European Journal of Surgical Oncology 45 (2019) 1005–1010

Contents lists available at ScienceDirect

European Journal of Surgical Oncology

journal homepage: www.ejso.com

UQCRC1 downregulation is correlated with lymph node metastasis and poor prognosis in CRC

Wenhua Li [a, b, 1], Gulinaizaier Wubulikasimu [c, 1], Xiaoying Zhao [a, b], Chenchen Wang [a, b], Rujiao Liu [a, b], Lei Wang [c, ***], Xiaodong Zhu [a, b, **], Zhiyu Chen [a, b, c, *]

[a] Department of Medical Oncology, Fudan University Shanghai Cancer Center, 270 Dong An Road, Shanghai, 200032, China
[b] Department of Oncology, Shanghai Medical College, Fudan University, 130 Dong An Road, Shanghai, 200032, China
[c] Department of Oncology, Kashgar Prefecture Second People's Hospital of Kashi, 844000, Xinjiang, China

ARTICLE INFO

Article history:
Accepted 19 February 2019
Available online 26 February 2019

Keywords:
UQCRC1
Colorectal cancer
lymph node metastasis
VEGF-C
Survival

ABSTRACT

Background: Mitochondrial dysfunction is common in cancer. UQCRC1 is a nuclear-encoded protein localized to the inner mitochondrial membrane; however, little is known about it in colorectal cancer (CRC). The purpose of this study was to investigate the expression pattern and the possible clinical significance of UQCRC1 in CRC.

Methods: A total of 197 patients with CRC were enrolled in this study. Immunohistochemistry was used to evaluate the expression pattern of UQCRC1. The relationship between UQCRC1 and clinical characteristics, especially lymph node metastasis, was also assessed. In addition, we evaluated the significance of UQCRC1 in the prognosis for CRC patients.

Results: UQCRC1 was downregulated in 28.9% (57/197) of human CRCs. Downregulation of UQCRC1 was correlated with increased lymph node metastasis ($p < 0.001$) and decreased disease-free survival (DFS) and overall survival (OS). Multivariate analysis revealed that downregulation of UQCRC1 was an independent prognostic factor both for DFS (HR 3.009; 95% CI: 1.613–8.548, $P = 0.009$) and OS (HR 4.062; 95% CI: 2.835–8.910, $P = 0.001$). In addition, downregulation of UQCRC1 was correlated with increased VEGF-C expression ($P = 0.002$).

Conclusion: UQCRC1 was downregulated in human CRC. Downregulation of UQCRC1 was correlated with increased lymph node metastasis and finally associated with decreased survival in CRC.

Introduction

Colorectal cancer (CRC) represents the third leading cause of cancer death worldwide with 5-year overall survival rates ranging from 40% to 60% [1,2]. Despite great improvements in curative resection and adjuvant chemotherapy, the relapse rate remains high. Approximately 40–50% of patients relapse within the first year after initial resection [3]. Furthermore, approximately 50% of CRC patients will develop liver metastasis during the course of their disease [4]. The challenges of considerable prognostic heterogeneity within each tumor stage need to be faced, as tumors at the same pathological stage can produce considerably different clinical outcomes [5]. Therefore, understanding the underlying molecular mechanisms of CRC malignancy has crucial significance for identifying potential biomarkers that may serve as new treatment targets.

Mitochondrial dysfunction is common in cancer, and the mitochondrial electron transport chain is often affected in carcinogenesis [6]. The ubiquinol-cytochrome c reductase complex, which is also called mitochondrial complex III, is the third complex in the mitochondrial electron transport chain and plays a crucial role in the synthesis of ATP [7]. Complex III is composed of 11 subunits that form a multisegment transmembrane protein. Among these subunits, UQCRC1 is a nuclear-encoded protein localized to the inner

* Corresponding author. Department of Medical Oncology, Fudan University Shanghai Cancer Center, 270 Dong An Road, Shanghai, China.
** Corresponding author. Department of Medical Oncology, Fudan University Shanghai Cancer Center, 270 Dong An Road, Shanghai, China.
*** Corresponding author. Department of Oncology, Kashgar Prefecture Second People's Hospital of Kashi, 1 Jian Kang Raod, Kashi, Xinjiang, China.
E-mail addresses: 48003504@qq.com (L. Wang), xddr001@163.com (X. Zhu), chanhj75@aliyun.com (Z. Chen).
[1] Wenhua Li and Gulinaizaier Wubulikasimu contributed equally to this work.

