Original Article

BRCA-associated protein 1 mutant cholangiocarcinoma: an aggressive disease subtype

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Background: BRCA-associated protein 1, an enzyme encoded by the *BAP1* gene, is commonly mutated in uveal melanoma, mesothelioma, and renal cancers. Tumors with *BAP1* mutation follow an aggressive course. *BAP1* mutations have also been observed in cholangiocarcinoma (CCA). The clinical phenotype of *BAP1* mutant CCA may yield useful prognostic and therapeutic information but has not been defined.

Methods: The records of CCA patients who underwent next-generation sequencing (NGS) were reviewed, and data on clinical, histopathological, genetic, and radiological features; response to therapy; time to progression; and survival were analyzed.

Results: Twenty-two cases of *BAP1*-mutation associated CCA were diagnosed from January 1, 2009, to February 1, 2015, at our center. Twenty patients had intrahepatic CCA and two had extrahepatic CCA. Tumor sizes (largest dimension) ranged from 2 to 16 cm (mean, 8.5 cm). Twelve patients had tumors that were poorly differentiated. Majority of the patients had advanced disease at presentation and 13 had bone metastases. Thirteen patients (59%) experienced rapidly progressive disease following primary therapy (chemotherapy or surgical resection). The mean time to tumor progression was 3.8 months after the first line chemotherapy.

Conclusions: *BAP1* mutation in CCA may be associated with aggressive disease and poor response to standard therapies. Therefore, *BAP1*-targeted therapies need to be investigated.

Keywords: *BAP1*-mutant cholangiocarcinoma; next-generation sequencing (NGS); Foundation Medicine; gemcitabine; cisplatin

Submitted Jan 05, 2016. Accepted for publication Feb 01, 2016.
doi: 10.21037/jgo.2016.03.05
View this article at: http://dx.doi.org/10.21037/jgo.2016.03.05

Introduction

Cholangiocarcinoma (CCA) is an uncommon but aggressive malignancy of the biliary tract. Although CCA is reported to be more prevalent in Asia (secondary to endemic liver fluke infestation), the incidence of CCA is increasing worldwide (1). Around 4,000–5,000 cases of CCA are diagnosed each year in the United States, with an annual age-adjusted incidence rate of 0.85 per 100,000 (2-4). No effective screening tests for CCA are available, and a significant proportion of CCA patients present with locally advanced and/or metastatic disease, which precludes curative surgery and leads to a poor 5-year rate of survival after diagnosis (5). New strategies to enable earlier diagnosis, treatment, and prognosis are needed.

Information on genomic mutations and molecular pathways associated with CCA appears to have potential

use in such strategies but is still evolving. Next-generation sequencing (NGS) studies have identified *TP53*, *KRAS*, *ARID1A*, *IDH1*, *MCL1*, *PBRM1*, *ERBB2*, *SMAD4*, *FBXW7*, and *CDKN2A* genes as being frequently aberrant in CCA (5). *KRAS*, *TP53*, and *MAPK/mTOR* aberrations in intrahepatic CCA are associated with worse prognosis (5). Genomic information may become increasingly important in the management of CCA and may explain why tumors with seemingly identical histopathology have significantly different clinical courses and responses to treatment.

Recent findings indicate that germline mutations in *BAP1* (the gene encoding BRCA-associated protein 1) are associated with a higher incidence of melanoma, mesothelioma, breast cancer, ovarian cancer, lung cancer, and renal carcinoma (5). Furthermore, in comparison with malignancies not associated with *BAP1* mutations, malignancies associated with *BAP1* mutations develop in individuals at an earlier age (6,7). However, the clinical phenotype of *BAP1*-mutant CCA has been inadequately described in the literature. In this study, we explore the clinicopathological features and treatment outcomes of CCA harboring *BAP1* mutation, which may help guide treatment decisions.

Methods

Patients selection

We retrospectively reviewed the records of CCA patients with *BAP1* mutation treated at The University of Texas MD Anderson Cancer Center from January 1, 2009, to February 1, 2015. Patients with a histological diagnosis of CCA plus *BAP1* mutation confirmed by NGS were identified. The electronic records of patients were used to collect and retrospectively analyze demographic, clinical, radiological, histopathological, and genetic characteristics; treatment received; time to progression; and survival. The study was approved by the Institutional Review Board.

