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Original Article

Low expression of microRNA-143 is related to degenerative scoliosis possibly by regulation of cyclooxygenase-2 expression

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Abstract: Aims: This study is to determine if expression level of microRNA-143 (miR-143) and cyclooxygenase-2 (COX-2) are relatedto the occurrence and development of degenerative scoliosis. Methods: A total of 30 patients with degenerative scoliosis, 30 patients with adolescent idiopathic scoliosis were enrolled in this study. For control, 30 patients with spinal burst fractures were also enrolled in this study. Real-time PCR and western blotting was performed to measure the expression levels of COX-2 in intervertebral disc tissues, peripheral blood and cerebrospinal. Expression levels of miR-143 in intervertebral disc tissues, peripheral blood and cerebrospinal were detected by real-time PCR. Results: The expression levels of COX-2 were increased in intervertebral disc tissues, peripheral blood and cerebrospinal of patients with degenerative scoliosis when compared with those of patients with adolescent idiopathic scoliosis and spinal burst fractures ($P < 0.05$). However, the expression levels of miR-143 were decreased in intervertebral disc tissues, peripheral blood and cerebrospinal of patients with degenerative scoliosis when compared with those of patients with adolescent idiopathic scoliosis and spinal burst fractures ($P < 0.05$). Conclusions: COX-2 is highly expressed whereas miR-143 is lowly expressed in patients with degenerative scoliosis. Decreased expression of miR-143 may be related to the aggravation of degenerative scoliosis by regulation of COX-2.

Keywords: Degenerative scoliosis, cyclooxygenase-2, microRNA-143, intervertebral disc tissues

Introduction

Degenerative scoliosis (DS), also known as adult scoliosis, occurs lateral bending in the straight spine after skeletal maturation with a Cobb angle greater than 10° [1]. Organic disease such as spinal cord trauma, scoliosis caused by tumor, and et al are excluded from DS. Lesions in spine commonly occur in the lumbar spine and a small part of thoracolumbar spine, accompanied by reduced thrust forward in lumbar spine, slipped vertebrae, spinal stenosis, and so on [2]. The elderly population is high-risk groups for this disease. With the progress of the global population aging, the proportion of the elderly population is increased year by year, the incidence of DS is also increased significantly [3]. Spinal degenerative diseases firstly occur in the intervertebral disc. Degenerative intervertebral disc can result in spinal stenosis, spinal instability, cervical spondylosis, low back pain, spinal cord compression [2]. Degenerative disc disease refers to the tissue components of the disc occurs biochemical reactions under the influence of various factors, which make these components change in the property, number and structure, eventually leading to the dysfunction [4]. However, mechanism of degenerative disc disease is unclear. With the development of molecular biology, studies of the mechanism of degeneration are no longer limited to traditional mechanical factors, but from the perspective of molecular biology [5].

Cyclooxygenase-2 (COX-2) is an enzyme that is responsible for formation of important biological mediators called prostaglandins. COX-2 can transform arachidonic acid into of various prostaglandins product, which involve in variety of pathophysiological processes, such as inflammation, fever, and blood clotting mechanism [6-8]. Regulation of COX-2 expression mainly depends on the level of transcription. COX-2

