BENHA MEDICAL JOURNAL bmj
Print ISSN 1110-208X.
Online ISSN 2357-0016

Original article

Significance of Stathmin1 and Acetyl-CoA Synthetase 2 Expression and Morphometric Analysis in Colorectal Carcinoma and Precancerous Lesions

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Received:
Accepted:

Abstract:

Background: Colorectal cancer (CRC) is the third leading cause of cancer death in the world. Precancerous lesions that may progress to CRC include inflammatory bowel disease (IBD) and adenoma This study aims to clarify the role of STMN1 and ACeCS2 and the morphometric changes in colorectal carcinogenesis. **Material and methods:** This is a retrospective study on 100 cases; control (n=8) IBD (n=29), convetional adenoma (n=19), serrated polyp/adenoma (n=9) and CRC (n=35), All cases were stained with STMN1 and ACeCS2 antibodies using Avidin-biotin complex. In morphometry, we assessed nuclear morphometric parameters and gland/stromal ratio among studied groups. **Results:** There was statistically significant correlation between Stathmin-1 and dysplasia in IBD (p=0.008), with dysplasia (p=0.05), types (p-0.003) of conventional adenoma, staging (p=0.015) and node metastasis (p=0.04) of CRC cases. There was statistically significant correlation between ACeCS2 among studied groups (p=0.006), with dysplasia in IBD (p=0.002), staging (p=0.001) and node metastasis (p=0.004) of CRC. There was statistically significant correlation between morphometric parameters among studied groups (P<0.001), with dysplasia in IBD(P<0.001), types and dysplasia of adenoma (P<0.001) and grading of carcinoma (P<0.001). **Conclusion:** STMN1 and ACeCS2 might have a key role in pathogenesis and aggressiveness of colorectal carcinoma. Morphometric parameters have significant role in detection the progression from IBD, adenoma and colorectal carcinoma and detection of high-grade dysplasia among IBD and colorectal adenoma.

Keywords: premalignant lesions or precancerous lesions, colorectal carcinoma, STMN1, ACeCS2 immunohistochemistry and morphometry.

Introduction

Colorectal cancer (CRC) is the third leading cause of cancer death in the world. CRC usually emerges from the glandular, epithelial cells of the large intestine [(1)].

According to the World Health Organization, colorectal cancer (CRC) is the third common cancer, comprising 11% in the world and is steadily rising because of the western lifestyle. It is 3-fold higher in developed compared to developing countries [(2)].

In Egypt, according to National Cancer Institute registry (NCI), Cairo University, colorectal carcinoma represents 35% from total GIT tumors and 6.49% from total malignancy [(3)(4)].

Environmental and genetic factors can increase the likelihood of developing CRC. Although inherited susceptibility results in the most striking increases in risk, the majority of CRCs are sporadic rather than familial [(5)].

Environmental factors associated with risk of CRC include smoking, alcohol intake, high red meat and processed meat consumption, high fat and protein diet intake, physical inactivity, and overweight [(6)]. Precancerous lesions that may progress to CRC include inflammatory bowel disease and adenoma [(7)].

The main prognostic factors in CRC are lymph node involvement, size of the tumor and stage of disease. However, these factors do not fully predict individual clinical outcome [(8)]. Several molecular and genetic signatures have emerged as promising biomarkers with enormous clinical value and can be used to predict prognosis in colorectal carcinoma [(9)].

Stathmin1 (STMN1) also known as oncoprotein 18 is a cytosolic phosphoprotein that regulates cellular microtubule dynamics [(10)]. STMN1 is a molecular target in several cancers such as distal oesophageal adenocarcinoma, oesophageal squamous cell carcinoma, gastric cancer, breast cancer, hepatocellular carcinoma, cholangiocarcinoma, prostate cancer, colorectal cancer and non-small cell lung cancer [(11-13)]. STMN1 was suggested as a possible prognostic marker and a novel target for cancer treatment [(14)].

Alteration in cellular metabolism is one of the hallmarks of cancers. Cancer-associated metabolic changes include the deregulated uptake of glucose and amino acids, use of glycolysis/tricarboxylic. The main goal of these alterations is to maintain cell proliferation in the nutrient-poor environment [(15)].

Acetyl-CoA synthetase-2 (AceCS2) is a key enzyme for cancer metabolism, which supplies acetyl-CoA for tumor cells [(15)]. AceCS2 overexpression has been reported in several types of cancers, including hepatocellular carcinomas, gliomas, and breast carcinomas, and the overexpression of acetyl-CoA synthetase-2 has been associated with a worse prognosis in these malignancies [(16)(17)].

