Clinical characterization of patients with metastatic colorectal cancer depending on the *KRAS* status

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This retrospective study investigated the clinical characteristics of patients with metastatic colorectal cancer (mCRC) depending on the *KRAS* status, thereby differentiating *KRAS* exon 2 mutations in codon 12 versus codon 13. In total, 273 patients with mCRC receiving first-line therapy were analyzed. One hundred patients were treated within the FIRE-3 trial (FOLFIRI plus cetuximab or bevacizumab), 147 patients within the AIO KRK-0104 trial (cetuximab plus CAPIRI or CAPOX), and further 26 patients received therapy outside the study. Thirty-eight tumors with *KRAS* mutation in codon 13, 140 tumors with mutation in codon 12, and 95 tumors with *KRAS* wild type as a comparison were included in this analysis. Bivariate analyses demonstrated significant differences between *KRAS* wild-type, codon 12-mutated, and codon 13-mutated tumors with regard to synchronous lymph node metastasis ($P=0.018$), organ metastasis (76.8% vs. 65.9% vs. 89.5%, $P=0.009$), liver metastasis (89.5% vs. 78.2% vs. 92.1%, $P=0.025$), lung metastasis (29.5% vs. 42.9% vs. 50%, $P=0.041$), liver-only metastasis (48.4% vs. 28.8% vs. 28.9%, $P=0.006$), and metastases in two or more organs (49.5, 61.4, 71.1, $P=0.047$). Regression models indicated a significant impact of *KRAS* mutations in codon 12 versus codon 13 for synchronous organ and nodal metastasis ($P=0.01$, 0.03). This pooled analysis indicates that mCRC is a heterogeneous disease, which seems to be defined by *KRAS* mutations of the tumor. Compared with *KRAS* codon 12 mutations, codon 13-mutated mCRC presents as a more aggressive disease frequently associated with local and distant metastases at first diagnosis. *Anti-Cancer Drugs* 22:913–918 © **2011 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Introduction

KRAS mutation was identified as a negative prognostic factor for disease recurrence more than a decade ago in patients undergoing surgery for primary colorectal cancer (CRC) [1–4]. First indications of the therapeutic impact of this mutation were described during the last years when mutations in the *KRAS* protooncogene were identified as determinants of poor response to anti-EGFR treatment for metastatic colorectal cancer (mCRC). Therefore, patients with *KRAS* mutations were excluded from treatment with cetuximab or panitumumab [5–9].

Although the *KRAS* mutational status has become a widely accepted tool to guide anti-EGFR therapy, it seems that the group of patients with mCRC cannot longer be seen as a uniform collective. Specifically, *KRAS*-mutated tumors obviously represent a heterogeneous group with regard to their clinical outcome.

Evidence for clinically relevant subgroups of *KRAS* mutations was presented in 1998 when *KRAS* mutations in codon 13 were described as a negative prognostic marker during postoperative follow-up [1]. This observation was later confirmed by an analysis demonstrating that codon 13 mutation of the *KRAS* oncogene was associated with advanced Dukes' stage, lymph node metastasis, a higher recurrence rate, and shorter survival when compared with other *KRAS* mutations or *KRAS* wild type [10]. In advanced disease, the poor outcome of patients with *KRAS* codon 13 mutations was also described by other investigators [4,11], but in one case, it was not confirmed by a multivariate analysis [11]. However, two large studies focusing on all stages of colorectal cancer and only stage II and III, respectively, did not identify the *KRAS* codon 13 mutation to correlate with the clinical outcome [12,13].

In 2010, De Roock *et al.* [14] investigated patients with chemotherapy-refractory mCRC and identified the codon 13 mutation to be correlated with poor survival in patients undergoing the best supportive care. However, a benefit from treatment was noted when codon

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13-mutated patients received the anti-EGFR antibody cetuximab. This observation was substantiated by results obtained from in-vitro studies and from mouse models that clearly indicated that codon 13-mutated tumor cells were sensitive to cetuximab [14].

