Original paper

Association between Her2/neu status in colorectal carcinoma and clinicopathological features: a retrospective study using whole – tissue sections

Aydan Kiliçarslan[1], Hayriye Tatli Dogan[1], Nuran Süngü[1], Mehmet Dogan[2], Abdussamet Yalcin[3], Didem Şener Dede[4]

[1]Department of Pathology, Yıldırım Beyazıt University, Ankara, Turkey
[2]Department of Pathology, Dr. Abdurrahman Yurtaslan Oncology Education and Research Hospital, Ankara, Turkey
[3]Department of Surgery, Yıldırım Beyazıt University, Ankara, Turkey
[4]Department of Oncology, Yıldırım Beyazıt University, Ankara, Turkey

The human epidermal growth factor receptor 2 (Her2/neu) signal pathway plays a significant role in the occurance of various solid tumor types. The rate of Her2/neu in colorectal carcinoma (CRC) is not clearly elucidated. In this study, we discuss the association between Her2/neu overexpression and clinicopathological parameters in CRC. Her2/neu immunohistochemical (IHC) staining was performed on whole sections of formalin fixed paraffin embedded tumor tissues of 100 CRC resections. Cases with score 3+ and score 2+ expressions were further evaluated by silver *in-situ* hybridization (ISH) for the existence of Her2/neu gene amplification. Her2/neu membranous overexpression was observed in 12 of the 100 cases (6 cases with a score 3+ and 6 cases with a score 2+) and 6 of these were heterogenous. There were 10 cases with Her2/neu amplification (6/6 score 3+, 4/6 score 2+). Strong cytoplasmic staining was observed in 5 cases. Membranous scores were either 3+ or 2+ in 3 of these 5 cases. Moreover, there was Her2/neu amplification in 2 of these 3 cases. Her2/neu amplification status and overexpression was not related to clinicopathological parameters and overall survival. More clear results can be obtained from studies with Her2/neu IHC and ISH test on whole sections.

Key words: colorectal cancer, Her2/neu, immunohistochemistry, *in situ* hybridization.

Introduction

Colorectal carcinoma (CRC) is the third most common disease in the world and third in line concerning deaths related to cancer [1]. Even though adjuvant chemotherapy is an excellent strategy, it may not prevent the recurrence of the tumor [2]. For this reason, studies into the treatment regime are still in progress. Targeted cancer treatment is a promising way for CRC.

The human epidermal growth factor receptor 2 (Her2/neu) is essential for cell proliferation and differentiation [3, 4]. Her2/neu overexpression or amplification is considerable for cancer types including breast, gastric, lung, overian, colorectal and prostate [4, 5, 6]. The detection of Her2/neu protein

Table I. Association between Her2/neu positive cases and clinicopathological data of CRC

		*Her2/neu	**Her2/neu	Total	
		Negative n (%)	Positive n (%)	n (%)	p
n		90 (90)	10 (10)	100 (100)	
Age	Average ± SD	66.5 ±1.,5	66.3 ±10.7	66.5 ±11.4	0.95
Gender	Female	30 (33.7)	4 (40)	34 (34.3)	0.69
	Male	59 (66.3)	6 (60)	65 (65.7)	
Tumor size (cm)	Average ± SD	5.4 ±2.6	5.4 ±1.6	5.4 ±2.5	0.98
	RC	32 (32)	3 (30)	35 (35)	
Tumor localization	TC	3 (3)	0 (0)	3 (3)	1.00
	LC	40 (40)	4 (40)	44 (44)	
	REC	15 (15)	3 (30)	18 (14)	
Tumor type	Tubular ca.	79	10	89 (89)	0,33
	Mucinous ca.+ signet ring ca.	11	0	11 (11)	
LN metastases	N0	44 (48.9)	5 (50)	49 (49)	0.94
	N+	46 (51.1)	5 (50)	51 (51)	
Distant metastases	M0	45 (69.2)	7 (87.5)	52 (71.2)	0.28
	M1	20 (30.8)	1 (12.5)	21 (28.8)	
Grade	1	31 (34.4)	2 (20)	33 (33)	0.53
	2	43 (47.8)	5 (50)	48 (48)	
	3	16 (17.8)	3 (30)	19 (19)	
Stage	T1	3 (3.4)	1 (11.1)	4 (4.2)	0.57
	T2	5 (5.7)	0 (0)	5 (5.2)	
	T3	62 (71.3)	7 (77.8)	69 (71.9)	
	T4	17 (19.5)	1 (11.1)	18 (18.8)	
LVI	L0	42 (46.7)	6 (60)	48 (48)	0.42
	L1	48 (53.3)	4 (40)	52 (52)	
Survival state	Alive	58 (64.4)	8 (80)	66 (66)	N/A
	Dismissed	32 (35.6)	2 (20)	34 (34)	
Median	(Month)	86	48	93	0.68
Survival period	1 year	86%	90%	86%	
	3 years	72%	78%	72%	