Morphometry is the process of measuring cellular and tissue archeticture features with regards to cell and nuclear size, shape and epithelial to stromal ratio [(18)]. Morphometric features of malignant cells differ from that observed in nonmalignant cells. This observation has led to the hypothesis that such changes occur prior to the emergence of clinically detectable disease and the morphometry can be used as an indicator for estimating an individual's risk for cancer [(19)].

Also, there is increasing evidence of the value of the morphometry as a key of tumor behavior. This is known to be accurate, reproducible and efficient and has been used to predict the prognosis in breast cancer [(20)]. The aim of this work is to study the expression of stathmin1 and acetyl-CoA synthetase2 and the morphometric changes in colorectal carcinoma (CRC) and precancerous lesions to clarify their role in colorectal carcinogenesis and to evaluate their prognostic value in CRC.

Material and methods:

This is a retrospective study carried on 35 selected paraffin blocks of colorectal carcinoma and 57 paraffin blocks of non-malignant lesions with or without dysplasia. In addition to 8 paraffin blocks of apparently normal colonic tissue were used as control. The non-malignant lesions included 29 biopsies of inflammatory bowel disease (22 biopsies of ulcerative colitis and 7 biopsies of Crohn's disease), 9 biopsies of serrated lesions (6 biopsies of sessile serrated polyp and 3 biopsies of traditional serrated adenoma), 19 biopsies of colorectal adenoma (12 biopsies of tubular adenoma, 6 biopsies of tubulovillous adenoma and 1 biopsy of villous adenoma). All the examined cases were endoscopic biopsies except 3 cases of Crohn's disease and 19 cases of colorectal carcinoma were colectomy specimens.

This study was performed on archival formalin fixed paraffin embedded tissue specimens of colonoscopic biopsies diagnosed as IBD, adenoma and carcinoma. Cases were collected from Pathology Department and early cancer detection unit (ECDU), Faculty of Medicine, Benha University. They were collected from January 2018 through December 2023. This study was approved by the Ethical committee of Benha Faculty of Medicine, Benha University (15-1-2022).

For Histopathological studies: Sections of 4 μm thickness were cut, dewaxed in xylene and then rehydrated in descending grades of alcohol and stained by conventional hematoxylin and eosin (H& E) stain. IBD cases were assessed whether with dysplasia or not. Serrated polyps/adenoma were assessed whether with dysplasia or not. Colorectal adenomas are graded histologically into adenoma with low grade dysplasia and adenoma with high grade dysplasia [(21)]. Colorectal carcinoma is graded according to degree of glandular differentiation to well differentiated (Grade I), moderately differentiated (Grade II) and poorly differentiated carcinoma (Grade III) [(22)]. Tumors were staged according to TNM staging system according to[(23)]. Two experienced pathologists blindly and independently confirmed the histopathological diagnosis of each lesion and agreed on grading and staging.

Inclusion criteria: Cases with clinicopathological data obtained from the patients' files, including age, histopathological grade, tumor size, tumor location, M stage) were included Patients with CRC who had received chemotherapy prior to the study and those without available paraffin blocks or clinicopathological data- were excluded.

For Immunohistochemical studies: avidin-biotin complex technique was used following manufacture instructions (Dako, CA). For antigen retrival citrate monohydrate (PH 6.0) was used. Slides were incubated with an anti- stathmin-1 (Chongqing Biospes Co., Ltd Innovation Centre, High-tech Venture Park, Jiulongpo District, Chongqing, 400039, China) at 1:50 dilution, for 1 hour at room temperature. Slides were incubated with an anti-acetyl coA synthetase-2 (Chongqing Biospes Co., Ltd Innovation Centre, High-tech Venture Park, Jiulongpo District, Chongqing, 400039, China) at 1:50 dilution, for 1 hour at room temperature. Freshly prepared chromogen diaminobenzine (DAB) was used; it was incubated with slides for 3-5 minutes. In each staining session sections of breast carcinoma was used as positive control for STMN1 and ACeCS2. For negative control, the primary antibody was omitted and replaced by normal rabbit serum IgG.

Immunohistochemical assessment: Only cytoplasmic stathmin-1 expression and cytoplasmic acetyl coA synthetase-2 is regarded. The extent of immunostain was evaluated in random five fields under the power of 20 magnification**.**

For stathmin 1: IHC scoring was based on the percentage of positive cells and the staining intensity following previous study [(24)]**. The Same for acetyl CoA synthetase 2** regarding IHC scoring that was based

DOI: 10.21608/bmfj.2024.312447.2167

on the percentage of positive cells and the staining intensity as detailed[25] . The expression of ACSS2 was classified as low (score <5) or high (score ≥5) according to median value.

