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Short Report

KRAS alterations in colorectal liver metastases: shifting to exon, codon, and point mutations

Pim B. Olthof[1], Stefan Buettner[1], Nikolaos Andreatos[2], Jane Wang[3], Inger Marie Løes[4], Doris Wagner[5], Kazunari Sasaki[6], Andrea Macher-Beer[7], Carsten Kamphues[8], Ioannis Pozios[8], Hendrik Seeliger[8], Daisuke Morioka[9], Katsunori Imai[10], Klaus Kaczirek[11], Timothy M. Pawlik[12], George Poultsides[13], Richard Burkhart[3], Itaru Endo[9], Hideo Baba[10], Peter Kornprat[5], Federico N. Aucejo[6], Per Eystein Lønning[4], Katharina Beyer[8], Matthew J. Weiss[14], Christopher L. Wolfgang[15], Martin E. Kreis[8] and Georgios A. Margonis[8,16,*]

[1]Department of Surgery, Erasmus University, Rotterdam, the Netherlands
[2]Division of Medical Oncology, Mayo Clinic, Rochester, Minnesota, USA
[3]Department of Surgery, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA
[4]Department of Clinical Science, University of Bergen, and Department of Oncology, Haukeland University Hospital, Bergen, Norway
[5]Department of General Surgery, Medical University of Graz, Graz, Austria
[6]Department of General Surgery, Digestive Disease Institute, Cleveland Clinic, Cleveland, Ohio, USA
[7]Department of Pathology, Medical University of Vienna, Vienna, Austria
[8]Department of General and Visceral Surgery, Charité Campus Benjamin Franklin, Berlin, Germany
[9]Department of Gastroenterological Surgery, Yokohama City University Graduate School of Medicine, Yokohama, Japan
[10]Department of Gastroenterological Surgery, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan
[11]Department of General Surgery, Medical University of Vienna, Vienna, Austria
[12]Department of Surgery, Ohio State University Wexner Medical Center, Columbus, Ohio, USA
[13]Department of Surgery, Stanford University School of Medicine, Stanford, California, USA
[14]Department of Surgery, Zucker School of Medicine at Hofstra, Northwell Health Cancer Institute, Lake Success, New York, USA
[15]Department of Surgery, New York University School of Medicine, New York, New York, USA
[16]Department of Surgery, Memorial Sloan Kettering Cancer Center, New York, New York, USA

Correspondence to: Georgios A. Margonis, Department of Surgery, Memorial Sloan Kettering Cancer Center, 1275 York Avenue, New York, New York 10065, USA (e-mail: margonig@mskcc.org)

Introduction

Complete resection of colorectal liver metastases (CRLMs) is considered potentially curative, but recurrence is common[1]. Vauthey and colleagues[2] were among the first to shift from reporting on the prognostic impact of clinicopathological features to focusing on *KRAS* as a marker of tumour biology in CRLMs. Little attention has been paid to potential differences between the specific mutations that give rise to the activated oncoproteins with respect to their different prognostic impact. In fact, data on the three levels that categorize *KRAS* mutations (exon, codon, and specific nucleotide mutations) are not only scarce, but also controversial[3–6]. Thus, the current practice of using *KRAS* mutations as a binary variable may be problematic. This study aimed to test this hypothesis by analysing the prognostic impact of various mutations based on their location in different exons or codons and, even more specifically, the effect of the different point mutations within each codon in patients who underwent surgery for CRLMs.

Methods

The International Genetic Consortium for Colorectal Liver Metastasis database, which was built to include only patients with CRLMs and known *KRAS* mutational status, was queried for adult patients who underwent surgery between 2000 and 2017. Patients were excluded if the codon and point mutation status were unknown. Further details on mutational testing, the inclusion of patients with extrahepatic disease, and variables included in the analyses can be found in the *supplementary material*. The study was conducted in accordance with the ethical standards of the participating institutions, and was approved by the appropriate ethical committees of the leading institution (Johns Hopkins University).

Survival was defined as the time between liver resection and death or last follow-up. To generate risk groups, *KRAS* point mutations were ranked based on associated median overall survival rates. Mutations associated with median overall survival below that of the overall *KRAS*-mutated group were classified as high risk, whereas those with a higher survival rate comparable to that of the overall *KRAS* group were classified as low risk. Median overall survival for the three patients with G13R mutations was not reached, and these were classified as high risk based on poor survival of one patient and early recurrence in two.