**Her2/neu IHC 0, 1+, IHC 2+ and ISH negative*

***Her2/neu IHC 3+, IHC 2+ and ISH positive*

RC – right colon; TC – tranvers colon; LC – left colon; REC – rectum; LVI – lymphovascular invasion

overexpression has been routine practice in breast and gastric cancer [7, 8]. Her2/neu overexpression ratios were reported to be between 0.16 and 47% and Her2/neu gene amplification was found to be between 2.5 and 7.4% in CRCs [9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22]. There are also inconsistent reports in terms of survival; some have reported Her2/neu overexpression to be related with short survival [12, 20, 21, 22, 23, 24] and others could not find any relationship between Her2/neu overexpression and survival [12, 25, 26, 27, 28].

Since 2004, monoclonal antibodies have been used to target the epidermal growth factor receptor (such as cetuximab and panitumumab) and the vascular endotelhial growth factor receptor (such as bevacizumab) in metastatic CRC (mCRC) and have been accepted by the US Food and Drug Administration (FDA) [29, 30]. Despite the presence of anti-EGFR receptor, response to monotherapy with cetuximab in metastasic CRC is only 10% [31]. Nowadays combined treatment strategies are targeting multiple members of the EGF receptor family to strengthen the antibody effects. Trastuzumab is an anti Her2/neu

monoclonal antibody and it is used as adjuvant, neoadjuvant therapy and for advanced stage metastatic breast carcinoma. For Her2/neu positive advanced stage gastric or gastro-oesophageal carcinomas, trastuzumab in combination with chemotherapy is a standard option [32]. Adjuvant chemotheraphy accompanied with trastuzumab and lapatinib is active and well tolerated in treatment-refractory patients with KRAS codon 12/13 wild-type, Her2/neu positive metastatic CRC [33].

In primary and metastatic breast cancer, the existence of heterogeneity of Her2/neu overexpression/amplification is a significant predictor for the effects of trastuzumab treatment [34]. Xenograft studies show a meaningful correlation between Her2/neu amplification and cetuximab resistance [35]. Remarkably, the evaluation of Her2/neu amplification is valuable for both treatment alternatives as well as the prediction of cetuximab resistance [33, 36].

Our aim in this study was to investigate Her2/neu expression frequency and heterogeneity of 100 CRC patients. Additionally we analyzed the relationship between the clinicopathological parameters and prognoses of CRC patients with Her2/neu overexpression/amplification.

Material and methods

Study population

Tissue samples of 100 primary colorectal carcinomas were obtained retrospectively from the archive of the pathology department. Resection specimens were reevaluated for histopathological parameters including tumor type, stage, lymph node status, and differentiation. Patient characteristics including gender, age, recurrence and follow-ups were summarized in Table I. Tumor type and grade were defined according to the WHO 2010 criteria. The TNM status was determined according to the seventh edition of the UICC guidelines. This retrospective cohort consisted of 66 men and 34 women. The median age of the patients at the time of diagnosis was 66.5 ±11.4 (41-93).

