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## Retrospective Study

# Significance of HER2 protein expression and *HER2* gene amplification in colorectal adenocarcinomas

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## Abstract

### BACKGROUND

Human epidermal growth factor receptor 2 (HER2) is an oncogenic driver, and a well-established therapeutic target in breast and gastric cancers. While the role of HER2 as a prognostic biomarker in colorectal adenocarcinomas (CRCs) remains uncertain, its relevance as a therapeutic target has been established. We undertook the present study to evaluate the frequency of HER2 expression in CRC and to correlate it with various clinicopathological variables.

### AIM

To correlate HER2 protein expression and *HER2* gene amplification with clinicopathological features and survival in surgically resected CRC.

### METHODS

About 1195 consecutive surgically resected CRCs were analyzed by immunohistochemical staining (IHC) to assess HER2 protein expression, and 141 selected tumors were further evaluated by fluorescence *in situ* hybridization (FISH) to assess *HER2* gene amplification. Follow-up information was available

informed consent to the study because the analysis used anonymous clinical data that were obtained after each patient agreed to treatment by written consent.

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for 1058 patients, and using this information we investigated the prevalence of HER2 protein overexpression and gene amplification in a large series of surgically resected CRCs, and evaluated the relationship between overexpression and clinicopathological parameters and prognosis.

## RESULTS

HER2 IHC scores of 3+, 2+, 1+, and 0 were seen in 31 (2.6%), 105 (8.8%), 475 (39.7%), and 584 (48.9%) tumors, respectively. *HER2* gene amplification was seen in 24/29 tumors with an IHC score of 3+ (82.8%; unreadable in 2/31), 12/102 tumors with an IHC score of 2+ (11.8%; unreadable in 2/104), and 0 tumors with IHC score of 1+ (0/10). *HER2* gene amplification was seen in 36/1191 tumors (3.0%; unreadable in 4/1195). Among the tumors with HER2 IHC scores of 3+ and 2+, the mean percentage of tumor cells with positive IHC staining was 90% (median 100%, range 40%-100%) and 67% (median 75%, range 5%-95%), respectively ( $P < 0.05$ ). Among tumors with IHC scores of 2+, those with *HER2* gene amplification had a higher number of tumors cells with positive IHC staining ( $n = 12$ , mean 93%, median 95%, range 90%-95%) than those without ( $n = 90$ , mean 70%, median 50%, range 5%-95%) ( $P < 0.05$ ). *HER2* gene status was significantly associated with distant tumor metastasis and stage ( $P = 0.028$  and  $0.025$ ). HER2 protein overexpression as measured by IHC or *HER2* gene amplification as measured by FISH was not associated with overall survival (OS) or disease-specific survival for the overall group of 1058 patients. However, further stratification revealed that among patients with tubular adenocarcinomas who were 65 years old or younger ( $n = 601$ ), those exhibiting *HER2* gene amplification had a shorter OS than those without (mean: 47.9 mo *vs* 65.1 mo,  $P = 0.04$ ). Among those patients with moderately to poorly differentiated tubular adenocarcinomas, those with positive HER2 tumor IHC scores (2+, 3+) had a shorter mean OS than those with negative HER2 IHC scores (0, 1+) (47.2 mo *vs* 64.8 mo,  $P = 0.033$ ). Moreover, among patients with T2 to T4 stage tumors, those with positive HER2 IHC scores also had a shorter mean OS than those with negative HER2 IHC scores (47.1 mo *vs* 64.8 mo,  $P = 0.031$ ).

## CONCLUSION

HER2 protein levels are correlated with clinical outcomes, and positive HER2 expression as measured by IHC confers a worse prognosis in those patients 65 years old or younger with tubular adenocarcinomas.

**Key words:** Human epidermal growth factor receptor 2; Immunohistochemical staining; Fluorescent *in situ* hybridization; Prognosis; Colorectal cancers

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**Core tip:** The manuscript investigated the prevalence of human epidermal growth factor receptor 2 (HER2) protein overexpression and gene amplification in a large series of surgically resected colorectal adenocarcinomas (CRCs), and evaluated their relationship with clinicopathological parameters and prognosis. We found that HER2 overexpression [immunohistochemical staining (IHC) score 2+ and 3+] is seen in a small percentage of colorectal adenocarcinomas and *HER2* gene amplification occurs in the vast majority of tumors with 3+ IHC score but in a much lower percentage of tumors with 2+ IHC score. In addition, although the prevalence of HER2 overexpression by IHC in CRCs is low, HER2 protein status is correlated with clinical outcomes and positive HER2 expression by IHC confers a worse prognosis in 65 years or younger patients with tubular adenocarcinomas.

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## INTRODUCTION

Colorectal adenocarcinoma (CRC) is one of the most common malignancies and remains one of the leading causes of cancer-related death in the world<sup>[1]</sup>. The vast majority of colorectal cancers are adenocarcinomas. Until recently, the role of chemotherapy in treating CRC has been fairly limited, and as such there is a need to develop more effective therapeutic regimes for CRC.