Next-generation sequencing (NGS)

Genomic analysis for CCA was performed with NGS by Foundation Medicine (Cambridge, MA, UK) using archival fresh frozen paraffin-embedded tumor blocks of surgically resected or biopsied tumors. The library was constructed using 50–200 ng of DNA sheared to B100–400 bp before end repair, addition of deoxyadenylic acid, and ligation of indexed Illumina sequencing adaptors. Enrichment of target sequences was achieved by solution-based hybrid capture with custom Agilent SureSelect biotinylated RNA bait set. The genes selected for analysis were obtained from The Cancer Genome Atlas (TCGA) or the Catalogue of Somatic Mutations in Cancer. The selected libraries were sequenced on an Illumina HiSeq 2000 platform using 49,149 paired-end reads. Sequence data from genomic DNA were mapped onto the reference human genome (hg19) using the Burrows-Wheeler Aligner and processed using the open-source packages SAMtools, Picard, and Genome Analysis Toolkit (8,9). Point mutations were identified by a Bayesian algorithm, short insertions and deletions by local assembly, gene copy number alterations by comparing with process-matched normal controls, and gene fusions/rearrangements by clustering chimeric reads mapped to targeted introns (10).

Results

Patient demographic and clinical characteristics, histopathology findings, and stages of disease [according to the American Joint Committee on Cancer (AJCC) Cancer Staging Manual, Seventh Edition 2010] (11) are listed in *Table 1*. There were 10 men and 12 women. Twenty patients had intrahepatic CCA and two had extrahepatic CCA. Sixteen patients had stage IV disease (Any T, Any N, and M1, where M1 is any distant metastasis), two had stage III disease, and four had stage II disease. Underlying hepatic diseases included cirrhosis (n=1), hepatitis C (n=1), and nonalcoholic steatohepatitis (n=1). Patients had various presenting symptoms, including chest and upper abdominal pain (n=8), elevated/abnormal liver enzymes (n=3), and weight loss (n=2). Twelve patients (56%) had poorly differentiated tumors and nine (41%) had moderately differentiated tumors. In four patients, the tumor was discovered as an incidental finding on a computed tomography (CT) scan. In one patient, the tumor was identified by CT performed for the surveillance of hepatitis C-associated liver disease. Diagnosis of CCA was made in all 22 patients by biopsy and histopathology and the presence of *BAP1* mutation was identified by NGS.

All patients underwent clinical disease staging by CT of the chest, abdomen, and pelvis. Tumor sizes, the presence or absence of vascular invasion and biliary dilatation, and distant metastases are listed in *Table 2*. Tumor size (greatest dimension) varied from 2 to 16 cm (mean, 8.5 cm). Seven patients had both vascular invasion and biliary dilatation. Twenty patients had liver metastases and 16 had nodal metastases. Thirteen patients had bone metastases.

Table 1 Characteristics of patients with *BAP1* mutation-associated CCA

Patient	Age (years)	Sex	Presenting symptoms	CCA type	Tumor differentiation	Disease stage at presentation
1	61	Female	Weight loss, right-sided pain	Intrahepatic	Poor	IV
2	79	Female	None; tumor was discovered on a CT scan performed for an unrelated reason	Intrahepatic	Poor	III
3	72	Female	Elevated liver function	Intrahepatic	Poor	IV
4	62	Male	Chest pain	Extrahepatic	Poor	IV
5	80	Female	Elevated liver function	Extrahepatic	Moderate	IV
6	57	Male	Chest pain	Intrahepatic	Poor	II
7	31	Female	Chest pain	Intrahepatic	Moderate	II
8	62	Male	None; tumor was discovered on a CT scan performed for an unrelated reason	Intrahepatic	Poor	II
9	61	Male	None; tumor was discovered on CT imaging for a hepatitis C follow-up	Intrahepatic	Poor	III
10	56	Male	Postprandial RUQ pain after meals	Intrahepatic	Moderate	II
11	79	Female	Upper abdominal discomfort, nausea	Intrahepatic	Poor	IV
12	58	Female	Pain in the upper left buttock, pain and tightness in the upper neck	Intrahepatic	Poor	IV
13	67	Male	Fatigue associated with weight loss	Intrahepatic	Moderate	IV
14	80	Male	Kidney stones; a CT scan of the abdomen showed a mass in the liver	Intrahepatic	Poor	IV
15	52	Female	None; tumor was discovered on CT imaging for uterine cancer surveillance	Intrahepatic	Well	IV
16	56	Female	Elevated liver function	Intrahepatic	Moderate/poor	IV
17	67	Female	2 weeks of RUQ pain	Intrahepatic	Poor	IV
18	84	Male	Palpitations; ultrasonography of the aorta identified a mass in the liver	Intrahepatic	Poor	IV
19	40	Male	RUQ pain	Intrahepatic	Moderate	IV
20	35	Female	Abdominal pain, indigestion, nausea, vomiting, postprandial diarrhea	Intrahepatic	Moderate	IV
21	56	Female	Abdominal pain, jaundice	Intrahepatic	Moderate	IV
22	81	Male	Acute-onset abdominal pain	Intrahepatic	Moderate/poor	IV

CCA, cholangiocarcinoma; CT, computed tomography; RUQ, right upper quadrant.

