

## Original Research Article

# Effect of dexmedetomidine on miR-144-3p expression and epithelial mesenchymal transition in gastric cancer cells

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### Abstract

**Purpose:** To investigate the effect of dexmedetomidine (DEX) on epithelial mesenchymal transition (EMT) in gastric cancer cells, and the role of microRNA-144-3p (miR-144-3p) in the process.

**Methods:** The effect of DEX on miRNA expression profile was analyzed using GEO database (<https://www.ncbi.nlm.nih.gov/gds/>). Human gastric cancer cells were cultured in vitro, and one group of cells was treated with saline for 48 h (control group). Cells treated with DEX at doses of 0.01, 0.1 and 1.0  $\mu\text{mol/L}$  for 48 h were marked as low-, medium- and high-DEX concentration groups. The mRNA expression levels of miR-144-3p, ZEB1, E-cadherin and vimentin were determined using real-time quantitative polymerase chain reaction (RT-PCR), while the protein expressions of ZEB1, E-cadherin and vimentin were assayed with Western blotting. Cell proliferation was determined with CCK-8 assay, while metastasis was measured using Transwell assay.

**Results:** The GEO database demonstrated that the expression of miR-144-3p in rat cardiomyocytes was significantly decreased after DEX treatment ( $p < 0.05$ ). The expression of miR-144-3p was decreased in all groups, when compared to the control group, but the expressions of ZEB1 and vimentin were increased, while that of E-cadherin was down-regulated ( $p < 0.05$ ). Cell proliferation in the high-DEX concentration group was decreased ( $p < 0.05$ ). The degrees of cell invasion and migration were increased in the medium- and high-DEX concentration groups ( $p < 0.05$ ).

**Conclusion:** DEX promotes the metastasis of gastric cancer cells by regulation of epithelial mesenchymal transition (EMT) and the expression of miR-144-3p. This finding provides a new insight into the treatment of gastric cancer.

**Keywords:** Gastric cancer, Dexmedetomidine, E-cadherin, Vimentin, MiR-144-3p, Tumor metastasis, Epithelial mesenchymal transition

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## INTRODUCTION

Gastric cancer is second only to lung cancer as a leading cause of death in the world [1]. The onset of most gastric cancer cases is insidious, but

they progress very rapidly. An understanding of the key factors that affect the malignant behavior of gastric cancer cells is very important guide in the clinical diagnosis and treatment of the disease. Dexmedetomidine (DEX), a frequently-

used intravenous anesthetic, affects different biological processes in the body by regulating a variety of signal pathways [2,3]. The importance of DEX in malignant tumors has been progressively revealed. Many studies have indicated that DEX is involved in regulating the biological behavior of tumor cells [4,5]. However, the effect of DEX on gastric cancer cells, and its exact mechanism remain to be elucidated.

Micro-RNAs (miRNAs) are important post-transcriptional regulatory factors which participate in various biological processes, including the pathogenesis of malignant tumors [6,7]. In this study, bioinformatics methods and *in vitro* experiments were used to investigate the regulatory effect of DEX on gastric cancer cell-related miRNA and malignant behavior, in order to provide a new insight into the use of DEX in malignant tumors.

## EXPERIMENTAL

### Materials

Dexmedetomidine was purchased from Absin Bioscience Inc (Shanghai, China), while RPMI-1640 medium and Gibvco FBS were bought from ThermoFisher Scientific ThermoFisher Scientific (Shanghai, China). Trypsin was product of Invitrogen (Shanghai, China). Reverse transcription kit was obtained from Luoqi Biotechnology (Henan, China). Fluorescent dye SYBR Green I was purchased from Takara (Beijing, China). Protein lysate and protease inhibitor were products of Beyotime Biotechnology, (Shanghai, China). Antibodies for ZEB1, E-cadherin, Vimentin,  $\beta$ -actin were got from Proteintech Company (Proteintech, Wuhan, China), while Cell Counting Kit (CCK-8) was obtained from Topscience Co. Ltd (Shanghai, China).

