Journal of Gastrointestinal Cancer (2019) 50:485–492
https://doi.org/10.1007/s12029-018-0104-5

ORIGINAL RESEARCH

Immunohistochemical Expression of Fatty Acid Synthase and Vascular Endothelial Growth Factor in Primary Colorectal Cancer: a Clinicopathological Study

Asmaa Hussein Mohamed[1] · Nelly Mohamed Said[1]

Published online: 21 April 2018
© Springer Science+Business Media, LLC, part of Springer Nature 2018

Abstract

Background Fatty acid synthase (FAS) is a valuable lipid enzyme involved in lipid biosynthesis and suggested to contribute in tumor carcinogenesis. Vascular endothelial growth factor (VEGF) is considered a serious angiogenic growth factor in the angiogenic pathway which is a very important in tumor growth and metastasis. Thus, inhibition of lipid biosynthesis and tumor angiogenesis can be new goals for colorectal cancer (CRC) treatment.

Aim of the Work The assessment of the expression of FAS and VEGF protein and the relationship between them in CRC with the clinicopathological parameters.

Methods The present retrospective study included 63 paraffin blocks previously diagnosed as primary cases of CRC. The slides were subjected to FAS and VEGF immunohistochemical staining using a streptavidin-biotin-peroxidase. The relationships among FAS and VEGF expression and clinicopathological parameters were statistically analyzed.

Results The expression rate of FAS was 81% and VEGF was 84.1% in the studied cases. FAS expression was significantly associated with histopathological type ($p = 0.02$) and grade ($p = 0.04$), and highly associated with lymph node metastasis and stage ($p < 0.001$).VEGF was significantly associated with histopathological type ($p = 0.01$) and tumor depth ($p = 0.02$); highly associated with grade, lymph node metastasis, and stage ($p < 0.001$). There was a positive association between FAS and VEGF expression in CRC ($p < 0.001$).

Conclusion FAS and VEGF showed a highly significant expression in the studied primary CRC cases. A significant association was observed between their expressions, suggesting the involvement of FAS in tumor angiogenesis. So they constitute potential targets in cancer prevention and treatment and make FAS an attractive antiangiogenic target.

Keywords Colorectal cancer · Fatty acid synthase · Vascular endothelial growth factor · Immunohistochemical study

Introduction

Colorectal cancer (CRC) is the second most frequent cancer in women and the third one in men worldwide [1]. The incidence of CRC in the USA is approximately 135,000 and the mortality is 50,200 per year. High mortality of CRC can be related to the delayed diagnosis of the majority of patients with advanced stages of the disease, mostly stage IV with a 5-year survival rate 13% [2]. The incidence of CRC in Egypt constitutes about 6.5% of all malignancies [3], 3.14% of total malignancies in men, and 3.35% of total malignancies in women with a male to female ratio about 1 [4]. Treatment protocols for high stages of CRC are limited and improved survival is hoped with developing new therapeutic targets.

Fatty acid synthase (FAS) is a critical enzyme of lipid biosynthesis [5]. The expression of FAS enzyme in normal tissues is very minimal due to its inhibition by lipid intake. However, high expression of FAS was found in various metaplastic, dysplastic, and malignant tumors as intestinal metaplasia, adenoma, and carcinoma of the stomach; breast cancer; prostate cancer; and squamous cell carcinoma of the lung [6–9].

The relation between lipid synthesis and metastatic behavior of cancer has been accepted, but still, it is not clear how FAS regulates metastasis. The metastatic procedure is

✉ Asmaa Hussein Mohamed
drasmaahussein@yahoo.com

[1] Department of Pathology, Faculty of Medicine, Zagazig University, Ash Sharqia Governorate, Zagazig 44519, Egypt

considered a complex one and undergoes multiple steps. It demands that cancer cells develop an aggressive behavior with tumor microenvironment modulation, including its valuable component, the vascular niche [10, 11].

Angiogenesis has an impressive role in tumor development, progressive course, and metastatic behavior [12]. Vascular endothelial growth factor (VEGF) is the most effective angiogenic growth factor in angiogenesis pathway [13]. It induces endothelial cell proliferation, blood vessels permeability, and migratory potential of the endothelial cells [14, 15]. Also, it suppresses apoptosis, whereas its inhibition reduces tumor growth [16].

The aim of the current study is to assess FAS and VEGF immunohistochemical expression in primary CRC cases, their association with the clinicopathological parameters, and whether there is a correlation between the expression of FAS and VEGF in primary CRC.

Material and Methods

The current retrospective study involved 63 formalin-fixed paraffin blocks previously diagnosed as cases of primary CRC. The blocks were randomly selected including tumor tissue with the adjacent part of normal mucosa as a control, from the archives of Pathology Department, Faculty of Medicine, Zagazig University (September 2016–September 2017). The patients who had preoperative chemotherapy or radiotherapy were excluded. The clinical and pathological information assessed were age, sex, tumor site, histopathological type, tumor grade, depth of invasion, lymph node involvement, and tumor stage.

The histopathological types of colorectal cancer were conventional adenocarcinoma, mucinous carcinoma, and signet ring carcinoma. Tumors of the conventional type were graded as well (grade I) and moderately (grade II) differentiated adenocarcinoma, while mucinous carcinoma and signet ring carcinoma were assessed as grade III. The staging of colorectal tumors was done according to the WHO classification 2010 [17].

