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KRAS G>A Mutation Favors Poor Tumor Differentiation but May Not be Associated with Prognosis in Patients with Curatively Resected Duodenal Adenocarcinoma

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Abstract

KRAS mutations have been found in duodenal adenocarcinomas and may have prognostic significance. The purpose of this study was to classify clinicopathological characteristics, microsatellite instability, and *KRAS* mutations and identify possible prognostic role of *KRAS* mutations in duodenal adenocarcinomas. Demographics, tumor characteristics and survival were recorded for 78 patients with duodenal adenocarcinomas (stages I to III). *KRAS* mutations were detected in 27 (34.6%) cases, of which the majority (74.1%) were G>A transitions. Multivariate logistic regression analysis showed that *KRAS* G>A mutation was significantly associated with late stage ($p = 0.025$) and poor tumor differentiation ($p = 0.035$), when compared with wild-type and other than G>A mutations. *KRAS* G>A mutation carriers were at increased risk for distant relapse ($p = 0.022$) and had significantly shorter overall survival (OS; log-rank $p = 0.045$) and a trend toward shorter relapse-free survival (RFS; log-rank $p = 0.062$) when compared with those who did not carry the *KRAS* G>A mutation. In multivariate analyses, there was a significant correlation between ≥ 3 positive lymph nodes and poor OS ($p < 0.001$) and RFS ($p = 0.001$) and *KRAS* G>A mutation carriers demonstrated no effect on clinical outcome. In conclusion, *KRAS* G>A mutation correlates significantly with late stage and poor tumor differentiation in duodenal adenocarcinoma. Among patients who undergo a curative resection of duodenal adenocarcinoma, *KRAS* G>A mutation carriers will more likely experience distant relapse but may not exhibit a poor prognosis. Number of positive lymph nodes should be incorporated in future staging systems.

Keywords

KRAS; mutation; duodenal adenocarcinoma; prognosis

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Cancer of the small bowel is rare and accounts for less than 3% of gastrointestinal malignancies, with an estimated 7570 new cases and 1100 deaths in the United States in the year 2011.[1] Adenocarcinoma is the second most commonly diagnosed histologic subtype of small bowel tumors.[2] Approximately, 58.8% of the small bowel adenocarcinomas are found in the duodenum.[2] Outcomes for patients with duodenal adenocarcinoma are poor, with data from a single institute reported demonstrating 5-year overall survivals of 68.6%, 43.5% and 24.6%, for stage I, stage II, and stage III, respectively.[3] Curative resection does improve survival but many patients will experience local or distant recurrence.

The Ras superfamily of small GTPases comprises a group of molecular switches that regulate a large diversity of cellular functions.[4] The *RAS* gene family consists of three genes, *HRAS*, *NRAS*, and *KRAS*, which encode highly homologous 21 kDa proteins.[5] *RAS* genes become oncogenic by single point mutations, mainly in codons 12 or 13, which alter the guanine nucleotide–binding region, rendering Ras unresponsive to GTPase activating proteins and resulting in constitutive activation of Ras and aberrant downstream signaling.[6] Oncogenic mutations have been found in all three members of the *RAS* gene family with *KRAS* being the most frequently mutated. *KRAS* mutations are found at high frequencies in pancreatic, colorectal, small intestine, lung, and liver cancers.[7]

KRAS mutations may also play a prognostic role in colorectal cancer (CRC).[8] Previous studies have described *KRAS* mutations in duodenal cancers.[9–12] However, these studies included a small number of patients, neither frequency assessment of *KRAS* mutations nor further information with regard to their prognostic significance in this disease, was available.

The aims of this study, therefore, were to assess the mutational status of the *KRAS* gene in the largest series of duodenal adenocarcinomas reported to date and to establish whether or not any or specific mutations of *KRAS* might have prognostic value or be associated with clinicopathological characteristics and microsatellite instability (MSI).

