Characterization of HER2 gene/protein and Ki67 protein expressions in colorectal carcinoma variants with relation to clinicopathological parameters and prognosis: An immunohistochemical and fluorescence in situ hybridization study

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ABSTRACT

Objective: The data on the frequency and pattern of human epidermal growth factor receptor 2 (HER2) expression in colorectal carcinoma (CRC) and its clinical significance are ambiguous. In addition, little is known about HER2 status in CRC variants and its relation with proliferative activity and clinical outcome. Such knowledge may be of potential value for therapeutic decision making in CRCs. Material and Methods: The HER2 gene/protein status was assessed by fluorescence in situ hybridization and immunohistochemistry (IHC) in a tissue microarray of 150 CRCs and correlated with the expression of the proliferation marker Ki67, clinicopathological factors, and prognosis. Results: CRCs were categorized into conventional adenocarcinoma (CA), 47 cases; adenocarcinoma with mucinous component (AMC), 28 cases; mucinous adenocarcinoma (MA), 56 cases; and signet ring cell carcinoma (SRC), 19 cases. Compared to other variants, CA was significantly associated with favorable clinicopathological features, higher overall survival (OS), and disease-free survival (DFS), while SRCC and MA were significantly associated with ominous clinicopathological features, lower OS, and DFS. Cytoplasmic HER2 overexpression was detected in 14.2% of CRC cases and showed a significant agreement with gene amplification. HER2 overexpression was significantly associated with favorable clinicopathological features, notably early stages. High Ki67 expression was detected in 48% of CRC cases. HER2 and Ki67 were significantly different among CRC variants with AMC showing the greatest frequency of HER2 overexpression and Ki67 high expression than the other variants. Conclusions: We conclude that mucinous histology infers an adverse prognosis in CRC. A subset of early stage CRC patients with HER2 overexpression, and possibly of a distinct variant, may benefit from HER2 targeted therapy. IHC can be used as a method of screening for HER2 gene amplification in CRCs.

KEY WORDS: Colorectal carcinoma, HER2, Ki67, mucinous, signet ring, variants

INTRODUCTION

Colorectal carcinoma (CRC) is one of the most prevalent cancers worldwide and it represents the fourth most common cause of cancer-related mortality. The incidence rates of CRC vary widely with approximately 60% of cases diagnosed in developed countries. In developing countries, the transition to a more Western diet has been associated with increasing rates of the disease [1,2].

Histologically, more than 90% of CRCs are adenocarcinomas. Several histopathological variants are recognized such as mucinous, signet ring cell, medullary, micropapillary, serrated, and cribriform comedo-type [3,4].

Conventional adenocarcinoma (CA) is characterized by glandular formation, which is the basis for histologic tumor grading. Mucinous adenocarcinoma (MA) features extracellular mucin pools with floating malignant epithelium
constituting more than 50% of the tumor. The designation “adenocarcinoma with mucinous component (AMC)” is used for adenocarcinomas with mucinous areas constituting <50%. Signet ring cell carcinoma (SRCC) is defined by the presence of >50% of tumor cells with prominent intracytoplasmic mucin and signet ring morphology [3, 4].

Although adequate surgery is the main treatment for CRCs, chemotherapy has proved to be an efficient strategy for adjuvant therapy; however, long-term results have been less than satisfactory. Therefore, there is extensive ongoing research on alternative therapeutic targets and agents. One of these targets is the human epidermal growth factor receptor 2 (HER2), which is primarily associated with breast cancer [5, 6].

HER2, (also known as HER2/neu, ErbB2 or p185) is a member of the epidermal growth factor receptor family that plays a central role in the pathogenesis of several human cancers [7, 8].

Similar to other members of the family, HER2 comprise a cysteine-rich extracellular ligand binding site, a transmembrane lipophilic segment, and an intracellular domain with tyrosine kinase catalytic activity [7, 9]. Activation of HER2 mediates cell proliferation, cell differentiation, inhibition of apoptosis, and tumor progression [9].

The advent of HER2 directed therapies has dramatically affected the outcome of patients with HER2 positive breast and gastric or gastroesophageal cancers; however, the results have been discouraging in other HER2 overexpressing cancers [6, 7].

Previous studies on HER2 expression in CRCs have yielded conflicting results regarding the frequency and pattern of expression, and its impact on prognosis. Thus, it is currently unclear whether HER2 is a potential therapeutic target in patients with CRCs [5, 6].

