

# Dexmedetomidine represses proliferation and promotes apoptosis of esophageal cancer cells by regulating C-Myc gene expression *via* the ERK signaling pathway

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**Abstract.** – **OBJECTIVE:** The aim of this study was to investigate the effects of dexmedetomidine (DEX) on proliferation and apoptosis of esophageal cancer (EC) cells, and to explore the possible underlying mechanism.

**MATERIALS AND METHODS:** EC cells (Eca109) were randomly divided into two groups, namely, Control group and DEX group. The viability, proliferation, and apoptosis of Eca109 cells were detected using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, 5-Ethynyl-2'-deoxyuridine (EdU) staining and terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) staining, respectively. Meanwhile, the messenger ribonucleic acid (mRNA) and protein expression levels of extracellular signal-regulated kinase (ERK) 1/2 and c-Myc in Eca109 cells were measured by quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR) and Western blotting, respectively.

**RESULTS:** The viability of Eca109 cells was remarkably weakened in DEX group when compared with Control group ( $p < 0.05$ ). DEX could significantly inhibit the proliferation and promote the apoptosis of Eca109 cells ( $p < 0.05$ ). Moreover, the mRNA and protein levels of ERK1/2 and c-Myc in Eca109 cells declined notably ( $p < 0.05$ ).

**CONCLUSIONS:** DEX represses the proliferation and facilitates the apoptosis of Eca109 cells prominently. The possible underlying mechanism may be associated with the inhibition of c-Myc gene expression through the ERK signaling pathway.

## Key Words:

Dexmedetomidine, Esophageal cancer cells, Proliferation and apoptosis, ERK signaling pathway, c-Myc.

## Introduction

Esophageal cancer (EC), a common digestive tract disease, has been recognized as one of the

eight major malignant tumors in the world<sup>1,2</sup>. Currently, EC has attracted increasingly more attention. About 300,000 people die of EC every year worldwide. Statistics have shown that China is one of the regions with highest incidence of EC, with approximately 150,000 deaths annually<sup>3</sup>. A study has indicated that EC is correlated with age, gender, occupation, region, living environment, etc. Meanwhile, chemical factors, biological factors, and vitamin deficiencies are all pathogenic factors of EC<sup>4</sup>. The typical symptoms of EC are progressive dysphagia, specifically, difficulty in swallowing dry food at first. Semi-liquid food, and water and saliva occur afterwards<sup>5,6</sup>. Therefore, it is of extremely importance to prevent, diagnose, and cure EC. Particularly, how to reduce the mortality rate of EC is an urgent problem to be resolved by clinical and medical workers.

Dexmedetomidine (DEX) is a highly selective  $\alpha_2$  receptor agonist. It exerts sedative, analgesic, anxiolytic, and anti-sympathetic effects by selectively binding to adrenoreceptor<sup>7-9</sup>. Modern pharmacological studies have demonstrated that DEX (chemical formula: C<sub>13</sub>H<sub>16</sub>N<sub>2</sub>, molecular weight: 200.28) plays crucial roles in the growth, proliferation, and apoptosis of tumor cells. Wang et al<sup>10</sup> have discovered that DEX affects the activity of glioblastoma and lung cancer cells and regulate their biological functions. Lavon et al<sup>11</sup> have revealed that DEX can modulate multiple pathological processes, such as metastasis of breast cancer, lung cancer, and colon cancer in rodents. Zhang et al<sup>12</sup> have found that DEX can improve the cognitive function of EC patients. Li et al<sup>13</sup> have further indicated that DEX is capable of ameliorating postoperative stress-induced pain. However, the regulatory role of DEX in EC cell proliferation and apoptosis still remain unknown.

Extracellular signal-regulated kinase (ERK) 1/2 is not only a member of the mitogen-activated protein kinase (MAPK) family, but also one of the most important representative kinases. When cells are stimulated by external factors, the ERK signaling pathway is activated, and ERK1/2 is persistently excited. Meanwhile, c-Myc, a key downstream molecule of the ERK signaling pathway, accelerates the proliferation and malignant transformation of tumor cells upon the stimulation. Nevertheless, whether DEX can exert its therapeutic effects on EC through the signaling pathway has rarely been reported.