Material and Methods

Study population

Patients were identified from the Johns Hopkins Hospital Oncology Clinical Information System from October 1997 to December 2009. Formalin-fixed, paraffin-embedded tissue blocks were collected from the patients who had curative surgical resection for duodenal adenocarcinomas with stages I to III. Tissue sections from all cases in this study were reviewed by an expert gastrointestinal pathologist. Ascertainment of survival was performed by using the Johns Hopkins electronic health records, the Cancer Registry and mortality was confirmed also within the Social Security Death Index. The Johns Hopkins Hospital Institutional Review Board approved this research protocol.

Analyses of *KRAS* mutations

Genomic DNA from paraffin-embedded tissue was extracted by phenol-chloroform, and polymerase chain reaction (PCR) targeted for *KRAS* codons 12 and 13 was performed as previously described.[13] PCR products were sequenced in both directions by use of a M13F primer (5′-GTAAAACGACGGCCAGT-3′) and a M13R primer (5′-CAGGAAACAGCTATGACC-3′) that were incorporated into the forward and reverse primer of each primer pair, respectively (Agencourt Bioscience Corporation, Beverly, MA). Sequence data were analyzed with SequencherTM 4.8 software (Gene Codes, Ann Arbor, MI). Verification of all mutations was accomplished by bidirectional sequencing of a second PCR product derived independently from the original template.

Analyses of microsatellites

MSI status was determined using D2S123, D5S346, D17S250, BAT25, and BAT26.[14] Microsatellite sizes were compared with those of normal adjacent tissue, and tumors with 2 or more of the markers exhibiting instability were classified as high MSI (MSI-H). Tumors with only one marker exhibiting instability or no markers with instability were classified as low MSI (MSI-L) or microsatellite stable (MSS) respectively. In this study, MSI-low and MSS tumors were grouped together and henceforth are referred to as MSS, and MSI-H is referred to as MSI.

Statistics

Differences in categorical variables between study groups were analyzed using χ^2 test for homogeneity of Fisher's exact test. Multivariate relationships were estimated by fitting logistic regression models. Survival was estimated by using the Kaplan-Meier method and log-rank statistics computed to test for differences between survival curves for various prognostic factors. Univariate and Multivariate Cox proportional hazard regression models included *KRAS* mutations, sex, age, stage or positive lymph nodes, tumor differentiation, chemotherapy and/or radiotherapy, and MSI status. Results of Cox regression are reported as hazard ratio (HR) with corresponding 95% confidence intervals (CI). All hypotheses tests were two-sided, and results were considered statistically significant for p values < 0.05. Calculations were performed using SPSS 16.0 software (SPSS Inc, Chicago, IL).

Results

KRAS mutations in duodenal adenocarcinoma

Amongst 78 patients who had underwent a curative resection of stages I to III duodenal adenocarcinomas, *KRAS* mutations were found in 27 (34.6%) patients. *KRAS* mutations were seen in 14.8% of early stage (stages I and II) cancers and 85.2% of late stage (stage III) cancers (Table 1). Twenty-one of the 27 patients with *KRAS* mutations had codon 12 mutations. Of the 21 mutations, 66.7% (14 of 21) were G to A transitions, 4.8% (1 of 21) were G to C transitions, and 28.6% (6 of 21) were G to T transitions. Conversely, mutations in *KRAS* codon 13 were detected only in 6 patients, and all of these were G to A transitions (Table 2). The overall frequency of G to A transition was 25.6% (20 of 78) and comprised 74.1% (20 of 27) of patients with *KRAS* mutations.

Clinicopathologic correlation of *KRAS* mutations

KRAS mutations occurred more frequently in late stage tumors ($p = 0.022$), and were more common in poorly differentiated tumors although this association failed to reach statistical significance ($p = 0.167$). No significant association was found between *KRAS* mutations and age, sex, extent of resection, undergoing chemotherapy/radiotherapy and MSI status (Table 1).

