RESEARCH ARTICLE

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The Role of *Beclin 1* and *HER2* in Colorectal Carcinoma; An Immunohistochemical Study

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Abstract

Objective: This study aimed to evaluate the expression of *Beclin 1* and *HER2* proteins using immunohistochemistry in CRC tissues compared to colonic adenoma, and to investigate the correlation of their expression with clinicopathological parameters and survival outcomes in CRC patients. **Methods:** The study utilized paraffin-embedded blocks from 17 colonic adenoma and 81 CRC cases. Immunohistochemical analysis was performed to assess the expression of *Beclin 1* and *HER2* proteins. **Results:** The cytoplasmic expression of *Beclin 1* was significantly higher in CRC tissues compared to adenoma specimens (P=0.051). High Beclin 1 expression was significantly associated with distal colon location (P=0.028). High *HER2* cytoplasmic expression was significantly associated with vascular invasion (P=0.05), perineural invasion (P=0.03), and shorter overall survival (P=0.035). **Conclusions:** The findings suggest that *Beclin 1* plays a role in colorectal carcinogenesis, with higher expression observed in CRC cases compared to adenoma cases. Furthermore, *HER2* carries poor prognostic impact in CRC cases.

Keywords: Autophagy- epidermal growth factor receptor- survival- colorectal carcinogenesis- target therapy

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Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed cancer and the third most common cause of cancer-related death in both men and women worldwide with 1931590 newly diagnosed cases in 2020 and mortality rate of 935173 [1]. In Egypt, cancer colon ranks the ninth among all tumors with 3430 newly diagnosed cases in 2020. Number of deaths due to cancer colon is 1910 with percent of 2.1 from all tumor's deaths in Egypt [1].

Despite advances in treatment options such as surgery, chemotherapy, and radiotherapy, the prognosis for CRC remains poor, particularly for patients with advanced disease as the five year survival rate reached 10-15% and about 40% of early staged cases experienced recurrence [2]. Consequently, there is a pressing need to develop innovative therapeutic strategies for CRC, including targeted receptor therapy and immune-oncology approaches, as well as the identification of molecular biomarkers for early detection [3].

Autophagy, a cellular process involved in maintaining cellular homeostasis, has been implicated in both tumor promotion and suppression, depending on the context [4]. Autophagy appears to be notably enhanced in colorectal cancer, and it has been linked to poor prognosis and drug resistance [5], however, autophagy studies in colorectal cancer show conflicting results, particularly in terms of chemotherapy resistance [4,6].

Beclin 1 is a critical regulator of autophagy and has been implicated in cancer development. However, its effects in cancer are still poorly understood. Studies have shown that *Beclin 1* deficiency promotes tumor growth in breast cancer by regulating WNT1 [7], while others have demonstrated its requirement for carcinogenesis in breast cancer progenitor cells [8]. Low expression of *Beclin 1* has been associated with a malignant phenotype and poor prognosis in gastric cancer [9]. These findings suggest that *Beclin 1* may have autophagy-independent functions, highlighting its potential distinct role in cancer.

The significance of *Beclin 1* in the development of colorectal cancer is still controversial. Although *Beclin 1* knockdown has been shown to decrease epithelial-mesenchymal transition and reduce CRC cell invasiveness. [10], ectopic expression of *Beclin 1* leads to growth retardation of the CRC cells [11]. So, the ability of *Beclin 1* in regulating cell cycle progression of colorectal cancer cells remains unknown.

HER2 pathway activation is an essential route of resistance for anti-epidermal growth factor receptor (EGFR) therapy, which is one of the most important treatments for a variety of cancers. The proto-oncogene *HER2* belongs to the EGFR family and is also known as *ERBB2* or HER2/neu. *HER2*-positive malignancies develop via a mechanism that is tightly linked to *HER2*

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gene amplification on chromosome 17q. *HER2* is a 185-kDa transmembrane receptor tyrosine kinase that promotes cell proliferation and opposes apoptosis by stimulating the RAS- and PI3K-AKT signaling pathways [12].

Gain-of-function mutations in *HER2* have the potential to cause uncontrolled cell proliferation and division, angiogenesis stimulation, and tumor development [13]. *HER2* is expressed in several tissues including epithelial cells and mammary tissue, and hence its presence is strongly implicated in breast and stomach cancers. Tratuzumab, a monoclonal antibody (MAB), has been demonstrated to have an overall excellent prognosis in patients with HER2-positive breast cancer. Additionally, *HER2* targeted therapy has been used more frequently for metastatic gastric cancer [14].

