K-ras Mutation in Adenomas and Carcinomas of the Ampulla of Vater

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ABSTRACT

The role of K-ras mutations in the progression of tumors of the ampulla of Vater is not well understood. To study the frequency and timing of K-ras mutations in ampullary tumors, areas of invasive carcinoma and adjacent adenomas were microdissected from paraffin blocks from 96 resected tumors. DNA was extracted, PCR amplification of K-ras exon 1 was performed, and PCR products were sequenced. Statistical analysis of K-ras mutations with respect to patient survival and clinicopathological factors was performed using the \( \chi^2 \) test, log-rank test, and Cox proportional hazard model. Thirty-four of 92 ampullary carcinomas (37.0%) and 25 of 46 adenomas (54.3%) had mutations in K-ras exon 1. Twenty-two of 23 (95.7%) adenomas adjacent to carcinomas with K-ras mutations also had K-ras mutations. The only clinicopathological factor significantly associated with K-ras mutation was tumor size >2 cm \( (P = 0.035) \). Patient survival did not correlate with the K-ras mutation status \( (P = 0.31) \). We conclude that K-ras mutations are frequent in both adenomas and carcinomas of the ampulla of Vater and appear to occur as an early genetic event. The spectrum of mutations is similar to that observed in colorectal neoplasms, and these do not significantly correlate with patient survival.

INTRODUCTION

Carcinoma of the ampulla of Vater is a relatively uncommon neoplasm, accounting for approximately 6% of periampullary tumors (1). The 5-year survival of patients with these tumors is significantly better than that for pancreatic adenocarcinoma, and may exceed 40% (2). This may be because even small lesions tend to obstruct the common bile duct and lead to jaundice, but it is also possible that distinct biological and genetic factors affect this improved prognosis. The ampulla of Vater represents the junction of pancreaticobiliary ductal epithelium and duodenal mucosa, and at present it is unknown whether adenocarcinomas in this region are biologically more similar to those of the pancreas, extrahepatic bile ducts, or gastrointestinal tract. Histologically, most ampullary carcinomas resemble colorectal carcinomas, and adenomatous polyps frequently precede the development of invasive cancer (3). It is of further interest that patients with FAP\(^2\) commonly develop ampullary tumors, suggesting that a common genetic predisposition may affect the genesis of both colonic and ampullary tumors.

K-ras mutations are frequent in both colorectal carcinomas (approximately 40%) and adenomas (4–9), and therefore these changes are thought to occur as an early event in the adenoma-carcinoma sequence (5). The mutation rate in pancreatic ductal adenocarcinoma is even higher, approaching 80–90% (10–15). Knowledge of the K-ras mutation status in these tumors has been of limited clinical usefulness because this does not appear to correlate with patient survival in either pancreatic (16, 17) or colorectal adenocarcinomas (18, 19).

Genetic studies of ampullary tumors have been limited thus far by the rarity of these lesions and their small size. K-ras gene mutations have been reported in anywhere from 0 to 75% of ampullary carcinomas (11, 20–23), but the numbers in each study have been too small and the results too variable to draw meaningful conclusions about the importance of these mutations. In this study, we have taken advantage of the large clinical experience with periampullary neoplasms at the Memorial Sloan-Kettering Cancer Center to study K-ras mutations in tumors of the ampulla of Vater. Because the majority of carcinomas appear to arise from adenomas, the frequency of these mutations in both carcinomas and adenomas derived from the same patients was examined to determine the timing of these mutations in the adenoma-carcinoma sequence.