Immunohistochemical Staining

Immunohistochemical staining was carried out using indirect streptoavidin-biotin peroxidase technique. Tissue sections (3–5 μm) were deparaffinized in xylene and rehydrated in graded alcohol. Slides were incubated for 10 min in 0.3% hydrogen peroxide in absolute methanol to block the activity of endogenous peroxidase enzyme. Antigen retrieval was performed using Dako target retrieval solution (pH 6.0).The slides were then incubated for 60 min at room temperature using a rabbit polyclonal FAS antibody (Santa Cruz Biotechnology, Cat#sc-20140, CA, USA) with a 1:150 dilution and a rabbit polyclonal [ready to use] VEGF antibody (Thermo Fisher Scientific, Cat#RB-222-R7, Fremont, USA), then washed with two changes of phosphate buffered saline. After that, the slides were stained again with secondary antibody for 15 min at room temperature and then rinsed in the buffer again. After conjugation with streptavidin-biotin-peroxidase complex (broad-spectrum LAB-SA detection system, Invitrogen), 3, 3-diaminobenzidine (DAB, Sigma-Aldrich, MO, USA) was used as a chromogen and Mayer's hematoxylin was used as a counterstain.

Appropriate positive and negative controls were included in each run. Negative controls were prepared by replacing the primary antibody with phosphate buffered saline. An internal positive control in the tissue samples of adipose tissue was evaluated for FAS expression, and of blood vessels, to assess VEGF expression.

Evaluation of FAS Expression

FAS expression was evaluated as positive if found in the cell cytoplasm. The evaluation method for FAS expression was modified after the method of Wang et al. 2016 [18]. The extent and the intensity of positive staining were estimated. The extent of FAS was scored according to positive cells percentage (1, 0–5%; 2, 6–50%; 3, 51–75%; 4, ≥ 76%). The intensity was graded as (1, no staining; 2, weak staining; 3, moderate staining; 4, strong staining). The score is finally calculated from 1 to 16 by multiplying the extent and the intensity scores, then evaluated as: negative expression (1–4), weak expression (5–8), moderate expression (9–12), or strong expression (>12). FAS expression is considered positive if the score is more than 8.

Evaluation of VEGF Expression

VEGF expression was detected in the cell cytoplasm. A modulated method of Hafez and Tahoun 2016 [19] was used for evaluation. The extent and the intensity of positive staining were assessed. The extent of VEGF was scored according to positive cells as follows: (0, < 10%; 1, 10–25%; 2, 26–50%; 3, ≥ 51%). Then, the staining intensity of staining for VEGF was assessed as 0 for negative staining, 1 for weak intensity, 2 for moderate intensity, and 3 for strong intensity. The sum of the two parameters ranged from 0 to 6, and then we considered: negative or weak expression, for scores between 0 and 2; moderate expression, for scores 3 and 4; strong expression, for scores 5 and 6. VEGF expression is considered positive if the score is more than 2.

Statistical Analysis

The collected data were computerized and statistically analyzed using SPSS program (Statistical Package for Social Science) version 18.0. Qualitative data were represented as frequencies and relative percentages. Chi-square test was used

to calculate the difference between qualitative variables. Fisher's exact test was used to calculate the difference between qualitative variables in different groups when one or more of the studied cells were less than 5. Quantitative data were expressed as mean ± SD. The significance level for all mentioned tests was done. The p value of < 0.05, < 0.01 indicates significant and highly significant results, respectively.

Results

In the present study, patients' ages of 63 cases of CRC ranged from 40 to 65 years old with a mean age = 53.4 ± 9.46. Most cases (84.1%) were present in the age group between 40 and 60 years. The male to female ratio was about 1.2.

The Relationship Between FAS Expression and Clinicopathological Data in Primary CRC Cases

In normal colonic mucosa adjacent to tumor, there was no expression of FAS **(**Fig. 1a**)**, but it was highly expressed in 81% of the studied CRC cases. The majority of cases showing negative or weak FAS expression were well-differentiated (grade I) adenocarcinomas (50%) **(**Fig. 1b**)**, tumors with no lymph node metastasis [N0] (91.7%), and stage I (83.3%). Most cases of moderately differentiated (grade II) adenocarcinomas (14/18) and mucinous carcinoma (grade III) (16/19) showed moderate positive FAS expression **(**Fig. 1c, d**)**. Cases with strong positive FAS expression were mostly signet ring (grade III) carcinomas (50%) **(**Fig. 1e**)**, showed lymph node metastasis [N2] (100%), and stage III (100%).

FAS expression was significantly associated with histopathological type ($p = 0.02$), and tumor grade ($p = 0.04$), and showed a highly significant association with lymph node metastasis ($p < 0.001$) and tumor staging ($p < 0.001$), while there was no significant association between FAS expression and patient age, sex, tumor site, or depth of invasion **(**Table 1**)**.

