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Overexpression of UQCRC2 is correlated with tumor progression and poor prognosis in colorectal cancer

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ABSTRACT

Ubiquinol-cytochrome c reductase complex core protein 2 (UQCRC2) is an important subunit of mitochondrial respiratory complex III. However, its role in tumorigenesis and tumor progression remains unknown, especially with regards to colorectal cancer (CRC). In this research, we measured the expression of UQCRC2 protein by immunohistochemistry assay in 89 paired paraffin-embedded tumor tissues and corresponding adjacent normal tissues from patients with colorectal adenocarcinoma and investigated possible correlations of UQCRC2 expression with clinicopathological parameters and prognosis. We found that UQCRC2 was significantly upregulated in CRC tissues compared with adjacent normal tissues, and immunohistochemical UQCRC2 status was correlated to the depth of invasion (T), lymph node metastasis (N), advanced TNM stage. Multivariate analysis indicated that UQCRC2 remained an independent prognostic factor for poorer overall survival. Furthermore, we determined the role of UQCRC2-knockdown in CRC cells (RKO and HCT116) using lentivirus-mediated small hairpin RNAs (shRNAs). The effects of UQCRC2 knockdown on CRC cells (RKO and HCT116) proliferation were analyzed by cell proliferation and colony formation assay, and cell cycle and apoptosis were assessed by flow cytometry. We found that silencing UQCRC2 suppressed cell proliferation and colony formation in RKO and HCT116 cells, led to a cell cycle arrest and induced cell apoptosis *in vitro*. These results provided novel insights into the potential role of UQCRC2 in the tumorigenesis and progression of CRC, and revealed that UQCRC2 may serve as a new prognostic and therapeutic target in CRC.

1. Introduction

Colorectal cancer (CRC) is recognized as the third most frequently diagnosed malignancy and the fourth most common cause of cancer-related death worldwide [1]. In China, CRC ranks fifth for both cancer incidence and mortality and there estimated to be 376,300 newly-diagnosed cases and 191,000 cancer-specific mortalities in 2015 [2]. Despite of encouraging improvements made in the diagnosis and treatment, including precancerous screening, surgical procedures, multidisciplinary treatment, chemotherapy and radiotherapy [3], a significant portion of patients with CRC, especially those in advanced stages, exhibits poor 5-year survival rate [4]. During the multi-stage process of CRC tumorigenesis and progression, multiple genomic mutations and epigenetic alterations accumulate, and many aberrant gene expression patterns have been determined to regulate cell proliferation, survival and related signaling events [5,6]. Therefore, it is a continuing concern to investigate the molecular mechanisms underlying CRC progression and identify potential therapeutic targets in CRC patients.

Mitochondria are gaining an increasing interest in cancer research in recent years because of their role in carcinogenesis [7]. Alterations in mitochondria protein and number have been confirmed to involved in prognosis and progression in many human cancers [8,9]. Here we focus on an important subunit of mitochondrial respiratory complex III, named ubiquinol-cytochrome c reductase complex core protein 2 (UQCRC2). UQCRC2, also known as Core protein II, is one of the 11 structural subunits of mitochondrial complex III, which constitutes a part of mitochondrial respiratory chain [10]. Mitochondrial complex III deficiency caused by UQCRC2 mutation can lead to neonatal onset recurrent hepatocellular insufficiency, lactic acidosis, hypoglycemia, ketosis and hyperammonemia [11,12]. UQCRC2 has been proved to participate in oxidative stress and intracellular reactive oxygen species (ROS) production, and deficiency of UQCRC2 induces increased cellular

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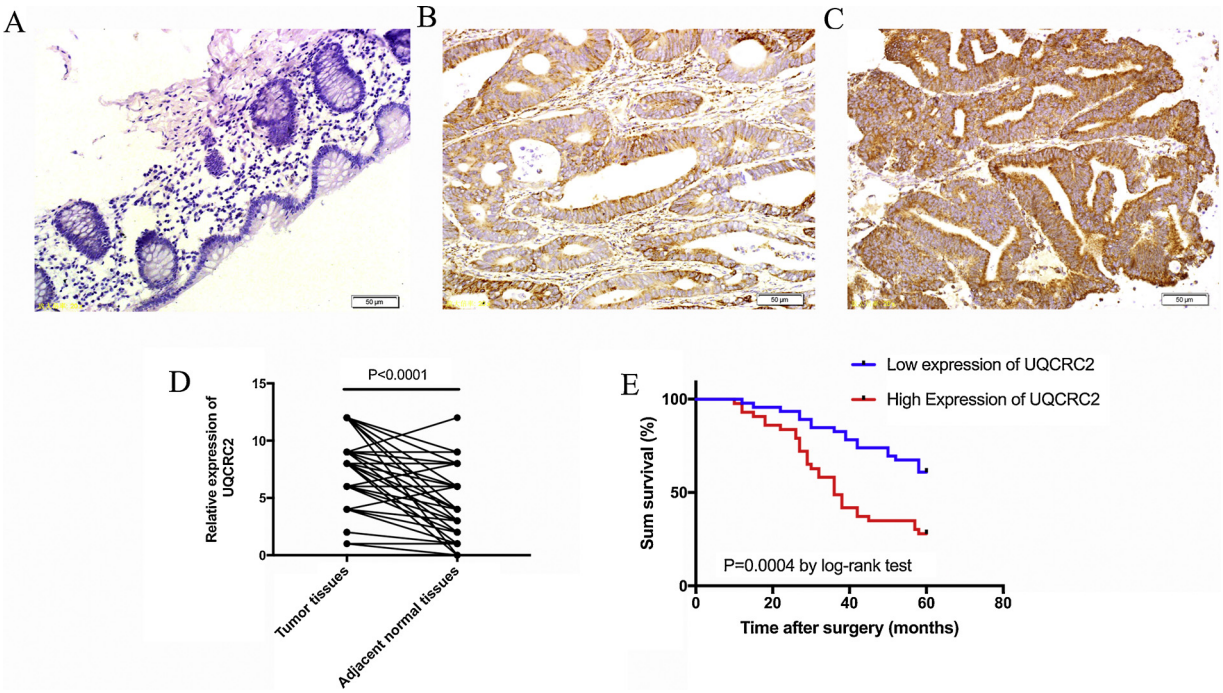

