Cancer Management and Research

Dovepress
open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

Comprehensive assessment of *HER2* alteration in a colorectal cancer cohort: from next-generation sequencing to clinical significance

This article was published in the following Dove Press journal:
Cancer Management and Research

Yu-Min Yeh[1,2]
Chun-Hui Lee[2]
Shang-Hung Chen[2,3]
Chung-Ta Lee[4]
Yi-Lin Chen[4]
Bo-Wen Lin[5]
Shao-Chieh Lin[5]
Ren-Hao Chan[5]
Jenq-Chang Lee[5]
Meng-Ru Shen[6,7]
Peng-Chan Lin[2]

[1]Graduate Institute of Clinical Medicine, National Cheng Kung University Hospital, College of Medicine, National Cheng Kung University, Tainan City, Taiwan; [2]Department of Internal Medicine, National Cheng Kung University Hospital, College of Medicine, National Cheng Kung University, Tainan City, Taiwan; [3]National Institute of Cancer Research, National Health Research Institutes, Hsinchu, Taiwan; [4]Department of Pathology, National Cheng Kung University Hospital, College of Medicine, National Cheng Kung University, Tainan City, Taiwan; [5]Division of Colorectal Surgery, Department of Surgery, National Cheng Kung University Hospital, College of Medicine, National Cheng Kung University, Tainan City, Taiwan; [6]Department of Pharmacology, National Cheng Kung University Hospital, College of Medicine, National Cheng Kung University, Tainan City, Taiwan; [7]Department of Obstetrics and Gynecology, National Cheng Kung University Hospital, College of Medicine, National Cheng Kung University, Tainan City, Taiwan

Correspondence: Peng-Chan Lin
Department of Internal Medicine, National Cheng Kung University Hospital, No. 138, Sheng-Li Road, Tainan 704, Taiwan
Tel +886 6 235 3535, ext. 4620
Email pengchanlin@gmail.com

Purpose: Human epidermal growth factor receptor 2 (HER2) is an emerging therapeutic target in colorectal cancer (CRC). Currently, immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) have been used to determine HER2-positive CRCs; however, the clinical utility of next-generation sequencing (NGS)-based techniques for determining HER2 status in CRC has been limited. Here, we detail our experience regarding the assessment of *HER2* alterations in a CRC cohort.

Materials and methods: We prospectively enrolled 73 CRC patients who underwent surgery and received adjuvant oxaliplatin treatment. We then examined *HER2* alterations using the Oncomine Comprehensive Assay version 1, as well as clinical outcomes, in this cohort.

Results: Using the NGS-based assay, *HER2* copy number gains in 12 of 73 CRCs were determined to range from 2.74 to 92.62. Of these 12 tumors, 6 had *HER2* high-level copy number gain (92.6, 57.9, 57.0, 52.0, 35.2, and 8.42) and were all defined as HER2-positive CRC using HERACLES Diagnostic Criteria. Nevertheless, other 6 patients with low-level copy number gain (ranging from 2.74 to 3.04) and the remaining 61 patients without increase in *HER2* copy number were all HER2-negative. Among the 6 HER2-positive CRCs, *KRAS* and *PIK3CA* mutations were detected in 1 (17%; G13D) and 2 (33.3%; 1 Q546R and 1 H1047R) patients, respectively. Moreover, 2 of the 6 (33.3%) HER2-positive patients had recurrent disease, while one patient had a partial response after anti-HER2 therapy.

Conclusion: NGS-based tools could assist in the simultaneous detection of *HER2* and other genomic alterations in patients with CRC. Only CRCs with *HER2* high-level copy number gain were HER2-postive by current diagnostic criteria.

Keywords: HER2, colorectal cancer, next generation sequencing, *PIK3CA*

Introduction

Colorectal cancer (CRC) still remains a major cause of cancer-related death worldwide with approximately 1.2 million new cases diagnosed yearly and 600,000 deaths attributed to the disease.[1] Around 25% of patients with CRC develop distant metastases at diagnosis, while 10–30% of patients with stage I–III disease develop recurrence after curative surgery. Despite aggressive surgical resection for limited metastases and recent advances in chemotherapy and targeted therapy, survival rates of patients with metastatic CRC have remained poor with a 5-year survival rate of approximately 12% among patients with stage IV CRC. Large-scale sequencing analyses have been performed to uncover important genetic alterations and

submit your manuscript | www.dovepress.com
DovePress
http://doi.org/10.2147/CMAR.S213247

© 2019 Yeh et al. This work is published and licensed by Dove Medical Press Limited. The full terms of this license are available at https://www.dovepress.com/terms.php and incorporate the Creative Commons Attribution – Non Commercial (unported, v3.0) License (http://creativecommons.org/licenses/by-nc/3.0/). By accessing the work you hereby accept the Terms. Non-commercial uses of the work are permitted without any further permission from Dove Medical Press Limited, provided the work is properly attributed. For permission for commercial use of this work, please see paragraphs 4.2 and 5 of our Terms (https://www.dovepress.com/terms.php).

potential therapeutic targets.[2,3] Clinical trials testing such novel treatment targets, such as the anti-PD1 antibody for mismatch repair deficient CRC,[4] the combination of an anti-EGFR antibody, MEK inhibitor, and BRAF inhibitor for CRC with BRAF mutations,[5] and a Trk inhibitor for CRC with *NTRK* gene fusions,[6] have shown promising results. Human epidermal growth factor receptor 2 (HER2), a well-established and standard therapeutic target in breast and gastric cancer, has also been one of the emerging therapeutic targets in CRC.