https://doi.org/10.1016/j.ejso.2019.02.025

mitochondrial membrane [8]. Recent studies have suggested that UQCRC1 is dysregulated in human cancers. For example, UQCRC1 expression increased in osteosarcoma [9] as well as breast and ovarian tumors [10], but it decreased in ccRCC [11] and gastric cancer [12], suggesting that UQCRC1 may be involved in carcinogenesis and that its dysregulation depends on the tumor entity.

The expression pattern and possible clinical significance of UQCRC1 expression in colorectal cancer has not been investigated. Therefore, the purposes of the current study are to examine the expression of UQCRC1 in surgical specimens of colorectal carcinoma and to determine the prognostic value of UQCRC1 expression. We found that UQCRC1 was downregulated in human CRC. Downregulation of UQCRC1 correlated with increased lymph node metastasis. Furthermore, UQCRC1 downregulation was an independent factor for poor prognosis in CRC.

Materials and methods

Patients and specimens

This study was approved by the Ethics Committee of the Fudan University Shanghai Cancer Center (Shanghai, China), and written informed consent was obtained from each participant according to institutional guidelines.

This study enrolled 197 CRC patients from the Affiliated Hospital of Jiangnan University, Wuxi, China, between March 2003 and December 2008. Only patients with fully characterized tumors, intact overall survival (OS) or disease-free survival (DFS) were included. All patient data were collected, including age at diagnosis, tumor localization, diagnostic year, tumor diameter, histological grade, number of lymph nodes retrieved, postoperative multimodal treatment (adjuvant chemotherapy or radiation), surgical procedure details, complication rates, postoperative histopathology, and follow-up information (date of last visit, tumor relapse, tumor-related or unrelated death, overall survival: OS and disease-free survival: DFS). Cancer tissue specimens were collected from all 197 patients after informed consent had been obtained. Formalin fixed paraffin-embedded (FFPE) samples were obtained for immunohistochemical analysis. Patient characteristics are provided in Table 1.

Tissue microarray (TMA) construction and immunohistochemistry (IHC) staining

Tissue microarrays were constructed from a representative core of each FFPE specimen. Tissue cylinders with a diameter of 1.5 mm were punched from marked areas of each sample and incorporated into a recipient paraffin block. Sections with 4 μm thickness were placed on slides coated with 3-aminopropyltriethoxysilane. All H&E-stained slides were reviewed by experienced pathologists, and the representative cores were premarked in the paraffin blocks.

For IHC, the sections were then deparaffinized in xylene and ethanol, placed in 0.1 mol/L citrate buffer (pH 6.0) and irradiated with microwaves (750 W) for 15 min. The sections were then incubated with primary antibodies for UQCRC1 (goat polyclonal, Santa Cruz Laboratory, Santa Cruz, CA, USA; dilution 1:100), Ki-67 (goat polyclonal, Santa Cruz Laboratory, Santa Cruz, CA, USA; dilution 1:200), vimentin (mouse monoclonal, R&D Systems, Minneapolis, MN, USA; dilution 1:200) and Ecadherin (mouse monoclonal, R&D Systems, Minneapolis, MN, USA; dilution 1:200). Negative controls were obtained by omitting the primary antibody. Briefly, the sections were incubated for 1 h at room temperature with the first antibody, rinsed five times with PBS, and then incubated with peroxidase-conjugated anti-mouse/goat immunoglobulins (DAKO EnVisionTM System; Dako Corporation, Carpinteria, CA). The sections were then developed with 3,3′-diaminobenzidine tetrahydrochloride and counterstained with hematoxylin. All immunostained sections were evaluated independently by 2 investigators without knowledge of the clinical or pathological background of the patients. Ten fields (inside the tumor and in the area exhibiting tumor invasion) were selected, and expression was evaluated in 1000 tumor cells (100 cells per field) with high power ($\times$ 200) microscopy.