The Relationship Between VEGF Expression and Clinicopathological Data in Primary CRC Cases

From the studied cases, 53/63 (84.1%) showed positive VEGF expression. Negative/weak VEGF expression was mostly found in cases of well-differentiated (grade I) adenocarcinomas (60%) **(**Fig. 2a**)**, depth of invasion [T2] (50%), and negative lymph node metastasis [N0] (60%). However, most cases showing strong positive VEGF expression were moderately differentiated (grade II) adenocarcinomas (36.4%)

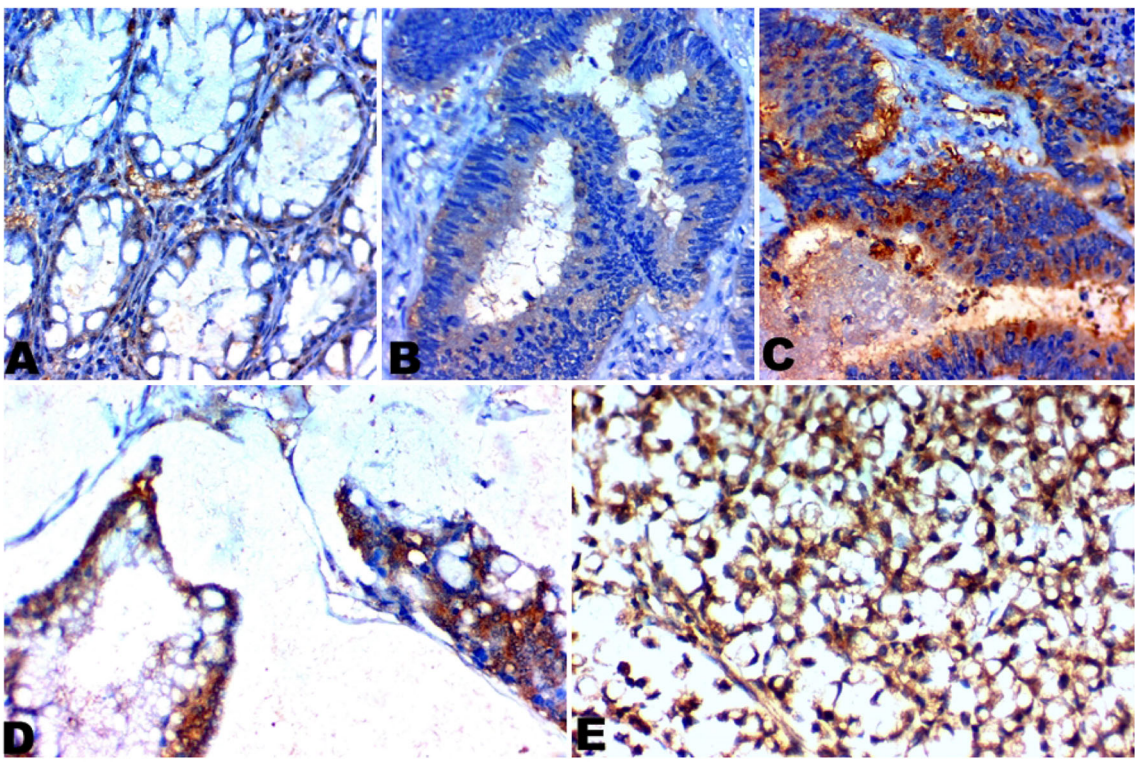

Fig. 1 Immunohistochemical expression of FAS using indirect streptoavidin-biotin immunoperoxidase technique (original magnification × 400). Negative FAS expression was found in **a** normal colonic mucosa and **b** well-differentiated (grade I) CRC. Moderate positive FAS expression with strong staining intensity was found in the neoplastic epithelium of **c** moderately differentiated CRC (grade II) and **d** mucinous carcinoma surrounded by mucin lakes (grade III). **e** Signet ring CRC carcinoma (grade III) showing strong positive expression in the cytoplasm of neoplastic signet ring cells

Table 1 The correlation between FAS expression and clinicopathological parameters in primary CRC cases

Parameters		Negative/weak expression		Moderate expression		Strong expression		*p*
		(*n* = 12)		(*n* = 45)		(*n* = 6)		
		No	%	No	%	No	%	
Age group	40–60 years	10	83.3	38	84.4	5	83.3	0.99
	> 60 years	2	16.7	7	15.6	1	16.7	
Sex	Male	5	41.6	26	57.8	3	50	0.60
	Female	7	58.4	19	42.2	3	50	
Histopathological type	Well-differentiated adenocarcinoma	6	50	13	28.9	1	16.7	*0.02**
	Moderately differentiated adenocarcinoma	3	25	14	31.1	1	16.7	
	Mucinous carcinoma	2	16.7	16	35.6	1	16.7	
	Signet ring carcinoma	1	8.3	2	4.4	3	50	
Tumor site	Left-sided	6	50	27	60	4	66.7	0.76
	Right-sided	6	50	18	40	2	33.3	
Tumor grade	Grade I	6	50	13	28.9	1	16.7	*0.04**
	Grade II	3	25	14	31.1	1	16.7	
	Grade III	3	25	18	40	4	66.7	
Depth of invasion	T2	4	33.3	6	13.3	1	16.7	0.50
	T3	5	41.7	29	64.4	3	50	
	T4a	3	25	10	22.2	2	33.3	
Lymph node metastasis	N0	11	91.7	19	42.2	0	0	*< 0.00***
	N1	1	8.3	22	48.9	0	0	
	N2	0	0	4	8.9	6	100	
Stage	I	10	83.3	2	4.4	0	0	*< 0.001**
	II	1	8.3	17	37.8	0	0	
	III	1	8.3	26	57.8	6	100	

*Indicates a significant statistical relation

**Indicates a highly significant statistical relation

(Fig. 2b) and mucinous (grade III) carcinomas (39.4%) (Fig. 2c), tumors with [T3] depth of invasion (69.7%) and lymph node metastasis [N1] (54.5%), and stage III (81.8%). Most cases of signet ring carcinoma 5/6 showed strong positive VEGF expression (Fig. 2d). VEGF expression was significantly associated with histopathological type ($p = 0.01$) and depth of invasion ($p = 0.02$); and a highly significant relation was found between VEGF expression and tumor grade ($p = 0.002$), lymph node metastasis ($p < 0.001$), and staging ($p < 0.001$). There was no significant association between VEGF expression and patient age, sex, or tumor site (Table 2).

