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Genomic Profiling of Intrahepatic Cholangiocarcinoma: Refining Prognosis and Identifying Therapeutic Targets

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Abstract

Background—The molecular alterations that drive tumorigenesis in intrahepatic cholangiocarcinoma (ICC) remain poorly defined. We sought to determine the incidence and prognostic significance of mutations associated with ICC among patients undergoing surgical resection.

Methods—Multiplexed mutational profiling was performed using nucleic acids that were extracted from 200 resected ICC tumor specimens from 7 centers. The frequency of mutations was ascertained and the effect on outcome was determined.

Results—The majority of patients (61.5 %) had no genetic mutation identified. Among the 77 patients (38.5 %) with a genetic mutation, only a small number of gene mutations were identified with a frequency of >5 %: *IDH1* (15.5 %) and *KRAS* (8.6 %). Other genetic mutations were identified in very low frequency: *BRAF* (4.9 %), *IDH2* (4.5 %), *PIK3CA* (4.3 %), *NRAS* (3.1 %), *TP53* (2.5 %), *MAP2K1* (1.9 %), *CTNNB1* (0.6 %), and *PTEN* (0.6 %). Among patients with an

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IDH1-mutant tumor, approximately 7 % were associated with a concurrent *PIK3CA* gene mutation or a mutation in *MAP2K1* (4 %). No concurrent mutations in *IDH1* and *KRAS* were noted. Compared with ICC tumors that had no identified mutation, *IDH1*-mutant tumors were more often bilateral (odds ratio 2.75), while *KRAS*-mutant tumors were more likely to be associated with R1 margin (odds ratio 6.51) (both $P < 0.05$). Although clinicopathological features such as tumor number and nodal status were associated with survival, no specific mutation was associated with prognosis.

Conclusions—Most somatic mutations in resected ICC tissue are found at low frequency, supporting a need for broad-based mutational profiling in these patients. *IDH1* and *KRAS* were the most common mutations noted. Although certain mutations were associated with ICC clinicopathological features, mutational status did not seemingly affect long-term prognosis.

Biliary tract cancers include a spectrum of invasive carcinomas encompassing cancers arising in the intrahepatic, perihilar, or distal biliary tree (cholangiocarcinoma), as well as carcinomas arising from the gallbladder. Intra-hepatic cholangiocarcinoma (ICC) represents a unique entity with particular clinical challenges. ICC is the second most common form of liver malignancy, with an incidence and mortality that have steadily increased over the last decade.[1] Although a subset of individuals with ICC have identifiable risk factors such as primary sclerosing cholangitis or liver fluke infestation, the majority have no underlying risk factors that can be used to develop screening strategies for early detection. Although resection remains the sole curative treatment option, surgery is only feasible in the 10–20 % of patients who present with early-stage disease.[1,2] For those patients with advanced disease, treatment typically includes systemic therapy with gemcitabine and cisplatin combination chemotherapy. However, the median survival of patients with locally advanced or metastatic disease continues to be less than 1 year.[3]

There remains an unmet need to identify novel molecular signatures in cholangiocarcinoma with prognostic and therapeutic implications. Recently, data on the genetic signatures and molecular mechanisms underlying the pathogenesis of ICC have begun to emerge.[4,5] For example, some groups have reported somatic alterations in the *KRAS*, *TP53*, *CDKN2A*, and *SMAD4* (*DPC4*) genes in cholangiocarcinoma.[6–9] Other investigators have identified mutations in genes encoding for molecules of the phosphatidylinositide 3-kinase (PI3K) cell-signaling pathway (e.g., *PIK3CA*, *PTEN*, and *AKT1*)[6,8,9], as well as for isocitrate dehydrogenase (*IDH*) 1 and 2.[6,10,11] Most data on the topic of ICC genetic profiling come from small, single-institution experiences. In addition, some previous reports included data on cholangiocarcinoma from various anatomic locations, including hilar, distal lesions, or gallbladder cancer, with some even including various stages of tumors, making the data heterogeneous and difficult to interpret.[6,12] Furthermore, the prognostic significance of newly identified genetic signatures in a well-defined ICC population remains unknown or controversial. Therefore, the aim of the current study was to characterize the genomic profile of ICC among a multi-institutional, international cohort of patients using a broad-based mutational profiling platform. Specifically, we sought to define the frequency of well-established cancer gene mutations, assess the association of these mutations with clinical and morphologic features, and correlate mutations with long-term oncologic outcomes in patients with resected ICCs.

