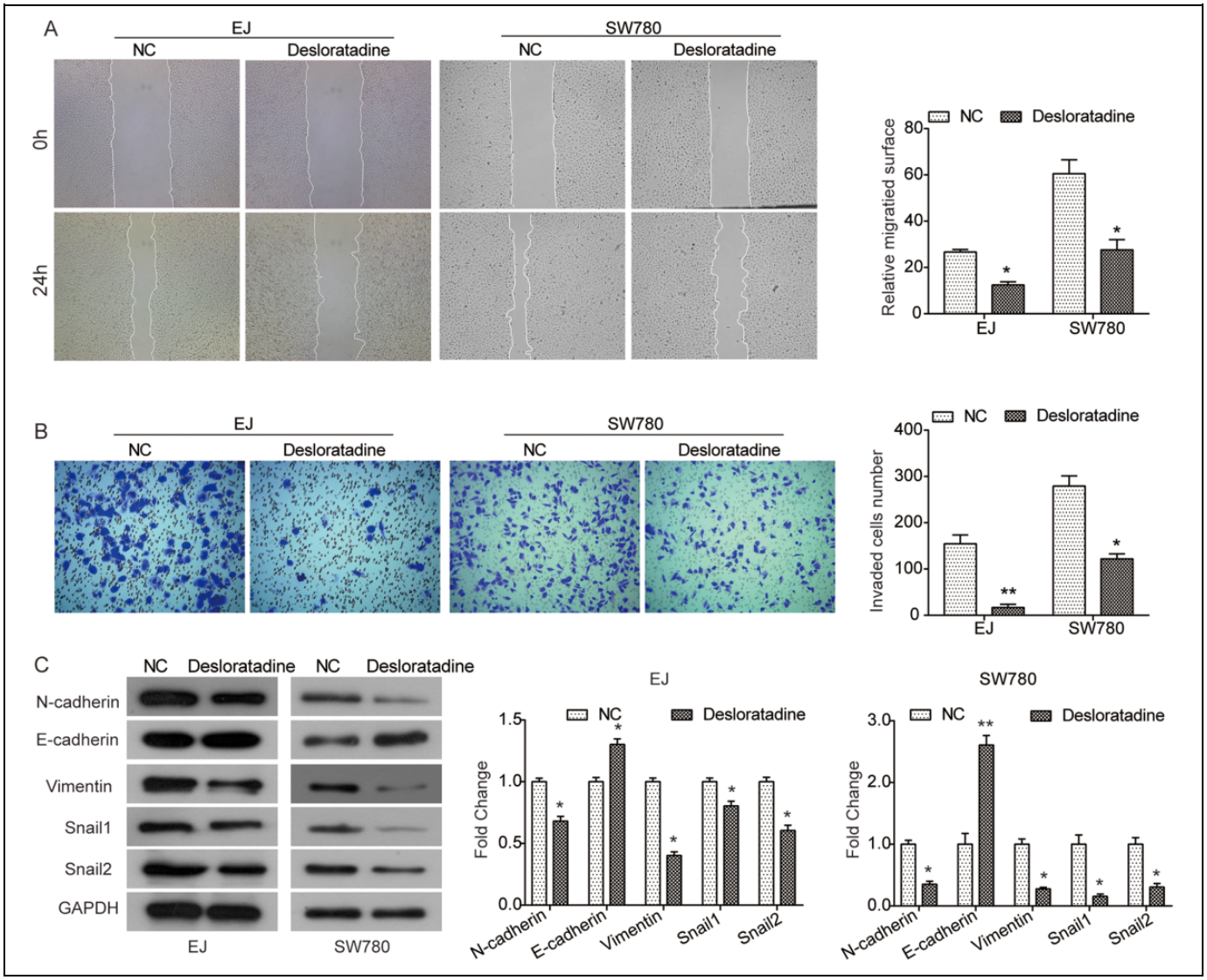

Figure 3. Desloratadine suppresses cell migration, invasion, and EMT in bladder cancer cells. A, A wound-healing assay was carried out to detect cell migration ability of EJ and SW780 cells treated with desloratadine. B, Transwell assay was performed to assess cell invasion of EJ and SW780 cells treated with desloratadine for 24 hours. C, EJ and SW780 cells treated with desloratadine for 24 hours, Western blot was performed to detect the expression of EMT-related markers, N-cadherin, E-cadherin, Vimentin, Snail1, and Snail2. Data are expressed as the mean ± SD from 3 independent experiments. $*P < .05$, $**P < .01$ versus the control group. EMT indicates epithelial-to-mesenchymal transition; SD, standard deviation.

