Original Article

Expression of Septin 2 and Her2/neu in Colorectal Cancer

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Abstract

Background: Colorectal cancer (CRC) is a common and lethal disease. Septin 2 belongs to the same class of GTPases as the *RAS* oncogenes influence the invasion and metastasis of many types of tumor cells. Furthermore, HER2/neu is involved in the tumor genesis and progression of various types of tumors. The role of both molecules is still questionable in CRC. **Aim:** The aim of the study is to examine the expression of septin 2 and Her2/neu in patients with CRC. **Materials and Methods:** The study was conducted on 2 groups; the first group consisted of 70 paraffin blocks for CRC patients and the second group was formed of 24 blocks from patients diagnosed as colorectal adenoma. For each adenoma and carcinoma case, a section was immunohistochemically stained using antihuman SEPT2 polyclonal antibody. For each carcinoma case, another section was immunostained using monoclonal anti-HER2/neu. The results were statistically analyzed and compared with the collected clinicopathologic data of the cases. **Results:** For the carcinoma patients, there was a significant association between SEPT2 staining intensity and histologic type ($P = 0.001$) and grade ($P < 0.001$), tumor T ($P = 0.001$) and N ($P = 0.011$) stages and the presence of lymphovascular invasion ($P < 0.001$) and a significant association between Her2/neu immunoreactivity scores (IRSs) and histologic grade ($P = 0.048$), tumor T ($P < 0.001$) and N ($P = 0.019$) stages and the presence of perineural ($P = 0.004$) and lymphovascular ($P = 0.003$) invasion. In colonic adenoma patients, there was a significant relation between septin 2 IRSs and the grade of dysplasia in the adenoma ($P < 0.001$) and significant relation with its expression in carcinoma group ($P < 0.001$). **Conclusion:** A potential prognostic role of septin 2 and Her2/neu for patients with CRC is suggested as expression of both markers was associated with many important prognostic clinicopathologic variables in patients of CRC.

Keywords: Adenoma, colorectal cancer, Her2/neu, Septin 2

Introduction

Septins are GTP-binding proteins that form filamentous structures, which function primarily in the spatial organization of membrane and cytoplasmic proteins and categorization of many cellular functions.[1] They belong to the same class of GTPases as the *RAS* oncogenes.[2] In human cells, septins encompass a family of 13 genes, which encode 13 types of septins (SEPT1-SEPT12, SEPT14) with multiple isoform variants.[3]

Mutations and abnormal expression of septins have been observed in many hematological malignancies as well as solid tumors; the most frequent mutations have been reported in cancers of the skin, large intestine, endometrium, and stomach.[4] Alterations in expression of septin have been observed in glioblastoma, cutaneous squamous cell carcinoma and melanoma, renal cell carcinoma, colorectal carcinoma, lung cancer, prostatic carcinoma, cancer breast, ovarian, and endometrial carcinoma. In most of these cancers, septins are overexpressed but occasional downregulation has been also reported.[5-7]

Carcinoma of the colon or rectum (colorectal cancer [CRC]) is a common and lethal disease. Approximately 148 thousand new cases are diagnosed each year in the United States, of which colon cancers are much more common than rectal cancers.[8]

CRC develops slowly and starts as polyp which with time acquires more mutations giving rise to carcinoma.[9] Hence, the presence of adenomas is considered a marker of CRC risk, especially with microscopic high-grade dysplasia.

In colon, SEPT9 is highly expressed in normal surface and glandular epithelia, and the expression is markedly reduced

Received: 14-04-2021 **Revised:** 15-05-2021
Accepted: 05-07-2021 **Published:** 22-11-2021

Access this article online

Quick Response Code:

Website: http://www.jmau.org/

DOI: 10.4103/jmau.jmau_38_21

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How to cite this article: El Hanbuli HM, Amer SI, Ibrahim HA. Expression of septin 2 and her2/neu in colorectal cancer. J Microsc Ultrastruct 2022;10:197-203.

in adenoma and completely diminished in CRC indicating its progressive decrease in tumorigenesis.[10] SEPT4 also showed abnormal expression when studied in CRC.[11]

SEPT2 may promote tumor angiogenesis and growth through cancer-associated fibroblasts.[12] Its overexpression results in cytokinesis failure, aneuploidy, centrosome amplification, and multipolar mitoses, which are all frequent events in cancer cells.[13]