PATIENTS AND METHODS

Patients and Samples

Using an international multi-institutional database, 200 patients with ICC who underwent surgical resection with curative intent between October 1973 and February 2013 at one of seven institutions were identified (Massachusetts General Hospital, Boston, MA; Johns Hopkins School of Medicine, Baltimore, MD; University of Virginia, Charlottesville, VA; Fundeni Clinical Institute of Digestive Disease, Bucharest, Romania; Medical College of Wisconsin, Milwaukee, WI; Cliniques universitaires Saint-Luc, Brussels, Belgium; Queen Mary Hospital at The University of Hong Kong, Hong Kong, China). The institutional review board of each respective institution approved this study. Only patients with histologically confirmed ICC who received their initial treatment for ICC at a study center were included.

Genotype Analysis

After independent pathologic review (VD) of the tumor samples to confirm the diagnosis of ICC, micro-dissection was performed to obtain only tumor samples for DNA extraction. Total nucleic acids were then extracted from formalin-fixed, paraffin-embedded diagnostic tumor tissue obtained from ICC patients using a custom automated platform based on the Agencourt FormaPure System on a Biomek NXP workstation (Beckman Coulter Genomics, Danvers, MA). Mutational profiling was performed on these nucleic acids, which simultaneously queried for over 150 previously described hotspot mutations across 15 cancer genes, including *AKT1*, *APC*, *BRAF*, *CTNNB1*, *EGFR*, *ERBB2*, *IDH1*, *IDH2*, *KIT*, *KRAS*, *MAP2K1*, *NOTCH1*, *NRAS*, *PIK3CA*, *PTEN*, and *TP53*. This was performed using a custom modified ABI Prism SNaPshot Multiplex System on an ABI Prism 3730 DNA Analyzer (Life Technologies/Applied Biosystems), as previously described.[13] The SNaPshot genotyping assay is a fast, high-throughput, multiplex mutational profiling method that has the advantage over conventional dideoxy-nucleotide (Sanger) sequencing in that mutations can be detected when mutant DNA comprises as little as 5 % of the total DNA. The specific mutations that were assessed using this SNaPshot approach are listed in Supplemental Table 1. Of note, testing of the tumor suppressor genes *TP53*, *APC* and *PTEN* was limited to only the most common mutation sites, where approximately 30, 15, and 15 %, of all known somatic mutations in these genes were covered. Mutational profiling was performed at the Translational Research Laboratory, Massachusetts General Hospital Cancer Center.

Data Collection

Standard demographic and clinicopathologic data were collected, including sex, age, and primary tumor characteristics. Specifically, data were collected on primary tumor location, size, and number as well as morphologic subtype and presence of vascular invasion, defined as minor and/or major. Data on treatment-related variables, such as type of surgery, receipt of lymphadenectomy, and adjuvant therapy, were also obtained. Resection was classified as less than hemi-hepatectomy, hemi-hepatectomy, or extended hepatectomy. Margin and nodal status were ascertained on the basis of final pathologic assessment. Date of last follow-up and vital status were collected on all patients.

Statistical Analysis

Summary statistics were obtained using established methods. Discrete variables were described as medians with interquartile range (IQR). Categorical variables were described as totals and frequencies. Univariate comparisons were assessed using the chi-squared or analysis of variance test as appropriate. Overall survival time was calculated from date of surgery to date of death or date of last follow-up. Cox proportional hazards models were developed using relevant mutations to determine the association of each with overall survival. Cumulative event rates were calculated using the Kaplan–Meier method. Univariate and multivariate logistic regression models were constructed to determine the association of relevant clinicopathologic factors with any identified mutation. Each mutation was tested for any possible association with clinical characteristics or tumor morphology using logistic regression models. Relative risks were expressed as hazard ratios (HR) with 95 % confidence intervals (CI). Significance levels were set at $P < 0.05$; all tests were two sided. All analyses were carried out with Stata version 12.0 (StataCorp, College Station, TX).

