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INTRAHEPATIC CHOLANGIOCARCINOMAS HAVE HISTOLOGICALLY AND IMMUNOPHENOTYPICALLY DISTINCT SMALL AND LARGE DUCT PATTERNS

Carlie S. Sigel, MD¹, Esther Drill, MS², Yi Zhou, MD, PhD³, Olca Basturk, MD¹, Gokce Askan, MD¹, Linda M. Pak, MD⁴, Efsevia Vakiani, MD, PhD¹, Tao Wang, MD¹, Thomas Boerner, MD⁵, Richard K.G. Do, MD PhD⁶, Amber L. Simpson, PhD⁵, William Jarnagin, MD⁵, David S. Klimstra, MD¹

¹Department of Pathology, Memorial Sloan Kettering Cancer Center, New York, NY

²Department of Epidemiology and Biostatistics, Memorial Sloan Kettering Cancer Center, New York, NY

³Department of Pathology, Wake Forest School of Medicine, Winston-Salem, NC

⁴Department of Surgery, Brigham and Women's Hospital, Boston, MA

⁵Department of Surgery, Memorial Sloan Kettering Cancer Center, New York, NY

⁶Department of Radiology, Memorial Sloan Kettering Cancer Center, New York, NY

Abstract

Intrahepatic cholangiocarcinomas are histologically heterogeneous. Using a cohort of 184 clinically defined, resected intrahepatic cholangiocarcinomas, we retrospectively classified the histology into four subtypes: large duct (LD), small duct (SD) (predominantly tubular (SD1) or predominantly anastomosing/cholangiolar, (SD2)), or indeterminate. Then, we tested the four subtypes for associations with risk factors, patient outcomes, histology, and immunophenotypic characteristics. SD was the most common (84%; 24% SD1 and 60% SD2) with lower proportions of LD (8%), and indeterminate (8%). Primary sclerosing cholangitis was rare (2%), but correlated with LD ($P=.005$). Chronic hepatitis, frequent alcohol use, smoking, and steatosis had no histologic association. LD was associated with mucin production ($P<.001$), perineural invasion ($P=.002$), CA19-9 staining ($P<.001$), CK7+, CK19+, CD56- immunophenotype ($P=.005$), and negative albumin RNA in situ hybridization ($P<.001$). SD was histologically nodular ($P=.019$), sclerotic ($P<.001$), hepatoid ($P=.042$), and infiltrative at the interface with hepatocytes ($P<.001$). Albumin was positive in 71% of SD and 18% of LD ($P=.0021$). Most albumin positive tumors (85%) lacked extracellular mucin ($P<.001$). S100P expression did not associate with subtype ($P>.05$). There was no difference in disease specific or recurrence free survival among the subtypes. Periductal infiltration and AJCC 8th edition pT stage predicted survival by multivariable analysis accounting for gross configuration, pT stage, and histologic type. pT2 had worse outcome relative to other pT

Corresponding author: Carlie Sigel 1275 York Ave New York, NY 10065 sigelc@mskcc.org phone: 212-639-5251 fax: 212-639-6318.

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stages. Significant differences in histology and albumin expression distinguish LD from SD, but there is insufficient evidence to support further subclassification of SD.

Keywords

intrahepatic cholangiocarcinoma; albumin; periductal infiltration

INTRODUCTION

Carcinomas arising from the bile ducts are divided into intrahepatic, peri-hilar, and extrahepatic types based on site of anatomic origin. Intrahepatic cholangiocarcinomas (ICCs) have an incidence of 1 per 100,000 in the United States.(1) Intrahepatic cholangiocarcinomas putatively arise from cholangiocytes beyond the second order of branching ducts of the intrahepatic biliary tree, and variations in cell function and morphology along this bile conduit may account for the well known heterogeneity of architectural patterns seen in these neoplasms.

Macroscopic classification of intrahepatic cholangiocarcinoma as mass forming, periductal infiltrating, and intraductal types has significance for resectability.(2) Histologically, it is clear that the anatomic distribution of the tumor does not rigidly equate with the microscopic morphology, with some ICCs resembling the carcinomas of the hilar and extrahepatic bile ducts. The relevance of classifying intrahepatic cholangiocarcinoma into histologic subtypes is less certain, particularly given the inconsistent and imprecise use of terminology.(3–6) One proposed binary histologic classification divides ICC into large duct (LD) ICC (a.k.a. peri-hilar, hilar, bile duct) and small duct (SD) subtypes (a.k.a. peripheral, cholangiolar, bile ductular), based on resemblance of the more well differentiated areas to benign bile ducts of similar size and cholangiocyte morphology. (7) The neoplastic cells of the SD subtype are cuboidal to polygonal and line either small well-defined tubules resembling interlobular bile ducts or small anastomosing glands resembling bile ductular reaction or canals of hering (so-called cholangiolocellular carcinoma).(8, 9) The LD subtype is composed of columnar cells lining irregularly infiltrating large glands, more commonly producing mucin and associated with a desmoplastic stromal response, and the neoplasms tend to be located closer to the large bile ducts in the perihilar portions of the liver.(10)

Immunohistochemistry has been used to investigate theories of ICC carcinogenesis and the results could inform subtyping. For example, the similarity between SD ICC and the bile ductular/cholangiolocellular components of so-called mixed hepatocellular-cholangiocarcinomas with stem cell features has led to the theory that both tumors arise from hepatocellular progenitor cells in the bile ductules that possess the potential for either hepatocytic or cholangiocytic differentiation. Hepatocellular progenitor cells label with CD56 (NCAM), CK7, SALL4, Okt4, CRP, and CK19 and neoplasms with bile ductular-like growth patterns stain similarly.(7, 11–13) In contrast, the LD subtype is negative for CD56 and has S100P expression.(5, 7, 10) Albumin RNA in situ hybridization is a sensitive and highly specific method for diagnosing carcinomas of intrahepatic origin but this marker has not been explored in ICC subtypes.(14)

Most affected patients have no known risk factors for ICC. However, disease associations, including chronic hepatitis, parasitic infections, and autoimmune disease, have an effect on the incidence of disease worldwide. If specific risk factors are associated with histologic subtypes it could influence the prevalence of subtypes in different populations.