Morphometric analysis: Slides were stained by H& E and were digitized using Olympus® digital camera installed on Olympus® microscope with 1/2 X photo adaptor, using 40 X objective. The result images were analyzed on Intel® Core I7® based computer using VideoTest Morphology® software (Russia) with a specific built-in routine for particle analysis and counting. 2 slides from each case were used, 5 random field from each slide were analyzed. We measured nuclear area, nuclear perimeter, nuclear length, nuclear width, nuclear roundness and gland stromal ratio and compared the results in each group. The measurement unit was µm2.

Statistical analysis: Results were analysed using the computer program Statistical package for social science (SPSS version 20 for windows; SPSS Inc., Chicago, Illinois, USA)**.** Statistically significance of the tests was expressed in P-value. A P value <0.05 was considered statistically significant. P value <0.01 was considered highly significant.

Results:

The mean age in all studied cases is recorded as 51.56±16.39. In all studied cases 55 (59.8%) cases were male and 37 (40.2%) cases were female.

Stathmin-1 immunohistochemical expression among the studied groups & its relations with clinicopathological parameters in colorectal carcinoma

Regarding the control group 3 biopsies (37.5%) were negative and 5 biopsies (62.5%) showed weak positive cytoplasmic expression.

In the current study, there is increased expression of Sathmin-1 gradually from IBD to serrated polyp/adenoma, conventional adenoma to carcinoma but the correlation was statistically insignificant (p>0.05) Figure (1).

Stathmin-1 was overexpressed in cases of IBD with dysplasia with significant correlation (p=0.008). However no significant correlation between Stathmin-1 and types of IBD or types or serrated polyp/adenoma Table (1).

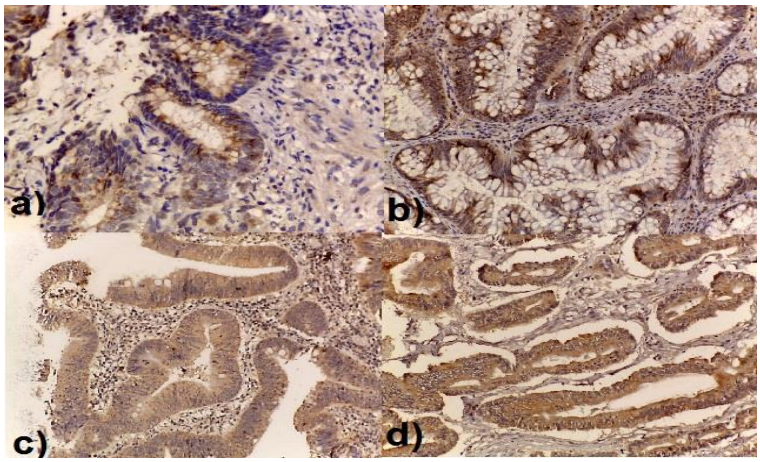

Figure (1): Stathmin 1 expression; a) ulcerative colitis with dysplasia: STMN1 positive cytoplasmic expression in more than 50% of glandular epithelial cells with moderate intensity (score 6/9) (ABC, X400), b) traditional serrated adenoma with dysplasia: STMN1 positive cytoplasmic expression in more than 50% of glandular epithelial cells with moderte intensity (score 6/9) (ABC, X200), c) tubulovillous adenoma with high grade dysplasia: STMN1 positive cytoplasmic expression in more than 50% of glandular epithelial cells with moderate intensity (score 6/9) (ABC, X200), d) Moderated differentiated carcinoma (GII): STMN1 positive cytoplasmic expression in more than 50% of malignant epithelial cells with strong intensity (score 9/9) (ABC, X200).

Table (1): STMN1 and ACeCS2 expression in non-malignant cases and correlation with dysplasia.