RESULTS

Clinical and Treatment Characteristics

Among the 200 patients, there were 111 men (55.5 %) and 89 women (44.5 %). The median patient age was 63 years (IQR 53–70). Median tumor size was 6.0 cm (IQR 4.5–8.5) and most patients had a solitary tumor (n = 156, 78.0 %). At the time of surgery, the extent of resection was less than a hemi-hepatectomy in 54 patients (29.0 %), a hemi-hepatectomy in 99 patients (53.2 %), and an extended hemi-hepatectomy in 33 patients (17.8 %). Surgical margins were R0 in the majority of patients (n = 179, 90.4 %), while a smaller number of patients had an R1 margin (n = 19, 9.6 %). Lymphadenectomy was performed in 86 patients (43.0 %). On final pathology, a majority of patients had T1 tumors (n = 59, 50.4 %), while smaller subsets had T2 (n = 33, 28.2 %) or T3/T4 (n = 25, 21.4 %) tumors. Among the 86 patients who had at least one lymph node evaluated, 32 patients (16.0 %) had lymph node metastasis. As such, 33 patients (38.8 %) were classified as having stage III disease, while 36 (42.4 %) and 14 (16.5 %) were classified as having stage I or II disease, respectively. Microscopic and major vascular invasion was present in 37 patients (18.5 %) and 33 patients (16.5 %), respectively, while 23 patients (11.5 %) had perineural invasion (Table 1).

In the postoperative setting, about one-third of patients (n = 60, 30.0 %) received adjuvant therapy. At a median follow-up of 23.2 months, 1-, 3-, and 5-year survival was 80.8, 46.7, and 34.8 %, respectively; median overall survival was 31.4 months. Several factors were associated with overall survival. Specifically, tumor size ≥5 cm (HR 1.73, 95 % CI 1.11–2.71), nodal status (HR 3.52, 95 % CI 2.14–5.78), microscopic/major vascular invasion (HR 1.71, 95 % CI 1.16–2.52), satellite lesions/intrahepatic metastasis (HR 2.91, 95 % CI 1.95–4.36), and perineural invasion (HR 1.80, 95 % CI 1.06–3.05) were all associated with a worse long-term prognosis (Supplemental Table 2).

Mutation Analyses

Of the 200 tumor samples evaluated, 162 tumors (81 %) were available for full mutational profiling. The majority (n = 92, 56.8 %) had no genetic mutation identified. Among the 70 patients (43.2 %) who had a tumor with an identified genetic mutation, only a small number of gene mutations were identified with a frequency of greater than approximately 5 % (Fig. 1). Specifically, well-known tumor associated genes such as *KRAS* (8.6 %) and *BRAF* (4.9 %) were mutated in roughly 5–10 % of patients. A concurrent *KRAS* and *BRAF* mutation was not noted in any patient. Alternations were also identified in the *PI3K* pathway. Although only one tumor (0.6 %) was found to have a mutation in the most common hotspot regions of *PTEN*, the incidence of *PIK3CA* mutations was higher (n = 7, 4.3 %). Genetic mutations in other pathways were identified in very low frequency: *NRAS* (3.1 %), *TP53* (2.5 %), *MAP2K1* (1.9 %), and *CTNNB1* (0.6 %) (Table 2).

Regarding IDH mutational analyses, 200 tumors samples were available for mutational profiling. A genetic mutation in *IDH1* was identified in 31 samples (15.5 %), compared with only 9 samples (4.5 %) for *IDH2*. Of note, among patients with an *IDH1*-mutant tumor, approximately 7 % were associated with a concurrent *PIK3CA* gene mutation, and to a much lower extent, a mutation in *MAP2K1* (4 %). No concurrent mutations in *IDH1* and *KRAS* were noted (Table 3).