This retrospective study evaluated patients receiving first-line chemotherapy for mCRC; the aim of this investigation was to correlate *KRAS* mutational status (wild type, codon 12 mutations, codon 13 mutations) with specific clinical patient characteristics associated with the presentation or course of the disease. The results obtained are clinically and scientifically relevant, as only limited information is currently available regarding the clinical course of mCRC patients with codon 13-mutated tumors. We tested the hypothesis that codon 13 mutations are correlated with synchronous metastasis in both lymph nodes and distant organs at the time of first diagnosis and with adverse allocation of metastasis in mCRCs when compared with codon 12 mutations or wild-type tumors.

Methods

KRAS mutation detection

KRAS testing was performed centrally in a German reference laboratory for *KRAS* analysis (Institute of Pathology, University of Munich). After dissection of tumor-containing areas, DNA was isolated using Qiagen DNA Micro-Amp kits (Qiagen, Venlo, Netherlands). Detection of mutations in codons 12 and 13 of the *KRAS* protooncogene then was performed by pyrosequencing using Qiagen's PyroMark Gold kits (Qiagen) together with a Q24 pyrosequencer device. This procedure resulted in a specificity of 0.98 and a sensitivity of 0.99 for the detection of mutations in the *KRAS* protooncogene.

Patients

For this study, we analyzed 273 patients suffering from mCRC with a proven *KRAS* status. One hundred patients were treated within the FIRE-3 trial (NCT00433927-comparing FOLFIRI plus cetuximab to FOLFIRI plus bevacizumab) before an amendment determined that only patients with *KRAS* wild type could be included into the study. A further 147 patients received first-line treatment within the AIO KRK-0104 trial (NCT00254137; comparing CAPIRI plus cetuximab to CAPOX plus cetuximab [15]), whereas 26 patients were treated outside clinical trials at the University of Munich. All patients had a histologically confirmed diagnosis and received first-line treatment for metastatic disease. The protocols of the clinical trials were approved by an independent ethics committee and governmental authorities. The trials were conducted in accordance with the Declaration of Helsinki (1996). All patients provided written informed consent to be treated within a clinical trial.

End points

This investigation was performed as an exploratory, retrospective pooled analysis. Initial tumor stage was documented according to the tumor nodes metastasis classification of malignant tumors developed by the international union against cancer. pT and pN stage were noted if surgery was performed on the primary tumor or cT and cN stages in rare cases if radiologic images gave a clear impression of these parameters. For this investigation, clinical and pathological stages of tumor and lymph nodes were summarized. Allocations of metastasis at the beginning of palliative treatment were detected by spiral computed tomographic scans of the thorax and abdomen. The performance status was determined using the Eastern Cooperative Oncology Group (ECOG) scale. When performance status was indicated as the Karnofsky index, values of 90–100% were as attributed to ECOG 0, 80% to ECOG 1, and 70% to ECOG 2. Patient characteristics were presented as valid percentages based on nonmissing data.

Statistical analysis

Data were summarized by adequate measures of location and spread for continuous variables and by proportions for discrete variables. Adequate tests for continuous data (Mann–Whitney *U*-test) and for discrete data (χ^2 test) were used. For modelling the dichotomous outcomes 'synchronous organ metastasis', 'liver metastasis', 'pulmonary metastasis', 'liver-only metastasis', 'synchronous nodal metastasis', and 'two or more organs with metastasis', we used the logistic regression model where covariates were selected from a set of candidate variables (*KRAS* status, sex, age, ECOG, grading, and 'primary tumor site') relying on a backward elimination algorithm using likelihood ratio tests. For each outcome, we used complete cases and the variable selection level for all variables was set to 0.05. For each final model, the *P* values of the likelihood ratio tests are reported as they especially allow assessing the impact of a covariate that has several categories. In addition, the odds ratio, 95% confidence intervals and *P* values resulting from the Wald tests are reported as they allow a *P* value to be provided for each category (except the chosen reference) of a covariate with several categories. All statistical tests were performed two-sided, and a *P* value of 0.05 or less was considered as statistically significant. All statistical analyses were performed by using *R* (version 2.12.2).