progression is thought to be an effective approach to inhibit tumor growth and destroy tumor cells.[13] Therefore, further study was drived to investigate the effect of desloratadine on cell cycle of EJ cells. Our data from flow cytometry revealed a remarked cell cycle arrest at G1 phase induced by desloratadine in EJ cells. Cyclin D1 is an indispensable regulator of cell cycle G1 to S phase transition. We observed that desloratadine treatment led to significant decrease in the expression of cyclin D1. P70S6K is also revealed to be upregulated in cancers, which functions in promoting protein synthesis and cell proliferation.[15] In addition, the expression of P70S6K was also inhibited by desloratadine in EJ cells. Together, these data indicated that desloratadine may inhibit cell growth of bladder cancer through inducing cell cycle arrest.

Apoptosis, a type of strict programmed cell death to control cell growth, is frequently dysregulated in tumor cells. Promoting tumor cell apoptosis is a pivotal hallmark of cytotoxic antitumor agents.[16-18] In the current study, we observed that another important feature of desloratadine was the induction of apoptosis in EJ and SW780 cells. Apoptosis process involves a series of proteolytic events which are mainly initiated by cysteine proteases, especially the caspase family, a pivotal executioner to trigger apoptosis.[19] Bcl-2 family is the key regulator of apoptosis process, in which the increased Bax/Bcl-2 results in the activation of caspase 9 and caspase 3.[20] As indicated by Western blot results, the Bax, cleaved caspase 3, and cleaved caspase 9 were upregulated by desloratadine treatment, whereas the expression of Bcl-2 was downregulated in EJ and

