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Original contribution

Fibroblast growth factor receptor 2 translocations in intrahepatic cholangiocarcinoma☆

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Summary Patients with cholangiocarcinoma often present with locally advanced or metastatic disease. There is a need for effective therapeutic strategies for advanced stage cholangiocarcinoma. Recently, *FGFR2* translocations have been identified as a potential target for tyrosine kinase inhibitor therapies. This study evaluated 152 cholangiocarcinomas and 4 intraductal papillary biliary neoplasms of the bile duct for presence of *FGFR2* translocations by fluorescence in situ hybridization and characterized the clinicopathologic features of cases with *FGFR2* translocations. Thirteen (10 women, 3 men; 8%) of 156 biliary tumors harbored *FGFR2* translocations, including 12 intrahepatic cholangiocarcinomas (12/96; 13%) and 1 intraductal papillary neoplasm of the bile duct. Histologically, cholangiocarcinomas with *FGFR2* translocations displayed prominent intraductal growth (62%) or anastomosing tubular glands with desmoplasia (38%). Immunohistochemically, the tumors with *FGFR2* translocations frequently showed weak and patchy expression of CK19 (77%). Markers of the stem cell phenotype in cholangiocarcinoma, HepPar1 and CK20, were negative in all cases. The median cancer-specific survival for patients whose tumors harbored *FGFR2* translocations was 123 months compared to 37 months for cases without *FGFR2* translocations (P = .039). This study also assessed 100 cholangiocarcinomas for *ERBB2* amplification and *ROS1* translocations. Of the cases tested, 3% and 1% were positive for *ERBB2* amplification and *ROS1* translocation, respectively. These results confirm that *FGFR2*, *ERRB2*, and *ROS1* alterations are potential therapeutic targets for intrahepatic cholangiocarcinoma.
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1. Introduction

Cholangiocarcinoma is a malignant tumor of the biliary epithelium which is often classified as extrahepatic, perihilar or intrahepatic based on the anatomic origin of the tumor. The incidence of cholangiocarcinoma continues to increase [1], and at presentation, these tumors are often not amenable to curative resection [2,3]. Gemcitabine plus cisplatin, the current standard of care for advanced cholangiocarcinoma, is a non-curative therapy with a progression-free survival of 8 months [4]. No second-line therapy is of proven benefit. There is an urgent need for more effective therapeutic strategies to treat patients with cholangiocarcinoma.

Molecular techniques have led to the identification of therapeutic targets for a number of tumor types. Recently, our group [5] and others [6] identified fibroblast growth factor receptor gene (*FGFR2*) translocations in cholangiocarcinomas which benefited from FGFR-targeted therapy (eg, ponatinib). However, only a small number of cases from the United States have been studied to date, and there have been no detailed morphologic characterization of cholangiocarcinomas reported in association with *FGFR2* status. In addition, *FGFR2* translocations have not been widely studied in perihilar and extrahepatic cholangiocarcinoma. Lastly, previous studies identified *FGFR2* rearrangements using large scale genomic analyses (ie, RNA-Seq) not widely used in clinical laboratories. The goal of this study was to assess a large series of intrahepatic, perihilar and extrahepatic cholangiocarcinomas for *FGFR2* translocations and clinicopathologic correlates using a fluorescence in situ hybridization (FISH) assay that could be routinely performed in a clinical laboratory. We also undertook investigation of these tumors for *ROS1* translocations and *ERBB2* (*HER2*) amplification since the tyrosine kinases encoded by these genes have also been reported in cholangiocarcinoma [7,8].

2. Methods and materials

2.1. Patients and specimens

This study was approved by the Mayo Clinic Institutional Review Board. We retrieved archived formalin-fixed paraffin-embedded diagnostic material from surgical cases of cholangiocarcinoma and intraductal papillary neoplasms of the bile duct (IPNB) diagnosed at Mayo Clinic between September 1993 and October 2009. Glass slides were reviewed by pathologists (R.P.G and L.Z.) to confirm the diagnosis, evaluate morphologic features, determine the tumor grade and presence of metastases, and select an appropriate paraffin block for ancillary studies. Cases with insufficient tumor for testing were excluded. Clinical data including patient age, gender, tumor location from surgical operative notes, radiological and pathologic reports, and clinical follow-up were also obtained when available.

2.2. *FGFR2* and *ROS1* FISH

Using the hematoxylin and eosin–stained slides as a guide, unstained 5-μm-thick glass slides from a selected paraffin block were etched to indicate the areas of tumor for subsequent molecular testing. Slides were placed in an oven at 90°C for 10 minutes and then pretreated with xylene at room temperature for two consecutive 15 minute intervals to remove paraffin. Slides were then immersed in 100% ethanol for 5 minutes and allowed to air dry at 30°C for 3 minutes. Acid treatment was then performed for 45 minutes using 10 mM of citric acid at 80°C. This was followed by saline-sodium citrate (SSC) pretreatment for 5 minutes at 37°C and pepsin digestion (0.2%) for 48 minutes. The slides were then dehydrated in serial ethanol baths of increasing concentration and air dried for 5 minutes. Three to 10 μL of *FGFR2* break-apart FISH probe mix (Abbott Molecular Diagnostics, Des Plaines, IL), containing Spectrum Orange (spanning 353.6 kb targeting the centromeric part of gene) and Spectrum Green (spanning 491.3 kb targeting the telomeric part of gene) probes in hybridization buffer, was then applied to the etched area of the slide and cover slip applied. Hybridization was performed on a HYBrite (Abbott Molecular Inc.) by denaturing at 80°C for 3 minutes and hybridizing for 12 hours at 37°C. The slides were then removed from the HYBrite, placed in 0.1%NP40/2x SSC at 74°C for 2 minutes and transferred to a room temperature solution of 0.1% NP40/2x SSC for an additional 2 minutes. DAPI-I counterstain was applied to the sections, and the slides were cover slipped.

FISH analyses were then performed in blinded fashion by an experienced FISH technologist (E.F.). In order to be considered positive, separate Spectrum Orange and/or Spectrum Green signals had to be present in greater than 20% of nuclei throughout the tumor. Cases with good FISH probe signal quality not meeting these criteria were considered negative. All cases with *FGFR2* translocation and a subset of cases without translocation were reviewed blindly by a second reviewer (B.R.K). For the *ROS1* break-apart probe, the same method was used. Previously reported [5] cholangiocarcinomas (N = 3) with *FGFR2* translocations and FGFR2 overexpression from transcriptome sequencing along with cholangiocarcinomas without *FGFR2* translocations (N = 5) were also evaluated as control specimens in a blinded fashion with this FISH strategy to verify accuracy of the FISH probes.

2.3. HER2 immunohistochemistry and FISH

Five-micrometer unstained sections from the chosen paraffin block were used for HER2 immunohistochemistry (HercepTest kit; Dako, Carpinteria, CA) following the manufacturer-provided protocol. The slides were reviewed by 2 pathologists and classified as negative, 1+, 2+ or 3+ based on previously published guidelines by the College of American Pathologists and American Society of Clinical Oncology [9]. In all 2+ or 3+ positive cases, the invasive

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