The purpose of this study was to undertake histologic subclassification of ICCs and assess their correlations with immunophenotype, clinical features, and outcome, using one of the largest North American cohorts of resected neoplasms.

MATERIALS AND METHODS

Case selection

We selected ICCs resected at Memorial Sloan Kettering Cancer Center from a prospectively maintained institutional database (January 1993–2014) with institutional review board approval. All patients were determined on a clinical basis to have a primary liver cancer by routine investigations including clinical characteristics, imaging studies, and upper and lower gastrointestinal endoscopy when indicated, and surgical findings. The study population included a subset of patients reported in an analysis of recurrence patterns reported previously by our institution.⁽¹⁵⁾ Patients with hilar, extrahepatic cholangiocarcinoma, purely intraductal neoplasms, invasive carcinomas arising in association with mucinous cystic neoplasms, and combined hepatocellular-cholangiocarcinoma were excluded. Patients with embolization therapy or neoadjuvant therapy were included as long as the majority of tumor was viable. All available tumor slides (total of 1 to 19 slides per patient, median=6 slides) were reviewed. Clinical data including history of cirrhosis, autoimmune disease, primary sclerosing cholangitis, smoking, and alcohol use were obtained retrospectively from the electronic medical record. The presence or absence of viral hepatitis was recorded only for patients with a clinical history or laboratory record of prior serologic testing for chronic hepatitis B (HBsAg+) or C (HCV-Ab). Frequent alcohol use history was defined as >15 drinks/week for men and >8 drinks/week for women.

Gross and histologic variables

The following histologic parameters were assessed on all available tumor slides (whole tissue section) with consensus among three pathologists (C.S.S., O.B, Y.Z.): nodular growth (Figure 1A and B), zones of histologic patterns (Figure 1C), intracellular and extracellular mucin, an infiltrative interface (Figure 1D), hepatoid cytology (Figure 1E), and the character of intratumoral stroma. “Zones of histologic patterns” was defined as the presence of abrupt transitions between areas of distinct architectural patterns (Figures 1C, 2A, 2F). “Nodularity” was defined at low power when aggregates of proliferating glands had a rounded contour (Figure 1A–B). An infiltrative interface by tumor was defined as tumor cells infiltrating the liver plates at the junction between liver and tumor (Figure 1D). Hepatoid cytology was defined as polygonal cells with eosinophilic cytoplasm in the absence of immunohistochemical evidence of actual hepatocellular differentiation (Figure 1E). Intratumoral stroma was categorized as scar (broad area of stroma with no viable tumor), desmoplastic stroma (prominent interglandular pale stroma rich in myofibroblast-

like cells (Figure 3A), sclerotic (prominent dense, sclerotic eosinophilic hypocellular interglandular stroma) (Figure 1C, 1F), or stroma poor (minimal interglandular stroma/back to back glands) (Figures 1E, 2D, 2H).

AJCC 8th edition pT stage was assigned to each case using histopathologic parameters.⁽¹⁶⁾ Gross configuration (periductal infiltrating versus mass forming) was determined using the American Joint Committee on Cancer TNM classification, 7th edition (Figures 2I and 3D). (16, 17) “Mixed” mass forming and periductal infiltrating tumors were classified as periductal infiltrating. Lymphovascular invasion included small and large vessel invasion. Background steatosis was defined as >50% steatosis.

Criteria for classification into large duct, small duct, and indeterminate subtypes

Two pathologists (C.S.S., T.W.) assigned histologic grade and subtype by reviewing all available tumor slides (whole tissue sections) and estimating the percentage of solid, large tubular, small tubular, and anastomosing glands. From this raw data (not shown), histologic grade was assigned using this definition: well differentiated had <75% solid growth pattern; poorly differentiated had ≥ 25% solid growth pattern. LD was assigned to tumors with a predominant component of large irregularly infiltrating glands lined by columnar cells (Figure 3A–F). Tumors with limited areas of micropapillary growth and/or focal small tubular patterns were allowed in this category. SD was assigned to tumors with a predominant component of cuboidal to polygonal cells lining predominantly small to intermediate sized tubules and/or anastomosing glands (Figure 2). Tumors with foci of dilated tubular ducts were included if the cells lining the tubules were cuboidal (Figure 2A). Given that a spectrum of morphology was anticipated, areas of solid, confluent, or cribriform growth of glands were acceptable in SD and LD subtypes and quantified for the purposes of grading as described previously; the subtype was thus defined based on the better differentiated regions of the neoplasms. SDs were sub-classified as SD1 (a.k.a. bile duct type) if the well differentiated component contained predominantly non-anastomosing tubules resembling interlobular bile ducts (Figure 2B); and SD2 (a.k.a. bile ductular type, cholangiolar) if the well differentiated component contained predominantly anastomosing tubules focally resembling a bile ductular reaction (Figure 2 E). Neoplasms with areas resembling a ductal plate malformation (Figure 2F) or cholangiocellular carcinoma pattern (Figures 1F, 2E) were included in SD2 because the glands in that pattern are anastomosing . (18) An estimated percentage of the cholangiocellular carcinoma pattern component was recorded, defined as a proliferation resembling the canals of Hering (Figure 1F, 2E). Cases with features that did not meet criteria for LD or SD, including adenosquamous carcinoma, were placed in an indeterminate category (IND) (Figure 3G–I).