We next assessed the correlation of *KRAS* mutation subtypes with clinicopathologic variables and MSI. On univariate association analysis stratified by *KRAS* mutations subtypes (Table 3), *KRAS* G>A mutation was significantly more frequent in late stage tumors ($p = 0.014$) and poorly differentiated tumors ($p = 0.009$) when compared with *KRAS* wild-type and other than G>A mutations. Most of the patients with *KRAS* G>A mutation (18/20, 90.0%) were seen in late stage cancers, while just 2 (2/20, 10%) of the patients with *KRAS* G>A mutation were seen in early stage tumors. Amongst 35 patients with poorly differentiated tumors, there were 14 patients with G>A mutation, 20 patients with *KRAS* wild-type, and only 1 patient with other than G>A mutation. In 43 patients with well and moderately differentiated tumors, there were 31 patients with *KRAS* wild-type, 6 patients with G>A mutation, and 6 patients with other than G>A mutation. In a multivariate logistic

model containing age, sex, stage, tumor differentiation, and MSI status (Table 3), *KRAS* G>A mutation remained significantly more frequent in late stage [odds ratio (OR) = 6.42, 95% CI: 1.26–32.79; $p = 0.025$] and poorly differentiated tumors (OR = 3.51, 95% CI: 1.09–11.26; $p = 0.035$).

Pattern of relapse

Relapses were observed in 27 patients (34.6%). Relapses were local in 10 patients (37%), and distant in 17 patients (63%; including 13 cases with distant failure only, and 4 cases with both local recurrence and distant metastases). Local relapse occurred in 2 patients with *KRAS* G>A mutation and in 8 patients with *KRAS* wild-type, respectively. Distant relapse occurred in 8 patients with *KRAS* G>A mutation status and in 9 patients with wild-type, respectively. None of 10 patients with other than G>A mutations experienced relapse. *KRAS* G>A mutation carriers were not more likely to recur ($p = 0.094$) but prone to experience distant relapse ($p = 0.022$), when compared with those without *KRAS* G>A mutation.

Prognostic role with *KRAS* mutations

We next assessed the influence of *KRAS* mutation status on clinical outcomes in this cohort of duodenal adenocarcinomas. With median follow-up of 46.8 months, there were 27 events for relapse-free survival (RFS) analysis, and 32 events for overall survival (OS) analysis. In Kaplan-Meier analysis, there were no significant differences in survival time distributions between patients with *KRAS* mutations and those with wild-type *KRAS* (log-rank $p = 0.299$ for OS, Figure 1*a*; log-rank $p = 0.646$ for RFS, Figure 1*b*).

We then compared OS and RFS for patients stratified by mutation status. The median OS of all *KRAS* G>A mutation carriers was significantly shorter at 64.9 months compared with that of *KRAS* wild-type patients and other than G>A mutations carriers at 148.4 months (log-rank $p = 0.045$, Figure 2*a*). *KRAS* G>A mutation carriers were also associated with shorter RFS (median 67.1 years) when compared with *KRAS* wild-type patients and other than G>A mutations carriers (median RFS had not been reached), but this did not reach statistical significance (log-rank $p = 0.062$, Figure 2*b*).

To correct for possible prognostic factors for OS and RFS, a Cox proportional hazards model that included *KRAS* mutation status, sex, age, stage, tumor differentiation, chemotherapy/radiotherapy, and MSI status, was used (Table 4). In univariate Cox regression analysis, *KRAS* G>A mutation, advanced stage, poor tumor differentiation, and undergoing chemotherapy and/or radiotherapy are associated with reduced OS, and young age, advanced stage, poor differentiation, and undergoing chemotherapy and/or radiotherapy are associated with reduced RFS. In multivariate analysis, there was no correlation between *KRAS* G>A mutation and poor OS (HR = 1.14, 95% CI: 0.51–2.53, $p = 0.748$) and RFS (HR = 1.59, 95% CI: 0.65–3.88, $p = 0.309$). Only undergoing chemotherapy and/or radiotherapy was significantly associated with poor OS (HR = 2.80, 95% CI: 1.02–7.69, $p = 0.046$). Late stage, poor differentiation and MSS were also associated with poor OS although these associations approached but failed to reach statistical significance. Poor differentiation (HR = 3.04, 95% CI: 1.28–7.19, $p = 0.011$) and MSS (HR = 3.62, 95% CI: 1.06–12.29, $p = 0.039$) were found to be independent prognostic factors for RFS. Young age, late stage and undergoing chemotherapy and/or radiotherapy were also associated with poor RFS though these were not statistically significant.

