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ORIGINAL ARTICLE

KRAS discordance between primary and metastatic tumor in patients with metastatic colorectal carcinoma

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Summary

Purpose: *Adding targeted therapies to chemotherapy in metastatic colorectal cancer (CRC) improves response rates and survival. KRAS is a predictive indicator for anti-epidermal growth factor receptor (EGFR) treatments. The most important reasons for KRAS discordance are intratumoral heterogeneity and incorrect mutation analysis. Evaluating the status of KRAS in primary and metastatic lesions becomes even more crucial to ensure efficient usage of anti-EGFR treatments.*

Methods*: Patients with metastatic CRC, whose primary disease and liver and/or lung metastases were operated, were retrospectively evaluated, and KRAS assessment was performed on 31 patients who were suitable for DNA analysis. Pyrosequencing with polymerase chain reaction (PCR) was used for KRAS analysis.*

Results: *The median age of 31 patients diagnosed with rectal cancer (N=13) and colon cancer (N=18) was 63 years (range 33-73). Metastasectomy locations included the liver (N=27), lung (N=3), and both lung and liver (N=1). KRAS discordance was detected in 22% (7/31) of the patients. While 3 patients with detected discordance had mutated KRAS in the primary material, wild type KRAS was detected in their liver or lung lesions. On the other hand, while 4 patients had wild type KRAS in the primary material, mutated KRAS was determined in their liver or lung lesions. The McNemar test revealed no significant discordance between primary and metastatic disease (p=1.00). No progression free survival (PFS) difference was detected between patients with determined discordance and patients with undetermined discordance (10.6 vs 14.7 months, p=0.719).*

Conclusion: *This is the first study to evaluate KRAS discordance between primary and metastasis in CRC patients, who underwent metastasectomy, together with survival data. In the literature and recent studies with large patient numbers in which modern KRAS tests were used, the KRAS discordance rate varies between 3-12%. In our study, a higher KRAS discordance (22%) was detected, and no survival difference was determined between patients with or without discordance. In recent years, the rising interest in borderline resectable disease may bring forward discussions related to which material the KRAS status should be analyzed.*

Key words: *colorectal carcinoma, discordance,KRAS, survival*

Introduction

Chemotherapy and targeted therapy combinations in patients with metastatic CRC prolong survival by more than 2 years, with a 5-year overall survival (OS) of 19% [1]. Recently, survival benefit of using anti-epidermal growth factor receptor (EGFR)-based treatments in metastatic CRC has been shown. Also, survival benefit of metastasectomy in patients with low tumor load limited to the liver and lung was shown [2,3]. Preoperative treatments may change borderline resectable or unresectable disease into resectable disease [4,5]. In the CELIM study, the addition of cetuximab, which is an anti-EGFR agent, to conventional cytotoxics increased the chance of resection from 32 to 60% in KRAS wild type patients [6]. This made the discordance of the KRAS mutation, which is

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a predictive indicator for the use of cetuximab, between primary and metastasis even more important. KRAS analyses are mainly performed on primary materials since the tissue is more easily accessible. In the literature, there are studies which have analysed the KRAS discordance between metastasis and primary tumors with different results [7-11].

In this study, we aimed to analyse KRAS discordance and its significance in primary tumors and metastasectomy material in patients with metastatic CRC.

Methods

The study included patients who underwent primary tumor operation and subsequently metastasis resection at the Dr A.Y. Ankara Oncology Training and Research Hospital from May 2007 to January 2013. Patients whose pathology preparations were performed in other centres or whose pathology preparations were not appropriate for DNA analysis were not included in the study. Available KRAS results of patients who were followed-up in our clinic and the KRAS studies performed in our hospital or 3 other accredited centres (Istanbul Genetics Laboratory, Hacettepe University, Ankara University and Gazi University) were evaluated. KRAS test was performed on patients without KRAS result in their primary tumor and metastatic material. Data including patient demographics (age, gender, diagnosis, age), location of the primary tumor, tumor (T) and lymph node (N) stage, whether the diagnosis of metastasis was metachronous or synchronous, and whether the metastasectomy was performed with curative intent were recorded.