Her2/neu immunohistochemistry

The Her2/neu immunohistochemistry procedure was applied to 4 μm-thick tumor whole sections obtained from formaline fixed paraffin embedded blocks of resection specimens. The rabbit monoclonal antibody 4B5 for Her2/neu was performed on the Benchmark GX automated platform (Ventana medical systems, Tuscon, AZ, US). Antigen retrieval was standardised at 37°C for 16 minutes on the stainer. The antibody was used as Ventana prediluted form.

Both the immunostaining of membrane and cytoplasm were scored. Her2/neu membranous staining was evaluated as a score of 0 to 3 according to the consensus of panel recommendations for gastric cancer [37]. Scores of 3+ were reported as positive, scores of 2+ as equivocal and scores of 0 or 1+ as negative. Cytoplasmic staining was defined as negative (score of 0), weak (score of 1), and strong (score of 2).

Her2/neu *in situ* hybridization

Tumors with membranous scores of 2+, 3+ and only strong cytoplasmic staining were tested by silver *in situ* hybridization on large sections. ISH score was estimated according to ASCO/CAP 2013 [38]. A minimum number of 20 tumor cells per sample were evaluated. For dual-probe ISH, HER2 signal to chromosome 17 centromere signal (HER2/CEP17) ratio adopting 2.0 or greater was considered as a positive result. Cases with a HER2/CEP17 ratio less than 2.0 were considered as negative if the HER2/nucleus count is less than 4.0, equivocal if the HER2/nucleus ratio is 4.0 to less than 6.0, and positive with a HER2/nucleus ratio of 6.0 or greater.

Statistical analysis

Mean ± standard deviation (SD) values are used to describe the quantitative variables. Median survival, one, three and 5-year survival were also reported for the survival data. Pearson χ^2 test without Yates continuity correction and Fisher-exact test were used to compare proportions; and Student T-test was used to compare the means of tumor characteristics. Kaplan-Meier survival curves and log-rank Test were used for univariate and Cox-Regression analyses were used for multivariate survival analyses. For all analyses IBM-SPSS version 20 was used and the statistical significance was set at $p < 0.05$.

Results

Overexpression of Her2/neu

In all 100 CRC cases the immunohistochemical expression status of Her2/neu had been assessed on whole sections. Membranous staining was observed in 33 cases whereas in 67 cases no membranous staining was observed (Table II). 21 of the 33 cases had a membranous score of 1+, 6 of the cases had a score of 2+ and 6 of the cases had a score of 3+ (Fig. 1A-C).

Heteregenous staining was detected in, one case with membranous score of 3+ and 5 cases with membranous score of 2+ (Table III, Fig. 1D).

There was cytoplasmic staining in 51 (51%) cases. Five of the 51 cases showed strong cytoplasmic staining. Two of the 5 cases with strong cytoplasmic

Table II. Her2/neu immunohistochemical membranous and cytoplasmic staining patern

	Membr. score 0+	Membr. score 1+	Membr. score 2+	Membr. score 3+	Total
No cytoplasmic staining	39	6	2	2	49
Weak cytoplasmic staining	26	15	2	3	46
Strong cytoplasmic staining	2	0	2	1	5
Total	67	21	6	6100	

Table III. Her2/neu immunohistochemical expression pattern and ISH

	Membranous staining pattern	Cytoplasmic staining pattern	ISH
Score 3+			
1	Homogeneous	Weak	Amplification
2	Homogeneous	Weak	Amplification
3	Homogeneous	None	Amplification
4	Homogeneous	None	Amplification
5	Heterogeneous	Weak	Amplification
6	Homogeneous	Strong	Amplification
Score 2+			
1	Homogeneous	None	Amplification
2	Heterogeneous	None	Amplification
3	Heterogeneous	Weak	Amplification
4	Heterogeneous	Weak	No amplification
5	Heterogeneous	Strong	Amplification
6	Heterogeneous	Strong	No amplification
Only cytoplasmic staining			
1	None	Strong	No amplification
2	None	Strong	No amplification

staining had a membranous score of 0. The remaining 2 cases had a membranous score of 2+ and one case had a membranous score of 3+. 46 of the cytoplasmic stained cases displayed weak cytoplasmic staining; 3 cases with weak cytoplasmic staining had a membranous score of 3+ and another 2 of these cases had a score of 2+ (Table II).