MATERIALS AND METHODS

Archival Tissue. Patients undergoing complete resection of carcinomas or adenomas of the ampulla of Vater were identified from the databases in the departments of Surgery and Pathology. Slides from these cases were reviewed to differentiate between tumors arising at the ampulla from those originating in the pancreas, bile duct, or duodenum. Paraffin blocks were retrieved, and ten to fifteen 10-\(\mu\)m sections were prepared from each. One of these slides was stained with H&E, and areas of normal tissue, adenoma, or carcinoma were identified on the slide. Microdissection of these areas from corresponding unstained sections was performed using razor blades and a \( \times2.5 \)

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\(^2\)The abbreviation used is: FAP, familial adenomatous polyposis.
K-ras Mutations in Ampullary Carcinoma

**RESULTS**

A total of 93 ampullary carcinomas and 46 ampullary adenomas were microdissected from 96 resected patients. Fifty patients had only carcinomas microdissected, 43 patients had both adenomas and invasive carcinomas, and 3 patients had adenomas only. K-ras mutations were found in 34 of 92 (37.0%) invasive ampullary carcinomas, and the sequence at K-ras codon 12 could not be determined in one carcinoma. Thirty of 34 mutations (88.2%) in ampullary carcinomas were in codon 12, and 4 (11.8%) were in codon 13. Mutations in codon 12 resulted in six different amino acid substitutions for the wild-type glycine at codon 12, whereas aspartate was the only change seen resulting from mutations at codon 13. The most common mutation was Aspartate 12 (35.3%), followed by Valine 12 (26.5%), Aspartate 13 (11.8%), Arginine 12 (11.8%), Cysteine 12 (8.8%), and Alanine 12 (5.9%).

Twenty-five of 46 (54.3%) ampullary adenomas were found to have K-ras mutations. In three patients with adenomas only, 1 tumor had a K-ras mutation. Twenty-four of 43 (56%) adenomas from patients with both adenomas and carcinoma harboring K-ras mutations, and adenomas adjacent to carcinomas without K-ras mutations. In two patients with adenomas from patients with both adenomas and carcinoma harboring K-ras mutations, and adenomas adjacent to carcinomas without K-ras mutations.

**Statistical Analysis.** A variety of clinicopathological variables were compared with the K-ras mutation status by the χ2 test using the Statview 4.1 statistical software package (Abacus Concepts, Berkeley, CA). Univariate survival analyses were performed by the Kaplan-Meier method using the log-rank test, and variables approaching significance by the log-rank test were then analyzed as covariates using a Cox proportional hazard regression model.

**Table 1** The frequency of K-ras mutations in ampullary adenomas, divided into three groups: adenomas only (no adjacent carcinoma), adenomas adjacent to carcinomas harboring K-ras mutations, and adenomas adjacent to carcinomas without K-ras mutations.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mutations (%)</th>
<th>No mutations (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenoma only</td>
<td>1 (33.3)</td>
<td>2 (66.7)</td>
<td>3 (100)</td>
</tr>
<tr>
<td>Ca. with mut.</td>
<td>22 (95.6)</td>
<td>1 (4.4)</td>
<td>23 (100)</td>
</tr>
<tr>
<td>Ca. without mut.</td>
<td>2 (10.0)</td>
<td>18 (90.0)</td>
<td>20 (100)</td>
</tr>
<tr>
<td>Total</td>
<td>25 (54.3)</td>
<td>21 (45.7)</td>
<td>46 (100)</td>
</tr>
</tbody>
</table>

*a Ca., carcinomas; Mut., mutations.*

**Fig. 1** Sequencing gel from patient 87-23569, demonstrating the wild-type codon 12 (GGT) in normal tissue, a Val 12 mutation (GTT) in an adenoma with mild dysplasia, and an Asp 12 mutation (GAT) in the carcinoma.
component with mild dysplasia had a Val 12 mutation (Fig. 1). Normal tissue microdissected from the same slides revealed no K-ras mutations in 10 cases in which mutations were found in both invasive carcinomas and adenomas. In 20 carcinomas without mutations, 2 cases (10%) harbored mutations in adjacent adenomas (Valine 12 and Aspartate 12), and 18 cases had mutations neither in the carcinomas nor adenomas (Table 1). The concordance rate for mutations in adenomas and carcinomas was 93% (40 of 43; \( \chi^2 = 31.823, P < 0.0001 \)), or 90.7% (39 of 43) when the specific mutations were considered. Mutations were found in 68% (15 of 22) of adenomas with severe dysplasia, 40% (4 of 10) with moderate dysplasia, and 50% (7 of 14) with mild dysplasia.

