RESEARCH ARTICLE

Clinicopathological value of epidermal growth factor receptor (EGFR) and Ki-67 expression in colorectal adenoma and adenocarcinoma

Eman Mohamed Ahmed Nor[1], Eman Mohamed Saied[2], Samir Nemr Mina[3], Mohamed Mostafa Shareef[4], Dareen Mohamed Abdelaziz[5].

Abstract

Objective: To evaluate epidermal growth factor receptor and Ki-67 immunohistochemical expression in colorectal adenoma and carcinoma cases, and to relate their expression with the available clinicopathological data.

Methods: The retrospective study was conducted at the Faculty of Medicine, Kafrelsheikh University, Egypt, from September 2019 to October 2020, and comprised formalin-fixed and paraffin-embedded specimens related to cases of colorectal adenoma and those of colorectal carcinoma, who had no previous radiation or chemotherapeutic treatment. Immunohistochemical staining of all thecases was done using anti-epidermal growth factor receptor and anti-Ki-67 antibodies. Data was analysed using SPSS 20.

Results: Of the 70 cases, 20(28.5%) were of colorectal adenoma; mean age 52.95±13.47 years, and male-to-female ratio 1:1. The remaining 50(71.5%) cases had colorectal carcinoma; mean age 51.08±13.49 years, and male-to-female ratio 1.17:1. Epidermal growth factor receptor and Ki-67 overexpression related significantly to villous histopathological type and high-grade dysplasia in colorectal adenoma cases ($p<0.05$). In colorectal carcinoma cases, epidermal growth factor receptor overexpression related significantly to tumour grade ($p<0.05$). Ki-67 overexpression related significantly to increased pathological stage ($p<0.05$).

Conclusions: Overexpression of epidermal growth factor receptor and Ki-67 was found to be an ominous sign of colorectal adenoma aggressiveness, and the risk of progression to colorectal carcinoma.

Keywords: Antigen, Adenoma, Colorectal neoplasms, Receptors, Carcinoma. **DOI: 10.47391/JPMA.EGY-S4-26**

Introduction

The third most prevalent cancer in the world is colorectal carcinoma (CRC).[1] It is the third leading cause of cancer-related death in both men and women in the United States, after lung and breast cancer in women, and prostate and lung cancer in men.[2] CRC is the seventh most prevalent cancer in Egypt, accounting for 3.47% of male cancers, 3% of female cancers, and the top malignancy of the digestive tract.[3] Egypt has a high rate of young-aged CRC, as nearly 35% of Egyptian CRC patients are age <40 years and have a three-fold risk to die within 5 years compared to those who have CRC and are aged >50 years.[4] The existence of adenomas is considered an important risk factor of CRC which develops as slowly growing tumour in the luminal wall, acquiring an increasing degree of dysplasia before progressing to malignancy.[5]

Although the most important and independent prognostic factor approved in CRC cases remains the tumour-node-metastasis (TNM) staging,[6] molecular biomarkers with prognostic or predictive value are also beneficial in selecting patients for preferred therapy and to prevent toxic side effects of the treatment.[7] Epidermal growth factor receptor (EGFR) is a glycoprotein transmembrane which is a part of the ErbB tyrosine kinase receptors, expressed by the proto-oncogene c-ErbB. Activation of EGFR stimulates carcinogenesis by increasing cell proliferation, migration, angiogenesis and apoptosis inhibition. It is also associated with metastasis, bad outcome and poor response to adjuvant post-operative therapy.[8] Currently, EGFR inhibitors are of value in metastatic CRC therapy and they have effective treatment options in combination with chemotherapy in EGFR-positive patients.[9] Ki-67 is a proliferation biomarker used in measuring the growth rate of cells in human tumours. Its expression is highly associated with tumour proliferation and is used in routine investigations as a prognostic and predictive biomarker in cancer. In CRC, Ki-67 expression has an undefined prognostic value. The high Ki-67 expression is related to bad prognosis and metastasis.[10]

The current study was planned to evaluate EGFR and Ki-67 immunohistochemical (IHC) expression in colorectal adenoma and carcinoma cases, and to relate their

[1, 2]Department of Pathology, Kafrelsheikh University, Egypt.
[3-5]Department of Pathology, Tanta University, Egypt.
Corrospondience: Eman Mohamed Ahmed Nor
email: dr.eman.nor@gmail.com

expression with the available clinicopathological data.