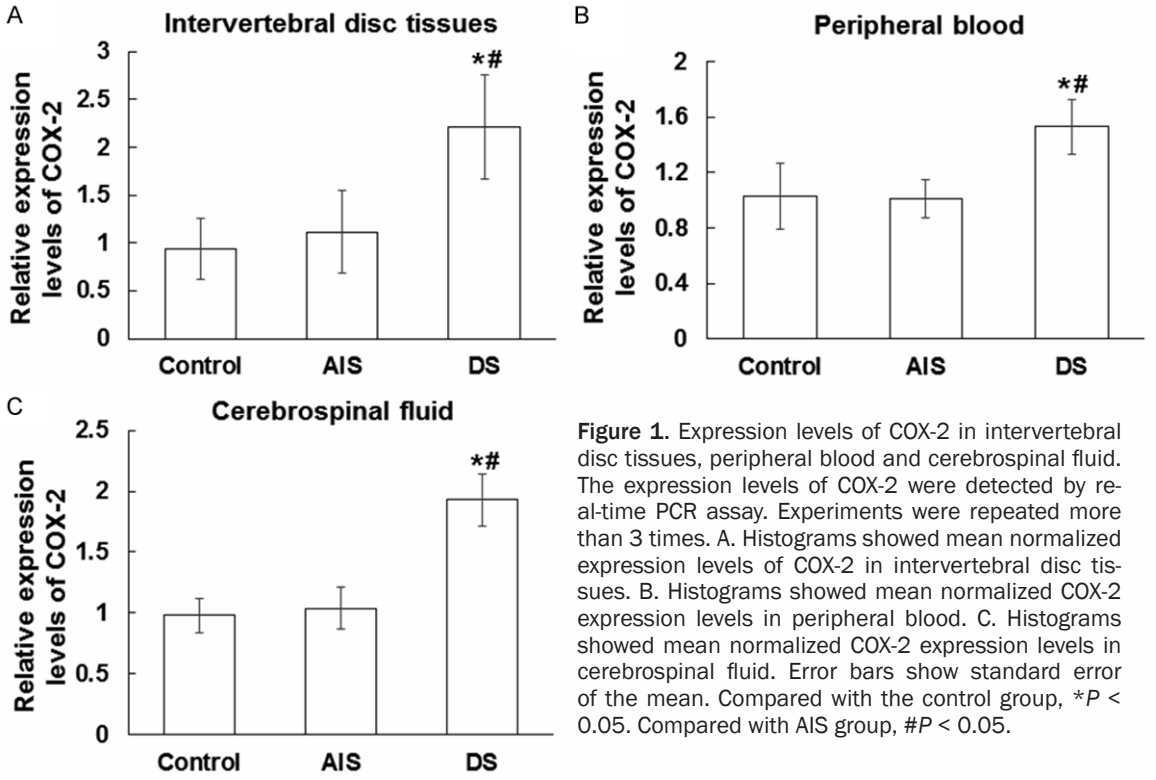

Figure 1. Expression levels of COX-2 in intervertebral disc tissues, peripheral blood and cerebrospinal fluid. The expression levels of COX-2 were detected by real-time PCR assay. Experiments were repeated more than 3 times. A. Histograms showed mean normalized expression levels of COX-2 in intervertebral disc tissues. B. Histograms showed mean normalized COX-2 expression levels in peripheral blood. C. Histograms showed mean normalized COX-2 expression levels in cerebrospinal fluid. Error bars show standard error of the mean. Compared with the control group, $*P < 0.05$. Compared with AIS group, $\#P < 0.05$.

