



# A comparative study of RTK gene status between primary tumors, lymph-node metastases, and Krukenberg tumors

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## Abstract

Krukenberg tumor (KT) refers to a rare ovarian tumor that has metastasized from a primary site. Patients with KTs have a poorer prognosis and worse survival. Thus far, little is known about the frequency of receptor tyrosine kinase (RTK) gene amplification and the concordance of gene amplification between primary tumors, lymph-node metastases, and KTs. Herein, 50 paired samples, including primary cancers, metastatic lymph nodes, and KTs were collected, and RTK gene amplification was tested by fluorescence in situ hybridization (FISH). There were four cases positive for human epidermal growth factor receptor type 2 (HER2) amplification, all of which showed conversion of HER2 status between different lesions. Of the two cases with c-mesenchymal–epithelial transition (c-MET) amplification, the primary tumors and lymph nodes were negative while the right involved ovaries were positive. Inconsistent fibroblast growth factor receptor 2 (FGFR2) status in different lesions was observed in three of the six FGFR2-amplified cases. Co-amplification of RTK genes was identified in only one patient for primary cancer and two for KTs. Collectively, there were 46, 48, 50, and 44 cases negative for HER2, c-MET, EGFR, and FGFR2 amplification in all lesions, respectively. There was no significant difference in overall survival between KTs of gastric origin and colorectal origin. However, of all synchronous cancers, KTs of colorectal origin had a better prognosis than those of gastric origin. In conclusion, the positive rate of RTK gene amplification in KTs was low. Intratumoral heterogeneity was frequent in KTs with RTK gene amplification. A mutually exclusive pattern of RTK gene amplification was dominant in primary cancers, lymph-node metastases, and KTs. There was no survival difference between KTs of gastric origin and colorectal origin. However, of all synchronous cancers, KTs of colorectal origin had a better prognosis than those of gastric origin.

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## Introduction

Krukenberg tumor (KT), named after Friedrich Krukenberg (1871–1946), a German gynecologist and pathologist, refers to a rare ovarian tumor that has metastasized from a primary site. KTs account for 1–2% of all tumors of the ovary [1]. The stomach is reported to be the most common site of origin of KTs (76%), followed by the colorectum (11%), breast (4%), biliary system (3%), appendix (3%), and other sites (e.g., pancreas, uterine cervix, urinary bladder, or renal pelvis) [2, 3]. Compared with primary ovarian cancer, patients with KTs have a poorer prognosis and worse survival, as KTs represent an advanced stage of the disease [4].

Although several routes have been suggested to explain the formation of KTs, such as lymphatic spread [1], peritoneal spread [5, 6], and hematogenous diffusion [1, 6–8], the underlying molecular mechanism is still under investigation. One mechanism of tumor pathogenesis and progression has been linked to the amplification of receptor

tyrosine kinase (RTK) genes, such as human epidermal growth factor receptor type 2 (HER2), c-mesenchymal–epithelial transition (c-MET), epidermal growth factor receptor (EGFR), and fibroblast growth factor receptor 2 (FGFR2) [9–12]. Clinical trials have shown that new treatment modalities targeting these molecules are promising, yielding robust efficacy and a durable response [13–15]. Nevertheless, a subgroup of patients do not benefit from the targeted therapy due to drug resistance. Two potential resistance mechanisms have been proposed, one of which is the co-amplification of RTK genes [16–18]. The second mechanism may be related to genetic heterogeneity [18, 19].

Owing to the limited number of studies that have investigated the expression pattern of RTK genes in ovary metastases, we collected 50 matched cases of KTs and conducted a comparative study of RTK gene status between primary tumors, lymph-node metastases, and KTs.

