CK20 and Lymph Node Involvement Predict Adverse Outcome of Malignant Intraductal Papillary Neoplasm of the Bile Duct

Authors: Jie Shi, Xueshuai Wan, Yuan Xie, Jianzhen Lin, Junyu Long, Weiyu Xu, Zhiyong Liang, Xinting Sang and Haitao Zhao

DOI: 10.14670/HH-18-179
Article type: ORIGINAL ARTICLE
Accepted: 2019-10-28
Epub ahead of print: 2019-10-28

This article has been peer reviewed and published immediately upon acceptance. Articles in "Histology and Histopathology" are listed in Pubmed.
Pre-print author's version
CK20 and Lymph Node Involvement Predict Adverse Outcome of Malignant
Intraductal Papillary Neoplasm of the Bile Duct.

Running head: Prognostic factors of IPNB.

Jie Shi MD,1 Xueshuai Wan MD,2 Yuan Xie MD,3 Jianzhen Lin MD,2 Junyu Long MD,2 Weiyu Xu MD,2 Zhiyong Liang MD,1 Xinting Sang MD,2 and Haitao Zhao MD2

1Department of Pathology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China;

2Department of Liver Surgery, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China;

3Department of Hepatobiliary Surgery II, Zhujiang Hospital, Southern Medical University, Guangzhou, China

Jie Shi and Xueshuai Wan contributed equally to this work.

Correspondence to
Xinting Sang, MD, Department of Liver Surgery, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College (CAMS & PUMC), 1 Shuaifuyuan, Wangfujing, Beijing 100730, China. Tel: 86-10-69156042, Fax: 86-10-69156043, Email: sangxt@pumch.cn.
Abstract

Objectives: To identify prognostic factors of malignant intraductal papillary neoplasm of the bile duct (m-IPNB).

Materials and Methods: We included 38 consecutive cases which underwent surgical resection and diagnosed as IPNB with malignant component from January 2003 to January 2017. Clinicopathological variables were collected to conduct survival analysis and identify prognostic factors.

Results: The median OS of m-IPNB was 76.0 months, with 1-, 3-, and 5-year survival rates of 97.2%, 73.5%, and 59.8%, respectively. The median RFS was 48.0 months with 1-, 3-, and 5-year RFS rate was 83.2%, 59.8%, and 44.6%, respectively. Univariate analysis showed that elevation of CEA, lymph node involvement, resection margin status, degree of periductal invasion, and positive expression of CK20 were associated with both OS and RFS of m-IPNB. After multivariate Cox models analysis, lymph node involvement and positive expression of CK20 were identified as independent prognostic factors for OS, while lymph node involvement and resection margin status were independent prognostic factors for RFS. The median OS of patients with m-IPNB involving lymphatic metastases and positive expression of...
CK20 was 27.0±8.8 months and 51.0±12.4 months, respectively. The median RFS of cases with lymph node involvement and R1 resection was 10.0±3.3 months and 25.0±6.9 months, respectively. However, there was no significant difference in OS or RFS between cases of pancreaticobiliary and intestinal subtype.  

**Conclusions:** Lymph node involvement and positive expression of CK20 are independent prognostic factors for shorter OS of m-IPNB, while patients with lymph node involvement and positive resection margin are at higher risk of tumor recurrence.

Keywords: CK20; lymph node; papillary neoplasm; bile duct; prognostic factors

**Introduction**

Intraductal papillary neoplasm of the bile duct (IPNB) was added to World Health Organization classification in 2010. It is a rare tumor, which may originate from intrahepatic or extrahepatic biliary tract, characterized by single or multiple lesions protruding into the lumen, with or without gross mucus secretion, and can be recognized as papillary cholangiocarcinoma or its benign precursors, pathologically. (Yeh et al., 2005; Kim et al., 2012)

The published literature examining pathobiology, management, and long-term outcome of IPNB is mounting, and suggests that it is a tumor with high malignant potential. According to previous studies, about 40-80% of IPNBs are with invasive carcinoma, which we call malignant intraductal papillary neoplasm of the bile duct.
(m-IPNB) in the present study (Wan et al., 2013; Gordon-Weeks et al., 2016). Although m-IPNB is known to be associated with more favorable outcome compared to other conventional types of cholangiocarcinoma, the reported prognostic factors are various and contradictory (Jung et al., 2012; Kim et al., 2016; Luvira et al., 2017; Wan et al., 2017; Harada et al., 2018). Therefore, we conducted the present study and expected to identify its independent prognostic factors, and thereby direct management decisions.