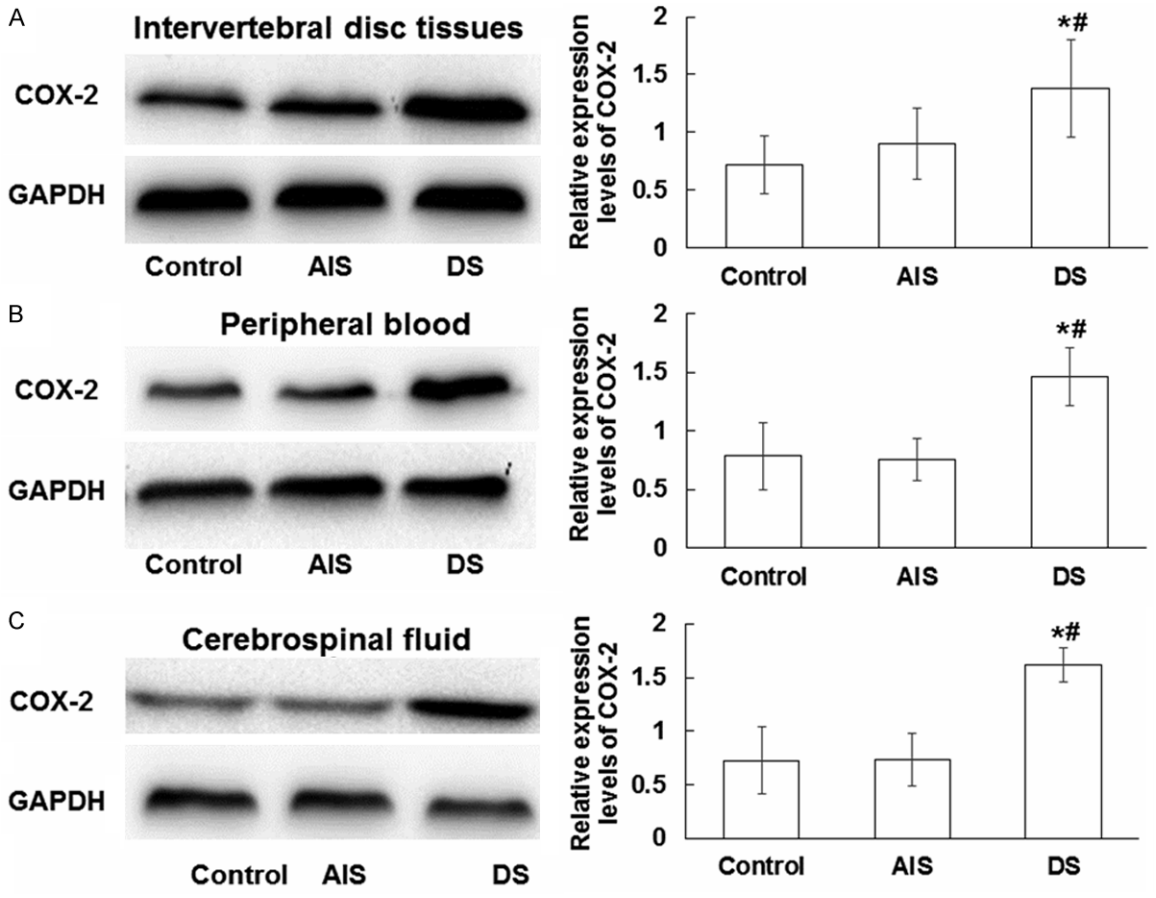

Figure 2. Expression levels of COX-2 in intervertebral disc tissues, peripheral blood and cerebrospinal fluiddetected by western blotting. Total proteins of intervertebral disc tissues, peripheral blood and cerebrospinal fluid were harvested and separated on 12% SDS/PAGE gels, and then analyzed by western blotting. GAPDH was used as a loading control. Expression levels of COX-2 were detected by rabbit anti-human COX-2 polyclonal antibodies (Abcam, Cambridge, MA, USA). A. Expression levels of COX-2 in intervertebral disc tissues. Left panel: representative western blotting results. Right panel: quantitative western blotting results. B. Expression levels of COX-2 in peripheral blood. Left panel: representative western blotting results. Right panel: quantitative western blotting results. C. Expression levels of COX-2 in cerebrospinal fluid. Left panel: representative western blotting results. Right panel: quantitative western blotting results. Compared with the control group, $*P < 0.05$. Compared with AIS group, $\#P < 0.05$.

expression is increased in many malignant tumors, which is closely associated with tumor cell proliferation, apoptosis, and angiogenesis [9-11]. COX-2 plays an important role in the regulatory of angiogenesis [9]. There is no blood vessel in the normal intervertebral disc, whereas new blood vessels are more common in the degenerative intervertebral disc [12]. Previous study showed that expression of COX-2 was increased in the degenerative intervertebral disc when compared with that in the normal intervertebral disc, which suggest that COX-2 may be related to the progress of angiogenesis in the degenerative intervertebral disc [13].

MicroRNA (miRNA), widely involves in the gene expression through post-transcriptional control, regulating the stability of mRNA [14]. MiRNA plays an important role in the regulation of many physiological and pathological processes However, miRNA expression profiles in tissues of patients with DS and its function are unclear. Studies showed that COX-2 expression was inhibited by microRNA-143 (miR-143) in the cells of bladder cancer, stomach cancer, and pancreatic cancer [15-17]. For patients with DS, whether miR-143 involves in the progression of DS through COX-2 is unknown. In this study, expression of miR-143 and COX-2 in the degenerative intervertebral disc tissues, peripheral blood, and cerebrospinal fluid of patients with DS has been studied. The relation of miR-143 and COX-2 expression in patients with DS has been discussed.