Furthermore, the majority of studies on HER2 status in CRCs have focused on conventional adenocarcinoma with only a few studies additionally investigating a limited number of mucinous adenocarcinoma [10-12]. To the best of our knowledge, this is the first study addressing HER2 expression in histopathological variants of CRCs with emphasis on mucin-producing variants.

Proliferation and cell cycle control are central processes in the biology of cancer [13]. The proliferative marker, Ki67, recognizes an antigen present in the nuclei of cells in all phases of the cell cycle except G0. High proliferative activity is a poor prognostic factor in many tumors, and it correlates with the expression of other poor prognostic factors. Nevertheless, the exact prognostic value of proliferative and cell cycle-associated markers in colon cancer remains vague [15-15].

Moreover, the interrelation between HER2 and Ki67 expressions in CRCs is still unclear. We, therefore, conducted this study to determine the frequency and clinical significance of HER2 and Ki67 expressions in CRC variants in a cohort of Egyptian patients.

**MATERIALS AND METHODS**

**Patients and Tissue Samples**

A total of 150 cases of CRCs were collected from the files of the Surgical Pathology Laboratory at Gastroenterology Center, Mansoura, Egypt. The subjects of the study were Egyptian patients (age range = 20-80 years, mean = 52.7, standard deviation [SD] = 13.094, and M:F ratio = 1.6:1), who underwent surgical resection for histopathologically confirmed CRCs between January 2007 and January 2012. Clinicopathological and follow-up data were available for all patients.

The studied clinicopathological variables included: age, gender, location, size, shape, multiplicity, histopathological variant, grade, tumor advancing edge (pushing versus infiltrating microscopically), lymphovascular or perineural invasion, peri- and intra-tumoral lymphocytic infiltration, extent of neutrophilic infiltrate, preexisting adenoma, depth of invasion (T), number of lymph node metastases (N), distant metastasis (M), TNM staging, state of surgical margins, and associated schistosomiasis.

H&E stained sections were examined to evaluate histopathological parameters and choose representative areas for tissue microarray (TMA) construction. Grading and TNM staging were done according to established criteria [3].

The study groups were categorized, according to the histopathological variants of CRCs, into 47 cases of CA, 28 cases of AMC, 56 cases of MA, and 19 cases of SRCC. In addition, 15 specimens of normal colonic mucosa and 15 adenomas were also included in the study.

Exclusion criteria included: Preoperative (neoadjuvant) chemotherapy, incomplete clinicopathological and follow-up information, and non-representative or insufficient tissue for immunostaining, or fluorescence in situ hybridization (FISH) analysis in microarray sections.

The local Scientific Ethical Committees at Mansoura and Alexandria Universities approved the study and REMARK criteria [16] were applied.

**TMA Construction**

Three manual TMA blocks were constructed using the modified mechanical pencil tip method as previously described by Foda [17]. Three representative cores of 0.8 mm diameter were punched out from each case. Cores of various normal tissues were included to serve as positive and negative controls. 4 µm thick sections from the TMA blocks were cut on ordinary slides for routine H&E evaluation and charged slides for immunohistochemistry (IHC) and FISH studies.
Immunohistochemical Assessment of HER2 and Ki67 Expressions

The slides were deparaffinized in xylene, rehydrated through graded alcohol and brought to water. Endogenous peroxidase was blocked with peroxide block for 15 min. Antigen retrieval was done using epitope retrieval 1 (Leica) for 30 min. The slides were then incubated for 30 min at room temperature with primary antibodies; monoclonal rabbit anti-HER2 (Clone GR011, Genemed Biotechnologies, Inc, CA, USA) in a 1:100 dilution and monoclonal mouse anti-Ki67 (Clone 4A1, Thermo Fisher Scientific, Lab Vision Corporation, CA, USA) in a 1:200 dilution. Immunoperoxidase method was performed using InnuoPure Ultra-Sensitive ABC Peroxidase Kit (Thermo Scientific, UK), and diaminobenzidine as the chromogen. Positive (breast carcinoma) and negative controls were included.