	Stathmin1				P	ACeCS2		P
According to types	negative	Weak positive	positive	Strong positive		Low	High	
IBD								
Ulcerative colitis	2(9.1%)	9(40.9%)	11(50.0%)	0	0.212	7(31.8%)	15(68.2%)	0.871
Crohn's	1(14.3%)	5(71.4%)	1(14.3%)	0		2(28.6%)	5(71.4%)	
Serrated polyp/adenoma (9)	0	1(16.7%)	5(83.3%)	0		3(50%)	3(50%)	
Sessile serrated polyp	0	0	3(100%)	0	0.453	1(33.3%)	2(66.7%)	1.0
Traditional serrated								
Conventional adenoma (19)	1(8.3)	4(33.3%)	7(58.3%)	0		5(41.7%)	7(58.3%)	
Tubular	0	0	1(100%)	0	0.003*	0	1(100%)	0.440
Villous	0	2(33.3%)	3(50%)	1(16.7%)		1(16.7%)	5(83.3%)	
Tubulovillous								
According to with or without dysplasia								
IBD								
Without dysplasia	3(10.3%)	13(61.9%)	5(23.8%)	0	0.008*	3(14.3%)	18(85.7%)	0.002*
With dysplasia	0	1(12.5%)	7(87.5%)	0		6(75%)	2(25%)	
Serrated polyp/adenoma (9)	0	0	3(100.0%)	0		1(33.3%)	2(66.7%)	0.343
Without dysplasia	0	1(16.7%)	5(83.3%)	0	1.0	2(33.3%)	4(66.7%)	
With low grade dysplasia								
Conventional adenoma (19)	0	6(54.5%)	5(45.5%)	0	0.05*	3(27.3%)	8(72.7%)	0.636
Low grade dysplasia	1(12.5%)	0	6(75.0%)	1(12.5%)		3(37.5%)	5(62.5%)	
High grade dysplasia								

In relation to CRC stathmin-1 expression was significantly increased with advanced stage (p=0.015) and lymph node metastasis (p=0.04). However, no statistically significant correlation with other clinicopathological data Table (2).

ACeCS2 IHC expression among the studied groups & its correlations with clinicopathoogical parameters in colorectal carcinoma

Regarding the control group all biopsies showed high cytoplasmic expression.

In contrast to Stathmin-1, ACeCS2 was downregulated gradually from Premalignant to invasive colorectal carcinoma (p=0.006) Figure (2)

There was statistically significant correlation between ACeCS2 and IBD with dysplasia cases (p=0.002)**.** However**,** no significant correlation between ACeCS2, types of IBD, types and dysplasia of adenoma Table (1). ACeCS2 was lower expressed in cases with advanced stage (p=0.001) and lymph node metastasis (p=0.004) of colorectal carcinoma Table (2).

Correlation of Stathmin-1 and ACeCS2 expression among the studied cases

There was no statistically significant correlation between ACeCS2 expression and Stathmin1 expression among the studied cases.

Results of Morphometeric parameters among the studied groups

There was statistically significant direct correlation between the morphometric parameters (N. perimeter, N. length, N width, N. roundness, N. area and gland stromal ratio) among studied cases as they increased from premalignant to colorectal carcinoma (p value <0.001) Table (3), Figure (3).

DOI: 10.21608/bmfj.2024.312447.2167

Nuclear morphometric parameters were increased from non-dysplastic to dysplastic IBD and from adenoma with low grade to high grade dysplasia with significant direct correlation (p value <0.001).

There was statistically significant direct correlation between the morphometric parameters and degree of diffrentiation (p <0.001), lymph node metastasis (p=0.045) in colorectal carcinoma.

Table (2): STMN1 and ACeCS2 expression in carcinoma cases and correlation with clinicopathological data.

Carcinoma	Stathmin1				P	ACeCS2		P
	Negative (1)	**Weak positive (11)**	**Positive (19)**	**Strong positive (4)**		**Low N=22**	**High N=13**	
Site								
Right	0	6(33.3%)	9(50%)	2(11.1%)	0.549	9(50%)	9(50%)	0.105
Left	1 (5.9%)	5 (29.4%)	10 (58.8%)	2(11.8%)		13(76.5%)	4(32.5%)	
Grade								
I	0	3(60%)	2(40%)	0	0.495	2(40%)	3(60%)	
II	1(4.2%)	5(20.8%)	14(58.3%)	4(16.7%)		16(66.7%)	8(33.3%)	0.521
III	0	3(50%)	3(50%)	0		4(66.7%)	2(33.3%)	
Colectomy cases	**N=1**	**N =4**	**N =11**	**N=3**		**Low N=12**	**High N=7**	
Size (CM)								
6 <	1(11.1%)	0	6(66.7%)	2(22.2%)	0.146	4(44.4%)	5(55.6%)	0.109
≥6	0	4(40%)	5(50%)	1(10%)		8(80%)	2(20%)	
Stage								
I, II	1(20%)	3(60%)	1(20%)	0	0.015*	0	5(100%)	0.001*
III, IV	0	1(7.1%)	10(71.4%)	3(21.4%)		12(85.7%)	2(14.3%)	
T Stage								
T2	1(10%)	3(30%)	4(40%)	2(20%)	0.839	6(60%)	4(40%)	
T3	0	1(16.7%)	4(66.7%)	1(16.7%)		3(50%)	3(50%)	0.446
T4	0	0	3(100%)	0		3(100%)	0	
N stage								
N 0	1(16.7%)	3(50%)	1(16.7%)	1(16.7%)	0.04*	1(16.7%)	5 (83.3%)	0.004*
N 1, N 2	0	1(7.7%)	10(76.9%)	2(15.4%)		11(84.6%)	2(15.4%)	
M stage								
M 0	1(7.7%)	3(23.1%)	6(46.1%)	3(23.1%)	0.78	7(53.8%)	6(46.2%)	0.220
M 1	0	1(16.7%)	5(83.3%)	0		5(83.3%)	1(16.7%)	
Perineural invasion								
+VE	**0**	1(16.7%)	4(66.7%)	1(16.7%)	0.882	3(50%)	3(50%)	0.419
-VE	1(7.7%)	3(23.1%)	7(53.8%)	2(15.4%)		9(69.2%)	4 (30.8%)	
L.V invasion					0.572			
+VE	1(12.5%)	1(12.5%)	5(62.5%)	1(12.5%)		5(62.5%)	3(37.5%)	P=1.0
-VE	0	3(27.3%)	6(54.5%)	2(18.2%)		7(63.6%)	4(36.4%)	