Materials and methods

Reagents

Trizol used for RNA extraction was purchased from Invitrogen (Life Technologies, Carlsbad, CA, USA). Takara PrimeScript RT Regent Kit and SYBR PrimeScript RT-PCR Kit II (Perfect Real Time) were purchased from TaKaRa Biotechnology (Tokyo, Japan). The rabbit anti-human COX-2 polyclonal antibodies were purchased from Abcam (Cambridge, MA, USA). The total protein extraction reagent was purchased from BioTeke Corporation (Beijing, China).

Patients

A total of consecutive 30 patients with DS (DS group) and 30 patients with adolescent idiopathic scoliosis (AIS) (AIS group) were enrolled in this study. The age of patients with DS ranged from 50 to 65 years, with an average age of 55.3 years. The age of patients with AIS ranged from 10 to 15 years, with an average age of 13.4 years. Intervertebral disc tissues collected from patients with DS was classified as degenerative intervertebral disc. Intervertebral disc tissues collected from patients with AIS was classified as normal intervertebral disc. For control, a total of consecutive 30 patients with spinal burst fractures (control group) were enrolled in this study. The age of these patients with spinal burst fractures ranged from 45 to 61 years, with an average age of 51.9 years. Peripheral blood and cerebrospinal fluid were collected from every patient. Prior written and informed consent were obtained from every patient. The study was approved by the ethics review board of the 117 Hospital of People's Liberation Army.

Real-time PCR assay

Total RNA of intervertebral disc tissues, peripheral blood and cerebrospinal fluid were extracted with Trizol reagent. Quality of RNA was detected by RNA electrophoresis and spectrophotometer at optical density 260/280. All RNA was reversely transcribed into cDNA with the PrimeScript RT Regent Kits. Real-time PCR assay was conducted using SYBR PrimeScript RT-PCR Kit II (Perfect Real Time) in accordance with the manufacturer's instructions. GAPDH was used as an internal control for COX-2. The primers for COX-2 are 5'-ATAACCCCGCCAAAAGGGG-3' and 5'-CTGAGTACCAGGTCTGCAGTG-