Ancillary studies

Paraffin embedded tissue microarrays (TMA) were constructed using 2 to 4 1.0 mm diameter punches from each neoplasm, targeted to represent the various morphologic patterns that were present. Immunohistochemistry for antibody (clone, manufacturer, dilution) were as follows: HepPar1 (OCH1E5, Dako, 1:2000), Arginase-1 (Cell Marque, 1:1000), Glypican-3 (Santa Cruz, 1:100); S100P (Dako 1:5000); CD56 (MRQ42, Cell Marque, 1:50); CK19 (RCK108, Dako, 1:400); CA19.9 (Ventana); and CK7 (OV-TL12130,

Dako, 1:1000) were performed using standard immunoperoxidase methods and manufacturer instructions. Immunohistochemical stains with >20% strong tumor cell labeling were scored as positive.

Extra- and intracellular mucin production was assessed by two different methods: by Hematoxylin and eosin stain whole tissue section or mucicarmine stains (TMA). Mucin was scored as extracellular if any mucin was identified in the luminal spaces or intracellular if within the confines of the cytoplasm.

Albumin RNA in situ hybridization was performed using two automated platforms: Albumin RNA ISH by ViewRNA™ ISH cell assay kit (ThermoFisher Scientific; Waltham, Massachusetts) and Advanced cell diagnostics system by Leica Biosystems (Buffalo Grove, Illinois), performed on the Leica BOND III autostainer. Both methods were performed according to manufacturers' instructions for the assay kits. All cases stained with ViewRNA™ ISH had verification of positive housekeeping mRNA-targeting Probe set, Pan HKG (ThermoFisher Scientific; Waltham, Massachusetts) and negative DAP B controls (ThermoFisher Scientific; Waltham, Massachusetts). Staining in each case was evaluated on 2 to 4 1.0 mm diameter punches prepared in a formalin fixed paraffin embedded tissue microarray. Positive staining by either method was scored as positive, using a threshold of granular staining in the cytoplasm of at least 5% of tumor cells. Negatively staining tumors on tissue microarray had repeat staining performed on whole tissue sections as feasible.

Clinical outcome data collection

Recurrence-free survival (RFS) was defined as the interval between resection and the date of disease recurrence which was defined as radiographic appearance of a new lesion (intrahepatic recurrence or metastasis) compatible with ICC or date of disease specific death for patients with no clinical date of recurrence. Patients without recurrence were censored at the date of last clinical encounter. Disease specific survival (DSS) was defined as the interval between resection and death from ICC. Patients without ICC-related death were censored at the date of the last follow up or non-cholangiocarcinoma-related death. Thirteen patients with a history of neoadjuvant therapy and 4 patients resected for recurrent liver disease were excluded from survival analysis.

Statistical analysis

To ascertain differences in the rates of clinical characteristics and histologic features among the 4 histologic subtypes we performed 3 analyses: comparison SD1 vs. SD2, SD vs. LD, and all types (SD1 vs. SD2 vs. LD vs. indeterminate). Categorical variables were compared using the Fischer exact test and Chi square test. All P values were based on 2-tailed statistical analysis with a threshold of <0.05 for statistical significance. Cox regression for survival analysis was performed to compare hazard rates of histologic types and other characteristics. Analysis was performed using R statistical software version 3.4.1.

RESULTS

Clinical characteristics of ICC

A total of 184 intrahepatic cholangiocarcinoma patients were included with a median age of 66 years. Demographics and clinical characteristics are summarized in Table 1. Women outnumbered men (103 women: 81 men) and there was an ethnic predominance of white patients (89%). Hepatitis serology was available for 110 patients, with a 17% prevalence of chronic viral hepatitis. The cohort had a high prevalence of smoking (46%) and low prevalence of frequent alcohol use (9%), cirrhosis (8%), autoimmune disease (8%), steatosis (3%), and primary sclerosing cholangitis (2%). There were no instances of major risk factors for cholangiocarcinoma in other geographic settings such as hepatic lithiasis, parasitic infection, and anatomic malformations.

Correlation between histologic type and clinical risk factors

The only risk factor for cholangiocarcinoma that showed an association with a histologic subtype was primary sclerosing cholangitis, which was rare, but had a significantly increased association comparing LD vs SD types ($P=0.018$) (Supplemental digital content table 1). Comparison of SD1 vs SD2 showed no statistically significant association ($P>.05$) with clinical risk factors (chronic hepatitis, steatosis, cirrhosis, autoimmune disease, primary sclerosing cholangitis, alcohol, and smoking).

Distribution of histopathologic features and gross configuration by histologic subtype

The distribution of histologic subtypes in this cohort was 24% SD1, 60% SD2, 8% LD, and 8% IND. Table 2 summarizes the results of analyses performed to identify whether certain histopathologic features were seen at higher rates among the histologic subtypes. The vast majority, 155 (84%) of the cases, were SD and only one histologic feature distinguished SD1 from SD2 when these two subtypes were compared: fewer cases of SD1 had zones of histologic patterns (20% versus 46%; $P=0.003$).

In contrast, several histologic features occurred in LD at rates distinctly different from the other types and although there were a few exceptions, the differences were significant when analysis included all types and when restricted to LD versus SD (Table 2 and Supplemental digital content table 2). This finding supports the greater similarities between SD1 and SD2 compared to LD. Comparing LD vs SD, LD had significantly higher rates of perineural invasion (71%), extracellular mucin (79%), intracellular mucin by H&E (79%), and lower rates of hepatoid cytology (none), infiltrative interface (29%), and sclerotic stroma (29%). The rate of desmoplastic stroma was similar between LD (57%) and IND (53%), but significantly different from SD1 (18%) and SD2 (13%) in analysis of all types (Supplemental digital content table 2). Nodularity was present in fewer cases of LD compared to SD (7% vs 39% $P=0.019$). No difference in AJCC 8th edition pT stage was detected among subtypes.