Human epidermal growth factor receptor 2 (HER2) represents a promising therapeutic target. The *HER2* gene is a proto-oncogene located on chromosome 17q21 encoding HER2 (ErbB-2), which is involved in cell proliferation and differentiation<sup>[2]</sup>. The HER2 protein is a transmembrane receptor tyrosine kinase and a member of the family of the epidermal growth factor receptors<sup>[3]</sup>. Oncogenic activation of HER2 commonly occurs through gene amplification, which results in protein overexpression on the cell membrane that is involved in signal transduction to regulate cell growth<sup>[4]</sup>. HER2 overexpression/amplification is linked to trastuzumab response in breast/gastric cancers. For breast cancer in particular, HER2 serves as both a prognostic factor and also a therapeutic target, with targeted therapy against HER2 using the monoclonal antibody Trastuzumab (Herceptin) having become the standard of care for patients whose breast carcinomas exhibit *HER2* gene amplification<sup>[5-7]</sup>. A recent randomized phase III trial (ToGA) also revealed that the addition of trastuzumab to chemotherapy improved response rates, median progression-free survival, and overall survival in patients with advanced gastric or gastroesophageal junction cancer with HER2 overexpression<sup>[8]</sup>.

Compared to breast and gastric cancers, the data regarding HER2 protein overexpression and gene amplification in CRCs are limited<sup>[9-18]</sup>. The numbers of cases in these previous studies were relatively small, and their analyses were primarily reliant upon tissue microarrays<sup>[9-18]</sup>. In addition, the reported rates of HER2 protein overexpression ranged widely from 3% to 47.4% across studies, indicating that the clinical significance of HER2 in CRC has not yet been fully clarified. In this study, we assessed HER2 overexpression in 1195 CRC patients *via* immunohistochemical staining (IHC) in 1195 patients and analyzed *HER2* gene status *via* fluorescence *in situ* hybridization (FISH) in a subset of these patients (all tumors with IHC scores of 2+ and 3+, and some a score of 1+). We then correlated HER2 protein overexpression and *HER2* gene status with clinicopathologic features and prognosis to gain insight into the importance of this marker in the context of CRC.

## MATERIALS AND METHODS

### *Patients and tissue specimens*

This investigation was performed after approval was obtained from the Ethics Committee of Peking University Cancer Hospital.

A total of 1195 colorectal cancer (545 rectal cancer and 650 colon cancer) patients who underwent curative surgery between April 2009 and March 2012 in the Peking University Cancer Hospital and Institute were included in this study. None of these patients had undergone preoperative radiation or chemotherapy. Clinicopathologic parameters including age, gender, tumor site, tumor type, tumor differentiation, localization and type of the tumor, tumor T stage, lymph node status, and distant metastasis were retrieved from clinical and pathological databases. Follow-up data was available for a total of 1058 CRC patients, and was retrieved from hospital records by interview, telephone, or mailed letters. Follow-up time started on the day of primary tumor surgical operation. The end point for the disease-associated overall survival (OS) analysis was the time of death of the patient or our last review. Disease free survival (DFS) was defined as the time from the date of operation to the date of diagnosis of metastatic disease or recurrence.

### *Immunohistochemical analysis*

IHC was performed with the Ventana pathway rabbit monoclonal antibody to HER2 (clone 4B5; prediluted) on a Ventana Benchmark automated stainer (Ventana, Tucson, Arizona) following the manufacturer's protocol. Antigen retrieval was performed using Cell Conditioning 1 citrate buffer (pH 6.0; Ventana) for 30 min. Immunostaining was scored by two pathologists using a 4-step scale (0, 1+, 2+, 3+) according to the consensus panel recommendations regarding HER2 scoring for gastric cancer<sup>[19,20]</sup>. The IHC staining was scored: 0 (no staining or membranous staining in less than 10% of tumor cells), 1+ (faint/barely visible membranous staining in at least 10% of cells or staining in parts of their membrane), 2+ (weak to moderate complete or basolateral membranous staining in at least 10% of tumor cells), 3+ (strong complete or

basolateral membranous staining in at least 10% of tumor cells). HER2 IHC scores of 2+ and 3+ were considered as being “HER2 positive”, while IHC scores of 0 and 1+ were considered as being “HER2 negative”.

### FISH

FISH was carried out using the PathVysion HER2 DNA probe kit and procedure (Vysis/Abbott, Abbott Park, Illinois). The kit contains 2 fluorescently labeled DNA probes, HER2 (labeled with Spectrum-Orange) and CEP17 (chromosome 17 enumeration probe labeled with Spectrum-Green). The total numbers of HER2 and CEP17 signals were counted in at least 20 tumor cell nuclei in 2 different areas. FISH staining was evaluated by two different investigators to ensure consistency. The criteria for positive gene amplification were: a HER2/CEP17 signal ratio of 2.0 or higher, or the presence of tight gene clusters as previously reported; otherwise samples were defined as being negative for gene amplification<sup>[14]</sup>.