Table (3): Comparison of morphometric parameters among different studied groups.

	Control **N=6**	**Inflammatory bowel disease N=29**	**Conventional adenoma N =19**	**Serrated polyp/adenoma N =9**	**Carcinoma N=35**	P
Nuclear area	129.56±28.05	148.43±27.02	204.07±5.09	175.01±26.21	242.09±10.44	P<0.001*
N. perimeter	18.08±2.15	19.69±2.24	25.27±1.69	22.84±2.89	30.45±1.66	P<0.001*
N. length	5.93±3.65	8.38±3.0	15.25±1.49	11.36±2.52	21.88±1.44	P<0.001*
N. width	4.98±0.75	5.78±0.86	5.23±8.39	3.50±6.70	13±12.07	P<0.001*
N. roundness %	0.506±0.34	0.528±0.046	0.742±0.051	0.569±0.051	0.870±0.04	P<0.001*
Gland stromal ratio	21.24±9.85	29.62±8.87	42.41±1.04	38.49±5.77	48.89±2.13	P<0.001*

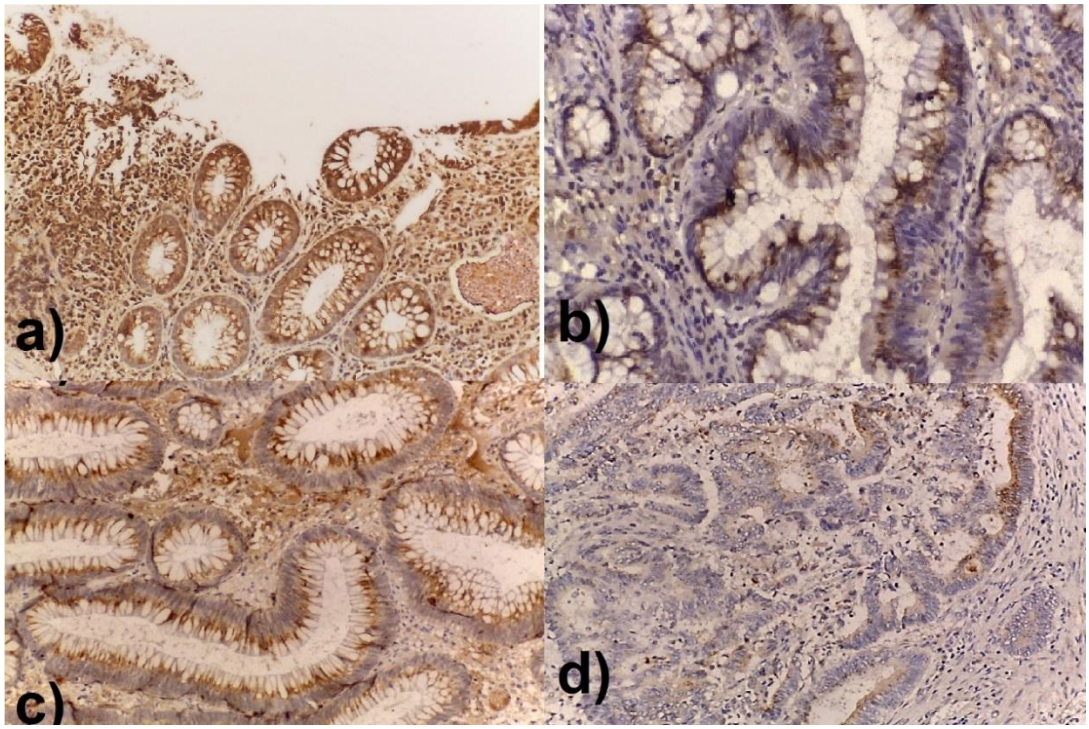

Figure (2): ACeCS2 expression; a) ulcerative colitis: ACeCS2 positive cytoplasmic expression in more than 70% of glandular epithelial cells with strong intensity (score 8/8) (ABC, X200), b) traditional serrated adenoma with dysplasia: ACeCS2 positive cytoplasmic expression in more than 70% of glandular epithelial cells with moderate intensity (score 7/8) (ABC, X200), c) tubulovillous adenoma with low grade dysplasia: ACeCS2 positive cytoplasmic expression in more than 70% of glandular epithelial cells with strong intensity (score 8/8) (ABC, X200), d) moderate differentiated carcinoma (GII): ACeCS2 positive cytoplasmic expression in less than 30 % of malignant epithelial cells with moderate intensity (score 4/8) (ABC, X200).

