European Journal of Surgical Oncology 45 (2019) 167–173

Contents lists available at ScienceDirect

European Journal of Surgical Oncology

journal homepage: www.ejso.com

HER2 as a potential biomarker guiding adjuvant chemotherapy in stage II colorectal cancer

Yang Feng [a, b, 1], Yaqi Li [a, b, 1], Dan Huang [b, c], Sanjun Cai [a, b, *], Junjie Peng [a, b, **]

[a] Department of Colorectal Surgery, Fudan University Shanghai Cancer Center, Shanghai 200032, China
[b] Department of Oncology, Shanghai Medical College, Fudan University, Shanghai 200032, China
[c] Department of Pathology, Fudan University Shanghai Cancer Center, Shanghai 200032, China

ARTICLE INFO

Article history:
Received 26 August 2018
Received in revised form
19 September 2018
Accepted 17 October 2018
Available online 19 October 2018

Keywords:
HER2
Colorectal cancer
Stage II
Adjuvant chemotherapy
Mismatch repair status

ABSTRACT

Background: HER2 is a well-established therapeutic target in breast and gastric cancers, while the role of HER2 in colorectal cancer is unclear, and no studies have explored the impact of HER2 on the outcome of stage II colorectal cancer patients treated with 5-fluorouracial based adjuvant chemotherapy.
Methods: We analyzed HER2 mRNA expression of 206 patients in GSE39582 dataset and explored the impact of HER2 expression on benefit from adjuvant chemotherapy for stage II colon cancer patients. We further validated the finding by retrospectively analyzing HER2 detection of immunohistochemistry in a cohort of 282 patients in Fudan University Shanghai Cancer Center (FUSCC).
Results: In GSE39582 dataset, chemo-treated HER2-high patients had a better overall survival (OS) and relapse-free survival (RFS) versus chemo-naïve HER2-high patients (5-year OS: 100% *vs* 69.5%, 5-year RFS: 100% and *vs* 64%, $P = 0.027$ and 0.025, respectively). On the contrary, chemo-treated HER2-low patients had a worse RFS compared with chemo-naïve HER2-low patients (5-year RFS: 65.6% *vs* 82.1%, $P = 0.022$). In FUSCC cohort, chemo-treated HER2-positive patients exhibited better OS *vs* chemo-naïve HER2-positive patients (5-year OS: 100% *vs* 73.8%, $P < 0.001$), and showed marginal evidence of a lower probability of recurrence (5-year RFS: 74.4% *vs* 58.7%, $P = 0.072$). After stratifying by mismatch repair (MMR) status, the results only kept consistency in patients with pMMR status.
Conclusions: HER2-positve patients with stage II colorectal cancer can benefit from 5-fluorouracial based adjuvant chemotherapy, especially for patients with pMMR status.

Introduction

The concept of 5-fluorouracial based adjuvant chemotherapy in stage III colorectal cancer (CRC) is well established. For patients with stage II CRC, its use is still a matter of debate considering the limited survival benefit and concomitant toxicity, cost, and inconvenience [1–4]. The mismatch repair (MMR) status has been regarded as a basis for risk classification in stage II CRC patients, as patients with deficient MMR (dMMR) status exhibited a better survival and could not benefit from 5-fluorouracil based adjuvant chemotherapy, thus only patients with proficient MMR (pMMR) status are recommended for adjuvant chemotherapy [5,6]. To date, numerous clinicopathological features correlated with prognosis in stage II disease have been proposed assisting the decision for adjuvant chemotherapy, including poor differentiation, intestinal obstruction or perforation, T4 stage, inadequate lymph nodes harvested (<12 nodes), or the presence of lymphovascular or perineural invasion [1,7,8]. While, only T4 disease has been validated help identify the group of stage II patients that benefit from adjuvant chemotherapy [9]. Some microarray-based signatures have also been constructed to identify high-risk stage II patients [10,11], but it's difficult to put these signatures into clinical practice. Identifying novel biomarkers that could reliably predict the likelihood of responsiveness to chemotherapy in patients with stage II CRC is in urgent need.

Abbreviations: Colorectal cancer, CRC; Mismatch repair, MMR; Deficient MMR, dMMR; Proficient MMR, pMMR; Robust multi-array average, RMA; Immunohistochemistry, IHC; National Comprehensive Cancer Network, NCCN.

* Corresponding author. Department of Colorectal Surgery, Fudan University Shanghai Cancer Center, 270 Dong'an Road, Shanghai 200032, China.
** Corresponding author. Department of Colorectal Surgery, Fudan University Shanghai Cancer Center, 270 Dong'an Road, Shanghai 200032, China.
E-mail addresses: caisanjun_sh@163.com (S. Cai), pengjj@shca.org.cn (J. Peng).
[1] Yang Feng and Yaqi Li contributed equally to this work.

https://doi.org/10.1016/j.ejso.2018.10.059
0748-7983/

Table 1
Baseline characteristics of HER2-low and HER2-high patients in GSE39582 cohort.
P values are from Chi-square test and were significant at < 0.05. MMR: mismatch repair; CIMP: CpG island methylator phenotype; CIN: chromosomal instability.