## Materials and Methods

### Reagents

DEX (Sigma-Aldrich, St. Louis, MO, USA), culture medium, fetal bovine serum (FBS), dual antibodies, and first-strand complementary deoxyribonucleic acid (cDNA) reverse transcription kit (Thermo Fisher Scientific, Waltham, MA, USA), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) kit (Beyotime Biotechnology Co., Ltd., Shanghai, China), cell culture plate and flask (Corning Incorporated, Corning, NY, USA), primary antibodies of ERK1/2, c-Myc and  $\beta$ -actin (Abcam, Cambridge, MA, USA), horseradish peroxidase (HRP)-labeled secondary antibodies (Boster Biological Technology Co., Ltd., Wuhan, China), and ribonucleic acid (RNA) extraction kit [Sangon Biotech (Shanghai, China) Co., Ltd.].

### Instruments

Inverted fluorescence microscope (Nikon, Tokyo, Japan), gel electrophoresis apparatus (Bio-Rad, Hercules, CA, USA), microplate reader (Shanghai Flash Spectrum Biotechnology Co., Ltd., Shanghai, China),  $-80^{\circ}\text{C}$  ultra-low-temperature refrigerator (Thermo Fisher Scientific, Waltham, MA, USA), and pure water distiller (Millipore, Billerica, MA, USA).

### Cell Culture

EC cell lines (Eca109) were purchased from the Cell Bank of Type Culture Collection Committee of Chinese Academy of Sciences (Shanghai, China). The cells were cultured in complete medium containing 10% fetal bovine serum (FBS; Gibco, Rockville, MD, USA) and 1% dual antibodies, and maintained in an incubator with 5%  $\text{CO}_2$  at  $37^{\circ}\text{C}$ . The medium was replaced, and the cells were sub-cultured according to their survival.

### Detection of Influence of DEX on Eca109 Cell Viability Via MTT Assay

Eca109 cells were reseeded into 96-well plates at a density of  $5 \times 10^3$  cells/well. After adherence, the cells were added with 1, 10, 100, and 1000 ng/mL DEX solution, respectively, followed by culture for 24 h. Three replicate wells were set for each concentration. Subsequently, 10  $\mu\text{L}$  of MTT solution and 100  $\mu\text{L}$  of Formazan dissolving solution were added into each well and mixed evenly. Absorbance at the wavelength of 570 nm was finally measured using a micro-plate reader.

### Detection of Influence of DEX on Proliferative Capacity of Eca109 Cells Via 5-Ethynyl-2'-Deoxyuridine (EdU) Staining

EdU is a kind of thymidine analogue that can infiltrate into DNA molecules being synthesized and precisely reflect DNA replication activity. Firstly, cells in the 96-well plates were fixed with 4% paraformaldehyde and sealed in 3% bovine serum albumin (BSA) for 15 min. Next, the cells were incubated with EdU staining solution in the dark for 30 min. Finally, the staining was observed at the wavelength of 495 nm under a microscope.

### Detection of Influence of DEX on Eca109 Cell Apoptosis Via Terminal Deoxynucleotidyl Transferase-Mediated dUTP Nick end Labeling (TUNEL) Assay

Eca109 cells in 96-well plates were fixed in 4% paraformaldehyde for 15 min and permeabilized with 0.3% Triton X-100 solution for 15 min. Next, TUNEL reaction mixture was prepared and added with 50  $\mu\text{L}$  of mixed solution of TdT and fluorescein-labeled dUTP. Then, the cells were covered with cover glass and incubated in dark for 60 min, followed by washing with phosphate-buffered saline (PBS). Finally, cell apoptosis was observed under a microscope.