Immunohistochemical (IHC) staining, fluorescent in situ hybridization (FISH) and chromogenic in situ hybridization have been the standard methods used for identifying patients with HER2 overexpression. Accordingly, the HERACLES Diagnostic Criteria, which integrates IHC and FISH analyses, has been proposed to determine HER2-postive CRCs.[7] A phase II trial revealed that 5% of *KRAS* wild-type metastatic CRCs were found to be HER2-positive by the HERACLES Diagnostic Criteria. Dual HER2 blockade therapy with trastuzumab and lapatinib has demonstrated an overall response rate of 30% in treatment-refractory HER2-positive metastatic CRCs.[8] The MyPathway trial has also reported similar response rates in patients with *HER2*-amplified/overexpressed metastatic CRC who received anti-HER2 therapy with trastuzumab and pertuzumab.[9] Based on these results, HER2 testing, as well as integration of HER2-directed therapy into the treatment algorithm, has been recommended in patients with metastatic *KRAS* exon 2 wild-type CRC.[9]

Recent advances in next-generation sequencing (NGS) have led to the identification of numerous somatically mutated genes, including single nucleotide variations (SNVs), copy number alterations (CNAs), small insertion/deletions (Indels), and fusion genes, in a single assay. Several NGS approaches have been successfully implemented in clinical practice.[10–12] Accordingly, NGS-based techniques have been a practical tool for detecting various genomic alterations that can offer treatment options in patients with CRC. However, the studies correlating NGS-based *HER2* CNAs with IHC and FISH results in patients with CRC have been limited.[13] Therefore, the present study utilized a targeted NGS assay to analyze *HER2* CNAs in 73 primary tumor tissues from patients with high-risk CRC receiving oxaliplatin treatment after surgery. HER2 expression levels were determined using the HERACLES Diagnostic Criteria. The correlations between HER2 overexpression and CNAs, as well as clinical outcomes in patients with HER2-positive CRC, were also examined.

Materials and methods

Study population

This study was approved by the institutional review board of National Cheng Kung University Hospital (NCKUH) and conducted in accordance with the Declaration of Helsinki. Written informed consent to review the medical records and use tissue samples was obtained from all patients. The confidentiality of patient data was confirmed. A total of 73 patients with pathologically confirmed stage III or high-risk stage II CRC who underwent standard surgical resection and received post-operative adjuvant chemotherapy with mFOLFOX6 (oxaliplatin, 5-fluorouracil, and leucovorin) at NCKUH were enrolled. All patients had adequate primary tumor tissues for genomic analysis. Clinicopathological characteristics, including age, gender, primary tumor location, stage at diagnosis, treatment courses, and clinical outcomes, were obtained from medical records.

HER2 immunohistochemical staining

Colon cancer specimens were fixed in 10% formalin solution and embedded in paraffin according to routine procedures at the Department of Clinical Pathology, NCKUH. Tissue sections were cut from the paraffin block, deparaffinized, and rehydrated with decreasing ethanol grades. HER2 IHC staining was performed using anti-HER2/neu monoclonal antibody (VENTANA 4B5) on an automatic immunostainer (BenchMark XT, Ventana Medical Systems) according to the manufacturer's instruction. According to the HERACLES Diagnostic Criteria,[7] the HER2 status of IHC staining was defined as follows: Positive, intense (3+) in >10% of the tumor cells; equivocal, moderate (2+) in ≥50% of the tumor cells; and negative, intense (3+) ≤10% of the tumor cells, moderate (2+) in <50%, faint (1+) in any cellularity, or no staining.

HER2 FISH

The *HER2* FISH assay was performed using PathVysion *HER2* DNA Probe Kit II (Abbott Laboratories, Des Plaines, IL, USA). In brief, tumor specimen slides were deparaffinized, dehydrated using 100% ethanol, and then pretreated with Vysis Paraffin Pretreatment Reagent Kit (Abbot Molecular, Des Plaines, IL, USA) according the manufacturer's instructions. After slide pretreatment, the

specimen DNA was denatured, dehydrated in serial ethanol solutions, and then hybridized with probe mixture at 37 °C overnight. After hybridization, a series of washes were performed to remove unbound probes, and nuclei were counterstained with 4′,6 diamidino-2-phenylindole. A fluorescence microscope was used to detect hybridization of HER2/neu (orange) and CEP 17 DNA (green) probes. HER2/neu and CEN17 signals from 100 nuclei per case were counted with a HER2/CEP17 ratio ≥2.0 indicating amplification.

Next-generation sequencing

The use of Oncomine™ Comprehensive Assay version 1, a targeted NGS assay, to detect relevant SNVs, CNAs, fusion genes, and Indels from 143 genes has been previously described and validated.[10] Genomic analysis using Oncomine™ Comprehensive Assay was performed on formalin-fixed, paraffin-embedded primary colon tumor samples obtained through surgical resection. Sequencing was performed on the Ion Torrent NGS platform with the average sequencing depth of 500×. Germline DNA was isolated from peripheral blood. The entire genome was sequenced using NGS on the Illumina HiSeq® 2500 system to analyze germline genetic variants used to determine somatic mutations. Genomic data were processed and analyzed using several different bioinformatics platforms as described.[10]

Statistical analysis

Kaplan–Meier survival analysis was used to estimate survival among patients with HER2-positive and HER2-negative CRC. A log-rank test was performed to compare the difference between both groups. A p-value <0.05 was considered statistically significant.