### Statistical analysis

All statistical analyses were performed using the SPSS software version 20.0 statistical package (SPSS Inc., Chicago, IL, USA). The correlation between HER2 status and patient clinicopathological characteristics was tested *via* the  $\chi^2$  test or Fisher's exact test. Survival curves were fitted using the Kaplan-Meier method, and the differences in survival were assessed by the log rank test. The effects of different factors on patient survival were assessed *via* multivariate analysis with the Cox proportional hazards regression model, and the hazard ratio and associated 95%CI were calculated for each factor. *P*-values < 0.05 (two-sided) were considered as being statistically significant.

## RESULTS

### Clinicopathological features of colorectal cancer patients

Among these 1195 CRC patients, 718 were males and 477 were females. The patient ages ranged from 29 to 92 years (mean: 60.8 years). The tumors were staged as pT1 in 32 patients, pT2 in 175 patients, pT3 in 937 patients, and pT4 in 51 patients (Table 1). Lymph node metastasis was evident in 602 patients (N1 in 296 patients, N2 in 306 patients). Follow-up data was available for a total of 1058 CRC patients, including 640 males and 418 females. These patients ranged in age from 24 to 89 years (mean 61 years). 568 patients had colon cancer, while 490 had rectal cancer patients. The histopathological diagnosis was tubular adenocarcinoma in 971 (91.8%) patients and mucinous adenocarcinoma or other types in 87 (8.2%) patients. According to the TNM classification system, 198 (18.7%) of CRC patients had stage I and II tumors, while 860 (81.3%) of patients had stage III and IV disease. Clinicopathological data are summarized in Table 1.

### HER2 protein overexpression by IHC analysis

Among these 1195 CRC tumors, HER2 IHC scores of 3+, 2+, 1+ and 0 were observed in 31 (2.6%), 105 (8.8%), 475 (39.7%) and 584 (48.9%) tumors, respectively (Table 2, Figure 1). Among the tumors with IHC 3+ and 2+ scores, the mean percentage of tumor cells with positive staining was 90% (median 100%, range 40%-100%) and 67% (median 75%, range 5%-95%), respectively (*P* < 0.05).

### Correlation between HER2 protein overexpression and HER2 gene amplification

All 31 tumors with positive 3+ HER2 staining were further evaluated by FISH to assay for HER2 gene amplification. The FISH results in 29 tumors (29/31; unreadable in 2/31) were evaluable and 24 (24/29, 83%) showed HER2 gene amplification (Table 3, Figure 2). HER2 gene amplification was seen in 12/102 (11.8%; unreadable in 2/104) tumors with HER2 IHC scores of 2+, and 0 (0/10) tumors with IHC scores of 1+ (Table 3, Figure 1). Among the 104 tumors with IHC scores of 2+, those with HER2 gene amplification had a higher number of tumor cells with positive IHC staining (*n* = 12; mean 93%, median 95%, range 90%-95%) than did those without (*n* = 102; mean 70%, median 50%, range 5%-95%) (*P* < 0.05) (Figure 2).

### Correlation of HER2 gene status with clinicopathological features

HER2 gene amplification was significantly associated with tumor depth of invasion, distant metastasis, and stage (*P* = 0.001, 0.028, and 0.012, respectively; Table 4), while there was no significant association between HER2 gene status and age, sex, tumor site, grade, histology, or lymph node metastasis (*P* > 0.05 for all). And we didn't find any observable differences between right/left-sided tumors in this study (*P* = 0.514).

**Table 1 Clinicopathological features of colorectal cancers**

Clinical / pathological features	<i>n</i>
Gender	
Male	718
Female	477
Age (yr)	
≤ 60	578
> 60	617
Tumor grade	
G1	42
G2	857
G3	296
Tumor stage	
pT1	32
pT2	175
pT3	937
pT4	51
Nodal status	
pN0	593
pN1	296
pN2	306
Tumor type	
Tubular carcinoma	1089
Mucinous carcinoma	106
Localization	
Ascending colon	273
Transverse colon	32
Descending colon	56
Sigmoid	289
Rectum	545
Total number	1195