Materials and Methods

Case selection

We searched our surgical pathology files, and those cases diagnosed as IPNB were retrieved and reconfirmed. Diagnoses and classifications of histological subtypes of IPNB were performed according to intraductal papillary mucinous neoplasms of the pancreas (IPMN), which is well known as its pancreatic analogue (Zen et al., 2006; Zen et al., 2006; Rocha et al., 2012).

When the same tumor contained cells of two or more subtypes, the subtype with the largest proportion determined tumor subtype. Tumors were graded according to the highest level of tumor cells when they contained cells of different pathological levels in different parts of the lesion.
Clinicopathological data

Demographic and clinicopathological variables were retrieved from inpatient and outpatient records, including age; gender; symptoms such as abdominal pain, jaundice and fever; concomitant biliary stones; preoperative liver function such as alanine aminotransferase (ALT); total bilirubin (TBil); gamma-glutamyl transpeptidase (GGT); alkaline phosphatase (ALP); tumor markers such as carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9); and pathological data such as visible mucus, multifocality, lymph node involvement, resection margin status, degree of periductal invasion, recurrence-free survival (RFS) and overall survival (OS). The finding of stones in the gallbladder, intrahepatic or extrahepatic bile duct or a biliary stone history was considered concomitant biliary stones. The highest values of preoperative liver function and tumor markers were adopted, and patients who exceeded the upper limit of normal reference were identified as positive. Degree of periductal invasion was classified as 0mm (confined to the bile duct lumen), 1-5mm, and >5mm. Resection margin status was defined as nonresidual tumor (R0), microscopic (R1), and macroscopic (R2) residual tumor. RFS was defined as the time interval from the date of surgery to the first time of recurrence, while OS was to patient death or last follow-up. All study procedures were approved by Peking Union Medical College Hospital Ethics Committee.
**Immunohistochemical staining**

One or two representative formalin-fixed and paraffin-embedded tissue blocks from each case were subjected to immunohistochemical staining for mucin 1 (MUC1), MUC2, MUC5AC, MUC6, cytokeratin 7 (CK7), CK20 and caudal-type homeobox transcription factor 2 (CDX2). According to the proportion of positive cells in the lesion, the expression of the above markers was semi-quantitatively divided into two scores: negative (-), 0 to 10%; positive (+), more than 10%.

**Statistical analysis**

All continuous data are presented as means ± standard deviation, and comparison was performed using Student’s t test between different groups. The Pearson chi-square test and Fisher's exact test were employed to compare difference of categorical variables between multiple groups. Survival analysis was conducted by using Kaplan-Meier curves and the log-rank tests. Multivariate Cox models analysis was used to identify independent prognostic factors. P value less than 0.05 is defined as statistically significant.

**Results**

**Clinicopathological characteristics**

After re-reading the pathological sections, a total of 48 cases were recognized as IPNB from January 2003 to January 2017. Of these, 38 cases with malignancy (m-IPNB) were included to conduct survival analysis and identify prognostic factors
The mean ages of m-IPNB were 58.4±13.6 (median, 60; range, 28–78) years old with a minor female preponderance (female, 21; male, 17). The most common presenting symptoms were jaundice (20/38), abdominal pain (17/38) and fever (11/38). Nine cases of m-IPNB were asymptomatic at diagnosis (23.7%). Ten cases of m-IPNB had concomitant biliary stones (26.3%). Serum CA19-9 and CEA level increased in 21 and 6 of 37 patients with m-IPNB, respectively.

Seven cases had macroscopic mucus secretion and 6 were multifocal on pathological examination of surgical specimens. Twenty five and 13 patients with m-IPNB received R0 and R1 resection, respectively. Two of the 6 cases with multifocal lesions received R0 resection and the other 4 received R1 (P=0.068). Only 5 of 38 cases with m-IPNB had lymph node involvement, of whom 2 received R0 and 3 received R1 resection. Ten cases were with malignant component which was confined to the bile duct lumen, while 22 with periductal invasion were within 5mm, and 6 with more than 5mm. Of the 5 cases with lymph node involvement, 3 were with periductal invasion within 5mm and 2 with more than 5mm, respectively. The distribution of the degree of periductal invasion in the two groups of R0 and R1 resection was similar. Clinical and pathological variables are summarized in Table 1.