Prognostic role with positive lymph nodes

We next assessed the prognostic role with positive lymph nodes. In Kaplan-Meier analysis, the median OS of ≥ 3 positive lymph nodes patients was significantly shorter at 20.8 months

compared with that of none positive lymph nodes (stages I and II) patients and < 3 positive lymph nodes patients (median OS had not been reached; log-rank $p = 0.001$; Supporting Information, Figure *a*). Patients with ≥ 3 positive lymph nodes were also associated with shorter RFS (median 16.7 months) when compared with none positive lymph nodes patients and < 3 positive lymph nodes patients (median RFS had not been reached; log-rank $p < 0.001$; Supporting Information, Figure *b*).

In multivariate analysis including positive lymph nodes, *KRAS* mutation status, sex, age, tumor differentiation, chemotherapy/radiotherapy, and MSI status, there was a significant correlation between ≥ 3 positive lymph nodes and poor OS (HR = 4.46, 95% CI: 2.03 – 9.77, $p < 0.001$) and RFS (HR = 4.79, 95% CI: 1.85–12.45, $p = 0.001$). *KRAS* G>A mutation was not associated with OS (HR = 1.12, 95% CI: 0.53–2.39, $p = 0.769$) and RFS (HR = 1.63, 95% CI: 0.68–3.88, $p = 0.273$; Supporting Information, Table).

Discussion

Approximately 30–40% of duodenal adenocarcinoma patients undergoing curative-intent surgery for primary cancer will relapse.[15–17] More than half of the recurrences occur distantly, and the most common sites of distant failure are liver and lung.[18, 19] Several studies have tried to better define clinicopathologic variables associated with the risk of recurrence with the goal of being able to supply effective therapeutic strategies in patients with high risk small bowel adenocarcinoma.[15, 19–24] Risk classification based on clinicopathologic criteria and the use of radiotherapy and/or chemotherapy has been proven unsuccessful.[25–27] Therefore, molecular characterization offers an attractive alternative to direct conventional adjuvant therapies and promote the development of novel targeted treatments for this malignancy.

KRAS mutation represents one of the most common molecular defects in colorectal cancer and small bowel cancer, and tumors with these defects are readily identifiable through direct sequencing. But due to rarity of duodenal adenocarcinomas, the distribution or contribution of *KRAS* mutation in this disease remains unknown. In CRC, there has been a considerable controversy in the literature regarding prognostic value of *KRAS* mutations. It was demonstrated that patients with G>A mutations and G>C mutations tended to have a worse prognosis than those with G>T mutations.[28] In addition, it was found that the presence of *KRAS* mutations increased risk of recurrence and death. In particular, any mutation of G>T but not G>A or G>C increased the risk of recurrence and death.[8] A subsequent analysis found only the codon 12 mutation leading to a glycine to valine substitution (G12V) of *KRAS* to be prognostic in stage III only.[29] More recently, several large randomized trials have failed to consistently demonstrate a meaningful effect of *KRAS* mutations on prognosis in CRC.[30–32] *KRAS* mutations are, however, now widely recognized as a predictive marker of resistance to EGFR-targeted therapy in CRC.[33–35]

The discovery of possible prognostic implications for *KRAS* mutations in colorectal cancer prompted this *KRAS* mutations study in duodenal adenocarcinoma. To our knowledge, the current analysis included the largest number of patients with duodenal adenocarcinoma in any single study to date.

There have been few studies on *KRAS* mutations in duodenal adenocarcinomas. Younes *et al.* first reported that 4 out of 12 patients with duodenal adenocarcinoma exhibited *KRAS* mutations, and all detected mutations were glycine to aspartate on codon 12 (G12D).[9] Rashid and colleagues found 3 out of 8 tumors with one G12D, one glycine to aspartate on codon 13 (G13D) and one glycine to serine on codon 12 (G12S).[11] Achille *et al.* detected 4 tumors with G12D and one tumor with glycine to alanine on codon 12 (G12A) in 12

tumors.[12] Muneyuki *et al.* found 2 cases with G12V in 10 cases.[10] Taken together, the frequency of *KRAS* mutations is nearly 30% and the predominant mutations are G>A transitions. However, all these studies included a small number of patients and had no classification of clinicopathological characteristics, MSI, and clinical outcomes in regard to *KRAS* mutations.