### Detection of Influences of DEX on Messenger RNA (mRNA) Levels of ERK1/2 and c-Myc in Eca109 Cells Via quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR)

Total RNA was extracted using TRIzol<sup>®</sup> reagent (Invitrogen, Carlsband, CA, USA; Thermo Fisher Scientific, Inc. Waltham, MA, USA) from Eca109 cells in 6-well plates, and its concentration was determined. Subsequently, extracted RNA was transcribed into complementary deoxyribose

**Table I.** Primer sequences.

Primer		Sequence
ERK1/2	Forward	GCAGTTCTGGAATGGAAGGGT
	Reverse	GGGTTTGAATGAGATGAGGG
c-Myc	Forward	TCCAGCTTGTACCTGCAGGAT
	Reverse	CCTCCAGCAGAAGGTGATCCA
GAPDH	Forward	AGGTCGGTGTGAACGGATTTG
	Reverse	TGTAGACCATGTAGTTGAGGTCA

nucleic acid (cDNA) using the PrimeScript RT reagent kit (Takara Biotechnology Co., Ltd., Dalian, China). Real-time PCR was performed with a FastStart Universal SYBR Green Master kit (Roche, Basel, Switzerland). Primer sequences used in this study were shown in Table I. GAPDH was served as the internal control. Finally, gene expression level was analyzed by software. The relative expression of the genes was calculated using the  $2^{-\Delta\Delta Ct}$  method.

#### **Detection of Influences of DEX on Protein Levels of ERK1/2 and c-Myc in Eca109 Cells Via Western Blotting**

Total proteins were extracted from Eca109 cells, and the concentration in each group was measured. 50  $\mu$ g of proteins were loaded for electrophoresis at 90 V for 110 min and transferred to polyvinylidene difluoride (PVDF) membranes. After sealing with 5% skimmed milk powder solution for 1 h, the membranes were separately incubated with 1% BSA-diluted primary antibodies of ERK1/2, c-Myc, and  $\beta$ -actin (1:1000) overnight. On the next day, the membranes were incubated with HRP-labeled secondary antibodies at room temperature for 1 h. Color development was performed using diaminobenzidine (DAB) developer (Solarbio, Beijing, China). Finally, the optical density of bands was subjected to statistical analysis using Image J software (NIH, Bethesda, MD, USA).

#### **Statistical Analysis**

Statistical Product and Service Solutions (SPSS) 16.0 software (SPSS Inc., Chicago, IL, USA) was used for all statistical analysis. Measurement data were presented as mean  $\pm$  standard deviation. Spearman correlation analysis was adopted for gene and protein expressions. One-way ANOVA was conducted to compare the differences among different groups, followed by post-hoc test (Least Significant Difference).  $p < 0.05$  was considered statistically significant.

