Journal on Surgery

www.journalonsurgery.org

Research Article

Open Access, Volume 4

High Expression of ZNF692 Impairs Overall Survival in Patients with Colorectal Cancer

Xiaosong Ge; Fen Liu; Xiaoyuan Liu; Xiang Gao; Yong Mao; Xiaosong Ge*

Department of Oncology, Affiliated Hospital of Jiangnan University, Wuxi 214122, Jiangsu, China.

Abstract

Background: Zinc Finger Protein 692 (ZNF692) promotes tumor progression as a potential oncogene, but its role in Colorectal Cancer (CRC) is not fully understood. We therefore carried out the present study to explore the correlation between ZNF692 expression and the progression of CRC.

Results: In this study, Tissue microarray and Immunohistochemistry (IHC) staining was applied to detect the expression of ZNF692 protein in Formalin-Fixed, Paraffin Embedded (FFPE) tissues. The colorectal cancer data in TCGA database is analyzed and verified using R language. Our results showed that ZNF692 expression in CRC tissues was significantly upregulated compared with the matched normal tissues. ($p<0.05$). Among 248 colorectal cancer tissues, 126 had high expression of ZNF692. In addition, there was a significant association between ZNF692 expression and age, M stage and histologic grade between the two groups, but not other clinical characteristics. Multivariate analysis showed that high expression of ZNF692 was an independent predictor of prognosis in colorectal cancer patients. The results of bioinformatics analysis also confirmed that the expression level of ZNF692 affects the prognosis of patients with CRC.

Conclusions: Our results suggest that the high expression of ZNF692 is associated with the poor prognosis and could be a novel biomarkers in colorectal cancer patients.

Keywords: ZNF692; Colorectal cancer; Bioinformatics; Immunohistochemistry.

Abbreviations: ZNF692: Zinc Finger Protein 692; CRC: Colorectal Cancer; TCGA: The Cancer Genome Atlas; TMA: Tissue Microarray; IHC: Immunohistochemistry Staining.

Introduction

Colorectal cancer (CRC) is one of the most common malignant tumor with high migration and invasion capacity. It is estimated that accounting for 9.0% of deaths worldwide [1]. In 2020, the number of new cases of colorectal cancer in China exceeded 550,000 and the number of deaths exceeded 280,000 [2]. Despite advancements in treatment over the few past decades, the 5-year survival rate of Metastatic CRC was still under 12%. Several factors were reported to influence CRC prognosis, such as lifestyle [3], genome instability [4] aberrant gene expression, etc. However, the mechanism underlying CRC survival remains elusive, which impedes the improvement of CRC prognosis.

Manuscript Information: *Received: Mar 27, 2023; Accepted: Apr 24, 2023; Published: May 01, 2023*

Correspondance: *Xiaosong Ge, Department of Oncology, Affiliated Hospital of Jiangnan University, Wuxi 214122, Jiangsu, China. Tel: +86-13506198192; Email: 85410328@qq.com*

Citation: *Ge X, Liu F, Liu X, Gao X, et al. High Expression of ZNF692 Impairs Overall Survival in Patients with Colorectal Cancer. J Surgery. 2022; 3(1): 1094.*

ZNF692 also known as AREBP or Zfp692 is located on chromosome 1q44. This gene has 30 transcripts (splice variants), 136 orthologues and 36 paralogues and ZNF692 protein was first identified as a transcription factor bound to the promoter elements of phosphoenolpyruvate carboxykinase [5]. Recent research showed that ZNF692 has different RNA splicing events within various types of carcinoma [6,7]. Additionally, overexpression of ZNF692 has been reported to be related with the worse overall survival of lung adenocarcinoma [8]. However, the expression profiles and molecular functions of ZNF692 in CRC remain unclear.

The Cancer Genome Atlas (TCGA) is a public funded project that aims to catalog and discover major cancer-causing genomic alterations [9,10]. So far, TCGA researchers have analyzed large cohorts of over 30 human tumors through large scale genome sequencing and integrated multi-dimensional analyzes. Studies of individual cancer types have extended current knowledge of tumorigenesis.