Fig. 1. Upregulation of UQCRC2 in CRC correlated with poor patient survival. Representative images of UQCRC2 expression were shown in normal colorectal tissues (A), relatively low-level expression (B) and high-level expression (C) in CRC tissues. Magnification for all photomicrographs was ×200. (D) Two-point line graph of the final H-score (mean ± SEM) in paired CRC tissues and adjacent normal tissues was shown. The difference was detected by using the paired t-test. (E) Kaplan-Meier overall survival curves for all 89 patients with CRC were shown stratified by high and low expression of UQCRC2.

Table 1
Correlations between UQCRC2 expression and clinicopathological para-meters of patients with CRC.

Clinical pathological parameters		UQCRC2 expression		
	N	High	Low	P-value
Age (y)				
≥60	50	24	26	0.946
< 60	39	19	20	
Gender				
Male	49	23	26	0.774
Female	40	20	20	
Location				
Rectum	38	18	20	0.605
Sigmoid	24	10	14	
colon	27	15	12	
Differentiation				
poorly	22	11	11	0.855
Moderately + well	67	32	35	
Depth of invasion (T)				
T1	2	0	2	0.015[a]
T2	23	7	16	
T3	32	14	18	
T4	32	22	10	
Lymph node metastasis (N)				
N0	46	12	34	< 0.001[a]
N1 + N2	43	31	12	
Distant metastasis (M)				
M0	77	38	39	0.62
M1	12	5	7	
TNM stage				
I + II	44	12	32	< 0.001[a]
III + IV	45	31	14	

[a] Statistically significant difference. UQCRC2, ubiquinol-cytochrome c reductase complex core protein 2. CRC, colorectal cancer.

ROS level [13]. Other studies have revealed that the UQCRC2 expression was associated with male fertility [14,15]. To date, the role of UQCRC2 in human cancers remains unclear. Preliminary evidences have shown that UQCRC2 is overexpressed in human lung adenocarcinoma as compared to adjacent normal tissues identified by bioinformatics analysis of public expression profile data [16]. Depressed UQCRC2 expression level has been detected in mitochondria isolated from a breast infiltrating ductal carcinoma cell culture (human mammary carcinoma, HMC-1) reference to epithelial mammary cell line (HMEC) using western blot and densitometric analysis [17]. However, no expression and function information has been available about UQCRC2 involved in tumorigenesis and tumor progression, especially in CRC.

The aims of the present study were to elucidate the correlation between the UQCRC2 expression and clinicopathological parameters in CRC and to determine the prognostic value of UQCRC2. Furthermore, after having investigated the expression of UQCRC2 in human CRC cell lines, we employed lentivirus-mediated RNAi in RKO and HCT116 cells to evaluated the UQCRC2 knockdown effects on cell growth, colony-formation, cell cycle distribution and apoptosis *in vitro*.

2. Materials and methods

2.1. Tissue specimens and cancer cell lines

We retrospectively studied 89 patients diagnosed with colorectal cancer and underwent radical surgery at the Department of General Surgery in Qingdao Municipal Hospital (Group) during the period of January 2009 to January 2012. Paraffin-embedded tumor tissues and corresponding adjacent normal tissues (> 3 cm away from the edge of tumor) were collected. Histopathological type of all samples was determined as colorectal adenocarcinoma by at least two experienced pathologists. Clinicopathological parameters, including age, gender, location, differentiation, depth of invasion (T), lymph node metastasis (N), distant metastasis (M), TNM stage, survival status during 5 years of follow-up and other information were obtained. Colorectal tissues had been staged according to the seventh edition of TNM classification revised by the Union for International Cancer Control (UICC) /American