Criteria for assessing the immunohistochemical results

Data were assessed by two independent single-blinded pathologists. An immunohistochemical scoring system employed in our study followed criteria that were reported previously [13]. The staining intensity was scored as 0 (negative), 1 (weak), 2 (medium) or 3 (strong). The extent of staining was scored as 0 (<5%), 1 (5–25%), 2 (26–50%), 3 (51–75%) and 4 (>75%) according to the percentage of the positive staining areas in relation to the whole carcinoma area. All cases were sorted into two groups according to the immunoreactivity score. Positive expression was defined as a final score $\geq$3 and negative expression was defined as a final score $\leq$2.

Statistical analyses

Group differences were statistically analyzed using the x^2 test and *t*-test. DFS was defined as time from the date of surgery to the date of disease recurrence. OS was defined as the interval between the dates of surgery and death. For patients who had not experienced recurrence at the time of death or last follow-up, the DFS was censored on the date of death or last follow-up. For patients who had not died by their last follow-up, OS was censored at the date of the last follow-up. The Kaplan–Meier method was used to analyze survival, and the log-rank test was used to estimate differences in survival. Prognostic factors were examined using univariate and multivariate analyses (Cox proportional hazards regression model). P-values less than 0.05 were considered statistically significant.

Results

UQCRC1 downregulation correlated with increased lymph node metastasis

We performed IHC on a TMA that included 197 CRCs. The immunostaining showed that the UQCRC1 protein was predominantly expressed in the cytoplasm (Fig. 1). The staining of UQCRC1 was weaker in the cytoplasm of CRC compared to normal tissue. Overall, 28.9% (57/197) specimens exhibited negative UQCRC1 expression. UQCRC1 negative expression was significantly correlated with clinicopathological parameters, including cancer stage ($P = 0.004$), vascular invasion ($P = 0.048$), and especially lymph node metastasis ($P < 0.001$). However, there was no significant association between UQCRC1 expression and age, gender, histological grade, or distant metastasis. There was also no association between UQCRC1 expression and perineural invasion (Table 1).

UQCRC1 downregulation predicted poor prognosis

We compared the survival time between patients with different UQCRC1 expression levels. By performing Kalan-Meier survival analysis, we identified that downregulated UQCRC1 expression was significantly associated with decreased disease-free survival and overall survival time ($p = 0$ 0.001 and $P < 0.001$, respectively; Fig. 2A and B).

We next performed univariate and multivariate analyses to

Table 1
Correlation between UQCRC1 expression levels and clinicopathological factors in CRC (N = 197).

	No. of patients	Gene2 expression*		*p*-value
		High expression n = 140	Low expression n = 57	
Age (years)				
Mean ± SD		61.6 ± 11.9	62.1 ± 8.4	0.762*
Gender				
Male	101	75	26	0.311
Female	96	65	31	
Histopathological grade				
G1/2	153	113	40	0.107
G3	44	27	17	
Cancer stage				
I	12	9	3	0.004
II	63	55	8	
III	107	68	39	
IV	15	8	7	
T stage				
T2	28	19	9	0.493
T3	43	27	16	
T4	126	84	32	
N stage				
N0	78	68	10	<0.001
N1	66	42	24	
N2	53	30	23	
M stage				
M0	182	131	51	0.325
M1	15	9	6	
Histological type				
Adenocarcinoma	184	132	52	0.433
Mucinous	13	8	5	
Lymph node examined				
Median		14 + 6	14 + 10	0.841
Perineural invasion				
Positive	35	22	13	0.238
Negative	162	118	44	
Vascular invasion				
Positive	54	44	10	0.048
Negative	143	96	47	
CEA (ng/ml)		54.1 + 14	55.0 + 11.5	0.723

Abbreviations: CRC, colorectal carcinoma. *Compared with Student's t-test; the other P-values were obtained with Pearson's χ^2 test.