Results

Clinical characteristics of patients with CRCs

The clinical characteristics of the patients included herein are shown in Table 1. The mean age of all 73 patients was 58 years (range 30–78) among whom 30 (41.1%) were males and 43 (58.9%) were females. All patients underwent standard surgical resection followed by adjuvant chemotherapy with mFOLFOX6. Among the 73 patients, 68 (93.2%) were diagnosed with pathological stage III disease and 5 (6.8%) with high-risk stage II disease. The primary tumor sites analyzed included the ascending colon (12.3%), transverse colon (4.1%), descending colon (12.3%), and rectal and sigmoid colon (69.9%). One (1.4%) patient simultaneously developed primary lesions over the cecum, descending colon, and rectum. *KRAS,*

Table 1 Clinical characteristics of the 73 patients with colorectal cancer

Clinical characteristic	HER2 (−) CRC N (%)	HER2 (+) CRC N (%)	Total N (%)
Age			
Mean ± SD	57.5±11.1	59.4±9.8	57.7±10.9
Gender			
Male	29 (43.3)	1 (16.7)	30 (41.1)
Female	38 (56.7)	5 (80.3)	43 (58.9)
Stage			
II	5 (7.5)	0	5 (6.8)
IIIA	8 (11.9)	0	8 (11.0)
IIIB	40 (59.7)	5 (83.3)	45 (61.6)
IIIC	14 (20.9)	1 (16.7)	15 (20.6)
Location			
Ascending	9 (13.4)	0	9 (12.3)
Transverse	3 (4.5)	0	3 (4.1)
Descending	7 (10.4)	2 (33.3)	9 (12.3)
Rectosigmoid	47 (70.2)	4 (66.7)	51 (69.9)
Multiple	1 (1.5)	0	1 (1.4)
KRAS			
Wild type	37 (55.2)	5 (83.3)	42 (57.5)
Mutant	30 (44.8)	1 (16.7)	31 (42.5)
G12D	9 (13.4)	0 (0)	9 (12.3)
G13D	5 (7.5)	1 (16.7)	6 (8.2)
G12V	3 (4.5)	0 (0)	3 (4.1)
Others*	13 (19.4)	0 (0)	13 (17.8)
NRAS			
Wild type	65 (97.0)	6 (100)	71 (97.3)
Mutant	2 (3.0)	0 (0)	2 (2.7)
G12D	1 (1.5)	0 (0)	0 (0)
G12V	1 (1.5)	0 (0)	0 (0)
BRAF V600E			
Wild type	61 (91.0)	6 (100)	67 (91.8)
Mutant	6 (9.0)	0 (0)	6 (8.2)
PIK3CA			
Wild type	55 (82.1)	4 (66.7)	59 (80.8)
Mutant	12 (17.9)	2 (33.3)	14 (19.2)
E545K	4 (6.0)	0 (0)	4 (5.5)
Others+	8 (11.9)	2 (33.3)	10 (13.7)

Notes: *Including 2 A146T, 2 Q61H, 2 G12C, 2 G12S, 1 D132N, 1 Q61L, 1 G15D, 1 G12W and 1 G13C mutation; +Including 1 N345I, 1 N345K, 1 G363R, 1 C420R, 1 E542K, 1 E545D, 1 E689K and 1 E726K mutation.
Abbreviations: CRC, colorectal cancer; SD, standard deviation.

NRAS, BRAF, and *PIK3CA* mutations were detected in 43%, 3%, 8%, and 19% of the 73 patients with CRC, respectively.

Correlation between *HER2* expression levels and copy number alterations in CRCs

Through the Oncomine Comprehensive Assay, *HER2* copy number gains (>2) were identified in 12 of the 73 CRCs (16.4%) with copy numbers ranging from 2.74 to 92.62 (Table 2 and Figure 1A). Among these 12 patients, 6 had high-level copy number gains (92.6, 57.9, 57.0, 52.0, 35.2, and 8.42). All of them had intense (3+) HER2 IHC staining in >50% tumor cells, which can be categorized as HER2-positive CRC according to the HERACLES Diagnostic Criteria as shown in Figure 1B. For cases with copy numbers of 92.62 and 8.42, the HER2/CEP17 ratio using FISH was 6.26 and 4.51, respectively, which confirmed the *HER2* amplification status. The mean age of the 6 patients with HER2-postive CRC was 59 years, among whom 5 (80%) were female. All primary tumors were located within the distal colon (Table 1). Six cases with low-level copy number gain (ranging from 2.74–3.03) and the remaining 61 patients without increased *HER2* copy number all showed negative (0+) HER2 IHC staining, which can be categorized as HER2-negative CRC using the HERACLES Diagnostic Criteria (Figure 1A and C). No *HER2* mutation was detected using the Oncomine Comprehensive Assay in our CRC cohort.

Table 2 Patients with colorectal cancer having increased *HER2* copy number

Case No.	CNA	FISH	IHC staining	HER2 status*
1	92.62	6.26	3+, >50%	Positive
2	57.86	ND	3+, >50%	Positive
3	57.04	ND	3+, >50%	Positive
4	52.04	ND	3+, >50%	Positive
5	35.16	ND	3+, >50%	Positive
6	8.42	4.51	3+, >50%	Positive
7	3.04	1.45	0+	Negative
8	2.98	ND	0+	Negative
9	2.86	ND	0+	Negative
10	2.82	ND	0+	Negative
11	2.76	ND	0+	Negative
12	2.74	ND	0+	Negative

Note: *Determined using the HERACLES Diagnostic Criteria.
Abbreviations: CRC, colorectal cancer; HER2, human epidermal growth factor receptor 2; CNA, copy number alteration; FISH, fluorescence in situ hybridization; IHC, immunohistochemical; ND, not done.