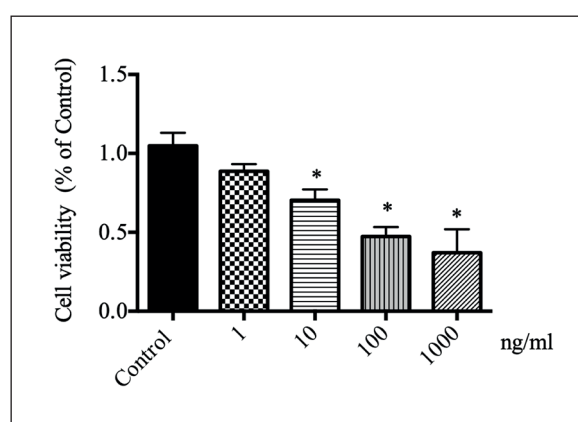
## **Results**

### **DEX Could Inhibit the Viability of Eca109 Cells**

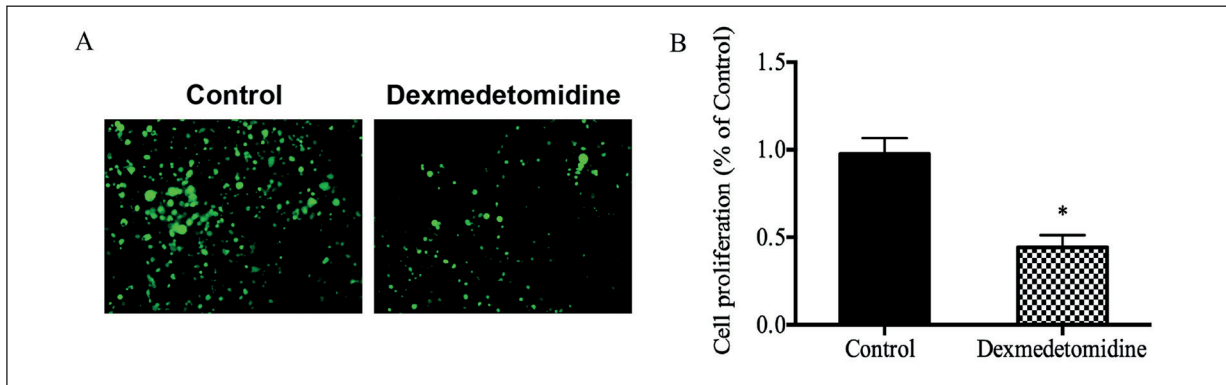
MTT assay (Figure 1) showed that the viability of Eca109 cells decreased remarkably at the concentration of 10 ng/mL in DEX group when compared with the Control group ( $*p < 0.05$ ). It also clearly decreased at the concentrations of 100 and 1000 ng/mL ( $*p < 0.05$ ,  $*p < 0.05$ ), but no significant difference was detected. Therefore, the concentration of 100 ng/mL was selected for subsequent experiments. These results indicated that DEX could notably repress the viability of Eca109 cells.

### **DEX Could Inhibit the Proliferation of Eca109 Cells**

EdU staining results (Figure 2A) manifested that DEX group exerted significantly weakened proliferative capacity in comparison with the Control group ( $*p < 0.05$ ) (Figure 2B). These findings suggested that DEX was able to inhibit the proliferation of Eca109 cells.



**Figure 1.** Comparison of Eca109 cell viability among groups. (\*DEX group vs. Control group,  $p < 0.05$ ).



**Figure 2.** Comparison of proliferative capacity of Eca109 cells among groups *via* EdU staining. (magnification: 20×). **A**, EdU staining results, **B**, Statistical chart of cell proliferation rate. (\*DEX group vs. Control group,  $p < 0.05$ ).

### **DEX Could Promote the Apoptosis of Eca109 Cell**

TUNEL staining results (Figure 3A) illustrated that DEX group exhibited remarkably higher cell apoptosis rate than the Control group ( $*p < 0.05$ ) (Figure 3B), illustrating that DEX could promote the apoptosis of Eca109 cells.

### **DEX Could Repress the mRNA Expressions of ERK1/2 and c-Myc in Eca109 Cells**

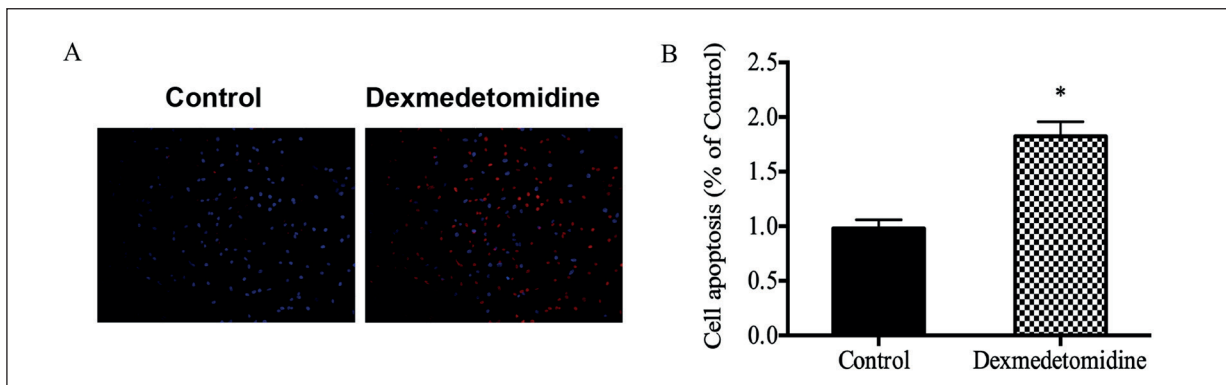
The influences of DEX on the mRNA expression levels of ERK1/2 and c-Myc in Eca109 cells were explored through qRT-PCR. As shown in Figure 4, the mRNA expression levels of ERK1/2 and c-Myc decreased evidently in the DEX group when compared with those in the Control group ( $*p < 0.05$ ,  $*p < 0.05$ ). Our findings suggested that DEX was able to prominently suppress the mRNA expressions of ERK1/2 and c-Myc in Eca109 cells.