Semi-quantitative evaluation of the immunostained slides was performed blindly and independently by the authors. For HER2 immunostaining, a modified scheme of Gill et al. [10] was followed. The intensity and extent of membranous or cytoplasmic staining were evaluated. Intensity of staining was graded as; 0 (negative), 1 (weak), 2 (moderate), and 3 (strong), and the extent (percentage) of positively stained cells was graded as; 0 (<10%), 1 (10-50%), and 2 (>50%). The final score for HER2 expression was determined by the sum of the two scores (0-5), then categorized as negative or low expression (scores 0-3) and overexpression (scores 4, 5).

For Ki67, nuclear immunostaining was assessed, and the percent of positively stained tumor cells was determined for each case, and then the median value was used as a cut-off point to divide the cases into low expression group (< median) and high expression group (≥ median). [18]

FISH Assessment of HER2 Expression

FISH was performed using the US FDA approved PathVysion® HER2 DNA Probe (Vysis (Abbott) Molecular Inc., Des Plaines, IL, USA), which is a dual-colored probe comprising a locus specific identifier HER2/neu SpectrumOrange which spans HER2 and a centromere enumeration probe (CEP)17 SpectrumGreen which hybridizes to the alpha satellite DNA located at the centromere of the chromosome.

The test was carried out on thin TMA sections using the paraffin pretreatment reagent kit II (Vysis Inc.). FISH procedure was carried out as per manufacturer’s instructions. Hybridization procedure was performed using co-hybridization method using the HYbrine denaturation/hybridization unit (Vysis Inc). The melt temperature of the HYbrite system was set to 73°C and the melt time to 5 min. The hybridization temperature was set to 37°C and the hybridization time to 20 h.

FISH signals were analyzed using BX51/61 Olympus fluorescent microscope equipped with a suitable set of filters including: DAPI single band pass, dual band pass (FITC/TRITC), and triple band pass (FITC/TRITC/DAPI) (Olympus, UK LTD). The fluorescent microscope is attached to a digital camera, and the results were interpreted using cytovision FISH software (Applied Imaging).

As regards interpretation of FISH results, a minimum of 100 interphase nuclei for each case were counted. Fields showing excess background signals or auto-fluorescence masking the nuclear signals were not evaluated. The total number of red and green signals counted in the nuclei was recorded and then ratio of the HER2 (red) to CEP17 (green) signals was calculated. A ratio of HER2 to CEP17 signals ≥ 2 was reported as amplification while a ratio <2 was reported as no-amplification.

Statistical Analysis

Data were analyzed, applying SPSS, version 16.0 for Windows (SPSS Inc, IBM, Chicago, Illinois). Chi-square (χ²) test was used to test significant differences in categorical variables among various groups. Survival data were analyzed using Kaplan–Meier analysis. A comparison of survival curves was carried out using the log-rank test. A two-tailed P ≤ 0.05 was considered significant in all tests.

RESULTS

In total, 150 CRC cases were analyzed. Ages ranged from 20 to 80 years (mean = 52.7, SD = 13.094). The patients were 93 males and 57 females with a M: F ratio of 1.6:1.

Relation between CRC Variants and Clinicopathological Parameters and Prognosis

CA was significantly associated with older age, lower nodal, and TNM stages, and absent perineural invasion compared to all other variants; conversely, SRCC was significantly associated with younger age, higher nodal and TNM stages, and positive perineural invasion than all other variants (P = 0.014, 0.020, 0.032, and < 0.001, respectively). SRCC and MA were significantly associated with greater depth of invasion than other variants (P = 0.022). For the remaining clinicopathological factors, there were no significant differences between CRC variants.

Overall survival (OS) and median OS were significantly different between various CRC variants (P<0.001). The 5-year OS rate and median OS were the highest for CA patients (55.3% and 61 months, respectively) and the lowest in patients with SRCC (10.5% and 19 months, respectively) [Figure 1a]. Similarly, disease-free survival (DFS) and median DFS were significantly different between CRC variants (P < 0.001). The 3-year DFS and median DFS were the highest for patients with CA (65% and 61 months, respectively) and the lowest in patients with SRCC (11.1% and 14 months, respectively) [Figure 1b].

