Origin and pathological characteristics of Klatskin tumor: a case report and literature review

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Hilar cholangiocarcinomas involving the bifurcation of the hepatic duct are called Klatskin tumors. A resected specimen of the hilar hepatic region with Klatskin tumor was analyzed. The lining epithelium of major biliary ducts was regular, while the majority of epithelial cells lining the excretory ducts of peribiliary glands (PBGs) exhibited malignant features. The connective tissue surrounding the PBGs was infiltrated by mucinous malignant epithelial cells, sometimes in a signet-ring cell form, with perineural invasion. The tumor epithelial cells showed distinct CK 7 and CA 19-9 positivity. The described cholangiocarcinoma was classified as the Bismuth-Corlette type IIIb and originated from the excretory ducts and acinar cells of PBGs.

Key words: cholangiocarcinoma, Klatskin tumor, biliary tract, cytokeratin 7, CA 19-9.

Introduction

Cholangiocarcinoma (CC) is adenocarcinoma of the bile ducts that may arise anywhere along the biliary tree [1]. Its annual incidence is constantly increasing. In the Western countries, it is 1-2 cases per 100 000, and in the northeastern Asia, 96 per 100 000 [2]. It comprises 10-15% of hepatobiliary malignancies [3, 4], approximately 13% of primary liver cancers and accounts for 1.3% to 2.6% of annual cancer-related deaths worldwide [2]. The CC can be intrahepatic, hilar or extrahepatic. Most common ones are hilar CCs, involving the bifurcation of the common hepatic duct, where they are referred to as Klatskin tumors [5]. The difficulties with the classification of Klatskin tumor pose a problem of an adequate assessment of the incidence [3] although it is known that Klatskin tumors account for 67% of all CCs [1].

Klatskin tumors usually show signs of biliary obstruction with jaundice and pale stools [6]. A combination of serum tumor markers, carbohydrate antigen (CA 19-9) and carcinoembryonic antigen (CEA), was recommended as one of the elements in the diagnosis of CC [6]. Most commonly used modalities of the preoperative imaging evaluation are X-rays, ultrasound, computed tomography (CT), magnetic resonance cholangiopancreatography (MRCP) and endoscopic retrograde cholangiopancreatography (ERCP) [2, 5, 6].

The surgical treatment of hilar CC has been shifted from local excision to more extensive resections combined with a major liver resection [7] in order to achieve a R0 resection, i.e. the complete excision with negative margins [6]. There are several staging systems for hilar CC. The most commonly used is the American Joint Committee on Cancer (AJCC) TNM staging system based on the pathological criteria, and the Bismuth-Corlette classification based on the biliary ducts' involvement by the tumor [5, 8, 9]. The Bismuth-Corlette classification distinguishes the following types: type I: tumors below the confluence of the left and right hepatic duct; type II: tumors reaching the confluence; type IIIa and IIIb: tumors involving the common hepatic duct and spreading to either the right or the left hepatic duct, respectively; type IV: tumors involving the confluence and both the right and left hepatic ducts [5, 9].

Hilar cholangiocarcinomas are often accompanied by both lymphatic (30-50%) and direct invasion into the periductal tissues along with perineural invasion [9]. These histological changes appear to cause symptoms of narrowing and obstruction of the bile duct [1, 2, 5].

The biliary tree may be regarded as an excretory duct of two secretory units: hepatocytes and peribiliary glands (PBGs) distributed in the wall of extrahepatic and large intrahepatic bile ducts [10-13]. The PBGs are subdivided into intramural and extramural, and the latter drain into the lumen of the bile ducts via their own conduit [12, 14].

It is generally stated that CC arises from the biliary epithelia, meaning both the lining epithelia of the bile ducts (cholangiocytes) and the cells lining PBGs and their ducts [12]. Cholangiocytes are mitotically dormant cells [15], while PBGs contain multipotent stem cells [15, 16]. The PBGs may show a spectrum of pathological changes such as inflammation, cystic dilatation, papillary hyperplasia, atypical hyperplasia and neoplasia [10, 11, 14, 17]. Terada *et al.* were the first to report an autopsy case of an invasive hilar cholangiocarcinoma (Klatskin tumor) arising from the intrahepatic peribiliary glands, with gradual transition from normal peribiliary glands, papillary and atypical hyperplasia to adenocarcinoma [18], and cases reporting the similar origin of biliary tumors followed [19].