In this study, a total of 248 CRC patients were enrolled. Table 1 presents the relationship between clinical factors and ZNF692 expression in patients with CRC. Age, M stage, and histological grade were significantly associated with ZNF692 expression. The proportion of high expression of ZNF692 for younger patients was significantly greater than older patients (62.7% vs. 42.0%, *P*=0.001). M1 stage patients with a higher rate for high expression of ZNF692 compared with M0 stage patients (68.4% vs. 48.1%, *P*=0.021). Middle/high grade patients showed a higher rate of ZNF692 high expression (55.8% vs. 38.6%, *P*=0.021). Relative higher expression of ZNF692 was associated with inferior overall survival. Furthermore, ZNF692 expression was proved to be an independent negative prognosis factor for Overall Survival (OS) in multivariate survival analysis as shown in table 2. Moreover, the adverse effect of high expression of ZNF692 on the prognosis of patients was also verified by TCGA data analysis of CRC.

Materials and methods

Patients

The colorectal cancer specimens involved in this study are all selected from patients undergoing surgical treatment in the Affiliated Hospital of Jiangnan University in China from August 2013 to December 2014. Case inclusion criteria: The primary tumor specimen was colorectal cancer, and all specimens were verified by two pathologists after the operation. The patients did not receive radiotherapy or chemotherapy before surgery. There are complete clinical, pathological and follow-up data, the cause of death is only tumor recurrence or metastasis. There was no second malignant tumor other than colorectal cancer within 5 years. Here, a total of 248 patients met the above criteria. There were 133 males and 115 females; with an average age of 63 years; According to the 8th edition of the AJCC colorectal cancer staging, there are a total of 29 cases in stage I , 99 cases in stage II, 82 cases in stage III, and 38 cases in stage IV. This study was approved by the medical ethics committee of our hospital, and all patients gave informed consent.

Tissue microarray (TMA) and immunohistochemistry staining (IHC)

The method of TMA and IHC were carried out in accordance with our previous standard operations [11]. ZNF692 polyclonal antibodies were purchased from Beijing Boaosen Technology (Cat: bs-4360R), GTVision™ III anti-rat/rabbit universal immunohistochemistry detection kit were purchased from Shanghai Gene Technology (GK500705), tissue chip blank wax blocks were purchased from UNITMA Quick-Ray (UB06-1, South Korea). Perforate the corresponding part marked on the donor wax block to collect the tissue core, with a diameter of 2 mm, transfer the tissue core to the hole of the acceptor module, and perforate each specimen twice. Then perform routine immunohistochemistry sectioning, staining and scoring. Briefly, sections of formalin-fixed, paraffin-embedded tissue were immunostained using ZNF692 polyclonal antibodies. The staining results revealed that ZNF692 was positively located in the nucleus and cytoplasm. Score by staining intensity and percentage of stained cells. A score of 0(no staining), 1(the tumor cells are light yellow stained without obvious granules or less than 10% of the tumor cells are yellow stained with obvious granules), 2(more than 10% of tumor cells are yellow stained with obvious granules or less than 10% of tumor cells are brown stained with obvious granules), 3(More than 10% of tumor cells are brown stained with obvious granules) within carcinomatous areas. A score of <2 is judged as low expression, and a score ≥2 is judged as high expression. Results were independently double-blind reading assessment by physicians in the pathology department

Bioinformatics analysis

The data of 512 colorectal cancer patients (COAD) in TCGA (The Cancer Genome Atlas) were downloaded using UCSC Xena (https://xenabrowser.net/datapages/), by which the genes differential expression analysis was also conducted. The genes with FDR 0.05 and |log FC| 1 were defined as differentially expressed genes (DEGs) and were further analyzed (R software 4.1.2). Overall survival of TCGA-COAD patients was determined by Kaplan-Meier analysis in 487 patients with survival data. Hazard ratio (HR) and corresponding 95% confidence interval (CI) was calculated with an optimal cutoff value, and log-rank *P*<0.05 was considered statistically significant for a difference.

Statistical analysis

Statistical analyses were conducted using SPSS, version 18.0. We performed Chi-square tests to examine the associations between ZNF692 expression and clinical characteristics. Hazard ratios (HRs) and corresponding 95% confidence intervals (CIs) for association of clinical characteristics and overall survival (OS) were estimated using univariate and multivariate survival analysis using Cox's regression model respectively. Differences were considered statistically significant at *P* <0.05.

Results

Expression of ZNF692 in colorectal cancer tissues

By immunohistochemistry staining, we analyzed the expression of ZNF692 in colorectal cancer tissues and adjacent normal tissues (Figure 1). The results showed that among 248 colorectal cancer specimens, 126 had high expression (IHC score greater than or equal to 2), 57 had low expression (IHC score less than 2), and 66 had no expression (score 0). The expression level of ZNF692 in tumors is much higher than that in adjacent normal tissues (Figure 1E), 1.492 ± 0.07091 VS 0.2727 ± 0.1408.

