NM_001013649.3 gene is down-regulated in human colorectal adenocarcinoma

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Abstract. The present study aimed to evaluate the expression of *NM_001013649.3* in human colorectal adenocarcinoma tissue and its correlation with clinicopathological aspects of the disease. Through the combination of a cDNA subtractive library and cDNA microarray analysis, a differentially expressed sequence tag *ES274081* was identified whose full-length cDNA sequence (*NM_001013649.3*) was obtained through BLASTn analysis on NCBI. Reverse transcription quantitative real-time PCR was used to assess the expression levels of *NM_001013649.3* in cancer and adjacent normal colorectal tissues from 30 patients with colorectal adenocarcinoma. The mRNA levels of *NM_001013649.3* were significantly lower in the cancer tissues compared to the adjacent normal colorectal tissues of 25 out of 30 (83%) patients ($p<0.005$). Furthermore, the rate of down-regulation was significantly higher in moderately differentiated samples (21 out of 23; 91%) compared to poorly differentiated samples (3 out of 6; 50%) ($p<0.05$). The mRNA levels of *NM_001013649.3* are significantly down-regulated in colorectal adenocarcinoma tissue, and this down-regulation is most likely correlated with the differentiation status of the cancer, indicating that *NM_001013649.3* is potentially involved in the pathogenesis of colorectal adenocarcinoma.

Introduction

The prevalence of of colorectal adenocarcinoma has increased in recent years, and it is the second most common cancer and the second most common cause of cancer-related death worldwide (1-3). The causes and molecular mechanisms of colorectal adenocarcinoma are largely unknown, although they may involve multiple factors and multiple genes in multiple pathways. We have recently identified 86 differentially expressed sequences from human colorectal adenocarcinoma tissue compared with normal colorectal tissue, through cDNA subtractive library and cDNA microarray. In this study, we further investigate the mRNA expression levels in human colorectal adenocarcinoma tissues by using reverse transcription quantitative real-time (RT-qPCR) for one of the most significant differentially expressed sequences (*ES274081*) (4-6), whose full-length cDNA is *NM_001013649.3* (NCBI), with the aim of assessing its association with clinicopathological aspects of human patients.

Materials and methods

Tissue samples. With informed consent from patients and approval from the Medical Research Ethics Committee at Sichuan University, fresh colorectal adenocarcinoma tissues and adjacent normal colorectal tissues (>5 cm away from the edge of the tumor, and no cancer cells under pathological examination) were collected from 30 patients with colorectal adenocarcinoma who were admitted to West China Hospital of Sichuan University. The pathology was confirmed for all samples. The tissues were immediately frozen and kept in liquid nitrogen until use.

RNA isolation. TRIzol reagent (Invitrogen, Carlsbad, CA, USA) was used for total RNA isolation according to the manufacturer's protocol. RNA yield was determined by measuring absorbency at 260 nm on a spectrophotometer, and total RNA was isolated according to the manufacturer's instructions. RNA integrity was judged by sharp 28S and 18S rRNA bands and 2:1 intensity ratio of the two bands (28S:18S) on 1% agarose gel.

Synthesis of first-strand cDNA. The isolated RNA from each sample was treated with DNase I to remove genomic DNA contamination prior to reverse transcription. Total RNA (1 μg) was reverse-transcribed using the M-MuLV reverse transcriptase kit (Fermentas, Canada) for first-strand cDNA synthesis according to the manufacturer's instructions. The synthesized cDNA was immediately used for quantitative real-time PCR, or kept at -20˚C until use.

Quantitative real-time PCR. Quantitative real-time PCR was performed for cDNA amplification using SYBR Premix

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Table I. Primers.

Gene	Primer sequences (5'-3')
NM_001013649.3	F: ACGCAACCCAGACTATGAAGAG R: CACCTTCTCACTCACCTTTCCT
GAPDH	F: GGAAGGTGAAGGTCGGAGT R: TGAGGTCAATGAAGGGGTC

ExTaq (Takara, Japan) and primers listed in Table I based on the manufacturer's instructions and related international standards (7-9), using the Bio-Rad C1000 real-time system (Bio-Rad, USA). cDNA (2 μl) from 1 μg RNA template was used for each PCR reaction. GAPDH was used as the internal control. The primers were designed in the open reading frame of *NM_001013649.3* using Primer Premier 5.0 software.