Treatment and time to progression and survival data are listed in *Table 3*. As of October 2015, six patients had died, and one was lost to follow-up. Therapeutic management varied according to the extent of disease and presence of comorbidities. Four patients presented with early-stage disease (stage II). Two patients, one with stage III and one with stage IV disease, underwent primary surgical resection followed by adjuvant chemotherapy and chemoradiotherapy, respectively, and two others, both with stage II disease, underwent neoadjuvant chemotherapy followed by surgery and postoperative chemotherapy. In all four patients, the disease radiologically progressed as per Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 (12) within 4 months of resection (*Table 3*). Eighteen patients had metastatic disease at presentation. Twelve of these patients underwent chemotherapy and the remaining six patients underwent chemoradiation (*Table 3*). All patients with metastasis had progressive disease; the mean time to progression was 3.8 months (*Figure 1*). First-line chemotherapy regimens administered included gemcitabine

Table 2 Disease characteristics in patients with *BAP1* mutation-associated CCA

Patient	Tumor size (cm)	Biliary dilatation	Vascular invasion	Sites of metastases that developed during the course of the disease
1	10.5	Yes	Yes	Liver, node, bone, peritoneum, lung
2	7.0	Yes	Yes	Node
3	14.0	Yes	Yes	Liver, node, bone, abdominal wall, lung
4	6.5	Yes	Yes	Liver, node, peritoneum, lung
5	6.0	Yes	No	Liver, node
6	7.0	Yes	Yes	Liver, node, bone
7	6.5	Yes	Yes	Liver, bone
8	14.7	No	No	Liver, node, bone
9	6.1	No	Yes	Peritoneum, lung, bone
10	16.0	No	No	Liver, node, lung
11	9.0	No	Yes	Liver, bone
12	12.2	Yes	No	Liver, node, bone
13	17.0	No	No	Liver, node, bone
14	9.0	No	Yes	Liver
15	2.4	No	No	Liver, abdominal wall, lung
16	2.0	No	No	Liver, lung
17	7.9	No	No	Liver, node, bone
18	9.2	No	Yes	Liver, node, lung, bone
19	10.0	No	No	Liver, node, lung
20	5.7	No	No	Liver, node, bone
21	3.1	Yes	No	Liver, node
22	5.0	Yes	Yes	Liver, bone

CCA, cholangiocarcinoma.

plus cisplatin (n=16); gemcitabine, cisplatin, and erlotinib (n=2); and gemcitabine plus capecitabine (n=1). Bone metastases was common (n=13; 59%) (*Table 2*).

The aggressive nature of CCA was particularly evident in two patients. Patient 6, who presented with poorly differentiated primary intrahepatic CCA, underwent neoadjuvant chemotherapy followed by extended left hepatectomy, but hepatic metastasis developed within 8 weeks after surgery. Patient 7, who presented with moderately differentiated primary intrahepatic CCA, underwent orthotopic liver transplantation, but the disease recurred within 8 months after the procedure.

Discussion

The objective of this study was to describe the clinical features of *BAP1*-mutant CCA to help guide treatment decisions. This case series had the largest number of CCA patients with *BAP1* mutation described to date. Our findings show that *BAP1*-mutant CCA is an aggressive disease, with time to progression in our study being much lower than that reported for patients with advanced CCA treated with gemcitabine plus cisplatin (8 months in the Advanced Biliary Cancer trial) (13). As in uveal melanoma, wherein *BAP1* mutation is seen more frequently (14), the presence of this mutation in the patients in our case series indicated poor prognosis at both surgically resectable and advanced stages of disease.