Variables	Subgroup	No. (%) of patients		
		Total Patients (n = 206)		
		HER2-low (n = 137)	HER2-high (n = 69)	*P*
Gender	Male	81(59.1)	45(65.2)	0.397
	Female	56(40.9)	24(34.8)	
Age	<60	34(24.8)	15(21.7)	0.624
	≥60	103(75.2)	54(78.3)	
Location	Proximal	57(41.6)	29(42.0)	0.954
	Distal	80(58.4)	40(58.0)	
pT	3	102(74.5)	58(84.1)	0.214
	4	27(19.7)	7(10.1)	
	NA	8(5.8)	4(5.8)	
KRAS mutation	No	86(62.8)	44(63.8)	0.874
	Yes	45(32.8)	23(33.3)	
	NA	6(4.4)	2(2.9)	
BRAF mutation	No	117(85.4)	58(84.1)	0.968
	Yes	9(6.6)	5(7.2)	
	NA	11(8.0)	6(8.7)	
TP53 mutation	No	49(35.8)	26(37.7)	0.002
	Yes	35(25.5)	31(44.9)	
	NA	53(38.7)	12(17.4)	
MMR Status	dMMR	25(18.2)	8(11.6)	0.219
	pMMR	112(81.8)	61(88.4)	
CIMP status	−	99(72.3)	49(71.0)	0.904
	+	23(16.8)	11(15.9)	
	NA	15(10.9)	9(13.0)	
CIN status	−	33(24.1)	13(18.8)	0.461
	+	77(56.2)	45(65.2)	
	NA	27(19.7)	11(15.9)	
Chemotherapy	Yes	32(23.4)	12(17.4)	0.324
	No	105(76.6)	57(82.6)	

The HER-2/neu oncogene is a member of transmembrane tyrosine kinase receptors which has been extensively studied in breast cancer [12,13]. Up to 30% breast cancer are presented with overexpression and/or amplification of HER2, which is verified related to aggressive tumor behavior, increased risk of recurrence and poorer survival [14,15]. HER2 overexpression has also been identified in other cancers, such as gastric cancer, and similar function of HER2 was recognized [16]. Adding trastuzumab (Herceptin) to standard chemotherapy has been verified to significantly improve response rates and survival in both diseases [17–19]. In CRC, the role of HER2 in prognosis predicting is still unclear and controversial [20–22]. No studies have yet explored the role of HER2 in guiding adjuvant chemotherapy in stage II CRC. Our study aimed to identify the group of stage II CRC patients who may benefit from 5-fluorouracial based adjuvant chemotherapy by detecting HER2 expression.

Methods

Acquisition of GSE39582 dataset

Raw microarray data were available via the NCBI Gene Expression Omnibus database (http://www.ncbi.nlm.nih.gov/geo/). The transcriptome arrays were determined on Affymetrix U133 Plus 2.0 chips and normalized expression values were obtained using the robust multi-array average (RMA) algorithm. We matched all probes to specific EntrezGeneID. When multiple probes were mapped to the same EntrezGeneID, the median value was used. Only stage II patients were included in our study, and patients with unknown MMR status or recurred within 3 months were excluded. We defined the upper tertile of HER2 mRNA level as HER2-high subgroup, and the lower and middle tertiles were defined as HER2-low subgroup.

Table 2
Baseline characteristics of HER2-negative and HER2-positive patients in FUSCC cohort.
P values are from Chi-square test and were significant at < 0.05. MMR: mismatch repair; LNH: lymph node harvested.

Variables	Subgroup	No. (%) of patients		
		Total Patients (n = 282)		
		HER2-neagative (n = 209)	HER2-positive (n = 73)	*P*
Gender	Male	124(59.3)	47(64.4)	0.447
	Female	85(40.7)	26(35.6)	
Age	<60	108(51.7)	32(43.8)	0.249
	≥60	101(48.3)	41(56.2)	
Location	Right	58(27.8)	25(34.2)	0.273
	Left	57(27.3)	23(31.5)	
	Rectum	94(45.0)	25(34.2)	
Pathological grading	Poorly differentiated	9(4.3)	4(5.5)	0.917
	Moderately differentiated	186(89.0)	64(87.7)	
	Well differentiated	14(6.7)	5(6.8)	
Histology type	Adenocarcinoma	193(92.3)	67(91.8)	0.877
	Mucinous adenocarcinoma	16(7.7)	6(8.2)	
pT	pT3	148(70.8)	58(79.5)	0.152
	pT4	61(29.2)	15(20.5)	
MMR Status	dMMR	77(36.8)	21(28.8)	0.212
	pMMR	132(63.2)	52(71.2)	
Vascular invasion	Yes	16(7.7)	2(2.7)	0.23
	No	193(92.3)	71(97.3)	
Neural invasion	Yes	29(13.9)	10(13.7)	0.97
	No	180(86.1)	63(86.3)	
Chemotherapy	Yes	141(67.5)	45(61.6)	0.366
	No	68(32.5)	28(38.4)	
LNH	<12	44(21.1)	8(11.0)	0.056
	≥12	165(78.9)	65(89.0)	