Association of Mutation Status with Clinicopathological Factors and Survival Outcomes

When patients were stratified according to whether “any” mutation was or was not identified, there were no differences in most clinicopathological and treatment characteristics (Table 1). Certain mutations were, however, associated with specific morphologic and pathologic findings. For example, compared with ICC tumors that had no identified mutation, *IDH1*-mutant tumors were more often bilateral [odds ratio (OR) 2.75, 95 % CI 1.17–6.44], while *KRAS*-mutant tumors were more likely to be associated with adjacent organ involvement (OR 10.00, 95 % CI 1.29–77.51), and R1 margin status (OR 6.51, 95 %CI 1.63–26.11) (all $P < 0.05$). Although other clinicopathological features such as tumor size and number as well as nodal status were associated with survival, no specific mutation was associated with these prognostic factors (Table 4).

Median survival among patients with no identified mutation was the same as survival among patients with “any” mutation (both 31.4 months) (Table 2; Supplemental Fig. 1a). Compared with wild-type tumors, median survival was 20.3 months for *KRAS* mutant cases ($P = 0.07$) and 25.5 months for *BRAF* mutant cases ($P = 0.92$). Cases with either *KRAS* or *BRAF* mutations had a median overall survival of 20.3 months ($P = 0.17$) (Supplemental Fig. 1b). Mutations in the PI3K pathway had a median survival comparable to wild-type cases (*PIK3CA* mutation only: 37.3 months vs. *PIK3CA* or *PTEN* mutation: 43.3 months) (all $P > 0.05$) (Supplemental Fig. 1c). Similarly, no association with mutations in IDH was noted (*IDH1* mutation only: 39.3 months vs. *IDH2* mutation only: 25.3 months vs. *IDH1* or *IDH2* mutation: 31.3 months) (all $P > 0.05$) (Supplemental Fig. 1d).

DISCUSSION

There has been an emerging interest in the molecular mechanisms of many different gastrointestinal malignancies. For example, data have suggested an important role in the mutation of the two proto-oncogenes, *KRAS* and *BRAF*, among many patients with colon cancer.[14–16] For hepatocellular carcinoma, genetic events such as gene mutation (e.g., *TP53*, *CTNNB1*, *KRAS*), DNA methylation, and other gene expressions (e.g., *IGF*, *VEGFR*, *CD24*) have been implicated in the multi-step process of hepatocarcinogenesis.[17–19] Fewer data are available regarding the molecular underpinnings of ICC. Cholangiocarcinoma is a heterogeneous malignancy with probable varied gene signatures for intrahepatic, proximal, and distal cancers.[20] Although previous reports on the genetic profiling of ICC have been published, these data were based on small, single-institution cohorts. The current study is important because we utilized a broad, multi-institutional cohort of patients who underwent surgery for ICC. Genetic profiling was performed at a single center (MGH) and revealed that only a small number of gene mutations were identified with a frequency of approximately 5 %. Specifically, genes such as *KRAS* and *BRAF*, as well as those such as *PIK3CA*, *IDH1*, and *IDH2* were mutated in about 5–15 % of patients with ICC. Interestingly, although we found that certain mutations (e.g., *KRAS* and *IDH1*) were associated with specific clinicopathologic and pathologic tumor characteristics, no mutation was a strong predictor of long-term survival.

Mutations in *KRAS* and *BRAF* have been noted to be important drivers of tumorigenesis in colon cancer and, to a lesser extent, ICC.[10,14–16,21] Although mutations in *KRAS* and *BRAF* have been reported in ICC, the frequency of these mutations has varied considerably, ranging from 5 to 50 %.[21–23] The reasons for the reported wide-ranging incidence of *KRAS* and *BRAF* mutations in ICC are likely multifactorial, including possible large variations that can result from deriving proportions from low sample sizes. In addition, some previous studies reported combined data on both ICC and extrahepatic cholangiocarcinoma, which may have a different incidence of *KRAS* mutations—thereby further confounding these reports.[6,12] In the current study we identified mutations in *KRAS* in 8.6 % of cases and *BRAF* mutations in an additional 4.9 % of cases. The identification of *KRAS* and *BRAF* mutations in a small subset of patients is consistent with several previous studies.[21–23] Interestingly, although it did not reach significance perhaps as a result of lack of statistical power, *KRAS* mutation tended to be associated with a worse outcome (Supplemental Fig. 1b). Specifically, the median survival of patients with ICC characterized by a *KRAS* mutation (20.3 months) was about 50 % shorter than the survival of patients with no identified mutation (31.4 months) ($P = 0.07$) (Table 2). Interestingly, in a separate smaller study, Robertson et al.[21] previously reported a comparable difference in survival among patients with *KRAS* (13.5 months) and wild-type (37.3 months) ICC cases. The identification of *KRAS* and *BRAF* mutated tumors may help inform future targeted therapy for ICC. For example, agents such as vemurafenib have antitumor activity in patients with *BRAF* mutations, whereas patients with *KRAS* or *BRAF* mutations are unlikely to be good candidates for *EGFR* inhibitor therapy.[15,16,21,24] In another study with an integrated genetic and genomic analysis, Andersen et al.[12] also identified genetic alterations of key signaling molecules and the relevance of *EGFR* and *HER2* targeting in ICC.