In the present study, *KRAS* mutations were found in 27 (34.6%) of the 78 patients with duodenal adenocarcinoma and most of them (74.1%) were G to A transitions, similar to what has been shown for CRC.[29, 30] Analysis of the *KRAS* mutation subtypes and patterns of histopathology revealed that *KRAS* G>A mutation was significantly more common in tumors with late stage and poor differentiation as compared with wild-type and other than G>A mutations. Remarkably, *KRAS* G>A mutation not only correlates with the loss of tumor differentiation but also shows a significant association with the presence of distant metastases when compared with those with wild-type and other subtypes of *KRAS* mutations. Therefore, presence of *KRAS* G>A mutation in tumor may have prognostic value.

In Kaplan-Meier analysis, using OS and RFS, *KRAS* G>A mutation carriers correlated with a worse prognosis as compared with those wild-type patients and other than G>A mutation carriers, whereas multiple mutations did not have an influence on survival. All *KRAS* mutation subtypes identified in this study have been shown to contribute to malignant transformation in various malignancies.[36–39] We speculate that the causes of *KRAS* G>A mutation, rather than the mutation itself, are more likely to lead to poor differentiation and worse prognosis in duodenal adenocarcinomas. If this is true, then perhaps tumors with *KRAS* G>A mutation arising from duodenum develop from a different pathway. The etiology of *KRAS* mutations is unclear and this may partially explain the failure to develop effective anti-*KRAS* therapies. Success in this endeavor would hinge on an in-depth analysis of distinct mechanisms for differences in *KRAS* mutations within a specific cancer type. It was reported that inactivation of *MGMT* by promoter hypermethylation was theoretically associated with the presence of *KRAS* G>A transitions in CRC.[40] We do not know whether this is true in duodenal cancers. Future studies of relevant lesions will be necessary to address these questions.

A large proportion of patients with a *KRAS* G>A mutation presented with late stage and poorly differentiated disease, and therefore to ensure that the prognostic effect associated with *KRAS* G>A mutation was not just a reflection of later stage and poor tumor differentiation, we carried out a multivariate analysis. We developed models correcting for age, sex, stage, tumor differentiation, chemotherapy/radiotherapy, and MSI status and analyzed the data using Cox regression. On multivariate analysis, undergoing chemotherapy/radiotherapy seemed to be associated with both poor OS and RFS. It is reasonable because patients who had undergone chemotherapy/radiotherapy were usually at advanced stages and had more lymph node involvement. As anticipated, late stage, poor differentiation and MSS were independently associated with, or at least, showed a trend toward worse OS and RFS. There was no correlation between *KRAS* G>A mutation and both OS and RFS. The *KRAS* G12V mutation, which has been associated with poor survival in CRC,[29] was present in six of our patients, five of them were still alive at the end of this study (data not shown), indicating that there are differences between duodenal adenocarcinoma and CRC.

It was reported that stratifying patients with stage III small bowel adenocarcinomas into those with < 3 positive lymph nodes and ≥ 3 positive lymph nodes significantly improved prognostication.[22] We looked into whether this is true in this cohort of patients with duodenal adenocarcima. Our results validated that patients with ≥ 3 positive lymph nodes

have worst prognosis in patients with stages I to III tumor. Future staging systems should incorporate the number of positive lymph nodes.[22]

In summary, this is the first study to classify clinicopathological characteristics, MSI, and *KRAS* mutations and examine possible prognostic role of *KRAS* mutations in duodenal adenocarcinomas. We show that *KRAS* mutations are present in 34.6% of duodenal cancers. The predominant *KRAS* mutations are G>A transitions (74.1%). Furthermore, *KRAS* G>A mutation is associated with late stage and poor tumor differentiation. Among patients who undergo a curative resection of duodenal adenocarcinoma, *KRAS* G>A mutation carriers will more likely experience distant relapse but may not exhibit a poor prognosis. Patients with ≥ 3 positive lymph nodes have worst prognosis, and number of positive lymph nodes should be incorporated in future staging systems.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