Case report

From a female patient aged 66 years, with the clinical diagnosis of the hepatic duct tumor (Klatskin tumor) and obstructive jaundice, the following surgical material was obtained: a resected specimen of the liver hilar region, gallbladder including a part of the cystic duct, 11 lymph nodes, and a band of connective tissue containing large bile ducts, blood vessels and nerves. The surgical data confirmed the left hepatectomy with extrahepatic bile ducts resection. The operative material was entirely embedded. The histological sections were stained by the routine hematoxylin-eosin (HE) method, Alcian-blue-PAS, Giemsa, and the additional immunohistochemical methods using CK7, CK20, CA19-9 and CEA.

The clinical data were obtained from the patient's files at the Clinic for Abdominal, Endocrine and Transplantation Surgery, Clinical Center of Vojvodina.

Clinical and macroscopic findings

A 66-year-old female patient first presented to a small district hospital in April 2008, with a history of nausea, vomiting, abdominal pain, weakness and fatigue. The MR with MRCP was inconclusive. For 2 years, the patient had no symptoms, but in April 2010, laboratory tests were as follows: alkaline phosphatase (ALP) – 467 IU/l; alanine aminotransferase (ALT) – 69 IU/l; aspartate transferase (AST) – 60 IU/l; γ-glutamyl transpeptidase (GGT) – 619 IU/l; negative for hepatitis B and C virus; CA19-9 – 80.9 U/ml. The MRCP in May 2010 showed signs of cholangitis, but otherwise was the same as in 2008.

In June 2010, the patient presented with symptoms similar to those in 2008 and the signs of jaundice of the skin and sclera. The liver enzymes were elevated (AST – 57 IU/l, ALT – 85 IU/l, ALP – 440 IU/l, GGT – 396 IU/l) and CA19-9 rose up to 166.0 (normal: < 33.0). The patient was referred to abdominal surgery, where ERCP revealed a stenosis of the left hepatic duct at its beginning, above the confluence (approximately 4 cm long) (Fig. 1 A). During ERCP, a plastic biliary stent (7 Fr × 12 cm) was placed. The findings pointed to Klatskin tumor, and at the end of July 2010, the patient underwent left hepatectomy (with the resection of the entire extrahepatic biliary tree including the gallbladder) and the dissection of the hepatoduodenal ligament (Fig. 1 B). The signs of the tumor spread were not observed. The negativity of the resection margin of the right hepatic duct was confirmed during the resection. The Roux-en-Y cholangiojejunostomy was performed at the level of the segmental ducts of the right lobe. The gross surgically extracted specimen of the left liver lobe measured 12 cm × 8 cm × 4 cm. The specimen included the extrahepatic biliary ducts and the gallbladder. The tumor was visible at the surface of the extracted lobe and it was not encapsulated; on the cut surfaces, it was poorly circumscribed and composed of solid areas, grey-yellow in color (Fig. 1 C, Fig. 1 D).

The immediate postoperative course was uneventful, but the patient died on the sixteenth postoperative day due to the multiorgan failure and blood loss caused by intra-abdominal bleeding (5^{th} postoperative day) and bleeding into the left pleural cavity (after the attempt to place a central venous catheter on the 7^{th} postoperative day).

Pathohistological findings

Histologically, the majority of the epithelial cells lining the excretory ducts of the extramural PBGs around the left hepatic duct and its branches exhibited malignant features. The malignant epithelial cells were found within the epithelium of the PBG ducts (Fig. 2) and partly as individual malignant mucinous cells (Fig. 3).

In contrast, the carcinoma cells were not identified on the surface of biliary epithelium. The connective tissue between some PBGs was focally hyalinized and infiltrated by mucinous malignant epithelial cells, different in size, partly of a signet-ring cell form, located individually or in small groups. Among adjacent PBG, the changes ranged from completely preserved cubical acinar cells, hyperplasia, grade I and II dysplasia, to malignant cells. There were signs of the perineural invasion by the tumor, and intensive tumor tissue propagation towards the