### Cell culture

HGC-27 cells (Shanghai Institute of Cell Biology, China, Shanghai) were cultured at 37 °C in 90 % RPMI-1640 containing 10 % FBS in a 5 % CO<sub>2</sub>

cell incubator at relative humidity > 95 %. Cells in logarithmic growth phase were divided into control group treated with normal saline, and three groups treated with DEX at doses of 0.01, 0.1 and 1  $\mu$ mol/L. All treatments lasted for 48 h.

### Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from the cells in each group with TRIzol reagent. Then, the total RNA was reverse-transcribed to cDNA using reverse transcription kit (Takara, Japan) according to the manufacturer's instructions. The primers for miR-144-3p, ZEB1, E-cadherin, Vimentin and  $\beta$ -actin which were used for qPCR (Table 1) were synthesized by Sangon Biotech (Sangon, Shanghai, China). The qRT-PCR reaction system was constituted in line with the operating instructions in a 7500 real-time fluorescence quantitative PCR instrument (Applied Biosystems, American, CA). The reaction steps were as follows: 50 °C for 2 min, 95 °C for 10 min, 95 °C for 15 min x 40 cycles; 95 °C for 15 sec, 60 °C for 1 min, 95 °C for 30 min, and 60 °C for 15 sec. The mRNA expression levels of the genes were calculated using the 2<sup>- $\Delta\Delta$ Ct</sup> method.

### Western blot assay

Total protein was extracted with IP lysing solution containing protease inhibitor, and the protein concentration of the lysate was determined using BCA kit. Equal amounts of protein (50- $\mu$ g) were resolved on 10 % polyacrylamide gel electrophoresis, followed by transfer to PVDF membrane. The membrane was blocked with 10 % skimmed milk at room temperature for 2 h, prior to incubation with primary antibodies for ZEB1, E-cadherin and Vimentin (each at 1:1000 dilution) at 4 °C overnight. Thereafter, the membrane was incubated with secondary antibody at room temperature for 1 h. The blots were subjected to enhanced chemiluminescence (ECL) analysis for 1 h. Image analysis was done with ChemiDoc XRS Chemiluminescence Imager (Bio-Rad, USA).

**Table 1:** Primer sequences for qRT-PCR

Gene	5' - 3'	3' - 5'
miR-144-3p	GCGCGCTACAGTATAGATGATG	GCTGTCAACGATACGCTACG
ZEB1	GCTTCTCACACTCTGGGTCTTA	CCTCATTCTCTGCCTCTTCTACC
E-cadherin	CCCGCCTTATGATTCTCTGCTCGTGTCC	TCCGTACATGTCAGCCAGCTTCTTGAA
Vimentin	TTTCTCCACGCCTCCAGTT	ATGTCTTCGGCCAGGTTGT
$\beta$ -Actin	GGCAGGTCATCACCATCGG	CGTGTGGCGTAGAGGTCTTT

## Determination of cell proliferation

Cells in each group were inoculated in 96-well plate at 0, 12, 24, 48 and 72 h, with 5 replicates at each time point. After adherence, 10  $\mu$ L CCK-8 solution was added to each well. The wells were cultured in the dark for 2 h, after which the optical density (OD) of each well was read at 450nm in a microplate reader.

## Transwell assay

Matrigel glue (50 mg/L) was diluted in serum-free RPMI-1640 medium at a volume ratio of 8:1. It was evenly spread in the center of Transwell chamber, and air-dried at 37 °C for 2 h as basement membrane reservoir. Cells in each group were digested, centrifuged and re-suspended in RPMI-1640 containing 1 % FBS in serum-free RPMI-1640 hydrated basement membrane. In each group,  $3 \times 10^4$  cells were inoculated on the basement membrane as invasion model, while  $1 \times 10^4$  cells were inoculated in Transwell chamber without Matrigel glue (migration model). After addition of 600  $\mu$ L of RPMI-1640 containing 10 % FBS solution to the lower chamber, the cells were cultured in a constant temperature incubator for 24 h. Thereafter, the cells were fixed with 4 % paraformaldehyde for 15 min, and stained with 0.1 % crystal violet for 30 min. Then, the cells were examined under a light microscope and counted using ImageJ software.