There were 2 signet ring carcinomas and 9 mucinous adenocarcinomas in our series of cases. None of the signet ring carcinomas overexpressed either membranous or cytoplasmic staining. Nine of the mucinous adenocarcinomas showed weak cytoplasmic staining but no membranous staining.

Her2/neu gene amplification

Her2/neu amplification was observed in 10 cases; 6 of them had 3+ membranous score and 4 of them had 2+ membranous score (Fig. 1E, F). Apart from that, in 6 cases heterogenity was observed. Four of the 6 heterogeneous cases (1 case with membranous score 3+ and 3 cases with membranous score of 2+) showed amplification by ISH (Table III). Two of the 5 cases with only strong cytoplasmic staining (membranous score 0) had no amplification. Two of the 5 cases with strong cytoplasmic staining (one with membranous score 3+ and homogeneous, one with membranous score 2+ and heterogeneous) showed amplification. The remaining strong cytoplasmic staining case (membranous score 2+ and heterogeneous) showed no amplification.

We detected Her2/neu amplification in 6/6 (100%) of the cases with homogeneous membranous score 2+ or score 3+. And Her2/neu amplification was found in 4/6 of cases with heterogeneous membranous score 2+ or score 3+. We performed reflex immunohistochemical test on a different tumor block for the single equivocal case. We found 1+ immunohistochemical score and HER/CEP17 ratio < 2 with HER2/nucleus ratio less than 4.

Clinicopathological correlation

Her2/neu positivity was identified in cases which displayed Her2/neu overexpression (score 2+ and score 3+) with Her2/neu amplification. No relationship was found between age, gender, tumor size, tumor grade, tumor localization, tumor type, tumor stage, lymph node metastases, distant metastases, lymphovascular invasion, survival state and survival period in cases indicating Her2/neu overexpression ($p > 0.05$). Her2/neu positive cases and clinicopathological data are shown in Table I. On the other hand no relation was found between tumor localization and Her2/neu amplification.

Her2/neu ISH was positive in 8.8% of the CRC on the right colon and 10.6% of the CRC on the left colon. No statistical difference was found between left and right colon tumors ($p = 1.00$).

Discussion

In this study we have investigated Her2/neu expression and the amplification on the whole sections of 100 primary CRC cases. Membranous overexpression of Her2/neu was found in 12 (12%) of the cases.