The Relationship Between FAS and VEGF Expression in Primary CRC Cases

In order to evaluate the relation between the immunohistochemical expression of FAS and tumor angiogenesis, we have evaluated the association between FAS and VEGF expression. All cases with strong positive FAS expression showed also strong positive VEGF expression, and negative/ weak FAS expression was found mostly with negative/ weak VEGF expression (83.3%). There was a highly significant relationship between FAS and VEGF expression in the studied cases of primary CRC ($p < 0.001$) (Table 3).

Discussion

The metastatic potentiality of cancer comprises several changes in the cellular oncogenic and metabolic pathways. The malignant cells produce high levels of fatty acids despite the level of extracellular lipids. This may explain the probable role of fatty acids overexpression in progression of malignancy [18].

Overexpression of FAS has been reported in advanced stages of CRC [18], but the role of FAS in metastasis is not well understood. And since tumor angiogenesis is crucial for the metastatic progression of the tumor [20], we investigated the expression of FAS and VEGF in CRC.

Several studies have demonstrated that FAS expression is elevated in primary CRC when compared with control normal mucosa [18, 21–23]. Our results revealed that FAS was highly expressed in 81% of our studied cases of primary CRC, which were concordant with the prior observations [18, 24, 25],

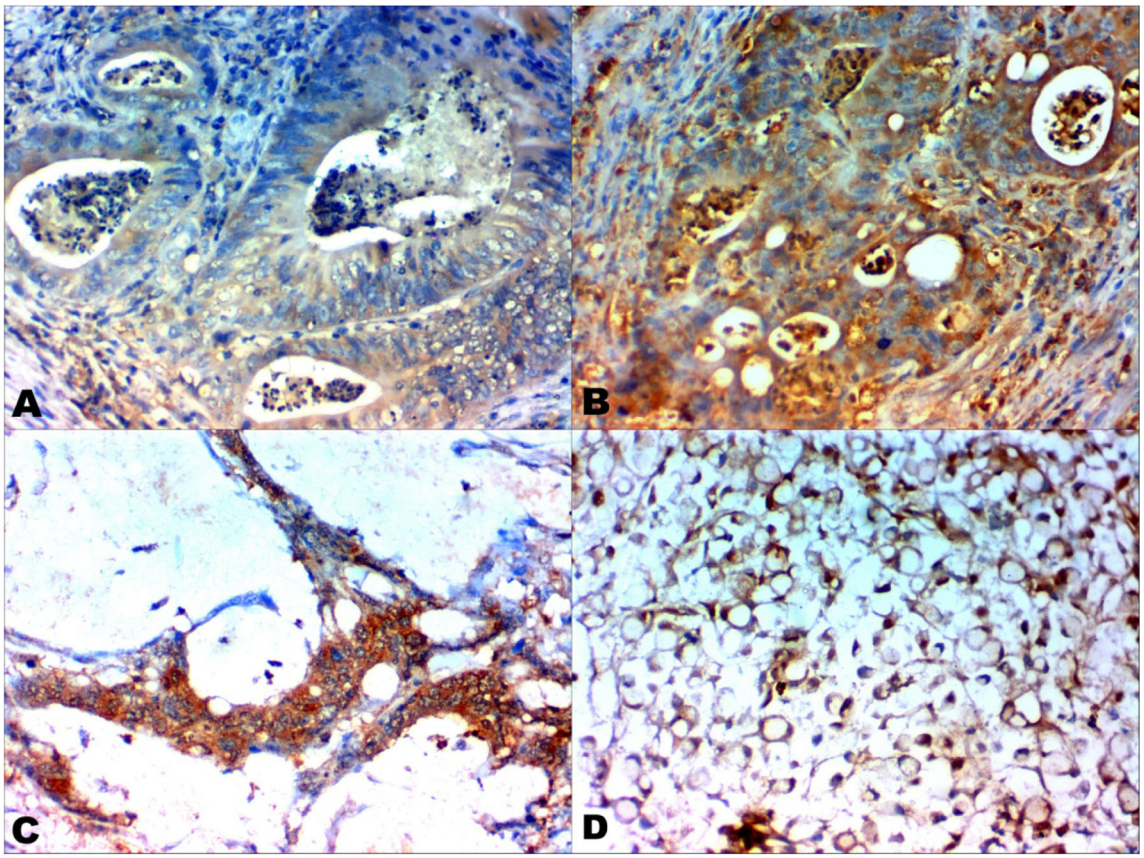

Fig. 2 Immunohistochemical expression of VEGF using indirect streptoavidin-biotin immunoperoxidase technique (original magnification × 400). **a** Negative VEGF expression with weak staining intensity was found in the glandular epithelium of well-differentiated CRC (grade I). Strong positive VEGF expression was found in the cytoplasm of the neoplastic cells of **b** moderately differentiated CRC (grade II), **c** mucinous carcinoma (grade III) surrounded by mucin lakes, and **d** Signet ring CRC carcinoma (grade III)

confirming that FAS plays an important role in the carcinogenic pathway of CRC. Higher and lower expressions of FAS were reported by others [5, 22, 23, 26, 27]. These discrepancies may be related to the use of different scoring systems, change in specificity, and sensitivity of antibodies employed in immunohistochemistry or patient heterogeneity.