### **DEX Could Repress the Protein Expressions of ERK1/2 and c-Myc in Eca109 Cells**

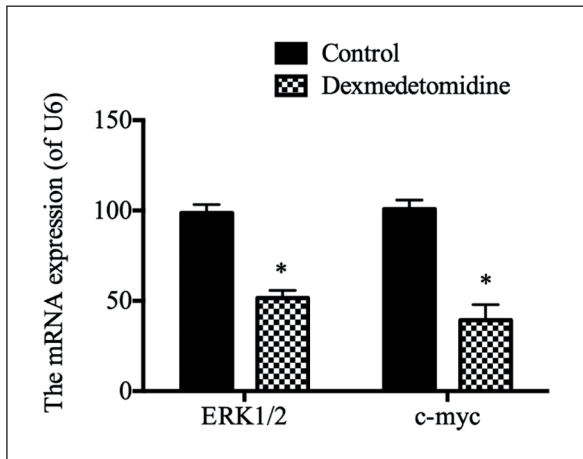
Similarly, the effects of DEX on the protein expression levels of ERK1/2 and c-Myc in Eca109 cells were investigated *via* Western blotting. The results (Figure 5A) manifested that the protein levels of ERK1/2 and c-Myc were significantly lower in the DEX group than the Control group ( $*p < 0.05$ ,  $*p < 0.05$ ) (Figure 5B). The above results demonstrated that DEX could significantly inhibit the protein expressions of ERK1/2 and c-Myc in Eca109 cells.

## **Discussion**

Currently, there are over 400,000 new cases of EC worldwide every year, 65% of which occur in China. Statistics have found that the incidence of EC exerts evident genetic predisposition. Mean-



**Figure 3.** Comparison of apoptosis of Eca109 cells among groups *via* TUNEL staining. (magnification: 20×). **A**, TUNEL staining results, **B**, Statistical chart of cell apoptosis rate. (\*DEX group vs. Control group,  $p < 0.05$ ).



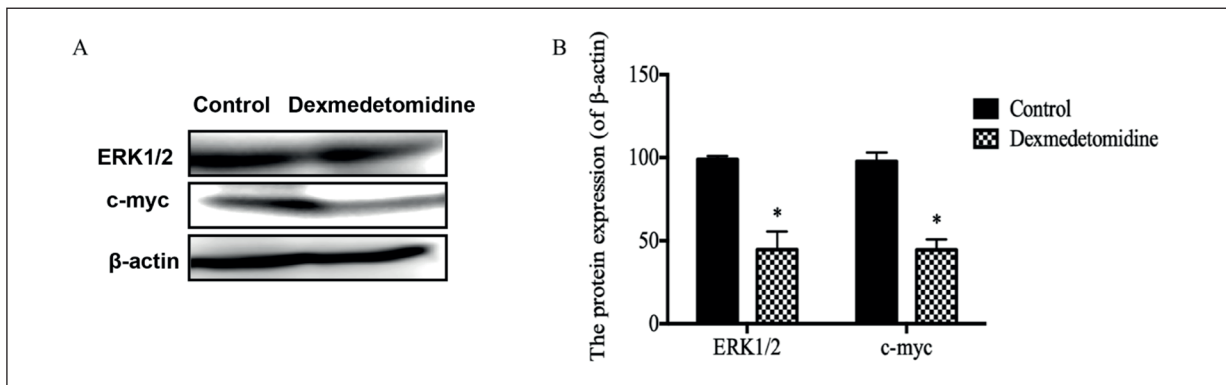
**Figure 4.** Comparisons of mRNA expression levels of ERK1/2 and c-Myc in Eca109 cells through qRT-PCR. (\*DEX group vs. Control group,  $p < 0.05$ ).

while, genetic factor may be an important risk factor of EC onset, followed by region or eating habit<sup>14</sup>. There are no apparent early symptoms of EC, which are hard to be detected by patients in the early stage. However, early detection and treatment will greatly improve the cure rate of EC. Clinically, surgery, radiotherapy, and chemotherapy are the major therapeutic methods for EC. However, these patients suffer from enormous pain during the treatment, seriously affecting their quality of life and bringing economic burdens to the family. Therefore, it is of important significance to seek for safe and efficacious therapeutic drugs for EC<sup>15</sup>.