## Materials and methods

### Patients

A total of 50 paired samples, including primary cancers, metastatic lymph nodes, and KTs, were collected between October 2007 and December 2017 from the Department of Pathology, the First Affiliated Hospital of Zhejiang University. A block with the highest number of tumor cells per area was selected from the primary tumor and KT. For patients with lymph-node metastases, a block with the highest number of tumor cells per area was also selected [20]. Clinicopathological parameters were obtained from pathological reports. For survival analysis, patients were followed-up from the date of surgery until death. Median follow-up time was 23 months (range, 1 month to 10 years). Twenty cases of gastric origin KTs were previously investigated in a study published in 2017 [21]. This research was approved by the Ethics Committee of the First Affiliated Hospital of Zhejiang University. The Ethics Committee waived the need for consent because the data were analyzed anonymously.

### Tissue microarray

Three representative tissue cores with a diameter of 2 mm were removed from the original paraffin blocks. Because

there was insufficient tumor for the tissue microarrays (TMAs), two cores were sampled for five lymph-node metastases and one core for two lymph-node metastases and one KT. Moreover, one core was taken for samples earlier than 2010. TMAs were constructed using an array (TMA Grand Master, 3D Histech). Sections that were 4- $\mu$ m thick were cut from each TMA block, and stained with hematoxylin and eosin (H&E) to confirm the presence of tumor cells. If an inconsistent status of RTK gene amplification between different lesions in one case was observed, then the whole section from each lesion was used to retest the gene status to determine the presence of intratumoral heterogeneity.

### FISH

Sections 4- $\mu$ m thick were cut for fluorescence in situ hybridization (FISH) analysis. The sections were baked overnight at 60 °C. After deparaffinization and rehydration, the slides were boiled in distilled water at above 90 °C for 40 min and incubated with protease solution at 37 °C for 18 min. Probes (Zytovision, Germany, Table 1) were applied, and the slides were transferred to the hybridization oven (S500–24, Abbott Molecular, USA). The procedure was as follows: denature at 75 °C for 10 min and hybridize overnight at 37 °C. The next morning, after three washes with wash buffer (Zytovision, Germany) at 37 °C, the slides were counterstained with 15  $\mu$ L of DAPI. A ratio of target probe/centromere enumeration probe signal  $\geq 2$  was defined as gene amplification.

### Statistical analyses

Statistical analyses were performed using SPSS for Windows version 22.0. Comparisons of the clinicopathological features of each group were analyzed by  $\chi^2$  test or Fisher's exact test. Survival analysis was estimated using the Kaplan–Meier method, and significance was determined by the log-rank test. *P*-values < 0.05 were considered statistically significant.

## Results

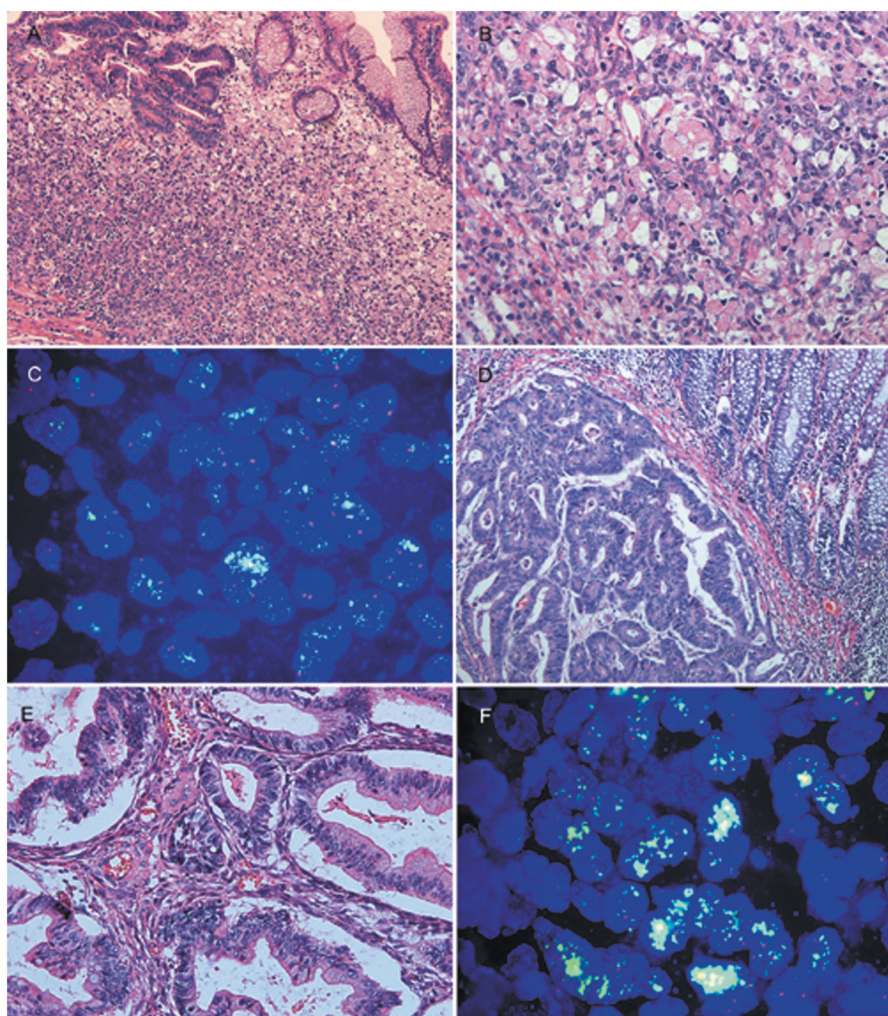
### Characteristics of the paired samples