FAS expression was significantly associated with the histopathological type ($p = 0.02$), tumor grade ($p = 0.04$), and had a highly significant association with lymph node metastasis ($p < 0.001$) and tumor staging ($p < 0.001$), suggesting that this enzyme might be involved in the tumor differentiation and metastasis. These results agreed with other reports [18, 22–24], however contradicted with other ones [21, 26–28]. In agreement with other studies [18, 23, 27], increased expression of FAS was observed in mucinous carcinomas and signet ring carcinomas with the extensive metastatis for lymph nodes. Therefore, FAS expression could correlate with poor prognosis of CRC through an association with lymph node metastasis and aggressive histopathological subtypes of CRC. No significant relation was found between FAS expression and patient age, sex, and tumor site, in agreement with some studies [18, 22, 25].

The increased expression of FAS in tumor cells is critical for the function of endoplasmic reticulum, the main site for phospholipid synthesis to maintain the membrane biogenesis [29]. FAS inhibitors, like C75 and orlistat, have been shown to exhibit an antitumor activity [30–32] through downregulation of FAS by means of its enhanced proteasomal degradation leading to increased apoptosis [33]. These data support that FAS acts as an oncoprotein. Importantly, FAS overexpression confers chemoresistance in breast cancer cells, and inhibition of FAS may enhance the effect of chemotherapy [34, 35]. Thus, inhibition of fatty acid synthesis by inhibiting the enzymatic function with metabolic analogs or by decreasing transcription of the FAS gene may be eventually a useful strategy to treat CRC or to inhibit progression of the tumor.

In many human cancers, the angiogenesis pathway of the tumors and its clinical importance has been evaluated. VEGF was confirmed to be a useful marker for the assessment of angiogenesis [36, 37].

Based on our immunohistochemical evaluation, cytoplasmic staining for VEGF was detected in 84.1% of the studied CRC cases. Our findings were homogeneous with many

Table 2 The correlation between VEGF expression and clinicopathological parameters in primary CRC cases

Parameters		Negative/weak expression		Moderate expression		Strong expression		*p*
		(*n* = 10)		(*n* = 20)		(*n* = 33)		
		No	%	No	%	No	%	
Age group	40–60 years	8	80	18	90	27	81.8	0.68
	> 60 years	2	20	2	10	6	18.2	
Sex	Male	6	60	9	45	19	57.6	0.62
	Female	4	40	11	55	14	42.4	
Histopathological type	Well-differentiated adenocarcinoma	6	60	11	55	3	9.1	*0.01**
	Moderately differentiated adenocarcinoma	2	20	4	20	12	36.4	
	Mucinous carcinoma	2	20	4	20	13	39.4	
	Signet ring carcinoma	0	0	1	5	5	15.2	
Tumor site	Left-sided	5	50	13	65	19	57.6	0.72
	Right-sided	5	50	7	35	14	42.4	
Tumor grade	Grade I	6	60	11	55	3	9.1	*0.002***
	Grade II	2	20	4	20	12	36.4	
	Grade III	2	20	5	25	18	54.5	
Depth of invasion	T2	5	50	4	20	2	6.1	*0.02**
	T3	2	20	12	60	23	69.7	
	T4a	3	30	4	20	8	24.2	
Lymph node metastasis	N0	6	60	18	90	6	18.2	*< 0.001 ***
	N1	4	40	1	10	18	54.5	
	N2	0	0	1	10	9	27.3	
Stage	I	4	40	5	25	3	9.1	*< 0.001***
	II	2	20	13	65	3	9.1	
	III	4	40	2	10	27	81.8	

*Indicates a significant statistical relation

**Indicates a highly significant statistical relation

studies [20, 38], but higher than the results of others [39–41]. Mohamed et al. (2016) [42] reported a higher positivity rate of VEGF (94.7%).

In the current study, there was a statistically significant relationship between VEGF expression and histopathological type, which was confirmed by some previous studies [41, 43, 44], while a study held up by Vahedi et al. (2015) [39] found a negative significance with the tumor type.