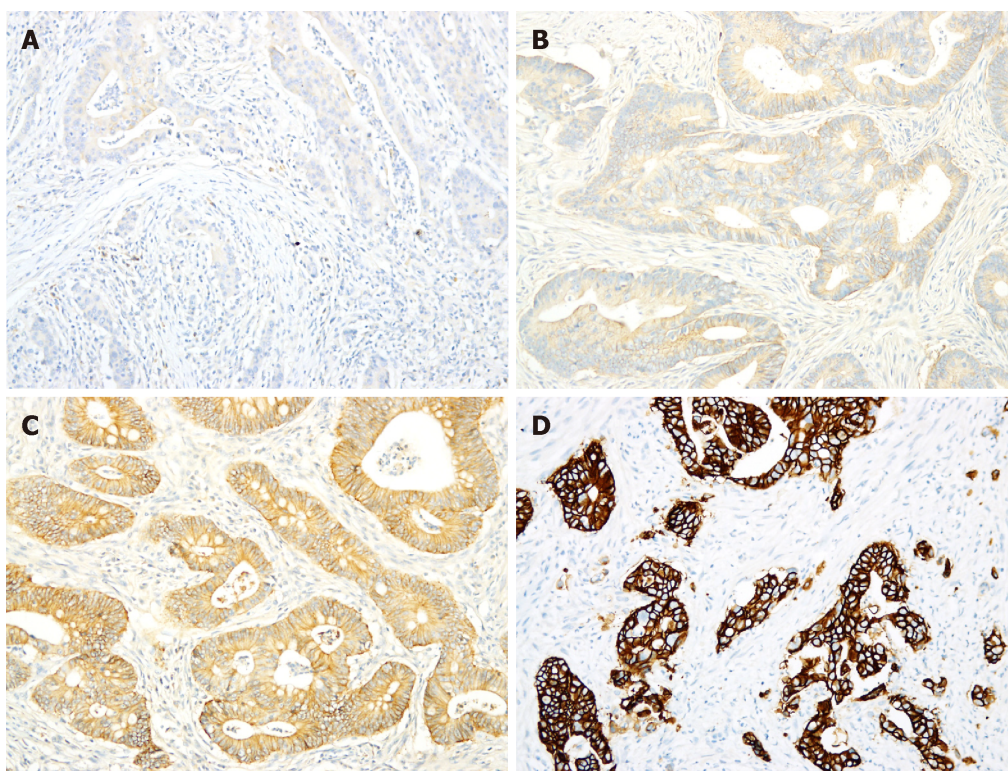
### Correlation of HER2 status with clinical prognosis

A total of 1058 CRC patients with available follow-up data were further assessed for overall survival. Median OS was 49.35 mo (1.1 to 77.0 mo) and median DFS was 48.80 mo (1.0 to 77.0 mo) for the patients without evidence of metastasis before surgery ( $n = 941$ ).

Neither HER2 protein overexpression nor *HER2* gene amplification were associated with OS ( $P = 0.220$  and  $0.458$ , respectively; [Figure 3A](#)). Tumor grade, histologic type, lymph node metastasis, and tumor stage were each significantly associated with OS ( $P < 0.05$  for all; [Table 5](#)). Neither HER2 protein overexpression nor *HER2* gene status was associated with DFS ( $P = 0.320$  and  $0.662$ , respectively).

There was no association between *HER2* gene amplification and OS or DFS among the 971 patients with tubular adenocarcinomas, this was not the case among the 797 patients who were 65 years old or younger. Among this group of patients, HER2 protein overexpression was associated with a shorter mean OS for those with tubular adenocarcinoma (47.1 mo for positive *vs* 65.1 mo for negative,  $P = 0.025$ ,  $n = 601$ ; [Figure 2B](#)). In a particular subpopulation of patients, those with moderately to poorly differentiated tubular adenocarcinomas ( $n = 573$ ), the mean OS in those patients whose tumors did not exhibit *HER2* gene amplification was significantly longer than those with evidence of *HER2* gene amplification (mean: 64.8 mo *vs* 46.4 mo,  $P = 0.020$ ; [Figure 2C](#)). Moreover, among the subgroup of patients with stage T2 to T4 tubular adenocarcinomas ( $n = 580$ ), those tumors had *HER2* gene amplification had a shorter OS compared to those without *HER2* gene amplification (mean: 47.1 mo *vs* 64.8 mo,  $P = 0.031$ ; [Figure 2D](#)).





**Figure 1** Immunohistochemical staining of human epidermal growth factor receptor 2. A,B: Immunohistochemical staining of human epidermal growth factor receptor 2 in most colorectal adenocarcinomas (CRCs) was scored as either 0 (A) or 1+ (B); C: Approximately 9% of CRCs exhibited 2+ staining; D: Only 2.6% of CRCs exhibited 3+ staining.