There were 50 matched cases in the study. The mean age of the patients at diagnosis was 44.6 years (range, 24–70

**Table 1** Summary of the probes used in the study.

Probe	Product number	Length of targeted probe	Source
ERBB2/CEN17 dual-color probe	Z-2020–5	610 kb	Zytovision, Germany
MET/CEN7 dual-color probe	Z-2087–50	795 kb	Zytovision, Germany
EGFR/CEN7 dual-color probe	Z-2033–50	635 kb	Zytovision, Germany
FGFR2/CEN10 dual-color probe	Z-2122–200	775 kb	Zytovision, Germany

**Fig. 1 Representative images for KTs.** Hematoxylin and eosin (H&E) staining for a KT of gastric origin, including the primary cancer (**a**,  $\times 100$ ) and ovarian metastasis (**b**,  $\times 200$ ). The primary tumor was positive for FGFR2 amplification (green: FGFR2; red: CEP10) (**c**,  $\times 1000$ ). H&E staining for another KT of colorectal origin, including the primary cancer (**d**,  $\times 100$ ) and ovarian metastasis (**e**,  $\times 200$ ). The primary tumor was positive for HER2 amplification (green: HER2; red: CEP17) (**f**,  $\times 1000$ ).



years) with 88% of patients <60 years old. Among all patients, 41 were premenopausal, and 9 were postmenopausal. The primary cancers originated in the stomach ( $n = 27$ ) (Fig. 1a) and colorectum ( $n = 23$ ) (Fig. 1d). Of all primary gastric cancers, there were three cases of intestinal type by Lauren's classification and 24 cases of diffuse type. According to the fifth edition of WHO classification (digestive system tumor volume), 18 primary colorectal cancers were adenocarcinoma (nonspecific type), three were mucinous adenocarcinoma, and two were signet-ring cell carcinoma. In cases of gastric origin KTs, ovarian metastases were confirmed at the time of initial primary cancer diagnosis in 15 patients (Fig. 1b), and were bilateral at presentation in 17 patients. Signet-ring cells were present in 21 cases. Poor differentiation was dominant ( $n = 26$ ). Among KTs of colorectal origin, 12 patients presented with synchronous detection (Fig. 1e) and 16 with bilateral involvement. Signet-ring cells were detected in two cases. Poor differentiation was more common ( $n = 14$ ). The clinicopathological details of the patients are summarized in Table 2.