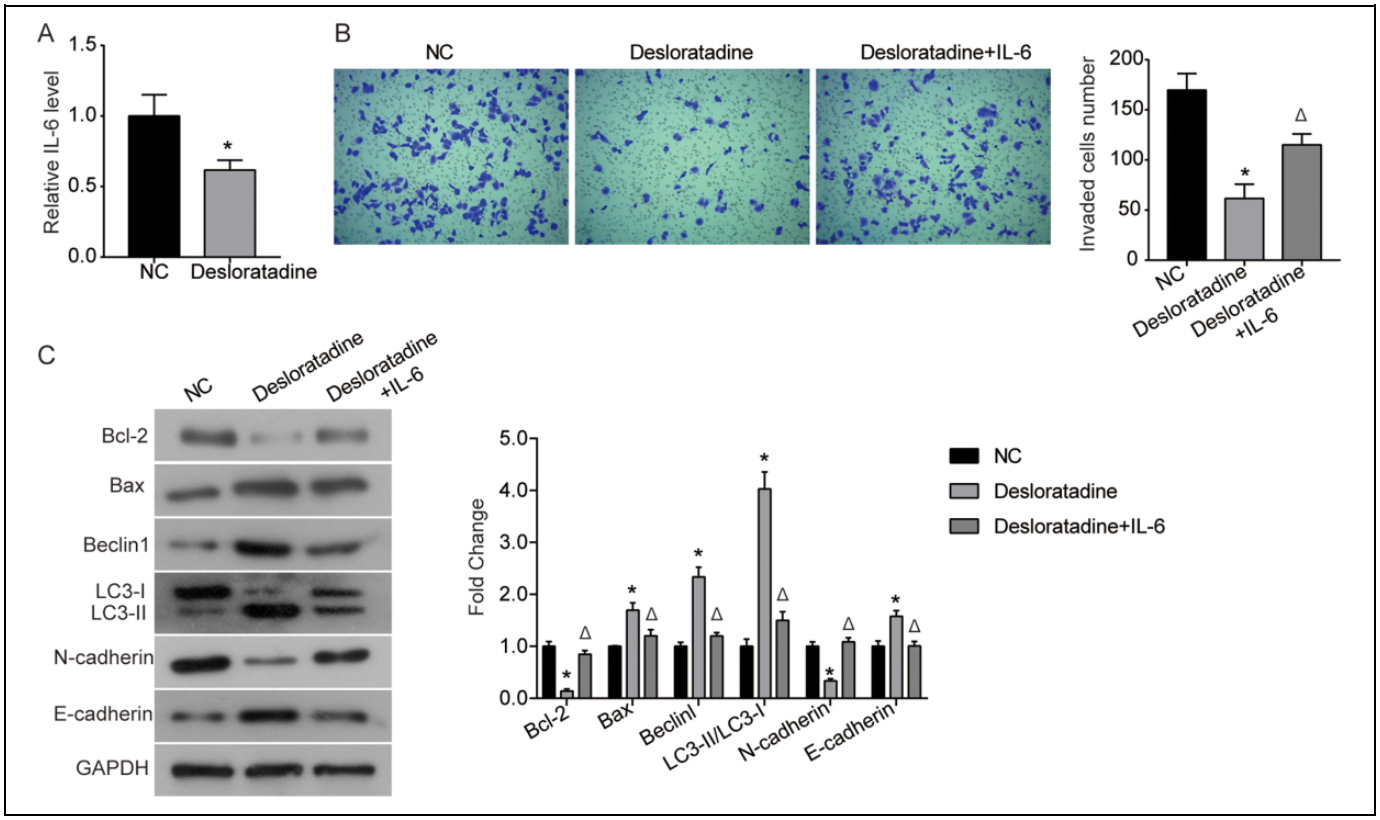

Figure 4. Upregulation of IL-6 partially restores the anticancer activity of desloratadine in bladder cancer cells A, ELISA assay was performed to measure the IL-6 level in EJ cells treated with desloratadine. B, Cell invasion was examined by transwell assay in EJ cells after indicated treatment. C, Expression of apoptosis-, autophagy- and EMT- related proteins was detected by Western blot. Data are expressed as the mean ± SD from 3 independent experiments. *$P < .05$ versus the control group. ELISA, enzyme-linked immunosorbent assay; IL, interleukin.

SW780 cells, indicating that desloratadine promotes apoptosis of bladder cancer cells in mitochondrial pathway through regulating the Bcl-2/Bax axis and caspase cascade.

Autophagy is often related with the stress and is another complex cellular process to cause cell death, which plays important roles in tumor progression.[21,22] Autophagy can work together with apoptosis and act as a backup mechanism to induce cell death in the case of apoptosis defect. The apoptosis and autophagy pathways are interrelated and regulate each other.[23] Studying and utilization of these interactions will be helpful to further reveal the pathogenesis of tumors and find effective targets for tumor treatment. Autophagy and apoptosis cooperation are frequently reported in tumors, such as ceramide could promote apoptosis and autophagy in breast and colon cancer cells.[24] It is also found that both of them are activated in the clinical trials of arsenic trioxide treatment of T-lymphocytes.[25] Maranhão *et al* found that PJOV56, a new quinoxalinyl-hydrazone derivative, induces both autophagy and apoptosis in colorectal cancer cells.[26] Therefore, we examined whether desloratadine impacted autophagy in bladder cancer. During autophagy, the cytoplasmic LC3I is translocated to the autophagosome membrane form LC3II, as a result, the ratio of LC3-II/I can estimate the level of autophagy.[27,28] p62 is the first described selective autophagy receptor in mammals,[29] which is decreased during autophagy and accumulates when autophagy is inhibited.[30] Beclin 1 is also reported to be a key regulator of autophagy. Herein, our data confirmed that desloratadine increased the expression of Beclin 1 and the ratio of LC3II/LC3I, while reduced the expression of P62 in EJ and SW780 cells, suggesting that desloratadine treatment causes autophagy in bladder cancer cells. Moreover, treatment with autophagy inhibitor LY294002 could rescue desloratadine-induced autophagy in bladder cancer cells. These results reveal that the activity of desloratadine in suppressing tumor cell survival might be mediated by inducing apoptosis and autophagy in bladder cancer. However, it is not clear whether apoptosis or autophagy is the main cause of desloratadine induced-cell death in bladder cancer, which is also the focus of our next study.