## DISCUSSION

In this study we assessed HER2 protein overexpression and *HER2* gene amplification in a large series of 1195 colorectal adenocarcinomas. 11.4% (136/1195) of these colorectal adenocarcinomas were positive for HER2 staining by IHC (scores of 2+ and 3+). *HER2* gene amplification was identified in 11.8% (12/102) of tumors with HER2 IHC scores of 2+ and in 82.8% (24/29) of tumors with HER2 IHC scores of 3+. No tumors with HER2 IHC scores of 1+ showed evidence of *HER2* gene amplification by FISH. Our findings indicate that HER2 protein overexpression occurs in a small percentage of colorectal adenocarcinomas, similarly to what has been reported in previous studies. There are only 9 prior studies regarding HER2 status in colorectal adenocarcinomas in the English literature<sup>[9-17]</sup>. Three of these nine studies used tissue microarrays (104, 518, and 1851 patients, respectively) and the remaining 6 used full tissue blocks (ranging from 138 to 317 patients)<sup>[9-17]</sup>. Our study is the largest to date to employ full tumor tissue blocks for analysis (1195 patients). The reported rates of HER2 protein overexpression in the literature ranged from 2.7% to 15.5%, with the exception of one study in which the authors reported 47.4% positive HER2 staining in 137 colorectal adenocarcinomas<sup>[9-17]</sup>. One explanation for this dramatic range in HER2 overexpression positivity across studies may be that antibodies used for staining varied among research groups. The HER2 antibody used in the study with highest positive HER2 staining (47.4%) was a polyclonal rabbit antibody. Even though those authors demonstrated a high HER2 overexpression rate, the percentage of cases exhibiting *HER2* gene amplification was only 8% in those with 3+ HER2 staining, and 0% in 38 cases with 2+ HER2 staining, raising questions regarding the validity of the IHC scoring. The positive rates of HER2 protein staining in studies using the DAKO HER2 antibody were 2.7% to 15.5%, while it was 8% in the study using Ventana pathway antibody by Kavanagh *et al*<sup>[14]</sup> and was 11.4% in our study using this same Ventana pathway.

Our study also indicates that there is a high concordance between HER2 IHC 3+ staining and *HER2* gene amplification in colorectal adenocarcinomas. In our study 83% of tumors (24/29) with 3+ HER2 staining exhibited *HER2* gene amplification. There were 6 other studies in the literature on this topic, however the number of cases analyzed in 5 of these 6 studies was small (less than 10). Marx *et al*<sup>[15]</sup> found that 100% of tumors (27/27) with 3+ HER2 staining (as measured with the DAKO antibody) harbored *HER2* gene amplifications. In the study by Park *et al*<sup>[10]</sup>, only 2 of 27 tumors

**Table 2** Distribution of human epidermal growth factor receptor 2 staining in 1195 colorectal adenocarcinomas

Staining pattern	Number of cases	Positive rate
IHC 3+	31	2.6%
IHC 2+	105	8.8%
IHC 1+	475	39.7%
IHC 0	584	48.9%

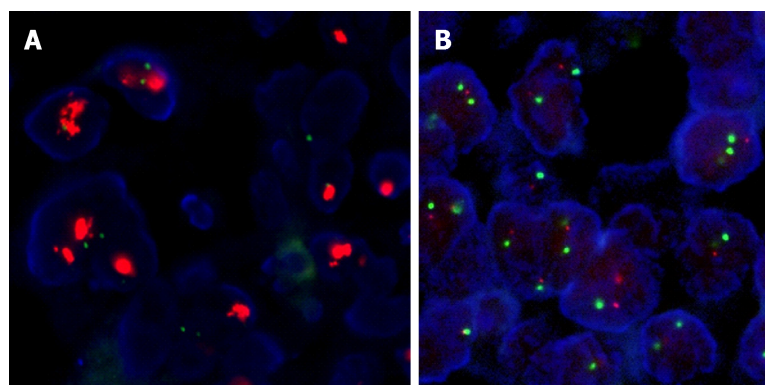
IHC: Immunohistochemical staining.

with 3+ HER2 staining showed evidence of *HER2* gene amplification, however, as described above, the IHC staining in that study was performed using a polyclonal antibody of uncertain reliability. In 3 other smaller studies (2-6 cases with 3+ HER2 staining), this concordance rate with gene amplification was high (100%). Variations in the antibodies used likely led to differential staining and scoring, contributing to the disagreement in *HER2* gene amplification in tumors with 3+ HER2 scores. Nevertheless, these studies, including ours, have shown that HER2 IHC 3+ staining is highly predictive of *HER2* gene amplification in colorectal adenocarcinomas. In contrast, the percentage of tumors with HER2 IHC 2+ staining showing evidence of *HER2* gene amplification was highly variable. In our study, such amplification was only evident in 20% of tumors, similar to what has been reported by Kavanagh *et al*<sup>[14]</sup> (2/9 or 22%, using the Ventana Pathway antibody) and Nathanson *et al*<sup>[9]</sup> (1/3 or 33%, using the DAKO antibody). In contrast, Marx *et al*<sup>[15]</sup> observed *HER2* gene amplification in 75% of tumors with HER2 scores of 2+ (using the DAKO antibody). In the study by Park *et al*<sup>[10]</sup>, using the Zymed antibody, no gene amplification events were observed in such tumors. In the study by Ooi *et al*<sup>[11]</sup>, 2/2 tumors with 2+ HER2 staining showed evidence of *HER2* gene amplification (using an antibody from Nicheri, Japan). Similarly, variations in the antibodies used likely led to differential staining and scoring, contributing to the observed high levels of reported variation in *HER2* gene amplification in tumors with 2+ HER2 scores.