**Table 2** Clinicopathological features of the paired samples.

	Stomach	Colorectum	<i>P</i>
Menopause			
Pre	23 (85.2%)	18 (78.3%)	0.715 <sup>a</sup>
Post	4 (14.8%)	5 (21.7%)	
Chronology			
Synchronous	15 (55.6%)	12 (52.2%)	0.811
Metachronous	12 (44.4%)	11 (47.8%)	
Bilaterality			
Bilateral	17 (63.0%)	16 (73.9%)	0.408
Unilateral	10 (37.0%)	7 (26.1%)	
Signet-ring cells			
Present	21 (77.8%)	2 (8.7%)	0.000
Absent	6 (22.2%)	21 (91.3%)	
Differentiation			
Poor	26 (96.3%)	14 (60.9%)	0.003 <sup>a</sup>
Well/moderate	1 (3.7%)	9 (39.1%)	

<sup>a</sup>Fisher's exact test.

**Table 3** Comparison of RTK gene status between primary tumors, lymph-node metastases, and KTs.

Gene status	Stomach			Colorectum		
	Primary cancers	LN metastases	Ovaries	Primary cancers	LN metastases	Ovaries
HER2	– 26	24	25	22	18	23
	+ 1	0	2	1	0	0
c-MET	– 27	24	25	23	18	23
	+ 0	0	2	0	0	0
EGFR	– 27	24	27	23	18	23
	+ 0	0	0	0	0	0
FGFR2	– 23	20	22	23	18	23
	+ 4	4	5	0	0	0

LN lymph node.

Lymph-node metastases were identified in 24 gastric cancer cases and 18 colorectal cancer cases, respectively.

**Table 4** Groups showing conversion of RTK gene amplification between primary tumors, lymph-node metastases, and KTs.

RTK gene	Origin of KT	Primary tumor	Lymph node	Ovaries	
				Left	Right
HER2					
Case 1	Stomach	–	–	+	+
Case 2	Stomach	+	–	–	NA
Case 3	Stomach	–	–	+	–
Case 4	Colorectum	+	–	–	–
c-MET					
Case 3	Stomach	–	–	NA	+
Case 5	Stomach	–	–	–	+
FGFR2					
Case 6	Stomach	–	–	+	+
Case 7	Stomach	+	+	–	NA
Case 8	Stomach	–	–	NA	+

NA not applied.

### Comparison of RTK gene status between primary tumors, lymph-node metastases, and KTs

Analysis failed in 15 tissue spots due to lack of tumor cells (primary gastric cancer  $n = 4$ ; primary colorectal cancer  $n = 2$ ; lymph-node metastasis  $n = 6$ ; KT  $n = 3$ ).

There were four cases positive for HER2 amplification; three gastric cancers and one colorectal cancer (Table 3). There was a conversion of HER2 status between different lesions in these four cases. In the two cases of gastric origin, the primary tumors and lymph nodes were negative for HER2 amplification, but KTs were positive, one of which was positive for bilateral ovaries and the other was positive for only the left ovary. In the third case of gastric origin, the primary tumor was positive, but negative in the lymph node and left ovary. The primary tumor with KT of colorectal origin was HER2-positive, but negative for lymph node or bilateral ovaries (Fig. 1f, Table 4). The remaining 46 cases

were HER2-negative in all lesions. The concordance rate was 92% (Table 3). The positive rates of HER2 for primary gastric and colorectal cancer were 3.7% and 4.3%, respectively.

The c-MET amplification was identified in two cases of gastric origin. Inconsistent status of c-MET between different lesions was observed. The primary tumors and lymph nodes were negative, but the right involved ovaries were positive (Table 4). The c-MET status was consistent in the remaining 48 cases, all of which were negative (Table 3). The concordance rate was 96%. EGFR amplification was not detected in any of the paired samples (Table 3).

FGFR2 amplification was identified in six cases of gastric origin, three of which were positive for all lesions (Fig. 1c, Table 3). Two of the other three cases were negative for FGFR2 amplification in primary tumors and lymph nodes, but positive in involved ovaries, while the other was positive in the primary tumor and lymph node,



but negative in the left ovary (Table 4). Lesions in the remaining 44 cases were all negative for FGFR2. The positive rate of FGFR2 amplification for primary gastric cancer was 14.8%.