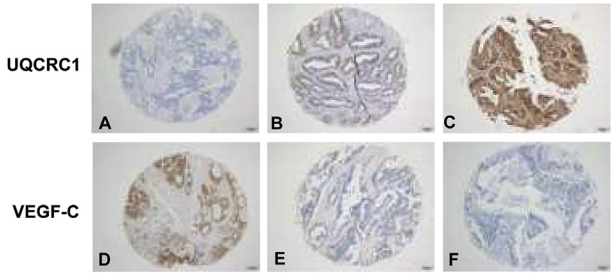

Fig. 1. Immunohistochemical staining for UQCRC1 and VEGF-C in CRC. (A–C) represent UQCRC1 negative, UQCRC1 positive (+), and UQCRC1 positive (++), respectively. (D–F) represent VEGF-C staining in UQCRC1-negative, -positive (+) and −positive (++) tumors. Original magnification: 400 × .

evaluate the prognostic significance of UQCRC1 in CRC patients (Tables 2 and 3). In univariate analysis, cancer stage (HR = 7.127; 95% CI 2.865–11.337; P < 0.001), T (HR = 8.857; 95% CI 3.463–16.217; P < 0.001), N (HR = 5.504; 95% CI 2.028–7.796; P = 0.002), M (HR = 7.022; 95% CI 4.865–14.217; P < 0.001), perineural invasion (HR = 2.204; 95% CI 1.318–3.177; P = 0.048), vascular invasion (HR = 1.984; 95% CI 1.667–5.876; P = 0.007), CEA (HR = 3.158; 95% CI 2.004–4.891; P < 0.001) and UQCRC1 downregulation (HR = 4.466; 95% CI 2.017–9.328; P < 0.001) were associated with DFS, whereas cancer stage (HR = 8.669; 95% CI 3.005–16.218; P < 0.001), T (HR = 5.024; 95% CI 2.911–8.524; P < 0.001), N (HR = 6.046; 95% CI 2.217–11.258; P < 0.001), M (HR = 5.879; 95% CI 3.892–9.687; P < 0.001), lymph node examination (HR = 1.668; 95% CI 1.112–4.339; P = 0.039), vascular invasion (HR = 1.339; 95% CI 1.192–3.110; P = 0.049), CEA (HR = 4.205; 95% CI 2.008–6.789; P = 0.007) and UQCRC1 downregulation (HR = 6.817; 95% CI 2.157–11.323; P < 0.001) were associated with OS. In the multivariate analysis, only cancer stage (HR = 2.106; 95% CI 1.499–4.057; P = 0.017 for DFS and HR = 5.559; 95% CI 2.987–14.089; P = 0.004 for OS, respectively) and UQCRC1 downregulation (HR = 3.009; 95% CI 1.613–8.547; P = 0.009 for DFS and HR = 4.062; 95% CI 2.835–8.910; P = 0.001 for OS, respectively) were associated with DFS and OS. Therefore, these results suggested that UQCRC1 is an independent prognostic predictor for both DFS and OS.

UQCRC1 downregulation correlated with increased VEGF-C expression

Enhanced expression of VEGF-C is prevalent in lymph node positive tumors and is associated with high lymphatic vessel density and poor survival in colon cancer [14]. Since we have observed that UQCRC1 downregulation was correlated with increased lymph node metastasis, we next examined the correlation between UQCRC1 and VEGF-C expression in patient specimens with CRC. Overall, 46.2% (91 out of 197) of tested specimens exhibited positive expression for VEGF-C. In Fig. 1 (D-F), a typical pattern of VEGF-C expression is shown as an example of the different expression

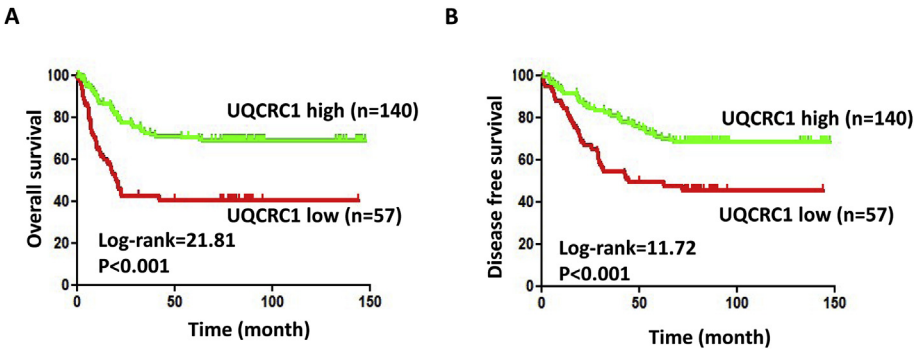

Fig. 2. Kaplan–Meier survival curves for patients with different UQCRC1 expression. (A) disease-free survival (DFS) and (B) overall survival of patients according to positive or negative UQCRC1 expression. The p-value was tested with a log-rank test.