Metastasis is the leading cause of cancer-related death in bladder cancer. In our study, we observed a significant suppression of migration and invasion in EJ and SW780 cells following the desloratadine treatment. As well known, EMT is the main mechanism for promoting tumor metastasis and invasion.[31] During this process, epithelial cells lose their properties, which are characterized by downregulation of epithelial marker proteins such as E-cadherin, and acquire mesenchymal phenotypes, which are characterized by upregulation of mesenchymal marker proteins such as Vimentin and N-cadherin.[32-34] Our data indicated that desloratadine blocked the protein expression

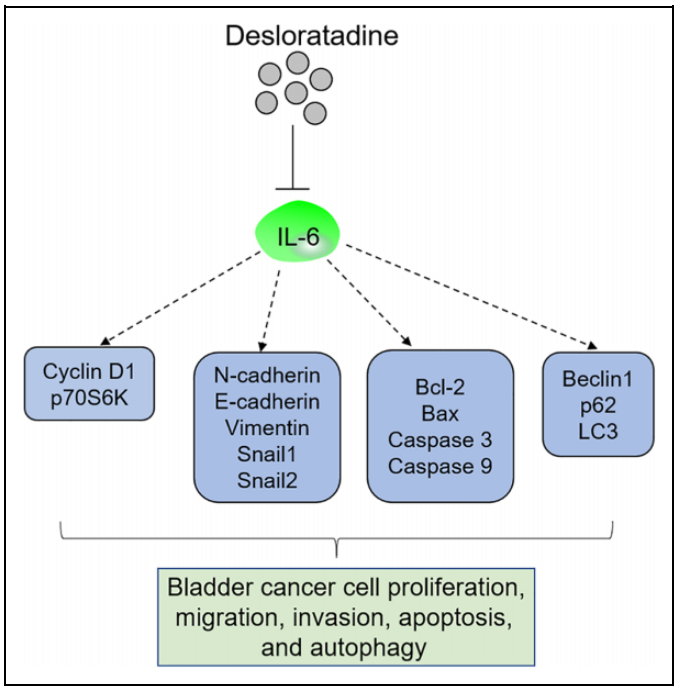

Figure 5. The model of desloratadine inhibiting in the progression of bladder cancer.

of acquired markers of EMT, such as N-cadherin and Vimentin, and upregulated the expression of attenuated marker of EMT in EJ and SW780 cells such as E-cadherin, suggesting that desloratadine suppresses the EMT process in bladder cancer cells. The transcription factors Snail1 and Snail2 are well-known suppressor of E-cadherin, and their activation is involved in EMT events in tumors.[35,36] The decrease in expression of Snail1 and Snail2 provided further evidence for the suppression of EMT in bladder cancer cells. Herein, these results indicate that desloratadine might inhibit cell migration and invasion through diminishing EMT process in bladder cancer cells.

Previous study report that IL-6 is overexpressed in bladder cancer and associated with poor prognosis of patients with bladder cancer.[11] Downregulation of IL-6 inhibits tumor growth and invasive capability and EMT in bladder cancer.[11] It has been revealed that desloratadine could inhibit the release of IL-6 from basophils and mast cells.[7] Herein, we found that IL-6 was downregulated in desloratadine-treated cells, it's upregulation abolished the suppressive effect of desloratadine on cell invasion, as well as expression of apoptosis-, autophagy-, and EMT-related proteins. Therefore, desloratadine might inhibit the growth and invasion of bladder cancer cells by downregulating IL-6 (Figure 5).

In conclusion, we for the first time demonstrated the anticancer activity of desloratadine on cell growth through inducing cell cycle arrest at G1 phase, apoptosis and autophagy, and desloratadine inhibits cell invasion via suppressing EMT in human bladder cancer cells. Further, IL-6 may be involved in the anticancer activity of desloratadine in bladder cancer (Figure 5). Our findings might provide a novel anticancer agent for therapy of bladder cancer.