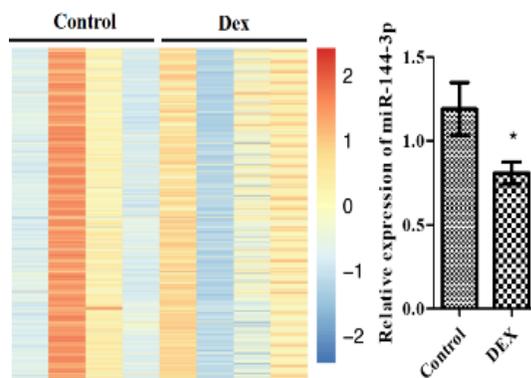
## Statistical analysis

The SPSS 23.0 software was used for statistical analysis. Statistical differences in measurement data amongst the groups were analyzed using analysis of variance, while Student's *t*-test was used for pairwise comparison. Statistical significance was assumed at  $p < 0.05$ .

## RESULTS

### Influence of DEX on miRNA expression profile

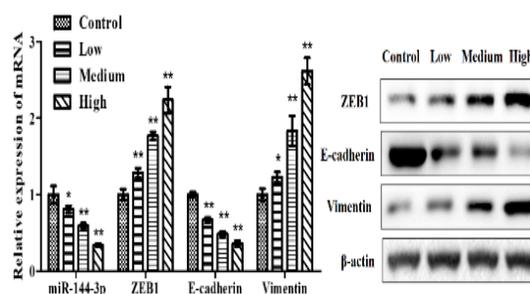
Results from GSE126106 chip analysis (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE126106>) showed that the expressions of miR-871-5p and miR-881-3p were significantly up-regulated after DEX treatment, while the expressions of miR-451-5p and miR-144-3p were markedly reduced ( $p < 0.01$ ). These results are presented in Figure 1.



**Figure 1:** Effect of DEX on miRNA expression profiles in rat cardiomyocytes, as analyzed using GSE126106 chip analysis. \* $P < 0.05$ , compared to the DEX group

### Effect of DEX on the proliferation of HGC-27 cells

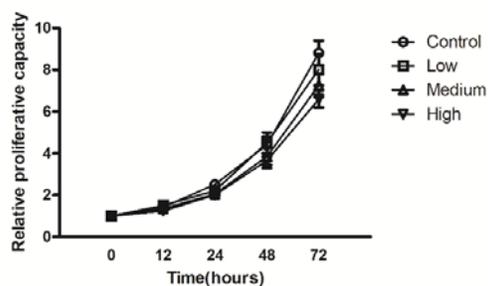
The results of RT-qPCR and Western blot assays showed that, compared with the control group, the expressions of miR-144-3p in low, medium and high DEX concentration groups were decreased significantly, while the mRNA and protein expressions of ZEB1, vimentin were markedly up-regulated ( $p < 0.05$ ). Moreover, the expression of E-cadherin was significantly decreased ( $p < 0.05$ ). These data are shown in Figure 2.



**Figure 2:** Expression levels of miR-144-3p and EMT-related molecules in each group of cells. \* $P < 0.05$ , compared to the control group; \*\* $p < 0.001$ , compared to the control group

### Effect of DEX on the proliferation of HGC-27 cells

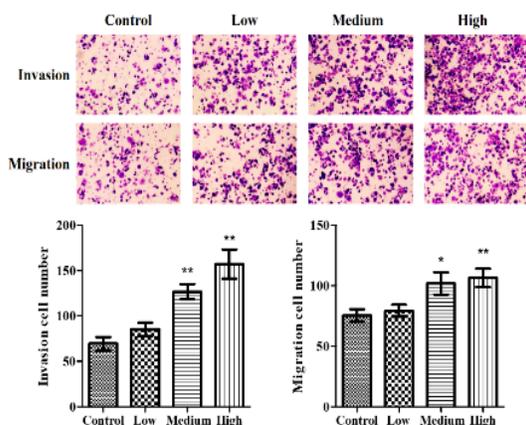
Compared with the control group, the proliferative potential of HGC-27 cells in high DEX concentration group was decreased significantly at 48 h and 72 h, while there were no significant changes in cell proliferation at the low and middle DEX concentration groups ( $p > 0.05$ ; Figure 3).