Histological subtype and immunohistochemical staining

Histologically, 21, 14, 3 and 0 of the 38 cases of m-IPNB were classified as intestinal, pancreaticobiliary, oncocytic, and gastric subtype, respectively. However,
some extent of gastric component was recognized in two cases of intestinal and one of pancreaticobiliary subtype of m-IPNB. Twelve of 14 cases of pancreaticobiliary, 16 of 21 cases of intestinal, and all 3 oncocytic subtype were positive for MUC1 expression. Likewise, 31 of 38 cases of m-IPNB had more than 10% of tumor cells expressed CK7. Six of 14 cases of pancreaticobiliary, 12 of 21 cases of intestinal, and 2 of 3 oncocytic subtype were positive for CK20 expression. Expression pattern of all markers except for CDX2 examined in the present study were similar between pancreaticobiliary and intestinal subtype. Nineteen cases of 21 intestinal subtype showed positive CDX2 expression, while only half of those of pancreaticobiliary subtype were positive (P=0.015). Meanwhile, the expression of CK20 and MUC2 were positively related to CDX2 (P=0.043, 0.042, respectively). Typical identified histological subtype component and expression of immunohistochemical markers are shown in Table 2 and Figure 1.

**Outcome and prognostic factors**

No surgery-related deaths were observed in all the 38 cases of m-IPNB. Thirty six patients completed the follow-up (94.7%) with a median follow-up time of 38.0 months (range, 9.0-161.0 months). The 1-, 3-, and 5-year survival rate of present cohort was 97.2%, 73.5%, and 59.8%, respectively, with a median OS of 76.0 months (95% CI, 28.2-123.8 months). We also reached a 10-year OS rate of 38.0%, suggesting the favorable long outcome of m-IPNB (Figure 2A). Seventeen cases experienced recurrence at the time of preparation of this paper, with a median RFS of
48.0 months (95% CI, 11.5-140.5 months). The 1-, 3-, and 5-year RFS rate was 83.2%, 59.8%, and 44.6%, respectively (Figure 2D).

Univariate analysis showed that elevation of CEA, lymph node involvement, resection margin status, degree of periductal invasion, and positive expression of CK20 was associated with both OS and RFS of m-IPNB. However, the OS or RFS was not statistically different between groups of pancreaticobiliary and intestinal subtype (P=0.289, 0.376, respectively). The above five variables were entered into multivariate Cox models, and lymph node involvement and positive expression of CK20 were identified as independent prognostic factors for OS, while lymph node involvement and resection margin status were independent prognostic factors for RFS. The median OS of patients with m-IPNB involving lymphatic metastases was 27.0±8.8 months (95% CI, 9.8-44.2 months) with an OR of 5.6 (P=0.007), while the OR of positive expression of CK20 was 3.9 (P=0.044) with median survival of 51.0±12.4 months (95% CI, 26.7-75.3 months) (Figure 2B, 2C). The median RFS of cases with lymph node involvement was 10.0±3.3 months (95% CI, 3.6-16.4 months) with an OR of 4.6 (P=0.008), while it was 25.0±6.9 months (95% CI, 11.6-38.4 months) of cases with R1 resection with an OR of 4.2 (P=0.009) (Figure 2E, 2F).

Discussion

Accumulating evidence showed that IPNB tumors demonstrated a stepwise progression to malignancy associated with mutation in various key genes, suggesting that with time most IPNB will reach an invasive phenotype (Adsay et al., 2004;
Nakanishi et al., 2008; Kloek et al., 2011; Fujikura et al., 2018). A recent systematic review presented that 63% of IPNB demonstrated a malignant component, either invasive or carcinoma in situ (Gordon-Weeks et al., 2016). They also found that pancreaticobiliary type tumors exhibited higher possibility to be with invasive disease compared with other tumor subtypes, and higher expression of MUC1. Besides, gastric subtype was reported to be less common and invasive compared to other subtypes. This may partly explain why no gastric subtype was found in the present cohort of 38 malignant cases. However, it is still not clear whether the expression of immunohistochemical markers and histological subtype classification could predict the prognosis of m-IPNB.