Statistical analyses were performed using SPSS® version 25 (IBM, Armonk, NY, USA) and R version 4.0.3 (R Foundation for Statistical Computing, Vienna, Austria). Further details can be found in *supplementary material*.

Results

Among the 1567 patients included, median follow-up was 58.4 (i.q.r. 95 per cent c.i., 54.8–62.1) months. Median and 5-year overall survival were 55.3 (51.0–59.6) months and 46.6 per cent respectively. *KRAS* mutations were found in 562 patients (35.9

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Table 1 Clinicopathological and treatment characteristics of patients with low- or high-risk KRAS mutations who underwent liver resection for colorectal liver metastases

	Wild-type ($n = 1005$)	High risk ($n = 254$)	Low risk ($n = 308$)	P†
Age (years)*	61.2 (53.0–68.9)	63.0 (55.0–71.0)	62.0 (54.0–71.0)	0.031‡
Sex				0.056
M	357 (36)	109 (43)	124 (40)	
F	647 (64)	145 (57)	184 (60)	
T3–4 primary tumour				0.179
No	125 (13)	42 (18)	39 (13)	
Yes	838 (87)	198 (82)	259 (87)	
Primary lymph node status				0.014
Negative	338 (34)	106 (43)	124 (41)	
Positive	646 (66)	142 (57)	179 (59)	
Primary tumour location				<0.001
Right colon	224 (22)	93 (37)	125 (41)	
Left colon	422 (42)	85 (34)	72 (24)	
Rectum	352 (35)	73 (29)	107 (35)	
Synchronous CRLM				0.724
No	426 (49)	111 (49)	142 (51)	
Yes	449 (51)	115 (51)	134 (49)	
CEA (ng/mL)*	7.1 (3.1–23.0)	7.6 (3.5–29.2)	9.1 (3.6–25.8)	0.117‡
Tumour size (cm)*	2.5 (1.6–4.0)	2.4 (1.5–3.7)	2.5 (1.5–3.9)	0.425‡
Chemotherapy before hepatic resection				0.221
No	383 (38)	111 (44)	125 (41)	
Yes	621 (62)	142 (56)	181 (59)	
Extrahepatic disease				0.502
No	896 (89)	221 (87)	269 (87)	
Yes	109 (11)	33 (13)	39 (13)	
No of tumours*	2.0 (1.0–3.0)	2.0 (1.0–3.0)	2.0 (1.0–3.0)	0.619‡
Bilobar disease				0.094
No	621 (62)	174 (69)	201 (66)	
Yes	376 (38)	77 (31)	105 (34)	
Intraoperative concurrent ablation				0.084
No	787 (88)	184 (82)	239 (86)	
Yes	110 (12)	40 (18)	40 (14)	
R0 resection				0.013
No	729 (74)	201 (81)	239 (80)	
Yes	258 (26)	46 (19)	61 (20)	
Adjuvant chemotherapy				0.001
No	491 (51)	105 (46)	110 (39)	
Yes	473 (49)	123 (54)	173 (61)	
Recurrence-free survival				0.006
1 year	354 (36)	76 (31)	78 (26)	
3 years	642 (64)	172 (69)	222 (74)	
Site of recurrence				<0.001
Intrahepatic	301 (51)	55 (39)	66 (32)	
Extrahepatic	170 (29)	45 (32)	72 (35)	
Both	122 (21)	42 (30)	66 (32)	

Values in parentheses are percentages unless indicated otherwise; *values are median (i.q.r.). CRLM, colorectal liver metastasis; CEA, carcinoembryonic antigen. †χ^2 or Fisher's exact test, except ‡Mann Whitney U test.

per cent). Exon 3 mutation was omitted from the analysis, as few patients harboured it. Clinicopathological and treatment characteristics, as well as outcomes, are summarized in *Table 1* and *supplementary material*.

There was no difference in survival when mutations were analysed according to location in exons 2 *versus* 4 ($P = 0.374$) (*Fig. 1a*). Overall survival was also similar for patients with exon 2 codon 12 and 13 mutations ($P = 0.282$) (*Fig. 1b*), with median overall survival rates of 42.4 (34.9–49.9) and 36.5 (24.3–48.8) months respectively.