Results

Study population

This pooled analysis included 273 patients with mCRC who received first-line treatment. Among this population, 38 patients showed a mutation in codon 13 (c.38G > A) of exon 2, whereas the other 140 patients presented with mutations in codon 12 (c.34G > A, c.34G > C c.34G > T, c.35G > A, c.35G > C, c.35G > T). In addition, 95 patients with *KRAS* wild-type mCRC were included for comparison. The mutation frequency of codon 13 mutations within the *KRAS*-mutated subgroup of our study population was 21.3%, whereas a mutation in codon 12 was observed in 78.7% of the tumors. Demographical, pathological, and

Table 1 Patient characteristics

	KRAS wild type		*KRAS* mutation codon 12		*KRAS* mutation codon 13		
Baseline characteristics	*N*	%	*N*	%	*N*	%	*P* value
Number of patients	95		140	78.7	38	21.3	χ^2 test
Age (years)							
Median	62.0		64.0		64.0		NS
Range	33–75		36–76		28–76		
Sex							
Female	30	31.6	42	30.4	14	36.8	NS
Male	65	68.4	96	69.6	24	63.2	
Not reported	0	0	2		0	0	
ECOG							
0	71	76.3	80	58.0	20	52.6	
1	19	20.4	53	38.4	14	36.8	0.009
2	3	3.2	5	3.6	4	10.5	
Not reported	2		2		0		
Primary tumor site							
Colon	66	69.5	82	59.4	27	71.1	
Rectum	29	30.5	55	39.9	11	28.9	NS
Colon and rectum	0	0	1	0.7	0	0	
Not reported	0	0	2		0	0	
Prior therapy							
Adjuvant chemotherapy	14	14.7	36	26.3	4	10.5	0.03
Not reported	0	0	3		0	0	
Prior radiotherapy	7	7.4	27	19.9	1	2.7	0.003
Not reported	0	0	4		1		

Percentages are calculated based on non-missing data.
ECOG, Eastern Cooperative Performance Score; NS, not significant.

clinical data were documented in all patients. Baseline characteristics of the patients analyzed in this study do not show significant differences with regard to age, sex, and location of the primary tumor when compared between patients with wild-type, codon 12%-mutated, or codon 13-mutated tumors. Patients with wild-type tumors seemed to present in a better performance status (ECOG) at treatment initiation for mCRC compared with patients with *KRAS*-mutated tumors ($P = 0.009$) (Table 1).

Impact of *KRAS* mutation status on initial tumor nodes metastasis status and tumor grading

Although the *KRAS* mutation status did not correlate with the status of the primary tumor, patients with a *KRAS* mutation in codon 13 had a statistically significant higher rate of synchronous nodal metastasis (93.6% vs. 71.3%, $P = 0.03$) and organ metastasis (89.5% vs. 65.9%, $P = 0.01$) at the time of first diagnosis of CRC compared with patients with codon 12-mutated tumors (Tables 2 and 3). Correspondingly, the rate of patients previously treated with systemic chemotherapy ($P = 0.03$) or local radiation therapy ($P = 0.003$) was significantly lower for patients with *KRAS* codon 13-mutated tumors (Table 1). No association of *KRAS* status with tumor grading was observed in our patient population (Table 2). A higher rate of synchronous nodal and organ metastasis was detected when *KRAS* wild-type tumors were compared with codon 12-mutated tumors (Table 2).

Impact of the *KRAS* mutation status on distant metastasis

Patients with *KRAS* wild-type or codon 13-mutated tumors had a higher rate of liver metastasis at the beginning of palliative treatment compared to the subgroup of patients with codon 12-mutated tumors (codon 12 vs. codon 13, $P = 0.1$; codon 12 vs. wild type, $P = 0.02$) (Tables 2 and 3). Moreover, patients with *KRAS*-mutated tumors tended to have a higher rate of at least two organs involved with metastasis when starting palliative treatment ($P = 0.047$; Tables 2 and 3). Correspondingly, the rate of pulmonary metastasis was significantly higher for *KRAS*-mutated patients ($P = 0.041$), showing no difference between codon 12-mutated and codon 13-mutated tumors (Tables 2 and 3). Despite the high frequency of synchronous metastasis, patients with *KRAS* wild-type tumors had a significantly higher probability of having hepatic metastasis only ($P = 0.006$) (Tables 2 and 3).

Discussion

This analysis was stimulated by a recent study indicating that patients with *KRAS* codon 13-mutated mCRC may belong to a specific subgroup of mCRC. Chemotherapy-refractory patients with *KRAS* codon 13 mutations had a very poor survival when treated with best supportive care only [14]. However, a marked benefit was obtained from anti-EGFR-directed treatment with cetuximab, whereas this effect was not observed in *KRAS* codon 12-mutated patients. Accordingly, it may be concluded that different mutations of the *KRAS* oncogene may confer specific patterns of response to treatment. This investigation was set out to define the phenotype of mCRC disease associated with *KRAS* codon 13 mutation.