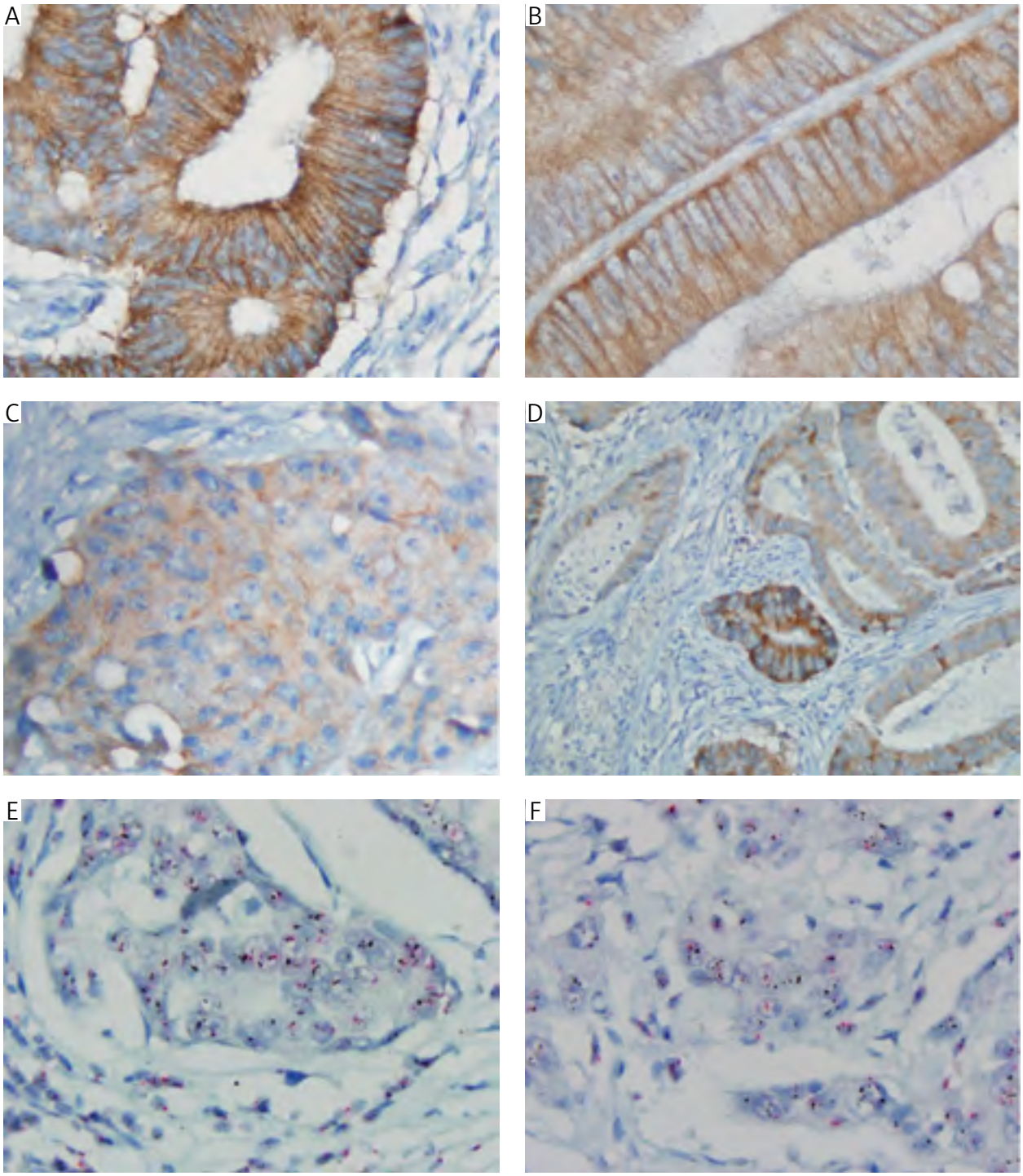

Fig. 1. Her2/neu immunohistochemical staining and ISH test. Her2/neu membranous score 3+ strong cytoplasmic staining (400×) (A), membranous score 2+ staining and strong cytoplasmic staining (1000×) (B), membranous score 1+ and weak cytoplasmic staining (1000×) (C), heterogeneous staining patern (400×) (D) and ISH test amplification (1000×) (E, F)

Her2/neu amplification was found in 10 out of 12 cases which showed membranous overexpression. According to these results the Her2/neu amplification rate was 10% out of 100 cases.

A number of studies on CRC indicates different rates of Her2/neu overexpression which might be caused by factors such as the tissue fixation period, primary antibody selection, antigen retrieval method, variation in detection systems and especially the size of the tissue in which immunohistochemical test evaulated on [39, 40]. These might be the reason for the difference in Her2/neu overexpression rates.

Previous studies with paraffin block of CRC tumors on whole sections reported higher rates of Her2/neu

expression compared with tissue microarray. The rates of expression on whole sections were between 11 and 47,4% [19, 20, 41, 42], on the other hand expression rates were 1-7,5% on tissue microarray [9, 18, 26, 25, 43]. The rate of expression was 12% in our whole section based study and similarly it was higher than tissue micraarray based studies. It is emphasized that comparatively low rates of Her2/neu expression monitored in studies including the tissue microarray method is caused by tumor heterogeneity. Tissue microarray method might be the reason for increase in number of false negative results. In our study, with using larger tumor tissues, we were able to overcome the false negative effect, which may be caused by heterogeneity.