CRC	colorectal cancer
MSI	microsatellite instable
MSS	microsatellite stable
PCR	polymerase chain reaction
HR	hazard ratio
OR	odds ratio
CI	confidence interval
RFS	relapse-free survival
OS	overall survival

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Study highlights

What is current knowledge

- *KRAS* mutations have been described in duodenal adenocarcinomas but have not been further characterized due to the rarity of this disease.
- The relevance of *KRAS* mutations to clinicopathological characteristics and prognosis remains unclear in duodenal adenocarcinomas.

What is new here

- *KRAS* is mutated in 34.6% of duodenal adenocarcinomas based on our analysis in the largest cohort described, to date. The predominant *KRAS* mutations are G>A transitions.
- *KRAS* G>A mutation correlates significantly with late stage and poor tumor differentiation in duodenal adenocarcinomas.
- *KRAS* G>A mutation favors distant relapse but may not be associated with poor prognosis in patients who undergo a curative resection for duodenal adenocarcinoma.

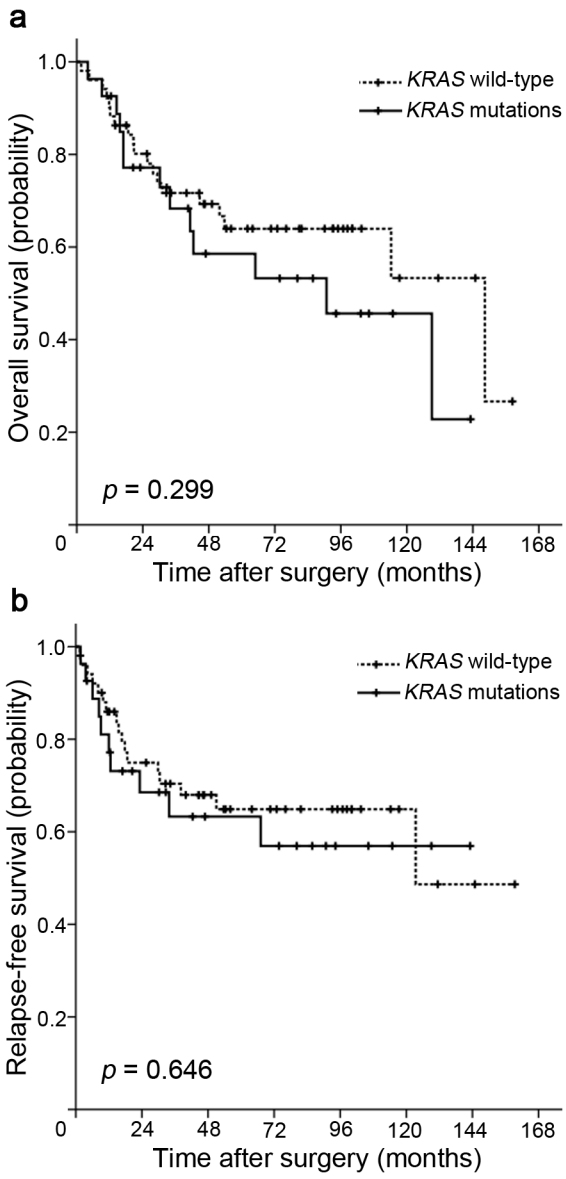

Figure 1.

Kaplan-Meier survival estimates between patients with duodenal adenocarcinomas with *KRAS* mutations and those with wild-type *KRAS*: (*a*) overall survival, (*b*) relapse-free survival. *P* values were based on the log-rank test.