Fudan University Shanghai Cancer Center cohort of stage II CRC patients

Recurrent patients were 1:2 matched by gender to patients with no recurrence for stage II CRC patients diagnosed between 2008 and 2013 in the Fudan University Shanghai Cancer Center (FUSCC). All patients were pathologically confirmed and received radical resection. None of the patients had received chemotherapy or radiation before surgery. Patients who could not tolerate adequate courses of 5-fluorouracial based adjuvant chemotherapy were excluded. After surgery, all patients were regularly followed-up and survival data was obtained by phone and the out-patient records. The median length of follow-up was 44.3 months (range 6.1–95.8 months). Immunohistochemical (IHC) results for HER2/neu, hMLH1, hMSH2, hMSH6 and PMS2 were retrieved from pathological report conducted by our pathology department. Only membrane staining was considered positive for HER2. HER2 scores 1+/2+/3 + were counted as positive, scores 0 as negative. Any deficiency of the four biomarkers (hMLH1, hMSH2, hMSH6 and PMS2) were defined as dMMR status, otherwise pMMR status. Informed consent had been obtained and this study was approved by the institutional review board of the Fudan University Shanghai Cancer Center.

Statistical analysis

Patient data based on HER2 expression were summarized and compared using Chi-square tests. Survival data were shown in Kaplan-Meier curves and analyzed with log-rank tests. All statistical analyses were performed with the R 2.15.3 software. All computed P values were two-sided, and statistical significance was accepted at $P < 0.05$.

Results

Baseline characteristics of patients in GSE39582 and FUSCC cohorts

As shown in Table 1, in GSE39582 cohort, HER2-low ($n = 137$) and HER2-high ($n = 69$) patients showed significant difference in *TP53* mutation ($P = 0.002$), where HER2 high-expression had a significantly higher incidence of *TP53* mutation (44.9% vs 25.5%). No significant differences were observed for gender, age, location, pT