Mucins play an important role in epithelial renewal and differentiation, epithelial integrity, carcinogenesis, and metastasis (Hollingsworth & Swanson, 2004). MUC1 is a membrane-bound mucin and is widely expressed in epithelial cells of human organs and primarily expressed in apical membranes of epithelial cells under normal circumstances. Previous research reported that MUC1 was expressed in IPNB, and was associated with pancreaticobiliary subtype. Therefore, MUC1 is considered a marker of pancreaticobiliary differentiation in IPNB (Zen et al., 2006; Yonezawa et al., 2008; Naito et al., 2012; Rocha et al., 2012; Sclabas et al., 2012; Sasaki et al., 2013). In our present study, 12 of 14 (85.7%) cases positively expressed MUC1 in pancreaticobiliary subtype, while the MUC1 positive cells were more than 10% in 16 of 21 (76.2%) cases of intestinal subtype, with no significant difference between the two subtypes (P=0.676). The strikingly high proportion of cases expressing MUC1 in
this series compared to previous reports which included non-invasive IPNB probably supports the association between MUC1 expression and invasive behavior in IPNB. However, neither pancreaticobiliary subtype nor positive MUC1 expression was recognized as prognostic factor in the present cohort of m-IPNB. This finding suggested that histological subtype and MUC1 expression may be of less benefit for determining prognosis, although they may participate in tumor progression along the adenoma-carcinoma sequence.

CK7 is a biliary marker that is expressed in tumor lesions, including IPNB and non-papillary cholangiocarcinoma, and normal epithelial cells of the bile duct (Shimonishi et al., 2000). As expected, 31 of 38 cases of m-IPNB had more than 10% of tumor cells expressing CK7 in the present cohort. CK20 is primarily confined to the epithelium of the stomach, small intestine and colon (Nakanuma et al., 2010). However, Shimonishi et al. found that intraductal papillary neoplasms expressed the gastrointestinal marker CK20 more frequently than non-neoplastic bile duct epithelia and other types of cholangiocarcinomas in the liver. Moreover, the expression of CK20 turned out to be positively correlated with tumor progression (Shimonishi et al., 2002). Consistently, patients with positive expression of CK20 were more likely to receive R1 resection, and it was identified as an independent prognostic factor for m-IPNB with an OR of 3.9 in the present study.

CDX2 is recognized as an intestinal epithelia-specific transcription factor which is expressed in the small intestine and colon (Silberg et al., 2000). MUC2 is expressed and regulated by the CDX2 gene in the goblet cells of the small intestinal and colonic
epithelia. Meanwhile, CDX2 is not expressed in the normal biliary tract, and its ectopic expression may be related to intestinal metaplasia (Moskaluk et al., 2003). Previously, Ishikawa et al. studied intrahepatic intraductal papillary neoplasms on the basis of hepatolithiasis, and found that aberrant expression of CDX2 was strongly linked to MUC2 and intestinal metaplasia (Ishikawa et al., 2004). Hong et al. found that CDX2 and MUC2 were present in all intestinal-type extrahepatic bile duct adenocarcinomas and 71% and 76% of papillary carcinomas, respectively, which suggested the presence of intestinal differentiation in papillary subtypes of extrahepatic bile duct carcinoma (Hong et al., 2005). Zen et al. investigated biliary papillary tumors and indicated that they commonly expressed MUC2, CDX2 and CK20 (Zen et al., 2006). In summary, intestinal metaplasia and CDX2, MUC2 and CK20 expression may play an important role in tumorigenesis of IPNB.

The pancreatic duct and common bile duct join to form Vater’s ampulla, which connects with the duodenum through duodenal papillae. Intestinal or pancreatic juice flows back into the biliary tract under certain circumstances and causes chemical damage, which induces metaplasia of the biliary epithelium (Ohike et al., 2010). Consistent with the literature (Shimonishi et al., 2002; Ishikawa et al., 2004; Hong et al., 2005; Yeh et al., 2005; Zen et al., 2006; Zen et al., 2006), the expression of CDX2 was correlated with intestinal subtype, and MUC2 and CK20 expression in the present cohort of m-IPNB, suggesting that intestinal metaplasia participated in tumorigenesis of IPNB. Furthermore, m-IPNB with positive CK20 expression showed worse OS than those negative, suggesting that molecules involved in process of CK20
expression or regulation may also take part in tumor progression.