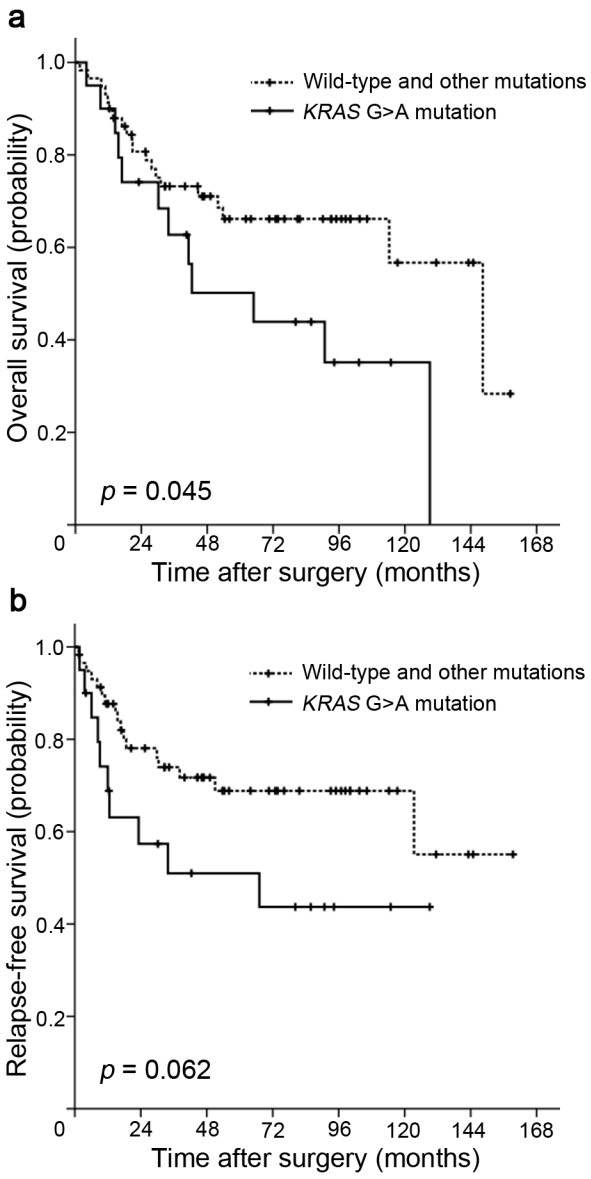

Figure 2.

Kaplan-Meier survival estimates between patients with duodenal adenocarcinomas with *KRAS* G>A mutation and those with wild-type *KRAS* and other than G>A mutations: (*a*) overall survival, (*b*) relapse-free survival. *P* values were based on the log-rank test.

Table 1

Patient characteristics by genetic and molecular groups

	Total (%)	*KRAS* wild-type (%)	*KRAS* mutated (%)	*p* *
No. of patients	78	51 (65.4)	27 (34.6)	
Age at surgery				0.614
<60	26 (33.3)	18 (35.3)	8 (29.6)	
≥60	52 (66.7)	33 (64.7)	19 (70.4)	
Sex				0.555
Male	44 (56.4)	30 (58.8)	14 (51.9)	
Female	34 (43.6)	21 (41.2)	13 (48.1)	
Stage				0.022†
Early stage (T1–4 N0 M0)	25 (32.1)	21 (41.2)	4 (14.8)	
Late stage (anyT N1-2 M0)	53 (67.9)	30 (58.8)	23 (85.2)	
Tumor differentiation				0.167
Well/Moderate	43 (55.1)	31 (60.8)	12 (44.4)	
Poor	35 (44.9)	20 (39.2)	15 (55.6)	
Extent of resection				0.086†
R0	68 (87.2)	47 (92.2)	21 (77.8)	
R1/R2	10 (12.8)	4 (7.8)	6 (22.2)	
Chemotherapy/radiotherapy				0.241
No	27 (34.6)	20 (39.2)	7 (25.9)	
Yes	51 (65.4)	31 (60.8)	20 (74.1)	
MSI status				0.390†
MSS	61 (78.2)	38 (74.5)	23 (85.2)	
MSI	17 (21.8)	13 (25.5)	4 (14.8)	

*$KRAS$ wild-type *vs.* $KRAS$ mutated, χ^2 test unless indicated otherwise;

†Fisher's exact test; MSS, microsatellite stable; MSI, microsatellite instability