Authors' Note

All authors contributed to conception and design, administrative support, provision of study materials or patients, collection and assembly of data, data analysis and interpretation, manuscript writing, and final approval of manuscript. Written informed consent was obtained from all individuals who participated in the study. Our study did not require an ethical board approval because it did not contain human or animal trials.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

ORCID iDs

Jianfeng Ma https://orcid.org/0000-0002-6667-5372
Jinchun Qi https://orcid.org/0000-0002-2662-7448

References

1. Weintraub MD, Li QQ, Agarwal PK. Advances in intravesical therapy for the treatment of non-muscle invasive bladder cancer (Review). *Mol Clin Oncol*. 2014;2(5):656-660.
2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. *CA Cancer J Clin*. 2016;66(1):7-30.
3. Fahmy O, Khairul-Asri MG, Schubert T, et al. Urethral recurrence after radical cystectomy for urothelial carcinoma: a systematic review and meta-analysis. *Urol Oncol*. 2017;36(2):54-59.
4. Tuo Z, Zhang J, Xue W. LncRNA TP73-AS1 predicts the prognosis of bladder cancer patients and functions as a suppressor for bladder cancer by EMT pathway. *Biochem Biophys Res Commun*. 2018;499(4):875-881.
5. Pei Z, Xian D, Song Y, et al. Down-regulation of lncRNA CASC2 promotes cell proliferation and metastasis of bladder cancer by activation of the Wnt/β-catenin signaling pathway. *Oncotarget*. 2017;8(11):18145-18153.
6. Fuchs R, Schwach G, Stracke A, et al. The anti-hypertensive drug prazosin induces apoptosis in the medullary thyroid carcinoma cell line TT. *Anticancer Res*. 2015;35(1):31.
7. Geha RS, Meltzer EO. Desloratadine: a new, nonsedating, oral antihistamine. *J Allergy Clin Immunol*. 2001;107(4):751-762.
8. Bousquet J, Bachert C, Canonica GW, et al. Efficacy of desloratadine in intermittent allergic rhinitis: a GA2LEN study. *Allergy*. 2009;64(10):395-402.
9. Shen W, Zhang X, Fu X, et al. A novel and promising therapeutic approach for NSCLC: recombinant human arginase alone or combined with autophagy inhibitor. *Cell Death Dis*. 2017;8(3):e2720.
10. Mcconkey DJ, Choi W, Marquis L, et al. Role of epithelial-to-mesenchymal transition (EMT) in drug sensitivity and metastasis in bladder cancer. *Cancer Metastasis Rev*. 2009;28(3-4):335-344.
11. Chen MF, Lin PY, Wu CF, Chen WC, Wu CT. IL-6 expression regulates tumorigenicity and correlates with prognosis in bladder cancer. *PloS One*. 2013;8(4):e61901-e61901.

