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Knockdown of lncRNA XIST inhibits retinoblastoma progression by modulating the miR-124/STAT3 axis

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ABSTRACT

Long non-coding RNA (lncRNA) X-inactive specific transcript (XIST) was reportedly to be tightly associated with tumorigenesis and progression of multiple cancers. However, the expression, biological function, and action mechanisms of XIST in retinoblastoma (RB) are still unknown. Here, we found that XIST expression was upregulated in RB tissues and cell lines, and that increased XIST expression was positively associated with advanced cTNM stage (III-V) and late differentiation status. We also revealed that knockdown of XIST inhibited RB cell proliferation, promoted cell cycle at G1/G0 phase, and induced cell apoptosis. Mechanistically, XIST directly bound to microRNA (miR)-124 in RB cells. *XIST* mRNA expression was inversely correlated with miR-124 in RB tissues. Importantly, miR-124 inhibition partially reversed the effect on cell proliferation, cycle arrest and apoptosis by XIST knockdown mediated. In addition, XIST could regulate expression of signal transducer and activator of transcription 3(STAT3), a directly target of miR-124 in RB. These findings implied that XIST promoted RB progression partially by modulating the miR-124/STAT3 axis.

1. Introduction

Retinoblastoma (RB) is the most common primary intraocular malignant tumor during childhood [1]. Due to the recent development of early tumor recognition and treatments, the survival rates of patients with RB have significantly improved [2]. But many retinoblastoma survivors are at risk of blindness and fatal eyes loss because of metastasis and resistant-chemotherapy or radiotherapy [2,3]. Therefore, understanding molecular mechanism involved in initiation and progression of RB are critical to develop molecular targeted therapeutic strategies for this disease.

Long non-coding RNAs (LncRNAs) were a class of RNA transcripts longer than 200 nucleotides in length without protein-coding capacity, which have been studied extensively in the past decade [4]. LncRNAs have been shown to be implicated in various physiological and pathological processes, such as cellular multipotency, differentiation, cellular apoptosis, X chromosome imprinting, and even tumorigenesis [5–7]. Many lncRNAs were identified to be frequently dysregulated in various cancers and function as critical tumor modulators or potential cancer biomarkers via complexes regulating mechanisms on oncogene and tumor suppressor [8,9]. In the past few years, several reports have highlighted the importance of LncRNAs in RB growth and metastasis, suggesting the involvement of lncRNAs in RB progression [10–12].

X-inactive specific transcript (XIST), located on chromosome Xq13.2 [13], has been found to be dysregulated in a variety of human cancers, and play crucial roles in the development and progression of tumors by regulating cancer cell proliferation, invasion and migration [14–16]. However, the roles and exact mechanism of XIST in the RB is still unknown.

A growing number of studies reported that microRNAs (miRNAs) are involved in development and progression of RB, and function as either oncogenes or tumor-suppressor genes [17].miR-124, an important miRNA, has been reported to serve as tumor suppressor in RB progression [18]. It has been shown that lncRNAs could serve as miRNA sponges and regulate the initiation and development of RB [10–12]. Therefore, we focused on the interaction between XIST and miR-124 in RB progression.

The aims of this study were to investigate the expression pattern, biological function XIST in RB progression. Moreover, the regulatory mechanism by which XIST interacted with miR-124 in the regulation of biological behaviors of RB cells was determined.

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Table 1
Correlation between clinicopathological features and XIST expression in 30 patients with RB. Patients was divided into high XIST expression and low XIST expression group based on mean value of XIST expression in RB tissues(n = 30).

Variables	No. of cases	XIST expression		P value
		High (n %)	Low (n %)	
Age(years)				$P > 0.05$
< 5	21	12(57.1)	9 (42.9)	
≥5	9	5(55.6)	4(43.4)	
Gender				$P > 0.05$
Male	16	9(56.3)	7(43.7)	
Female	14	8(57.1)	6(42.9)	
cTNM stage				$P < 0.01$
I-II	20	8(40.0)	12(60.0)	
III-IV	10	9(90.0)	1(10.0)	
Tumor size				$P > 0.05$
< 15 mm	18	10(55.5)	8(45.5)	
≥15 mm	12	7(58.4)	5(41.6)	
Differentiation				$P < 0.01$
Poor	13	11(84.6)	2(16.4)	
Moderate or well	17	6(35.3)	11(64.7)	

RB, retinoblastoma; cTNM, clinical staging of tumor (T), the extent of spread to the lymph nodes (N), and the presence of metastasis (M).

Table 2
Primers for qRT-PCR analysis.

Target gene	Prime(5'-3')
miR-124	F- CGGTA AGGCA CGCGG TGA
	R- AGTGC GAACT GTGGC GAT
U6	F- CTCGCTTCGGCAGCACA
	R- AACGCTTCACGAATTTGCGT
STAT3	F- GCACAGATTGCCTGCATTG
	R- CTGCTAATGACGTTATCCAGT
CyclinA1	F- GACGGCACCAACCACTA
	R- ACTCAGGCAAGGCACAA
CyclinB1	F- AATCCCTTCTTGTGGTTA
	R- CTTAGATGTGGCATACTTG
CyclinD2	F- ACCTTCCGCAGTGCTCCTA
	R- CCCAGCCAAGAAACGGTCC
GAPDH	F-TGCCAACGTGTCGGTTGT
	R-TGTCATCATATTTGGCAGGTTT

Abbreviations: F, forward; mRNA, messenger RNA; PCR, polymerase chain reaction; R, reverse.