The high concordance between HER2 IHC 3+ staining and *HER2* gene amplification indicates that gene amplification is the major factor accounting for HER2 protein overexpression in colorectal adenocarcinomas with HER2 IHC scores of 3+. However, *HER2* gene amplification was seen in only 12/102 (11.8%) tumors with HER2 IHC scores of 2+, and among these tumors, those with evidence of *HER2* gene amplification had a higher number of tumor cells with positive IHC staining ( $n = 12$ , mean 93%) than those without ( $n = 90$ , mean 70%) ( $P < 0.05$ ). Our study thus confirmed reported results regarding colorectal cancer, and these results were similar to those observed in breast cancer where IHC 3+ HER2 overexpression is highly correlated with *HER2* gene amplification, whereas 2+ staining is equivocal for gene amplification<sup>[21-24]</sup>.

In our present study, *HER2* gene amplification was higher in patients with a more advanced disease stage or distant metastases. This suggests that *HER2* may play some role in tumor progression and would thus be a valuable prognostic factor for certain CRC patients. In our study, the significance of *HER2* gene amplification was best observed in the subpopulation of patients who were 65 years or younger with tubular adenocarcinomas. However, we did not observe a significant difference in prognosis for all colorectal carcinoma patients, regardless of HER2 amplification or HER2 expression status.

Although we found a statistically significant association between *HER2* gene amplification and tumor depth of invasion, advanced stage, and distant metastasis, several other studies have failed to show such associations<sup>[15,19,25,26]</sup>. Those studies, however, reported a correlation between *HER2* gene amplification and high grade histology, higher tumor stage, and positive nodal status<sup>[27,28]</sup>. The role of HER2 as a prognostic factor in CRCs is still controversial, as some of the earlier studies have failed to identify any specific association with prognosis<sup>[28,29]</sup> while others reported a correlation between *HER2* gene amplification and poorer survival<sup>[30]</sup>. Similar observations have been made for gastric cancer, although a larger number of studies have supported the association between HER2 overexpression and poorer survival<sup>[31]</sup>. Possible explanations for such discrepancies include: Antibody resources, detection methods, scoring systems, and patient populations (different ethnic groups with different genetic backgrounds). It has been clearly demonstrated that *HER2* gene amplification differs significantly between right/left-sided and rectal carcinomas. We were not able to produce similar results, potentially due to differences in groups



**Figure 2 Fluorescence *in situ* hybridization results.** By fluorescence *in situ* hybridization, all tested tumors with 1+ human epidermal growth factor receptor 2 (HER2) staining and 80% of tumors with 2+ staining showed no evidence of *HER2* gene amplification (A) whereas 83% of tumors with 3+ HER2 staining harbored *HER2* gene amplifications (B).

analyzed, HER2 testing methods, and tumor biological characteristics<sup>[32,33]</sup>.

In summary, we investigated HER2 protein status and *HER2* gene status in a large series of colorectal adenocarcinomas. Our results show that HER2 protein overexpression is evident in 11.4% of colorectal adenocarcinomas. HER2 IHC scores of 3+ are highly correlated with *HER2* gene amplification, while this correlation is weaker in tumors with IHC 2+ staining. We also observed an association between *HER2* gene amplification and prognosis in patients with tubular adenocarcinomas who were 65 years or younger. Our study indicates that a certain population of patients with colorectal adenocarcinomas may benefit with HER2-targeted therapy.

**Table 3** Concordance between human epidermal growth factor receptor 2 overexpression by Immunohistochemical staining and human epidermal growth factor receptor 2 gene amplification by fluorescence *in situ* hybridization

IHC score	HER2 gene amplification (FISH)		% HER2 gene amplification
	Positive	Negative	
1+ ( <i>n</i> = 10)	0	10	0%
2+ ( <i>n</i> = 102)	12	90	11.8%
3+ ( <i>n</i> = 29)	24	5	82.8%

HER2: Human epidermal growth factor receptor 2; FISH: Fluorescence *in situ* hybridization; IHC: Immunohistochemical staining.