Although some studies suggest that HER2-positive CRC cases have a poor prognosis, some clinical trials targeting the *HER2* pathway have yielded promising results, with dual *HER2* blockade with MABs (trastuzumab with pertuzumab) or the combination of MABs with tyrosine kinase inhibitors (trastuzumab with lapatinib) inducing durable tumor response in approximately one-third of patients' refractory to standard systemic therapy [15].

Vega-Rubn-de-Celis et al. found that *Beclin 1* and autophagy are likely inhibited by HER2, which likely contributes to HER2-mediated tumorigenesis of breast cancer. They also found that strategies to block HER2/*Beclin 1* binding and/or increase autophagy may represent a new therapeutic approach for HER2-positive breast cancers [16].

Aim of the study

This study aimed to investigate the immunohistochemical expression of *Beclin 1* and *HER2* in colonic adenoma and CRC cases, and their correlation with clinicopathological parameters and survival outcomes. By elucidating the roles of *Beclin 1* and *HER2* in colorectal cancer, this research may contribute to the development of novel prognostic markers and targeted therapies for CRC patients.

Materials and Methods

This retrospective case control study was performed on 98 colonic surgical specimens obtained from Egyptian patients, including 81 cases of colorectal carcinoma (colectomy specimens) and 17 cases of colonic adenomas (colonoscopic biopsies). The specimens were collected between 2015 and 2019 from the Pathology Department at Menoufia University's Faculty of Medicine. The study was conducted in accordance with an approved Institutional Review Board (IRB) protocol. Data regarding clinical features and overall survival (OS) were extracted from patients' medical records. OS was calculated from the date of diagnosis to either the time of death or the last follow-up visit. Recurrence-free survival time was calculated from the date of surgery until the occurrence of a recurrence. Data regarding both of overall and recurrence free survival was available for only 54 cases.

**Histopathological evaluation*

Four 4-µm-thick hematoxylin and eosin (H&E) stained slides were reevaluated using the 2019 WHO classification of tumors of the digestive system [17]. The histopathological features assessed included TNM staging [18], stage grouping, lymph node ratio (LNR) [rN1: 0% < LNR ≤ 35%, rN2: 35% < LNR ≤ 69%, rN3: LNR > 69%] [19], histopathologic type [conventional adenocarcinoma, mucinous adenocarcinoma with mucinous component representing > 50%, and adenocarcinoma with mucinous differentiation ≤ 50% [17], tumor grade, lymphovascular invasion, perineural invasion [17], necrosis, mitotic count and tumor margin and it was available 79 cases [20].

*Construction of Tissue microarray (TMA) block: H&E stained slides from selected cases were carefully examined to identify viable and representative areas of each sample. A manual tissue arrayer's needle (Breecher Instrument, USA) was used to create tissue microarrays with a 2 mm punch size. Three cores from different areas of the tumor and stroma were sampled from each tumor specimen [21].

**Immunohistochemical staining*

Two sections were cut from each TMA block and immunostained using the streptavidin-biotin-amplified system. The primary antibodies used were polyclonal rabbit anti-human antibodies, including *Beclin 1* (concentrated with dilution of 1:100, Cat#ab114071) and *HER2* (concentrated with dilution of 1:100, Cat#ab214275), purchased from Abcam (Cambridge, UK).

**Evaluation of immunohistochemical results*

The expression of *Beclin 1* was either cytoplasmic or nucleo-cytoplasmic. The immunohistochemical cytoplasmic staining was evaluated based on both the percentage of stained cells and the immunostaining intensity [22].

Pattern of *HER2* expression: membranous/cytoplasmic/membranous + cytoplasmic was evaluated. The immunohistochemical cytoplasmic staining was evaluated based on both the percentage of stained cells and the immunostaining intensity [23].

Furthermore, H-score was used for both *Beclin 1* and *HER2* and it was calculated by adding (1 x mildly stained cells) + (2 x moderately stained cells) + (3 x strongly stained cells) [24]. cases were grouped using H-score into Low (≤median) and high (>median).

** Statistical analysis*

Data was collected, tabulated, and analyzed using IBM (statistical package for the social sciences (SPSS) software package version 20 (Armonk, NY: IBM Corp). Fisher's exact (FE) and chi-square (χ^2) tests were used for evaluation of qualitative data while Mann-Whitney (U) test was used for evaluation of quantitative data. Kaplan Meier curve and log rank test were constructed for survival analysis. Results with ($P \leq 0.05$) were considered as statistically significant [25].

Results

Clinicopathologic data of adenoma and colorectal carcinoma cases

The current retrospective study was carried out on 17 adenoma cases and 81 colorectal adenocarcinoma cases; the characteristics of both adenoma and carcinoma cases were presented in Table 1 and 2.