Heterogeneity was found in one of the 6 cases with membranous score 3+ and 5 of the cases with score 2+. Overall, in 6 cases out of 12 cases heterogenity was found.

We found amplification in 4 out of 6 heterogeneously membranous stained cases. Since we applied both Her2/neu and SISH tests on whole section tumors, clearer evaluation on tumor heterogeneity was possible. According to literature, the reason for comparatively higher rates of Her2/neu overexpression could be explained in this way.

On the other hand, taking our facts into consideration, we determined that Her2/neu with cytoplasmic staining rate (cytoplasmic weak and strong) was at a high rate of 51%. Only 2 of Her2/neu with cytoplasmic homogeneous strongly stained cases were tested with ISH and there was no gene amplification. However, cytoplasmic overexpression might not always be in relation with gene amplification [44]. The reason for this cytoplasmic overexpression could be proteins connected with promoters or the feedback mechanism at target mutations like KRAS at Her2/neu downstreams [35]. Cytoplasmic Her2/neu protein overexpression's pathophysiology should be enlightened because intracellular Her2/neu targeting treatment agents (ex.lapatinib) could be beneficial [33]. Additionally, in literature the reason for higher-level Her2/neu expression relative to amplification is due to an exaggeration of cytoplasmic staining [44].

In our study, there was no correlation between Her2/neu posivity and age, gender, tumor size, tumor site, tumor type, tumor grade, tumor stage, lymph node metastases, distant metastases, lymphovascular invasion, survival state and survival period.

There was no correlation found between Her2/neu overexpression and gender, age, tumor stage, tumor type, tumor localization, grading, TNM stage, recurrence, lymph node metastases and follow-up clinicopathological data in previous studies [25, 26, 42]. While Heppner *et al.* found relation between Her2/neu overexpression and UICC stage, lymph node metastases in 1,645 primer CRC cases however no relation was found between Her2/neu overexpression and gender, age, survival data, localization, tumor grade and lymphovascular invasion [9]. Pyo *et al*. found a relation between Her2/neu overexpression and lymph node and distant metastasis but no relation was found between Her2/neu overexpression tumor dept and overall survival [22].

Annually, about 1.36 million new cases are diagnosed with CRC [1]. Estimating that 5% of these patients will be diagnosed with Her2/neu membranous overexpression worldwide then 68.000 patients are candidates for targeted treatment strategy. There are many studies on CRC where Her2/neu status is seen as a predictive and prognostic factor and CRC patients benefit from anti-Her2/neu treatment like in breast and gastric carcinomas. However, tumor heterogeneity in CRC is a restrictive aspect when it comes to determine Her2/neu evaluation in CRC is most likely to become a part of routine pathology practice in the future. When Her2/neu overexpression and amplification are being evaluated for CRC, tumor heterogeneity should be taken under consideration.

The authors declare no conflict of interest.

References

1. Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 2015; 136: E359-386.
2. André T, Boni C, Mounedji-Boudiaf L, et al. Oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment for colon cancer. N Engl J Med 2004; 350: 2343-2351.
3. Gutierrez C, Schiff R. HER2 biology, detection and clinical implications. Arch Pathol Lab Med 2011; 135: 55-62.
4. Olayioye MA, Neve RM, Lane HA, et al. The ErbB signaling network. receptor heterodimerization in development and cancer. Embo J 2000; 19: 3159-3167.
5. Molina R, Jo J, Filella X, et al. C-erbB-2 oncoprotein, CEA, and CA 15.3 in patients with breast cancer. prognostic value. Breast Cancer Res Treat 1998; 51: 109-119.
6. Bernhard H, Salazar L, Schiffman K, et al. Vaccination against the HER-2/neu oncogenic protein. Endocr Relat Cancer 2002; 9: 33-44.
7. Slamon DJ, Clark GM, Wong SG, et al. Human breast cancer correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science 1987; 235: 177-182.
8. Ieni A, Barresi V, Rigoli L, et al. HER2 Status in premalignant, early, and advanced neoplastic lesions of the stomach. Dis Markers 2015; 2015: 234851.
9. Ingold Heppner B, Behrens HM, Balschun K, et al. HER2/neu testing in primary colorectal carcinoma. Br J Cancer 2014; 111: 1977-1984.
10. Lim SW, Kim HR, Kim HY, et al. Over-expression of Her-2 in colorectal cancer tissue, but not in serum, constitutes an independent worse prognostic factor. Cell Oncol (Dordr) 2013; 36: 311-321.
11. Demirbas S, Sucullu I, Yildirim S, et al. Influence of the c-erb B-2, nm23, bcl-2 and p53 protein markers on colorectal cancer. Turk J Gastroenterol 2006; 17: 13-19.