Material and Methods

The retrospective study was conducted at the Faculty of Medicine, Kafrelsheikh University, Egypt, from September 2019 to October 2020, and comprised formalin-fixed and paraffin-embedded (FFPE) specimens related to cases of colorectal adenoma and those of CRC, who had no previous radiation or chemotherapeutic treatment. The specimens were collected using comprehensive sampling technique from the Pathology Department and some private laboratories after approval from the institutional ethics review committee. Informed consent had been obtained from all the patients at the time of their treatment. Exclusion criteria comprised patients who had previously received radiotherapy or chemotherapy, insufficient tissue for immunostaining or poor quality of the blocks. Clinicopathological data of the patients regarding age, gender and clinical presentation was obtained from patient files, including surgery and oncology reports.

Sections were stained using haematoxylin and eosin (H&E), and optical microscope was used to examine them for the confirmation of the diagnosis and classification of histopathological types, tumour grade and other histological features, such as pT stage and lymphovascular invasion (LVI). The grade of dysplasia in adenoma cases was classified into high-grade (HG) or low-grade (LG). HG dysplasia included intra-mucosal carcinoma.[11] Colorectal carcinoma cases were graded and categorized according to American Joint Committee on Cancer (AJCC) staging system (2018) at the time of diagnosis.[12]

A representative haematoxylin and eosin (H&E) stained section was selected for each case and sections 3 micron-thick were cut from the corresponding block and mounted on positively-charged coated slides. The conventional manual IHC technique was performed using a method described in 2010.[13] The primary antibodies used were (Anti-EGFR, clone SP84, rabbit monoclonal antibody, SAB5500096-100UL [Cell Marque, Sigma-Aldrich Co., USA] and Anti-Ki-67, clone RM360, rabbit monoclonal antibody, SAB5600249-100UL [Cell Marque, Sigma-Aldrich Co., USA]).

Semi-quantitative analysis was used to determine the EGFR expression patterns. Following were the ratings for EGFR immunoreactivity intensity; 0 = no response; 1+ = poor intensity, weak membranous, cytoplasmic or both types of brown reactivity; 2+ = moderate intensity, brown stains in various shades and usually variegated reactive cell membranes; 3+ = high intensity for which a thick outline of the cell was created by a dark brown stain that covered the whole membrane pattern.[14] The mean proportion of EGFR-reactive cells per instance was used to calculate the degree of EGFR immunoreactivity, and the results were evaluated as: 1+ = 1-10%, 2+ = >10-50%)%, and 3+ = >50% of EGFR-reactive cells, respectively. The intensity and extent of staining for each tissue segment were multiplied to produce the EGFR IHC composite score, which varied from 0 to 9, and was then divided into composite scores of to differentiate between low and high EGFR expression.[15]

Nuclear staining that is uniform or granular is a sign of a good response to Ki-67. The position of the area with the highest marking was considered when choosing the hot spot For which 500 cells were counted and at least 5 high-power fields were selected. The number of Ki-67-positive nuclei divided by the total number of cells examined was used to construct the Ki-67 proliferative labelling index (LI), which was then represented as a percentage.[16] A cut-off value of 25% was chosen to categorise the samples into low (25% positively-stained tumour cells) or high nuclear Ki-67 expression (25% positively-stained tumour cells) based on the percentage of positively stained tumour cells in various representative visual fields.[17]

Data was analysed using IBM SPSS 20. Qualitative data was expressed as frequencies and percentages. Quantitative data was reported and mean and standard deviation, or as median and interquartile range (IQR), as appropriate. When more than 20% of the cells had an estimated count <5, Fisher's Exact or Monte Carlo correction for chi-square tests was utilised. To compare results across several groups, categorical variables were tested using the chi-square test. $P<0.05$ was considered statistically significant.

Results

Of the 70 cases, 20(28.5%) were of colorectal adenoma; mean age 52.95±13.47 years, and male-to-female ratio 1:1. The most common location was the left colon 9(45%), followed by rectum 7(35%). The most frequent histopathological type was tubulovillous adenomas 8(40%), followed by tubular adenomas 5(25%), villous adenomas 4(20%) and serrated adenomas 3(15%). Further, 9(45%) adenoma cases showed LG dysplasia, while 11(55%) showed HG features. The relation between the grade of dysplasia and the adenoma size was significant ($p<0.05$).

The remaining 50(71.5%) cases had CRC; mean age 51.08±13.49 years, and male-to-female ratio 1.17:1. The most common location was the left colon 23(46%), followed by proximal colon 16(32%). The most frequent histopathological type was conventional type 31(62%), followed by mucinous adenocarcinoma 12(24%), signet ring carcinoma 5(10%) and carcinoma with neuroendocrine differentiation 2(4%). CRCs were grade (G) II in 28(56%) cases and GIII in 13(26%). The most diagnosed stage was pT3 20(40%), followed by pT4 15(30%). The most frequent lymph node (N) status was N1 22(44%) cases and CRC showing LVI 10(20%) cases (Table 1).