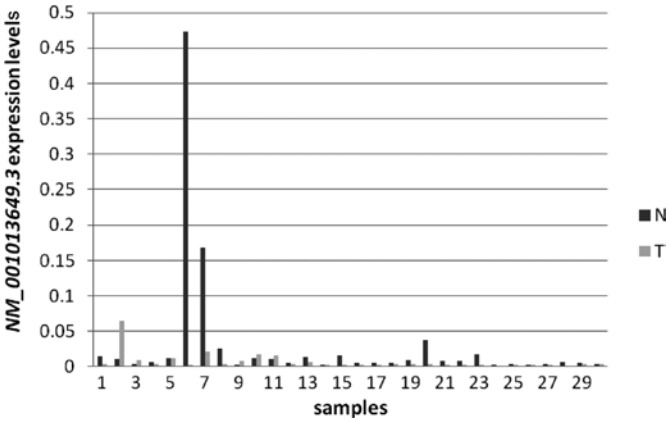

Figure 1. Expression levels of *NM_001013649.3*. T=374, P=0.004 by Wilcoxon signed-rank test. T, colorectal adenocarcinoma sample; N, matched normal sample.

Statistical analysis. All values are represented as means ± SEM. Statistical analysis was performed using the Wilcoxon signed-rank test and Fisher's exact probabilities test with SPSS17.0. A P-value <0.05 was considered significant.

Results

The mRNA levels of *NM_001013649.3* were found to be down-regulated in 83% of the tumor samples (25 out of 30), compared to those of the adjacent normal colorectal tissue samples. The expression levels of the samples are shown in Fig. 1, and the average expression levels in both the adenocarcinoma and normal colorectal tissues are shown in Table II. The mRNA expression levels of NM_001013649 in adenocarcinoma tissues were significantly lower compared to those of adjacent normal colorectal tissues (Wilcoxon signed rank test, p<0.005).

The effect of the patients' gender, age, Dukes' stage, and tumor differentiation on *NM_001013649.3* expression is shown in Table III. The down-regulation rate was as high as 91% in moderately differentiated samples (21 out of 23), but only 50% in poorly differentiated tissue samples (3 out of 6), indicating a significantly higher rate of down-regulation of *NM_001013649.3* in moderately differentiated samples (Fisher's exact probability test, p<0.05). One patient exhibited a markedly high expression level in the normal colorectal tissue (186.31-fold higher), compared to the adenocarcinoma tissue. Age, gender and tumor stage showed no significant effect on *NM_001013649.3* mRNA expression.

Discussion

Through the cDNA subtractive library and cDNA microarray, one differentially expressed EST fragment was identified between tumor and normal colorectal tissues from 4 patients with colorectal adenocarcinoma in Dukes' A stage, with a reverse hybridization ratio of 2.472 (4). This EST fragment has a length of 735 bp, and its GenBank accession number is *ES274081*. Full-length cDNA was obtained by a BLASTn search on the NCBI website (4283 bp, GenBank accession

Table II. Expression levels of *NM_001013649.3* in adenocarcinoma and normal tissues.

Name	Minimum	Maximum	Average	Standard error
Normal	0.00263	0.47323	0.3021930	0.8883944
Adenocarcinoma	0.00089	0.06425	0.0071123	0.1188477

Table III. Effect of gender, age, Dukes' stage and tumor differentiation on *NM_001013649.3* expression.

	Gender		Age (years)		Dukes' stage			Tumor differentiation		
	Male	Female	≥50	<50	B	C	D	Well	Moderately	Poorly
Patients (no.)										
Up-regulation	1	4	3	2	1	3	1	0	2	3
Down-regulation	16	9	23	2	5	14	6	1	21	3

number *NM_001013649.3*). This gene is located on human chromosome 2p11.2 and encodes a human hypothetical protein LOC388969. To date, there have been no reports regarding the function of this gene (10,11).

In the present study, we investigated the mRNA expression of the *NM_001013649.3* gene using RT-qPCR in tumor and adjacent normal colorectal tissues from 30 patients with colorectal adenocarcinoma.

We found that the mRNA levels of *NM_001013649.3* in the colorectal adenocarcinoma tissue were significantly lower than those in the adjacent normal colorectal tissue, and this down-regulation was observed in 83% of patients (25 out of 30), indicating potential involvement of this gene in the pathogenetic process of colorectal adenocarcinoma. Furthermore, the down-regulation was more frequently observed in moderately differentiated samples than in poorly differentiated samples ($p<0.05$), which may indicate a correlation of *NM_001013649.3* gene expression with tumor differentiation. We did not find any effects of age, gender and tumor stage on *NM_001013649.3* mRNA expression.

Further studies are required to investigate *NM_001013649.3* gene expression at the protein level and to probe the molecular pathways linking this gene and colorectal adenocarcinoma.

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