12. Kavanagh DO, Chambers G, O'Grady L, et al. Is overexpression of HER-2 a predictor of prognosis in colorectal cancer? BMC Cancer 2009; 9: 1.
13. Rossi HA, Liu Q, Banner B, et al. The prognostic value of invariant chain (Ii) and Her-2/neu expression in curatively resected colorectal cancer. Cancer J 2002; 8: 268-275.
14. Ramanathan RK, Hwang JJ, Zamboni WC, et al. Low overexpression of HER-2/neu in advanced colorectal cancer limits the usefulness of trastuzumab (Herceptin) and irinotecan as therapy. A phase II trial. Cancer Invest 2004; 22: 858-865.
15. Half E, Broaddus R, Danenberg KD, et al HER-2 receptor expression, localization and activation in colorectal cancer cell lines and human tumors. Int J Cancer 2004; 108: 540-548.
16. Kruszewski WJ, Rzepko R, Ciesielski M, et al. Expression of HER2 in colorectal cancer does not correlate with prognosis. Dis Markers 2010; 29: 207-212.
17. Kountourakis P, Pavlakis K, Psyrri A, et al. Clinicopathologic significance of EGFR and Her-2/neu in colorectal adenocarcinomas. Cancer J 2006; 12 : 229-236.
18. Kim JY, Lim SJ, Park K. Cyclooxygenase-2 and c-erbB-2 expression in colorectal carcinoma assessed using tissue microarrays. Appl Immunohistochem Mol Morphol 2004; 12: 67-70.
19. Li Q, Wang D, Li J, et al. Clinicopathological and prognostic significance of HER-2/neu and VEGF expression in colon carcinomas. BMC Cancer 2011; 11: 277.
20. Park DI, Kang MS, Oh SJ, et al. HER-2/neu overexpression is an independent prognostic factor in colorectal cancer. Int J Colorectal Dis 2007; 22: 491-497.
21. Uner A, Ebinc FA, Akyurek N, et al. Vascular endothelial growth factor, c-erbB-2 and c-erbB-3 expression in colorectal adenoma and adenocarcinoma. Exp Oncol 2005; 27: 225-228.
22. Pyo JS, Kang G, Park K. Clinicopathological significance and diagnostic accuracy of HER2 immnohistochemistry in colorectal cancer: a meta-analysis. Int Biol Markers 2016; 31: e389-e394.
23. Tavangar SM, Shariftabrizi A, Soroush AR. Her-2/neu over-expression correlates with more advanced disease in Iranian colorectal cancer patients. Med Sci Monit 2005; 11: Cr123-126.
24. Wu SW, Ma CC, Yang Y. The prognostic value of Her-2/neu overexpression in colorectal cancer: evidence from 16 studies. Tumour Biol 2014; 35: 10799-10804.
25. Song Z, Deng Y, Zhuang K, et al. Immunohistochemical results of HER2/neu protein expression assessed by rabbit monoclonal antibodies SP3 and 4B5 in colorectal carcinomas. Int J Clin Exp Pathol 2014; 7: 4454-4460.
26. Marx AH, Burandt EC, Choschzick M, et al. Heterogenous high-level HER-2 amplification in a small subset of colorectal cancers. Hum Pathol 2010; 41: 1577-1585.
27. Drecoll E, Nitsche U, Bauer K, et al. Expression analysis of heat shockprotein 90 (HSP90) and Her2 in colon carcinoma. Int J Colorectal Dis 2014; 29: 663-671.
28. Wu SW, Ma CC, Li WH. Does overexpression of HER-2 correlate with clinicopathological characteristics and prognosis in colorectal cancer? Evidence from a meta-analysis. Diagn Pathol 2015; 10: 144.