In adenoma cases, 12(60%) showed a low EGFR composite score (<6), while 8(40%) showed a high score (≥6) (Figure 1). The EGFR composite score showed a significant relationship with the histopathological type ($p<0.05$), as villous 4(100%) and tubulovillous 4(50%) types showed high expression. Also, the EGFR composite score showed a significant association with HG dysplasia ($p<0.05$).

Among adenoma cases, 13(65%) showed a low Ki-67 LI score (<25%), while 7(35%) showed a high score (≥25%). High score was associated with villous adenomas 3(75%), and HG dysplasia 6(654.6%). The Ki-67 LI score had a significant relationship with the histopathological type and grade of dysplasia ($p<0.05$).

In CRC cases, 29(58%) showed high EGFR composite score (≥6), and 21(42%) showed a low score (<6) (Figure 2). EGFR high composite score increased in tumours showing LVI 15(75%). The score had a significant relationship with the tumour grade and the presence of LVI ($p<0.05$), but it had no significant relationship with the histopathological type, the pT stage and the N status ($p>0.05$).

CRC cases showed a high score of Ki-67 LI 38(76%). The relation between Ki-67 LI score and tumour grade was significant ($p<0.05$). The relation between Ki-67 LI score and pT stage was significant ($p<0.05$). The Ki-67 LI score showed no significant relationship with the histopathological type, the N status and the presence of LVI ($p>0.05$) (Table 2).

The expression of EGFR and Ki-67 LI in CRC was increased compared to adenoma tissue, but the difference was not significant ($p>0.05$). EGFR expression demonstrated a significant connection to the expression of Ki-67 in adenoma and CRC ($p<0.05$) (Figure 3).

Table-1: Demographic and clinicopathological data (n = 70).

Demographic and clinicopathological data of studied colorectal adenoma cases (n = 20)	n (%)	Demographic and clinicopathological data of studied colorectal carcinoma cases (n = 50)	n (%)
Age (years)		**Age**	
≤40	5 (25%)	≤40	12 (24%)
40 – 60	8 (40%)	40 – 60	23 (46%)
>60	7 (35%)	>60	15 (30%)
Mean ± SD.	52.95 ± 13.47	Mean ± SD.	51.08 ± 13.49
Median (Min. – Max.)	55 (27 – 72)	Median (Min. – Max.)	53.5 (26 – 76)
Gender		**Gender**	
Male	10 (50%)	Male	27 (54%)
Female	10 (50%)	Female	23 (46%)
Tumour location		**Tumour location**	
Left colon	9 (45%)	Left colon	23 (46%)
Proximal colon	4 (20%)	Proximal colon	16 (32%)
Rectum	7 (35%)	Rectum	11 (22%)
Gross pattern		**Gross pattern**	
Sessile	9 (45%)	Ulcerating	21 (42%)
Pedunculated	11 (55%)	Infiltrating(annular)	13 (26%)
Number		Fungating	16 (32%)
Single	16 (80%)	**Histological type**	
Multiple	4 (20%)	Adenocarcinoma	31 (62%)
Size		Mucinous carcinoma	12 (24%)
≤1cm	7 (35%)	Signet ring carcinoma	5 (10%)
>1cm	13 (65%)	Neuroendocrine differentiation	2 (4%)
Mean ± SD.	1.71 ± 0.96	**Histological grade**	
Median (Min. – Max.)	1.5 (0.5 – 4)	Grade (I)Well differentiated	8 (16%)
Histological type		Grade (II)Moderately differentiated	28 (56%)
Tubulovillous	8 (40%)	Grade (III)Poorly differentiated	13 (26%)
Tubular	5 (25%)	Undifferentiated	1 (2%)

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Table-1: *continued from previous page*

Demographic and clinicopathological data of studied colorectal adenoma cases (n = 20)	n (%)	Demographic and clinicopathological data of studied colorectal carcinoma cases (n = 50)	n (%)
Villous	4 (20%)	**Primary tumour (T)**	
Serrated	3 (15%)	T1	4 (8%)
Grade of dysplasia		T2	11 (22%)
Low	9 (45%)	T3	20 (40%)
High	11 (55%)	T4	15 (30%)
		Lymph node status (N)	
		N0	19 (38%)
		N1	22 (44%)
		N2	9 (18%)
		Lymphovascular invasion (LVI)	
		Without lymphovascular invasion	30 (60%)
		With lymphovascular invasion	20 (40%)

SD: Standard deviation.

Table-2: Association of immunohistochemical (IHC) epidermal growth factor receptor (EGFR) and Ki-67 with different parameters (n = 70).