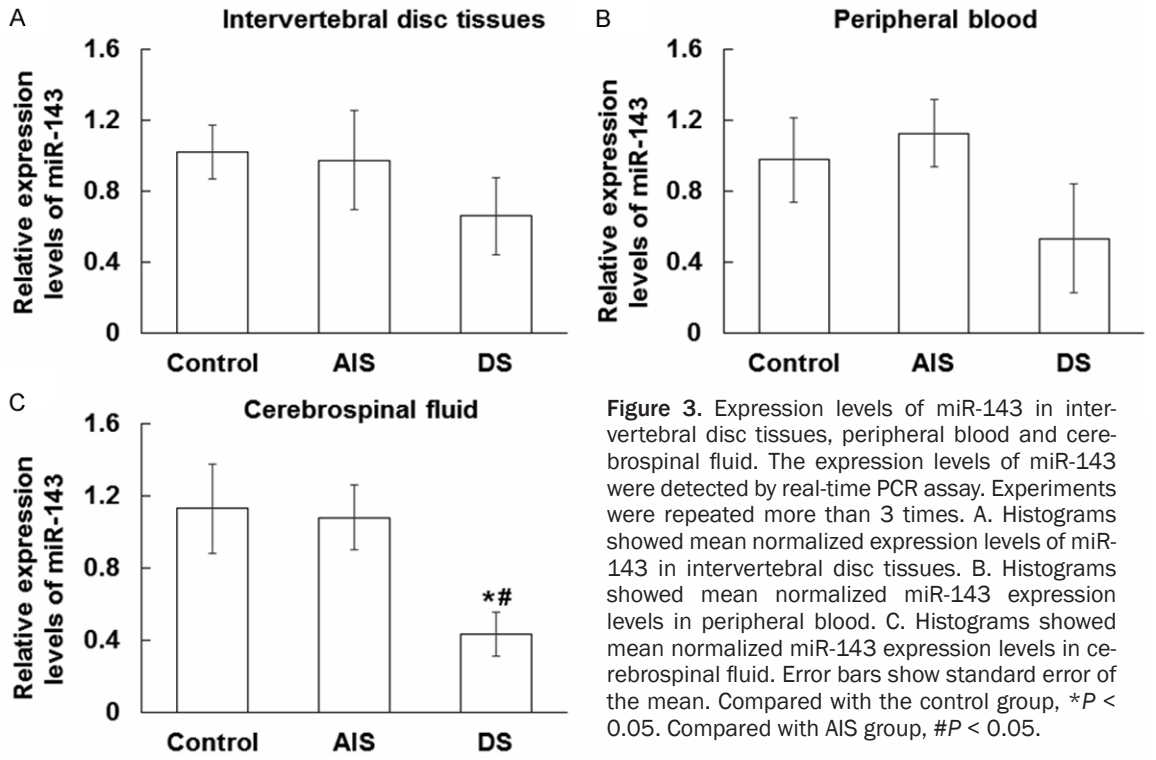

Figure 3. Expression levels of miR-143 in intervertebral disc tissues, peripheral blood and cerebrospinal fluid. The expression levels of miR-143 were detected by real-time PCR assay. Experiments were repeated more than 3 times. A. Histograms showed mean normalized expression levels of miR-143 in intervertebral disc tissues. B. Histograms showed mean normalized miR-143 expression levels in peripheral blood. C. Histograms showed mean normalized miR-143 expression levels in cerebrospinal fluid. Error bars show standard error of the mean. Compared with the control group, $*P < 0.05$. Compared with AIS group, $\#P < 0.05$.

3'. The primers for GADPH are 5'-GGGAGCCAAAAGGGTCATCA-3' and 5'-TGATGGCATGGACTGTGGTC-3'. U6 was used as an internal control for miR-143. The primers for miR-143 were 5'-GCTGAGATGAAGCACTGAAGCTC-3' and 5'-GTTGACCTACCTTTTGATGCC-3'. The primers for U6 were 5'-CAAAGTCAGTGCAGGTAGGCTTA and 5'-AACGCTTCACGAATTTGCGT-3'. Oligonucleotide primers of COX-2, miR-143, GAPDH, and U6 were synthesized from (Invitrogen, Shanghai, China). For each sample, PCR reaction was repeated for at least 3 times.

Western blotting

Intervertebral disc tissues were homogenized in RIPA buffer (50 mM Tris-base, 1 mM EDTA, 150 mM NaCl, 0.1% SDS, 1% TritonX-100, 1% Sodium deoxycholate) after liquid nitrogen grinding. Total proteins of peripheral blood and cerebrospinal fluid were extracted by the total protein extraction reagent (BioTeke Corporation, Beijing, China). Total proteins were separated by 12% SDS-PAGE gel and then analyzed by immunoblotting. GAPDH was used as an internal control. The primary antibodies were rabbit anti-human COX-2 polyclonal antibodies (1:800) and rabbit anti-human anti-GAPDH polyclonal antibodies (1:2000). The secondary antibodies used in this study were HRP-conjugated goat anti-rabbit antibodies (1:1000). The blots were detected using BeyoECL Plus enhanced chemiluminescence reagent. Image quantifications were performed using Image Lab software version 4.1 (Bio-Rad Laboratories, Hercules, CA, USA). The experiments were repeated for at least 3 times. Relative quantification of COX-2 expression was performed and expressed as fold relative to GAPDH expression.

Statistical analysis

All results were expressed as mean ± standard deviation. All statistical analyses were performed with SPSS version 16.0 for Windows (SPSS Inc., Chicago, IL, USA). Paired t-test was used to analyze comparisons between groups and analysis of paired data. *P* value less than 0.05 was considered to be significantly different.

Results

Expression levels of COX-2 detected by real-time PCR assay are increased in patients with DS

To determine the expression levels of COX-2 in intervertebral disc tissues, peripheral blood

and cerebrospinal fluid, real-time PCR assay was performed. As shown in **Figure 1**, the expression levels of COX-2 in intervertebral disc tissues of DS group (2.214 ± 0.55) was significantly increased when compared with those of AIS group (1.114 ± 0.43) ($P < 0.05$) and the control group (0.937 ± 0.32) ($P < 0.05$), respectively (**Figure 1A**). The expression levels of COX-2 in peripheral blood of DS group (1.53 ± 0.2) was significantly increased when compared with those of AIS group (1.11 ± 0.14) ($P < 0.05$) and the control group (1.032 ± 0.24) ($P < 0.05$), respectively (**Figure 1B**). The expression levels of COX-2 in cerebrospinal fluid of DS group (1.93 ± 0.214) was significantly increased when compared with those of AIS group (0.98 ± 0.214) ($P < 0.05$) and the control group (1.04 ± 0.172) ($P < 0.05$), respectively (**Figure 1C**). These results indicate that the expression levels of COX-2 are increased in patients with DS.