Immunohistochemical expression of Beclin 1 & HER2 in adenoma and CRC biopsies

In the adenoma cases, all samples exhibited cytoplasmic expression of *Beclin 1*, without any nuclear positivity. Among the colorectal carcinoma cases, cytoplasmic expression of *Beclin 1* was observed in 80.2% of cases, while nucleocytoplasmic expression was detected in only 22.2% of adenocarcinoma cases. Notably, a high H-score of *Beclin 1* was found in 58% of cases, primarily in conventional adenocarcinoma tumor types with grade 2 tumor differentiation (Plate 1).

Regarding *HER2* expression, the majority of adenoma cases (94.12%) demonstrated positive cytoplasmic expression, with 47.1% exhibiting a membranous + cytoplasmic staining pattern. In CRC cases, 96.3% displayed positive cytoplasmic expression of HER2, but only twenty-two cases (17%) showed a membranous +

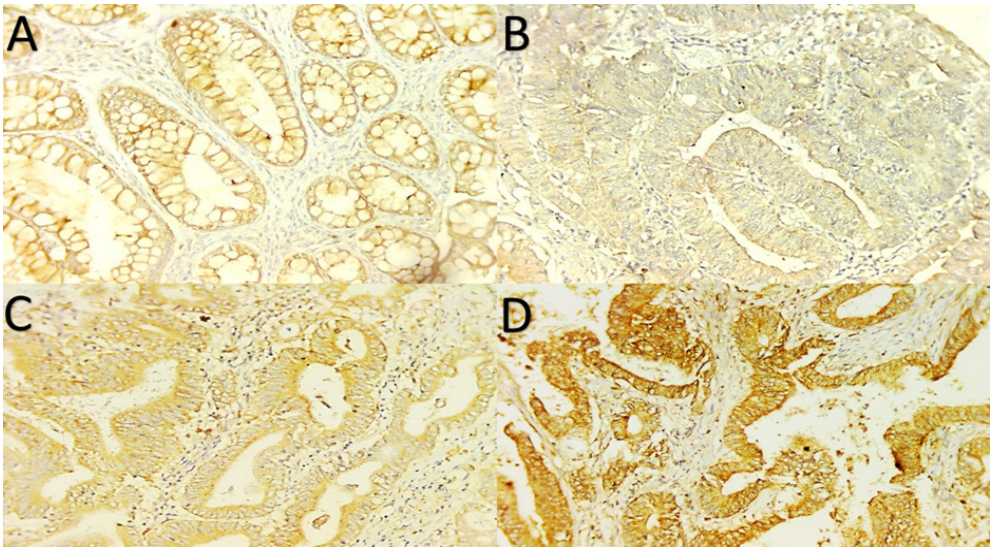

Plate 1. Immunohistochemical Expression of *Beclin 1* in Adenoma and Colorectal Carcinoma Shows Positivity in the Cytoplasme. Moderate expression of *Beclin 1* in colonic adenoma (A). Mild expression of *Beclin 1* in CRC (B). Moderate expression of *Beclin 1* in CRC (C). Strong expression of *Beclin 1* in CRC (D). (IHCx200)

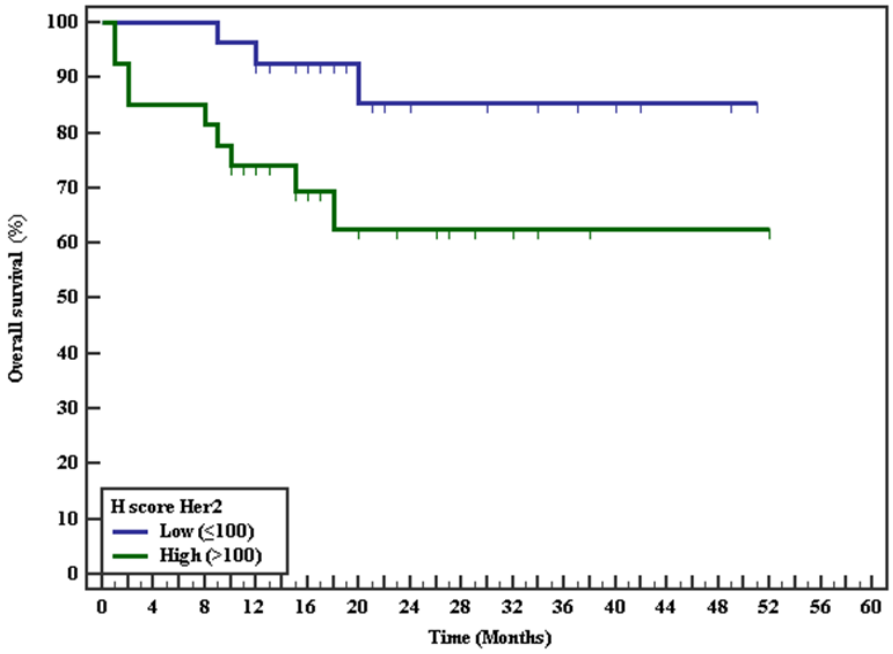

Figure 1. High-*HER2* CRC Cases (H-score ≥100) were Significantly Associated with Shorter Overall Survival than low-*HER2* CRC Cases (P = 0.035)