The relation between CRC variants and clinicopathological features and patients’ survival is illustrated in Table 1.
**HER2 Protein Expression and Gene Amplification**

HER2 was negative in all the 15 normal colorectal mucosa and the 15 adenomatous tissues by IHC and FISH. 127 CRC cases were analyzed by IHC and FISH as 23 cores were lost. Cytoplasmic HER2 overexpression was detected in 18/127 CRC cases (14.2%) which was significantly higher than the group of normal mucosa and adenomas ($P < 0.001$). Two cases additionally showed lateral membranous expression in $<10\%$ of tumor cells. None of the 18 cases showed pure membranous HER2 staining. The rest of the cases showed negative or low expression [Figure 2a and b]. HER2 immunoexpression in CRC cases was significantly different among the histopathological variants of CRCs with the AMC variant showing the highest frequency of HER2 overexpression (44.4%) compared to other variants ($P = 0.015$) [Table 2].

Among the 18 HER2 overexpressing CRC cases, 15 cases (83.33%) showed HER2 gene amplification by FISH and 3 cases were non-amplified [Figure 3a and b]. There was a statistically significant strong relation between the results of HER2 protein expression by IHC and gene amplification by FISH ($\chi^2 = 1.240; P < 0.001$).

* $P \leq 0.05$ is significant, CRC: Colorectal carcinoma, CA: Conventional adenocarcinoma, AMC: Adenocarcinoma with mucinous component, MA: Mucinous adenocarcinoma, SRCC: Signet ring cell carcinoma, DFS: Disease-free survival, OS: Overall survival, CI: Confidence interval

**Table 1: Relation between CRC variants and clinicopathological features and patients’ survival**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CA (n=47) (%)</th>
<th>AMC (n=28) (%)</th>
<th>MA (n=56) (%)</th>
<th>SRCC (n=19) (%)</th>
<th>Chi-square ($\chi^2$)</th>
<th>$P$ value</th>
</tr>
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<tbody>
<tr>
<td>Age (y)</td>
<td></td>
<td></td>
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<tr>
<td>$&lt;40$</td>
<td>6 (12.8)</td>
<td>4 (14.3)</td>
<td>13 (23.2)</td>
<td>9 (47.4)</td>
<td>$\chi^2=10.676$</td>
<td>$P=0.014^*$</td>
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<tr>
<td>$\geq40$</td>
<td>41 (87.2)</td>
<td>24 (85.7)</td>
<td>43 (76.8)</td>
<td>10 (52.6)</td>
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<tr>
<td>Depth of invasion (T)</td>
<td></td>
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<td></td>
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<tr>
<td>T1/T2</td>
<td>13 (27.7)</td>
<td>5 (17.9)</td>
<td>6 (10.7)</td>
<td>0 (0)</td>
<td>$\chi^2=9.609$</td>
<td>$P=0.022^*$</td>
</tr>
<tr>
<td>T3/T4</td>
<td>34 (72.3)</td>
<td>23 (82.1)</td>
<td>50 (89.3)</td>
<td>19 (100)</td>
<td></td>
<td></td>
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<tr>
<td>Nodal stage (N)</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>N0</td>
<td>27 (57.4)</td>
<td>11 (39.3)</td>
<td>24 (42.8)</td>
<td>3 (15.8)</td>
<td>$\chi^2=9.875$</td>
<td>$P=0.020^*$</td>
</tr>
<tr>
<td>N1</td>
<td>12 (25.5)</td>
<td>13 (46.4)</td>
<td>16 (28.6)</td>
<td>3 (15.8)</td>
<td></td>
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<tr>
<td>N2</td>
<td>8 (17.1)</td>
<td>4 (14.3)</td>
<td>16 (28.6)</td>
<td>13 (68.4)</td>
<td></td>
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<tr>
<td>TNM stage</td>
<td></td>
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<tr>
<td>I/II</td>
<td>26 (55.3)</td>
<td>11 (39.3)</td>
<td>24 (42.9)</td>
<td>3 (15.8)</td>
<td>$\chi^2=8.818$</td>
<td>$P=0.032^*$</td>
</tr>
<tr>
<td>III/IV</td>
<td>21 (44.7)</td>
<td>17 (60.7)</td>
<td>32 (57.1)</td>
<td>16 (84.2)</td>
<td></td>
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<tr>
<td>Perineural invasion</td>
<td></td>
<td></td>
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<tr>
<td>Negative</td>
<td>38 (80.9)</td>
<td>16 (57.1)</td>
<td>42 (75.0)</td>
<td>6 (31.6)</td>
<td>$\chi^2=17.927$</td>
<td>$P&lt;0.001^*$</td>
</tr>
<tr>
<td>Positive</td>
<td>9 (19.1)</td>
<td>12 (42.9)</td>
<td>14 (25.0)</td>
<td>13 (68.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 year DFS (%)</td>
<td>(63)</td>
<td>(60.7)</td>
<td>(28.3)</td>
<td>(11.1)</td>
<td>$\chi^2=37.283$</td>
<td>$P&lt;0.001^*$</td>
</tr>
<tr>
<td>Median DFS (months)</td>
<td>61 (95% CI=57.7,64.3)</td>
<td>45 (95% CI=30.7,59.3)</td>
<td>19 (95% CI=12.8,25.2)</td>
<td>14 (95% CI=9.8,18.1)</td>
<td>$\chi^2=37.657$</td>
<td>$P&lt;0.001^*$</td>
</tr>
<tr>
<td>5 year OS (%)</td>
<td>(55.3)</td>
<td>(42.9)</td>
<td>(23.2)</td>
<td>(10.5)</td>
<td></td>
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<tr>
<td>Median OS (months)</td>
<td>61 (95% CI=57.7,64.3)</td>
<td>45 (95% CI=32.0,58.0)</td>
<td>22 (95% CI=20.2,23.8)</td>
<td>19 (95% CI=14.7,23.3)</td>
<td>$\chi^2=37.657$</td>
<td>$P&lt;0.001^*$</td>
</tr>
</tbody>
</table>