DNA preparation

DNA was extracted from those 3 slides that were not stained. Tumor-containing sections were microdissected from regions corresponding to the stained slides. Further steps of DNA isolation were performed in accordance with the tissue protocol QIAamp DNA FFPE TISSUE KIT (Qiagen, Hilden, Germany).

Pyrosequencing

Preparation for sequencing reaction was done using the PyroMark *KRAS* kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Polymerase chain reaction (PCR) was performed with KRAS 12-13 and 61 primers. Single-strand preparation of PCR products was done by immobilization on streptavidin-coated Sepharose beads (General HealthCare, USA), using a vacuum prep tool (Qiagen, Hilden, Germany). After adding specific sequencing primers (PyroMark-*KRAS* kit) samples were run on a PyroMark Q24 pyrosequencer (Qiagen, Hilden, Germany) and subsequently analyzed by PyroMark Q24 MDx software (PyroMark Q24).

Statistics

Data were expressed with median values and ranges. Discordance was indicated as percentage. PFS was accepted as the period from the first chemotherapy course in the metastatic setting to the first progression, and overall survival (OS) was accepted as the period from the first chemotherapy course in the metastatic setting to death from any cause. Survival rates were calculated using Kaplan-Meier survival analysis and were compared with the log-rank test. The change in KRAS status in primary and metastatic disease was compared using the McNemar test. A p value <0.05 was characterised as statistically significant. For statistical analyses, SPSS 15.0 version (SPSS Inc., Chicago, ILL, USA) was used.

Results

Specific patient characteristics and treatments

Thirty one patients were evaluated, all of whom were diagnosed at the Dr A.Y. Ankara Oncology Training and Research Hospital during May 2007-January 2013 and whose primary operations and metastasectomies were performed at our hospital. The general characteristics of the patients are summarised in Table 1. Three patients (9.7%) had undergone only lung metastasectomy, 27 patients (87.0%) had undergone only liver metastasectomy, and 1 patient (3.2%) had undergone both lung and liver metastasectomy. While R0 resection of primary and metastatic disease could be performed in 20 patients (64.5%), R2 resection (macroscopic residual disease) could be performed in 5 patients (16.1%), and R1 resection (microscopic residual disease) could be performed in 6 patients (19.4%).

KRAS discordance

KRAS mutation was determined in the primary tumor in 13 patients (41.9%). The most frequent mutation was located in codon 12. KRAS mutation was determined in the metastatic material in 13 patients (41.9%); 7 patients had discordance and the discordance rate (DR) was 22%. The McNemar test revealed no statistically significant discordance between primary tumor and metastasis (p=1.00; Table 2). While the KRAS status was mutant in the primary material in 3 of the patients with determined discordance, the KRAS status in the liver or lung metastasis material was determined to be wild type; but in 4 patients where

Table 1. General characteristics of the primary tumor of patients with metastatic colorectal carcinoma

Characteristics	*N*	*%*
Number of patients	31	100
Age, years, median (range)	63	33-73
Gender		
Female	16	51.6
Male	15	48.4
Localization of primary tumor		
Rectum	13	41.9
Sigmoid colon	8	25.9
Left colon	5	16.2
Transverse colon	3	9.6
Right colon	2	6.4
T stage		
2	2	6.5
3	25	80.6
4	4	12.9
N stage		
0	10	32.2
1	11	35.6
2	10	32.2
Differentiation		
Good	3	9.7
Moderate	18	58.0
Low	7	22.6
Unknown	3	9.7
Metastasis		
Synchronous	25	80.6
Metachronous	6	19.4