HER2 protein (also known as HER2/neu, ErbB-2) is a 185-kDa transmembrane receptor tyrosine kinase that belongs to the four-member family of epidermal growth factor receptors.[14] Aberrant expression of HER2/neu leads to abnormal activation of multiple downstream signal transduction pathways, resulting in increased cellular proliferation and differentiation, decreased apoptosis, and enhanced tumor cell motility and angiogenesis.[15]

HER2/neu is involved in the tumor genesis and progression of various types of solid tumors, such as breast cancer, pulmonary adenocarcinoma, gastric cancer, and CRC.[16-18]

Several studies observed a questionable role for HER2/neu protein in CRC.[19,20] Whoever some studies suggested that HER2/neu is a potential therapeutic target and a biomarker in CRC.[21,22]

Few recent studies examined the expression of septin 2 in CRC and no one examined it in colonic adenoma with no one done on Egyptian population; also, to the best of our knowledge, no previous study has conducted to examine in a single study the immunohistochemical staining character of both septin 2 and Her2/neu in CRC, so it was the aim of this study.

Materials and Methods

Study sample and data collection

The study was conducted on 2 groups; the first group consisted of 70 paraffin blocks for patients diagnosed as colorectal carcinoma for which a variable colonic resection procedure was performed and the second group was formed of 24 blocks from patients diagnosed as colorectal adenoma. All cases were diagnosed in Cairo university hospital from 2016 to 2019. The relevant patients' data were retrieved from the files. The study attained approval by the ethical committee for the release of the archival medical records and the utilization of the patient samples for scientific research. Due to the retrospective nature of the study, no written informed consent was obtained.

Inclusion criteria

Samples with fulfilled data, resection specimens (for the first group).

Exclusion criteria

Insufficient data, colonoscopic biopsies (for the first group), necrosis, poorly fixed biopsies, and history of neoadjuvant therapy.

Histopathological evaluation

Re-evaluation of H and E stained sections was performed by 3 independent pathologists as follows:

1. The tumors histopathological features were assessed according to the WHO 2019 classification for the tumors of the gastrointestinal tract[23]
2. Categorization of the tumors was done: the adenomas were classified according to the histological pattern and to the grade of dysplasia. The carcinomas were classified according to the tumor subtype, with emphasis on the presence of perineural invasion and lymphovascular tumor emboli. For each carcinoma case, a peritumoral budding score was performed according to the recommendations adopted by the International tumor budding consensus conference 2016 (ITBCC): tumor budding was defined by the presence of a single tumor cell or a tumor cell cluster formed of up to 4 tumor cells, detected by H and E stain at the advancing border of the tumor. Counting of the tumor budding was done at a single tumor hot spot, using ×20 objective lens. The eyepiece diameter of the used microscope was 22 mm, so dividing the number of the tumor buds on a correction factor (1.210) from a conversion table was done. This conversion table was proposed by the ITBCC to get the number of tumor buds in the equivalent of a 0.785-mm^2 field. Tumor budding score was estimated using a 3-tiered system based on the number of tumor buds in a 0.785-mm^2 field (low, 0–5 tumor buds; intermediate, 6–9 tumor buds; high, 10, or more tumor buds).[24]

Finally, the tumor was staged according to the American Joint Committee on Cancer 8th edition.[25]

Immunohistochemical procedures

For each adenoma and carcinoma case, a section of formalin-fixed, paraffin-embedded tissue was immunohistochemically stained using antihuman SEPT2 polyclonal antibody (clone Q15019, Rabbit Ig G) prediluted at 1:50, manufactured by Abbexa LLC. Houston, TX, USA. For each carcinoma case, another section was immunostained using monoclonal anti-HER2/neu (4B5) rabbit monoclonal antibody (Roch, USA). The staining was done by Ventana Benchmark automated stainer, following the manufacturer protocol, and the reaction was carried out using the avidin-biotin immunoperoxidase system.