The IND type had few distinguishing features in the analysis of all types (Supplemental table 2). IND had a higher rate of high grade tumors (73%) compared to the other types (14–33%) and a low rate of zones of histologic patterns (20%). These findings are not surprising

because this category contained carcinomas with mostly solid architecture with gland formations too limited to categorize as LD or SD, and it also included adenosquamous carcinomas.

Histologic features that showed no significant associations ($P>0.05$) with the subtypes included lymphovascular invasion, lymph node metastasis, satellite nodules, stroma poor, and scar.

Most tumors (90%) were mass forming. Histologic subtypes did not show an association with mass forming or periductal infiltrating type ($P>0.05$), however this configuration was present in a minority of cases overall (9%). There were too few tumors associated with an intraductal neoplasm to evaluate for associations.

Mucin stain and albumin expression

Mucicarmine staining (on tissue microarray) detected extracellular mucin in 28% of SD1, 18% of SD2, 82% of LD, and 25% of IND subtypes ($P<0.001$, Table 3) which is consistent with the significant association between LD and extracellular mucin detected on H&E whole tissue section. Unexpectedly, intracellular mucin was identified in fewer LD type on the mucin stained tissue microarray compared to the whole tissue section H&E evaluation (27% vs 79% positive staining) and consequently we saw no significant association between intracellular mucin stain positive cases and subtype in all analyses. This discrepancy could be attributed to sampling since the glandular density in LD is greatly reduced compared to SD, with few glands represented on some samples in the tissue microarray.

By mucin stain there was no significant difference in intracellular or extracellular mucin comparing SD1 vs SD2 ($P>0.05$). Select histologic features were tested for associations with mucin staining (Table 4). Neoplasms negative for extracellular mucin correlated with a lack of periductal infiltration ($P=.038$). No correlation was seen between mucin (intracellular or extracellular) staining, grade, and $>10\%$ cholangiocellular carcinoma pattern ($P>0.05$).

Albumin expression was positive in 18% of LD, which was significantly lower compared to albumin expression by the other types (53–78% positive) ($P=0.001$). The higher rate of albumin expression in SD2 (78% positive) compared with SD1 (53% positive) was statistically significant ($P=0.018$). Albumin expression was also associated with a $>10\%$ cholangiocellular carcinoma pattern ($P=0.004$) (Table 4). Of the 44 cases testing negative for albumin on TMA, 30 (68%) were repeated on whole tissue section. Ten cases that were negative on TMA were positive on whole tissue section.

We identified a significant correlation between albumin expression and extracellular mucin production; of the 84 albumin positive tumors, only 13 (15%) were positive for extracellular mucin by mucin stain ($P<0.001$) (Table 4). However, there was no correlation between albumin expression and intracellular mucin by mucin stain ($P>0.05$).

Immunophenotype of the morphologic subtypes

Significant immunophenotypic differences were detected between LD and SD (Table 3). Compared with SD, LD showed higher rates of CA19–9 expression (73%) and the CK7+, CK19+, CD56- phenotype (91% of LD positive).

Comparing SD1 and SD2, there was no difference in expression of CA19–9, the CK7+, CK19+, CD56- phenotype, CD56, and S100P ($P>.05$). S100P was rarely positive in SD (SD1 4% and SD2 8%).

Select histologic features were tested for associations with immunophenotype (Table 4). CA19–9 expression correlated with periductal infiltration ($P=0.007$). The CK7+/CK19+/CD56- phenotype, CD56, and S100P expression did not show an association with periductal infiltration, >10% cholangiocellular carcinoma pattern, or grade. CA19–9 expression correlated with a lack of albumin staining ($P<0.001$).

We tested markers that are associated with hepatocellular differentiation: HepPar1 was focally positive in 1 of 128 tested cases; Arginase was negative in all 127 tested cases. Glypican-3 was negative in 127 tested cases.

Outcome after resection

The median follow up for this cohort was 37 months (interquartile range 19–73 months). AJCC 8th edition pT stage and periductal infiltration were significant predictors of reduced RFS and DSS by univariate and multiple variable analysis accounting for pT stage, gross configuration, and histologic subtype (combined LD/IND) (Tables 5 and 6). Periductal infiltration was seen in 17 cases and all would have been pT4 in the AJCC 7th edition, but in the 8th edition were distributed as follows: pT1 (2 cases), 8 pT2 (8 cases), 7 pT3 (7 cases). As a likely consequence, there were only 3 pT4 cases (this cohort was limited to resectable disease) and pT2 had the highest hazard ratio in time-to-event analysis. Time-to-event analysis for RFS and DSS across the histologic subtypes, using SD2 as a reference, only reached significance in univariate analysis if LD and IND were combined, however after accounting for multiple variables, the differences in time-to-event for LD/IND was not significant.

DISCUSSION

ICC has a higher prevalence in Asia over North America, but its incidence is rising in the western hemisphere.(19) In the 2010 WHO classification of tumors of the digestive system, there is no subtyping of intrahepatic cholangiocarcinoma (excluding rare variants).(20) However, several investigators have proposed dividing ICC into two or more histologic types.(4, 5, 7, 10, 21, 22) Our primary aim was to assess if architectural patterns in a large North American cohort of resected ICC would show clinical and pathologic associations that have been previously observed in other (primarily Asian) populations.