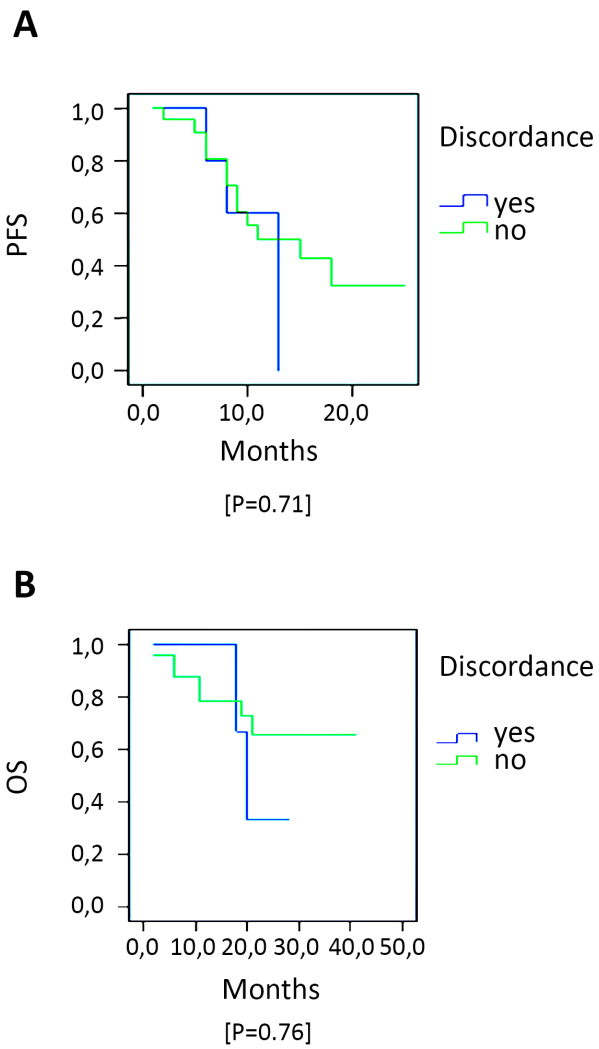


Figure 1. Progression free survival (PFS) **(A)** and overall survival (OS) **(B)** of patients with and without KRAS discordance in the primary and metastatic tissue.

the KRAS status in the primary material was wild type, the KRAS status in the liver or lung metastasis material was mutant. In 4 patients with wild type KRAS primary tumor and KRAS mutant metastasis, the median PFS was 11.2 months (range 8-13) and OS was 20.5 months (range 18-28), whereas in patients with mutant KRAS in the primary tumor and wild type KRAS in metastasis, the median PFS was 5.6 months (range 3-10) and the median OS was 12.3 months (range 10-14). In all of the patients with determined discordance, a mutation was detected in codon 12. Also, in liver and lung metastases of a patient whose KRAS status in the primary tumor was wild type, a mutation was detected both at codon 12 and codon 61. The general characteristics of patients with determined discordance are summarised in Table 3.

Survival analysis

Median follow-up duration was 19 months (range 2-41). A median of 2 lines of chemotherapy were administered (range 1-4). Twenty two (70%) individuals in the total patient cohort were still alive on July 2013. The PFS difference was not statistically significant in patients with discordance compared to patients without (PFS 10.6 vs 14.7 months, p=0.719). While OS was 22 months in patients with discordance, the median OS could not be determined in patients without discordance (Figure 1a-b). While the PFS of patients with wild type KRAS in the primary tumor tissue was 10.0 months, the PFS of patients with mutant KRAS was 15 months (p=0.23) (Figure 2a). While the PFS of patients with wild type KRAS in metastatic tissue was 8 months, the PFS of patients with a KRAS mutation was not statistically significant with a mean of 15.4 months (p=0.067) (Figure 2b).