Table 1. Clinco-Pathological Data of Colorectal Carcinoma Group

		Cancer colon group (n=81)	
		N	%
Age (years)	Mean ± SD.	54.99±13.33	
	Median (Min. – Max.)	57 (25-85)	
Gender	Male	31	38.3
	Female	50	61.7
Neo Adjuvant Therapy	No	75	92.6
	Yes	6	7.4
Tumor Location	Rectal	21	25.9
	Distal Colon	29	35.8
	Proximal Colon	31	38.3
Tumor size	Mean ± SD.	5.81±3.59	
	Median (Min. – Max.)	5 (2-21)	
Gross Morphology	Infiltrative thickness	25	30.8
	Fungating Mass	42	51.9
	Ulcerative	14	17.3
Perforation	No	71	87.7
	Yes	10	12.3
Dukes Stage	A	3	3.7
	B	41	50.6
	C	37	45.7
T Stage	T1	2	2.5
	T2	16	19.7
	T3	49	60.5
	T4a	6	7.4
	T4b	8	9.9
N Stage	N0	44	54.4
	N1(a&b)	23	28.4
	N1c	4	4.9
	N2a	3	3.7
	N2b	7	8.6
M Stage	M0	48	59.3
	M1	6	7.4
	Mx	27	33.3
Lymph Node Ratio	rN1	70	86.4
	rN2	7	8.6
	rN3	4	4.9
Death (n=54)*	Yes	12	14.8
	No	42	51.9
Recurrence (n=54)*	Yes	10	12.3
	No	44	54.3
Histological Type	Adenocarcinoma with mucinous differentiation≤ 50%	14	17.3
	Conventional adenocarcinoma	57	70.4
	Mucinous adenocarcinoma with mucinous differentiation>50%	10	12.3
Tumor differentiation grade	Poorly differentiated	19	23.5
	Moderate differentiated	59	72.8
	Well differentiated	3	3.7
Vascular Invasion	Negative	54	66.7
	Positive	27	33.3
Perineural Invasion	Negative	67	82.7
	Positive	14	17.3
Necrosis	Absent	65	80.2
	Present	16	19.8
Mitotic Count	Mean ± SD.		7.72±4.04
	Median (Min. – Max.)		7 (1-17)
Tumor Margin (n=79)*	Infiltrating	54	68.4
	Pushing	25	31.6

SD, Standard deviation; n; Number of cases; *, The available number of cases

Table 2. Clinico-Pathologic Data of Adenoma Group

Clinico-pathologic data		Adenoma group (n=17)	
		N	%
Age (years)	Mean ± SD.	59.00±7.63	
	Median (Min. – Max.)	60 (36-68)	
Gender	Male	14	82.4
	Female	3	17.6
Adenoma histologic type	Tubular	3	17.6
	Tubulovillous	11	64.8
	villous	3	17.6
Adenoma dysplasia degree	High grade	7	41.2
	Low grade	10	58.8

SD, Standard deviation; n, Number of cases

cytoplasmic staining pattern. Among the cases with high H-scores for *HER2* cytoplasmic expression (57.7%), most were conventional adenocarcinoma tumor types (73.3%) with grade 2 tumors (75.6%) (Plate 2).

Comparison between adenoma and colorectal carcinoma cases regarding expression of Beclin 1 and HER2 expression

A higher median H score of *Beclin 1* was observed in CRC specimens compared to adenoma specimens (P-value = 0.051). On the other hand, no statistical difference was found in the expression of *HER2* between the two groups (Table 3).

The relationship between Beclin 1 and HER2 with the studied clinicopathologic parameters of CRC cases

A high *Beclin 1* score showed a significant association with colorectal cancer located in the distal colon, followed by the rectum (P-value = 0.028). Additionally, there was a near-significant association between high *Beclin 1* score and older age (P-value = 0.066), infiltrative tumor margin (P-value = 0.075), and advanced Dukes' stage (P-value = 0.097) (Table 4). Regarding *HER2* expression, three cases showed negative expression and the correlation was done on the positive cases only which showed that high H score was significantly associated with vascular invasion (P-value = 0.05) and perineural invasion (P-value = 0.03) (Table 5).