Table 2 Results of statistical analysis in resected ampullary carcinomas of various clinicopathological factors versus survival using univariate (log-rank) and multivariate (Cox proportional hazard model) methods

<table>
<thead>
<tr>
<th>Factor</th>
<th>( P ) value (univariate)</th>
<th>( P ) value (multivariate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor size &gt;2 cm</td>
<td>0.666</td>
<td></td>
</tr>
<tr>
<td>Perineural invasion</td>
<td>0.479</td>
<td></td>
</tr>
<tr>
<td>Tumor grade</td>
<td>0.316</td>
<td></td>
</tr>
<tr>
<td>K-ras mutation</td>
<td>0.310</td>
<td></td>
</tr>
<tr>
<td>T-stage</td>
<td>0.236</td>
<td></td>
</tr>
<tr>
<td>Histological subtype</td>
<td>0.146</td>
<td></td>
</tr>
<tr>
<td>Vascular invasion</td>
<td>0.051</td>
<td>0.379</td>
</tr>
<tr>
<td>Nodal metastases</td>
<td>0.007</td>
<td>0.032</td>
</tr>
</tbody>
</table>

The only statistically significant association between K-ras mutation and clinicopathological factors was tumor size, in which tumors <2 cm in size \( (n = 51) \) had a 27.5% incidence of mutation and those >2 cm in size \( (n = 41) \) had a 48.8% incidence of mutation \( (\chi^2 = 4.44; P = 0.035) \). There was no significant association between K-ras mutation and tumor grade, histological subtype, depth of invasion, T-stage, perineural invasion, or nodal metastases. The median survival of the 34 patients with invasive carcinomas harboring K-ras mutations was 69.7 months (95% confidence interval of 14.2–125.3 months). Sixteen patients died during the follow-up interval, and 18 were alive or lost to follow-up (median follow-up of 22.9 months, mean of 36.9 months). The patients without K-ras mutations in their ampullary carcinomas had a median survival of 47.6 months (95% confidence interval of 8.0–87.2 months), with a median follow-up of 19.8 months (mean 36.1 months). These differences in survival were not significant as determined...
by the logrank test \((P = 0.31; \text{ Fig. 2})\). Patients with nodal metastases \((n = 42)\) had a median survival of 20.8 months, and those without \((n = 48)\) had a median survival of 63.2 months \((P = 0.007, \text{ log-rank test})\). The association between vascular invasion and survival approached statistical significance by the logrank test \((P = 0.051)\), with a median survival of 20.8 months in 30 patients with vascular invasion and 59.5 months in 63 patients without vascular invasion. Cox regression analysis performed using vascular invasion and nodal status as covariates revealed that only nodal status was significantly associated with survival \((\text{ Table 2})\).

**DISCUSSION**

A summary of the published series of \(K\text{-}ras\) mutations in ampullary carcinomas plus the present study is shown in \(\text{ Table 3}\). The range of mutations seen in these studies varies from 0 to 75%, but most contained only a small number of tumors. The current study accounts for three-quarters of the tumors reported in the table, for a mutation rate of 37.0% \((33.9\% \text{ for all studies combined})\). The rate of \(K\text{-}ras\) mutation in ampullary carcinoma is remarkably similar to that of colon cancer \((4, 5, 8, 9)\) and about half of that seen for pancreatic adenocarcinoma \((10-15, 17, 25-31)\). The most significant differences seen between these tumor types is the rarity of \(K\text{-}ras\) mutations in pancreatic cancer \((1.1\% \text{ overall; Ref. 32})\), whereas these mutations are relatively common in colorectal \((22.8\% ; \text{ Refs. 5 and 8})\) and ampullary carcinomas \((11.8\% \text{ in the present study})\). This overall pattern of mutations more closely resembles that of colorectal than pancreatic cancer, and histologically there is great similarity in the adenoma-to-carcinoma sequence in both the colon and ampulla \((3, 33)\).