12. Zhuo D, Wu Y, Luo J, Deng L, Niu X. CSTP1 inhibits IL-6 expression through targeting Akt/FoxO3a signaling pathway in bladder cancer cells. *Exp Cell Res*. 2019;380(1):80-89.
13. Otto T, Sicinski P. Cell cycle proteins as promising targets in cancer therapy. *Nat Rev Cancer*. 2017;17(2):93-115.
14. Li X, Xu P, Wang C, et al. Synergistic effects of the immune checkpoint inhibitor CTLA-4 combined with the growth inhibitor lycorine in a mouse model of renal cell carcinoma. *Oncotarget*. 2017;8(13):21177-21186.
15. Zhou H, Huang S. Role of mTOR signaling in tumor cell motility, invasion and metastasis. *Curr Protein Pept Sci*. 2011;12(1):30-42.
16. Tangutur AD, Kumar D, Krishna KV, et al. Microtubule targeting agents as cancer chemotherapeutics: an overview of molecular hybrids as stabilising and destabilising agents. *Curr Top Med Chem*. 2017;17(22):2523-2537.
17. Li N, Yang L, Deng X, Sun Y. Effects of isoliquiritigenin on ovarian cancer cells. *Oncotargets Ther*. 2018;11:1633-1642.
18. Huang L. Glaucocalyxin A induces G2/M cell cycle arrest and apoptosis through the PI3K/Akt pathway in human bladder cancer cells. *Int J Biol Sci*. 2018;14(4):418-426.
19. Gui D, Guo Y, Feng W, et al. Astragaloside IV, a novel antioxidant, prevents glucose-induced podocyte apoptosis in vitro and in vivo. *Plos One*. 2012;7(6):e39824.
20. Yamada H, Abe T, Li SA, et al. N'-[4-(dipropylamino)benzylidene]-2-hydroxybenzohydrazide is a dynamin GTPase inhibitor that suppresses cancer cell migration and invasion by inhibiting actin polymerization. *Biochem Biophys Res Commun*. 2014; 443(2):511-517.
21. White E. Deconvoluting the context-dependent role for autophagy in cancer. *Nat Rev Cancer*. 2012;12(6):401-410.
22. White E. The role for autophagy in cancer. *J Clin Invest*. 2015; 125(1):42-46.
23. Levine B, Sinha S, Kroemer G, Levine B, Sinha S, Kroemer G. Bcl-2 family members: dual regulators of apoptosis and autophagy. *Autophagy*. 2008;4(5):600-606.
24. Pattingre S, Bauvy C, Carpentier S, Levade T, Levine B, Codogno P. Role of JNK1-dependent Bcl-2 phosphorylation in ceramide-induced macroautophagy. *J Biol Chem*. 2008;284(5):2719-2728.
25. Qian W, Liu J, Jin J, Ni W, Xu W. Arsenic trioxide induces not only apoptosis but also autophagic cell death in leukemia cell lines via up-regulation of Beclin-1. *Leuk Res*. 2007;31(3): 329-339.
26. Maranhão SSA, Moura AF, Oliveira ACA, et al. Synthesis of PJOV56, a new quinoxalinyl-hydrazone derivative able to induce autophagy and apoptosis in colorectal cancer cells, and related compounds. *Bioorg Med Chem Lett*. 2019;30(2):126851.
27. Mizushima NOY, Yoshimori T. Autophagosome formation in mammalian cells. *Semin Immunopathol*. 2010;32(4):397-413.
28. Tan S, Shi H, Ba M, et al. miR-409-3p sensitizes colon cancer cells to oxaliplatin by inhibiting Beclin-1-mediated autophagy. *Int J Mol Med*. 2016;37(4):1030.
29. Bjørkøy G, Lamark T, Brech A, et al. p62/SQSTM1 forms protein aggregates degraded by autophagy and has a protective effect on huntingtin-induced cell death. *J Cell Biol*. 2005; 171(4):603-614.
30. Mathew R, Karp CM, Beaudoin B, et al. Autophagy suppresses tumorigenesis through elimination of p62. *Cell*. 2009;137(6): 1062-1075.
31. Kiselyov A, Bunimovichmendrazitsky S, Startsev V. Key signaling pathways in the muscle-invasive bladder carcinoma: clinical markers for disease modeling and optimized treatment. *Int J Cancer*. 2016;138(11):2562-2569.
32. Gonzalez DM, Medici D. Signaling mechanisms of the epithelial-mesenchymal transition. *Sci Signal*. 2014;7(344):re8.
33. Qureshi R, Arora H, Rizvi MA. EMT in cervical cancer: its role in tumour progression and response to therapy. *Cancer Lett*. 2015; 356(2):321-331.
34. Li X, Teng S, Zhang Y, et al. TROP2 promotes proliferation, migration and metastasis of gallbladder cancer cells by regulating PI3K/AKT pathway and inducing EMT. *Oncotarget*. 2017;8(29): 47052-47063.
35. Boutet A, Esteban MA, Maxwell PH, Nieto MA. Reactivation of Snail genes in renal fibrosis and carcinomas: a process of reversed embryogenesis? *Cell Cycle*. 2007;6(6):638-642.
36. Zeisberg M, Neilson EG. Biomarkers for epithelial-mesenchymal transitions. *J Clin Invest*. 2009;119(6):1429-1437.