Figure 1: Kaplan-Meier curves for overall (a) and disease-free (b) survival in colorectal carcinoma histopathological variants. CRC: Colorectal carcinoma, CA: Conventional adenocarcinoma, AMC: Adenocarcinoma with mucinous component, MA: Mucinous adenocarcinoma, SRCC: Signet ring cell carcinoma
Ki67 Protein Expression

127 CRC cases were analyzed for Ki67 immunohistochemical expression as 23 cores were lost. Median Ki67 expression value was 20% (cut-off point). Sixty-one out of 127 analyzed cases (48%) showed high Ki67 expression while 66/127 (52%) showed low Ki67 expression [Figure 2c and d]. Ki67 expression in the 15 normal colorectal mucosa and the 15 adenomatous tissues showed low expression which was significantly lower than CRCs cases (P < 0.001).

Nuclear Ki67 expression was significantly different among the histopathological variants of CRCs with the AMC variant showing the greatest frequency of high Ki67 expression (72.0%) (P = 0.028) [Table 2].

Interrelation between HER2 and Ki67 Immunohistochemical Expression in CRC Cases

Of the 18 CRC cases that showed HER2 overexpression, 12 cases (66.7%) showed concomitant high Ki67 expression and 6 cases (33.3%) showed low Ki67 expression. This was statistically non-significant (P = 0.100) [Table 3]. Similarly, the interrelation between HER2 and Ki67 expressions in CRC variants was not statistically significant (P = 0.633 for CA, P = 0.751 for AMC, and P = 0.382 for MA).

Relation of HER2 and Ki67 immunohistochemical expression in CRC cases with clinicopathological parameters and prognosis

The relation between HER2 expression and clinicopathological parameters of the studied CRC cases is summarized in Table 4. HER2 overexpression was significantly associated with fungating tumors (P = 0.027), negative lymphovascular emboli (P = 0.001), negative lymph node metastasis (P = 0.002), lower stages (P = 0.001), and absence of associated schistosomiasis (P = 0.042). On the other hand, high Ki67 expression was significantly associated with the absence of peritumoral lymphocytic infiltrate (Crohn’s-like response) (P = 0.002).

To clarify the prognostic impact of HER2 and Ki67 expressions on survival of CRC patients, univariate analysis was carried out for each variant separately and showed that neither HER2 nor Ki67 had any significant relation to OS or DFS in any of the CRC study groups [Table 5].
Discussion

Despite the advancement in modern therapies, colorectal carcinoma remains one of the main health problems worldwide. Therefore, identification of biological markers for targeted therapy continues to be a high priority in the field of cancer therapy [4].

Although conventional adenocarcinoma is the most frequently encountered variant of CRCs, other distinct variants with possible prognostic implications are recognized [4]. Some studies have linked MA and SRCC to poor prognosis while others did not show this association [19-22].

In the present study, conventional histology was significantly associated with favorable clinicopathological parameters and better prognosis, while SRCC and MA were significantly associated with less favorable parameters and worse prognosis emphasizing that mucinous histology infers a more adverse prognosis.