Immunohistochemical evaluation

1. Septin immunoreactivity: positive septin reaction was identified by brownish cytoplasmic staining within the tumor cells. Septin immunoreactivity was assessed according to the immunoreactivity score intensity (IRS), which was calculated by multiplication of the score and percentage of staining. Tissue staining intensity was graded as follows: (0; no staining), (1; weak staining), (2; moderate staining), and (3; strong staining). The percentage of the tissue staining was graded as follows: (0; no positive cell staining), (1; <25% positive cell staining), (2; 25%–50% positive cell staining), (3; 50%–75% positive cell staining), and

(4; >75% positive cell staining). IRS values were stratified as negative (−, 0 scores), weakly positive (+, 1–4 score), moderately positive (++, 5–8 score), or strongly positive (+++, 9–12 score).[26] For adenoma cases and when comparing CRC to adenoma, the staining was considered as positive (+, ++ and +++) or negative

2. HER2-neu immunoreactivity: HER2-neu interpretation was performed guided by the HERACLES diagnostic criteria; the HER2 status of immunohistochemistry (IHC) staining was defined as follows: positive, intense (3+) in >10% of the tumor cells; equivocal, moderate (2+) in ≥50% of the tumor cells; and negative, intense (3+) ≤10% of the tumor cells, moderate (2+) in <50%, faint (1+) in any cellularity, or no staining.[27] The following considerations were taken into accounts: Complete membranous staining was not essential for positivity scoring and only luminal surface staining in the absence of lateral and basal staining was considered negative.

HER2/neu IHC scores of 2+ (equivocal) were further evaluated using fluorescence *in situ* hybridization (FISH) technique for detection of amplification of CEP17.

The technique for (fluorescence *in situ* hybridization) procedure

Paraffin-embedded tissue sections were mounted on Superfrost/plus microscope slides, deparaffinized through Xylene immersion, then dehydrated through immersion in graded ethanol, and then preheated with a pretreatment solution for 15 min then allowed for enzymatic digestion for 10 min. Serial washes followed by dehydration were applied, then hybridized with HER2-neu probe mixture (Vysis, FDA approved PathVysion probe kit) at 37°C at hybridization chamber overnight. After hybridization, a series of washes were performed to remove unbound probes, followed by serial ethanol solutions, 10 µl of DAPI were applied. Slides were then incubated in the dark for 15 min before visualization.

Fluorescence microscope evaluation and interpretation of fluorescence *in situ* hybridization

The slides were visualized using a Zeiss Axioscope fluorescent microscope using orange, green, DAPI, and dual orange and green filters. Zeiss imaging software system was used. We evaluated Her-2 gene copy number through counting orange signals and CEP17 copy number through counting green signals. The scoring was performed in at least 20 nonoverlapping nuclei. A ratio of HER2 signal to CEP17 signal of ≥2 was considered as amplification of HER2 and hence HER2 positive.[28]

Statistical analysis

Microsoft Excel 2016 was used for data entry, and the statistical package for the social science (SPSS) version 24 (SPSS, Armonk, New York, USA: International Business Machines Corporation) was used for data analysis. Simple descriptive statistics (arithmetic mean and standard deviation) used for the summary of quantitative data and frequencies used for qualitative data. Bivariate relationship was displayed in cross tabulations, and comparison of proportions was performed using the Chi-square test or Fisher exact whenever appropriate. T-independent, one-way analysis of variance, and *post hoc* tests were used to compare normally distributed quantitative data. The level of significance was set at probability $P < 0.05$.

Results

Relevant CRC patients' data and tumor characteristics are shown in Table 1, and representative figures of septin 2 [Figure 1] and Her2/neu [Figure 2] IHC are also shown. For Her2/neu IHC

Table 1: Data and tumor characteristics in 70 colorectal cancer patients

Tumor characteristics	*n* (%)
Age (meam±SD)	57.9±13.8
Gender	
Male	35 (50)
Female	35 (50)
Tumor location	
Colon	58 (82.9)
Rectum	12 (17.1)
Histologic type	
Adenocarcinoma	60 (85.7)
Mucinous carcinoma	10 (14.3)
Histologic grade	
Grade II	51 (72.9)
Grade III	19 (27.1)
T stage	
T2	9 (12.9)
T3	49 (70)
T4	12 (17.1)
N stage	
N0	33 (47.1)
N1	19 (27.1)
N2	18 (25.7)
LVI	
Present	33 (47.1)
Absent	37 (52.9)
PNI	
Present	15 (21.4)
Absent	55 (78.6)
Budding score	
Low	35 (50)
Intermediate	24 (34.3)
High	11 (15.7)
Septin score	
0 (negative)	5 (7.1)
+ (weakly positive)	16 (22.9)
++ (moderately positive)	26 (37.1)
+++ (strongly positive)	23 (32.9)
Her2/neu	
Positive	24 (34.3)
Negative	46 (65.7)