Several studies have shown a highly significant association between VEGF expression and tumor grade in CRC, which are in harmony with our results [39, 45–47], but no significant difference was observed by Hashim et al. (2010) [48]. As regards the lymph node involvement and the tumor stage, we detected a highly significant relation with VEGF expression that matched with various works [39, 41, 42, 49, 50], who found an association between the tumor stage and lymph node

Table 3 The correlation between FAS and VEGF expression in primary CRC cases

		FAS expression						*p*
		Negative/weak expression (*n* = 12)		Moderate expression (*n* = 45)		Strong expression (*n* = 6)		
		No	%	No	%	No	%	
VEGF expression	Negative/weak express ion (*n* = 10)	10	83.3	0	0	0	0	*< 0.001***
	Moderate expression (*n* = 20)	1	8.3	19	42.2	0	0	
	Strong expression (*n* = 33)	1	8.3	26	57.8	6	100	

**Indicates a highly significant statistical relation

metastasis with VEGF expression. Our findings support that high VEGF expression is linked to advanced CRC. However, Hashim et al. (2010) [48] found no significant difference between the stages of CRC and VEGF expression, may be due to their use of a different scoring system that depends only on assessing of the percentages of positively stained cells.

Our results observed no statistically significant relation between VEGF expression and some clinicopathological features, such as patient age, sex, and tumor site ($p > 0.05$). These were confirmed by some researchers [28, 31, 39, 42], but in contrary with the results of van Triest et al. (2000) [47] who identified a significant relation between VEGF expression and patient age and sex.

In order to evaluate the contribution of FAS expression to tumor angiogenesis, we evaluated the relation between the immunohistochemical expression of FAS and VEGF. Our result revealed a highly significant association between them ($p < 0.001$). These data suggest that high level of FAS enzyme involved in the development of angiogenesis in CRC cases may be through VEGF upregulation. Our results are in agreement with Zaytseva et al. (2014) [20] who showed that a stable FAS knockdown in CRC cells leads to suppression of proliferation, migration, and the tubule formation of the endothelial cells and attenuation of vascular endothelial growth factor receptor-2 signaling in vitro.

In conclusion, our study detected that both FAS and VEGF show high expression in primary cases of CRC. A significant association was found between their expressions in primary CRC cases, proposing the involvement of FAS in tumor angiogenesis. So, FAS could be an attractive antiangiogenic target for advanced CRC warranting further works, using FAS inhibitors drugs that could inhibit CRC metastasis through tumor angiogenesis.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

References

1. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin. 2015;65(2):87–108. https://doi.org/10.3322/caac.21262.
2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. CA Cancer J Clin. 2017;67(1):7–30. https://doi.org/10.3322/caac.21387.
3. Mokhtar N, Salama A, Badawy O, Khorshed E, Mohamed G, Ibrahim M, Abdelazim H. Gastrointestinal tract tumors. In: Cancer Pathology Registry. A 12-year Registry (2000–2001). 2016a. pp, 32–58.
4. Mokhtar N, Salama A, Badawy O, Khorshed E, Mohamed G, Ibrahim M, Abdelazim H. General features of total series. In: Cancer Pathology Registry. A 12-year Registry (2000–2001) 2016b. pp, 1–6.
5. Kuhajda FP. Fatty acid synthase and cancer : new application of an old pathway. Cancer Res. 2006;66(12):5977–81. https://doi.org/10.1158/0008-5472.CAN-05-4673.
6. Kusakabe T, Nashimoto A, Honma K, Suzuki T. Fatty acid synthase is highly expressed in carcinoma, adenoma and in regenerative epithelium and intestinal metaplasia of the stomach. Histopathol. 2002;40(1):71–9. https://doi.org/10.1046/j.1365-2559.2002.01289.x.
7. Piyathilake C, Frost A, Manne U, Bell W, Weiss H, Heimburger D, et al. The expression of fatty acid synthase (FASE) is an early event in the development and progression of squamous cell carcinoma of the lung. Hum Pathol. 2000;31(9):1068–73. https://doi.org/10.1053/hupa.2000.9842.
8. Alò PL, Visca P, Marci A, Mangoni A, Botti C, Di Tondo U. Expression of fatty acid synthase (FAS) as a predictor of recurrence in stage I breast carcinoma patients. Cancer. 1996;77(3):474–82. https://doi.org/10.1002/(SICI)1097-0142(19960201)77:3<474::AID-CNCR8>3.0.CO;2-K.
9. Epstein JI, Carmichael M, Partin AW. OA-519 (fatty acid synthase) as an independent predictor of pathological stage in adenocarcinoma of the prostate. Urology. 1995;45(1):81–6. https://doi.org/10.1016/S0090-4295(95)96904-7.
10. Dvorak HF, Weaver VM, Tlsty TD, Bergers G. Tumor microenvironment and progression. J Surg Oncol. 2011;103(6):468–74. https://doi.org/10.1002/jso.21709.
11. Joyce JA, Pollard JW. Microenvironmental regulation of metastasis. Nat Rev Cancer. 2009;9(4):239–52. https://doi.org/10.1038/nrc2618.
12. Potente M, Gerhardt H, Carmeliet P. Basic and therapeutic aspects of angiogenesis. Cell. 2011;146(6):873–87. https://doi.org/10.1016/j.cell.2011.08.039.
13. Nowak DG, Woolard J, Amin EM, Konopatskaya O, Saleem MA, Churchill AJ, et al. Expression of pro- and anti-angiogenic isoforms of VEGF is differentially regulated by splicing and growth factors. J Cell Sci. 2008;121(Pt 20):3487–95. https://doi.org/10.1242/jcs.016410.
14. Lau T, Roslani A, Lian L, Lee P, Hilmi I, Goh K, et al. Association between EGF and VEGF functional polymorphisms and sporadic colorectal cancer in the Malaysian population. Genet Mol Res. 2014;13(3):5555–61. https://doi.org/10.4238/2014.July.25.9.
15. Hansen TF, Jakobsen A. Clinical implications of genetic variations in the VEGF system in relation to colorectal cancer. Pharmacogenomics. 2011;12(12):1681–93. https://doi.org/10.2217/pgs.11.118.
16. Jannuzzi A, Özhan G, Yanar H, Alpertunga B. VEGF gene polymorphisms and susceptibility to colorectal cancer. Genet Test Mol Biomarkers. 2015;19(3):133–7. https://doi.org/10.1089/gtmb.2014.0259.
17. Hamilton SR, Rubio CA, Vogelstein B, Sobin LH, Kudo S, Fogt F. Carcinoma of the colon and rectum. In: Bosman FT, Carneiro F, Hruban RH, Theise ND, editors. WHO classification of tumours of the digestive system. 4th ed. IARC: Lyon; 2010. p. 134–46.
18. Wang H, Xi Q, Wu G. Fatty acid synthase regulates invasion and metastasis of colorectal cancer via Wnt signaling pathway. Cancer Med. 2016;5(7):1599–606. https://doi.org/10.1002/cam4.711.
19. Hafez N, Tahoun N. Expression of cyclooxygenase 2 and vascular endothelial growth factor in gastric carcinoma: relationship with clinicopathological parameters. J Egypt Natl Canc Inst. 2016;28(3):149–56. https://doi.org/10.1016/j.jnci.2016.05.005.
20. Zaytseva YY, Elliott VA, Rychahou P, Mustain WC, Kim JT, Valentino J, et al. Cancer cell-associated fatty acid synthase activates endothelial cells and promotes angiogenesis in colorectal cancer. Carcinogenesis. 2014;35(6):1341–51. https://doi.org/10.1093/carcin/bgu042.
21. Zaytseva YY, Rychahou PG, Gulhati P, Elliott VA, Mustain WC, O'Connor K, et al. Inhibition of fatty acid synthase attenuates