Co-amplification of RTK genes in primary cancers was present in only one patient (HER2-FGFR2 co-amplification:  $n = 1$ ), while these genes exhibited a mutually exclusive pattern of amplification in another four patients (FGFR2 amplification only:  $n = 3$ ; HER2 amplification only:  $n = 1$ ).

Among all lymph-node metastases with RTK gene amplification, FGFR2 was the only positive biomarker ( $n = 4$ ).

For ovarian metastasis, co-amplification of RTK genes was present in two patients (HER2-FGFR2 co-amplification:  $n = 1$ ; HER2-c-MET co-amplification:  $n = 1$ ), while a mutually exclusive pattern of amplification was observed in another five patients (FGFR2 amplification only:  $n = 4$ ; c-MET amplification only:  $n = 1$ ).

### Association between RTK gene status and treatment of KTs

Among the 27 cases with synchronous detection of primary cancers and KTs, 8 patients received adjuvant chemotherapy prior to surgical resection. Inconsistent HER2 status between different lesions was identified in two cases, one of which was negative for primary gastric cancer and lymph node, but positive for bilateral ovaries (Table 4, Case 1), and the other was positive for primary gastric cancer but negative for lymph node and left ovary (Table 4, Case 2). The RTK gene status was consistent in the other six cases. Out of the remaining 19 cases who had never received chemotherapy before surgery, two cases showed conversion of RTK gene status between different lesions, one of which was negative for HER2 and c-MET in primary gastric cancer and lymph node, but positive in involved ovaries (Table 4, Case 3), and the other was positive for HER2 in primary colon cancer but negative in lymph node and bilateral ovaries (Table 4, Case 4). The RTK gene status was consistent in the other 17 cases.

Among the 23 cases with metachronous detection of primary cancers and KTs, 19 patients underwent radical resection for primary cancers, followed by chemotherapy and then metastasectomy for KTs. Inconsistent RTK gene status between different lesions was identified in three cases, one of which was negative for c-MET in primary gastric cancer and lymph node but positive in the right ovary (Table 4, Case 5), one was negative for FGFR2 in primary gastric cancer and lymph node but positive in bilateral ovaries (Table 4, Case 6), and the other was positive for FGFR2 in primary gastric cancer and lymph node but negative in the left ovary (Table 4, Case 7). For another three patients who received adjuvant chemotherapy

**Table 5** Comparison between HER2 status and clinicopathological features in KTs of gastric origin.

	Positive	Negative	<i>P</i>
Menopause			
Pre	3 (100%)	20 (83.3%)	1.0 <sup>a</sup>
Post	0 (0)	4 (16.7%)	
Chronology			
Synchronous	3 (100%)	12 (50%)	0.231 <sup>a</sup>
Metachronous	0 (0)	12 (50%)	
Bilaterality			
Bilateral	2 (66.7%)	15 (62.5%)	1.0 <sup>a</sup>
Unilateral	1 (33.3%)	9 (37.5%)	
Signet-ring cells			
Present	2 (66.7%)	19 (79.2%)	0.545 <sup>a</sup>
Absent	1 (33.3%)	5 (20.8%)	
Differentiation			
Poor	3 (100%)	23 (95.8%)	1.0 <sup>a</sup>
Well/moderate	0 (0)	1 (4.2%)	

<sup>a</sup>Fisher's exact test.

and then radical resection for primary cancers and metastasectomy for KTs, one showed negative for FGFR2 in primary gastric cancer and lymph node but positive in the right ovary (Table 4, Case 8). The RTK gene status was consistent between different lesions in the last patient who underwent cytoreductive surgery for KT, followed by chemotherapy and then radical resection for primary gastric cancer.

### Association between clinicopathological parameters and RTK gene status

Due to the limited number of positive cases in KTs of colorectal origin, comparisons between RTK gene status and clinicopathological parameters were only conducted in KTs of gastric origin. Statistical analyses showed there were no significant differences between HER2 or FGFR2 status and clinicopathological features (Tables 5 and 6).