DEX is a type of colorless and odorless drug that can specifically activate  $\alpha$  receptors, following clonidine. Recently, DEX is widely applied in

the perioperative period due to its sedative and analgesic effects, thus reducing the consumption of anesthetics and analgesics during operation. Xing et al<sup>16</sup> have indicated that the benefits of DEX in the perioperative period of tumor patients can be conjectured on the basis of its sedative and analgesic characteristics. Other studies have found that the injection of DEX into the spinal cord of tumor patients during operation can alleviate the pain induced by tumor. Besides, a study has revealed that DEX inhibits the proliferation and migration of osteosarcoma cells by regulating miR-520a-3p. The mechanism of action may be associated with the regulation of the AKT signaling pathway<sup>17</sup>. However, the regulatory role of DEX in EC cells has rarely been reported. Hence, MTT assay was first utilized to detect the regulatory effect of DEX on the viability of Eca109 cells. The results discovered that DEX could remarkably repress the survival of Eca109 cells (Figure 1). To further investigate the impacts of DEX on the proliferation and apoptosis of Eca109 cells, EdU and TUNEL staining assays were adopted, respectively. The results revealed that DEX was capable of inhibiting the proliferation and promoting the apoptosis of Eca109 cells.

The regulatory mechanism of DEX was further explored in our study. Firstly, the regulatory effect of DEX on the gene level of ERK1/2 in the ERK signaling pathway in EC cells was investigated *via* qRT-PCR. The ERK signaling pathway, a member of the MAPK family, has been found to mediate stress response in cells and participate in modulating cell proliferation, differentiation, apoptosis, and intercellular interaction. ERK, located in p34-35 of chromosome 1, has three functional areas. ERK1/2 is a subfamily that has been



**Figure 5.** Comparisons of protein expression levels of ERK1/2 and c-Myc in Eca109 cells *via* Western blotting. **A**, Western Blotting bands. **B**, Statistical chart of bands (\*DEX group vs. Control group,  $p < 0.05$ ).

widely studied. In the case of stimulation on cells, external stimuli can convey the stimulation to the nucleus by means of receptors, further activating crucial effector molecules on other signal transduction pathways, such as c-Myc and NF- $\kappa$ B. Ciccarelli et al<sup>18</sup> have found that proto-oncogene c-Myc is abnormally accumulated in prostate cancer cells. This can facilitate the occurrence and development of the disease. However, when the ERK signal transduction pathway is repressed or destroyed, c-Myc level decreases markedly and is conducive to the recovery of patients with prostate cancer. Zhao et al<sup>19</sup> have discovered that the ERK/c-Myc signaling pathway plays key roles in osteosarcoma proliferation and metastasis. In addition, some researchers have found that shikonin and its derivatives can suppress the expression of c-Myc *via* the ERK signaling pathway, thus killing leukemic cells<sup>20</sup>. These findings all suggest that the ERK signaling pathway have vital functions in the pathological processes of tumors. Therefore, the mRNA levels of ERK1/2 and c-Myc in Eca109 cells were measured by qRT-PCR in this study. The results indicated that DEX could restrain the mRNA levels of ERK1/2 and c-Myc in Eca109 cells (Figure 4). Furthermore, Western blotting demonstrated that DEX significantly inhibited the protein levels of ERK1/2 and c-Myc in Eca109 cells.

## Conclusions

*In vitro* these studies illustrated that DEX could prominently inhibit the viability and proliferation, while stimulating the apoptosis of Eca109 cells. In this study, only preliminary investigations at the cellular level were conducted. Hence, in order to deeply explore the therapeutic effect of DEX in EC *in vivo*, a mouse model should be established. The novelty of this research was that our findings might provide a theoretical basis for the research and development of new-generation drugs.

## Conflict of Interest

The Authors declare that they have no conflict of interests.

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