The only clinical risk factor that showed an association with histologic subtype was primary sclerosing cholangitis which was rare (2%) but correlated with the LD subtype. SD has been reported to associate with chronic hepatitis but we did not detect this.(8) Prevalence of

chronic hepatitis B and C in this study group (16%) is higher than the United States population (<2% hepatitis B and 1.3% hepatitis C, respectively) but lower than the prevalence in other studies of ICC histology (range: 29–57%).(5, 7, 8, 11, 21, 23, 24)

In this study, where subtyping was performed purely based on histologic parameters, we provide additional support for histopathologic and immunophenotypic differences distinguishing LD from SD. Compared to SD, LD has higher rates of intracellular and extracellular mucin, desmoplastic stroma, and perineural invasion, while SD has higher rates of an infiltrative tumor/liver interface, nodularity, sclerotic stroma, and hepatoid cytology. The two subtypes also show distinct staining patterns: in LD, albumin is less likely detected by ISH and CA19–9 is more often positive than in SD. Other authors have shown differences between LD and SD including a unique LD immunophenotype (S100P, TFF1, galectin-3, and AGR2 expression, absent N-cadherin) and a higher prevalence of *KRAS* mutation.(8, 21, 22, 25, 26)) Many features of LD are also seen in extrahepatic and hilar cholangiocarcinoma. We did not reproduce a previously reported association between S100P expression and LD type but showed a high proportion of the “mature cholangiocyte” CK7+, CK19+, CD56- phenotype.(11)

The majority (84%) of ICC in this cohort was SD and this finding is divergent from generalized descriptions that most ICC resemble adenocarcinomas of the hilar, extrahepatic, or pancreatobiliary type.(20) In fact, LD type (9%) in this study was vastly underrepresented compared to other studies of ICCs that report ranges from 41–59%.(5, 7, 10, 21, 22) The proportion of periductal infiltrating type (10%) in our study was also low in comparison to others (range: 12–28%).(7, 21, 27) Reasons for these differences include case selection, epidemiology, variation in histologic criteria between studies, and use of immunohistochemistry for diagnosis.(7, 10) In the present study, case selection was not entirely consecutive and excluded tumors determined to be of hilar origin on a clinical basis. Clinical distinction between intra- versus extrahepatic peri-hilar origin is known to be challenging, particularly in large tumors involving the hilum.(28) Epidemiologic factors also contributed to this difference. For example, a Taiwanese cohort had 59% LD (“bile duct” type in their study) and a 13% rate of hepatic lithiasis which is a risk factor associated with LD type that was absent in our population.(21) Another factor is the lack of consensus among pathologists for large and small duct classification. Unlike some studies, we did not include mucin production as a criterion for categorization because it can be present in both types, although in different proportions.(7, 10) Other authors have used S100P to increase recognition of LD.(7, 27) We did not apply S100P results to classification but it would be unlikely to have reclassified many SD cases because only 8 (7%) non-large duct cases (1 SD1 cases, 6 SD2 cases) expressed S100P. We tested for S100P on a TMA but that is not a major limitation because S100P positive cholangiocarcinomas tend to have diffuse reactivity.(29) We did not detect a significant difference in survival for LD vs SD, but the analysis was likely limited by the low proportion of LD.

AJCC 8th edition pT stage is a predictor of survival, but pT2 had the highest hazard ratio among T stage categories in our time-to-event analysis. Our finding that periductal infiltration was also an independent predictor of survival would argue against the recent

removal of this criterion from the pT4 category in the 8th edition of the AJCC cancer staging manual.

Recent improvements in automated technology for detecting albumin mRNA have increased the clinical applicability of this marker for the diagnosis of ICC but it is still not widely used in practice. Prior studies on albumin detection in ICC have reported variable results, which is likely due to differences in methodologies and case selection.(30–33) Ferrone and Shahid reported on the same ICC cohort, suggesting albumin RNA ISH may help in distinguishing ICC peri-hilar (extra-hepatic) cholangiocarcinoma from intrahepatic carcinomas because 99% of ICC had 5–100% albumin staining on TMA. In contrast, Coulouarn et al detected albumin in a much smaller proportion (33%) of ICC.(33) Neither study described histomorphology of the ICC tested.(14, 32) Our study provides some insight into potential limitations of the albumin stain as a marker for working up a liver biopsy containing an unknown primary neoplasm. Although we found albumin expression in 66% of ICCs overall, we conclude that albumin staining is unlikely to be useful to support intrahepatic origin for neoplasms with a LD morphology, as 82% are negative. Negative albumin staining on small biopsies of tumors with a SD pattern should not exclude intrahepatic origin. Since the IND subtype stained with albumin in 64% of cases, it may still be useful to rule in intrahepatic origin in the context of neoplasms lacking a well differentiated SD pattern suggesting cholangiocarcinoma. The significant correlation between >10% cholangiocellular carcinoma pattern and positive albumin expression suggests that this histologic pattern, when present, may be helpful in suggesting intrahepatic origin when albumin staining is not available.

Like Hayashi et al, our comparison of SD1 and SD2 ICC does not support subdividing the SD category because there are very limited clinical, histological, or immunophenotypic differences distinguishing them.(7) Distinction of SD1 from SD2 can be difficult when there are only small amounts of well differentiated glands. Compact, angulated, and dilated glands can also be difficult to consistently classify as anastomosing vs tubular.

Nomenclature for subtypes of ICC is confusing because terms used to describe the anatomy of the liver and bile ducts overlap with terms used to describe histologic subtypes. For example, ICC is often termed, “peripheral” cholangiocarcinoma because it arises peripheral to the second order bile ducts at the hilar biliary confluence, but this terminology should be discouraged because these tumors occur throughout the liver parenchyma including central or adjacent to the liver hilum.(34) Conversely, some clinically intrahepatic carcinomas resemble the carcinomas of the larger bile ducts of the hepatic hilum or extrahepatic bile ducts. Using the terms “hilar” or “peri-hilar” for these histologic patterns is also problematic because these terms are very specific to the anatomic sites of extrahepatic cholangiocarcinoma and these neoplasms have different staging parameters. Since it is accepted that tumors with an LD pattern can arise peripheral to the second order bile ducts, we advocate for the use of *small duct* and *large duct* types in describing the histology of ICC to avoid confusion between histologic pattern and site of tumor origin.