29. Cunningham D, Humblet Y, Siena S, et al. Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. N Engl J Med 2004; 351: 337-345.
30. McIntire M, Redston M. Targeted therapies and predictive markers in epithelial malignancies of the gastrointestinal tract. Arch Pathol Lab Med 2012; 136: 496-503.
31. Ciardiello F, Normanno N. HER2 signaling and resistance to the anti-EGFR monoclonal antibody cetuximab. a further step toward personalized medicine for patients with colorectal cancer. Cancer Discov 2011; 1: 472-474.
32. Bang YJ, Cutsem EV, Feyereislova A, 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 2010; 376: 687-697.
33. Sartore-Bianchi A, Trusoline L, Martino C, et al. Dual-targeted therapy with trastuzumab and lapatinib in treatment-refractory, KRAScodon 12/13 wild-type, HER2-positive metastatic colorectal cancer (HERACLES): a proof-of-concept, multicentre, open-label, phase 2 trial. Lancet Oncol 2016; 17: 738-746.
34. Simon R, Nocito A, Hubscher T, et al. Patterns of her-2/neu amplification and overexpression in primary and metastatic breast cancer. J Natl Cancer Inst 2001; 93: 1141-1146.
35. 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: 508-523.
36. Fuchs EM, Köstler WJ, Horvat R, et al. High-level ERBB' gene amplification is associated with a particulary short time-to-metastasis, but result in high rate of complete response once trantuzumab-based therapy is offered in the metastatic setting. Int J Cancer 2014; 135: 224-231.
37. Josef R, Wedad H, Michael B, et al. HER2 testing in gastric cancer: a partical approach. Modern Pathology 2012; 25: 637-650.
38. Long TH, Lawce H, Durum C, et al. The New Equivocal. Changes to HER2 FISH Results When Applying the 2013 ASCO/CAP Guidelines. Am J Clin Pathol 2015; 144: 253-262.
39. Lee AH, Key HP, Bell JA, et al. The effect of delay in fixation on HER2 expression in invasive carcinoma of the breast assessed with immunohistochemistry and in situ hybridisation. J Clin Pathol 2014; 67: 573-575.
40. Tong LC, Nelson N, Tsourigiannis J, et al. The effect of prolonged fixatiom on the imminihistochemical evaluation of estrogen receptor, progestrone receptor, and HER2 expression in invasive est cancer: a prospective study. Am J Surg Pathol 2011; 35: 545-552.
41. Dursun A, Poyraz A, Suer O, et al. Expression of Bcl-2 and c-ErbB-2 in colorectal neoplasia. Pathol Oncol Res 2001; 7: 24-27.
42. Tu J, Yu Y, Liu W, et al. Significance of human epidermal growth factor receptor 2 expression in colorectal cancer. Exp Ther Med 2015; 9: 17-24.
43. Nathanson DR, Culliford ATt, Shia J, et al. HER 2/neu expression and gene amplification in colon cancer. Int J Cancer 2003; 105: 796-802.
44. Blok EJ, Kuppen PJ, van Leeuwen JE, et al. Cytoplasmic overexpression of HER2. a key factor in colorectal cancer. Clin Med Insights Oncol 2013; 7: 41-51.

Address for correspondence

Aydan Kılıçarslan
Department of Pathology
Yıldırım Beyazıt University
Eskişehir yolu
Lodumlu Mevkii No.2
Ankara, Turkey
e-mail: dr.kilicaydan@gmail.com