	Composite score of EGFR staining		Ki-67 proliferation labelling index	
	Low <6 (n=21/42%)	high≥6 (n=29/58%)	Low score (n=12/24%)	High score (n=38/76%)
Colorectal adenoma cases (n = 20)				
Histological type				
Tubulovillous	4 (50%)	4 (50%)	5 (62.5%)	22 (71.0%)
Villous	0 (0.0%)	4 (100%)	1 (25.0%)	10 (83.3%)
Serrated	3 (100%)	0 (0.0%)	3 (100%)	4 (80.0%)
Tubular	5 (100%)	0 (0.0%)	4 (80.0%)	2 (100%)
Test of Sig. (p)	χ2= 10.580*,MCp=0.006*	χ2=9.638*,p=0.022*		
Histological grade				
Low	8 (88.9%)	1 (11.1%)	8 (88.9%)	1 (11.1%)
High	4 (36.4%)	7 (63.6%)	5 (45.5%)	6 (54.5%)
Test of Sig. (p)	χ2= 5.690*,FEp=0.028*	Z=3.348*,p=0.001*		
Colorectal carcinoma cases (n = 50)				
Histological grade				
GI (Well differentiated)	6 (75.0%)	2 (25.0%)	6 (75.0%)	2 (25.0%)
GII (Moderately differentiated)	12 (42.9%)	16 (57.1%)	3 (10.7%)	25 (89.3%)
GIII (Poorly differentiated)	2 (15.4%)	11 (84.6%)	2 (15.4%)	11 (84.6%)
GIV (Undifferentiated)	1 (100%)	0 (0.0%)	1 (100%)	0 (0.0%)
Test of Sig. (p)	χ2=8.500*,MCp=0.022*	χ2=10.708*,p=0.013*		
Primary tumour (T)				
T1	2 (50.0%)	2 (50.0%)	3 (75%)	1 (25%)
T2	8 (72.7%)	3 (27.3%)	3 (27.3%)	8 (72.7%)
T3	6 (30.0%)	14 (70.0%)	4 (20.0%)	16 (80.0%)
T4	5 (33.3%)	10 (66.7%)	2 (13.3%)	13 (86.7%)
Test of Sig. (p)	χ2=5.888,MCp=0.114	χ2=12.880*,p=0.005*		
Lymph node status (N)				
N0	9 (47.4%)	10 (52.6%)	7 (36.8%)	12 (63.2%)
N1	9 (40.9%)	13 (59.1%)	3 (13.6%)	19 (86.4%)
N2	3 (33.3%)	6 (66.7%)	2 (22.2%)	7 (77.8%)
Test of Sig. (p)	χ2=0.546,p=0.774	χ2=0.635,MCp=0.728		
Lymphovascular invasion (LVI)				
Tumours without lymphovascular invasion	16 (53.3%)	14 (46.7%)	7 (23.3%)	23 (76.7%)
Tumours with lymphovascular invasion	5 (25.0%)	15 (75.0%)	5 (25.0%)	15 (75.0%)
Test of Sig. (p)	χ2=3.955*,p=0.047*	Z=1.867*,p=0.042*		

χ2: Chi square test, MC: Monte Carlo, p: p value for association between different categories. *: Statistically significant at $p \leq 0.05$.

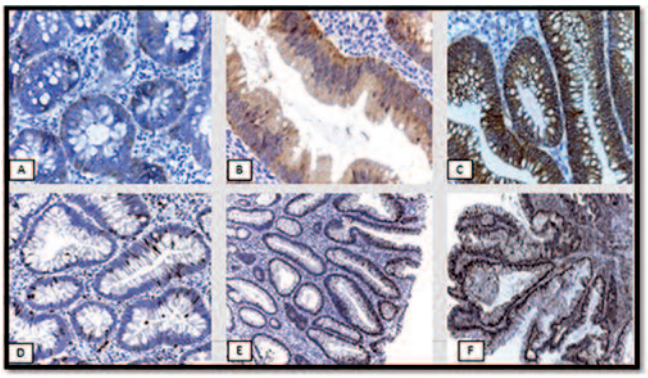

Figure 1: Figure 1: Colorectal adenoma. A: Low-grade tubular type, showing positive epidermal growth factor receptor (EGFR) immunostaining score +1 (faint cytoplasmic), low composite score<6 (x400); B: Low-grade tubulovillous type, showing positive EGFR immunostaining score +2 (variegated membranous), high composite score≥6 (x400); C: High-grade tubulovillous type, showing positive EGFR immunostaining score +3 (thick outline membranous), high composite score≥6 (x400); D: Low-grade tubular type, showing positive nuclear immunostaining Ki-67 labelling index (LI) (<25%) low score (x400); E: Low-grade tubulovillous type, showing positive nuclear immunostaining Ki-67 LI (≥25%) high score (x100); F: High-grade villous type, showing positive nuclear immunostaining Ki-67 LI (≥25%) high score (x100), immunoperoxidase.