Table 3. Comparison between Adenoma and Carcinoma Groups According to *Beclin 1* and *HER2* Expression

	Cancer colon (n = 81)	Adenoma (n = 17)	Test of Sig.	p
Beclin 1				
H score				
Mean ± SD.	107.3 ± 55.79	106.5 ± 61.94	U=	0.55
Median (Min. – Max.)	100 (10 – 250)	90 (20 – 250)	625	
Low (≤100)	41 (50.6%)	13 (76.5%)	χ2=	0.051
High (>100)	40 (49.4%)	4 (23.5%)	3.796	
HER2				
H score				
Mean ± SD.	111.9 ± 64.75	100.6 ± 78.22	U=	0.34
Median (Min. – Max.)	100 (0 – 300)	80 (0 – 300)	587	
Low (≤100)	44 (54.3%)	12 (70.6%)	χ2=	0.218
High (>100)	37 (45.7%)	5 (29.4%)	1.518	

SD, Standard deviation; U, Mann Whitney test; χ^2, Chi square test; n, number; p, p value for comparing between the two studied groups

The impact of Beclin 1 and HER2 immuno-expression on overall and recurrence free survival of CRC patients

Higher *HER2* expression was found to be significantly associated with shorter overall survival in CRC patients (P = 0.035) (Figure 1). However, neither *Beclin 1* nor *HER2* showed a significant correlation with recurrence-free survival.

Correlation between immunohistochemical expressions of Beclin 1 and HER2 in CRC cases

Unfortunately, there was no significant association between *Beclin 1* and *HER2* expression in the studied CRC cases.

Discussion

This study aimed to investigate and analyze the expression of *Beclin 1* and *HER2* in colorectal cancer (CRC) in relation to various clinicopathological variables, including survival data. Our results revealed that the immunohistochemical expression of *Beclin 1* was mainly cytoplasmic. However, it is worth noting that several studies have demonstrated both nuclear and cytoplasmic localization of Beclin-1. In a cohort study of CRCs, nearly half of the cases exhibited a significant nuclear Beclin-1 staining pattern [26].

Cytoplasmic expression of *Beclin 1* was significantly higher in CRC cases compared to the adenoma group, which is consistent with previous studies [27]. Zhang et al. found that *Beclin 1* overexpression suppressed tumor growth of colon cancer cells in a xenograft model by inhibiting proliferation (assessed by Ki67 expression) and inducing apoptosis (assessed by TUNEL) [28].

Furthermore, *Beclin 1* did not show a significant

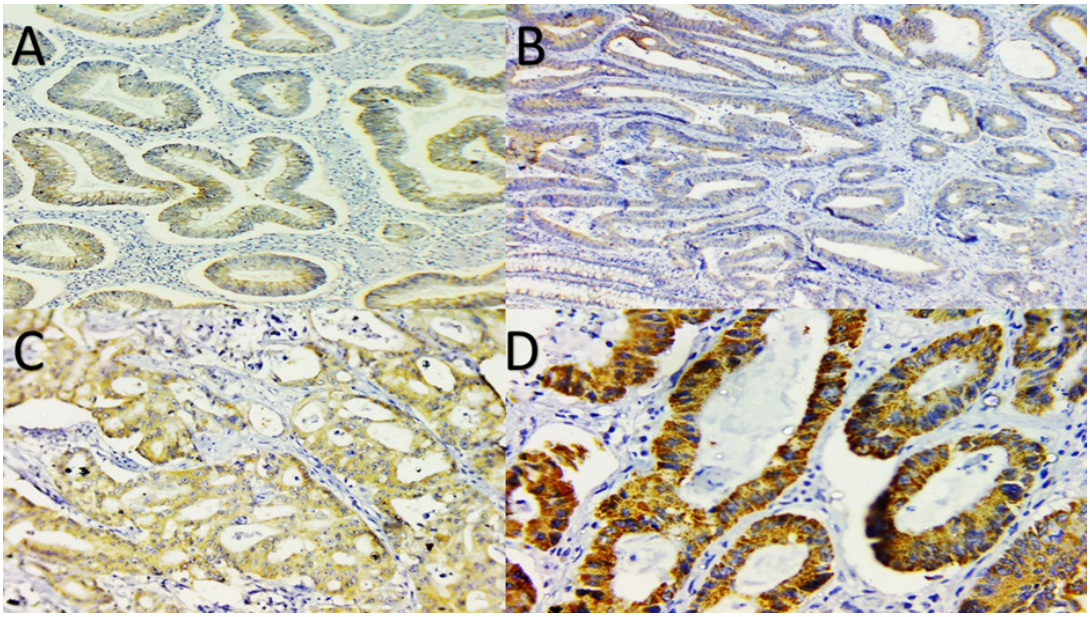

Plate 2. Immunohistochemical Expression of *HER2* in Adenoma and CRC Shows Positivity in the Cytoplasme. Mild expression of HER2 in colonic adenoma (A)(IHCx200). Mild expression of *HER2* in conventional CRC (B) (IHCx200). Moderate expression of HER2 in CRC (C)(IHCx200). Strong expression of *HER2* in CRC (D)(IHCx400).