CD44-associated signaling and reduces metastasis in colorectal cancer. Cancer Res. 2012;72(6):1504–17. https://doi.org/10.1158/0008-5472.CAN-11-4057.
22. Kearney KE, Pretlow TG, Pretlow TP. Increased expression of fatty acid synthase in human aberrant crypt foci: possible target for colorectal cancer prevention. Int J Cancer. 2009;125:249–52. https://doi.org/10.1002/ijc.24356.
23. Visca P, Alo PL, Nonno FD, Botti C, Trombetta G, Marandino F, et al. Immunohistochemical expression of fatty acid synthase, apoptotic-regulating genes, proliferating factors, and ras protein product in colorectal adenomas, carcinomas, and adjacent nonneoplastic mucosa. Clin Cancer Res. 1999;5(12):4111–8.
24. Ogino S, Nosho K, Meyerhardt JA, Kirkner GJ, Chan AT, Kawasaki T, et al. Cohort study of fatty acid synthase expression and patient survival in colon cancer. J Clin Oncol. 2008;26:5713–20. https://doi.org/10.1200/JCO.2008.18.2675.
25. Visca P, Sebastiani V, Botti C, Diodoro MG, Lasagni RP, Romagnoli F, et al. Fatty acid synthase (FAS) is a marker of increased risk of recurrence in lung carcinoma. Anticancer Res. 2004;24:4169–73.
26. Uddin S, Hussain AR, Ahmed M, Abubaker J, Al-Sanea N, Abduljabbar A, et al. High prevalence of fatty acid synthase expression in colorectal cancers in Middle Eastern patients and its potential role as a therapeutic target. Am J Gastroenterol. 2009;104(7):1790–801. https://doi.org/10.1038/ajg.2009.230.
27. Rashid A, Pizer ES, Moga M, Milgraum LZ, Zahurak M, Pasternack GR, et al. Elevated expression of fatty acid synthase and fatty acid synthetic activity in colorectal neoplasia. Am J Pathol. 1997;150:201–8.
28. Zhan Y, Ginanni N, Tota MR, Wu M, Bays NW, Richon VM, et al. Control of cell growth and survival by enzymes of the fatty acid synthesis pathway in HCT-116 colon cancer cells. Clin Cancer Res. 2008;14(18):5735–42. https://doi.org/10.1158/1078-0432.CCR-07-5074.
29. Li JN, Gorospe M, Chrest FJ, Kumaravel TS, Evans MK, Han WF, et al. Pharmacological inhibition of fatty acid synthase activity produces both cytostatic and cytotoxic effects modulated by p53. Cancer Res. 2001;61(4):1493–9.
30. Kridel SJ, Axelrod F, Rozenkrantz N, Smith JW. Orlistat is a novel inhibitor of fatty acid synthase with antitumor activity. Cancer Res. 2004;64(6):2070–5.
31. Alli PM, Pinn ML, Jaffee EM, McFadden JM, Kuhajda FP. Fatty acid synthase inhibitors are chemopreventive for mammary cancer in neu-N transgenic mice. Oncogene. 2005;24(1):39–46. https://doi.org/10.1038/sj.onc.1208174.
32. Little JL, Wheeler FB, Fels DR, Koumenis C, Kridel SJ. Inhibition of fatty acid synthase induces endoplasmic reticulum stress in tumor cells. Cancer Res. 2007;67(3):1262–9. https://doi.org/10.1158/0008-5472.CAN-06-1794.
33. Graner E, Tang D, Rossi S, Baron A, Migita T, Weinstein LJ, et al. The isopeptidasUSP2a regulates the stability of fatty acid synthase in prostate cancer. Cancer Cell. 2004;5(3):253–61.
34. Menendez JA, Lupu R, Colomer R. Inhibition of tumor-associated fatty acid synthase hyperactivity induces synergistic chemosensitization of HER-2/neu-overexpressing human breast cancer cells to docetaxel (taxotere). Breast Cancer Res Treat. 2004;84:183–95. https://doi.org/10.1023/B:BREA.0000018409.59448.60.
35. Furuta E, Pai SK, Zhan R, Bandyopadhyay S, Watabe M, MoYY HS, et al. Fatty acid synthase gene is up-regulated by hypoxia via activation of Akt and sterol regulatory element binding protein-1. Cancer Res. 2008;68(4):1003–11. https://doi.org/10.1158/0008-5472.CAN-07-2489.