IDH1 and *IDH2* are genes that have gained considerable interest in patients with ICC.[6,8,11] *IDH1* and *IDH2* (*IDH1*/*2*) normally function to catalyze the oxidative carboxylation of isocitrate to α-ketoglutarate. The recurrent cancer mutations in these enzymes confer neomorphic activity through the reduction of α-ketoglutarate to the metabolite *R*(−)-2-hydroxyglutarate (2HG), resulting in 2HG accumulation in the tumor tissue.[25,26] High intracellular levels of 2HG are sufficient for promoting the tumorigenic effects of mutant *IDH* activity that are associated with enhanced proliferation and impaired differentiation.[27] Several previous studies found these mutations to be a significant molecular feature present in approximately 20 % of ICC cases.[6,11,28] In the current study, we found a comparable mutation rate for *IDH1* (15.5 %), but a lower incidence in the mutation of *IDH2* (4.5 %). For *IDH* mutations, earlier reports suggested that mutations in *IDH1* or *IDH2* were associated with a longer overall survival and time to tumor recurrence after resection.[11] However, a recent study showed the opposite trend; subjects with *IDH1* or *IDH2* mutations had 3-year survival of 33 % compared with 3-year survival of 81 % for subjects with wild-type *IDH* genes ($P = 0.0034$).[10] In the current study, we failed to find any association of *IDH1*/*2* mutation with survival (Supplemental Fig. 1d). Collectively, these data suggest that although *IDH1* and *IDH2* may be one of the more commonly identified genetic mutations in ICC tumors, its effect on patient prognosis remains uncertain.

More recently, exome sequencing has also identified frequent inactivating mutations in multiple chromatin-remodeling genes including *BAP1* and *ARID1A*.[10,29,30] Interestingly, comparisons between fluke-related and non-fluke-related ICCs demonstrated statistically significant differences in some mutation patterns including *BAP1*, which was more frequently mutated in non-fluke-related ICCs.[29] In addition to the mutations, *FGFR* gene fusions have also emerged as a frequent molecular event in ICC.[31] In the current study, we did identify low frequency mutations in some other genes including *NRAS* (3.1 %), *TP53* (2.5 %), *MAP2K1* (1.9 %), and *CTNNB1* (0.6 %), as well as genes in the *PI3K* pathway. Activation of *EGFR* can activate downstream pathways such as *PI3K*.[32] Previous reports on mutations in the *PI3K* pathway among patients with ICC are rare. Compared with the 4 % incidence of *PIK3CA* mutation noted in the current study, two previous smaller studies reported mutations of 9 and 32 %.[9,33]

The current study had several limitations. Despite being one of largest series of ICC patients undergoing molecular profiling reported in the literature, the current study still had a relatively small sample size. Given the overall low frequency of genetic mutations found in the cohort, statistical tests assessing differences in the subgroups may have been underpowered. Because only 162 out of 200 patients in our cohort underwent complete mutation analyses, the assessment of concurrent mutations with *IDH* mutations may be underestimated. Although the multi-institutional study design did offer the benefits of increased generalizability, collaborating with multiple institutions limited the ability to easily standardize all diagnostic and treatment criteria. Furthermore, given that all patients included in the study had undergone surgical resection, these data may not be representative of other patients with more advanced, unresectable ICC. Last, the inherent limitations of multiplexed mutational profiling platform exclude the assessment of other mutations or gene fusions that are potentially relevant in ICC. SNaPshot genotyping platform has inherent

limitations and this may explain, at least in part, why only 57 % of tumors had mutations identified in our study compared with a recent report by Ross et al.[34] that noted an average of 2.9 alterations per ICC tumor. We are planning to expand our initial findings from this study and use more comprehensive genomic technology [i.e., Next-Generation Sequencing (NGS)] for a future study to assess the prevalence and prognostic significance of other mutations including those in the ARID family and others that have been recently reported.

