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Long noncoding RNA kcna3 inhibits the progression of colorectal carcinoma through down-regulating YAP1 expression

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ABSTRACT

Long non-coding RNAs (lncRNAs) regulate diverse cellular processes, and their anomalous expression exert an essential role in the progression of many kinds of cancers, including colorectal carcinoma (CRC). The objective of this study was to investigate the role of lncRNA kcna3 and its underlying mechanism in CRC progression. The expression of lncRNA kcna3 in human CRC tissues and the adjacent non-tumor tissues was evaluated by RT-PCR. The correlations between lncRNA kcna3 expression levels and the overall survival (OS), as well as the clinicopathological features of CRC patients were analyzed. Gain-of-function and loss-of-function experiments were used to evaluate the effects of lncRNA kcna3 on the proliferation, apoptosis, migration, invasion and tumorigenesis of colon cancer SW620 cells. We found that lncRNA kcna3 was lowly expressed in CRC tissues, and its low expression was closely associated with patients' higher TNM grade and the higher occurrence rate of lymphatic metastasis and distant metastasis, as well as shorter OS. Enhanced expression of lncRNA kcna3 inhibited SW620 cells' proliferation, migration and invasion, and induced cell apoptosis in vitro, and repressed CRC tumor growth in vivo. Whereas knockdown of lncRNA kcna3 showed the opposite results. Mechanistically, up-regulation of lncRNA kcna3 decreased YAP1 protein expression and accelerated its degradation. The effects of lncRNA kcna3 overexpression on cell growth and tumorigenesis inhibition and apoptosis promotion were weakened when the expression of YAP1 was up-regulated. In conclusion, this study revealed that lncRNA kcna3 exerts a tumor-inhibit role in CRC progression through down-regulating YAP1 expression, indicating that lncRNA kcna3/YAP1 might be served as a new prognostic biomarker and therapeutic target for CRC.

1. Introduction

CRC is the third most common malignant tumor in men and second in women [1]. In 2012, there were approximately 694,000 CRC-related deaths all over the world [2]. The 5-year relative survival rate of CRC patients with stage I is greater than 90%, but it is sharply dropped to slightly greater than 10% in patients with stage IV disease [3,4]. Although it is well documented that multiple known carcinogens and genetic backgrounds are contributed to the occurrence of CRC, the regulatory mechanisms of pivotal genes and pathways involved in the tumorigenesis of CRC are still indistinct.

Genomic studies have confirmed that only approximately 2% of human gene transcripts can stably be translated into proteins, producing a number of non-protein-coding ribonucleic acids called noncoding RNA (ncRNAs) [5]. Among the ncRNAs, lncRNAs are a class of transcripts of more than 200 nt [6,7] and are strongly implicated in epigenetic regulation, chromosome remodeling and gene expression [8,9], and promotes the advanced progression of multiple malignancies [10], including CRC [11]. For example, Zhang et al. [12] demonstrated that lncRNA CPS1-IT1 was lowly expressed in CRC tissues and cell lines, and patients with low CPS1-IT1 expression always had poor survival outcomes as compared with that of high CPS1-IT1 expression patients. They also revealed that up-regulation of CPS1-IT1 obviously repressed cell proliferation, migration and invasion and enhanced cell apoptosis in CRC. Han et al. [13] revealed that lncRNA CRNDE was up-regulated in CRC tissue samples, and knockdown of lncRNA CRNDE significantly inhibited cell proliferation and reduced chemoresistance of CRC cells. Wang et al. [14] suggested that the expression of lncRNA AB073614 was elevated in CRC tissues, and lncRNA AB073614 up-regulation significantly improved the proliferation, migration, and invasion of SW480 cells. Nonetheless, the potential functions of majority of lncRNAs in CRC progression remain completely unclear.

LncRNA KCNA3 gene (NCBI Reference Sequence: NR_109846.1) is clustered together with KCNA2 and KCNA10 genes on chromosome 1,

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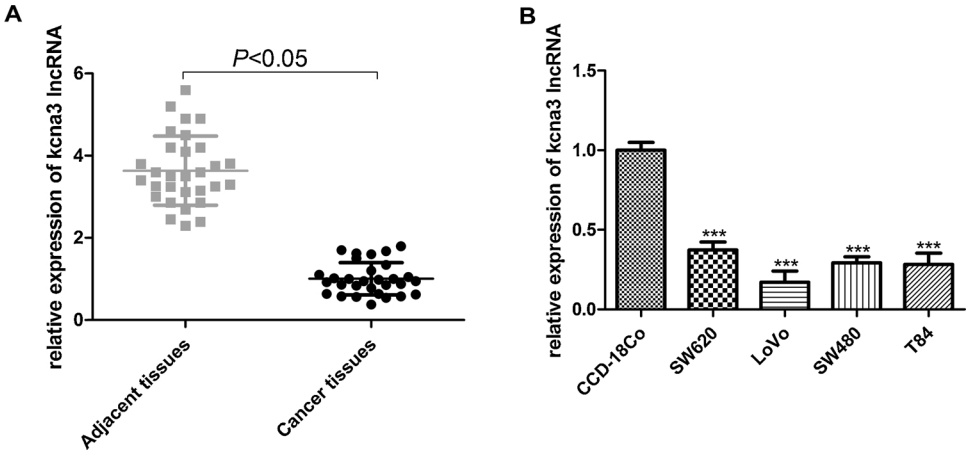

Fig. 1. Determination of the expression of lncRNA kcna3 in CRC tissues. A. RT-PCR analysis of the mRNA level of lncRNA kcna3 in 30 paired CRC tissues and adjacent non-tumor tissues, and the expression of lncRNA kcna3 in CRC tissues was lower than that of the adjacent non-tumor tissues, and the difference was statistically significant ($P < 0.05$). B. RT-PCR was performed to detect the mRNA level of lncRNA kcna3 in normal colon cell line CCD-18Co and CRC cancer cell lines SW480, SW620, T84 and LoVo, and the differences between CCD-18Co group and other group was statistically significant (n = 3, $^{***}P < 0.001$).