Table 2
Univariate and multivariate Cox regression analyses of UQCRC1 expression for DFS of patients with CRC (N = 197).

Variables	Case number	Univariate Analysis		Multivariate Analysis	
		HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Age (<70 years vs. ≥70 years)		1.194 (0.833–1.762)	0.452		
Gender (male vs. female)		1.356 (0.715–1.827)	0.861		
Histolopathological grade (G3 vs. G1/G2)		1.440 (0.889–2.941)	0.115		
Cancer Stage (IV vs. I/II/III)		7.127 (2.865–11.337)	<0.001	2.106 (1.499–4.057)	0.017
T stage (T4 vs. T1/2/3)		8.857 (3.463–16.217)	<0.001	1.447 (0.793–2.359)	0.571
N stage (N2 vs. N0/1)		5.504 (2.028–7.796)	0.002	1.533 (0.602–3.830)	0.245
M stage (M1 vs. M0)		7.022 (4.865–14.217)	<0.001	1.257 (0.838–1.972)	0.806
Histological type (Mucinous vs. Adenocarcinoma)		0.781 (0.833–4.218)	0.657		
Lymph node examined (>12 vs. <=12)		0.839 (0.519–1.994)	0.269		
Perineural invasion (Positive vs. Negative)		2.204 (1.318–3.177)	0.048	1.428 (0.706–2.544)	0.581
Vascular invasion (Positive vs. Negative)		1.984 (1.667–5.876)	0.007	0.957 (0.627–4.097)	0.488
CEA (>10 vs. <=10)		3.158 (2.004–4.891)	<0.001	1.588 (0.714–3.066)	0.752
UQCRC1 (Negative vs. Positive)		4.466 (2.017–9.328)	<0.001	3.009 (1.613–8.547)	0.009

Abbreviations: OS, overall survival; PDAC, pancreatic duct adenocarcinoma; pT, pathologic T stage; pN, pathologic lymph node metastasis; CI, confidence interval.

Table 3
Univariate and multivariate Cox regression analyses of UQCRC1 expression for OS of patients with CRC (N = 197).

Variables	Case number	Univariate Analysis		Multivariate Analysis	
		HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Age (<70 years vs. ≥70 years)		1.055 (0.762–1.833)	0.732		
Gender (male vs. female)		1.244 (0.811–2.004)	0.851		
Histolopathological grade (G3 vs. G1/G2)		1.399 (0.641–3.827)	0.218		
Cancer Stage (IV vs. I/II/III)		8.669 (3.005–16.218)	<0.001	5.559 (2.987–14.089)	0.004
T stage (T4 vs. T1/2/3)		5.024 (2.911–8.524)	<0.001	1.278 (0.822–2.042)	0.647
N stage (N2 vs. N0/1)		6.046 (2.217–11.258)	<0.001	1.446 (0.765–3.091)	0.883
M stage (M1 vs. M0)		5.879 (3.892–9.687)	<0.001	0.957 (0.744–1.928)	0.982
Histological type (Mucinous vs. Adenocarcinoma)		1.217 (0.545–2.008)	0.912		
Lymph node examined (>12 vs. <=12)		1.668 (1.112–4.339)	0.039	1.242 (0.839–2.057)	0.449
Perineural invasion (Positive vs. Negative)		1.247 (0.812–3.441)	0.118		
Vascular invasion (Positive vs. Negative)		1.339 (1.192–3.110)	0.049	1.402 (0.557–2.268)	0.770
CEA (>10 vs. <=10)		4.205 (2.008–6.789)	0.007	1.164 (0.651–3.009)	0.214
UQCRC1 (Negative vs. Positive)		6.817 (2.157–11.323)	<0.001	4.062 (2.835–8.910)	0.001