Expression levels of COX-2 detected by western blotting are increased in patients with DS

To further verify the expression of COX-2 in intervertebral disc tissues, peripheral blood and cerebrospinal fluid, western blotting was performed. The expression levels of COX-2 in intervertebral disc tissues of DS group were significantly increased when compared with those of AIS group and the control group ($P < 0.05$) (**Figure 2A**). The expression levels of COX-2 in peripheral blood of DS group were significantly increased when compared with those of AIS group and the control group ($P < 0.05$) (**Figure 2B**). The expression levels of COX-2 in cerebrospinal fluid of DS group were significantly increased when compared with those of AIS group and the control group ($P < 0.05$) (**Figure 2C**). The western blotting results suggest that the expression levels of COX-2 are increased in patients with DS.

Expression levels of miR-143 are decreased in patients with DS

To investigate the relationship between miR-143 and COX-2, the expression levels of miR-143 in intervertebral disc tissues, peripheral blood and cerebrospinal fluid were examined by real-time PCR assay. The expression levels of miR-143 in intervertebral disc tissues of DS group (0.435 ± 0.12) was significantly decreased when compared with those of AIS group (1.082 ± 0.18) ($P < 0.05$) and the control group (1.131 ± 0.25) ($P < 0.05$), respectively (**Figure 3A**). The expression levels of miR-143 in peripheral blood of DS group (0.657 ± 0.22) was significantly decreased when compared with those of AIS group (0.973 ± 0.28) ($P < 0.05$) and the control group (1.02 ± 0.15) ($P < 0.05$), respectively (**Figure 3B**). The expression levels of miR-143 in cerebrospinal fluid of DS group (0.532 ± 0.31) was significantly decreased when compared with those of AIS group (1.124 ± 0.19) ($P < 0.05$) and the control group (0.976 ± 0.24) ($P < 0.05$), respectively (**Figure 3C**). These results indicate that the expression levels of miR-143 are decreased in patients with DS. The expression of miR-143 may be related to the increase expression of COX-2.

Discussion

In many malignant tumors, COX-2 expression is increased, which is associated with tumor cell proliferation, apoptosis, and angiogenesis [9-11]. Previous study showed that angiogenesis occurred in degenerative intervertebral disc, especially in tissues of herniated intervertebral disc, whereas no blood vessel was seen in normal intervertebral disc [18]. Expression of COX-2 can be positively regulated by prostaglandin. Levels of prostaglandin are significantly increased in degenerative intervertebral disc when compared with those in normal intervertebral disc [13]. As a rate-limiting enzyme in the synthesis of prostaglandin, COX-2 is closely related to the spinal degenerative disease.

Previous study showed that miR-143 could specifically bind to 3′-untranslated regions of COX-2 mRNA, promoting formation of RNA-induced silencing complex and COX-2 mRNA degradation in human bladder cancer cells. Therefore, the expression of COX-2 and functions of tumor cells were inhibited [15]. In this study, the expression of miR-143 in intervertebral disc tissues, peripheral blood and cerebrospinal fluid were determined. Real-time PCR results indicate that the expression of miR-143 in intervertebral disc tissues, peripheral blood and cerebrospinal fluid of DS group are significantly decreased when compared with those of AIS group and the control group. In addition, real-time PCR and western blotting results indicate that the expression of miR-143 in intervertebral disc tissues, peripheral blood and cerebrospinal fluid of DS group are significantly increased when compared with those of AIS

group and the control group. These results suggest that miR-143 play an important role in the progression of DS through binding to COX-2. Decreased expression of miR-143 is related to the increased expression of COX-2. Our results provide evidences for the mechanism of abnormal COX-2 expression in the progression of DS. The studies on the function of miR-143 have a clinical significance in clinical diagnosis, treatment and prognosis of DS patients. In conclusion, miR-143 plays an important role in the in the progression of DS. Decreased expression of miR-143 may be related to the aggravation of DS.

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Disclosure of conflict of interest

None.

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