Table 1
Clinical pathologic characteristics of CRC patients with high or low lncRNA kcna3 expression.

	N	LncRNA kcna3 expression		*P* value
Age (years)		High	Low	0.599
≤60	53	21	32	
>60	38	13	25	
Gender				0.393
Male	62	25	37	
Female	29	9	20	
Tumor size (cm)				0.167
≤5	45	20	25	
>5	46	14	32	
TNM stage				0.011
I/II	33	18	15	
III/IV	58	16	42	
Lymphatic metastasis				0.001
No	38	22	16	
Yes	53	12	41	
Distant metastasis				0.024
No	56	26	30	
Yes	35	8	27	
Vessel invasion				0.101
No	60	26	34	
Yes	31	8	23	

and encodes a member of the potassium channel, voltage-gated, shaker-related subfamily. This member contains six membrane-spanning domains with a shaker-type repeat in the fourth segment. It belongs to the delayed rectifier class, members of which allow nerve cells to efficiently repolarize following an action potential. It plays an essential role in T-cell proliferation and activation. However, its effects on tumorigenesis remains unknown. This study aimed to explore the function of lncRNA kcna3 and its underlying mechanism in the progression of CRC, hoping to find new target for CRC treatment. We explored the expression patterns of lncRNA kcna3 in CRC tissues and paracancerous tissues, and evaluated the association between lncRNA kcna3 expression levels and patients' clinicopathologic feature and prognosis. Then, we investigated the effects and mechanism of lncRNA kcna3 in the progression of CRC through gain/loss-of function assays.

2. Methods and materials

2.1. Experimental sample collection

Thirty matched samples of fresh colon cancer tissues and the adjacent non-tumor tissues were obtained from patients with colon cancer from April 2016 to November 2016 in the Affiliated Hospital of Southwest Medical University. All samples were confirmed by pathological diagnosis without any chemotherapy, radiotherapy or other

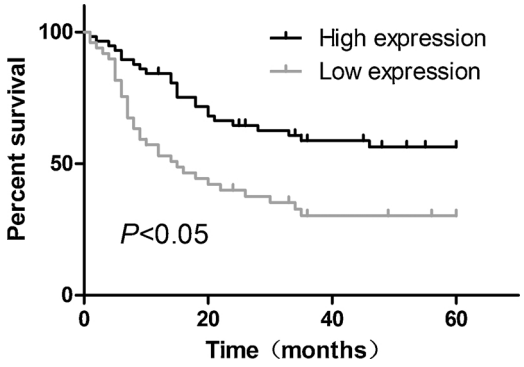

Fig. 2. Kaplan–Meier survival curves with log-rank test to evaluate the OS of CRC patients after surgical resection. Patients with high lncRNA kcna3 expression level exhibited significantly better survival than patients with the low lncRNA kcna3 expression ($P < 0.05$).

treatments before surgery. All tissue samples were placed in liquid nitrogen for further study. Experiments involving human samples in this study were approved by the Medical Ethics Committee of the Affiliated Hospital of Southwest Medical University and the written informed consent documents were signed by every subject.

2.2. Cell culture

Human normal colon cell line CCD-18Co, and CRC cell lines LoVo, SW480 and T84 were purchased from BeNa Culture Collection (Beijing, China); colon cancer cell line SW620 was purchased from the American Type Culture Collection (ATCC, VA, USA). CCD-18Co, SW480 cells were cultured in DMEM (Gibco, CA, USA); T84 cells were cultured in DMEM/F12 medium (Gibco, CA, USA); LoVo cells were cultured in Ham's F-12 K (Kaighn's) Medium (Invitrogen, CA, USA) and SW620 cells were cultured in ATCC-formulated Leibovitz's L-15 medium (No. 30-2008, ATCC, Manassas, VA, USA), with all culture medium containing 10% fetal bovine serum (FBS; Gibco, CA, USA), and was maintained in a humidified incubator with 5% CO_2 at 37 °C.

2.3. Construction of siRNA and lentivirus plasmid

Three siRNA interference sequences targeting lncRNA kcna3 and their control sequences were all synthesized by Gemma Gene Company (Shanghai, China), and the sequences were as follows: si-kcna3-1: 5′-TCGCTTCATAATGCGTGCATATAAA-3′, si-kcna3-2:5′-GACATTTCAG CAGAATGGTAGCTAT-3′, si-kcna3-3: 5′-CAGCAGAATGGTAGCTATTT GTATA-3′, si-Scramble: 5′-CATCAATTGAACCGAGCCTTACGTA-3′. For transfection of siRNAs-kcna3, 2 mL of serum-free medium without