DOI: 10.21608/bmfj.2024.312447.2167

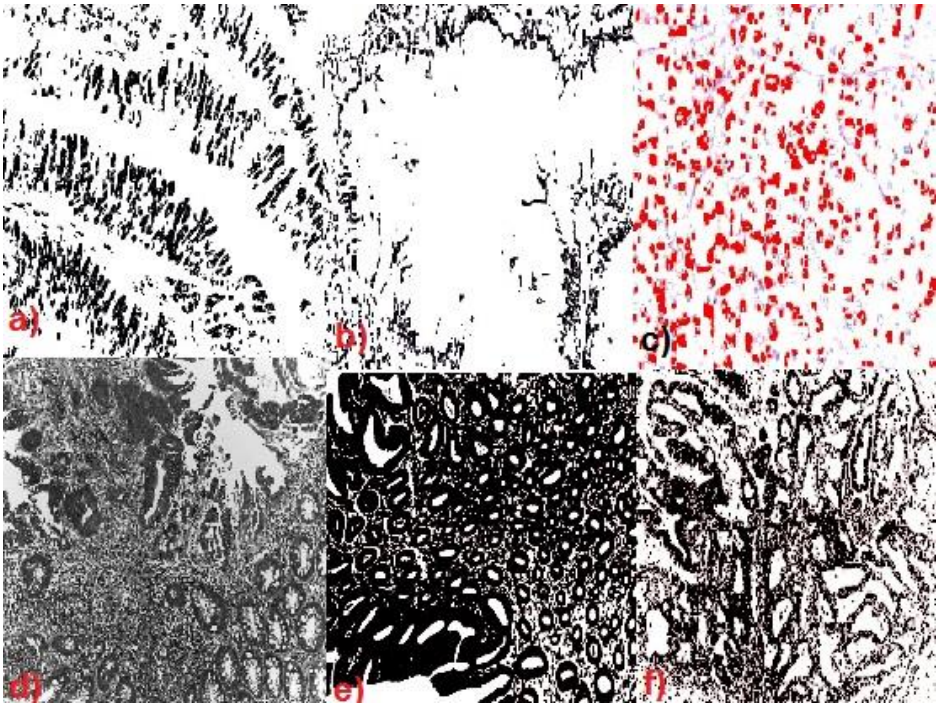

Figure (3): Morphometric analysis; a) Tubulovillous adenoma with high grade dysplasia, Mean N. area = 209.22±1.92, Mean N. perimeter = 27.18±0.26, Mean N. length = 16.96±0.13, Mean N. width = 8.98±0.07, Mean N. roundness = 0.800±0.002, b) Sessile serrated adenoma with low grade dysplasia, Mean N. area = 165.8±28.12, Mean N. perimeter = 21.56±2.72, Mean N. length = 10.50±2.74, Mean N. width=6.51±0.57, Mean N. roundness=0.55±0.05, c) Poor differentiated adenocarcinoma (grade III), Mean N. area =251.25±2.76, Mean N. perimeter = 33.90±0.27, Mean N. length = 24.03±0.12, Mean N. width = 13.95±0.26, Mean N. roundness=0.90±0.0, d) Inflammatory bowel disease with dysplasia: Gland stromal ratio = 41.74±2.89, e) Tubular adenoma with low grade dysplasia: Gland stromal ratio = 40.23±4.09, f) Well differentiated adenocarcinoma (grade I): Gland stromal ratio = 44.86±1.97.

Discussion:

Colorectal cancer is the third common cancer in the world, inflammatory bowel disease and adenoma are precancerous lesions that may progress to CRC [(7)]. New markers are needed to differentiate between them and detect cancer progression.