Table 4. Relation between *Beclin 1* H Score and Clinicopathological Data in Cancer Colon Group. (% from raw)

Variables		*Beclin 1* H score in cancer colon group (n=81)		Test of Sig	p value
		Low (<100) (n =34) No (%)	High (≥100) (n = 47) No (%)		
Age (years)	Mean ± SD.	51.79 ± 13.25	57.30 ± 13.04	t=1.862	0.066
Gender	Male	10 (32.3%)	21 (67.7%)	χ^2=1.95	0.163
	Female	24 (41.3%)	26 (52.0%)		
Neo Adjuvant Therapy	No	31 (41.3%)	44 (58.7%)	FE=0.171	0.679
	Yes	3 (50.0%)	3 (50.0%)		
Tumor Location	Rectal	10 (34.5%)	19 (67.7%)	χ^2=7.127	0.028
	Distal Colon	10 (32.3%)	21 (89.7%)		
	Proximal Colon	14 (66.7%)	7 (33.3%)		
Tumor size	Mean ± SD.	5.40 ± 3.24	6.11 ± 3.82	U=0.895	0.371
	Median (Min. – Max.)	4.25 (2-16)	6 (2-21)		
Gross Morphology	Infiltrative thickness	12 (48.0%)	13 (52.0%)	χ^2=1.419	0.482
	Fungating Mass	18 (42.9%)	24 (57.1%)		
	Ulcerative	4 (28.6%)	10 (71.4%)		
Perforation	No	32 (45.1%)	39 (54.9%)	FE=2.62	0.133
	Yes	2 (20.0%)	8 (80.0%)		
Dukes Stage	A	3 (100.0%)	0 (0.0%)	FE=4.661	0.097
	B	15 (36.6%)	26 (63.4%)		
	C	16 (43.2%)	21 (56.8%)		
T Stage	T1+T2	6 (33.3%)	12 (66.7%)	χ^2=0.710	0.4
	T3+T4	28 (44.4%)	35 (55.6%)		
N Stage	N0	18 (40.9%)	26 (59.1%)	χ^2=0.045	0.832
		16 (43.2%)	21 (56.8%)		
M Stage	M0	21 (43.8%)	27 (56.2%)	FE=0.263	0.877
	M1	2 (33.3%)	4 (66.7%)		
	Mx	11 (40.7%)	16 (59.3%)		
Lymph Node Ratio	rN1	32 (45.7%)	38 (54.3%)	FE=3.079	0.215
	rN2	1 (14.3%)	6 (85.7%)		
	rN3	1 (25.0%)	3(75.0%)		
Histological Type	Adenocarcinoma with mucinous differentiation≤ 50%	6 (42.9%)	8 (57.1%)	FE=0.676	0.713
	Conventional adenocarcinoma	25 (43.9%)	32 (56.1%)		
	Mucinous adenocarcinoma with mucinous differentiation>50%	3 (30.0%)	7 (70.0%)		
Tumor differentiation grade	Poorly differentiated	7 (36.8%)	12 (63.2%)	FE=0.960	0.619
	Moderate differentiated	25 (42.4%)	34 (57.6%)		
	Well differentiated	2 (66.7%)	1 (33.3%)		
Vascular Invasion	Negative	22 (40.7%)	32 (59.3%)	χ^2=0.101	0.75
	Positive	12 (44.4%)	15 (55.6%)		
Perineural Invasion	Negative	27 (40.3%)	40 (59.7%)	χ^2=0.477	0.504
	Positive	7 (50.0%)	7 (50.0%)		
Necrosis	Absent	26 (40.0%)	39 (60%)	χ^2=0.527	0.468
	Present	8 (50.0%)	8 (50.0%)		
Mitotic Count	Mean ± SD.	7.50 ± 3.74	7.87 ± 4.28	U=0.197	0.844
	Median (Min. – Max.)	7 (1-17)	7 (2-17)		
Tumor Margin(n=79)	Infiltrating	25 (46.3%)	29 (53.7%)	FE=5.183	0.075
	Pushing	7 (28.0%)	18 (72.0)		