Considering the limited sample size included herein, the available TCGA database[2] was used to study the association between *HER2* expression and copy number alteration. *HER2* mRNA expression levels and putative copy number alterations estimated using the Genomic Identification of Significant Targets in Cancer (GISTIC) algorithm were available in 193 CRCs. Using the GISTIC algorithm, 7, 29, 144, and 13 of the 193 cases were categorized under *HER2* amplification, gain, diploid, and shallow deletion, respectively. As shown in Figure 1D, *HER2*-amplified CRCs had around 18.1–32.7-fold higher *HER2* mRNA expression levels than CRCs with *HER2* gain, diploid, or shallow deletion. Compared to CRCs with diploid *HER2*, those with *HER2* gain had 1.3-fold higher *HER2* mRNA expression level. CRCs with *HER2* shallow deletion had the lowest *HER2* mRNA expression levels. These results imply that CRCs with increased *HER2* amplification estimated using the GISTIC algorithm or high-level copy number gains are correlated with higher *HER2* transcripts or protein overexpression. HER2 expression levels of CRCs should however be interpreted judiciously in patients with modest copy number gains.

KRAS, NRAS, BRAF, and *PIK3CA* mutations in HER2-positive CRCs

The 143 genes analyzed by the Oncomine Comprehensive Assay, including *TP53, APC, KRAS, NRAS, BRAF*, and *PIK3CA*, were all crucial oncogenes reported for CRC tumorigenesis. *TP53* mutations were detected in nearly 100% of the 73 CRCs (Figure 2A). The frequency of *APC* mutations was 82% and 83% in HER2-negative and -positive CRCs, respectively. Among the 67 HER2-negative CRCs, *KRAS, NRAS*, and *BRAF* mutations were identified in 45%, 3%, and 9%, respectively (Table 1 and Figure 2A). Although previous studies reported that *HER2* amplification and *KRAS, NRAS*, and *BRAF* mutations were mutually exclusive in advanced CRCs,[2] *KRAS* G13D (p.Gly13Asp) mutations was detected in one of six HER2-postive CRCs (Figure 2B). No HER2-positive CRCs carrying *NRAS* or *BRAF* mutations were observed. *PIK3CA* mutations have been reported in around 25–30% of CRCs.[14] In the present study, 14 (19%) of the 73 CRCs carried *PIK3CA* mutations (Table 1). *PIK3CA* mutations were detected in 12 (18%) of the 67 HER2-negative cases. In contrast, 2 (33%) of 6 HER2-positive CRCs harbored *PIK3CA* mutations, including p.Gln546Arg and p. His1047Arg (Figure 2C).