In accordance with our data, the studies of Kanemitsu et al. [19] and Hugen et al. [20] reported that the 5-year survival rates in patients with MA and SRCC, respectively, were significantly worse than for those with non-mucinous adenocarcinoma.

Conversely, one large series has shown that the survival rates for patients with mucinous carcinomas were similar to those of conventional adenocarcinoma.
with adenocarcinomas, whereas the signet-ring cell subtype had poor outcomes [21].

With the rapid therapeutic advancement in the era of personalized medicine, the role of pathologists in the management of cancer patients has greatly expanded [4].

The prognostic and predictive roles of HER2 membranous expression in breast and gastric cancers are well established. To date, there is no consensus about the incidence, pattern, and significance of HER2 overexpression in CRCs [6,12].

Overall, it can be deduced that about 5% of colorectal tumors have a membranous overexpression of HER2 while the cytoplasmic overexpression varies strongly with an average around 30% [6,12]. The studies of Kruszewski et al. [12] and Osako et al. [23] revealed even higher percentages of HER2 cytoplasmic staining (66.3% and 68.5%, respectively) in CRCs. On the other hand, Arnaut et al. [24] observed cytoplasmic HER2 staining in 7-34% of CRCs.

In our study, a relatively low frequency of HER2 overexpression (14.2%) in CRCs was documented. Remarkably, the pattern of HER2 staining was cytoplasmic with no pure membranous staining.

The inconsistency of HER2 results between different studies might be attributed to differences in technical approaches, antibodies, and scoring protocols that emphasize the need for standardized staining procedures [6].

Other factors include sample size, racial differences, and intratumoral HER2 heterogeneity that has also been reported in studies of HER2 expression in gastric cancer [9].

The identity and clinical significance of cytoplasmic HER2 are still unclear. In colorectal cancer, accumulating evidence suggests that cytoplasmic HER2 could be implicated in tumor pathogenesis and prognosis. A plausible explanation would be that cytoplasmic HER2 is forming homodimers, leading to an intracellular activation of the tyrosine kinase domain [6].

This could be the basis for trials on the administration of lapatinib, an intracellular tyrosine kinase inhibitor, or other intracellular HER2-targeting compounds, which could indeed be a breakthrough in the treatment of CRC patients [25].

A number of publications have analyzed HER2 overexpression in CRCs with genomic techniques such as FISH and reverse transcription polymerase chain reaction and concluded that for colorectal cancers, there was a strong correlation between genomic amplification and membranous overexpression, whereas no genomic amplification was observed in cases with cytoplasmic HER2 expression [5,6,9].

In a stark contrast to previous studies, our study showed a significant agreement between cytoplasmic HER2 overexpression by IHC and gene amplification by FISH analysis suggesting that, unlike breast cancer, HER2 gene amplification in CRCs is not restricted to membranous expression. As a possible explanation, other mechanisms could exist and govern the ultimate localization of HER2 protein expression in CRC cases with HER2 gene amplification.

The studies of Braut et al. [26,27] on EGFR (another member of the epidermal growth factor receptor family) expression in glottic carcinoma showed a significant correlation between cytoplasmic EGFR staining and gene amplification in cancerous tissue. They concluded that their findings could be a new indicator of differently driven EGFR signaling in glottic cancer.

In the majority of HER2-positive cancers, HER2 protein overexpression is the result of gene amplification [28]. We encountered a minority of HER2 gene/protein discordant cases in this study. This could be attributed to unusual HER2 genotypes, such as polysomy of chromosome 17 and genomic heterogeneity, which can lead to discrepant non-correlating cases [28].

Nevertheless, our observation of high concordance between IHC and FISH results justifies the use of IHC as a method of screening for HER2 gene amplification in CRCs.

The prognostic significance of HER2 in CRCs has been debatable, with some publications associating HER2 overexpression to survival [23,29] while others failed to show such correlation [30,31].

To investigate the clinical relevance of HER2 status, we evaluated its association with clinicopathological variables and prognosis. HER2 overexpression was significantly associated with favorable clinicopathological features notably negative lymphovascular emboli, negative lymph node metastasis, and earlier stages which suggest that targeting HER2 is a possible treatment option for patients with early colon cancer. However, no significant relation was found between HER2 overexpression and patients’ survival.