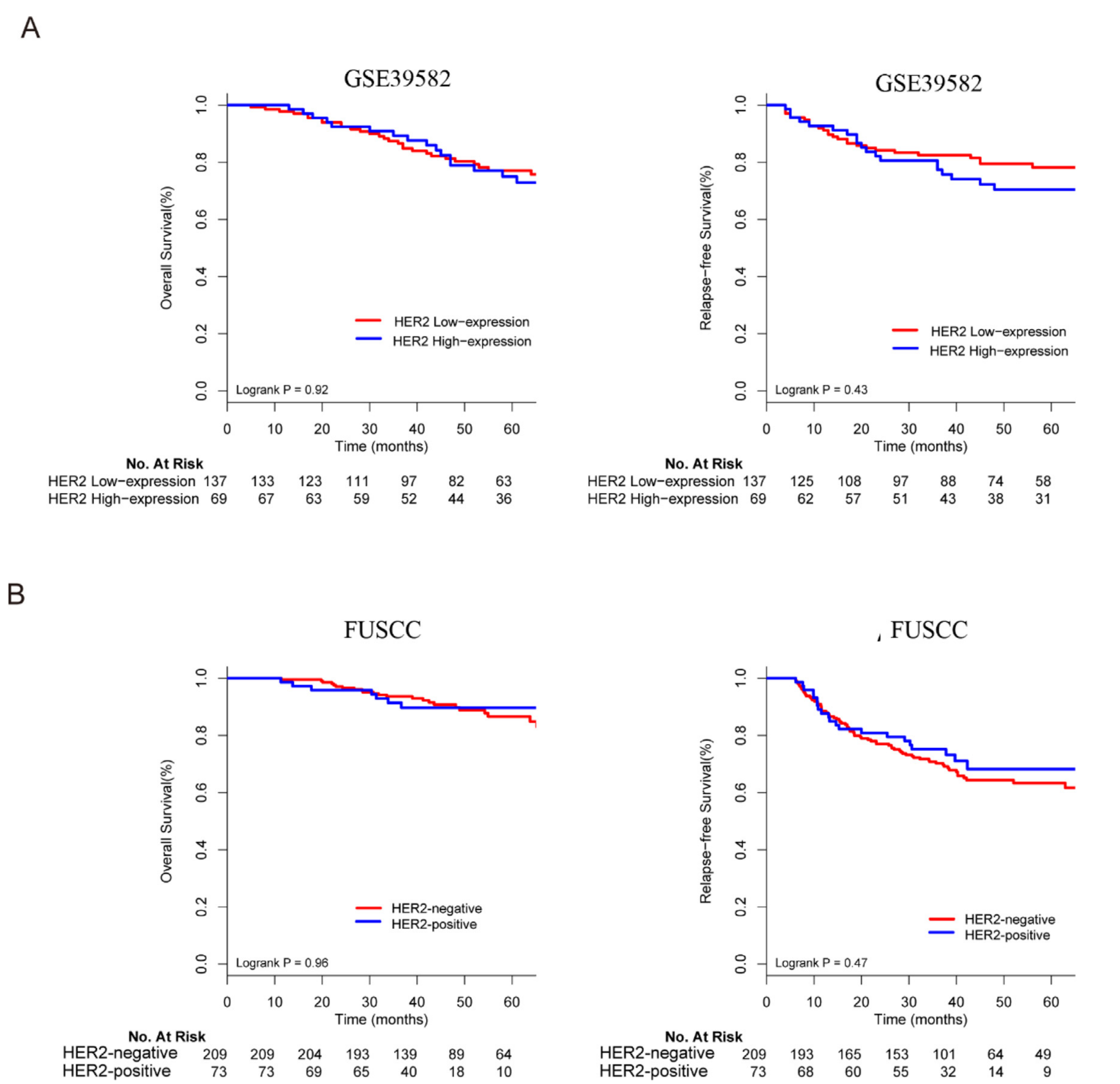

Fig. 1. Relationship between HER2 expression and overall survival and relapse-free survival in stage II patients. (A) In GSE39582 cohort; (B) In FUSCC cohort.

stage, *KRAS* mutation, *BRAF* mutation, MMR Status, CIMP status, CIN status and chemotherapy. In FUSCC cohort (see Table 2), all baseline characteristics were well-balanced and no statistical significances were achieved between HER2-negative (n = 209) and HER2-positive (n = 73) patients except for number of lymph nodes harvested (LNH), as HER2-positive patients had a slightly higher incidence of adequate lymph nodes retrieval (89.0% vs 78.9%, P = 0.056).

HER2 expression and benefit from adjuvant chemotherapy for stage II CRC

In GSE39582 cohort, during follow-up, 35 patients (25.5%) in the HER2-low group died comparing to 19 patients (27.5%) in the HER2-high group, and 29 patients (21.2%) in the HER2-low group recurred comparing to 19 patients (27.5%) in the HER2-high group. Log-rank test confirmed that HER2 was not a prognostic factor for OS or RFS (P = 0.92 and 0.43, respectively), as illustrated in Fig. 1. The relationship between HER2 expression and benefit from adjuvant chemotherapy was shown in Fig. 2. In HER2-high group, chemo-treated patients had a better OS and DFS compared with chemo-naïve patients (5-year OS:100% *vs* 69.5%, 5-year RFS: 100% *vs* 64%, P = 0.027 and 0.025, respectively). While, in HER2-low group, no significant difference was observed for OS between chemo-treated and chemo-naïve patients (5-year OS: 77.3% *vs* 77.0%, P = 0.873). Chemo-treated patients even exhibited a worse RFS compared with chemo-naïve HER2-low patients (5-year RFS: 65.6% *vs* 82.1%, P = 0.022).

In FUSCC, HER2 was not a prognostic factor for OS or RFS (p = 0.960 and 0.470, respectively). For HER2-positive group (see Fig. 3), chemo-treated patients had a better overall survival compared with chemo-naïve patients (5-year OS: 100% *vs* 73.8%, P < 0.001), and showed marginal evidence of a lower probability of recurrence (5-year RFS:74.4% *vs* 58.7%, P = 0.072). In HER2-negative group, no significant survival difference for OS or DFS could be achieved between chemo-treated and chemo-naïve patients (P = 0.480 and 0.950, respectively).

Subgroup analyses by MMR status and T stage

In GSE39582 cohort, after stratifying by MMR status (supporting information Fig. S1 and Fig. S2), the results only kept consistency in patients with pMMR status. Similar results could be drawn after stratifying as T3 and T4 tumors (supporting information Fig. S3 and Fig. S4). In FUSCC, after stratifying by MMR status (supporting information Fig. S5 and Fig. S6), the results only kept consistency in patients with pMMR status, and the association between HER2 status and benefit from adjuvant chemotherapy was not confounded by T stage (T3 vs. T4) in FUSCC (supporting information Fig. S7 and Fig. S8).