Lymphatic metastasis is less common in IPNB, even those with malignant component (Wan *et al*., 2013; Wan *et al*., 2017). Despite the high rate of malignancy in IPNB tumors, only 9% of cases were with lymphatic spread according to the present literature (Gordon-Weeks *et al*., 2016). However, several previous series of IPNB patients had consistently identified lymph node involvement as prognostic factor for adverse outcome (Yeh *et al*., 2006; Kloek *et al*., 2011; Jung *et al*., 2012). Consistently, in our series of 38 cases of m-IPNB, only 5 cases showed lymph node involvement, and all of them died at the end of follow-up with median survival of 27.0±8.8 months, which was significantly worse than those without lymph node involvement. The clinicopathological variables were similar between the two groups, and we failed to recognize any correlation between lymph node involvement and expression of any immunohistochemical markers. These findings suggested that lymph node involvement is a relatively late event in IPNB progression, while its molecular mechanism is still unknown.

Previous studies reported that resection margin status, lymph node involvement, and tumor multifocality were associated with RFS of IPNB (Jung *et al*., 2012; Rocha *et al*., 2012; Kang *et al*., 2013). Consistently, multivariate Cox models analysis showed that R1 resection and lymph node involvement predicted shorter RFS of m-IPNB in the present cohort. However, we failed to recognized the relation between tumor multifocality with patients’ outcome. This may be partly due to the relatively small sample of this study. More studies with large samples are needed to address this
point and investigate the detailed mechanisms of the progression of IPNB.

Conclusions

In conclusion, IPNB is a class of tumor with high malignant potential. Lymph node involvement and positive expression of CK20 are independent factors to predict shorter OS of IPNB with malignant component. Patients with lymph node involvement and positive resection margin are at higher risk of tumor recurrence.

Declarations

Ethics approval and consent to participate: All participants provided written informed consent, and the Peking Union Medical College Hospital Ethics Committee approved all study procedures.

Consent for publication: Not applicable.

Availability of data and material: Not applicable.

Funding: This work was supported by the International Science and Technology Cooperation Projects under Grant [2016YFE0107100]; the Capital Special Research Project for Health Development under Grant [2014-2-4012]; the Beijing Natural Science Foundation under Grant [L172055 and 7192158]; the National Ten-thousand Talent Program, the Fundamental Research Funds for the Central Universities under
Grant [3332018032]; and the CAMS Innovation Fund for Medical Science (CIFMS) under Grant [2017-I2M-4-003 and 2018-I2M-3-001].

Disclosure of interest: The authors report no conflict of interest.

Acknowledgements: Not applicable.

References


and intrahepatic location of intrahepatic cholangiocarcinoma and primary sites of metastatic adenocarcinoma of liver. Histopathology 37, 55-63.


Table I. Clinical and pathological characteristics of malignant IPNB.