The relationship between ZNF692 expression and pathological features in colorectal cancer patients

The relationship between ZNF692 expression and clinicopathological characteristics of colorectal cancer patients was further analyzed. The results showed that there were no significant differences between patients with low ZNF692 expression and those with high ZNF692 expression in terms of gender, T stage, N stage, neural and/or vascular invasion. (*P*>0.05, Table 1). However, there were significant differences in age, M stage and degree of differentiation between the two groups (*P*<0.05, Table 1). The proportion of high expression of ZNF692 for younger patients was significantly greater than older patients (62.7% vs. 42.0%, *P*=0.001). M1 stage patients with a higher rate for high expression of ZNF692 compared to M0 stage patients (68.4% vs. 48.1%, *P*=0.021). Middle/high grade patients showed a higher rate of ZNF692 high expression (55.8% vs. 38.6%, *P*=0.021).

The effect of ZNF692 expression on patient survival

Kaplan-Meier survival curve display that relative higher expression of ZNF692 was associated with inferior overall survival. The average survival months of patients with low ZNF692 expression was 47.58±1.36 months, while the survival months of patients with high ZNF692 expression was 38.83 ± 1.57 months. The difference between the two groups was statistically significant (Figure 2A, *P*=0.009). Furthermore, The Cox risk regression model was used to analyze whether ZNF692 protein expression can be used as an independent predictor of prognosis in colorectal cancer patients. Multivariate analysis showed that ZNF692 expression, age, gender, and M status were independent predictors of prognosis in colorectal cancer patients. As shown in Table 2.

Validation of the effect of ZNF692 on overall survival of patients with colorectal cancer in TCGA public data

We downloaded the data of 512 patients with colorectal cancer from TCGA database, 176 of whom had the data required for survival analysis and were used to draw Kaplan-Meier survival curve. The results showed that the survival rate of patients with high ZNF692 expression was significantly lower than that of patients with low ZNF692 expression (Figure 2B, *P*=0.0198).

Table 1: Correlation between ZNF692 expression and clinicopathological characteristics of CRC.

Characteristics	Cases	ZNF692 expression		*P* value
		Low	High	
Gender				0.82
male	133	64	69	
female	115	57	58	
Age (mean =63)				0.001*
< 63	110	41	69	
≥ 63	138	80	58	
Nerve & Vessel invasion				0.49
No	140	71	69	
Yes	108	50	58	
T status				0.078
T1-2	37	23	14	
T3-4	211	98	113	
N status				0.849
N0	144	71	73	
N1/2/3	104	50	54	
M status				0.021*
M0	210	109	101	
M1	38	12	26	
Histologic grade#				0.039*
Low	44	27	17	
Middle/high	197	87	110	

* *P* < 0.05; # The Histologic grade of 7 samples is missing

Table 2: Univariate and multivariate analysis for overall survival (Cox proportional hazards regression model).

Risk factors	Univariate			Multivariate		
	HR	P value	95% CI	HR	P value	95% CI
ZNF692 expression (low/high)	1.988	0.01*	1.18-3.36	1.859	0.034*	1.05-3.30
Age (<63/≥63)	2.364	0.003*	1.33-4.19	2.884	0.001*	1.57-5.29
Gender (male/female)	0.68	0.149	0.40-1.15	0.688	0.026*	0.31-0.93
T status (T1-2/T3-4)	5.939	0.013*	1.45-24.32	2.253	0.128	0.73-12.73
N status (N0/N1-2)	2.577	<0.001*	1.53-4.34	0.414	0.326	0.75-2.37
Nerve & Blood invasion(No/Yes)	1.297	0.16	1.05-1.60	1.631	0.171	0.85-2.59
Differentiation (moderate &well/poor)	0.722	0.3	0.39-1.34	0.639	0.181	0.33-1.23
M status (M0/M1)	10.027	<0.001*	5.98-16.82	7.491	<0.001*	4.27-13.14