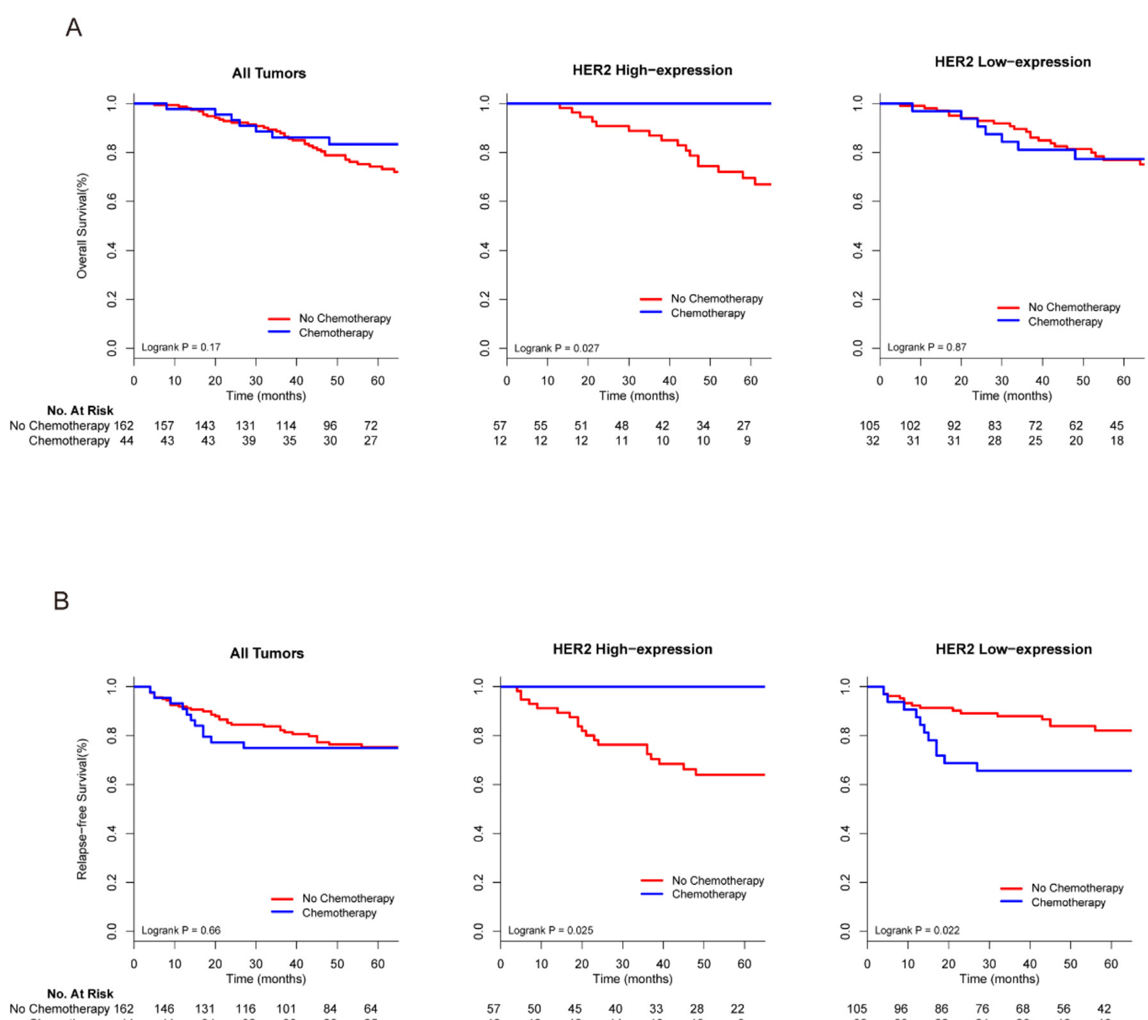

Fig. 2. Relationship between HER2 expression and benefit from Adjuvant Chemotherapy in stage II patients in GSE39582 cohort. (A) Overall survival; (B) Relapse-free survival.