In conclusion, two distinct patterns of ICC arise within the liver: LD, which more commonly produces mucin, resembles extrahepatic biliary neoplasms, has a low prevalence in this

North American population, has an association with primary sclerosing cholangitis, and tends to lack albumin expression; and SD, the more common pattern in this cohort, is composed of predominantly tubular or anastomosing small ducts and can also produce mucin. At this time we find no compelling argument such as a significant clinical association or known biological significance to justify further subtyping of SD. Since we were unable to demonstrate a difference in survival between SD and LD treated primarily by resection, it may not be clinically relevant at this time to report SD vs LD routinely, however recognition of these two morphologic patterns can aid in the interpretation of the albumin stain. Also, the marked difference in the histologic presentation of these two types prompts us to advocate that future investigations of the biologic and genetic characteristics of intrahepatic cholangiocarcinoma include correlation with these two distinct histologic phenotypes so that the biological significance can be better understood.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Histologic parameters included (A–B) nodularity, (C) zones of histologic patterns, (D) infiltrative interface with hepatocytes, (E) hepatoid cytology (negative for markers of hepatocellular differentiation), and (F) cholangiocellular carcinoma pattern with sclerotic stroma.

Figure 2.

Small duct 1 (A–D) and small duct 2 (E–I) subtypes. (A) Large caliber ducts can be present, however the cells lining the glands are cuboidal and similar to the background tubular ducts in a stroma poor SD1. (B) Small tubular ducts do not show anastomoses in SD1. (C) Extracellular mucin in the lumens of small glands with cuboidal cells of SD1. (D) Back to back and confluent tubular glands with minimal intervening stroma in SD1. (E) Angulated anastomosing glands lined by cuboidal epithelium in a low grade SD2 that was negative for albumin in situ hybridization on a whole tissue section. (F) This albumin negative SD2 has a zones of histologic patterns including a ductal plate malformation pattern (right) and increased complexity (left). (G) An SD2 with increased glandular complexity. (H) A stroma poor SD2 (I) SD2 with periductal infiltration.

Figure 3.

Examples of the large duct (A–D) and indeterminate subtypes (E–F). (A) Large caliber ducts lined by columnar cells were necessary to establish the large duct subtype. This example shows desmoplastic stroma. (B) Foci of crowded small tubular glands can be seen in large duct subtype. (C) Intracellular and extracellular mucin in a large duct subtype. (D) Large duct subtype with periductal infiltration. (E–F) A large duct subtype H &E stain (E) and positive albumin in situ hybridization (F). (G, H) Uniformly solid growth patterns were typed as indeterminate. (I) Adenosquamous carcinoma.

Table 1:

Clinical characteristics

Characteristic	Number tested	N
Age at diagnosis (years, median/IQR)	184	66 (57.75, 74)
Gender	184	
Female		103 (0.56)
Male		81 (0.44)
Ethnicity	184	
White		163 (0.89)
Asian		13 (0.07)
Black		7 (0.04)
Other		1 (0.01)
Hepatitis viral infection	110	19 (0.17)
HBV positive	101	10 (0.10)
HBV positive	99	8 (0.08)
Autoimmune disease (any)	184	14 (0.08)
Primary sclerosing cholangitis	184	4 (0.02)
Smoking history	183	
Current		22 (0.12)
Former		62 (0.34)
Never		99 (0.54)
Frequent alcohol use	183	16 (0.09)
Cirrhosis	178	15 (0.08)
Steatosis	184	6 (0.03)
Tumor size (median, IQR)	183	6 (4, 8.9)
Mass forming	184	165 (0.90)
Periductal infiltrating	182	17 (0.09)
Satellite nodules	184	54 (0.29)
Associated with an intraductal neoplasm	184	5 (0.03)

Table 2:

Pathologic features of intrahepatic cholangiocarcinoma by histologic type

	Small duct type 1 (tubular) N=45	Small duct type 2 (anastomosing) N=110	Large duct type N=14	Indeterminate N=15	Total N=184	P- value for SD1 vs. SD2	P-value for SD vs. LD
Grade						0.330	0.358
Low	30 (0.67)	82 (0.75)	12 (0.86)	4 (0.27)	128 (0.70)		
High	15 (0.33)	28 (0.25)	2 (0.14)	11 (0.73)	56 (0.30)		
Lymphovascular invasion						0.500	1.000
Negative	35 (0.78)	91 (0.83)	12 (0.86)	12 (0.8)	150 (0.82)		
Positive	10 (0.22)	19 (0.17)	2 (0.14)	3 (0.2)	34 (0.18)		
Perineural Invasion						0.840	0.002
Negative	30 (0.71)	73 (0.73)	4 (0.29)	9 (0.75)	116 (0.69)		
Positive	12 (0.29)	27 (0.27)	10 (0.71)	3 (0.25)	52 (0.31)		
Lymph node metastasis						1.000	0.498
Negative	17 (0.71)	33 (0.72)	7 (0.58)	3 (0.43)	60 (0.67)		
Positive	7 (0.29)	13 (0.28)	5 (0.42)	4 (0.57)	29 (0.33)		
Mass forming						0.767	0.174
Negative	5 (0.11)	10 (0.09)	3 (0.21)	1 (0.07)	19 (0.10)		
Positive	40 (0.89)	100 (0.91)	11 (0.79)	14 (0.93)	165 (0.90)		
Periductal infiltrating						1.000	0.136
Negative	40 (0.93)	100 (0.91)	11 (0.79)	14 (0.93)	165 (0.91)		
Positive	3 (0.07)	10 (0.09)	3 (0.21)	1 (0.07)	17 (0.09)		
Satellite nodules						0.444	1.000
Negative	30 (0.67)	80 (0.73)	10 (0.71)	10 (0.67)	130 (0.71)		
Positive	15 (0.33)	30 (0.27)	4 (0.29)	5 (0.33)	54 (0.29)		
Nodularity						0.368	0.019
Negative	30 (0.67)	63 (0.58)	13 (0.93)	11 (0.73)	117 (0.64)		
Positive	15 (0.33)	45 (0.42)	1 (0.07)	4 (0.27)	65 (0.36)		
Zones of histologic patterns						0.003	0.572
Negative	36 (0.8)	58 (0.54)	10 (0.71)	12 (0.8)	116 (0.64)		
Positive	9 (0.2)	50 (0.46)	4 (0.29)	3 (0.2)	66 (0.36)		
Extracellular mucin						0.194	<0.001
Negative	32 (0.71)	89 (0.82)	3 (0.21)	11 (0.73)	135 (0.74)		
Positive	13 (0.29)	20 (0.18)	11 (0.79)	4 (0.27)	48 (0.26)		
Intracellular mucin						0.757	<0.001
Negative	42 (0.93)	99 (0.91)	3 (0.21)	15 (1)	159 (0.87)		
Positive	3 (0.07)	10 (0.09)	11 (0.79)	0 (0)	24 (0.13)		
Hepatoid cytology						0.093	0.042
Negative	30 (0.67)	88 (0.81)	14 (1)	10 (0.67)	142 (0.78)		
Positive	15 (0.33)	21 (0.19)	0 (0)	5 (0.33)	41 (0.22)		