The patient survival analysis was studied in terms of the resection type of metastasis. Five pa-

Table 2. KRAS discordance rates between primary tumor and metastasis

			KRAS metastasis		
			Mutant	*Wild*	*Total*
KRAS primary	Mutant	Number of patients (%)	1 (76.9)	3 (23.1)	13 (100)
	Wild	Number of patients (%)	4 (22.2)	14 (77.8)	18 (100)
Total		Number of patients (%)	14 (45.2)	17 (54.8)	31 (100)

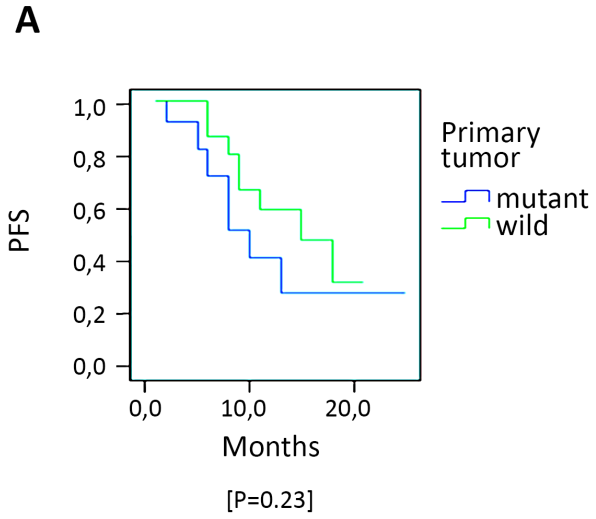
Table 3. Characteristics of patients with detected discordance between primary tumor and metastasis

No. of patients	1	2	3	4	6	7
Age, years	57	43	57	75	33	52
Gender	Female	Male	Male	Female	Female	Female
Localization of primary	Right colon	Left colon	Right colon	Transversecolon	Sigmoid	Rectum
Differentiation	Moderate	Low	Low	Low	Moderate	Low
Primary KRAS status	Wild	Wild	Wild	Mutant	Mutant	Wild
Metastasis KRAS status	Mutant	Mutant	Mutant	Wild	Wild	Mutant
Metastatic region	Liver	Liver	Liver	Liver	Liver	Liver /Lung
Primary operation	R0	R0	R0	R0	R2	R0
Metastasis time	Synchronous	Synchronous	Metachronous	Synchronous	Synchronous	Metachronous
Metastasis surgery time	At same session with primary	At same session with primary	At same session with primary	At same session with primary	At different time with primary	At different time with primary
Relapse time after metastasectomy (months)	13	13	11	10	3	8
Overall survival (months)	18	16	18	14	13	28

Table 4. KRAS status studies in primary tumor and its metastasis

First author [Ref]	*Year*	*Analyzed metastatic region*	*Mutant type patients in KRAS primary N (%)*	*Wild type patients in KRAS primary in mutant metastasis N (%)*	*Mutant patients in KRAS primary in wild type metastasis N (%)*	*Overall discordance rate N (%)*
Albanase [9]	2004	Liver	14 (47)	5/14 (36)	4/16 (25)	9/30 (30)
Artale [10]	2008	81% of liver	11 (23)	1/11 (9)	2/37 (5)	3/48 (6)
Baldus [8]	2010	Visceral metastasis	9 (45)	1/9 (11)	1/11 (9)	2/20 (10)
Italiano [11]	2009	Not indicated	23 (39)	1/23 (4)	2/36 (6)	3/59 (5)
Molinari [15]	2009	74% liver	16 (43)	2/16 (13)	1/21 (5)	3/37 (8)

tients underwent R2 resection, 6 patients underwent R1 resection, and 20 patients underwent R0 resection. Recurrence was determined during follow-up in 6 patients (30%) among the 20 patients who underwent R0 resection. In this group, the median PFS was 8 months (range 2-18). Among these patients, 4 had wild type KRAS in the primary tumor and metastatic material, 1 patient had wild type KRAS in the primary tumor material and mutation in metastatic material, and the other had mutant KRAS in the primary tumor material and wild type KRAS in the metastatic material. Recurrence was determined in 5 (84%) of the 6 patients who underwent R1 resection. PFS of pa-



