Biomedicine & Pharmacotherapy 99 (2018) 645–654

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

Solute carrier family 34 member 2 overexpression contributes to tumor growth and poor patient survival in colorectal cancer

Liguo Liu[a,1], Yi Yang[b,1], Xueming Zhou[c,1], Xuebing Yan[d,**], Zhenqian Wu[a,*]

[a] Department of General Surgery, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai, China
[b] Department of Oncological Surgery, Kunshan Traditional Chinese Medicine Hospital Affiliated to Nanjing University of Chinese Medicine, Kunshan, Jiangsu, China
[c] Department of Oncology, Kunshan Traditional Chinese Medicine Hospital Affiliated to Nanjing University of Chinese Medicine, Kunshan, Jiangsu, China
[d] Department of General Surgery, Shanghai Tenth People's Hospital, Tongji University School of Medicine, Shanghai, China

ARTICLE INFO

Keywords:
SLC34A2
Colorectal cancer
Prognosis
Biomarker

ABSTRACT

Solute carrier family 34 member 2 (SLC34A2) is a well-known sodium-dependent phosphate transporter that has recently been linked to cancer development. However, its specific oncogenic role remains controversial in numerous human malignancies, and is currently unknown in colorectal cancer (CRC). Therefore, in this study we firstly used Oncomine database to determine its expression in cancer tissues and found it is overexpressed in thyroid, ovarian and renal cancer, while it is opposite in lung, breast and pancreas cancer. Using qRT-PCR and western blot, we then demonstrated its overexpression in CRC tissues as compared with adjacent normal tissues (n = 20). In a retrospective cohort enrolling 190 CRC patients, we proved its expression was significantly correlated with N stage. Furthermore, high SLC34A2 expression is associated with higher postoperative metastasis rate and serves as an independent adverse factor affecting patient prognosis. In subgroup analysis, SLC34A2 expression could stratify the patient prognosis in stage II and III CRC, but failed in stage IV CRC. In cellular assays in vitro, knockdown of SLC34A2 dramatically inhibited the proliferation and colony formation, induced the apoptosis and arrests the cell cycle progression of HCT-116 CRC cells. In cellular assays in vivo, knockdown of SLC34A2 significantly inhibited the growth of xenografts, decreasing Ki-67 and proliferating cell nuclear antigen (PCNA) expression and increasing apoptosis rate. Taken together, our study indicates SLC34A2 plays a crucial promoting role in CRC development and therefore has great potential to be further developed as a reliable biomarker for CRC diagnosis and treatment.

1. Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed cancer in men and the second in females with more than 1.3 million new cases annually in the world [1]. In the United States, CRC is the third most common malignancy in both genders and there estimated to be 135,430 newly diagnosed cases and 50,260 cancer-specific deaths in 2017 [2,3]. The pathogenesis of CRC is a multistep process involving series of factors such as lifestyle, genetic and environmental changes [4]. Despite encouraging advances in early detection and individualized treatment during the past decade, only 39% of patients can be diagnosed at localized stage and those with metastatic disease have a poor 5-year survival rate of 14% [2]. In addition, current treatment modalities for CRC are mainly based on clinical and pathological characteristics, frequently resulting in undertreatment or overtreatment in patients within the same TNM stage [5]. Increasing studies have highlighted the potential clinical utilities of molecular biomarkers in prognostic prediction and treatment selection, but a considerable part of them fail to be further recommended into clinical detection due to insufficient validations or their inferior sensitivity/specificity [6]. Therefore, for better improving biomarker-directed individualized treatment, it is of great necessity to identify more reliable molecular biomarkers that participate in the malignant progression of CRC.

Inorganic phosphate (Pi) is a well-known chemical compound that is essential for numerous biological processes such as organ development and protein translation [7]. The Pi uptake is mainly regulated by sodium-dependent phosphate (NaPi) transporters, among which solute carrier family 34 (SLC34) appears to be the most sufficiently studied [8]. SLC34A2, also known as NaPi-IIb, is a member of SLC34 family that can be detected in various human organs such as lung, liver and small

* Corresponding author at: Department of General Surgery, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai 200233, China.
** Corresponding author at: Department of General Surgery, Shanghai Tenth People's Hospital, Tongji University School of Medicine, Shanghai 200072, China.
E-mail addresses: yyxxbb8904@163.com (X. Yan), wuzhenqian12741@163.com (Z. Wu).
[1] Contributed equally to this work.

https://doi.org/10.1016/j.biopha.2018.01.124
Received 19 September 2017; Received in revised form 14 January 2018; Accepted 24 January 2018

intestine [9]. The mutations of SLC34A2 gene are associated with the pathogenesis of pulmonary alveolar microlithiasis [10,11]. Recently, emerging evidences have suggested SLC34A2 is involved in the initiation and development of human malignancies. For example, Li et al. [12] found SLC34A2 promotes the growth and invasion of hepatocellular carcinoma cells through inducing epithelial-mesenchymal transition (EMT) phenotype and activating PI3K/AKT signaling pathway. In bladder cancer, SLC34A2 serves as an independent unfavorable prognostic factor and drives the malignant progression of cancer cells through upregulating c-Myc [13]. Furthermore, SLC34A2 is proved to enhance the cancer stem cell phenotypes of non-small cell lung cancer (NSCLC) cells via increasing Bmi1 expression [14]. However, despite those evidences supporting its oncogenic role, recent researches have suggested SLC34A2 may also exert its suppressive role in the development of several cancers such as osteosarcoma and NSCLC [15,16]. Therefore, the specific biological role of SLC34A2 in cancer development remains inconclusive and extensive related validations are needed.