SD, Standard deviation; U, Mann Whitney test; χ^2, Chi square test; FE, Fisher Exact; n, number; p, p value for comparing between the two studied groups

correlation with most clinicopathological parameters, except for tumor location, where it was higher in the distal colon and rectum. The regional distribution of CRC is important due to genetic factors, as well as regional diet and traditions that may affect CRC risk. The lack of significant association between *Beclin 1* expression

Table 5. Relation between *HER2* H score and Clinical Data in Cancer Colon Group. (% from raw)

Clinical data		*HER2* H score in cancer colon group (n=78)		Test of Sig	p value
		Low (<100) (n =33) No (%)	High (≥100) (n = 45) No (%)		
Age (years)	Mean ± SD.	54.55 ± 13.12	55.27 ± 13.7	t=0.234	0.816
Gender	Male	13 (43.3%)	17 (56.7%)	χ^2=0.021	0.885
	Female	20 (41.7%)	28 (58.3%)		
Neo Adjuvant Therapy	No	31 (43.2%)	41 (56.9%)	FE=0.214	0.643
	Yes	2 (33.3%)	4 (66.7%)		
Tumor Location	Rectal	8 (38.1%)	13 (61.9%)	χ^2=1.705	0.426
	Distal Colon	10 (35.7%)	18 (64.3%)		
	Proximal Colon	15 (51.7%)	14 (48.3%)		
Tumor size	Mean ± SD.	6.85±3.88	5.14±3.27	U=2.600	0.009
	Median (Min. – Max.)	6 (2-21)	4.5 (2-20)		
Gross Morphology	Infiltrative thickness	7 (31.8%)	15 (68.2%)	χ^2=1.479	0.477
	Fungating Mass	19 (45.2%)	23 (54.8%)		
	Ulcerative	7 (50.0%)	7 (50.0%)		
Perforation	No	29 (42.6%)	39 (57.4%)	FE=0.025	0.874
	Yes	4 (40.0%)	6 (60.0%)		
Dukes Stage	A	1 (33.3%)	2 (66.7%)	FE=2.574	0.276
	B	20 (51.3%)	19 (48.7%)		
	C	12 (33.3%)	24 (66.7%)		
T Stage	T1+T2	6 (35.3%)	11 (64.7%)	χ^2=0.438	0.508
	T3+T4	27 (44.3%)	34 (55.7%)		
N Stage	N0	21 (50.0%)	21 (50.0%)	χ^2=2.206	0.137
	N1+N2	12 (33.3%)	24 (66.7%)		
M Stage	M0	19 (41.3%)	27 (58.7%)	FE=2.265	0.322
	M1	1 (16.7%)	5 (83.3%)		
	Mx	13 (50.0%)	13 (50.0%)		
Lymph Node Ratio	rN1	29 (43.3%)	38 (56.7%)	FE=0.518	0.772
	rN2	3 (42.9%)	4 (57.1%)		
	rN3	1 (25.0%)	3 (75.0%)		
Pathological data		*Her2* H score in cancer colon group (n=81)		Test of Sig	p value
		Low (<100) (n =33) No (%)	High (≥100) (n =45) No (%)		
Histological Type	Adenocarcinoma with mucinous differentiation≤ 50%	5 (41.7%)	7 (58.3%)	FE=0.280	0.87
	Conventional adenocarcinoma	23 (41.1%)	33 (58.9%)		
	Mucinous adenocarcinoma with mucinous differentiation>50%	5 (50.0%)	5 (50.0%)		
Tumor differentiation grade	Poorly differentiated	9 (50.0%)	9 (50.0%)	FE=0.625	0.732
	Moderate differentiated	23 (40.0%)	34 (59.6%)		
	Well differentiated	1 (33.3%)	2 (66.7%)		
Vascular Invasion	Negative	26 (50.0%)	26 (50.0%)	χ^2=3.782	0.052
	Positive	7 (26.9%)	19 (73.1%)		
Perineural Invasion	Negative	31 (47.7%)	34 (52.3%)	χ^2=4.633	0.031
	Positive	2 (15.4%)	11 (84.6%)		
Necrosis	Absent	27 (42.2%)	37 (57.8%)	χ^2=0.002	0.963
	Present	6 (42.9%)	8 (57.1%)		
Mitotic Count	Mean ± SD.	8.24±4.16	7.47±4.01	U=0.915	0.36
	Median (Min. – Max.)	7 (2-17)	7 (1-16)		
Tumor Margin(n=79)	Infiltrating	23 (43.1%)	31 (56.9%)	FE=0.117	0.943
	Pushing	10 (40.0%)	15 (60.0%)		