SD: Standard deviation, LVI: Lymphovascular invasion, PNI: Perineural invasion

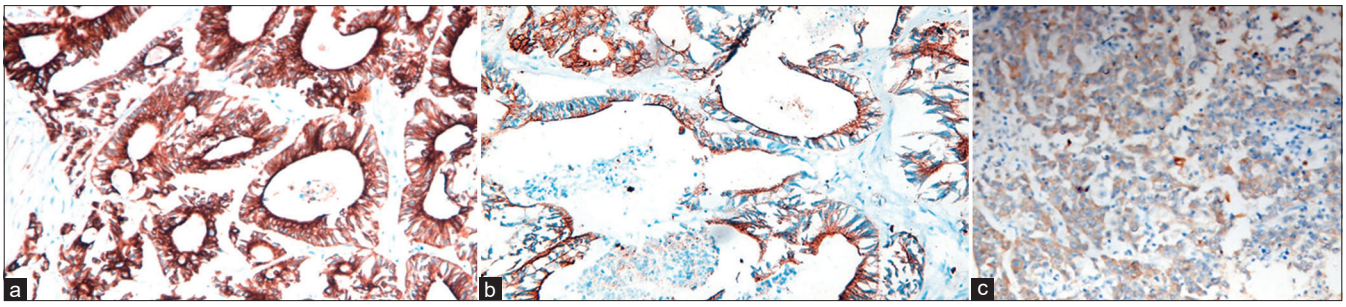

Figure 1: Septin 2 expression in colorectal cancer (×40): strongly positive (a), moderately positive (b), and weakly positive (c)

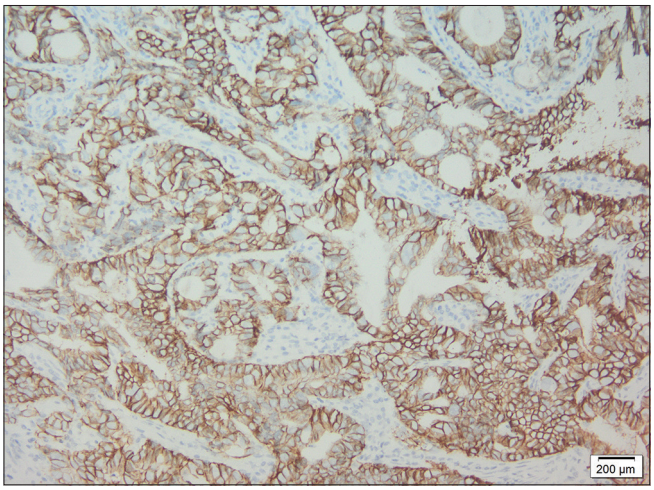

Figure 2: Her2/neu positive expression in colorectal cancer (×40)

9 cases showed equivocal, moderate (2+) immunoreactivity so were further evaluated using (FISH) technique for detection of amplification of CEP17 (as mentioned in the methodology section) that revealed 5 positive and 4 negative cases included in the total cases shown in Table 1.

Association between SEPT2 staining intensity and clinicopathological data of CRC patients used in this study revealed a significant association with histologic type ($P = 0.001$) and grade ($P < 0.001$), tumor T ($P = 0.001$) and N ($P = 0.011$) categories, and the presence of lymphovascular invasion ($P < 0.001$) [Table 2].

Association of Her2/neu IRSs and clinicopathological data of CRC patients used in this study revealed a significant association with histologic grade ($P = 0.048$), tumor T ($P < 0.001$) and N ($P = 0.019$) categories, and the presence of perineural ($P = 0.004$) and lymphovascular ($P = 0.003$) invasion [Table 3].

For the second group of patients diagnosed with colonic adenoma [Figure 3], there was a significant relation between septin 2 IRSs and the grade of dysplasia in the adenoma ($P < 0.001$) [Table 4].

For statistical reasons (due to small group of colonic adenoma patients), the study considered cases as positive or negative when detecting the relation between the cancer group and the adenoma group regarding septin 2 expression [Table 5] and the relation was statistically significant ($P < 0.001$).