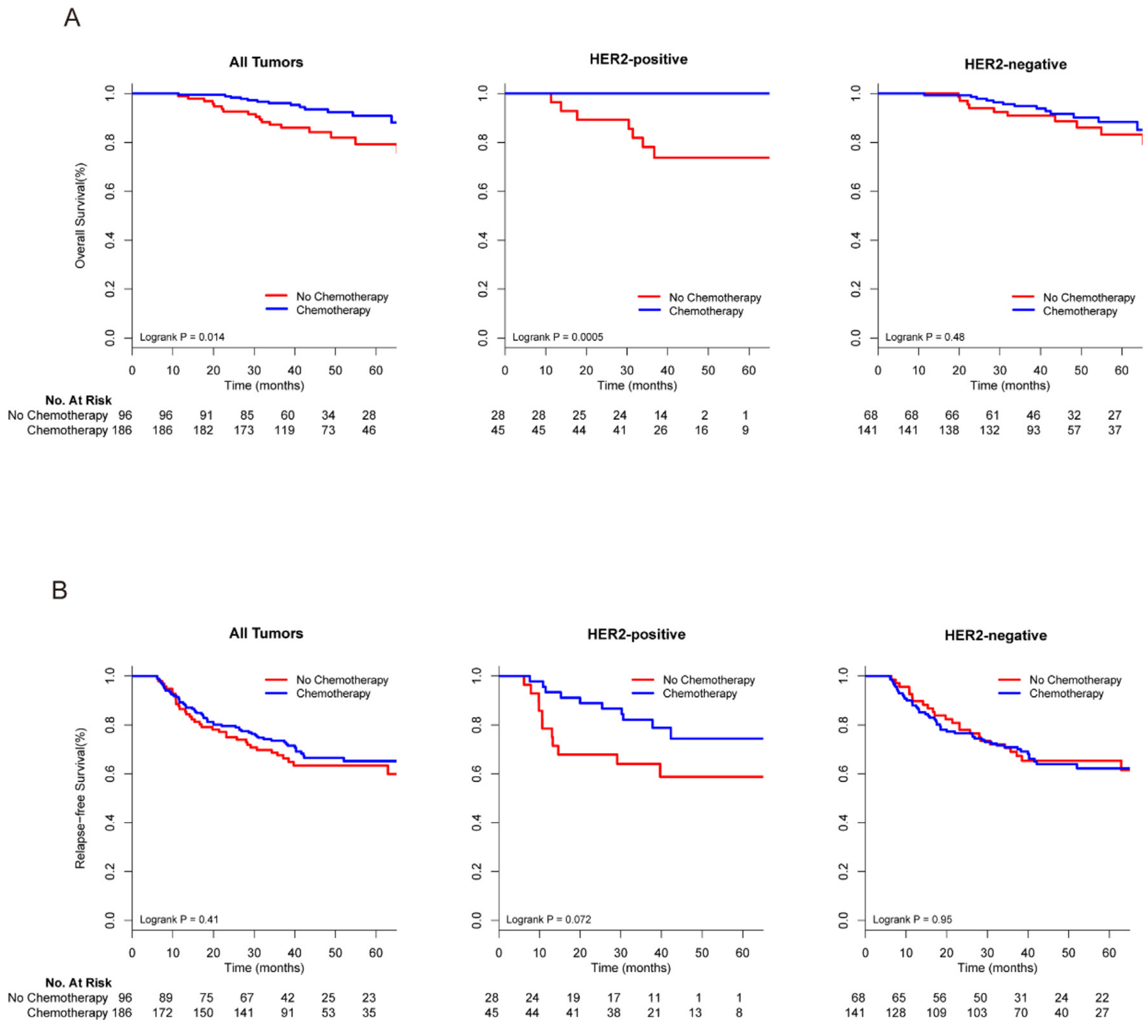

Fig. 3. Relationship between HER2 expression and benefit from Adjuvant Chemotherapy in stage II patients in FUSCC cohort. (A) Overall survival; (B) Relapse-free survival.

Discussion

5-fluorouracil based adjuvant chemotherapy has been widely accepted for the treatment of stage III colorectal cancer. However, this therapeutic success has not been analogized among patients with stage II disease [23,24]. The American National Comprehensive Cancer Network (NCCN) recommends that patients with high-risk stage II colon cancer could be considered for adjuvant chemotherapy [2], but most of these high risk factors are not well verified yet. Dalerba's study [25] revealed lack of CDX2 expression could identify a subgroup of patients with high-risk stage II colon cancer who appeared to benefit from adjuvant chemotherapy, however, CDX2 could only identify 87 of 2115 patients (4.1%). Rohr et al. [26] found pMMR/MACC1-low tumors have a similar favorable prognosis to those with dMMR with potential implications for the role of adjuvant therapy, but the clinical transition of this study is also impeded as few patients were identified with pMMR/MACC1-low status by immunohistochemistry (BIOGRID 1, 7%; BIOGRID 2, 5%). Gao et al. [10] developed a robust combinatory cancer hallmark-based gene signature sets (CSS sets) that identify a subset of patients with stage II CRC who could gain survival benefits from adjuvant chemotherapy, but it's difficult to put this signature into clinical practice considering the cost and operability. Our study first identified HER2 as a potential biomarker which could identify a considerable proportion (about 30%) of stage II CRC patients who may benefit from adjuvant chemotherapy, and the results were confirmed at both mRNA and protein level.