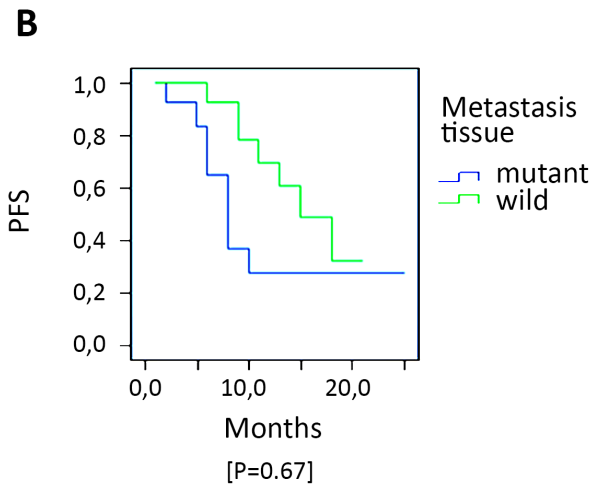
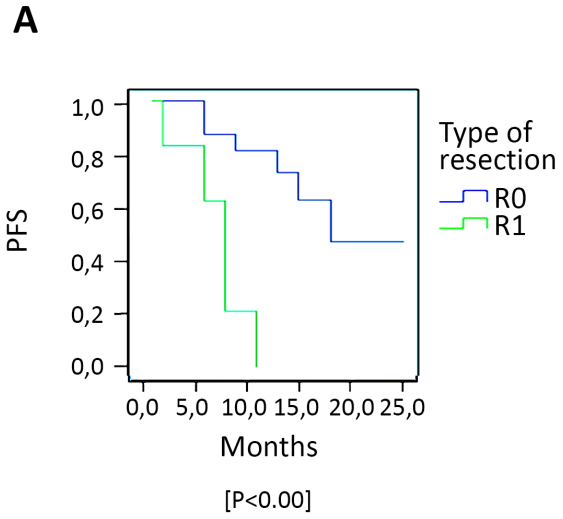


Figure 2. Progression-free survival (PFS) of patients whose KRAS status in the primary tissue **(A)** and metastatic tissue **(B)** was mutant or wild.



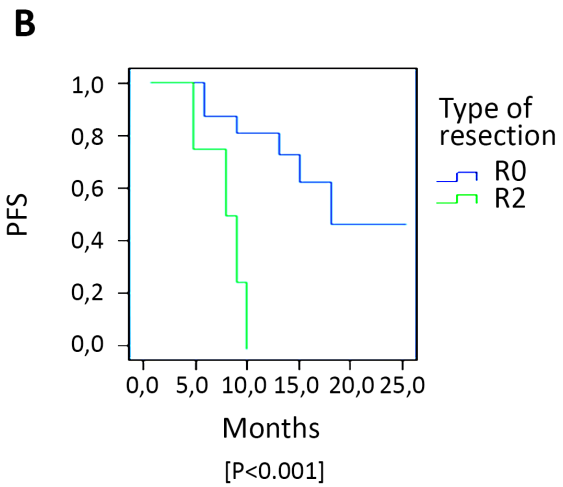


Figure 3. Progression-free survival (PFS) of patients who underwent R0 resection and patients who underwent R1 **(A)** and R2 **(B)** resection.

tients who underwent R0, R1 and R2 resection was 18, 7.2 and 8 months, respectively. A statistically significant difference in PFS was found between R0 and R2 resection (<0.001) (Figure 3a), and PFS was statistically different between R0 and R1 resection (p<0.001) (Figure 3b). Also, no statistically significant difference was determined in PFS in patients who underwent R1 and R2 resection (7.2 and 8 months, respectively; p=0.96) (Figure 4a). Median OS was 19 months in patients who underwent R1 resection and 20 months in patients who underwent R2 resection, whereas median OS could not be reached in patients who underwent R0 resection (Figure 4b).