**Figure 3:** Cell proliferation potential in each group.  $**P < 0.001$ , compared to the control group

### Effect of DEX on the invasion and migration of HGC-27 cells

As shown in Figure 4, the invasion and migration potential of cells in the middle- and high-DEX concentration groups were significantly higher than those in the control group ( $p < 0.05$ ).



**Figure 4:** Cell invasion and migration potential in each group.  $*P < 0.05$ , compared to the control group;  $**p < 0.001$ , compared to the control group

## DISCUSSION

Dexmedetomidine (DEX) is a frequently-used intravenous anesthetic in clinics. In recent years, it has been discovered that DEX regulates the malignant behaviors of tumor cells. A study revealed that DEX significantly upregulated the phosphorylation levels of the transmembrane protease serine 2 (TMPRSS2) and STAT3 Tyr705 in breast cancer cells i.e., MCF-7 and MDA-MB-231, and promoted their migration [4]. Moreover, it has been reported that DEX enhanced the invasion and metastasis of A549 lung cancer cells and HCT116 colorectal cancer cells, through a mechanism related to upregulation of matrix metalloproteinases MMP-2 and MMP-9, and hypoxia-induced factor-1 $\alpha$  (HIF-1 $\alpha$ ) [8]. Dexmedetomidine (DEX) up-regulated

the expression of miR-155 in ovarian cancer cells, thereby inhibiting the tumor-promoting effect mediated by HIF-1 $\alpha$  [9]. It has also been demonstrated that DEX promoted the expression of miR-143-3p and inhibited the malignant behavior of esophageal cancer cells [10]. These results indicate that DEX exerts different effects on different malignant tumor cells. However, its effect on gastric cancer cells, and the related mechanisms are still unclear. In the present study, it was found that DEX significantly down-regulated the expression level of miR-144-3p in rat myocardium in the GEO database chip (GSE126106). Thus, it can be reasonably speculated that miR-144-3p may serve as a potential target of DEX.

Low expression of miR-144-3p is often closely associated with malignant tumors [11-13]. It has been reported that in gastric cancer, the expression level of miR-144-3p was significantly lower than those of adjacent tissues, and it was negatively correlated with lymph node metastasis and TNM stage, suggesting that miR-144-3p may be a protective factor for gastric cancer [14]. *In vitro* studies have shown that miR-144-3p promoted the radiosensitivity of gastric cancer cells by inhibiting the expression of zinc finger transcription factor ZEB1 [15]. Moreover, it has been reported that miR-144-3p regulated the target molecules PBX3 and CBX4, and inhibited epithelial-mesenchymal transformation, proliferation and metastasis of gastric cancer cells [16,17].

In the present study, human gastric cancer cells HGC-27 were used as an *in vitro* model. It was found that the expression of miR-144-3p was significantly and dose-dependently reduced after DEX treatment. This finding was consistent with the sequencing data of the GSE126106 chip. At the same time, the expression of EMT-related molecules downstream of miR-144-3p were significantly changed, and the EMT process was enhanced. Moreover, the levels of invasion and migration were significantly increased. These results indicate that DEX inhibited the expression of miR-144-3p and promoted the EMT process of gastric cancer cells. Interestingly, cell proliferation was inhibited on treatment with the high concentration of DEX, due to the cytotoxicity produced the drug through other mechanisms.

## CONCLUSION

DEX promotes the metastasis of gastric cancer cells by regulation of miR-144-3p and EMT. This finding provides a new insight into the treatment of gastric cancer.

## DECLARATIONS

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### Conflict of Interest

No conflict of interest associated with this work.

### Contribution of Authors

We declare that this work was done by the authors named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Zong Chen and Yong Ding conceived and designed the study. Ying Zeng, Zhifeng Chen, Xueping Zhang and Jianyan Chen collected and analyzed the data, while Zong Chen wrote the manuscript which was approved by all authors. Zong Chen and Yong Ding contributed equally to this study.