Abbreviations: OS, overall survival; PDAC, pancreatic duct adenocarcinoma; pT, pathologic T stage; pN, pathologic lymph node metastasis; CI, confidence interval.

patterns of UQCRC1. The collective data indicate that VEGF-C expression was associated with UQCRC1 expression (P = 0.002; Table 4). These results suggested that UQCRC downregulation was correlated with increased lymph node metastasis that involved VEGF-C overexpression.

VEGF-C expression was correlated with increased lymph node metastasis in CRC

We further confirmed the correlation between VEGF-C expression and lymph node metastasis. We observed that positive staining of VEGF-C was associated with increased lymph node metastasis (P = 0.001, Table 4). Taken together, these results suggested that UQCRC1 downregulation is associated with a poor prognosis that involves increased lymph node metastasis.

Discussion

In this study, we investigated the expression of UQCRC1 in human CRC and found that UQCRC1 was downregulated in CRC cells compared to normal tissue. In addition, we found that downregulated UQCRC1 expression was correlated with decreased DFS

Table 4
Correlation between UQCRC1 expression and VEGF-C and lymph node metastasis in CRC.

	No. of patients	VEGF-C expression*		*p*-value*
		Positive n = 91	Negative n = 106	
UQCRC1				
Positive	140	55	85	0.002
Negative	57	36	21	
pN				
N0/1	144	55	89	0.001
N2	53	36	17	

Abbreviations: PDAC, pancreatic duct adenocarcinoma. P-values were obtained with Pearson's χ^2 test.

and OS. Therefore, our study suggested there was a tumor-inhibiting role for UQCRC1 in human CRC.

Mammalian cells use a complex network of redox-dependent processes necessary to maintain cellular integrity during oxidative metabolism as well as to protect against and/or adapt to stress. The disruption of these redox-dependent processes, including those in the mitochondria, creates a cellular environment that facilitates progression to a malignant phenotype and the development of resistance to commonly used anticancer agents. Recent studies have confirmed that mitochondrial dysfunction has been associated with increased invasiveness, metastatic potential, and drug resistance in cancer cells [15–17]. There are multiple precise mechanisms underlying mitochondrial dysfunction, and they may involve deregulated autophagic processes, an imbalance in reactive oxygen species (ROS) homeostasis, mutations in oxidative phosphorylation (OXPHOS) complexes, the electron transport chain (ETC), or Krebs' cycle (TCA) enzymes [18]. Mitochondria are the major producers of superoxide and other downstream ROS in the cell [19], and the main sources of mitochondrially derived superoxide are Complex I and III [20].

UQCRC1 is a subunit of complex III, which is one of the main sources of reactive oxygen species (ROS) [21]. Additionally, ROS has been demonstrated to play an important role in cancer. Furthermore, decreased UQCRC1 expression levels in a mitochondrial sample isolated from hearts was found to possibly contribute to contractile dysfunction in diabetic mice [22]. Recently, mitochondrial dysfunction was also associated with specific loss of UQCRC1 in cells with an epithelial origin [23] and mouse spermatocytes [24]. Results further showed that UQCRC1 overexpression significantly increased the $\Delta\Psi m$, which is an indicator of mitochondrial function. Based on these findings, we speculated that UQCRC1 can regulate mitochondrial function and its specific role in CRC cells needs to be explored. Therefore, we first evaluated the expression pattern in a total of 197 CRC specimens. We found that the staining of UQCRC1 was weaker in the cytoplasm of CRC compared to normal tissue, which suggests that downregulation of UQCRC1 may correlated with a malignant phenotype. Mitochondrial dysfunction is common in cancer, and the mitochondrial electron transport chain is often affected in carcinogenesis [6], however, UQCRC1 is downregulated in only 28.9% of CRC in our study. As mitochondrial complex III is composed of 11 subunits that form a multisegment transmembrane protein [8], it is suggested that other subunits such as UQCRFS1, UQCRH, UQCRB, UQCR9, etc., may play roles in CRC, which need further study. We also found that UQCRC1 negative expression was significantly correlated with clinicopathological parameters, including cancer stage, vascular invasion, and lymph node metastasis. Furthermore, we confirmed that downregulated UQCRC1 expression was significantly associated with decreased disease-free survival and overall survival time. Therefore, our study suggested there is a tumor-promoting role for UQCRC1 in human CRC, which deserves further functional study.