**Table 4** Association of human epidermal growth factor receptor 2 gene status with clinicopathological features *n* (%)

Clinicopathological features	HER2 status		P-value
	HER2 negative	HER2 positive	
Gender			0.441
Male	616 (96.2)	24 (3.8)	
Female	406 (97.1)	12 (2.9)	
Age (yr)			0.272
≤ 60	501 (96.0)	21 (4.0)	
> 60	521 (97.2)	15 (2.8)	
Tumor grade			0.784
1	39 (97.5)	1 (2.5)	
2	773 (96.7)	26 (3.3)	
3	210 (95.9)	9 (4.1)	
Histology			0.762
Tubular	937 (96.5)	34 (3.5)	
Mucinous/other types	85 (97.7)	2 (2.3)	
Tumor site			0.819
Colon	548 (96.5)	20 (3.5)	
Rectal	474 (96.7)	16 (3.3)	
Left-sided colon	203 (88.3)	27 (37.0)	0.514
Right-sided colon	292 (86.4)	46 (63.0)	
Depth of invasion			0.039
T1	31 (100.0)	0 (0.0)	
T2	165 (98.8)	2 (1.2)	
T3	815 (96.3)	31 (3.7)	
T4	11 (78.6)	3 (21.4)	
T1-2	196 (99.0)	2 (1.0)	0.001
T3-4	826 (96.0)	34 (4.0)	
Lymph node metastasis			0.260
N0	541 (96.3)	21 (3.7)	
N1	261 (98.1)	5 (1.9)	
N2	220 (95.7)	10 (4.3)	
No	541 (96.3)	21 (3.7)	0.524
Yes	481 (97.0)	15 (3.0)	
Distant metastasis			0.028
No	940 (96.9)	30 (3.1)	
Yes	81 (92.0)	7 (8.0)	
Tumor stage			0.012
(1)I-II	192 (98.5)	2 (1.0)	
III	749 (96.5)	27 (3.5)	
IV	81 (92.0)	7 (8.0)	
(3)I-II	192 (99.0)	2 (1.0)	0.044

III-IV	830 (96.1)	34 (3.9)
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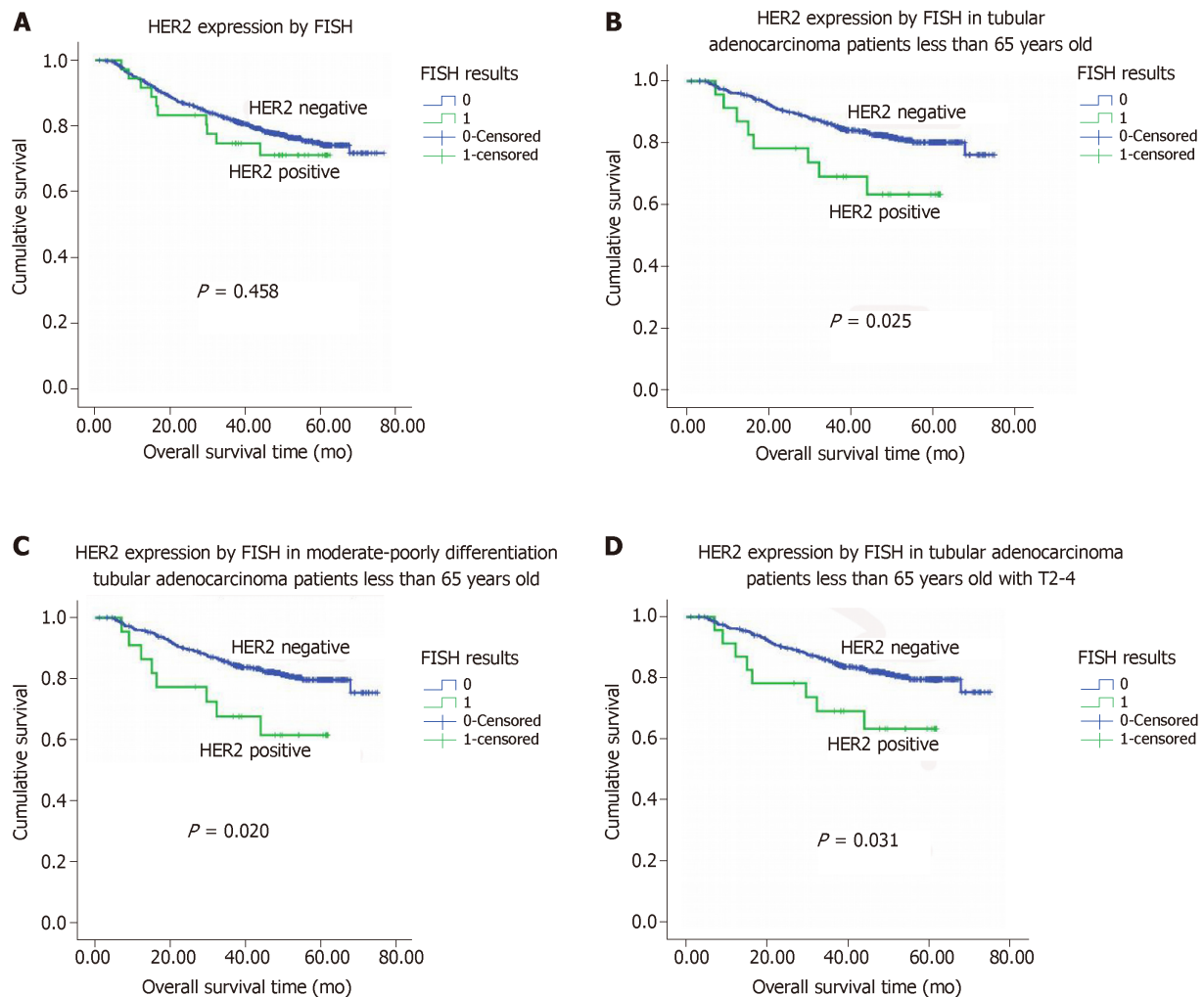
HER2: Human epidermal growth factor receptor 2.