According to the published literature, codon 13 mutations occur at an overall frequency of 8%, and at a rate of

Table 2 Tumor characteristics

	KRAS wild type		*KRAS* mutation codon 12		*KRAS* mutation codon 13		
Tumor characteristics	*N*	%	*N*	%	*N*	%	*P* value
Number of patients	95		140		38		χ^2 test
Initial T stage							
T1	3	3.3	3	2.4	0	0	
T2	2	2.1	13	10.6	4	13.3	NS
T3	59	65.6	73	57.7	16	53.3	
T4	26	28.9	36	29.3	10	33.3	
Not reported	5		17		8		
Initial N stage							
N0	17	19.3	35	28.7	2	6.5	
N1	23	26.1	42	34.4	11	35.5	0.018
N2	48	54.5	45	36.9	18	58.1	
Not reported	7		18		7		
Initial M stage							
M0	22	23.2	47	34.1	4	10.5	0.009
M1	73	76.8	91	65.9	34	89.5	
Not reported	0	0	0	0	0	0	
Tumor grading							
G1	0	0	2	1.7	0	0	
G2	59	67.8	87	71.9	27	81.8	NS
G3	28	32.2	32	26.4	6	28.2	
Not reported	8		19		5		
Metastatic disease site							
Liver	85	89.5	104	78.2	35	92.1	0.025
Liver only	46	48.4	38	28.8	11	28.9	0.006
Lung	28	29.5	57	42.9	19	50.0	0.041
Peritoneum	15	15.8	14	10.5	5	13.2	NS
Other including lymph nodes	37	38.9	50	37.6	11	28.9	NS
Two or more organs involved	47	49.5	81	61.4	27	71.1	0.047

Percentages are calculated based on non-missing data.
NS, not significant.

Table 3 Logistic regressions

Variables	*N*	Odds ratio (95% CI)	*P* value (Wald)	*P* value (LR)
Synchronous organ metastasis (initial M1 status)				
codon 12 vs. wild type	239	1.9 (1.0–3.6)	0.04	0.003
codon 12 vs. codon 13		5.5 (1.6–19.1)	0.01	
Synchronous nodal metastasis (initial N Status)				
codon 12 vs. wild type	219	2.0 (0.98–4.2)	0.06	0.02
codon 12 vs. codon 13		5.3 (1.2–24.3)	0.03	
ECOG 0 vs. 1		2.4 (1.1–5.4)	0.04	0.04
ECOG 0 vs. 2		0.4 (0.1–2.1)	0.3	
Liver metastasis at the beginning of palliative treatment				
codon 12 vs. wild type	234	2.8 (1.2–6.5)	0.02	0.01
codon 12 vs. codon 13		2.9 (0.8–10.2)	0.1	
Pulmonary metastasis at the beginning of palliative treatment				
codon 12 vs. wild type	234	0.48 (0.3–0.9)	0.02	0.02
codon 12 vs. codon 13		1.4 (0.6–3.1)	0.4	
PTS (colon vs. rectum)		2.4 (1.4–4.3)	0.003	0.002
Liver-only metastasis at the beginning of palliative treatment				
codon 12 vs. wild type	233	2.8 (1.5–5.2)	0.001	0.003
codon 12 vs. codon 13		1.3 (0.5–3.0)	0.6	
PTS (colon vs. rectum)		0.5 (0.2–0.8)	0.01	0.01
Two or more organs with metastasis at the beginning of palliative treatment				
codon 12 vs. wild type	233	0.5 (0.3–0.9)	0.02	0.03
codon 12 vs. codon 13		1.3 (0.5–3.0)	0.6	
ECOG 0 vs. 1		1.0 (0.6–1.8)	1.0	0.05
ECOG 0 vs. 2		7.8 (0.95–63.6)	0.06	
PTS (colon vs. rectum)		1.9 (1.0–3.3)	0.04	0.03