In the current study , there is increased expression of Stathmin-1 from non-neoplastic (IBD) to dysplastic (premalignant) lesions (serrated polyp/adenoma, conventional adenoma) to carcinoma however this correlation was statistically insignificant ($p>0.05$).These results were consistant with other studies which revealed that STMN1 was found to be highly expressed in colorectal cancer cases as compared to the adjacent normal colorectal tissues[(10,24,26)].

Also in another study, they did not observe STMN1 staining in normal fallopian tube epithelium and found strong STMN1 expression in TICs (tubal intraepithelial carcinoma) and invasive serous carcinomas which was similar to our results [(27)].

Stathmin1 is a key oncoprotein involved in cell proliferation by regulating cellular microtubule dynamics. STMN1 activity is important for both successful M-phase entry and M-phase exit [(26)].

In the present study there was statistically significant correlation only between

stathmin 1 expression and tumor stage (p=0.015) and node metastsis (p=0.04) of colorectal carcinoma with no correlation with other pathological parameters (p>0.05).

These results are in line with other studies which demonstrated that levels of stathmin-1 expression showed no correlation with primary tumor size, tumor grade, lymphovascular invasion while Stathmin-1expression has significant correlation with lymph node metastasis and with tumor stage [14,24]. Another study on gastric carcinoma was in line with our study [11].

Stathmin-1 play role in regulating cell division, motility, and migration, all of which are critical for tumorigenesis, it's depletion results in cell cycle arrest, induction of apoptosis and malignant cell death [28].

Regarding ACeCS2, it showed gradual decrease expression from normal, IBD with dysplasia, adenoma and carcinoma which is statistically significant (p= 0.006). Its expression was decreased with IBD with dysplasia rather than non-dysplastic ones (p =0.002*).

Similarly, on other study it was found that the normal mucosa exhibited strong cytoplasmic staining of ACeCS2, retained cytoplasmic expression in adjacent area with high-grade dysplasia whereas the carcinoma tissues exhibited the absence to low cytoplasmic staining (P= 0.001) [15].

In contrast, performed on non-small lung carcinoma which showed that the expression levels of ACSS2 were significantly higher in tissues than those in adjacent non-tumor tissues. This discrepancy may be due to diversity of tissue or cancer with different genetic alternation in lung [25].

Cells with an adequate oxygen and nutrient supply obtain energy from mitochondrial respiration via the conversion of pyruvate to acetyl-CoA [29]. However, cancer cells that exhibit a metabolic shift from mitochondrial respiration to aerobic glycolysis (Warburg effect) cannot efficiently obtain acetyl-CoA from pyruvate. Instead, cancer cells obtain acetyl-CoA from glutamine and short-chain fatty acids such as acetate, butyrate, and proprionate[30,31].

Acetyl-CoA synthetase-2 (AceCS2) is an emerging key enzyme for cancer metabolism, which supplies acetyl-CoA for tumor cells which is important in aerobic glycolysis (Warburg effect) [15].

It is highly expressed in the colon, adipose tissue, and skeletal muscle. In the intestinal lumen, microflora ferment dietary fibers and produce short-chain fatty acids. Thus, acetate can be used by acetyl-CoA synthetase-2 in normal colonocytes. However, acetyl-CoA synthetase-2 expression is markedly reduced in colorectal carcinomas, because cancerous colonocytes rely on glycolysis as their primary energy source [32].

The current study showed there was no statistically significant difference between ACeCS2 expression and dysplasia among the studied colorectal adenoma or types of colorectal adenoma (conventional or serrated) as all adenomas exhibited high expression.

These results were consistent with other study which illustrated that ACeCS2 expression in conventional adenomas, sessile serrated adenomas, and traditional serrated adenomas, exhibited moderate-to-strong cytoplasmic staining regardless the histologic type or grade of dysplasia [15].

There was statistically significant inverse correlation between ACeCS2 expression and lymph node metastasis (p=0.004) & staging of CRC (p=0.001). Although there is decreased ACeCS 2 expression in higher T stage and distant metastasis, the correlation was statistically insignificant (p=0.446 & p=0.263, respectively). No significant difference between ACeCS2 and other clinicopathological parameters (p>0.05)

The results agreed with other studies which revealed that ACeCS2 expression was independent to tumor size or tumor grade and was correlated to tumor stage,

DOI: 10.21608/bmfj.2024.312447.2167

advanced T category, an advanced N category, an advanced M category [15,33].

Similarly, another study on breast carcinoma in which reported that acetyl-CoA synthetase-2 expression was correlated with the stage [34].