HER2 is a plasma membrane protein with intrinsic tyrosine kinase activity [12]. Some important signaling pathways such as the RAS and PI3K pathways could be activated by HER2, resulting in cell duplication and regulation of apoptosis [27]. HER2 overexpression and/or amplification are associated with tumor initiation and progression, and may also predict the response to chemotherapy and hormonal therapy [28]. The role of HER2 in breast cancer and gastric cancer were well established, while for CRC, limited studies could be referred to. The immunohistochemically positive rate was 20% in D'Emilia's series [29], 47.4% in Park's series [20], and 35% in Lee's series [30]. In agreement with previous studies, our data showed that for HER2/neu expression, 70 (24.8%) were classified as '+', and 3 (1.1%) as '++'. Based on these studies, we defined upper tertile as HER2-high expression in GSE39582. Park et al. [20] found the association of HER2 expression with advanced tumor stage and poor survival, while D'Emilia's [29] and Lee's [30] studies could not demonstrate the role of HER2 in predicting prognosis. The largest QUASAR study concluded HER2 was not associated with OS or PFS [31], which is consistent with our finding.

Our study found that HER2-high group had a significantly higher incidence of TP53 mutation than HER2 low group (44.9% vs 25.5%). TP53 is a tumor-suppressor protein and TP53 gene mutation is widely known as one of the determinants of impaired chemo sensitivity. 5-FU-based adjuvant chemotherapy may provide survival benefit for patients whose tumors contained normal p53 in stage III CRC [32,33]. Liang's study [34] found stage IV colorectal cancers with p53 overexpression was associated with poorer chemo sensitivity. While in stage II CRC, studies regarding the chemo sensitivity and TP53 mutation is rare and Ahnen's series revealed there was no benefit of adjuvant therapy in stage II nor in any of the stage II subgroups defined by mutational status [35]. Whether TP53 mutation contribute to the chemo sensitivity of HER2 high-expression group remains unknown, further studies are merited to unmask the intrinsic mechanism.

There are several limitations in the present study. First, our study is retrospective in nature. Secondly, colon and rectal cancer are two heterogenous entities, but we didn't separate colon and rectal cancer due to limited sample size. Besides, we didn't validate our findings in other GEO datasets or TCGA database, as limited sample size, restricted endpoint events, or severe bias between chemo-naïve and chemo-treated groups could be outlined, which weakened the reliability of our study.

In summary, we have provided evidence that HER2 expression levels has utility to identify a considerable proportion of Stage II colorectal cancer patients who may benefit from adjuvant chemotherapy, especially in patients with pMMR status. Further studies are needed to validate our findings and uncover the mechanism of chemo-sensitivity generated by HER2.

Ethics approval and consent to participate

Informed consent had been obtained and this study was approved by the institutional review board of the Fudan University Shanghai Cancer Center.

Availability of data and material

GSE39582 raw microarray data were available via the NCBI Gene Expression Omnibus database (http://www.ncbi.nlm.nih.gov/geo/). As for FUSCC data, please contact author for data requests.

Consent for publication

Yes.

Competing interests

The authors have no conflicts of interest to disclose.

Funding

The work supported by the grants from Science and Technology Commission of Shanghai Municipality (No. 16411966300) and National Key Basic Research Program of China (2014CBA02000). The funders had no role in the study desigh, data collection and analysis, decision to publish, or preparation of the manuscript.

Author' contributions

Y.F. and J.J.P. planned the study. Y.F. and Y.Q.L. calculated statistics and analyzed the data. Y.F. and Y.Q.L. wrote the manuscript. S.J.C. and J.J.P. supervised the entire project. All authors reviewed the manuscript.

Acknowledgements

Not applicable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejso.2018.10.059.