Logistic regressions, only significant 'candidate variables' are shown.
Odds ratio >1 means the second parameter is more likely to be coexisting.
Codon 12, patients with a *KRAS* mutated tumors, mutation located in codon 12; codon 13, patients with a *KRAS* mutated tumors, mutation located in codon 13; ECOG, Eastern Cooperative Performance Score; LR, likelihood-ratio test; PTS, primary tumor site colon vs. rectum; Wald, Wald-test; wild type, patients with *KRAS* wild-type tumors.

approximately 20% in *KRAS*-mutated patients [16]. In this analysis of *KRAS*-mutated tumors, a frequency of 21.3% was observed. Ninety-five patients with *KRAS* wild-type tumors treated inside the AIO KRK 0104 trial were included in this investigation to provide a comparison for the mutant subgroups. Therefore, the rate of

KRAS mutations in our study population appears to be higher compared with a nonpooled analysis. *KRAS* mutation status was neither correlated to sex or age. ECOG performance status, however, was superior in *KRAS* wild-type patients when compared with *KRAS* mutant patients, which is possibly explained by the differing number of organs involved in the respective subgroups. This finding differs from the results reported by Yokota *et al.* [11], who – in a smaller analysis – failed to observe a correlation between *KRAS* mutation status and performance status.

In this study, we observed a higher rate of synchronous nodal and organ metastasis in *KRAS* codon 13-mutated patients compared with patients with codon 12 mutations. An increased rate of synchronous nodal metastasis was also observed in a study by Bazan *et al.* [10], who described an association of lymph node involvement and codon 13 mutations in a collective of patients undergoing surgery for primary CRC. In accordance with Yokota *et al.* [11], we did not find a correlation between *KRAS* mutation status and tumor grading.

In this study, patients with *KRAS* codon 13 mutation showed a trend for a higher rate of liver metastasis than patients with *KRAS* codon 12-mutated tumors (92% vs. 78%, $P = 0.1$). Possibly because of the smaller number of patients investigated, no correlation between the rate of liver involvement and the subtype of *KRAS* mutation was described by Yokota *et al.* [11].

Tie *et al.* [17] reported that in recurrent CRC *KRAS* mutations were specifically associated with lung and brain metastasis. Our study reveals a significantly higher rate of pulmonary metastasis for patients with *KRAS* mutant tumors compared with wild-type tumors. No significant difference between the subtypes of *KRAS* mutation was observed in our study, although the rate of pulmonary metastasis was higher in *KRAS* codon 13-mutated tumors (50% vs. 43%).

Within our study population, the metastatic involvement of at least two organs was significantly associated with a *KRAS* mutation with a higher rate for codon 13 compared with codon 12-mutated patients (71% vs. 61%). Again, this observation points to the high malignant potential derived from a *KRAS* oncogene alteration. A comparable observation was also reported by Yokota *et al.* [11]; they found the highest rate of more than two organs involved in patients with *KRAS* codon 13 mutations.

Although the character of *KRAS* codon 13-mutated mCRC seems aggressive in our analysis and codon 13 mutations were identified as a poor prognostic markers in several investigations [1,4,10,14], conflicting data exist [12,13]. Treatment regimens for codon 13-mutated mCRC may play an important role with regard to the outcome of these patients and may explain the inconsistent data. As De Roock *et al.* [14] suggest, the application of cetuximab changes the outcome of patients with codon 13 mutant mCRC significantly. The precise predictive and prognostic impact of codon 13 mutations in patients with mCRC has to be investigated by large studies with defined treatment strategies containing anti-EGFR treatment such as the PRIME study or the CRYSTAL study [9,18]. This analysis is limited by the retrospective nature, which only allows an explorative analysis of data. Moreover, this analysis included only patients with disease progression into a metastatic situation. The character of resectable codon 13-mutated tumors might differ from our population. Moreover, the number of codon 13-mutated patients was limited.

In conclusion, this study demonstrated that *KRAS* mutation status may significantly be associated with different clinical characteristics of mCRC. *KRAS* mutations in codon 13 were associated with a significantly higher rate of synchronous nodal and organ metastases compared with codon 12 mutations, and with a higher frequency of multiorgan involvement. Although patients with *KRAS* wild-type tumors also had a high rate of synchronous metastasis, they presented with a lower rate of multiorgan involvement. Baseline characteristics of patients with mCRC showed a trend toward a higher rate of liver metastasis in *KRAS* codon 13-mutated compared with codon 12-mutated mCRC.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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