<table>
<thead>
<tr>
<th>Variables</th>
<th>CK20 negative (n=18)</th>
<th>CK20 positive (n=20)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Mean±SD)</td>
<td>57.8 ±12.1</td>
<td>60.0 ±15.1</td>
<td>0.804</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td>0.054</td>
</tr>
<tr>
<td>Male</td>
<td>11</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>7</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Presenting symptoms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jaundice</td>
<td>10</td>
<td>10</td>
<td>0.732</td>
</tr>
<tr>
<td>Abdominal pain, fever</td>
<td>10</td>
<td>7</td>
<td>0.203</td>
</tr>
<tr>
<td>Concomitant biliary stones</td>
<td>6</td>
<td>5</td>
<td>0.572</td>
</tr>
<tr>
<td>Liver function test</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALT</td>
<td>10</td>
<td>14</td>
<td>0.357</td>
</tr>
<tr>
<td>TBil</td>
<td>9</td>
<td>11</td>
<td>0.758</td>
</tr>
<tr>
<td>GGT</td>
<td>13</td>
<td>14</td>
<td>0.920</td>
</tr>
<tr>
<td>ALP</td>
<td>10</td>
<td>13</td>
<td>0.420</td>
</tr>
<tr>
<td>Tumor markers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CEA</td>
<td>1</td>
<td>5</td>
<td>0.116</td>
</tr>
<tr>
<td>CA19-9</td>
<td>11</td>
<td>10</td>
<td>0.603</td>
</tr>
<tr>
<td>Tumor location</td>
<td></td>
<td></td>
<td>0.189</td>
</tr>
<tr>
<td>Intrahepatic</td>
<td>6</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Hilar</td>
<td>3</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Extrahepatic</td>
<td>9</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Visible mucus</td>
<td>4</td>
<td>3</td>
<td>0.566</td>
</tr>
<tr>
<td>Multifocality</td>
<td>3</td>
<td>3</td>
<td>0.888</td>
</tr>
<tr>
<td>Lymph node involvement</td>
<td>2</td>
<td>3</td>
<td>0.723</td>
</tr>
<tr>
<td>Resection margin status</td>
<td></td>
<td></td>
<td>0.031</td>
</tr>
<tr>
<td>R0</td>
<td>15</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>R1</td>
<td>3</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Periductal invasion degree</td>
<td></td>
<td></td>
<td>0.235</td>
</tr>
<tr>
<td>0mm</td>
<td>7</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>1-5mm</td>
<td>9</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>&gt;5mm</td>
<td>2</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: SD, Standard deviation; ALT, alanine aminotransferase; TBil, total bilirubin; GGT, gamma-glutamyl transpeptidase; ALP, alkaline phosphatase; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9.
Table II. Histological subtype classification and immunohistochemical expression pattern of malignant IPNB.

<table>
<thead>
<tr>
<th>Histological subtypes</th>
<th>MUC1</th>
<th>MUC2</th>
<th>MUC5AC</th>
<th>MUC6</th>
<th>CK7</th>
<th>CK20</th>
<th>CDX2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreaticobiliary (n=14)</td>
<td>12 (85.7%)</td>
<td>2 (14.3%)</td>
<td>10 (71.4%)</td>
<td>5 (35.7%)</td>
<td>10 (71.4%)</td>
<td>6 (42.9%)</td>
<td>7 (50.0%)</td>
</tr>
<tr>
<td>Intestinal (n=21)</td>
<td>16 (76.2%)</td>
<td>7 (33.3%)</td>
<td>11 (52.4%)</td>
<td>3 (14.3%)</td>
<td>18 (85.7%)</td>
<td>12 (57.1%)</td>
<td>19 (90.5%)</td>
</tr>
<tr>
<td>Oncocytic (n=3)</td>
<td>3 (100.0%)</td>
<td>0 (0.0%)</td>
<td>2 (66.7%)</td>
<td>1 (33.3%)</td>
<td>3 (100.0%)</td>
<td>2 (66.7%)</td>
<td>3 (100.0%)</td>
</tr>
<tr>
<td>Total (38)</td>
<td>31 (81.6%)</td>
<td>9 (23.7%)</td>
<td>23 (60.5%)</td>
<td>9 (23.7%)</td>
<td>31 (81.6%)</td>
<td>20 (52.6%)</td>
<td>29 (76.3%)</td>
</tr>
</tbody>
</table>

Figure 1. **Typical identified histological subtype component and expression of immunohistochemical markers.** A, pancreaticobiliary; B, intestinal; C, oncocytic; D, gastric. H&E staining 100×. MUC1 in the apical membrane (E) and cytoplasm of tumor cells (F); MUC2 (G), MUC5AC (H), MUC6 (I), CK7 (J), and CK20 (K) in the cytoplasm; CDX2 in the nucleus (L). Immunohistochemical staining 200×.

Figure 2. **The overall and recurrence-free survival of m-IPNB and survival curves of independent prognostic factors.** A, The median overall survival of m-IPNB was 76.0 months, with 1-, 3-, 5-, and 10-year survival rates of 97.2%, 73.5%, 59.8%, and 38.0%, respectively. Lymph node involvement (B) and positive expression of CK20 (C) were independent factors to predict adverse outcome of m-IPNB. The median RFS was 48.0 months with 1-, 3-, and 5-year RFS rate was 83.2%, 59.8%, and 44.6%, respectively (D). Lymph node involvement (E) and positive resection margin (F) suggested a higher risk of tumor recurrence.