In conclusion, most patients with resected ICC had no somatic mutation identified on multiplexed mutational profiling. *KRAS* and *IDH1* were the most common mutations noted. Although certain mutations were associated with ICC clinicopathological features, mutational status did not seemingly affect long-term prognosis. Future studies should strive to enhance our understanding of the molecular underpinnings of ICC with more advanced genomic testing platforms in order to refine the prognosis, as well as identify potential therapeutic targets, for patients with this disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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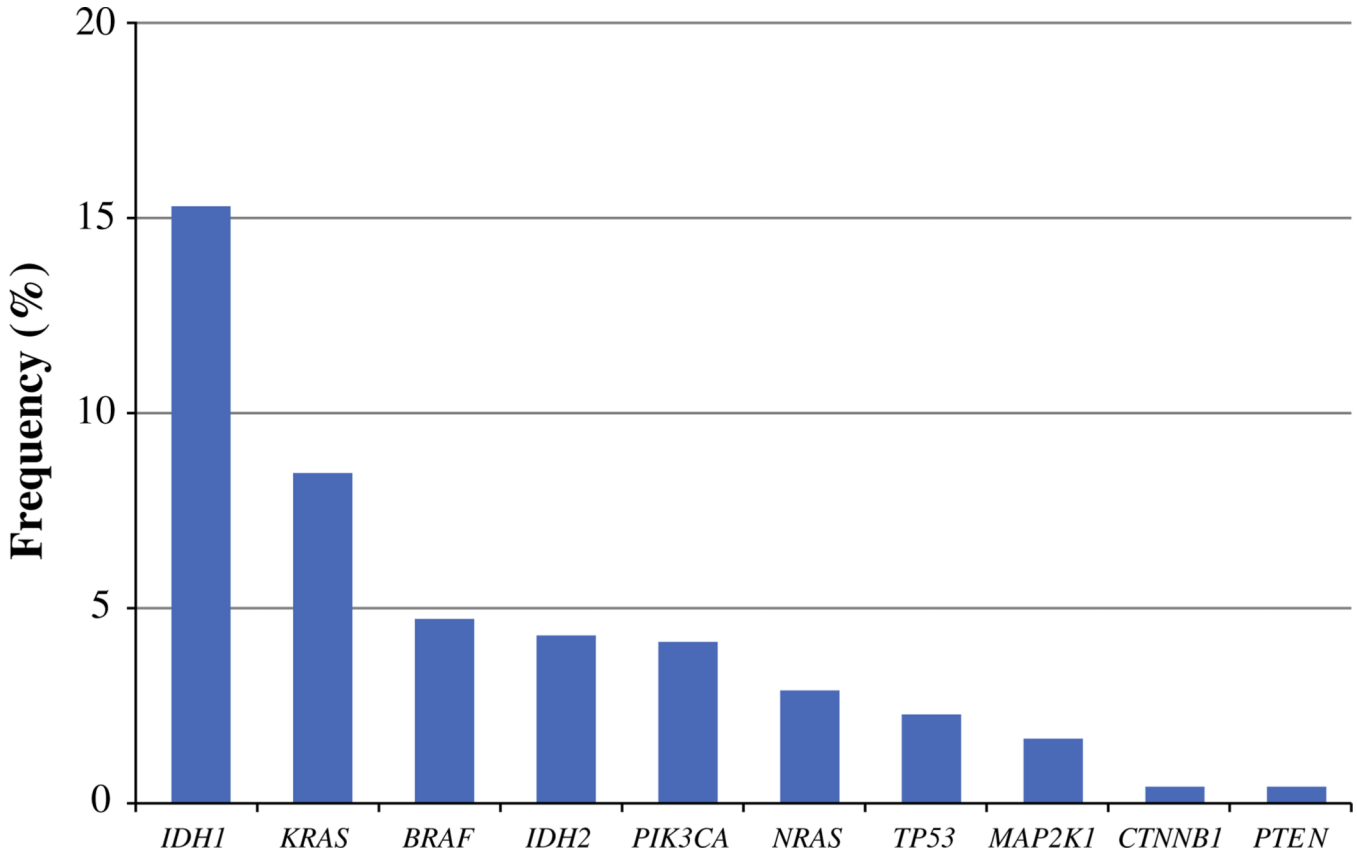

FIG. 1.