Table 2: Association of septins 2 immunoreactivity scores and clinicopathological parameters of 70 colorectal cancer patients

Variable	Septin score				
	0	+	++	+++	*P*
Gender					
Male	5	5	12	13	0.05
Female	0	11	14	10	
Tumor location					
Colon	5	14	20	19	0.589
Rectum	0	2	6	4	
Histologic type					
Adenocarcinoma	5	16	25	14	0.001*
Mucinous carcinoma	0	0	1	9	
Histologic grade					
Grade II	4	16	24	7	<0.001*
Grade III	1	0	2	16	
T category					
T2	2	5	1	1	0.001*
T3	3	11	22	13	
T4	0	0	3	9	
N category					
N0	3	13	12	5	0.011*
N1	1	3	8	7	
N2	1	0	6	11	
LVI					
Present	2	2	9	20	<0.001*
Absent	3	14	17	3	
PNI					
Present	0	0	8	7	0.042
Absent	5	16	18	16	
Budding score					
Low	3	12	10	10	0.313
Intermediate	1	3	10	10	
High	1	1	6	3	

*The level of significance was set at probability (*P*) value <0.05. LVI: Lymphovascular invasion, PNI: Perineural invasion

Discussion

The exact pathogenesis of CRC is unknown, but our recent

Table 3: Association of Her 2/neu immunoreactivity scores and clinicopathological parameters of 70 colorectal cancer patients

Variable	Her2/neu score		
	Positive	Negative	*P*
Gender			
Male	12	23	1
Female	12	23	
Tumor location			
Colon	19	39	0.554
Rectum	5	7	
Histologic type			
Adenocarcinoma	21	39	0.76
Mucinous carcinoma	3	7	
Histologic grade			
Grade II	14	37	0.048*
Grade III	10	9	
T category			
T2	1	8	<0.001*
T3	13	36	
T4	10	2	
N category			
N0	6	27	0.019*
N1	8	11	
N2	10	8	
LVI			
Present	17	16	0.004*
Absent	7	30	
PNI			
Present	10	5	0.003*
Absent	14	41	
Budding score			
Low	10	25	0.284
Intermediate	8	16	
High	6	5	

*The level of significance was set at probability (*P*) value <0.05. LVI: Lymphovascular invasion, PNI: Perineural invasion

Table 4: Association of septin 2 immunoreactivity scores and clinicopathological parameters of 24 patients diagnosed with colonic adenoma

Variable	Septin score		Total, *n* (%)	*P*
	Positive (+, ++, +++)	Negative (0)		
Gender				
Male	11	8	19 (79.2)	0.317
Female	1	4	5 (20.8)	
Site				
Colon	11	10	21 (87.5)	1
Rectum	1	2	3 (12.5)	
Type				
Tubular	2	4	6 (25)	0.64
Tubulovellous	10	8	18 (75)	
Grade of dysplasia				
Low grade	0	10	10 (41.7)	<0.001*
High grade	12	2	14 (58.3)	

*The level of significance was set at probability (*P*) value <0.05.

knowledge suggested that the development of CRC needs a complex interaction between environmental carcinogens, genetic alterations, and the host immune system.[29]

Since the early 2000s, when mammalian septins began to emerge as a new field of research, there have been major advances in our knowledge of the cellular functions of septins. In parallel, clear evidence has indicated that septin levels of expression are altered in a variety of cancers. A cause-and-effect relationship between these alterations and tumorigenesis is yet to be confirmed.[4]

It has been reported that SEPT2 influences the invasion and metastasis of many types of tumor cells.[30-32] In addition, it has been proved that overexpression of SEPT2 results in cytokinesis failure, aneuploidy, centrosome amplification and multipolar mitoses, which are all frequent in cancer cells.[13]

Although many reports have focused on the function of septin 2 in tumors, its role in CRC remains unclear. Only one study was carried to examine the immunohistochemical expression of septin 2 in CRC by He *et al*.[26] that found that higher SEPT2 staining was more frequent in CRC samples with lymph node metastasis compared with samples with no metastasis ($P < 0.05$). In addition, the staining intensity of SEPT2 was associated with the differentiation degree of tumor tissue ($P < 0.001$) and also associated with TNM staging ($P < 0.05$) and concluded that SEPT2 may be a potential prognostic marker and therapeutic target for patients with CRC. This study had nearly the same relations with different studied variables in patients with CRC.