HR; Hazard ratio, * *P*<0.05

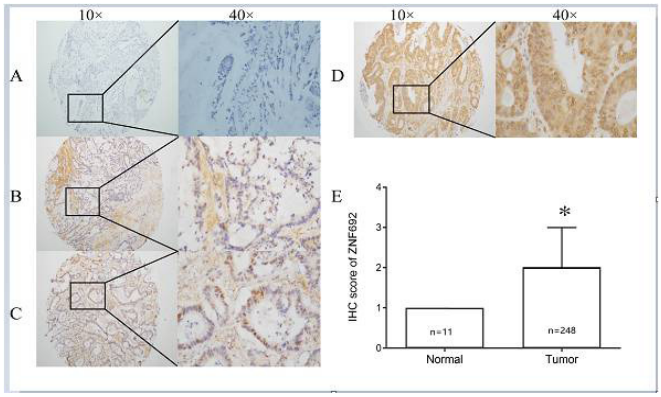

Figure 1: Immunohistochemical staining for ZNF692 expression in normal tissue and CRC tissues. Representative images of ZNF692 expression in normal tissues and CRC tissues are shown. (A) Score=0, ZNF692 expression in normal colonic epithelium. (B) Score=1, ZNF692 staining in CRC, (C) Score=2, ZNF692 staining in CRC and (D) Score=3 ZNF692 staining in CRC. ×10 represents the magnification of the objective lens is 10 times, ×40 represents the magnification of the objective lens is 40 times. ZNF692, 4. (E) Staining scores between normal colon tissues and CRC tissues Unpaired T-test, * p<0.05.

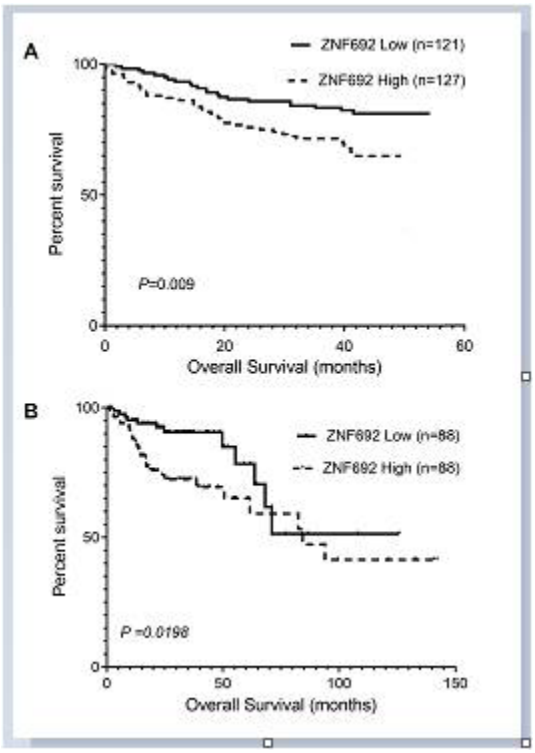

Figure 2: The expression level of ZNF692 affected the overall survival of the patients. **(A)** Overall survival analysis of 248 CRC patients in the affiliated hospital of Jiangnan University. **(B)** Overall survival analysis of 176 CRC patients in the TCGA database.

Discussion

Our study found that ZNF692 may play a regulatory role as an oncogene in the progression of colorectal cancers. Because we observed that ZNF692 is highly expressed in more than half of the colorectal cancer tissues, this is consistent with the reported results [6]. And the high expression of ZNF692 is closely related to the appearance of distant metastasis and the low degree of tumor differentiation. It is well known that distant metastasis and poor differentiation are important factors for poor tumor prognosis. Our survival analysis also confirmed that the survival of patients with high expression of ZNF692 was worse than that of patients with low expression. In fact, ZNF692 does promote tumor growth, Xing Y et al. reported that ZNF692 promotes colorectal adenocarcinoma cell growth and metastasis by activating the PI3K/AKT pathway [6]. However, the study was only validated from in vitro cytology tests, and they have not been validated on a large scale in clinical specimens and lack data for survival analysis.

By analyzing The Cancer Genome Atlas (TCGA) dataset, Zhang Q et al. confirmed ZNF692 as a potential oncogene in cervical cancer and promotes proliferation, migration and invasion of cervical cancer cells in vitro [12]. In order to verify the reliability of our results, we also performed a validation analysis of colorectal cancer public data in the TCGA database. In the analysis, we found that ZNF692 did affect the survival of patients.