Table 2

Non-synonymous *K-RAS* mutation status of patients with duodenal adenocarcinoma in stage I, II and III

Mutation Sequence	Amino Acid	Stage I	Stage II	Stage III
Codon 12				
GGT>GAT	G12D	0	1	12
GGT>AGT	G12S	0	0	1
GGT>CGT	G12R	0	0	0
GGT>GCT	G12A	0	0	1
GGT>GTT	G12V	0	2	4
GGT>TGT	G12C	0	0	0
Codon 13				
GGT>GAT	G13D	1	0	5
All				
G>A		1	1	18
G>C		0	0	1
G>T			2	4
Total		1	3	23

Table 3

Association between clinicopathologic features or microsatellite instability status and the presence of *KRAS* G>A mutation in 78 duodenal adenocarcinomas

Characteristics	Frequency analysis			Multivariate logistic regression analysis		
	Frequency					
	No.	%	*p**	OR	95% CI	*p*
Sex (female *vs.* male)	10/34 *vs.* 10/44	29.4 *vs.* 22.7	0.503	1.71	0.53, 5.49	0.371
Age (≥60 *vs.* <60)	15/52 *vs.* 5/26	28.8 *vs.* 19.2	0.359	2.00	0.56, 7.13	0.283
Stage (late *vs.* early)	18/53 *vs.* 2/25	34.0 *vs.* 8.0	0.014†	6.42	1.26, 32.79	0.025
Differentiation (poor *vs.* well/moderate)	14/35 *vs.* 6/43	40.0 *vs.* 14.0	0.009	3.51	1.09, 11.26	0.035
MSI (MSS *vs.* MSI)	17/61 *vs.* 3/17	27.9 *vs.* 17.6	0.536†	1.76	0.40, 7.77	0.454

OR, odds ratio; CI, confidence interval; MSS, microsatellite stable; MSI, microsatellite instability;

* *p* values were calculated by χ^2 test unless indicated otherwise;

† Fisher's exact test

Table 4

Univariate and multivariate Cox proportional hazard analysis of overall survival and relapse-free survival

Characteristic	OS				RFS			
	Univariate		Multivariate		Univariate		Multivariate	
	HR (95% CI)	*p*	HR (95% CI)	*p*	HR (95% CI)	*p*	HR (95% CI)	*p*
KRAS mutations (G>A *vs.* wild-type and not G>A)	2.06 (1.00, 4.25)	0.049	1.14 (0.51, 2.53)	0.748	2.07 (0.95, 4.54)	0.068	1.59 (0.65, 3.88)	0.309
Sex (Female *vs.* Male)	1.01 (0.50, 2.05)	0.986	1.16 (0.55, 2.48)	0.694	0.72 (0.33, 1.57)	0.408	0.90 (0.38, 2.16)	0.812
Age (≥60 *vs.* <60)	1.17 (0.55, 2.47)	0.685	1.37 (0.62, 3.04)	0.442	0.44 (0.21, 0.93)	0.032	0.44 (0.19, 1.00)	0.050
Stage (late *vs.* early)	3.09 (1.27, 7.55)	0.013	2.34 (0.88, 6.22)	0.089	4.31 (1.48, 12.59)	0.008	2.57 (0.80, 8.31)	0.115
Differentiation (Poor *vs.* Well/moderate)	2.68 (1.26, 5.71)	0.011	2.05 (0.94, 4.46)	0.071	3.67 (1.59, 8.48)	0.002	3.04 (1.28, 7.19)	0.011
Chemotherapy/radiotherapy (yes *vs.* no)	3.52 (1.33, 9.31)	0.011	2.80 (1.02, 7.69)	0.046	4.21 (1.41, 12.51)	0.010	2.88 (0.95, 8.73)	0.061
MSI status (MSS *vs.* MSI)	2.39 (0.83, 6.83	0.105	2.74 (0.95, 7.91)	0.063	2.97 (0.89, 9.87)	0.076	3.62 (1.06, 12.29)	0.039

OS, overall survival; RFS, relapse-free survival; HR, hazard ratio; CI, confidence interval; MSS, microsatellite stable; MSI, microsatellite instability