### Survival analyses

The median survival times of KTs of gastric and colorectal origin were 23.0 and 27.0 months, respectively. There was no significant difference in overall survival between KTs of gastric and colorectal origin (Fig. 2a). There was also no relationship between overall survival and clinicopathological features. However, of all synchronous cancers, KTs of colorectal origin had a better prognosis than those of gastric origin ( $P = 0.019$ ) (Fig. 2b).

The median survival time of HER2-positive KTs was 20.0 months, and 26.0 months for patients without HER2

amplification. Statistical analyses showed there was no significant difference in overall survival between these two groups (Fig. 2c).

The median survival time of FGFR2-positive KTs was 31.5 months, compared with 23.0 months in patients without FGFR2 amplification. Survival analyses revealed that there was no significant survival difference between these two groups (Fig. 2d).

**Table 6** Comparison between FGFR2 status and clinicopathological features in KT of gastric origin.

	Positive	Negative	<i>P</i>
Menopause			
Pre	6 (100%)	17 (81%)	0.545 <sup>a</sup>
Post	0 (0)	4 (19%)	
Chronology			
Synchronous	3 (50%)	12 (57.1%)	1.000 <sup>a</sup>
Metachronous	3 (50%)	9 (42.9%)	
Bilaterality			
Bilateral	3 (50%)	14 (66.7%)	0.638 <sup>a</sup>
Unilateral	3 (50%)	7 (33.3%)	
Signet-ring cells			
Present	4 (66.7%)	17 (81%)	0.588 <sup>a</sup>
Absent	2 (33.3%)	4 (19%)	
Differentiation			
Poor	6 (100%)	20 (95.2%)	1.000 <sup>a</sup>
Well/moderate	0 (0)	1 (4.8%)	

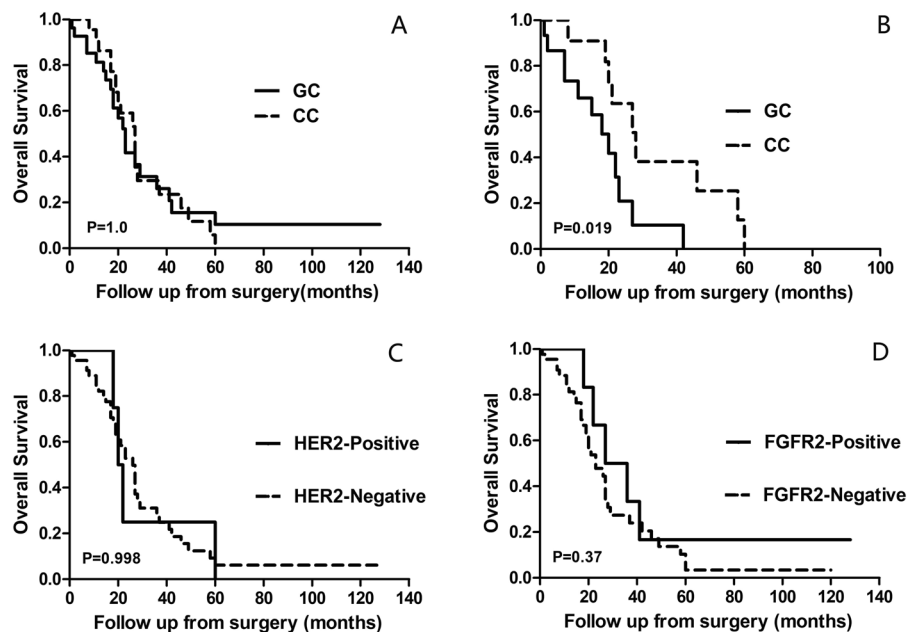
<sup>a</sup>Fisher's exact test.