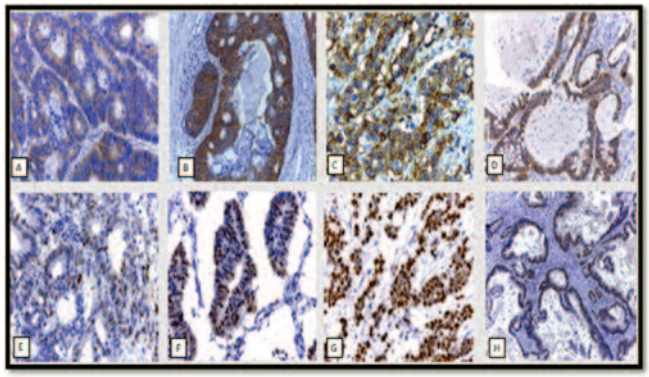

Figure 2: Colorectal adenocarcinoma. A: Well-differentiated grade 1 (GI), showing positive epidermal growth factor receptor (EGFR) immunostaining score +1 (faint membranous/ cytoplasmic), low composite score<6 (x200); B: Moderately-differentiated GII, showing positive EGFR immunostaining score +3 (thick outline membranous), high composite score≥6 (x200); C: Poorly-differentiated GIII showing positive EGFR immunostaining, score +3 (thick outline membranous), high composite score≥6 (x400); D: Mucinous adenocarcinoma showing positive EGFR immunostaining, score +2 (variegated membranous), high composite score≥6 (x200); E: Well-differentiated GI, showing positive nuclear immunostaining Ki-67 LI (<25%) low score (x400); F: Moderately-differentiated GII, showing positive nuclear immunostaining Ki-67 LI (≥25%) high score (x400); G: Poorly-differentiated GIII, showing positive nuclear immunostaining Ki-67 LI (≥25%) high score (x400); H: Mucinous adenocarcinoma, showing positive nuclear immunostaining Ki-67 LI (≥25%) high score (x200), immunoperoxidase.

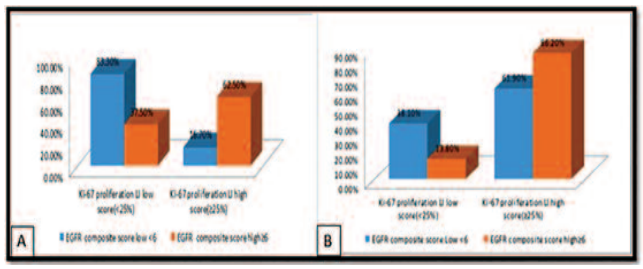

Figure 3: A: The relation between epidermal growth factor receptor (EGFR) immunostaining composite score and Ki-67 labelling index (LI) in colorectal adenoma cases (n=20); B: The relation between EGFR immunostaining composite score and Ki-67 LI in colorectal carcinoma cases (n=50).

Discussion

The EGFR IHC expression in colorectal adenoma case in the current study was in concordance with Williet et al.[18]. In cases of investigated adenoma, the grade and type of histopathological dysplasia were significantly correlated with the Ki-67 LI, which is close to earlier findings,[19], but findings in contrast have also been reported.[20]

In the current study, 58% CRC cases showed overexpression of EGFR compared to Spano et al.[15] who showed a higher percentage (80%). Most other studies showed variable ranges (25-82%) of EGFR overexpression in CRC.[21] Despite such issues with reproducibility and validation, IHC testing is still one of the most used ways to determine EGFR expression. In CRC cases, the EGFR composite score revealed a significant connection with tumour grade. High EGFR expression was mainly seen in moderately and poorly-differentiated HG cases. Similar[15] and conflicting[22,23] findings have been reported. High EGFR expression was more observed in tumours that showed LVI than tumours that did not show this feature, but Yun et al.[24] did not show such an association. The relation between EGFR expression and histopathological type of CRC in the present study was non-significant, which has also been reported by Brahim et al.[23] The EGFR expression showed a non-significant relationship with pT stage, which agreed with Azevedo et al.[22] and Brahim et al.[23], but not with Goldstein et al.[14] and Spano et al.[15]. Concerning the N stage in the current study, no relation was observed between EGFR expression and lymph node metastasis. Kapogiannatos et al.[25] found a significant relationship. The current study agreed with Azevedo et al.[22], who observed higher expression of EGFR in CRC compared to adenoma tissue. According to Liang et al.[26], overexpression of EGFR is related to bad prognosis and poor survival in many malignant tumours. Additionally, EGFR-positive CRC is associated with an advanced stage of the illness and can indicate a risk of metastatic spread.[27]