2. Materials and methods

2.1. Human tissue samples

A total of 30 human RB specimens (mean age 4.3 years) were collected from patients with RB during enucleation at the Department of Ophthalmology, the Second Hospital of Jilin University (Changchun, China). All tumor tissues were examined by two independent histopathologists, and graded in accordance with the guideline published by the 7 th edition of American Joint Committee on Cancer [19]. Normal retinal specimens as control group were harvested from the ruptured globes of six patients (mean age 6.3 years). All samples were immediately frozen in liquid nitrogen following surgery and stored at −80 °C for further analysis. The clinicopathological information of the patients was recorded and listed Table1. Consent forms were obtained from the donors or their family members. This study protocol was approved by the Ethics Committee of the Second Hospital of Jilin University (Changchun, China).

2.2. Cell culture and transfection

Four human RB cell lines (Y79, Weri-Rb1, SO-RB-50, and HXO-RB44) and human retinal pigment epithelial cell line(ARPE-19) were brought from the from the Institute of Biochemistry and Cell Biology of the Chinese Academy of Sciences (Shanghai, China) All cells grown in RPMI-1640 (Life Technologies, Waltham, MA, USA) containing 10% heat-inactivated fetal bovine serum(FBS, Gibco, Grand Island, NY, USA) and 1% penicillin-streptomycin and incubated at 37 °C in a humidified atmosphere containing 5% CO_2.

siRNAs against XIST (si-XIST), negative control siRNA (si-NC), miR-124 mimics, negative control mimics (miR-NC), miR-124 inhibitor (anti-miR-124) and corresponding negative control (anti-miR-NC) were all purchased from GenePharma(Shanghai, China). These constructs were transiently transfected into RB cells using lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA)according to manufacturers' instructions, respectively.

2.3. RNA isolation and qRT-PCR

Total RNA from tissue samples and cultured cells was extracted using Trizol (Invitrogen) following the manufacturer's instructions. Total RNA (1 μg) was reverse-transcribed to complimentary DNA (cDNA) using the TaqMan miRNA Reverse Transcription Kit (Applied Biosystems, Waltham, MA, USA) to detection for mature miR-124. Then miR-124 was quantified using the TaqMan miRNA kit (Applied Biosystems). The miR-124 expression level was normalized using U6 as an internal control. For detection of mRNA expression, complimentary DNA (cDNA) was reverse transcribed from 2 μg total RNA using a PrimeScript first-strand cDNA synthesis kit (Takara Biotechnology Co., Ltd., Dalian China). Quantitative real-time PCR (qRT-PCR) was performed using a SYBR® Premix Ex Taq™ II (Takara) according to the manufacturer's protocol. The primes used in this study were listed in Table2. Real-time PCR was performed on the ABI 7900 cycler (Applied

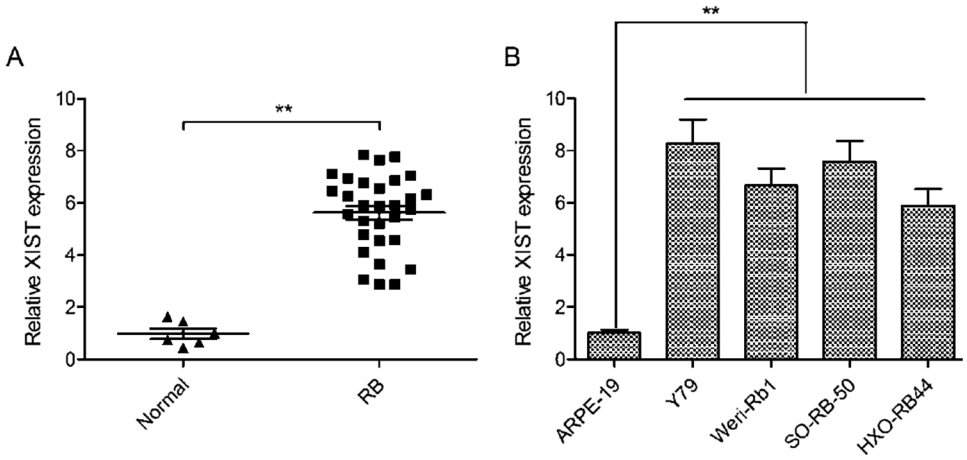

Fig. 1. Expression of XIST is higher in RB tissues and cell lines. (A) qRT-PCR analyses were performed to examine XIST expression in 30 RB tissues compared with normal tissues. (B) qRT-PCR analyses were performed to examine XIST expression in four RB cell line (Y79, Weri-Rb1, SO-RB-50, and HXO-RB44) and retinal pigment epithelial cell line, ARPE-19. GAPDH was used as internal control. *$P < 0.05$, **$P < 0.01$.