In addition to its controversial role in other human malignancies, to the best of our knowledge, the clinical significance and biological role of SLC34A2 in CRC are currently unknown. Therefore, in this study, we firstly determined the expression of SLC34A2 in cancer and normal tissues using Oncomine databases. Then, a retrospective study cohort enrolling 190 CRC patients was utilized to investigate its clinical correlations and prognostic value in CRC. Finally, cellular assays in vivo and in vitro were employed to clarify its specific biological role in the malignant progression of CRC cells. These efforts not only provide us with a more comprehensive understanding about SLC34A2 in oncology field, but also help identify a novel molecular biomarker used for CRC diagnosis and treatment.

2. Materials and method

2.1. Patients and tissue samples

A total of 190 primary CRC tumors and matched adjacent normal tissues were obtained from patients who received surgical treatment from October 2009 to June 2016, at the Department of Surgery, Shanghai Jiao Tong University Affiliated Sixth People's Hospital and Shanghai Tenth People's Hospital, Tongji University School of Medicine. None of them have received preoperative chemotherapy or radiotherapy. The standard first-line adjuvant chemotherapy (FOLFOX) was recommended to stage II patients with high risk factors and stage III/IV patients postoperatively. The tumor stage was classified according to the tumor-node-metastasis (TNM) classification of the American Joint Committee on Cancer Staging (7th edition). T and N stage was determined by the pathological detection, while M stage was determined by preoperative radiological examination and intra-operative observation. For evaluating clinical outcome, CRC-specific survival (CSS) and disease-free survival (DFS) was utilized. CSS was defined as the time period from the date of surgery to the date of death caused by CRC. DFS was defined as the time period from the date of surgery to the date of CRC recurrence or metastasis (stage IV patients received R0 resection). This study was reviewed and approved by the Ethics Committee of both the mentioned hospitals. Written informed consents were obtained from all the participants for using their tissue specimens and clinical data in scientific researches. The general clinical characteristics of the patients were provided in Table 1.

2.2. Bioinformatics analysis

The mRNA expression of SLC34A2 in cancer and normal tissues were compared using Oncomine™ database online (https://www.oncomine.org) according to our previous description [17]. The filtering conditions were used as follows: Gene: SLC34A2; analysis type: Cancer vs. Normal analysis; Data type: mRNA; p value < .0001; fold change > 2; gene rank: Top 10%.

Table 1
Correlations between SLC34A2 expression and clinicopathological features in CRC patients.

Characteristics	Total	SLC34A2 expression		*P* value
		Low	High	
Age				
≤60	59	24	35	.343
> 60	131	63	68	
Gender				
male	92	40	52	.536
female	98	47	51	
Tumor location				
Rectal	69	31	38	.857
Colon	121	56	65	
Tumor size				
≤5 cm	132	62	70	.622
> 5 cm	58	25	33	
Tumor differentiation				
Well/moderate	165	78	87	.292
Poor	25	9	16	
Tumor invasion				
T1	9	6	3	.626
T2	30	14	16	
T3	64	29	35	
T4	87	38	49	
Lymph node metastasis				
N0	87	40	47	< .001
N1	71	43	28	
N2	32	4	28	
Distant metastasis				
Absent	174	80	94	.864
Present	16	7	9	
Serum CEA level				
≤5 ng/ml	107	53	54	.240
> 5 ng/ml	83	34	49	
Ki-67 expression				
< 30%	59	28	31	.757
≥30%	131	59	72	

Abbreviation: CRC,colorectal cancer; SLC34A2,solute carrier family 34 member 2.

2.3. Cell lines and culture conditions

Four human CRC cell lines (SW620, HCT116, HT29 and LoVo) were purchased from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China), while the rest two (Caco-2 and SW480) were purchased from the American Type Culture Collection (ATCC, USA). The cell lines were incubated in culture mediums supplemented with 10% fetal bovine serum (FBS; Gibco, USA) and 100 U/ml penicillin and 100 μg/ml streptomycin in a 5% CO_2 and 95% humidified air atmosphere at 37 °C.

2.4. RNA interference and lentivirus infection

RNA interference (RNAi) technique was utilized to silence SLC34A2 expression in CRC cells and its sequence was designed as follows: 5′-CTCCCTGGATATTCT TAGTTT-3′. Meanwhile, a negative control was utilized and its sequence was designed as follows: 5′-TTCTCCGAACGT GTCACGT-3′. Then, double strand DNA oligo containing specific sequences was synthesized and connected to lentivirus vector. After validation by sequencing, the products were mixed with pHelper 1.0 and pHelper 2.0 vector (all purchased from Genechem, Shanghai, China). The obtained complexes was transfected into 293T cell line using Lipofectamine 2000 (Thermo Fisher Scientific). After 48 h incubation at