The Ki-67 LI in current CRC cases ranged from 2% to 93% with a mean of 25%. These results were close to those documented by Wang et al.[28] Petrowsky et al.[29] reported that the mean value of Ki-67 LI for CRC was 50%, and Terzi et al.[30] detected 84%. This variation may be caused by the difficulty and subjectivity involved in quantitatively assessing immunostaining for practical or diagnostic purposes. Ki-67 LI high score ≥25% showed a significant relationship with the tumour grade and the pT stage ($p<0.05$), and a high Ki-67 LI score was related to HG carcinoma. Likewise, Petrowsky et al.[29] found that HG tumours (G3) had a higher mean Ki-67 LI score. Also, Nayak, et al.[31] observed an increase in Ki-67 LI score in advanced grade. However, Wang et al.[28] observed no association. High Ki-67 LI was associated with a higher pT stage in the

current study, which agreed with the results of Wang et al.[28]

Ki-67 LI score did not relate significantly with the histopathological type in the current cases. This was in agreement with Ihmann et al.[32]. Also, its expression showed no significant relationship with N stage or LVI in the studied carcinoma cases. This agreed with Wang et al.[28] The most reliable information for a high Ki-67's unfavourable prognostic value was reported by Ignatiadis et al.[33] which was for breast cancer and sarcomas. The relationship between cell proliferation and clinical prognosis in CRCs is ambiguous, if not contradictory. CRC tumours with high proliferative activity have greater responses to conventional radiation and chemotherapy and better disease-free survival.[34]

In the current study, colorectal adenoma and CRC cases showed EGFR and Ki-67 expression having a significant association, as cases with high EGFR composite score showed a higher percentage of Ki-67 LI. In concordance, Rego et al.[35]reported that high EGFR expression was related to high cell proliferation and increased Ki-67 LI. Moreover, Chen et al.[36] stated that there was a positive correlation of advanced stage and bad prognosis with the two biomarkers.

In terms of limitations, the current study did not calculate the sample size which could have influenced the power of the study.

To find a potential meaningful cut-off value of Ki-67 expression between colorectal adenoma and carcinoma, more studies with larger sample sizes are require. The relevance of EGFR and Ki-67 biomarkers in the modification of therapy regimens requires further research.

Conclusions

The overexpression of EGFR and Ki-67 in adenomas, particularly those with villous components and HG dysplasia, is a risk factor for the development of CRC. Compared to colorectal adenomas, CRC cases had greater levels of EGFR and Ki-67 LI overexpression. The relationship between EGFR expression and Ki-67 LI in CRC was significant. Increased histological grade and tumour invasion in CRC were significantly correlated with EGFR and Ki-67 overexpression, which foretells a potential metastatic risk.

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Abbreviation

- ErbB: family of proteins contains four receptor tyrosine kinases, structurally related to the epidermal growth factor receptor (EGFR) and ErbB is used as alternative name for (EGFR).
- pT: primary tumour size of pathologic stage according to the American Joint Committee on Cancer (AJCC) system.
- SP84, SAB, RM: catalogue code number of the clone, which is used in research, named by manufacturing company.