Previous studies have suggested that VEGF-C expression was mediated through the PI3K/Akt and ROS dependent pathways [25–27]. Mitochondrial dysfunction can affect energy metabolism in cancer. For example, HADH is an enzyme located in the mitochondria, and its downregulation promoted gastric cancer progression via activation of Akt signaling pathway [28]. In addition, mitochondrial dysfunction is involved an imbalance in ROS homeostasis [19]. Therefore, in our study, we also observed a positive correlation between increased VEGF-C and downregulated UQCRC1 expression. Since the correlation between VEGF-C expression and lymphangiogenesis has previously been described [29], it is suggested that downregulation of UQCRC1 promotes lymphatic metastasis by inducing VEGF-C expression.

Taken together, our study suggested that UQCRC1 was downregulated in human CRC. Downregulation of UQCRC1 was correlated with increased lymph node metastasis and was ultimately associated with decreased survival in CRC.

Conflicts of interest statement

None.

Declarations of interest

None.

Acknowledgements

This study was supported by Natural Science Foundation of Shanghai, China (16ZR1406800).

References

[1] Bibbins-Domingo K. Colorectal cancer screening recommendations-reply. J Am Med Assoc 2016;316:1717.
[2] Bibbins-Domingo K, Grossman DC, Curry SJ, Davidson KW, Epling JJ, Garcia F, et al. Screening for colorectal cancer: US preventive services task force recommendation statement. J Am Med Assoc 2016;315:2564–75.
[3] Seo SI, Lim SB, Yoon YS, Kim CW, Yu CS, Kim TW, et al. Comparison of recurrence patterns between </=5 years and >5 years after curative operations in colorectal cancer patients. J Surg Oncol 2013;108:9–13.
[4] Biasco G, Derenzini E, Grazi G, Ercolani G, Ravaioli M, Pantaleo MA, et al. Treatment of hepatic metastases from colorectal cancer: many doubts, some certainties. Cancer Treat Rev 2006;32:214–28.
[5] Edge SB, Compton CC. The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM. Ann Surg Oncol 2010;17:1471–4.
[6] Zhu Y, Dean AE, Horikoshi N, Heer C, Spitz DR, Gius D. Emerging evidence for targeting mitochondrial metabolic dysfunction in cancer therapy. J Clin Invest 2018;128:3682–91.
[7] Sousa JS, D'Imprima E, Vonck J. Mitochondrial respiratory chain complexes. Subcell Biochem 2018;87:167–227.
[8] Kadenbach B, Huttemann M. The subunit composition and function of mammalian cytochrome c oxidase. Mitochondrion 2015;24:64–76.
[9] Liu X, Zeng B, Ma J, Wan C. Comparative proteomic analysis of osteosarcoma cell and human primary cultured osteoblastic cell. Cancer Invest 2009;27:345–52.
[10] Kulawiec M, Arnouk H, Desouki MM, Kazim L, Still I, Singh KK. Proteomic analysis of mitochondria-to-nucleus retrograde response in human cancer. Cancer Biol Ther 2006;5:967–75.
[11] Ellinger J, Gromes A, Poss M, Bruggemann M, Schmidt D, Ellinger N, et al. Systematic expression analysis of the mitochondrial complex III subunits identifies UQCRC1 as biomarker in clear cell renal cell carcinoma. Oncotarget 2016;7:86490–9.
[12] Wang X, Zhi Q, Liu S, Xue SL, Shen C, Li Y, et al. Identification of specific biomarkers for gastric adenocarcinoma by ITRAQ proteomic approach. Sci Rep 2016;6:38871.
[13] Hu X, Li YQ, Li QG, Ma YL, Peng JJ, Cai SJ. ITGAE defines CD8+ tumor-infiltrating lymphocytes predicting a better prognostic survival in colorectal cancer. EBIOMEDICINE 2018;35:178–88.
[14] Soumaoro LT, Uetake H, Takagi Y, Iida S, Higuchi T, Yasuno M, et al. Coexpression of VEGF-C and Cox-2 in human colorectal cancer and its association with lymph node metastasis. Dis Colon Rectum 2006;49:392–8.