At present, there are few experimental studies and bioinformatics analysis data on ZNF692, and the signal transduction pathway involved in ZNF692 is still not clear enough. Although our findings are the first to combine bioinformatics with clinical specimen data, it is only preliminary to clarify that ZNF692 may play a regulatory role in the progression of colorectal cancer, but the upstream and downstream molecules of the ZNF692 molecule involved are still unclear. Limited by experimental conditions and funding, we did not conduct cytological experiments to verify the molecular mechanism of ZNF692 that involved in colorectal cancer regulation, nor did we conduct large-scale public data validation of colorectal cancer, including cross-platform multi-group data integration analysis. Therefore, in the future, based on the existing results, we will continue to analyze the expression of ZNF692 in various cancer tissues, including serum, and actively explore the molecular mechanism of ZNF692 regulating the occurrence and development of colorectal cancer. By elucidating the role of ZNF692 in the occurrence and development of CRC, it may provide potential molecules for the target therapy or screening of patients with CRC, which has very important theoretical and clinical significance.

Statements & declarations

Funding This work was supported by Youth Fund of National Natural Science Foundation of China (Grant No. 81502042) and Natural Science Foundation for Young Scholars of Jiangsu Province, China (Grant No. BK20140171), and Top Talent Support Program for young and middle-aged people of Wuxi Health Committee (Grant No. HB2020056)

Competing interests: The authors declare that they have no financial interests

Author contributions Xiaosong Ge designed and carried out experiments, analyzed and interpreted part of the data, Xiaosong Ge drafted the manuscript. Fen Liu, Xiaoyuan Liu, Xiang Gao and Yong Mao analyzed and interpreted part of the data. We thanks Dr. Fang Wang for conducting the TCGA data analysis. All authors read and approval the final manuscript.

Ethics approval: The study was approved by the Ethics Committee of the Affiliated Hospital of Jiangnan University

Consent to participate: Informed consent was obtained from all individual participants included in the study.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. Ca-a Cancer Journal for Clinicians. 2021; 71: 209-249.

2. Cao W, Chen HD, Yu YW, Li N, Chen WQ. Changing profiles of cancer burden worldwide and in China: a secondary analysis of the global cancer statistics 2020. Chin Med J (Engl). 2021; 134: 783-791.

3. Heuchan GN, Lally PJ, Beeken RJ, Fisher A, Conway RE. Perception of a need to change weight in individuals living with and beyond breast, prostate and colorectal cancer: a cross-sectional survey. J Cancer Surviv. 2023.

4. Mei WJ, Mi M, Qian J, Xiao N, Yuan Y, et al. Clinicopathological characteristics of high microsatellite instability/mismatch repair-deficient colorectal cancer: A narrative review. Front Immunol. 2022; 13: 1019582.

5. Inoue E, Yamauchi J. AMP-activated protein kinase regulates PEPCK gene expression by direct phosphorylation of a novel zinc

finger transcription factor. Biochem Biophys Res Commun. 2006; 351: 793-799.

6. Xing Y, Ren S, Ai L, Sun W, Zhao Z, et al. ZNF692 promotes colon adenocarcinoma cell growth and metastasis by activating the PI3K/AKT pathway. Int J Oncol. 2019; 54: 1691-1703.

7. Shirai T, Tanioka Y, Furusho T, Yamauchi J. A Nuclear Factor Involved in Transcriptional Regulation of the AREBP Gene. J Nutr Sci Vitaminol (Tokyo). 2017; 63: 430-432.

8. Zhang Q, Zheng X, Sun Q, Shi R, Wang J, et al. ZNF692 promotes proliferation and cell mobility in lung adenocarcinoma. Biochem Biophys Res Commun. 2017; 490: 1189-1196.

9. Guo Y, Sheng QH, Li J, Ye F, Samuels DC, Shyr Y. Large Scale Comparison of Gene Expression Levels by Microarrays and RNAseq Using TCGA Data. Plos One. 2013; 8.

10. Liu JF, Lichtenberg T, Hoadley KA, Poisson LM, Lazar AJ, et al. An Integrated TCGA Pan-Cancer Clinical Data Resource to Drive High-Quality Survival Outcome Analytics. Cell. 2018; 173: 400-416.

11. Wu J, Wang F, Liu X, Zhang T, Liu F, et al. Correlation of IDH1 and B7H3 expression with prognosis of CRC patients. Eur J Surg Oncol. 2018; 44: 1254-1260.

12. Zhu B, Pan Y, Zheng X, Zhang Q, Wu Y, et al. A clinical, biologic and mechanistic analysis of the role of ZNF692 in cervical cancer. Gynecol Oncol. 2019; 152: 396-407.