Our results are in general agreement with Tu et al. [32] and Seo et al. [9]. Tu et al. [32] reported that HER2 overexpression was significantly associated with early stage CRC cases, yet it did not correlate with other clinicopathological data or the survival rate. Seo et al. [9] found that HER2 protein overexpression in CRCs was not associated with infiltrative tumor border, depth of invasion, lymph node metastasis, distant metastasis, and perineural invasion. They concluded that HER2 was not associated with aggressive behavior or worse prognosis in CRCs.

On the other hand, Osako et al. [23] have shown that cytoplasmic HER2 overexpression in CRCs correlated with subserosal invasion, liver metastasis, higher stage, and significantly lower survival rates. They concluded that cytoplasmic HER2 overexpression plays an important role in the progression of colorectal cancer and is considered to be an independent prognostic indicator for this tumor. Similarly, Gill et al. [10] and Elwy et al. [11] found a significant relationship between HER2 expression in CRC and metastatic lymph nodes and advanced tumor stage, respectively.
Other studies reported conflicting results in rectal tumors. Conradi et al. [33] found that HER2 positivity independently correlated with prolonged cancer-specific survival in rectal cancer patients, whereas Sclafani et al. [34] stated that HER2 positivity in rectal cancer had no association with clinicopathologic parameters and patient outcome.

The prognostic value of Ki67 in various malignancies has been extensively studied. The most consistent data for an adverse prognostic value of high proliferative activity have been reported for breast cancer, lung cancer, and sarcomas. In CRCs, data have, to some extent, been contradictory [18,35].

In this study, high proliferative activity was detected in 48% of CRC cases and was significantly associated with the absence of peritumoral lymphocytic infiltrates (Crohn’s-like response). No significant relation was found with any other clinicopathological parameter or patients’ survival.

Oshima et al. [15] found no significant correlation between Ki67 expression and sex, age, or clinical stage of CRCs. Nevertheless, they reported that in colorectal cancer, high proliferative activity was correlated with poor survival and concluded that Ki67 expression may be used as a marker of prognosis. Similarly, Kimura et al. [36] reported an adverse prognostic value of high Ki67 after curative resection for colorectal cancer.

On the other hand, Fluge et al. [18] and Allegra et al. [37] found that high Ki67 was associated with improved survival in CRCs patients who received adjuvant chemotherapy. They elaborated that more rapidly proliferating tumor cells may be more vulnerable to chemotherapy-induced tumor cell death.

It is noteworthy in our study that HER2 and proliferative activity were significantly different among CRC variants with the AMC variant featuring the greatest frequency of HER2 overexpression and high Ki67 expression compared to other variants. Our findings suggest that HER2 and Ki67 expression might play different roles in CRC variants.

Gill et al. [10] reported that mucinous carcinomas showed more positivity (71.4%) for HER2 as compared to conventional adenocarcinomas (64.5%). Other studies failed to document an association between HER2 expression and histopathological variants [11,12].

Nevertheless, our study did not show a significant relation between HER2 and Ki67 immunohistochemical expressions in CRC cases. Previous studies on the expression of both proteins in CRCs did not attempt to investigate their relation [13,38].

Interestingly, our study showed that normal colorectal mucosa and adenomas were negative for HER2 and had a low proliferative activity which was significantly different from CRCs. Our findings imply that the upregulation of HER2 and Ki67 reflects important events in colorectal oncogenesis.

In line with our study, Amaout et al. [24] reported negative HER2 expression in normal colonic mucosa and tubular adenomas and Backus et al. [39] found a higher Ki67 expression in CRCs compared to normal colonic mucosa. On the other hand, Uno et al. [9] found faint or weak HER2 expression in the membrane and/or cytoplasm of the normal colonic epithelium.

CONCLUSIONS

Our study showed that mucin-producing variants of CRC are associated with poor prognosis. The upregulation of HER2 and Ki67 appears to play an important role in colorectal oncogenesis.

Our finding of cytoplasmic HER2 expression in a subset of CRC patients with early disease and possibly a distinct histopathological variant (AMC) suggests that intracellular HER2-targeting compounds may be effective in these patients.

The documented high agreement between HER2 cytoplasmic expression and gene amplification implies that the latter is not restricted to membranous expression and justifies the use of IHC as a method of screening for HER2 gene amplification in CRCs.

We conclude that our findings together with previous studies may serve as a basis for future studies on candidate selection for HER2 targeted therapy in CRC patients.

REFERENCES