References

[1] Kucukzeybek Y, et al. Adjuvant chemotherapy and prognostic factors in stage II colon cancer–Izmir Oncology Group Study. Asian Pac J Cancer Prev APJCP: APJCP 2015;16:2413–8.
[2] Benson 3rd AB, et al. American Society of Clinical Oncology recommendations on adjuvant chemotherapy for stage II colon cancer. J Clin Oncol: Off J Am Soc Clin Oncol 2004;22:3408–19.
[3] O'Connor ES, et al. Adjuvant chemotherapy for stage II colon cancer with poor prognostic features. J Clin Oncol: Off J Am Soc Clin Oncol 2011;29:3381–8.
[4] Gray R, et al. Adjuvant chemotherapy versus observation in patients with colorectal cancer: a randomised study. Lancet (London, England) 2007;370:2020–9.
[5] Sargent DJ, et al. Defective mismatch repair as a predictive marker for lack of efficacy of fluorouracil-based adjuvant therapy in colon cancer. J Clin Oncol: Off J Am Soc Clin Oncol 2010;28:3219–26.
[6] Ribic CM, et al. Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. N Engl J Med 2003;349:247–57.
[7] Quah HM, et al. Identification of patients with high-risk stage II colon cancer for adjuvant therapy. Dis Colon Rectum 2008;51:503–7.
[8] Okada K, et al. The size of retrieved lymph nodes correlates with the number of retrieved lymph nodes and is an independent prognostic factor in patients with stage II colon cancer. Int J Colorectal Dis 2015;30:1685–93.
[9] Kumar A, et al. Adjuvant chemotherapy use and outcomes of patients with high-risk versus low-risk stage II colon cancer. Cancer 2015;121:527–34.
[10] Gao S, et al. Identification and construction of combinatory cancer hallmark-based gene signature sets to predict recurrence and chemotherapy benefit in stage II colorectal cancer. JAMA Oncol. 2016;2:37–45.
[11] Tian X, et al. Recurrence-associated gene signature optimizes recurrence-free survival prediction of colorectal cancer. Mol. Oncol. 2017;11:1544–60.
[12] Gschwind A, et al. The discovery of receptor tyrosine kinases: targets for cancer therapy. Nat Rev Canc 2004;4:361–70.
[13] Wells A. EGF receptor. Int J Biochem Cell Biol 1999;31:637–43.
[14] Slamon DJ, et al. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science (New York, NY) 1987;235:177–82.
[15] Press MF, et al. HER-2/neu gene amplification characterized by fluorescence in situ hybridization: poor prognosis in node-negative breast carcinomas. J Clin Oncol: Off J Am Soc Clin Oncol 1997;15:2894–904.
[16] Hynes NE, Stern DF. The biology of erbB-2/neu/HER-2 and its role in cancer. Biochim Biophys Acta 1994;1198:165–84.
[17] Slamon DJ, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med 2001;344:783–92.
[18] Burstein HJ, et al. Clinical activity of trastuzumab and vinorelbine in women with HER2-overexpressing metastatic breast cancer. J Clin Oncol: Off J Am Soc Clin Oncol 2001;19:2722–30.
[19] Bang YJ, et al. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. Lancet (London, England) 2010;376:687–97.
[20] Park DI, et al. HER-2/neu overexpression is an independent prognostic factor in colorectal cancer. Int J Colorectal Dis 2007;22:491–7.
[21] Kavanagh DO, et al. Is overexpression of HER-2 a predictor of prognosis in colorectal cancer? BMC Canc 2009;9:1.
[22] Osako T, et al. Immunohistochemical study of c-erbB-2 protein in colorectal cancer and the correlation with patient survival. Oncology 1998;55:548–55.
[23] Kohne CH. Should adjuvant chemotherapy become standard treatment for patients with stage II colon cancer? Against the proposal. Lancet Oncol 2006;7:516–7.
[24] Sobrero A. Should adjuvant chemotherapy become standard treatment for patients with stage II colon cancer? For the proposal. Lancet Oncol 2006;7:515–6.
[25] Dalerba P, et al. CDX2 as a prognostic biomarker in stage II and stage III colon cancer. N Engl J Med 2016;374:211–22.
[26] Rohr UP, et al. Prognostic value of MACC1 and proficient mismatch repair status for recurrence risk prediction in stage II colon cancer patients: the BIOGRID studies. Ann Oncol: Off J Eur Soc Med Oncol 2017;28:1869–75.
[27] Weigelt B, et al. PIK3CA mutation, but not PTEN loss of function, determines the sensitivity of breast cancer cells to mTOR inhibitory drugs. Oncogene 2011;30:3222–33.
[28] Scholl S, et al. Targeting HER2 in other tumor types. Ann Oncol: Off J Eur Soc Med Oncol 2001;12(Suppl 1):S81–7.
[29] D'Emilia J, et al. Expression of the c-erbB-2 gene product (p185) at different stages of neoplastic progression in the colon. Oncogene 1989;4:1233–9.
[30] Lee JC, et al. Investigation of the prognostic value of coexpressed erbB family members for the survival of colorectal cancer patients after curative surgery

(Oxford, England: 1990) Eur J Canc 2002;38:1065−71.
[31] Richman SD, et al. HER2 overexpression and amplification as a potential therapeutic target in colorectal cancer: analysis of 3256 patients enrolled in the QUASAR, FOCUS and PICCOLO colorectal cancer trials. J Pathol 2016;238: 562−70.
[32] Nehls O, et al. Studies on p53, BAX and Bcl-2 protein expression and microsatellite instability in stage III (UICC) colon cancer treated by adjuvant chemotherapy: major prognostic impact of proapoptotic BAX. Br J Canc 2007;96:1409−18.
[33] Elsaleh H, et al. P53 alteration and microsatellite instability have predictive value for survival benefit from chemotherapy in stage III colorectal carcinoma. Clin Canc Res: Off J Am Assoc. Canc. Res. 2001;7:1343−9.
[34] Liang JT, et al. P53 overexpression predicts poor chemosensitivity to high-dose 5-fluorouracil plus leucovorin chemotherapy for stage IV colorectal cancers after palliative bowel resection. Int J Canc 2002;97:451−7.
[35] Ahnen DJ, et al. Ki-ras mutation and p53 overexpression predict the clinical behavior of colorectal cancer: a Southwest Oncology Group study. Cancer Res 1998;58:1149−58.