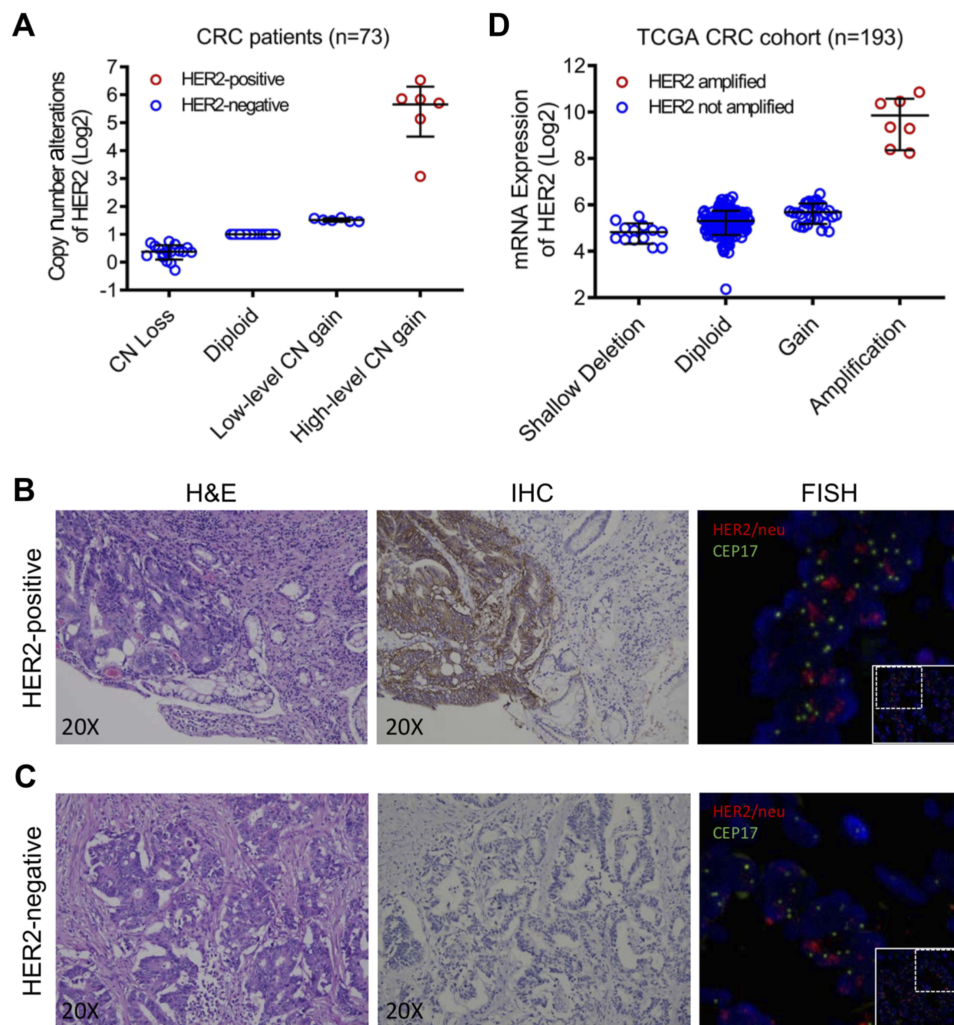

Figure 1 HER2 expression and copy number alterations in colorectal cancer (CRC). (**A**) Copy number (CN) alterations in the 73 CRCs included herein were analyzed using the Oncomine Comprehensive Assay. Red and blue circles represent HER2-positive and -negative CRCs, respectively, as determined using the HERACLES Diagnostic Criteria. (**B**) A representative histologic section of HER2-positive CRC showed moderately differentiated adenocarcinoma with a disoriented arrangement and confluent glandular structures (left). IHC staining with anti-HER2 antibody revealed intense (3+) HER2 expression (middle), while FISH analysis showed HER2 amplification with a HER2/CEN17 ratio of 6.26 (right). (**C**) A representative Hematoxylin and Eosin staining of HER2-negative CRC (left) with 0+ HER2 IHC staining (middle) and a non-amplified HER2/CEN17 ratio of 1.45 (right). (**D**) *HER2* mRNA expression in 193 CRCs obtained from the TCGA database was analyzed. Putative copy number alterations were estimated using the Genomic Identification of Significant Targets In Cancer (GISTIC) algorithm. Red and blue circle represent HER2-amplified and HER2-nonamplified CRCs determined using GISTIC.

Clinical impact of *HER2* amplification on CRC

Among the 73 CRC patients, 2 (33%) of the 6 HER2-positive and 20 (29.9%) of the 67 HER2-negative cases developed recurrence. In the 20 HER2-negative cases with recurrence, 15 cases had no *HER2* copy number alteration (copy number =2) and the remaining 5 cases had copy number loss (copy number <2). For 6 cases with low-level HER2 copy number gain, there was no recurrence detected. Although a small increase in the recurrence rate was observed in HER2-positive cases, HER2 positivity was not significantly associated with shorter recurrence-free survival [hazard ratio (HR) 1.18, 95% CI 0.25–5.53; p=0.8226], as shown in Figure 3A. Among the two HER2-positive patients who had recurrent disease, one had peritoneal seeding and needed surgical intervention to relieve the obstruction related to small-bowel ileus. This patient subsequently received FOLFIRI regimen in combination with bevacizumab as first-line treatment followed by salvage chemotherapy with irinotecan plus cetuximab when disease progressed. In the other patient, liver metastases developed 2 years after resection of the primary sigmoid colon cancer (Figure 3B) for which the patient underwent curative surgical resection without perioperative chemotherapy. HER2 IHC staining

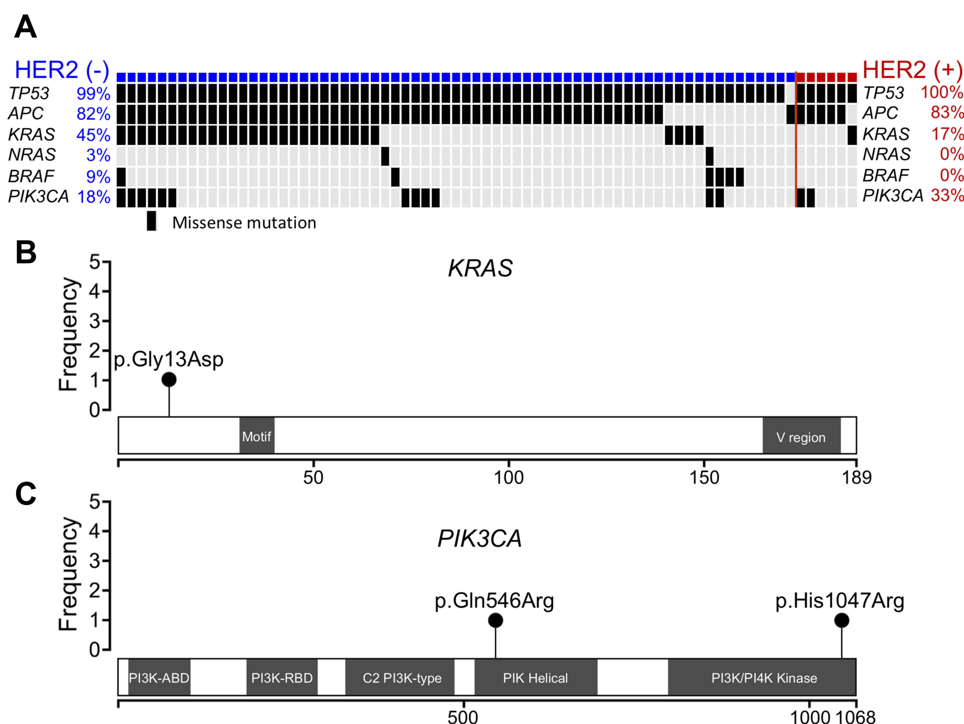

Figure 2 Genetic alterations of HER2-positive and -negative colorectal cancers (CRCs). (**A**) Mutation status of *TP53, APC, KRAS, NRAS, BRAF,* and *PIK3CA* in 6 HER2-positive and 67 HER2-negative CRCs. Lolliplots showing the distribution of *KRAS* (**B**) and *PIK3CA* (**C**) mutations in HER2-positive CRCs.

performed on recurrent tumor tissues from these two patients showed strong positive (3+) HER2 expression consistent with that in primary tumors. Based on the positive HER2 status of recurrent tumors, dual anti-HER2 therapy with trastuzumab and lapatinib was provided as salvage treatment in the former case after treatment progression with irinotecan and cetuximab. However, the tumors did not respond to the treatment. In the latter case, recurrent liver metastases developed 1 year after curative surgery of liver metastases. This patient subsequently received dual anti-HER2 therapy (trastuzumab 4 mg/kg loading followed by 2 mg/kg weekly and lapatinib 1000 mg daily) as the first-line treatment for tumor control. Abdominal CT scanning after eight weeks of anti-HER2 treatment showed significant regression of liver metastases (Figure 3B).