References

1. Weinberg BA, Marshall JL. Colon Cancer in Young Adults: Trends and Their Implications. Curr Oncol Rep 2019;21:3. doi: 10.1007/s11912-019-0756-8.
2. Islami F, Goding Sauer A, Miller KD, Siegel RL, Fedewa SA, Jacobs EJ, et al. Proportion and number of cancer cases and deaths attributable to potentially modifiable risk factors in the United States. CA Cancer J Clin 2018;68:31-54. doi: 10.3322/caac.21440.
3. Ibrahim AS, Khaled HM, Mikhail NN, Baraka H, Kamel H. Cancer incidence in egypt: results of the national population-based cancer registry program. J Cancer Epidemiol 2014;2014:e437971. doi: 10.1155/2014/437971.
4. Gado A, Ebeid B, Abdelmohsen A , Axon A. Colorectal cancer in Egypt is commoner in young people: is this cause for alarm?. Alexandria J. Med 2014;50:197-201. Doi: 10.1016/j.ajme.2013.03.003.
5. Shanmugam C, Katkoori VR, Jhala NC, Grizzle WE, Siegal GP, Manne U. p53 Nuclear accumulation and Bcl-2 expression in contiguous adenomatous components of colorectal adenocarcinomas predict aggressive tumor behavior. J Histochem Cytochem 2008;56:305-12. doi: 10.1369/jhc.7A7362.2007.
6. Feng RM, Zong YN, Cao SM, Xu RH. Current cancer situation in China: good or bad news from the 2018 Global Cancer Statistics? Cancer Commun (Lond) 2019;39:22. doi: 10.1186/s40880-019-0368-6.
7. Lech G, Slotwinski R, Krasnodebski IW. The role of tumor markers and biomarkers in colorectal cancer. Neoplasma 2014;61:1-8. DOI: 10.4149/neo_2014_003.
8. Schlessinger J. Receptor tyrosine kinases: legacy of the first two decades. Cold Spring Harb Perspect Biol 2014;6:a008912. doi: 10.1101/cshperspect.a008912.
9. Martinelli E, Ciardiello D, Martini G, Troiani T, Cardone C, Vitiello PP, et al. Implementing anti-epidermal growth factor receptor (EGFR) therapy in metastatic colorectal cancer: challenges and future perspectives. Ann Oncol 2020;31:30-40. doi: 10.1016/j.annonc.2019.10.007.
10. Li LT, Jiang G, Chen Q, Zheng JN. Ki67 is a promising molecular target in the diagnosis of cancer (review). Mol Med Rep 2015;11:1566-72. doi: 10.3892/mmr.2014.2914.
11. Kuijpers CC, Sluijter CE, von der Thüsen JH, Grünberg K, van Oijen MG, van Diest PJ. Interlaboratory variability in the grading of dysplasia in a nationwide cohort of colorectal adenomas. Histopathology 2016;69:187-97. doi: 10.1111/his.12923.
12. Loughrey MB, Quirke P, Shepherd NA. Standards and datasets for reporting cancers Dataset for histopathological reporting of colorectal cancer. [Online] 2018 [Cited 2019 January 30]. Available from URL: https://www.acpgbi.org.uk/_userfiles/pages/files/resources/g049datasetforhistopathologicalreportingofcolorectalcancer.pdf