## Discussion

Cytoreductive surgery plays an important role in the treatment of KTs, especially for patients with tumors of colorectal origin [22, 23]. Adjuvant chemotherapy following cytoreductive surgery is another approach to the management of KTs. This may provide a survival benefit for patients with KTs of gastric and colorectal origin [24]. However, the prognosis is still poor. In recent years, targeted therapy has emerged as an optional strategy to treat gastrointestinal (GI) tumors [25], which can complement conventional chemotherapy. Clinical trials have demonstrated that targeted therapy can yield better outcomes for GI cancer patients with RTK gene amplification [13, 14, 26]. Nevertheless, resistance against RTK-targeted therapy has been reported. The potential mechanism may be related to the co-amplification of RTK genes and genetic heterogeneity [16–19]. Herein, we aimed to investigate the expression pattern of RTK genes in ovary metastases.

Although a consensus on the definition of KT has not been reached worldwide, the term “Krukenberg tumor” is frequently used to describe all secondary tumors that metastasize to the ovaries. The stomach and colorectum have been reported to be the two major sources of KTs [2, 3]. During the period between October 2007 and December 2017 in our department, 56 cases involving the ovaries were identified, 27 and 23 of which were from the stomach and colorectum, respectively, followed by liver ( $n = 3$ ), breast ( $n = 1$ ), pancreas ( $n = 1$ ), and lung ( $n = 1$ ). Of the 50 cases of gastric and colorectal origin included in the present study, the mean age at diagnosis was 44.6 years

**Fig. 2 Kaplan–Meier overall survival curves.** **a** Comparison of overall survival between KTs of gastric and colorectal origin. **b** Comparison of overall survival for synchronous cancers between KTs of gastric and colorectal origin. **c** Comparison of overall survival between HER2-positive and HER2-negative cases. **d** Comparison of overall survival between FGFR2-positive and FGFR2-negative cases.



with 82% premenopausal, and 68% were bilateral at presentation, which is comparable to the epidemiology reported in the literature [2, 27, 28].

The prognosis of patients with KTs varied depending on the origin. Patients with KTs of genital tract origin have a better prognosis than those with tumors originating outside the genital tract [29]. Jeung et al. demonstrated that the mean survival time of patients with tumors of colorectal origin was significantly longer than that of patients with tumors of gastric origin [28]. Among all KTs, those originating from the pancreas and small intestine had the poorest prognosis [30]. Considering all the cases in this study, there was no statistical difference in overall survival between KTs of gastric and colorectal origin. However, of the synchronous cancers, KTs of colorectal origin had a better prognosis than those of gastric origin.

Gastric and colorectal cancers are complex diseases with heterogeneous expression of RTK genes. Heterogeneity implies both intertumoral and intratumoral heterogeneity [31]. The latter includes spatial heterogeneity in different tumor areas, and temporal heterogeneity, along progression from primary to recurrent and/or metastatic disease [31]. Intratumoral heterogeneity of RTK gene amplification is common in gastric and colorectal cancer. Cho et al. demonstrated that heterogeneous HER2 amplification could be detected in 23% of HER2-positive primary gastric carcinomas [32]. Marx et al. revealed heterogeneous findings in three of four HER2-positive colorectal cancers [33]. Stahl et al. conducted a systematic study, in which intratumoral heterogeneity was observed in nine of nineteen gastric cancers with HER2 amplification and five of seven cancers with EGFR amplification [34]. In addition, a large international multicenter study by Su et al. confirmed that intratumoral heterogeneity was observed in 24% of FGFR2-amplified gastric cancer cases [35]. Similarly, we found that all HER2-positive cases showed HER2 heterogeneous amplification between primary cancers, lymph-node metastases, and KTs, two with negative conversion and two with positive conversion. For the two KT cases of gastric origin with c-MET amplification, the involved ovaries were positive but negative in primary cancers and lymph-node metastases. Moreover, intratumoral heterogeneity of FGFR2 amplification was present in three of the six FGFR2-positive cases. In general, intratumoral heterogeneity is frequent in KTs with RTK gene amplification.