	Small duct type 1 (tubular) N=45	Small duct type 2 (anastomosing) N=110	Large duct type N=14	Indeterminate N=15	Total N=184	P- value for SD1 vs. SD2	P-value for SD vs. LD
Infiltrative interface						0.291	<0.001
Negative	8 (0.18)	12 (0.11)	10 (0.71)	9 (0.6)	39 (0.22)		
Positive	36 (0.82)	96 (0.89)	4 (0.29)	6 (0.4)	142 (0.78)		
Stroma poor						0.231	0.706
Negative	35 (0.78)	94 (0.86)	11 (0.79)	12 (0.8)	152 (0.83)		
Positive	10 (0.22)	15 (0.14)	3 (0.21)	3 (0.2)	31 (0.17)		
Desmoplastic stroma						0.453	0.001
Negative	37 (0.82)	95 (0.87)	6 (0.43)	7 (0.47)	145 (0.79)		
Positive	8 (0.18)	14 (0.13)	8 (0.57)	8 (0.53)	38 (0.21)		
Sclerotic stroma						0.205	<0.001
Negative	13 (0.29)	21 (0.19)	10 (0.71)	9 (0.6)	53 (0.29)		
Positive	32 (0.71)	88 (0.81)	4 (0.29)	6 (0.4)	130 (0.71)		
Scar						0.288	0.265
Negative	24 (0.53)	47 (0.43)	9 (0.64)	10 (0.67)	90 (0.49)		
Positive	21 (0.47)	62 (0.57)	5 (0.36)	5 (0.33)	93 (0.51)		
AJCC 8th ed. stage						0.830	0.296
pT1	17 (0.38)	42 (0.38)	4 (0.29)	6 (0.4)	69 (0.38)		
pT2	22 (0.49)	53 (0.48)	6 (0.43)	7 (0.47)	88 (0.48)		
pT3	6 (0.13)	13 (0.12)	3 (0.21)	2 (0.13)	24 (0.13)		
pT4	0 (0)	2 (0.02)	1 (0.07)	0 (0)	3 (0.02)		

Abbreviations: SD1=small duct type 1, SD2=small duct type 2; LD=large duct type; Ind=indeterminate type

Table 3.

Staining patterns for histologic subtypes

	Small duct type 1 (tubular) N=45	Small duct type 2 (anastomosing) N=110	Large duct type N=14	Indeterminate N=15	Total (n=184)	P-value for SD1 vs. SD2	P-value for SD vs. LD
CK7+ CK19+ CD56–						0.515	0.001
Negative	20 (0.67)	45 (0.59)	1 (0.09)	8 (0.73)	74 (0.58)		
Positive	10 (0.33)	31 (0.41)	10 (0.91)	3 (0.27)	54 (0.42)		
CA19–9						0.753	<0.001
Negative	27 (0.9)	65 (0.87)	3 (0.27)	10 (0.91)	105 (0.83)		
Positive	3 (0.1)	10 (0.13)	8 (0.73)	1 (0.09)	22 (0.17)		
CD56						0.630	0.064
Negative	21 (0.7)	57 (0.75)	11 (1)	8 (0.73)	97 (0.76)		
Positive	9 (0.3)	19 (0.25)	0 (0)	3 (0.27)	31 (0.24)		
Albumin ISH						0.018	0.001
Negative	14 (0.47)	17 (0.22)	9 (0.82)	4 (0.36)	44 (0.34)		
Positive	16 (0.53)	59 (0.78)	2 (0.18)	7 (0.64)	84 (0.66)		
S100P						0.670	0.571
Negative	27 (0.96)	68 (0.92)	10 (0.91)	10 (1)	115 (0.93)		
Positive	1 (0.04)	6 (0.08)	1 (0.09)	0 (0)	8 (0.07)		
Mucicarmine (extracellular)						0.299	<0.001
Negative	21 (0.72)	62 (0.82)	2 (0.18)	9 (0.9)	94 (0.75)		
Positive	8 (0.28)	14 (0.18)	9 (0.82)	1 (0.1)	32 (0.25)		
Mucicarmine (intracellular)						0.440	0.069
Negative	28 (0.97)	69 (0.91)	8 (0.73)	10 (1)	115 (0.91)		
Positive	1 (0.03)	7 (0.09)	3 (0.27)	0 (0)	11 (0.09)		

Abbreviations: SD1=small duct type 1, SD2=small duct type 2; LD=large duct type; ISH=in situ hybridization

Table 4.