Discussion

In this study, we analysed the discordance of KRAS status between primary tumor and metastasis in patients with CRC whose primary tumor and metastasis were operated. In our study, KRAS mutation was detected in primary material in 13 patients (41.9%) and in metastatic material in 14 patients (45.1%). This result is consistent with the RASCAL studies [12,13]. In our study, discordance was detected at a rate of 22% (7/31). In the literature, there are studies that have analysed the KRAS mutation discordance between primary and metastatic tissues in CRC [7-11]. However, this is the first study that has analysed KRAS discordance between primary tumor and metastasis, together with survival of CRC patients who underwent metastasectomy.

It is believed that KRAS mutation should display a low discordance, since it is a driver mutation which is observed in the early adenoma

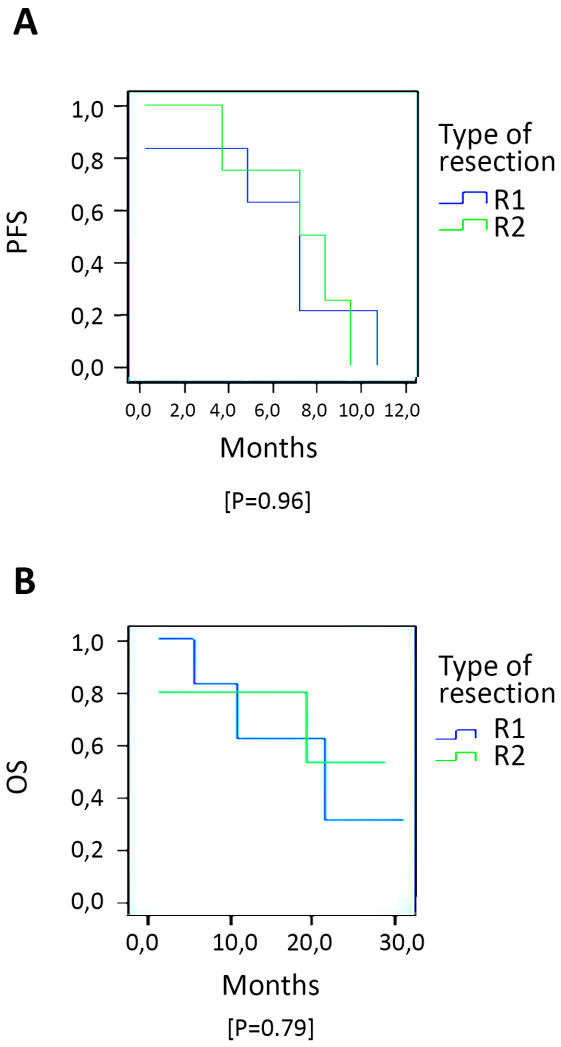


Figure 4. Progression-free survival (PFS) **(A)** and overall survival (OS) **(B)** of patients who underwent R1 and R2 resection.

period [14]. In the literature, there are many studies, the first being published 20 years ago; most of these have analysed a limited number of patients, but found discordance to vary between 5 and 30% [8-11,15] (Table 4). A great part of these studies belong to periods during which KRAS mutation was not yet used in clinical practice. In the first study where the PCR method was used, 99 patients with metastatic CRC were analysed [7]. Discordance was detected in only 4 patients (4%); in one of them, while wild type KRAS was determined in the primary mass, a KRAS mutation was determined in a peritoneal metastasis, diagnosed simultaneously with the primary tumor. In the other 3 patients, while the primary tumor was found to be mutant, wild type KRAS was detected in their liver metastases. In a study published by Watanabe et al. in 2011, the KRAS status was analysed in the primary tumors of 43 patients and in 113 metastatic materials from the same patients, as a result of which KRAS discordance was detected in 5 patients (12%) [16]. While in one of these patients the primary and liver metastasis were wild type, a KRAS mutation was detected in generalised peritoneal metastases. In the Watanabe et al. study, liver metastasis was present in 25 patients and discordance in the KRAS status was not detected in the same patient among the 76 materials analysed in these metastases. The same was also valid for the 26 materials of the 11 patients with lung metastasis. Fifty eight regions were analysed with microdissection in the primary tumor of 5 patients with detected discordance and it was observed that these patients had mixed KRAS status. When the same analysis was performed in the 10 patients without discordance for 91 microdissections, it was observed that all patients had homogeneous KRAS status. In the study of Knijn et al., which is the largest study reported in this topic, the primary and liver metastasis of 294 patients with CRC were analysed, and KRAS mutation was detected in 108 (36%) patients in the primary tumor [17]. KRAS discordance was detected in 11 (3.6%) patients. When they analysed the patients with discordance, they detected heterogeneity for the KRAS status in primary tumor in 2 patients. However, in this study, it was not indicated how many of the metastatic materials were surgical resections and how many were biopsies.