36. Zhao HC, Qin R, Chen XX, Sheng X, Wu JF, Wang DB, et al. Microvessel density is a prognostic marker of human gastric cancer. World J Gastroenterol. 2006;12(47):7598–603. https://doi.org/10.3748/wjg.v12.i47.7598.
37. Zhao ZQ, Yang S, Lu HS. Expression of midkine and vascular endothelial growth factor in gastric cancer and the association of high levels with poor prognosis and survival. Mol Med Rep. 2012;5(2):415–9. https://doi.org/10.3892/mmr.2011.649.
38. Xu T, Chen D, Chen J. Expression of vascular endothelial growth factor C and its correlation with lymph node metastasis in colorectal carcinoma. J Huazhong Univ Sci Technolog Med Sci. 2004;24(6):596–8.
39. Vahedi L, Ghasemi M, Yazdani J, Mehrabian-Fard M, Sajadi N. Evaluation of VEGF immunohistochemical expression and correlation with clinicopathologic features in colorectal Cancer. Govaresh. 2015;20(3):199–204.
40. 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(3):392–8. https://doi.org/10.1007/s10350-005-0247-x.
41. Xu Q, Chen RX, Wang ZW, Ni QC, Qian JJ, Sheng HX. Clinical research on angiogenesis in colorectal carcinoma and expression of CK20 mRNA in peripheral blood. Zhonghua Yi Xue Za Zhi. 2005;85(17):1205–8. https://doi.org/10.3760/j:issn:0376-2491.2005.17.015.
42. Mohamed HAD, Abd El All HS, Kamel AA, Yossef WT, Hammam MM. Correlation of vascular endothelial growth factor expression and neovascularization with colorectal carcinoma: a pilot study. J Adenocarcinoma. 2016;1:1–5. https://doi.org/10.21767/2572-309X.10005.
43. Jia Y, Li Z, He Y, Liang W, Yang H, Ma H. Expression of vascular endothelial growth factor-C and the relationship between lymphangiogenesis and lymphatic metastasis in colorectal cancer. World J Gastroenterol. 2004;10(22):3261–3. https://doi.org/10.3748/wjg.v10.i22.3261.
44. Zheng S, Han MY, Xiao ZX, Peng JP, Dong Q. Clinical significance of vascular endothelial growth factor expression and neovascularization in colorectal carcinoma. World J Gastroenterol. 2003;9(6):1227–30. https://doi.org/10.3748/wjg.v9.i6.1227.
45. Wen L, Wang R, Lu X, You C. Expression and clinical significance of vascular endothelial growth factor and fms-related tyrosine kinase 1 in colorectal cancer. Oncol Lett. 2015;9(5):2414–8. https://doi.org/10.3892/ol.2015.3013.
46. Hedaya M, Helmy A, H E, Hammam O. Cyclo-oxygenase-2 and vascular endothelial growth factor expression in colorectal cancer patients. The Egyptian Journal of Surgery. 2015;34(1):35–40. https://doi.org/10.4103/1110-1121.153369.
47. van Triest B, Pinedo HM, Blaauwgeers JL, van Diest PJ, Schoenmakers PS, Voorn DA, et al. Prognostic role of thymidylate synthase, thymidine phosphorylase/platelet-derived endothelial cell growth factor and proliferation markers in colorectal cancer. Clin Cancer Res. 2000;6(3):1063–72.
48. Hashim AF, Al-Janabi AA, Mahdi LH, Al-Toriahi KM, Yasseen AA. Vascular endothelial growth factor (VEGF) receptor expression correlates with histologic grade and stage of colorectal cancer. Libyan J Med. 2010;5(1):5059. https://doi.org/10.3402/ljm.v5i0.5059.
49. Minagawa N, Nakayama Y, Hirata K, Onitsuka K, Inoue Y, Nagata N, et al. Correlation of plasma level and immunohistochemical expression of vascular endothelial growth factor in patients with advanced colorectal cancer. Anticancer Res. 2002;22(5):2957–63.
50. White JD, Hewett PW, Kosuge D, McCulloch T, Enholm BC, Carmichael J, et al. Vascular endothelial growth factor-D expression is an independent prognostic marker for survival in colorectal carcinoma. Cancer Res. 2002;62(6):1669–75.