Staining patterns for selected histological features

	High grade	Low grade	P-value	Periductal infiltrating present	Periductal infiltrating absent	P-value	CLC pattern >10%	CLC pattern ≤10%	P-value	Albumin ISH positive	Albumin ISH negative	P-value
CK7+ CK19+ CD56-												
0.248												
Negative	49 (0.54)	25 (0.66)		7 (0.54)	66 (0.58)	0.774	26 (0.54)	48 (0.6)	0.581	51 (0.61)	23 (0.52)	0.451
Positive	41 (0.46)	13 (0.34)		6 (0.46)	47 (0.42)		22 (0.46)	32 (0.4)		33 (0.39)	21 (0.48)	
CA19-9												
0.213												
Negative	71 (0.8)	34 (0.89)		6 (0.5)	97 (0.86)	0.007	40 (0.85)	65 (0.81)	0.635	76 (0.92)	29 (0.66)	<0.001
Positive	18 (0.2)	4 (0.11)		6 (0.5)	16 (0.14)		7 (0.15)	15 (0.19)		7 (0.08)	15 (0.34)	
CD56												
0.179												
Negative	65 (0.72)	32 (0.84)		10 (0.77)	86 (0.76)	1.000	37 (0.77)	60 (0.75)	0.834	63 (0.75)	34 (0.77)	0.831
Positive	25 (0.28)	6 (0.16)		3 (0.23)	27 (0.24)		11 (0.23)	20 (0.25)		21 (0.25)	10 (0.23)	
Albumin ISH												
0.839												
Negative	30 (0.33)	14 (0.37)		5 (0.38)	37 (0.33)	0.759	9 (0.19)	35 (0.44)	0.004			
Positive	60 (0.67)	24 (0.63)		8 (0.62)	76 (0.67)		39 (0.81)	45 (0.56)				
S100P												
0.691												
Negative	82 (0.94)	33 (0.92)		12 (1)	101 (0.93)	1.000	44 (0.96)	71 (0.92)	0.709	76 (0.94)	39 (0.93)	1.000
Positive	5 (0.06)	3 (0.08)		0 (0)	8 (0.07)		2 (0.04)	6 (0.08)		5 (0.06)	3 (0.07)	
Mucicarmine (extracellular)												
0.370												
Negative	64 (0.72)	30 (0.81)		6 (0.46)	86 (0.77)	0.038	38 (0.79)	56 (0.72)	0.405	71 (0.85)	23 (0.55)	<0.001
Positive	25 (0.28)	7 (0.19)		7 (0.54)	25 (0.23)		10 (0.21)	22 (0.28)		13 (0.15)	19 (0.45)	
Mucicarmine (intracellular)												
1.000												
Negative	81 (0.91)	34 (0.92)		11 (0.85)	102 (0.92)	0.324	42 (0.88)	73 (0.94)	0.331	78 (0.93)	37 (0.88)	0.504
Positive	8 (0.09)	3 (0.08)		2 (0.15)	9 (0.08)		6 (0.12)	5 (0.06)		6 (0.07)	5 (0.12)	

Abbreviations: CLC=cholangiocellular; ISH=in situ hybridization

Table 5:

Univariate Cox regression for recurrence free survival and disease specific survival (n=163*, 127 RFS events, 95 DSS events)

Characteristic	RFS HR	RFS (95% CI)	RFS P value	DSS HR	DSS (95% CI)	DSS P value
Histological subtype			0.163			0.069
Small duct type 1	1.29	(0.85, 1.97)		1.24	(0.75, 2.05)	
Small duct type 2	1.00	Reference		1.00	Reference	
Large duct type	1.64	(0.82, 3.29)		1.98	(0.94, 4.18)	
Indeterminate type	1.84	(1.00, 3.39)		2.18	(1.16, 4.07)	
Combined types: Large duct/Indeterminate	1.63	(1.02, 2.61)	0.043	1.98	(1.21, 3.25)	0.007
High grade (reference: low grade)	1.13	(0.78, 1.64)	0.517	0.93	(0.60, 1.45)	0.756
Periductal infiltrating type (reference: mass forming)	2.73	(1.55, 4.80)	0.001	3.58	(2.01, 6.39)	<0.001
AJCC 8 th edition T Stage			0.013			0.007
pT1	1.00	Reference		1.00	Reference	
pT2	1.90	(1.29, 2.81)		2.15	(1.36, 3.38)	
pT3	1.37	(0.76, 2.47)		1.94	(1.01, 3.73)	
pT4	1.48	(0.36, 6.12)		1.02	(0.14, 7.47)	

* Individuals were excluded if chemotherapy was received prior to resection, metastasis was present at the time of resection, or the resection was for recurrent disease.

Abbreviations: AJCC= American Joint Committee on Cancer; CI= confidence interval; DSS=disease (cholangiocarcinoma) specific survival, HR=hazard ratio; recurrence free survival (RFS)

Table 6:

Multiple Cox regression for recurrence free survival and disease specific survival (n=163^{*}, 127 RFS events, 95 DSS events) Model: Gross type + AJCC 8th edition T Stage + histologic subtype

Characteristic	RFS HR	RFS (95% CI)	RFS P	DSS HR	DSS (95% CI)	DSS P
Periductal infiltrating type (reference: mass forming)	2.31	(1.29, 4.16)	0.010	2.65	(1.43, 4.90)	0.004
Large duct type/Indeterminate (reference: small duct type)	1.54	(0.94, 2.53)	0.103	1.70	(1.00, 2.89)	0.060
AJCC 8 th Edition T Stage			0.012			0.012
pT1	1.00	Reference		1.00	Reference	
pT2	1.92	(1.30, 2.84)		2.11	(1.33, 3.34)	
pT3	1.26	(0.70, 2.28)		1.73	(0.90, 3.35)	
pT4	1.65	(0.40, 6.86)		1.18	(0.16, 8.67)	

* Individuals were excluded if chemotherapy was received prior to resection, metastasis was present at the time of resection, or the resection was for recurrent disease.

Abbreviations: AJCC= American Joint Committee on Cancer; CI= confidence interval; DSS=disease (cholangiocarcinoma) specific survival, HR=hazard ratio; RFS= recurrence free survival.