Title

Hilar cholangiocarcinoma without macroscopic mass formation classified into extrahepatic cholangiocarcinoma based on molecular pathologic studies

Authors

Yuki Masuda[1 2], Naoto Kubota[2], Yasuhito Arai[5], Ryo Takemura[3], Yuta Abe[1], Minoru Kitago[1], Minoru Esaki[4], Tatsuhiro Shibata[5], Yuko Kitagawa[1], Hidenori Ojima[2,5]

Academic affiliations:

[1]Department of Surgery, Keio University School of Medicine, Shinanomachi 35, Shinjuku-ku, Tokyo, 160-8582, Japan

[2]Department of pathology, Keio University School of Medicine, Shinanomachi 35, Shinjuku-ku, Tokyo, 160-8582, Japan

[3]Biostatistics Unit, Clinical and Translational Research Centre, Keio University Hospital, Shinanomachi 35, Shinjuku-ku, Tokyo, 160-8582, Japan

[4]Hepatobiliary Pancreatic Surgery Division, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo, 104-0045 Japan

[5]Division of Cancer Genomics, National Cancer Center Research Institute, 5-1-1 Tsukiji, Chuo-ku, Tokyo, 104-0045 Japan

Corresponding author:

Hidenori Ojima, MD, PhD

Department of pathology, Keio University School of Medicine, Shinanomachi 35, Shinjuku-ku, Tokyo, 160-8582, Japan

Introduction

Recent genomic analysis of biliary tract carcinoma has revealed the distinct genetic features based on their primary sites and is expected to help develop primary site-specific treatments. However, the exact primary site of perihilar cholangiocarcinoma (phCCA), which is located between the intrahepatic and extrahepatic bile ducts, is virtually unknown. A precise diagnosis of the primary tumor site of phCCA is urgently necessary to select appropriate treatment and improve prognosis.

Aims & Methods

This study aims to identify the primary site of phCCA by molecular pathological analysis. We analyzed a total of 358 surgically treated invasive CCA operated at the National Cancer Centre Central Hospital Tokyo, Japan from 1990 to 2013. The primary site of the

tumor was evaluated based on bile duct stenosis location on preoperative image findings, detailed macroscopic observation in the resected specimen and histological tumor localization using elastic fiber staining. Hilar CCA (hCCA) was defined as a tumor originating in the upper common, right or left hepatic duct without macroscopic mass formation in liver. The analysis set included 39 cases of mass-forming type intrahepatic CCA (iCCA-MF) and 9 cases of extrahepatic CCA (eCCA) in which both frozen and formalin-fixed paraffin-embedded specimens were available. The validation set included 60, 100, 85, 14, 36, and 15 cases of iCCA-MF, eCCA, hCCA, periductal infiltrating intrahepatic CCA (iCCA-PI), periductal infiltrating plus mass-forming intrahepatic CCA (iCCA-PI+Mass), and iCCA-MF with perihilar invasion, respectively. First, transcriptome analysis was performed using the analysis set to identify candidate genes differentially expressed between eCCA and iCCA-MF. Second, immunohistochemical staining of the candidate genes was performed on the analysis set to determine the evaluation criteria. Finally, immunostaining of the candidate markers was performed on the validation set to evaluate the expression levels of each CCA subtype and statistically analyze the comparison between hCCA and the other CCA subtypes.

Results

Candidate genes included Serpin Family A Member 1 (SEPRINA1), Claudin18 (CLDN18), and Mesothelin (MSLN) as positive cases, with their immunohistochemical staining evaluation criteria of ≥33%, ≥5%, and ≥1+ (positive intensity), respectively. The immunohistochemical expression of each three molecules in the analysis set between eCCA and iCCA-MF revealed SERPINA1 at 0% vs. 82% ($P < 0.01$), CLDN18 at 100% vs. 41% ($P < 0.01$), and MSLN at 100% vs. 48% ($P < 0.01$). Similarly, a statistically significant difference was observed in the expression of each of the three molecules in the validation set. However, no significant difference was found in the immunohistochemical expression of the three molecules between eCCA and hCCA. Conversely, SERPINA1 expression in iCCA-MF cases with perihilar invasion was significantly different from that in cases of hCCA with microscopic hepatic parenchyma invasion, but not significantly different from that in cases of iCCA-PI+Mass. Furthermore, clustering analysis of the combination of three molecule expressions revealed that CCA was divided into three subgroups, consisting of iCCA-MF, iCCA-PI+Mass and iCCA-PI, and hCCA and eCCA.

Conclusion

We demonstrated molecular pathologically that the primary tumor site of phCCA can be identified and clinicopathologically classified in detail. In particular, we succeeded in showing that hCCA is classified into the same group as eCCA.