13. Buchwalow IB, Böcker W. Immunostaining enhancement. In: Immunohistochemistry: basics and methods. Heidelberg, Germany: Springer-Verlag Berlin Heidelberg, 2010; pp 47-59.
14. Goldstein NS, Armin M. Epidermal growth factor receptor immunohistochemical reactivity in patients with American Joint Committee on Cancer Stage IV colon adenocarcinoma: implications for a standardized scoring system. Cancer 2001;92:1331-46. doi: 10.1002/1097-0142(20010901)92:5<1331::aid-cncr1455>3.0.co;2-m.
15. Spano JP, Lagorce C, Atlan D, Milano G, Domont J, Benamouzig R, et al. Impact of EGFR expression on colorectal cancer patient prognosis and survival. Ann Oncol 2005;16:102-8. doi: 10.1093/annonc/mdi006.
16. Swiderska-Chadaj Z, Gallego J, Gonzalez-Lopez L, Bueno G. Detection of Ki67 hot-spots of invasive breast cancer based on convolutional neural networks applied to mutual information of H&E and Ki67 whole slide images. Appl Sci 2020;10:7761. doi: 10.3390/app10217761.
17. Tong G, Zhang G, Liu J, Zheng Z, Chen Y, Niu P, et al. Cutoff of 25% for Ki67 expression is a good classification tool for prognosis in colorectal cancer in the AJCC 8 stratification. Oncol Rep 2020;43:1187-98. doi: 10.3892/or.2020.7511.
18. Williet N, Petcu CA, Rinaldi L, Cottier M, Del Tedesco E, Clavel L, et al. The level of epidermal growth factor receptors expression is correlated with the advancement of colorectal adenoma: validation of a surface biomarker. Oncotarget 2017;8:16507-17. doi: 10.18632/oncotarget.14961.
19. Moriichi K, Fujiya M, Kobayashi Y, Murakami Y, Iwama T, Kunogi T, et al. Autofluorescence Imaging Reflects the Nuclear Enlargement of Tumor Cells as well as the Cell Proliferation Ability and Aberrant Status of the p53, Ki-67, and p16 Genes in Colon Neoplasms. Molecules 2019;24:1106. doi: 10.3390/molecules24061106.
20. Vernillo R, Lorenzi B, Banducci T, Minacci C, Vindigni C, Fei AL, et al. Immunohistochemical expression of p53 and Ki67 in colorectal adenomas and prediction of malignancy and development of new polyps. Int J Biol Markers 2008;23:89-95. doi: 10.1177/172460080802300205.
21. Gerber PA, Meller S, Eames T, Buhren BA, Schrumpf H, Hetzer S, et al. Management of EGFR-inhibitor associated rash: a retrospective study in 49 patients. Eur J Med Res 2012;17:4. doi: 10.1186/2047-783X-17-4.
22. Azevedo MA, Souza BD, Mader AM, Martins LC, Waisberg J. Immunohistochemical expression of the epidermal growth factor receptor (EGFR) in colorectal carcinoma: relation with clinicopathological parameters. J. Coloproctology 2011;31:225-32. doi: 10.1590/S2237-93632011000300001.
23. Brahim EB, Ayari I, Jouini R, Atafi S, Koubaa W, Elloumi H, et al. Expression of epidermal growth factor receptor (EGFR) in colorectal cancer: An immunohistochemical study. Arab J Gastroenterol 2018;19:121-4. doi: 10.1016/j.ajg.2018.08.002.
24. Yun S, Kwak Y, Nam SK, Seo AN, Oh HK, Kim DW, et al. Ligand-Independent Epidermal Growth Factor Receptor Overexpression Correlates with Poor Prognosis in Colorectal Cancer. Cancer Res Treat 2018;50:1351-61. doi: 10.4143/crt.2017.487.
25. Kapogiannatos G, Margaritis D, Alevizou R, Gogoulou I, Nomicos A, Digalakis M. Immunohistochemical expression of EGFR in colorectal adenocarcinoma and its correlation with the presence of lymph node metastases. Hell Cheirourgike 2015;87:364–7. Doi: 10.1007/s13126-015-0242-1.
26. Liang W, Wu X, Fang W, Zhao Y, Yang Y, Hu Z, et al. Network meta-analysis of erlotinib, gefitinib, afatinib and icotinib in patients with advanced non-small-cell lung cancer harboring EGFR mutations. PLoS One 2014;9:e85245. doi: 10.1371/journal.pone.0085245.
27. Xie YH, Chen YX, Fang JY. Comprehensive review of targeted therapy for colorectal cancer. Signal Transduct Target Ther 2020;5:22. doi: 10.1038/s41392-020-0116-z.
28. Wang L, Liu Z, Fisher KW, Ren F, Lv J, Davidson DD, et al. Prognostic value of programmed death ligand 1, p53, and Ki-67 in patients with advanced-stage colorectal cancer. Hum Pathol 2018;71:20-9. doi: 10.1016/j.humpath.2017.07.014.
29. Petrowsky H, Sturm I, Graubitz O, Kooby DA, Staib-Sebler E, Gog C, et al. Relevance of Ki-67 antigen expression and K-ras mutation in colorectal liver metastases. Eur J Surg Oncol 2001;27:80-7. doi: 10.1053/ejso.2000.1029.
30. Terzi C, Canda AE, Sagol O, Atila K, Sonmez D, Fuzun M, et al. Survivin, p53, and Ki-67 as predictors of histopathologic response in locally advanced rectal cancer treated with preoperative chemoradiotherapy. Int J Colorectal Dis 2008;23:37-45. doi: 10.1007/s00384-007-0376-x.
31. Nayak J, Mohanty P, Lenka A, Sahoo N, Agrawala S, Panigrahi SK. Histopathological and Immunohistochemical Evaluation of CDX2 and Ki67 in Colorectal Lesions with their Expression Pattern in Different Histologic Variants, Grade, and Stage of Colorectal Carcinomas. J Microsc Ultrastruct 2021;9:183-9. doi: 10.4103/JMAU.JMAU_69_20.
32. Ihmann T, Liu J, Schwabe W, Häusler P, Behnke D, Bruch HP, et al. High-level mRNA quantification of proliferation marker pKi-67 is correlated with favorable prognosis in colorectal carcinoma. J Cancer Res Clin Oncol 2004;130:749-56. doi: 10.1007/s00432-004-0612-5.
33. Ignatiadis M, Azim HA Jr, Desmedt C, Veys I, Larsimont D, Salgado R, et al. The Genomic Grade Assay Compared With Ki67 to Determine Risk of Distant Breast Cancer Recurrence. JAMA Oncol 2016;2:217-24. doi: 10.1001/jamaoncol.2015.4377.
34. Melling N, Kowitz CM, Simon R, Bokemeyer C, Terracciano L, Sauter G, et al. High Ki67 expression is an independent good prognostic marker in colorectal cancer. J Clin Pathol 2016;69:209-14. doi: 10.1136/jclinpath-2015-202985.
35. Rego RL, Foster NR, Smyrk TC, Le M, O'Connell MJ, Sargent DJ, et al. Prognostic effect of activated EGFR expression in human colon carcinomas: comparison with EGFR status. Br J Cancer 2010;102:165-72. doi: 10.1038/sj.bjc.6605473.
36. Chen YT, Tsao SC, Yuan SS, Tsai HP, Chai CY. Serine Protease Inhibitor Kazal Type 1 (SPINK1) Promotes Proliferation of Colorectal Cancer Through the Epidermal Growth Factor as a Prognostic Marker. Pathol Oncol Res 2015;21:1201-8. doi: 10.1007/s12253-015-9949-0.