BAP1 encodes deubiquitylating enzymes and is found in association with multiprotein complexes that regulate key cellular pathways in the cell cycle, cellular differentiation, cell death, gluconeogenesis, and the DNA repair process (6,7). The chromosome region, wherein *BAP1* is located, 3p21.1, is deleted in several malignancies (6,7). *BAP1* acts as a tumor suppressor through chromatin remodeling, DNA repair, and ubiquitin-proteasome pathways. Germline mutation of *BAP1* is associated with increased susceptibility to uveal melanoma, epithelioid atypical Spitz tumors,

Table 3 Treatments and outcomes in patients with *BAP1* mutation-associated CCA

Patient	Surgery	Chemotherapy (first line)	Months to progression[a]
1	Primary resection	Gemcitabine–cisplatin	3
2	No	Gemcitabine–cisplatin	3
3	No	Gemcitabine–cisplatin–erlotinib	4
4	No	Gemcitabine–cisplatin	3
5	No	Gemcitabine–cisplatin	3
6	Primary resection after neoadjuvant chemotherapy	Gemcitabine–capecitabine	3
7	Primary resection after neoadjuvant chemotherapy	Gemcitabine–cisplatin	3
8	No	Gemcitabine–cisplatin	2
9	Primary resection	Gemcitabine–cisplatin	4
10	No	Gemcitabine–cisplatin	6
11	No	Gemcitabine–cisplatin	2
12	No	Gemcitabine–cisplatin	3
13	No	Gemcitabine–cisplatin	7
14	No	Gemcitabine–oxaliplatin	1
15	Yes	Gemcitabine–cisplatin–erlotinib	4
16	Yes	Gemcitabine–cisplatin	2
17	No	Gemcitabine–cisplatin	7
18	No	Gemcitabine	1
19	No	Gemcitabine–cisplatin	12
20	No	Gemcitabine–cisplatin	Unknown
21	No	CEP-37250/KHK2804[b]	3
22	No	Gemcitabine–cisplatin	Unknown

[a], after the first course of therapy; [b], phase I study. CCA, cholangiocarcinoma.

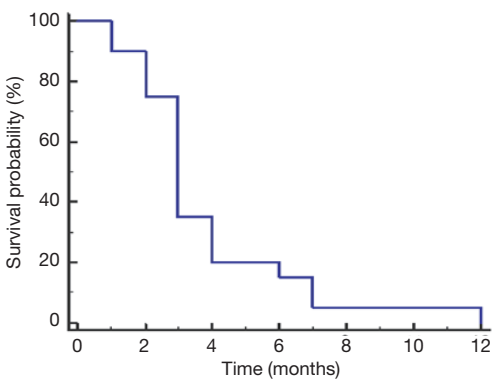

Figure 1 Time to progression of patients with *BAP1* mutation-associated cholangiocarcinoma (CCA) (the data of 20 subjects were included, 2 subjects were lost to follow up).

cutaneous melanoma, and mesothelioma (14,15).

The patients in our case series had no notable family history of CCA; therefore, the *BAP1* mutations were likely somatic. Somatic *BAP1* mutations, although rare, have been described in prostate, ovarian, colon, breast, and lung cancers and mesothelioma (7). Our finding that *BAP1*-mutant CCA follows an aggressive course is consistent with the findings of a study reporting that *BAP1* mutations are associated with an aggressive metastatic phenotype in uveal melanoma and renal cell carcinoma and that depletion of *BAP1* induces loss of differentiation ability and early dissemination in uveal melanoma (14,15).

Because *BAP1* mutation is a chromatin-remodeling mutation, histone deacetylase inhibitors such as vorinostat and panobinostat may have activity against *BAP1* mutation-associated disease (16-19). Some *BAP1*-associated tumors such as atypical Spitz tumors have abundant lymphocytic infiltrates, increasing the possibility that these tumors may respond to immunotherapy. Currently clinical trials are lacking for this orphan disease population. However, we must emphasize that the detection of *BAP1* mutation does not necessarily indicate relevance of the mutation as a

prognostic biomarker (or a potential therapeutic target). An integrated approach is required to expedite current efforts to identify prognostic markers and therapeutic targets for *BAP1*-mutant CCA.

Conclusions

Genomic sequencing can potentially identify distinct molecular subsets of CCA likely with prognostic and therapeutic implications. *BAP1* mutation confers poor response to standard therapies; some genotype–phenotype correlations should be established in CCA as carriers of *BAP1* mutations. Further extensive clinical, epidemiological, and functional studies are required to define the role of *BAP1* and its interaction in CCA and to identify therapeutic targets in CCA with *BAP1* mutation. Agents directed at these targets may be useful for the treatment of patients with *BAP1*-mutated CCA.

Acknowledgements

This work was supported by a grant from the Cholangiocarcinoma Foundation.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The study was approved by institutional ethics board (No. PA 13-0206)

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Cite this article as: Al-Shamsi HO, Anand D, Shroff RT, Jain A, Zuo M, Conrad C, Vauthey JN, Javle MM. BRCA-associated protein 1 mutant cholangiocarcinoma: an aggressive disease subtype. J Gastrointest Oncol 2016;7(4):556-561. doi: 10.21037/jgo.2016.03.05