The proto-oncogene HER-2/neu is a member of the growth factor receptor family with intrinsic protein tyrosine kinase activity[33] that plays a vital role in normal cell proliferation and tissue growth, as well as in the development of carcinoma through influencing cell migration, proliferation and differentiation, and apoptosis.[34] It has been shown to be an effective target for adjuvant therapy for especially breast cancer.[35]

The prognostic role ofHER2/neu in CRC remains controversial. A negative prognostic impact of HER2/neu overexpression was proposed by some studies,[36-38] but other trials have found no association between HER2/neu amplification and outcome.[39-43] Despite this controversy, Her2/neu has been investigated as a therapeutic target in metastatic CRC in several small studies during the last decade, but with differing outcomes.[44,45]

The significant association found in this study between most of the studied prognostic clinicopathologic features and Her2/neu expression is additional to the reports that suggested a role of Her2/neu in CRC.

It was clearly outlined from this study the significant differences in septin 2 protein expression between colorectal

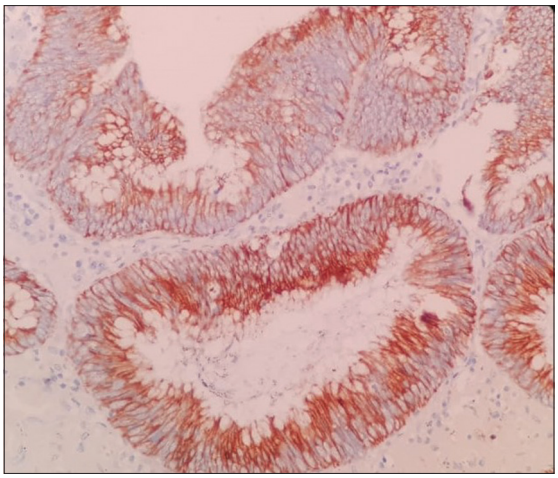

Figure 3: Septin 2 expression in colorectal in adenoma with high grade dysplasia (×40)

Table 5: Relation between colorectal cancer and colonic adenoma regarding septin 2 expression

Septin 2 expression	Colorectal carcinoma	Colonic adenoma	Total, *n* (%)	*P*
Positive (+, ++, +++)	65	12	77 (81.9)	<0.001*
Negative (0)	5	12	17 (18.1)	

*The level of significance was set at probability (*P*) value <0.05.

adenomatous polyps and CRC ($P < 0.001$) [Table 5]. It was clear that expression of such protein was present in benign and malignant cases as 50% and 92.9%, respectively, and in adenomatous polyps, the expression was limited to the cases with high-grade dysplasia. However, the present study did not investigate what pathways are altered by septin 2 expression in CRC; this finding refers to a possible role of this protein in early neoplastic transformation of such tumor especially in progression from adenoma to carcinoma with acquisition of further mutation as a part of multistep theory for malignant transformation.