Discussion

IHC staining and FISH analyses have currently been the standard methods for determining HER2 status, an emerging therapeutic target, in patients with CRC.[7] Through the targeted NGS assay, the presented study detected 12 CRCs with increased *HER2* copy numbers. Only CRCs with high-level copy number gain have been identified as HER2 positive using the HERACLES Diagnostic Criteria. Among the HER2-positive CRCs, the lowest copy number was 8.42, which was 4-fold greater than that of normal diploid *HER2*. In contrast, tumors with low-level copy number increase did not exhibit HER2 overexpression. The correlation between *HER2* mRNA expression and putative copy number alteration analyzed using the TCGA database also supported this finding. These results suggest that *HER2* CNA data generated using the NGS assay should be carefully interpreted to determine candidates for anti-HER2 therapy. Patients with low-level copy number gain may not benefit from HER2-targeted treatment. Only limited studies have reported a correlation between *HER2* CNAs and HER2 status in CRC. Although the ROC curve cutoff value of CNAs for the identification of HER2-positive CRC in our cohort was 5.7, the optimal value for HER2 determination remains unclear due to the utility of diverse platforms and tools in separate laboratories. In a prior study, thresholds >2.5 fold have been used to indicate *HER2* amplification.[13] Through such a definition, the study demonstrated 100% concordance between HER2 status and *HER2* amplification. However, the exact *HER2* copy number changes and the rationale for threshold selection have not been reported in this study. Considering that NGS-based genomic analyses are becoming widespread, their clinical utility for determining *HER2* amplification in patients with CRC needs further investigation.

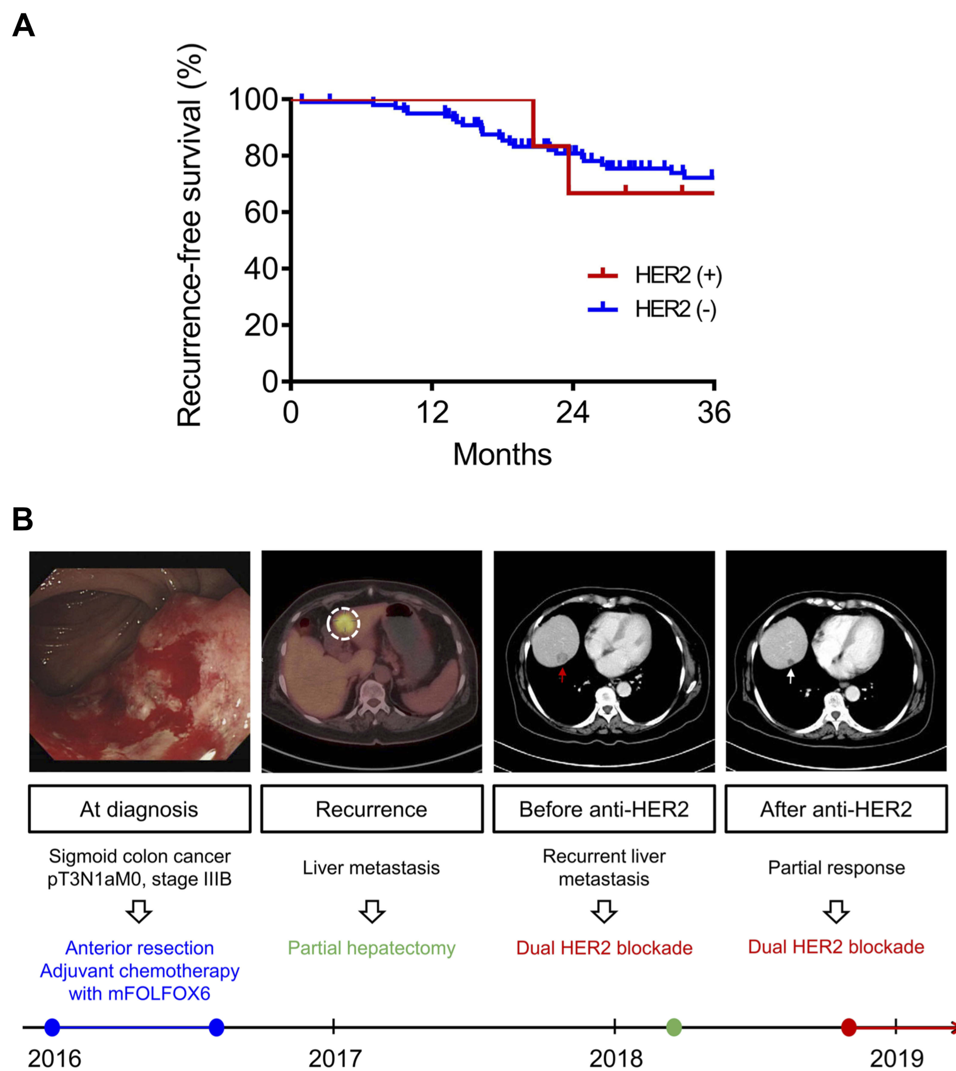

Figure 3 Clinical outcomes of HER2-positive and -negative colorectal cancers (CRCs). (**A**) Kaplan–Meier estimates of recurrence-free survival for HER2-positive and -negative patients with CRC. (**B**) A 75-year-old lady was diagnosed with stage III sigmoid colon cancer in the end of 2015. Standard surgical resection was performed at that time followed by adjuvant chemotherapy with mFOLFOX6. Liver metastases (white dotted circle) developed 2 years later. Despite curative surgical resection, recurrent liver metastases (red arrow) were detected soon after the surgery for liver metastases. Dual anti-HER2 blockade was initiated based on HER2 overexpression in both primary and recurrent tumors. Computed tomography scans obtained 2 months after anti-HER2 therapy demonstrated significant shrinkage of liver metastases (white arrow).