**Table 5 Overall survival based on clinicopathological features**

Clinicopathological features	Patients	Events	Median survival (95%CI), mo	P-value
Gender				0.707
Male	640	144	NA	
Female	418	100	NA	
Age (yr)				0.591
≤ 60	522	116	NA	
> 60	536	128	NA	
≤ 65	661	138	NA	0.027
> 65	397	106	NA	
Tumor site				0.457
Colon	568	133	NA	
Rectal	490	111	NA	
Tumor grade				0.000
1-2	839	176	NA	
3	219	68	NA	
Histology				0.000
Tubular	971	205	NA	
Mucinous/ Other types	87	39	54.90	
Lymph node metastasis				0.000
No	562	61	NA	
Yes	496	183	NA	
Tumor stage				0.000
I-II	198	13	NA	
III-IV	860	231	NA	
HER2 protein overexpression by IHC				0.220
IHC (-)	935	221	NA	
IHC (+)	123	23	NA	
HER2 gene amplification by FISH				0.458
Positive	1022	234	NA	
Negative	36	10	NA	

NA: Because the overall survival rates of these patients were more than 50%, we didn't get the median survival time. HER2: Human epidermal growth factor receptor 2; FISH: Fluorescence *in situ* hybridization; IHC: Immunohistochemical staining.





**Figure 3 Kaplan-Meier overall survival analysis.** A: Kaplan-Meier overall survival (OS) analysis of colorectal adenocarcinoma patients based on human epidermal growth factor receptor 2 (HER2) expression status (as determined by fluorescence *in situ* hybridization); B: Kaplan-Meier OS analysis of tubular adenocarcinoma patients age  $\leq 65$  based on HER2 expression status; C: Kaplan-Meier OS analysis of tubular adenocarcinoma patients with moderate-poor differentiation and age  $\leq 65$  based on HER2 expression status; D: Kaplan-Meier overall survival analysis of tubular adenocarcinoma patients with stage T2-4 tumors and age  $\leq 65$  based on HER2 expression status. HER2: Human epidermal growth factor receptor 2; FISH: Fluorescence *in situ* hybridization.

## ARTICLE HIGHLIGHTS

### Research background

The vast majority of colorectal cancers are adenocarcinomas. Until recently, the role of chemotherapy in treating colorectal adenocarcinomas (CRCs) has been fairly limited, and as such there is a need to develop more effective therapeutic regimes for CRC. Human epidermal growth factor receptor 2 (HER2) is an oncogenic driver; it's a well-established therapeutic target in breast and gastric cancers.

### Research motivation

The role of HER2 as a prognostic biomarker in CRCs remains uncertain, but its relevance as a therapeutic target has been established.

### Research objectives

In this study, the authors aim to evaluate the frequency of HER2 expression in CRC and to correlate it with various clinicopathological variables.

### Research methods

In this study, to assess HER2 protein expression, 1195 consecutive surgically resected CRCs were analyzed by immunohistochemical staining (IHC). And to assess HER2 gene amplification, 141 selected tumors were further evaluated by fluorescence *in situ* hybridization (FISH). The authors investigated the prevalence of HER2 protein overexpression and gene amplification in a large series of surgically resected CRCs, and evaluated the relationship between overexpression and clinicopathological parameters and prognosis.

## Research results

HER2 gene amplification was seen in 24/29 tumors with an IHC score of 3+, 12/102 tumors with an IHC score of 2+, and 0 tumors with IHC score of 1+ (0/10). HER2 gene amplification was seen in 36/1191 tumors. Among the tumors with HER2 IHC scores of 3+ and 2+, the mean percentage of tumor cells with positive IHC staining was 90% and 67%. Among tumors with IHC scores of 2+, those with HER2 gene amplification had a higher number of tumors cells with positive IHC staining than those without. HER2 gene status was significantly associated with distant tumor metastasis and stage. HER2 protein overexpression as measured by IHC or HER2 gene amplification as measured by FISH was not associated with overall survival (OS) or disease-specific survival for the overall group of 1058 patients. Among those patients with moderately to poorly differentiated tubular adenocarcinomas, those with positive HER2 tumor IHC scores (2+, 3+) had a shorter mean OS than those with negative HER2 IHC scores (0, 1+).

## Research conclusions

HER2 protein levels are correlated with clinical outcomes, and positive HER2 expression confers a worse prognosis in patients 65 years old or younger with tubular adenocarcinomas.

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