[15] Moro L, Arbini AA, Marra E, Greco M. Mitochondrial DNA depletion reduces PARP-1 levels and promotes progression of the neoplastic phenotype in prostate carcinoma. Cell Oncol 2008;30:307–22.
[16] Porporato PE, Payen VL, Perez-Escuredo J, De Saedeleer CJ, Danhier P, Copetti T, et al. A mitochondrial switch promotes tumor metastasis. Cell Rep 2014;8:754–66.
[17] Moro L, Arbini AA, Yao JL, di Sant'Agnese PA, Marra E, Greco M. Mitochondrial DNA depletion in prostate epithelial cells promotes anoikis resistance and invasion through activation of PI3K/Akt2. Cell Death Differ 2009;16:571–83.
[18] Guerra F, Arbini AA, Moro L. Mitochondria and cancer chemoresistance. Biochim Biophys Acta Bioenerg 2017;1858:686–99.
[19] Wang W, Gong G, Wang X, Wei-LaPierre L, Cheng H, Dirksen R, et al. Mitochondrial flash: integrative reactive oxygen species and pH signals in cell and organelle biology. Antioxidants Redox Signal 2016;25:534–49.
[20] Bae YS, Oh H, Rhee SG, Yoo YD. Regulation of reactive oxygen species generation in cell signaling. MOL CELLS 2011;32:491–509.
[21] Zhang L, Jaswal JS, Ussher JR, Sankaralingam S, Wagg C, Zaugg M, et al. Cardiac insulin-resistance and decreased mitochondrial energy production precede the development of systolic heart failure after pressure-overload hypertrophy. Circ Heart Fail 2013;6:1039–48.
[22] Essop MF, Chan WA, Hattingh S. Proteomic analysis of mitochondrial proteins in a mouse model of type 2 diabetes. CARDIOVASC J AFR 2011;22:175–8.
[23] Shibanuma M, Inoue A, Ushida K, Uchida T, Ishikawa F, Mori K, et al. Importance of mitochondrial dysfunction in oxidative stress response: a comparative study of gene expression profiles. Free Radic Res 2011;45:672–80.
[24] Huang S, Wang J, Cui Y. 2,2',4,4'-Tetrabromodiphenyl ether injures cell viability and mitochondrial function of mouse spermatocytes by decreasing mitochondrial proteins Atp5b and Uqcrc1. Environ Toxicol Pharmacol 2016;46:301–10.
[25] Zhu C, Qi X, Chen Y, Sun B, Dai Y, Gu Y. PI3K/Akt and MAPK/ERK1/2 signaling pathways are involved in IGF-1-induced VEGF-C upregulation in breast cancer. J Canc Res Clin Oncol 2011;137:1587–94.
[26] Xiang L, Xie G, Ou J, Wei X, Pan F, Liang H. The extra domain A of fibronectin increases VEGF-C expression in colorectal carcinoma involving the PI3K/AKT signaling pathway. PLoS One 2012;7. e35378.
[27] Huang YL, Lin YC, Lin CC, Chen WM, Chen B, Lee H. High glucose induces VEGF-C expression via the LPA1/3-akt-ROS-LEDGF signaling Axis in human prostate cancer PC-3 cells. Cell Physiol Biochem 2018;50:597–611.
[28] Shen C, Song YH, Xie Y, Wang X, Wang Y, Wang C, et al. Downregulation of HADH promotes gastric cancer progression via Akt signaling pathway. Oncotarget 2017;8:76279–89.
[29] Zong S, Li H, Shi Q, Liu S, Li W, Hou F. Prognostic significance of VEGF-C immunohistochemical expression in colorectal cancer: a meta-analysis. Clin Chim Acta 2016;458:106–14.