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Winter 2021, Volume 6, Issue 20 (18-20)

Expression Evaluation of Long Non-Coding RNA TLC6 in Renal Cell Carcinoma

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DOI: 10.22034/pmj.2021.243880

Submitted: 2020-12-02
Accepted: 2020-01-28

Keywords:
Renal Cell Carcinoma
Long Non-Coding RNA
Gene Expression

Abstract

Renal Cell Carcinomas (RCC) are a wide range of heterogeneous tumors that mainly originate in renal tubular epithelial cells. These carcinomas are more common in males older than 60. Studies have shown that overexpression of the Long Non-Coding RNA (lncRNA) TCL6, which is a lncRNA with roles in T-cell lymphoproliferative diseases, could suppress the proliferation and growth of Clear Cell RCC (ccRCC) cells. The present study intended to investigate the association of this specific lncRNA with ccRCC by comparing the expression levels in cancerous tissues with the adjacent normal tissues.

The study included 44 samples of ccRCC cells and healthy, adjacent tissue from the affected patients. The expression levels of TCL6 lncRNA were assessed in the cancerous tissues and the adjacent normal tissues using real-time PCR.

According to the results, TCL6 lncRNA was significantly underexpressed in the cancerous tissues than the adjacent normal tissues.

In conclusion, these findings indicated that TCL6 might have roles in the tumorigenesis regulation and development of RCC.

INTRODUCTION

LncRNAsLong Non-Coding RNAs (lncRNAs) are a group of non-coding RNAs composed of >200 nucleotides (1). Initially, lncRNAs were considered the product of evolutionary junk or transcriptional noise; however, it was demonstrated that they exhibit various functions, including chromatin remodeling, mRNA splicing, and editing and transcriptional regulations. Both transcriptionally and post-transcriptionally, lcnRNAs may up-regulate or down-regulate gene expression. They exert a key role in numerous cellular processes, including cell growth, apoptosis, and carcinogenesis (2-3). Thus, they are implicated in the progression of various types of human cancers. To investigate the association between a specific lncRNA and a certain type of cancer, the expression levels of lncRNA in adjacent normal tissues and cancer tissues as well as normal and cancer cell lines must be compared (4).

Renal Cell Carcinoma (RCC) is the 7th most common malignant tumor in men. In 2016, it was estimated to have causes 63,000 new cases and 14,000 deaths in the United States (5). Epidemiological researches have reported that more than half of the renal cell carcinoma cases have local invasive tumors at the time of diagnosis, while 17% of the patients present with distant metastasis (6). A lncRNA named T-Cell Leukemia/Lymphoma 6 (TCL6), located on a region on chromosome 14q, has been found to be involved in T-cell leukemia. The *TCL* gene could encode different open-reading frames with no homology through alternative splicing, thereby leading to T-cell lymphoproliferative diseases (7). Moreover, previous studies have shown that TCL6 overexpression could suppress the proliferation and growth of ccRCC cells, and that the TCL6 expression was negatively correlated with tumor stage based on the TNM criteria (8). The present study intended to investigate the expression levels of this lncRNA in the RCC tissues and adjacent normal tissues.

METHODS AND MATERIALS

The study included 44 samples from abnormal ccRCC tissues and 44 samples from normal, adjacent renal tissues. The samples were collected from 2018 to 2020. None of the patients whose samples were used had been under treatment for cancer before the surgery and specimen collection.

All samples were re-evaluated before the current study by two pathologists. Then, they were kept in liquid nitrogen and stored at -80° C for qRT-PCR. Total RNA from the cancerous tissue and adjacent normal tissue was extracted using the Trizol reagent (Invitrogen, Carlsbad, CA, USA) based on the manufacturer's protocols. Then, the total RNA concentration was measured using a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA) and reverse transcribed using the Reverse Transcription Kit (BioFact, Korea). PCR amplification was performed using the SYBR Quantitative PCR (BioFact, Korea) based on the instructions. Primer sequences used were as follows:
TCL6 forward primer: 5'-CTATCCATTCAGCATCAGAGA-3'
TCL6 reverse primer: 5'-CACATACTCACGCATCCTT-3'
GAPDH forward primer: 5'-ATGACATCAAGAAGGTGGTG-3'
GAPDH forward primer: 5'-CATACCAGGAAATGAGCTTG-3'
Reactions were performed in ABI Step one Real-time PCR (Applied Biosystems, USA), while data were analyzed using the 2^-dCT method.