In the sequence of neoplastic transformation in ampullary tumors, \(K\text{-}ras\) mutations appear to occur at the precancerous stage. \(\text{ Table 1}\) demonstrates that 96% of ampullary adenomas have these mutations when adjacent carcinomas are found to have \(K\text{-}ras\) mutations. The possibility that mutations found in adenomas were due to cross-contamination by adjacent carcinoma is unlikely, because the normal tissues microdissected from the same slides all had the wild-type \(K\text{-}ras\) sequence at codons 12 and 13 \((n = 10)\). Conversely, in cases in which ampullary carcinomas did not have \(K\text{-}ras\) mutations, only 10% of adjacent adenomas had \(K\text{-}ras\) mutations. Thus, the presence of \(K\text{-}ras\) mutation alone in these adenomas was insufficient for the progression to cancer, suggesting that mutations at other genetic loci may be necessary for this transformation. In this study, the majority of adenomas associated with carcinomas \((39 \text{ of 43}, \text{ or } 90.7\%)\) had the same codon 12 and 13 genotypes, and therefore it is reasonable to assume that most of these carcinomas developed from their neighboring adenomas. The fact that the incidence of mutation was similar in adenomas with mild \((50\%)\), moderate \((40\%)\), and severe dysplasia \((68\%)\) further supports the observation that \(K\text{-}ras\) mutation occurs as a relatively early event.

The rate of \(K\text{-}ras\) mutation in ampullary adenomas \((54.3\% )\) was very similar to that described in colonic adenomas \((39-75\% ; \text{ Refs. 5-7})\). In colonic adenomas, the mutation rate is related to the size of the tumor, with those <1 cm having a 9% rate of mutation \(versus\text{ 58}\%\) for those >1 cm in size \((P = 0.0001; \text{ Ref. 5})\). The size of adenomas was not recorded in this study, but there was an association between the size of carcinomas and \(K\text{-}ras\) mutations. Gallinger et al. \((34)\) found a 37% prevalence \((7 \text{ of 19})\) of \(K\text{-}ras\) mutations in periampullary adenomas >1 cm in size from FAP patients, but no mutations in 29 adenomas <1 cm in size. It is interesting that they found only one \(K\text{-}ras\) mutation in eight periampullary carcinomas \((34)\). In the present study, only one patient was documented to have FAP and was found to carry the wild-type \(K\text{-}ras\) sequence in both an adenoma and carcinoma.

Similar studies in pancreatic adenocarcinoma have been limited because precursor lesions are less well characterized. \(K\text{-}ras\) mutations have been found in 44% \((4 \text{ of 9})\) of intraductal papillary neoplasms \((28)\) and 50% \((5 \text{ of 10})\) of patients with mucous cell hyperplasia in the setting of chronic pancreatitis \((35)\), suggesting that these mutations may also occur at a preneoplastic stage in pancreatic cancer.

There was no difference in survival between patients with and without \(K\text{-}ras\) mutations \((\text{ Fig. 2})\), which could have been biased by selecting only completely resected tumors for this study, which is the most important predictor of survival in ampullary carcinoma \((2, 36)\). The specific mutations present have been suggested by some authors to be of clinical importance in colorectal cancer \((8, 37, 38)\). In this study, there was no significant difference in survival between patients with Asp 12, Val 12, and other \(K\text{-}ras\) mutations. Three of four patients with Asp 13 mutations in this study had nodal metastases, although Asp 13 mutations have been described as relatively indolent in colorectal cancer \((8, 37, 38)\). The only clinicopathological variable that was significantly associated with \(K\text{-}ras\) mutation in this study was the size of the primary tumor, and the only factor significantly correlated with survival was the presence of nodal metastases. The latter confirms the findings of other reports \((39-41)\). The lack of prognostic significance of \(K\text{-}ras\) mutations upon survival found in completely resected ampullary carcinomas is similar to results in series of colorectal \((18, 19)\) and pancreatic cancer \((13, 16, 17, 25, 27, 42)\). These findings suggest that although \(K\text{-}ras\) may be an important early molecular event leading to cell proliferation, other genetic alterations must also be important in determining the ultimate biological behavior of these neoplasms.

**REFERENCES**


K-ras mutation in adenomas and carcinomas of the ampulla of vater.

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