In contrast, another study on gliomas reported that acetyl-CoA synthetase-2 expression was increased along with the WHO grade. These variabilities among studies may be due to different tissues with different molecular signature or different tissue specificity [35].

The reason why decreased acetyl-CoA synthetase-2 expression exhibited poor clinical outcome in colorectal carcinomas remains unknown. In vitro study results have suggested that alterations in acetate metabolism may differentially contribute to cancer cell survival [36].

In the current results, Stathmin 1 is more sensitive (70.8%) and more specific (62.9%) than ACeCS2 (65.7% sensitivity and 46.2% specificity) in detection of colorectal carcinoma.

On other studies showed that the sensitivity for stathmin 1 was 97.4% and the specificity was 91.4% [26], a sensitivity of stathmin1 for solid tumors of 73% (70–76%) and a specificity of 77% (73–81%) [37], a sensitivity of 75% and a specificity of 92% in esophageal cancer for statmin1[38].

Regarding morphometric analysis, there was statistically significant difference in N. area ($p<0.001$), N. Perimeter ($p<0.001$), N. Length ($p<0.001$), N. Width ($p<0.001$) and N. Roundness ($p<0.001$) between studied groups as each of the parameters increases gradually from normal colon to inflammatory bowel disease to Colorectal adenoma to colorectal Carcinoma.

In agreement with other studies who studied different nuclear morphometric variables in normal colon, adenoma, and adenocarcinoma cases. They found that all variables differed significantly [39-41].

In these results, there was statistically significant difference in mean gland stromal ratio between all studied groups ($p<0.001$). The mean gland stromal ratio increases from normal colon 21.24±9.8 to inflammatory bowel disease (29.62±8.87) to Colorectal adenoma (41.15±3.75) to colorectal Carcinoma (48.89±2.13).

This was in line with other study which compared gland stromal ratio in normal colon and carcinoma and found that there was increased the mean ratio in colorectal carcinoma compared to normal colon [42].

There was statistically significant difference in morphometric parameters between dysplasia and non-dysplastic cases either in IBD or adenoma ($p<0.001$ & $p<0.001$, respectively). This was in agreement with other studies which concluded that Mucosal morphometry may be of use in confirming high grade dysplasia [43,44]. Also, other study concluded that the nuclear to cytoplasmic ratio, variation in nuclear area, and variation in nuclear height above the basement membrane showed significant differences between mild, moderate, and severely dysplastic epithelia in adenoma [40].

According to the current study, there was statistically significant difference in the nuclear morphometric parameters and glandular stromal ratio in grading of colorectal carcinoma ($p<0.001$) as it increases from grade I to grade II to grade III among colorectal carcinoma. However, no correlations were found with other clinicopathological variables in our current study.

Other study agreed with our results and found that mean nuclear shape was significantly correlated with tumor grade [45].

This agreed with other studies which revealed that the nuclear shape factor means values, which is statistically non-significant with tumor stage. tumor size [40,46].

In contrast, other studies declared that the nuclear area (NA) of tumor cells was enlarged according to the increase in the depth of tumor invasion, positive for lymphatic invasion, venous invasion,

lymph node metastases and liver metastasis [40,47]. Different number of cases, low number of LN in resected specimen and pathological diagnostic variability according to staging of colorectal carcinoma might explain this discrepancy.

This was consistent with other study which revealed that there was significant relation between gland stromal ratio and tumor grade, he found that low tumor stromal ratio was associated with medium or low-grade malignancy ($p<0.001$) [48].

In the current study, there were no statistically significant differences between gland stromal ratio and clinicopathological data (depth of invasion, LN metastasis, distant metastasis, perineural invasion, LV invasion or staging) of colorectal carcinoma. Other study revealed that stroma-high CRC tended to have higher T and N stage but there was no difference in tumor differentiation or venous invasion between the stroma-high and stroma-low groups [49].

Conclusion

STMN1 and ACeCS2 might have a key role in pathogenesis, aggressiveness of colorectal carcinoma. Morphometric parameters have significant role in detection the progression from IBD, adenoma and colorectal carcinoma and detection of high-grade dysplasia among IBD and colorectal adenoma.

Conflict of interest

None of the contributors declared any conflict of interest

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To cite this article: Hadeer M. Elshahat, Ahlam A. Abd El-maksoud, Magdy M. Nouh, Omneya Y.Bassyoni. Significance of Stathmin1 and Acetyl-CoA Synthetase 2 Expression and Morphometric Analysis in Colorectal Carcinoma and Precancerous Lesions. BMFJ XXX, DOI: 10.21608/bmfj.2024.312447.2167.