SD, Standard deviation; U, Mann Whitney test; χ^2, Chi square test; FE, Fisher Exact; n, number; p, p value for comparing between the two studied groups

and clinicopathologic parameters in the current study is consistent with other studies [27]. This could be explained by the findings of Schmitz et al., who failed to demonstrate the prognostic value of *Beclin 1* in the complete CRC cohort, particularly in the wild-type KRAS subgroup. However, in the mutated KRAS subgroup, increased nuclear Beclin-1 expression was significantly associated with decreased overall survival (OS). This suggests an alteration of the autophagy flux in the context of KRAS mutation [22]. It is also possible that nuclear *Beclin 1* has an autophagy-independent role in colorectal carcinogenesis, as it has been discovered that nuclear *Beclin 1* can influence RB protein expression, regulating cell cycle and colorectal cancer cell proliferation [29].

On the other hand, Koustas et al. found that CRC patients with low expression levels of *Beclin 1* protein had better overall survival than those with high expression levels [30]. In contrast, Wu et al. reported that high protein expression of *Beclin 1* was positively associated with prolonged survival, suggesting a potential tumor suppressor role [31]. These discrepancies among studies may be attributed to variations in sample sizes.

In this study, we also examined the expression pattern of *HER2* and found that out of the total 81 cases, 78 (96.3%) stained positive for HER2. All 78 positive cases showed a cytoplasmic staining pattern, while only 22 cases exhibited an additional membranous pattern. A similar finding was reported in a previous study, which observed cytoplasmic expression of *HER2* in the majority (93%) of CRC cases, with only one case showing both membranous and cytoplasmic expression [23].

The variability in *HER2* positivity in CRC and the diversity in expression patterns have been previously noted. The lack of a globally standardized protocol for reporting *HER2* expression in CRC, as well as differences between studies conducted in different geographical regions with varying confounding factors such as lifestyle and diet, contribute to the inconsistency in reported data [32].

The distinction between membranous and cytoplasmic expression of *HER2* is crucial in current monoclonal antibody (MAB) therapy for HER2-related breast cancer, as the cytoplasmic expression is not considered a clinically relevant target [3]. The specific cause of *HER2* cytoplasmic expression in CRC is unknown, but the overexpression of promoter-binding proteins, leading to increased *HER2* synthesis, suggests its presence [33]. Recent literature suggests that cytoplasmic *HER2* expression in colorectal cancer may be related to survival prognosis, which provides a reason for optimism [34].

Our results indicated that a high H-score of *HER2* expression was significantly correlated with poor prognostic factors such as vascular invasion (p=0.052), perineural invasion (p=0.031), and shorter overall survival (p=0.035). These findings are in agreement with Abdul Razzaq et al. and Hasan et al., who found that *HER2* overexpression was associated with more aggressive colorectal cancer [35, 36].

HER2 is a 185-kDa transmembrane receptor tyrosine kinase that promotes cell proliferation and inhibits apoptosis by activating the RAS- and PI3K-AKT signaling pathways. Gain-of-function mutations in *HER2* can lead to uncontrolled cell growth, angiogenesis stimulation, and tumor development [13]. However, contrary to our findings, Ezz El Din et al. observed no significant association between *HER2* expression and different clinicopathological parameters or overall survival [37].

Previous studies have demonstrated the presence of a *Beclin 1-HER2* complex on the cell surface of HER2-expressing breast cancer cells. However,in our study, we did not find a significant correlation between *Beclin 1* and *HER2* expression. This discrepancy could be attributed to the differences in tissue type and localization of both markers in our study.

In conclusion, *Beclin 1* plays a role in colorectal carcinogenesis, with higher expression observed in CRC compared to adenoma cases. *HER2* expression carries poor prognostic implications in CRC and can be used as a prognostic indicator and a potential target for therapy in CRC patients. However, further research is needed to better understand the complex interactions and roles of *Beclin 1* and *HER2* in colorectal cancer.

Author Contribution Statement

Marwa Salah Gadallah evaluated the slides and wrote the manuscript, Marwa Mohammed Dawood prepared the results and figures and Asmaa Gaber Abdou prepared the discussion, and all authors revised the paper.

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Ethical Approval

This paper was approved by ethical committee of faculty of medicine, Menofia university

Consent for publication

Not applicable

Ethics approval and consent to participate

All methods were carried out in accordance with relevant guidelines and regulations.

Informed consent was obtained from all subjects

Competing interests

No conflict of interest regarding this manuscript

Data Availability Statement

All data and results included in this paper are available with the corresponding author upon request

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