Previous studies have shown that *HER2* amplification is highly prevalent in distal colon cancer. Accordingly, reports have shown that HER2-positive CRCs are mutually exclusive with *KRAS, NRAS*, and *BRAF* mutations.[2,15] Therefore, patients with distal colon primary tumors and those without *KRAS, NRAS*, or *BRAF* mutations are good candidates for further HER2 expression examination. In the current study, genotyping revealed wild-type *NRAS* and *BRAF* in HER2-positive CRCs. However, 1 (20%) HER2-positive CRC harboring a *KRAS* G13D mutation has been identified. This suggested that examining HER2 expression in only *KRAS, NRAS*, and *BRAF* wild-type CRCs might lead to the underestimation of HER2-positive CRCs. Given that NGS approaches can extensively identify hundreds of genomic alterations in a single assay, *HER2* copy number alterations and other critical genetic information can be simultaneously determined in patients with CRC.

PIK3CA is an important oncogene in the development of human cancers. Somatic missense mutations leading to increased PIK3CA kinase activity have been reported to strengthen cell signaling, cell proliferation, and invasion[16] in many types of cancers, including CRC.[14] The present study observed that 19 (26%) of the 73 CRCs harbored PIK3CA mutations, consistent with results of previous reports.[14,17–20] Meanwhile, around 25% of HER2-negative patients in our cohort carried *PIK3CA* mutations.

Additionally, *PIK3CA* activating mutations have been detected in 2 (33.3%) of 6 HER2-positive CRCs. *PIK3CA* mutation has also been known as a mechanism mediating the resistance to HER2-targeted therapy in breast cancer.[21–23] The high incidence of *PIK3CA* mutations in HER2-positive CRCs in our cohort highlights the impact *PIK3CA* mutations have on anti-HER2 treatments in CRC. Interestingly, one of our patients with recurrent disease who responded to anti-HER2 treatment also carried the *PIK3CA* mutation. Therefore, further studies are necessary to clarify the correlation between *PIK3CA* mutations and effectiveness of anti-HER2 treatments in patients with CRC.

A recent study investigating the prognostic impact of HER2 in patients with stage III CRC from a FOLFOX-based trial showed that HER2 overexpression was associated with worse recurrence-free survival and overall survival.[24] However, the present study showed that patients with HER2-positive CRCs had only slightly higher recurrence rate after standard surgical resection and adjuvant mFOLFOX6 chemotherapy compared to those with HER2-negative CRCs. This inconsistency might have been deeply correlated with the small sample size of our CRC cohort. In the current study, two of the HER2-positive patients had recurrent diseases. Moreover, both recurrent tumor samples showed strong HER2 overexpression, consistent with that in primary tumors. Notably, one of the two patients responded to dual anti-HER2 blockade treatment, a finding comparable to the 30% anti-HER2 treatment response rate in metastatic CRC demonstrated in the HERACLES trial. Apart from the therapeutic value of HER2 alterations, our findings also suggest that HER2 might be a critical molecular driver conferring metastatic potential on CRC cells. These findings could provide the rationale for further studies evaluating the effectiveness of anti-HER2 therapy as an adjuvant treatment for patients with early CRC.

Conclusion

NGS-based diagnostic tools can be useful to comprehensively profile genomic alterations, including *HER2*, in patients with CRC. However, judicious interpretation of HER2 expression levels in CRC using such cancer assays is warranted for patients with modest copy number gains. Anti-HER2 therapies can offer clinical benefit to patients with CRC who have this specific molecular target. Moreover, studies investigating prognostic and therapeutic roles of HER-2 overexpression in patients with early CRC are warranted.

Acknowledgment

This work was supported by the National Cheng Kung University Hospital and E-Da Hospital (NCKUEDA10702), the Ministry of Science and Technology (MOST 108-2634-F-006-011), and Ministry of Health and Welfare (MOHW 107-TDU-B-211-114018).

Disclosure

The authors report no conflicts of interest in this work.