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## REFERENCES

- Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. *Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries.* *CA Cancer J Clin* 2018; 68(6): 394-424.
- Yu ZY, Geng J, Li ZQ, Sun YB, Wang SL, Masters J, Wang DX, Guo XY, Li M, Ma D. *Dexmedetomidine enhances ropivacaine-induced sciatic nerve injury in diabetic rats.* *Br J Anaesth* 2019; 122(1): 141-149.
- Zhao Y, He J, Yu N, Jia C, Wang S. *Mechanisms of Dexmedetomidine in Neuropathic Pain.* *Front Neurosci* 2020; 14: 330.
- Chi M, Shi X, Huo X, Wu X, Zhang P, Wang G. *Dexmedetomidine promotes breast cancer cell migration through Rab11-mediated secretion of exosomal TMPRSS2.* *Ann Transl Med* 2020; 8(8): 531.
- Wang Z, Shen Z, Wang H, Zhang L, Dong R. *Effect of dexmedetomidine on the cognitive function of patients undergoing gastric cancer surgery by regulating the PI3K/AKT signaling pathway.* *Oncol Lett* 2020; 19(2): 1151-1156.
- Wei L, Sun J, Zhang N, Zheng Y, Wang X, Lv L, Liu J, Xu Y, Shen Y, Yang M. *Noncoding RNAs in gastric cancer: implications for drug resistance.* *Mol Cancer* 2020; 19(1): 62.
- Zhou M, Wu Y, Li H, Zha X. *MicroRNA-144: A novel biological marker and potential therapeutic target in human solid cancers.* *J Cancer* 2020; 11(22): 6716-6726.
- Chen HY, Li GH, Tan GC, Liang H, Lai XH, Huang Q, Zhong JY. *Dexmedetomidine enhances hypoxia-induced cancer cell progression.* *Exp Ther Med* 2019; 18(6): 4820-4828.
- Zheng L, Jia R, Zhao J. *Dexmedetomidine Regulates Proliferation, Apoptosis, Migration, and Invasion in Ovarian Cancer Cells via MiR-155-HIF-1 $\alpha$  Axis.* *Med Sci Monit* 2019; 25: 10164-10172.
- Zhang P, He H, Bai Y, Liu W, Huang L. *Dexmedetomidine suppresses the progression of esophageal cancer via miR-143-3p/epidermal growth factor receptor pathway substrate 8 axis.* *Anticancer Drugs* 2020; 31(7): 693-701.
- Wu J, Zhao Y, Li F, et al. *MiR-144-3p: a novel tumor suppressor targeting MAPK6 in cervical cancer.* *J Physiol Biochem* 2019; 75(2): 143-152.
- Sun N, Zhang L, Zhang C, Yuan Y. *miR-144-3p inhibits cell proliferation of colorectal cancer cells by targeting BCL6 via inhibition of Wnt/beta-catenin signaling.* *Cell Mol Biol Lett* 2020; 25: 19.
- Chen YJ, Guo YN, Shi K, Huang HM, Huang SP, Xu WQ, Li ZY, Wei KL, Gan TQ, Chen G. *Down-regulation of microRNA-144-3p and its clinical value in non-small cell lung cancer: a comprehensive analysis based on microarray, miRNA-sequencing, and quantitative real-time PCR data.* *Respir Res* 2019; 20(1): 48.
- Sahami-Fard MH, Yazd EF, Jamalpoor Z, Nezhadi A. *Expression Levels of miR-127-3p and miR-144-3p in Gastric Cancer and their Relationships with Clinicopathological Parameters.* *Clin Lab* 2020; 66(6): 417.
- Gao ZY, Liu H, Zhang Z. *miR-144-3p increases radiosensitivity of gastric cancer cells by targeting inhibition of ZEB1.* *Clin Transl Oncol* 2020; 7: 618.
- Li B, Zhang S, Shen H, Li C. *MicroRNA-144-3p suppresses gastric cancer progression by inhibiting epithelial-to-mesenchymal transition through targeting PBX3.* *Biochem Biophys Res Commun* 2017; 484(2): 241-247.
- Yang Z, OuYang X, Zheng L, Dai L, Luo W. *Long intergenic noncoding RNA00265 promotes proliferation of gastric cancer via the microRNA-144-3p/Chromobox 4 axis.* *Bioengineered* 2021; 12(1): 1012-1025.