Discordance in KRAS status may be explained by intratumoral heterogeneity [16]. Baisse et al. analysed the loss of heterogeneity (LOH) for chromosomes 5q and 18q from 15 tumor samples, and searched for KRAS mutations and point mutations of the p53 gene [15]. They detected at least one genetic change in 10 patients. In the APC and DCC genes, they detected LOH to occur at a rate of 58%, and the heterogeneity rate for KRAS mutation or p53 mutation was 20%. The intratumoral heterogeneity detected in this preclinical study and in other discordance studies explains why some of the patients with wild type KRAS status do not benefit from anti-EGFR treatment. It is clear that the detection of a low KRAS discordance, KRAS heterogeneity remains unable to explain the resistance to all anti-EGFR treatments. The most probable explanation is that driver pathways, besides the KRAS pathway, are present, and these pathways are either more active in resistant patients, or newly developed in connection with treatment. Some patients with wild type KRAS who are resistant to anti-EGFR treatment may

have BRAF and NRAS mutation and these mutations may be responsible for the refractoriness to anti-EGFR treatment [18,19].

Another reason for the detection of discordance in the studies may be analytical errors of the KRAS test. The primary and metastasis operations of our 3 patients with detected discordance were performed in different times. However, we do not know whether the time lapse between the two operations affects the differentiation of the tumor biology. Also, another mechanism which may explain discordance is the programmed transformation of the tumor biology with its effect to the tumor microframe of the tissue it metastasises. The fact that Watanabe et al. detected a wild type KRAS status in the primary tumor and liver metastasis of a patient with detected discordance, and a mutant status in peritoneal metastasis, leads us to consider the microframe effect on host tissue [16].

In our study, in which the 'pyrosequencing' method (a sample of sequencing technology) was used, KRAS discordance was determined to be 22%. Through this method, mutation may be detected with a mutant load of 2-5%. Although the literature indicates a discordance rate of up to 30%, it is worth mentioning that in recent studies [7,8,15,16] where more patients were included and more sensitive methods were used KRAS discordance varied between 3 and 12%. In our series, this rate was higher. We believe that an important reason for this is the limited number of patients. A difference in PFS was determined between the primary tumor and metastasis in patients with or without discordance, which, however, did not reach statistical significance. Because the monitoring period was short, the median OS could not be reached. Although a statistical analysis could not be performed due to the limited number of patients, both PFS and OS were longer in patients when KRAS was wild type in the primary tumor and mutant in metastasis in comparison with patients with mutant primary tumor and wild type metastasis (median PFS 11.2 vs 5.6 months, median OS 20.5 vs 12.3 months). In addition, in our study, no difference in OS was detected between patients who underwent R0, R1 and R2 resection. This may be a result of the short median follow up period (19 months). However, PFS was significantly longer in patients who underwent R0 resection compared to patients who underwent R1 and R2 resection. No difference in PFS was determined between patients who underwent R1 and R2 resection. Our results are consistent with those found in the literature [20].

We do not know whether the patients with detected discordance have a different clinical course. Our study is one of the first to examine the KRAS discordance and survival results. It is a clinically accepted and reasonable approach to analyse KRAS from the material which is most easily accessible in metastatic patients. However, the rising interest in borderline resectable cases in recent years may bring forward discussions related to which material the KRAS status should be analysed.

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