References

1. Brenner H, Kloor M, Pox CP. Colorectal cancer. *Lancet*. 2014;383 (9927):1490–1502. doi:10.1016/S0140-6736(13)61649-9
2. Cancer Genome Atlas N. Comprehensive molecular characterization of human colon and rectal cancer. *Nature*. 2012;487(7407):330–337. doi:10.1038/nature11252
3. Yaeger R, Chatila WK, Lipsyc MD, et al. Clinical sequencing defines the genomic landscape of metastatic colorectal cancer. *Cancer Cell*. 2018;33(1):125–136 e123. doi:10.1016/j.ccell.2017.12.004
4. Le DT, Uram JN, Wang H, et al. PD-1 blockade in tumors with mismatch-repair deficiency. *N Engl J Med*. 2015;372(26):2509–2520. doi:10.1056/NEJMoa1500596
5. Corcoran RB, Andre T, Atreya CE, et al. Combined BRAF, EGFR, and MEK inhibition in patients with BRAF(V600E)-mutant colorectal cancer. *Cancer Discov*. 2018;8(4):428–443. doi:10.1158/2159-8290.CD-17-1226
6. Amatu A, Sartore-Bianchi A, Siena S. NTRK gene fusions as novel targets of cancer therapy across multiple tumour types. *ESMO Open*. 2016;1(2):e000023. doi:10.1136/esmoopen-2015-000023
7. Valtorta E, Martino C, Sartore-Bianchi A, et al. Assessment of a HER2 scoring system for colorectal cancer: results from a validation study. *Mod Pathol*. 2015;28(11):1481–1491. doi:10.1038/modpathol.2015.98
8. Sartore-Bianchi A, Trusolino L, Martino C, et al. Dual-targeted therapy with trastuzumab and lapatinib in treatment-refractory, KRAS codon 12/13 wild-type, HER2-positive metastatic colorectal cancer (HERACLES): a proof-of-concept, multicentre, open-label, phase 2 trial. *Lancet Oncol*. 2016;17(6):738–746. doi:10.1016/S1470-2045(16)00150-9
9. Siena S, Sartore-Bianchi A, Marsoni S, et al. Targeting the human epidermal growth factor receptor 2 (HER2) oncogene in colorectal cancer. *Ann Oncol*. 2018;29(5):1108–1119. doi:10.1093/annonc/mdy100
10. Hovelson DH, McDaniel AS, Cani AK, et al. Development and validation of a scalable next-generation sequencing system for assessing relevant somatic variants in solid tumors. *Neoplasia*. 2015;17 (4):385–399. doi:10.1016/j.neo.2015.03.004
11. Zehir A, Benayed R, Shah RH, et al. Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. *Nat Med*. 2017;23(6):703–713. doi:10.1038/nm.4333
12. Frampton GM, Fichtenholtz A, Otto GA, et al. Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing. *Nat Biotechnol*. 2013;31(11):1023–1031. doi:10.1038/nbt.2696
13. Shimada Y, Yagi R, Kameyama H, et al. Utility of comprehensive genomic sequencing for detecting HER2-positive colorectal cancer. *Hum Pathol*. 2017;66:1–9. doi:10.1016/j.humpath.2017.02.004
14. Samuels Y, Wang Z, Bardelli A, et al. High frequency of mutations of the PIK3CA gene in human cancers. *Science*. 2004;304(5670):554. doi:10.1126/science.1096502
15. Bertotti A, Migliardi G, Galimi F, et al. A molecularly annotated platform of patient-derived xenografts ("xenopatients") identifies HER2 as an effective therapeutic target in cetuximab-resistant colorectal cancer. *Cancer Discov*. 2011;1(6):508–523. doi:10.1158/2159-8290.CD-11-0109

16. Karakas B, Bachman KE, Park BH. Mutation of the PIK3CA oncogene in human cancers. *Br J Cancer*. 2006;94(4):455–459. doi:10.1038/sj.bjc.6602970
17. Velho S, Oliveira C, Ferreira A, et al. The prevalence of PIK3CA mutations in gastric and colon cancer. *Eur J Cancer*. 2005;41(11):1649–1654. doi:10.1016/j.ejca.2005.04.022
18. Kato S, Iida S, Higuchi T, et al. PIK3CA mutation is predictive of poor survival in patients with colorectal cancer. *Int J Cancer*. 2007;121(8):1771–1778. doi:10.1002/ijc.22890
19. Benvenuti S, Frattini M, Arena S, et al. PIK3CA cancer mutations display gender and tissue specificity patterns. *Hum Mutat*. 2008;29(2):284–288. doi:10.1002/humu.20648
20. Barault L, Veyrie N, Jooste V, et al. Mutations in the RAS-MAPK, PI(3)K (phosphatidylinositol-3-OH kinase) signaling network correlate with poor survival in a population-based series of colon cancers. *Int J Cancer*. 2008;122(10):2255–2259. doi:10.1002/ijc.23388
21. Majewski IJ, Nuciforo P, Mittempergher L, et al. PIK3CA mutations are associated with decreased benefit to neoadjuvant human epidermal growth factor receptor 2-targeted therapies in breast cancer. *J Clin Oncol*. 2015;33(12):1334–1339. doi:10.1200/JCO.2014.55.2158
22. Berns K, Horlings HM, Hennessy BT, et al. A functional genetic approach identifies the PI3K pathway as a major determinant of trastuzumab resistance in breast cancer. *Cancer Cell*. 2007;12(4):395–402. doi:10.1016/j.ccr.2007.08.030
23. Eichhorn PJ, Gili M, Scaltriti M, et al. Phosphatidylinositol 3-kinase hyperactivation results in lapatinib resistance that is reversed by the mTOR/phosphatidylinositol 3-kinase inhibitor NVP-BEZ235. *Cancer Res*. 2008;68(22):9221–9230. doi:10.1158/0008-5472.CAN-08-1740
24. Laurent-Puig P, Balogoun R, Cayre A. ERBB2 alterations a new prognostic biomarker in stage III colon cancer from a FOLFOX based adjuvant trial (PETACC8). *Ann Oncol*. 2016;27:suppl6; abstr 459O. doi:10.1093/annonc/mdw141

Cancer Management and Research Dovepress

Publish your work in this journal

Cancer Management and Research is an international, peer-reviewed open access journal focusing on cancer research and the optimal use of preventative and integrated treatment interventions to achieve improved outcomes, enhanced survival and quality of life for the cancer patient. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit http://www.dovepress.com/testimonials.php to read real quotes from published authors.

Submit your manuscript here: https://www.dovepress.com/cancer-management-and-research-journal