Overall survival by mutation-based subgroups based on the amino acid change is shown in *Table S1*. Median overall survival was 34.2 (28.7–39.8) months among the 244 patients with high-risk *KRAS* mutations, compared with 53.1 (41.8–64.3) months in the 295 patients with low-risk *KRAS* mutations, and 60.8 (55.1–66.6) months in the 996 patients with *KRAS* wild-type tumours ($P < 0.001$) (*Fig. 1c*). On multivariable analysis, *KRAS* risk groups were identified as independent prognostic factors (*Table S2*).

Of note, after recurrence, median survival was longer for patients with wild-type and low-risk *KRAS* mutations than for those with high-risk *KRAS* mutations (34.8, 26.5, and 22.0 months respectively ($P < 0.001$) (*Fig. S1*). High-risk patients were less often treated with curative intent (high risk: 78 (48.1 per cent); low risk: 130 (61.3 per cent); wild-type: 441 (70.1 per cent); $P < 0.001$). High-risk patients also underwent significantly fewer re-resections of metastases (high risk: 53 (35.1 per cent); low risk: 99 (47.1 per cent); wild-type: 327 (54.5 per cent); $P < 0.001$).

Discussion

Although there was no significant survival difference associated with exon 2 *versus* 4 mutations, the Kaplan–Meier curves did separate suggesting that patients with exon 4 mutations may have a distinct and less aggressive tumour biology. Saadat and co-workers[5] similarly found that, although there was no significant survival difference associated with exon 2, 3, and 4