A multiplexed mutational profiling platform was used to identify cancer gene mutations in diagnostic cholangiocarcinoma tissue. The frequency of cancer gene mutations identified are expressed as a percentage of all tumors that were tested. Mutational profiling was performed on 162 patient samples. An additional 38 samples were included for *IDH1* and *IDH2* analysis only (n = 200)

TABLE 1

Characteristics of patients with intrahepatic cholangiocarcinoma

Characteristic	Total (*n* = 200)	Any mutation (*n* = 77, 38.5 %)[a]	No mutation (*n* = 123, 61.5 %)	*P*
Age, median (IQR)	63 (53–70)	67 (55–72)	60 (53–68)	0.03
Male gender	111 (55.5)	35 (45.5)	76 (61.8)	0.01
White race	149 (74.5)	65 (84.4)	84 (68.3)	0.02
Histologic grade (*n* = 184)				0.48
1	22 (12.0)	7 (10.3)	15 (12.9)	
2	112 (60.9)	46 (67.6)	66 (56.9)	
3	49 (26.6)	15 (22.1)	34 (29.3)	
4	1 (0.5)	0	1 (0.9)	
Size, median (IQR)	6.0 (4.5–8.5)	6.0 (4.5–8.5)	6.0 (4.1–8.0)	0.33
No. of lesions, mean (SD)	1.7 (1.5)	1.6 (1.5)	1.8 (1.5)	0.41
Solitary lesion	156 (78.0)	58 (75.3)	98 (79.7)	0.47
Bilobar involvement	45 (22.5)	22 (28.6)	23 (18.7)	0.09
AJCC stage (*n* = 85)				0.46
1	36 (42.4)	19 (41.3)	17 (43.6)	
2	14 (16.5)	6 (13.0)	8 (20.5)	
3	33 (38.8)	19 (41.3)	14 (35.9)	
4	2 (2.3)	2 (4.4)	0	
AJCC T stage (*n* = 117)				0.42
1	59 (50.4)	34 (50.8)	25 (50.0)	
2	33 (28.2)	21 (31.3)	12 (24.0)	
3	21 (18.0)	9 (13.4)	12 (24.0)	
4	4 (3.4)	3 (4.5)	1 (2.0)	
Liver resection (*n* = 186)				0.34
Less than hemihepatectomy	54 (29.0)	18 (23.7)	36 (32.7)	
Hemihepatectomy	99 (53.2)	42 (55.3)	57 (51.8)	
Extended hepatectomy	33 (17.8)	16 (21.0)	17 (15.5)	
Margin (*n* = 198)				0.02
R0	179 (90.4)	65 (84.4)	114 (94.2)	
R1	19 (9.6)	12 (15.6)	7 (5.8)	
Lymphadenectomy	86 (43.0)	44 (57.1)	42 (34.1)	0.04
Lymph node metastases	32 (16.0)	16 (20.8)	16 (13.0)	0.56
Vascular invasion				
Microscopic	37 (18.5)	13 (16.9)	24 (19.5)	0.73
Major	33 (16.5)	13 (16.9)	20 (16.3)	0.85
Perineural invasion	23 (11.5)	10 (13.0)	13 (10.6)	0.66
Biliary invasion	22 (11.0)	9 (11.7)	13 (10.6)	0.76
Satellite lesions	45 (22.5)	19 (24.7)	26 (21.1)	0.58
Intrahepatic metastases	20 (10.0)	9 (11.7)	11 (8.9)	0.54