RESULTS

In thisThe study included 44 samples from abnormal ccRCC tissues and 44 samples from normal, adjacent renal tissues. According to the results, TCL6 lncRNA was significantly underexpressed in the cancerous tissue than the adjacent normal tissue. Therefore, these findings indicated that TCL6 might have roles in the tumorigenesis regulation and development of RCC.

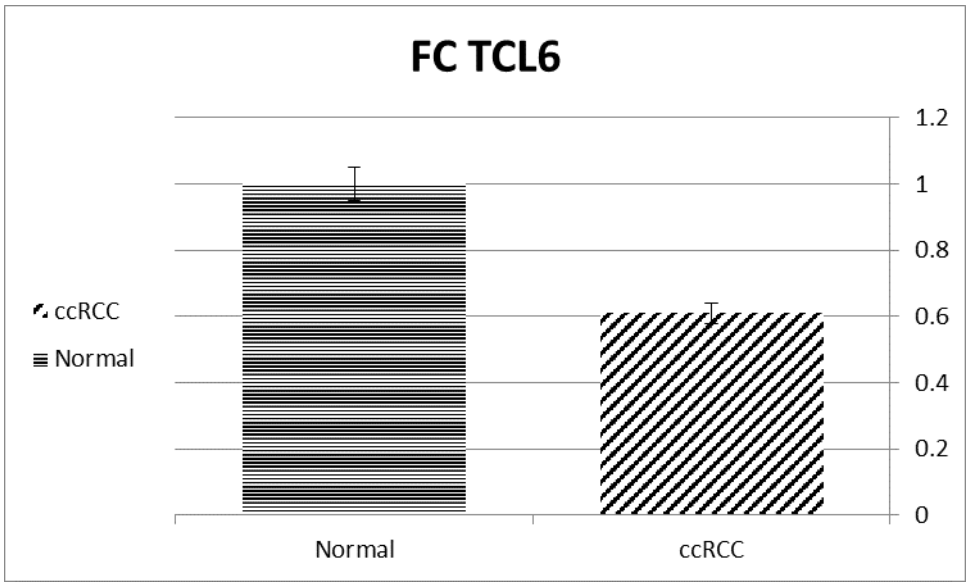

Fig 1. Fold change of TCL6 lncRNA in ccRCC tissue samples and normal adjacent samples

DISCUSSION

Renal Cell Carcinomas (RCC) are a range of heterogeneous tumors that mainly originate in renal tubular epithelial cells. These carcinomas are more common in males older than 60 (9). The most common pathological subtype of RCC is the Clear Cell RCC (ccRCC), which comprises a high number of cancer-related mortality due to urologic neoplasms (10). Therefore, it is of great importance to elucidate the genes and underlying molecular mechanisms involving in the ccRCC development and progression. On the other hand, increasing studies have shown the crucial biological functions of lncRNAs by being involved in the development and process of human diseases through epigenetic, transcription, and post-transcription regulations. A growing body of evidence revealed that lncRNAs harbored similar sequences to their targeted miRNAs to regulate mRNA expression in many tumors (11). A lncRNA named T-Cell Leukemia/Lymphoma 6 (TCL6), located on a region on chromosome 14q, has been found to be involved in T-cell leukemia. The *TCL* gene could encode different open-reading frames with no homology through alternative splicing, thereby leading to T-cell lymphoproliferative diseases (12). Moreover, previous studies have shown that TCL6 overexpression could suppress the proliferation and growth of ccRCC cells, and that the TCL6 expression was negatively correlated with tumor stage based on the TNM criteria (13), which was compatible with our results. The present study investigated the expression levels of this lncRNA in 44 samples of RCC tissue and 44 samples of adjacent normal tissue. According to the results, TCL6 lncRNA was significantly underexpressed in the cancerous tissue than the adjacent normal tissue.

Therefore, these findings indicated that TCL6 might have roles in the tumorigenesis regulation and development of RCC.

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