Conclusion

Septin 2 and Her2/neu expressions were associated with many important prognostic clinicopathologic variables in patients of CRC, and septin 2 was also expressed in colonic adenoma with only high-grade dysplasia referring to a possible role in tumor progression that needs further exploration in subsequent researches. This may point to a potential prognostic role and therapeutic target of both markers for patients with CRC.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Spiliotis ET, Gladfelter AS. Spatial guidance of cell asymmetry: Septin GTPases show the way. Traffic 2012;13:195-203.
2. Leipe DD, Wolf YI, Koonin EV, Aravind L. Classification and evolution of P-loop GTPases and related ATPases. J Mol Biol 2002;317:41-72.
3. Russell SE, Hall PA. Septin genomics: A road less travelled. Biol Chem 2011;392:763-7.
4. Angelis D, Spiliotis ET. Septin mutations in human cancers. Cell Dev Biol 2016;4:122.
5. Hall PA, Russell SE. The pathobiology of the septin gene family. J Pathol 2004;204:489-505.
6. Dolat L, Hu Q, Spiliotis ET. Septin functions in organ system physiology and pathology. Biol Chem 2014;395:123-41.
7. Montagna C, Bejerano-Sagie M, Zechmeister JR. Mammalian septins in health and disease. Res Rep Biochem 2015;5:59-73.
8. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin 2020;70:7.
9. Pino MS, Chung DC. The chromosomal instability pathway in colon cancer. Gastroenterology 2010;138:2059-72.
10. Toth K, Galamb O, Spisak S, Wichmann B, Sipos F, Valcz G, *et al*. The influence of methylated septin 9 gene on RNA and protein level in colorectal cancer. Pathol Oncol Res 2011;17:503-9.
11. Zieger B, Tran H, Hainmann I, Wunderle D, Zgaga-Griesz A, Blaser S, *et al*. Characterization and expression analysis of two human septin genes, PNUTL1 and PNUTL2. Gene 2000;261:197-203.
12. Calvo F, Ranftl R, Hooper S, Farrugia AJ, Moeendarbary E, Bruckbauer A, Batista F, *et al*. Cdc42EP3/BORG2 and septin network enables mechanotransduction and the emergence of cancerassociated fibroblasts. Cell Rep 2015;13:2699714.
13. Poüs C, Klipfel L and Baillet A. Cancerrelated functions and subcellular localizations of septins. Front Cell Dev Biol 2016;4:126.
14. Pappas A, Lagoudianakis E, Seretis C, Tsiambas E, Koronakis N, Toutouzas K, *et al*. Clinical role of HER2/neu expression in colorectal cancer. BUON 2013;18:98104.
15. Lutgens MW, van Oijen MG, van der Heijden GJ, Vleggaar FP, Siersema PD, Oldenburg B. Declining risk of colorectal cancer in inflammatory bowel disease: An updated meta-analysis of population-based cohort studies. Inflamm Bowel Dis 2013;19:789-99.
16. Chao WR, Lee MY, Lin WL, Chen CK, Lin JC, Koo CL, *et al*. HER2 amplification and overexpression are significantly correlated in mucinous epithelial ovarian cancer. Hum Pathol 2014;45:8106.
17. Zhang DY, Zhang YH, Sun HY, Lau CP, Li GR. Epidermal growth factor receptor tyrosine kinase regulates the human inward rectifier potassium K (IR) 2.3 channel, stably expressed in HEK 293 cells. Br J Pharmacol 2011;164:146978.
18. Shitara K, Yatabe Y, Matsuo K, Sugano M, Kondo C, Takahari D, *et al*. Prognosis of patients with advanced gastric cancer by HER2 status and trastuzumab treatment. Gastric Cancer 2013;16:2617.
19. Schell MJ, Yang M, Teer JK, Lo FY, Madan A, Coppola D, *et al*. Multigene mutation classification of 468 colorectal cancers reveals a prognostic role for APC. Nat Commun 2016. 15;7:11743.
20. Hankey W, Frankel WL, Groden J. Functions of the APC tumor suppressor protein dependent and independent of canonical WNT signaling: Implications for therapeutic targeting. Cancer Metastasis Rev 2018;37:159-72.
21. Lu Y, Jingyan G, Baorong S, Peng J, Xu Y, Cai S. Expression of EGFR, Her2 predict lymph node metastasis (LNM) associated metastasis in colorectal cancer. Cancer Biomark 2012;11:21926.
22. Heppner BI, Behrens HM, Balschun K, Haag J, Kruger S, Becker T, *et al*. HER2/neu testing in primary colorectal carcinoma. Br J Cancer 2014;111:197784.
23. Nagtegaal ID, Odze RD, Klimstra D, Rugge M, Schirmacher P, Washington KM, *et al*. The 2019 WHO classification of tumors of the digestive system. Histopathology 2020;76:182-8.
24. Lugli A, Kirsch R, Ajioka Y, Bosman F, Cathomas G, Dawson H, *et al*. Recommendations for reporting tumor budding in colorectal cancer based on the international tumor budding consensus conference (ITBCC) 2016. Mod Pathol 2017;30:1299-311.
25. Amin MB, Edge S, Greene F, Byrd DR, Brookland RK, *et al*., editors. Colon and Rectum. American Joint Committee on Cancer. AJCC Cancer Staging Manual. 8th ed. New York, NY: Springer; 2017.
26. He H, Li J, Xu M, Kan Z, Gao Y, Yuan C. Expression of septin 2 and