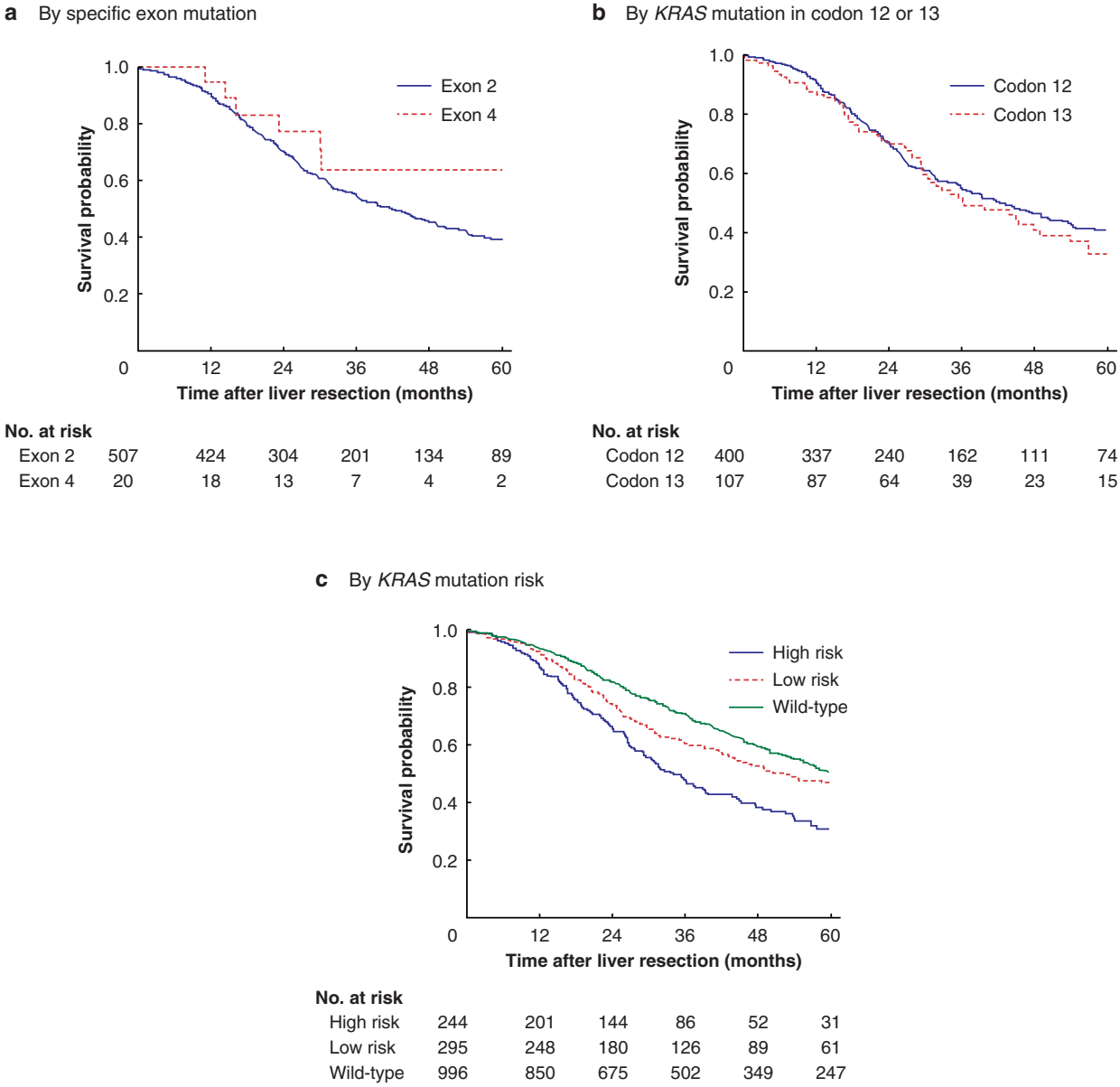

Fig. 1 Overall survival curves for patients who underwent liver resection for colorectal liver metastases according to *KRAS* mutation type

Overall survival according to **a** specific *KRAS* exon mutations, **b** *KRAS* mutation in codon 12 or 13, and **c** *KRAS* mutation risk *versus* wild-type. **a** $P = 0.374$, **b** $P = 0.282$, **c** $P < 0.001$ (log rank test).

mutations, the Kaplan–Meier curves separated. In contrast to a previous report[7], overall survival was also similar for patients with exon 2 codon 12 and 13 mutations. Of note, the previous study[7] on codon-specific mutations in CRLM included only 67 and 24 patients with codon 12 and 13 mutations respectively, whereas the present study included 415 and 111 patients respectively.

The clinical heterogeneity among *KRAS* variants was ultimately confirmed at the point-mutation level. In this cohort, G12V, G13D, and G12D mutations were most common and represented 70 per cent of all *KRAS* mutations, which is consistent with the current literature. G12V was associated with the worst survival across all point mutation groups at 31.7 months, whereas patients with a G12D mutation had a median survival of 49.2 months. The association of G12V with poor survival is consistent with previous studies in patients with non-metastatic colorectal cancer[7–9].

In contrast, the present findings for patients with G13D and G12S mutations are not consistent with existing studies. For example, another study[7] reported poor survival of patients with G12S mutations, although their sample size was only 7. By comparison, the present study included 34 patients with G12S mutations, who had a median survival of 80.3 months. This was not only higher than that of any other point-mutation group, but even higher (albeit not significantly) than the median survival of patients with wild-type tumours. Although this is the first study to suggest that certain *KRAS* subgroups may serve as positive prognosticators, similar findings have been found for *BRAF* mutations[10,11].

Patients with left-sided primary tumours were more likely to have high-risk mutations, whereas those with right-sided disease were more likely to have low-risk mutations. These differences may explain why others[12] found that *KRAS* status is prognostic for patients with left-sided but not for right-sided primary tumours.

Clinically, the differences in overall survival rates between patients with high- *versus* low-risk *KRAS* mutations were

partially driven by inferior postrecurrence survival of the former group. Indeed, patients with high-risk recurrences were less likely to receive treatment with curative intent. Interestingly, this cannot be attributed to different patterns of recurrence as the rates of intrahepatic, extrahepatic, and intrahepatic and extrahepatic recurrences were similar in the high- and low-risk *KRAS* groups. Instead, it can be speculated that patients with high-risk *KRAS* mutations experience recurrences with a higher tumour burden that are not amenable to potentially curative therapies. The limitations of the study are documented in the *supplementary material*.

This study has demonstrated that the prognosis of patients with *KRAS* mutations is not as uniform as previously believed. Instead, patients can be stratified into those with low- and high-risk mutations, which carry a 28 and 72 per cent increase in risk of death respectively compared with that associated with wild-type tumours. Aside from the obvious refinement of prognostication, the findings from this study may inform future trials. Finally, the need for knowledge regarding the different effects of specific mutations will inevitably increase as new drugs targeting mutated *KRAS* are developed[13].

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Supplementary material

Supplementary material is available at BJS online.

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