Characteristic	Total (*n* = 200)	Any mutation (*n* = 77, 38.5 %)[a]	No mutation (*n* = 123, 61.5 %)	*P*
Recurrence	98 (49.0)	42 (54.5)	56 (45.5)	0.33
Site of recurrence (*n* = 98)				0.30
Intrahepatic only	44 (44.9)	20 (48.8)	24 (42.1)	
Extrahepatic only	26 (26.5)	7 (17.1)	19 (33.3)	
Both intra- and extrahepatic	28 (28.6)	14 (34.1)	14 (24.6)	
Adjuvant therapy	60 (30.0)	25 (32.5)	35 (28.5)	0.83
Death	112 (56.0)	46 (59.7)	66 (53.7)	0.71

[a]Mutations: *IDH1*, *IDH2*, *BRAF*, *CTNNB1*, *KRAS*, *MAP2K1*, *NRAS*, *PIK3CA*, *PTEN*, and *TP53*. Thirty-eight patients were tested for *IDH1* or *IDH2* only

TABLE 2

Incidence of mutation and association with OS for individual and combinations of mutations

Characteristic	*n* (%)	HR	95 % CI	*P*	Median OS, mo
WT	92 (56.8)	Ref		–	31.35
Any mutations	70 (43.2)	1.10	0.72–1.68	0.66	31.38
BRAF	8 (4.9)	1.06	0.38–2.94	0.92	25.46
CTNNB1	1 (0.6)	1.17	0.16–8.55	0.88	–
KRAS	14 (8.6)	1.99	0.96–4.12	0.07	20.33
MAP2K1	3 (1.9)	1.74	0.54–5.64	0.36	26.94
NRAS	5 (3.1)	1.01	0.25–4.19	0.98	28.62
PIK3CA	7 (4.3)	0.55	0.17–1.76	0.31	37.34
PTEN	1 (0.6)	1.11	0.15–8.09	0.92	–
TP53	4 (2.5)	1.26	0.39–4.08	0.70	10.82
KRAS or *NRAS*	19 (11.7)	1.69	0.86–3.30	0.13	28.62
KRAS or *NRAS* or *BRAF*	27 (16.7)	1.44	0.80–2.59	0.22	25.46
MAP2K1 + *PIK3CA*	1 (0.6)	1.23	0.17–8.99	0.84	–
KRAS or *BRAF*	22 (13.6)	1.54	0.83–2.87	0.17	20.33
PIK3CA or *PTEN*	8 (4.9)	0.62	0.22–1.73	0.36	43.26

Full mutational profiling except for IDH (*n* = 162)

OS overall survival, *HR* hazard ratio, *CI* confidence interval, *WT* wild type

TABLE 3

Incidence of mutation and association with overall survival for individual and combinations of mutations for IDH mutation

Characteristic	*n* (%)	HR	95 % CI	*P*	Median OS, mo
WT	123 (61.5)	Ref	–	–	30.95
Any mutations	77 (38.5)	1.08	0.75–1.58	0.68	31.38
IDH1	31 (15.5)	0.98	0.58–1.65	0.94	39.31
IDH2	9 (4.5)	1.16	0.47–2.88	0.75	25.33
IDH1 or *IDH2*	40 (20.0)	1.01	0.63–1.63	0.96	31.25
IDH1 + *PIK3CA*	2 (1.0)	–	–	–	–
IDH1 + *MAP2K1*	1 (0.5)	1.81	0.25–13.15	0.56	–
IDH2 + *BRAF* + *PIK3CA*	1 (0.5)	2.57	0.35–18.76	0.35	–

IDH mutations (*n* = 200)
CI overall survival, *OS* overall survival

TABLE 4

List of mutations that is significantly associated with tumor morphology (compared to no-mutation group)

Clinical factor	Odds ratio	95 % CI	*P*
IDH1			
Bilobar invasion	2.75	1.17–6.44	0.02
KRAS			
R1 margin	6.51	1.63–26.11	0.01
Direct involvement of adjacent organ	10.00	1.29–77.51	0.03
NRAS			
Intrahepatic metastasis	6.73	1.01–44.68	0.05
IDH1 or *IDH2*			
Bilobar invasion	2.72	1.24–5.98	0.01
NRAS or *KRAS*			
R1 margin	5.82	1.63–20.81	0.01
NRAS, *KRAS*, or *BRAF*			
R1 margin	5.70	1.80–18.01	0.003

CI confidence interval