association with clinicopathological parameters in colorectal cancer. Oncol Lett 2019;18:2376-83.
27. Valtorta E, Martino C, Sartore-Bianchi A, Penaullt-Llorca F, Viale G, Risio M, *et al*. Assessment of a HER2 scoring system for colorectal cancer: Results from a validation study. Mod Pathol 2015;28:1481-91.
28. Bartley AN, Washington MK, Colasacco C, Ventura CB, Ismaila N, Benson AB 3rd, *et al.* HER2 testing and clinical decision making in gastroesophageal adenocarcinoma: Guideline from the College of American Pathologists, American Society for Clinical Pathology, and the American Society of Clinical Oncology. J Clin Oncol 2017;35:446.
29. Fiebelkorn IC, Foxe JJ, Butler JS, Mercier MR, Snyder AC, Molholm S. Ready, set, reset: Stimuluslocked periodicity in behavioral performance demonstrates the consequences of crosssensory phase reset. Neurosci 2011;31:997181.
30. Yu J, Zhang W, Tang H, Qian H, Yang J, Zhu Z, *et al.* Septin 2 accelerates the progression of biliary tract cancer and is negatively regulated by mir1405p. Gene 2016;589:206.
31. Cao LQ, Shao ZL, Liang HH, Zhang DW, Yang XW, Jiang XF, *et al*. Activation of peroxisome proliferatoractivated receptorγ (PPARγ) inhibits hepatoma cell growth via downregulation of SEPT2 expression. Cancer Lett 2015;359:12735.
32. Zhang N, Liu L, Fan N, Zhang Q, Wang W, Zheng M, *et al.* The requirement of SEPT2 and SEPT7 for migration and invasion in human breast cancer via MEK/ERK activation. Oncotarget 2016;7:61587600.
33. Yan SY, Hu Y, Fan JG, Tao GQ, Lu YM, Cai X, *et al*. Clinicopathologic significance of HER2/neu protein expression and gene amplification in gastric carcinoma. World J Gastroenterol 2011;17:15016.
34. Chen J, Li Q, Wang C, Wu J, Zhao G. Prognostic significance of cerbB2 and vascular endothelial growth factor in colorectal liver metastases. Ann Surg Oncol 2010;1:155563.
35. Gutierrez C, Schiff R. HER2: Biology, detection, and clinical implications. Arch Pathol Lab Med 2011;135:55-62.
36. Kapitanovi S, Radosevi S, Kapitanovi M, Andelinovic S, Ferencic Z, Tavassoli M, *et al*. The expression of p185(HER-2/neu) correlates with the stage of disease and survival in colorectal cancer. Gastroenterology 1997;112:1103-13.
37. Osako T, Miyahara M, Uchino S, Inomata M, Kitano S, Kobayashi M. Immunohistochemical study of cerbB- 2 protein in colorectal cancer and the correlation with patient survival. Oncology 1998;55:548-55.
38. Obrocea FL, Sajin M, Marinescu EC, Stoica D. Colorectal cancer and the 7th revision of the TNM staging system: Review of changes and suggestions for uniform pathologic reporting. Rom J Morphol Embryol 2011;52:53744.
39. McKay JA, Loane JF, Ross VG, Ameyaw MM, Murray GI, Cassidy J, *et al*. c-erbB-2 is not a major factor in the development of colorectal cancer. Br J Cancer 2002;86:568-73.
40. Kountourakis P, Pavlakis K, Psyrri A, Rontogianni D, Xiros N, Patsouris E, *et al*. Clinicopathologic significance of EGFR and Her-2/neu in colorectal adenocarcinomas. Cancer J 2006;12:229-36.
41. Kruszewski WJ, Rzepko R, Ciesielski M, Szefel J, Zielinski J, Szajewski M, *et al*. Expression of HER2 in colorectal cancer does not correlate with prognosis. Dis Markers 2010;29:207-12.
42. Song Z, Deng Y, Zhuang K, Li A, Liu S. Immunohistochemical results of HER2/neu protein expression assessed by rabbit monoclonal antibodies SP3 and 4B5 in colorectal carcinomas. Int J Clin Exp Pathol 2014;7:4454-60.
43. Richman SD, Southward K, Chambers P, Cross D, Barrett J, Hemmings G, *et al*. HER2 overexpression and amplification as a potential therapeutic target in colorectal cancer: Analysis of 3256 patients enrolled in the QUASAR, FOCUS and PICCOLO colorectal cancer trials. Pathol 2016;238:562-70.
44. Meric-Bernstam F, Hurwitz H, Raghav K, McWilliams R, Fakih, M, VanderWalde, A, *et al*. Pertuzumab plus trastuzumab for HER2-amplified metastatic colorectal cancer (MyPathway): an updated report from a multicentre, open-label, phase 2a, multiple basket study. The Lancet. Oncology, 2019;20:518-30. https://doi.org/10.1016/S1470-2045(18)30904-5.
45. Siena S, Sartore Bianchi A, Marsoni S, Hurwitz H, McCall S, Penault-Llorca F, *et al*. Final Results of the HERACLES Trial in HER2 Amplified Colorectal Cancer. Anti HER2 Treatment in HER2þ mCRC. Washington, DC: Oral presentation at the AACR Annual Meeting 2017..