In this study, the positive rates of HER2 for primary gastric and colorectal cancer were 3.7% and 4.3%, respectively, while the positive rate of FGFR2 for primary gastric cancer was 14.8%; none of the primary colorectal cancers were positive for FGFR2 amplification. Moreover, c-MET and EGFR amplification were not detected in any of the primary gastric or colorectal cancers, which might be due to the small number of cases included in the study. RTK genes

have been reported to be amplified in a mutually exclusive manner across different gastric cancers [36, 37]. In other words, a tumor exhibiting amplification of one biomarker rarely amplifies the other markers. Das et al. performed a multicolor FISH assay targeting FGFR2, HER2, and KRAS in gastric cancer and showed these amplified genes to be mutually exclusive [37]. A mutually exclusive pattern of FGFR2 and HER2 amplification was also described in patients with gastric cancer from China, Korea, and the UK [35]. Among all of the cases, co-amplification of HER2 and FGFR2 was observed in only one Chinese patient and two from the UK [35]. Furthermore, a four-color FISH assay demonstrated that low-level co-amplification was detected in the same tumor cells, while high level amplifications occurred in distinct areas [35]. Another comparative study found that high level RTK gene amplification in Japanese primary gastric cancer was mutually exclusive, and co-amplification happened in cases with low-level RTK amplification [20]. Similarly, our data concluded that a mutually exclusive pattern of RTK gene amplification was dominant in primary cancers, lymph-node metastases, and KTs. The targeted probe signals were presented with clusters in all positive cases, except two, one for HER2 from primary colorectal cancer and the other for c-MET from the involved ovary. However, frequent RTK gene co-amplification was reported by Kwak et al. [18], who found that 40–50% of MET-amplified esophagogastric cancer patients exhibited co-amplification of HER2 and/or EGFR within the same tumor cells. More significantly, one case displayed amplification of all three genes within the same tumor cells [18]. This unusual observation could be related to the findings that RTK gene amplification occurs predominantly in gastric cancer affiliated with the chromosomal instability subtype based on TCGA classification, which exhibits frequent somatic copy number alterations [18, 38]. Other potential explanations could be the pathway cross talk and the ability of MET to heterodimerize with HER family members [18, 39]. Collectively, the underlying reasons for the different results across different studies might be attributed to the number of cases included, patient ethnicity, experimental platforms, definition for an amplification, and molecular subtype of gastric cancers [18, 20].

This study is limited by the application of the TMA platform, which may not reflect potential heterogeneity within an individual tumor. Despite favorable results obtained with TMA analysis of breast cancers [40, 41], RTK gene assessment of GI cancers is more complex. Some researchers have suggested that TMA technology cannot be applied as a method equal to full-section investigations [34, 42]. However, Gasljevic et al. concluded that with adequate fixation, a higher concordance of IHC results for HER2 between the cores in TMAs and the whole sections could be expected [43]. In addition, Berlth et al. found that

TMA technology using a 2-mm sample core delivered a substantial agreement with the full-section slides in gastric cancer, but not in the cases of positive expression [42]. To address this concern, three representative tissue cores with a diameter of 2 mm were taken for each sample. When an inconsistent status of RTK gene amplification between different lesions in one case was observed, then the whole section from each lesion was used to retest the gene status to confirm the presence of intratumoral heterogeneity. The results of whole sections were consistent with those from the TMAs. Even so, we still have to be cautious when interpreting the results from TMAs. TMA is indeed an ideal tool for studying a large number of cases in a fast and effective manner, but it seems to be impossible to fully avoid a sampling bias. There is a potential for missing areas of amplification which could underestimate the true number of cases with RTK gene amplification in this study.

In conclusion, the positive rate of RTK gene amplification in KTs was low. Intratumoral heterogeneity was frequent in KTs with RTK gene amplification. A mutually exclusive pattern of RTK gene amplification was dominant in primary cancers, lymph-node metastases, and KTs. There was no survival difference between KTs of gastric origin and colorectal origin. However, of all synchronous cancers, KTs of colorectal origin had a better prognosis than those of gastric origin.

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### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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