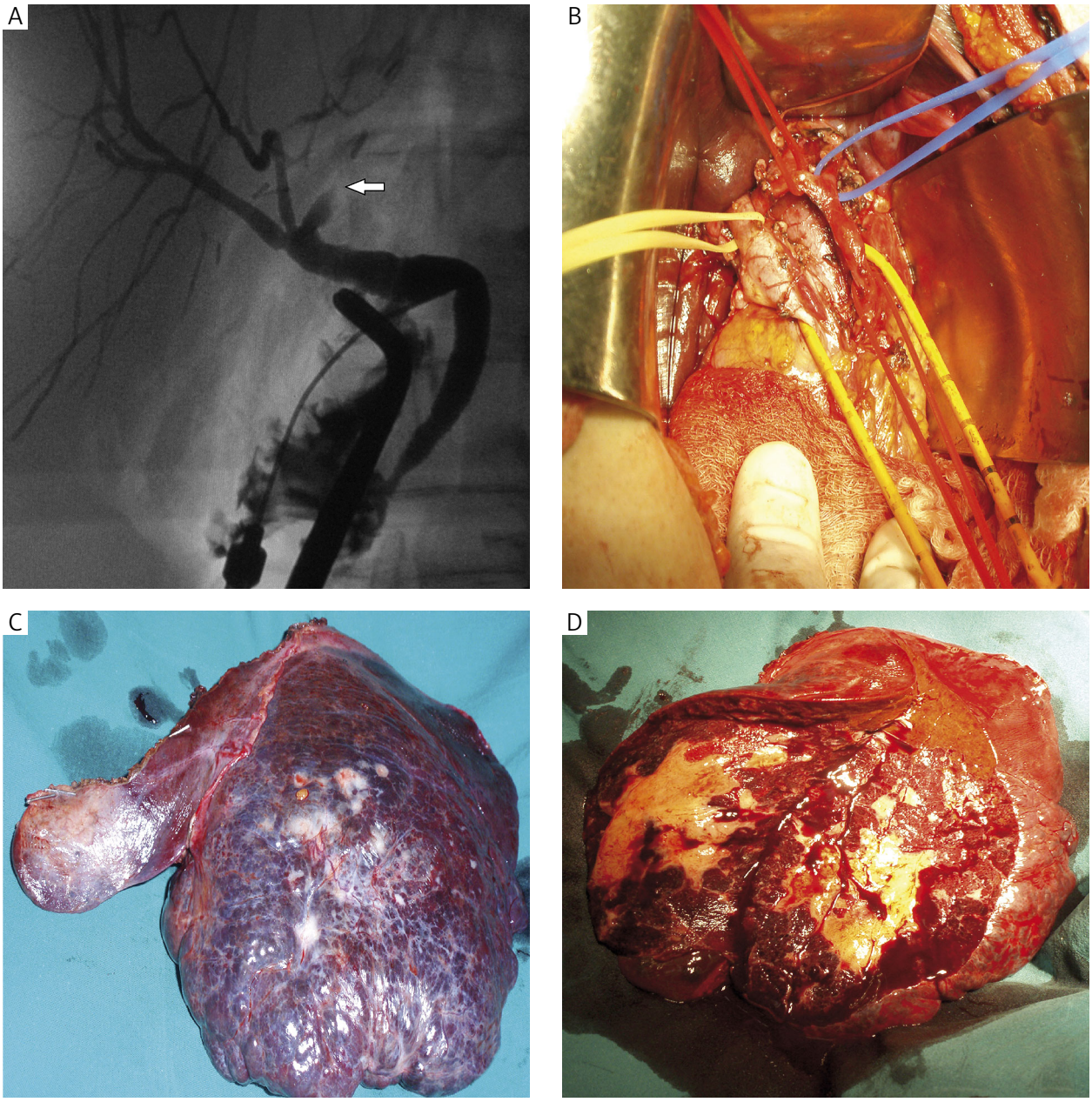

Fig. 1. Liver hilar region with the tumor. A – ERCP showing the obstruction in the left hepatic duct (arrow); B – surgical preparation of bifurcation, left and right hepatic duct; C – gross appearance of the surgically removed specimen; D – cross section of the left hepatic lobe with tumor

liver (prominent septal and portal canal infiltration) (Fig. 4), but lymph node metastases were not detected.

Cholangiocarcinoma cells showed distinct CK7 and CA 19-9 positivity, mild positivity for CEA staining and negativity to CK20. The preserved PBG acinar cells were distinctively positive to CK7, but only weakly positive to CA19-9.

Among the samples containing intrahepatic bile ducts, there was a fragment of the left hepatic duct and its branches with focal pseudostratification and slight papillary hyperplasia of the epithelium (Fig. 5). In these intrahepatic bile ducts, like in the cystic duct and extrahepatic bile ducts, PBGs had both intramural and extramural locations. The gallbladder with mucin-secreting glands situated in the neck, exhibited signs of phlegmonous-purulent inflammation. Tumor involvement of the extrahepatic bile ducts or the gallbladder was not found.

In the surrounding connective tissue, there was a small node of the ectopic pancreatic tissue made of the preserved exocrine pancreatic acini and ducts.

Discussion

The disappointing outcome in patients with Klatskin tumor arises from late and unspecific symptoms and

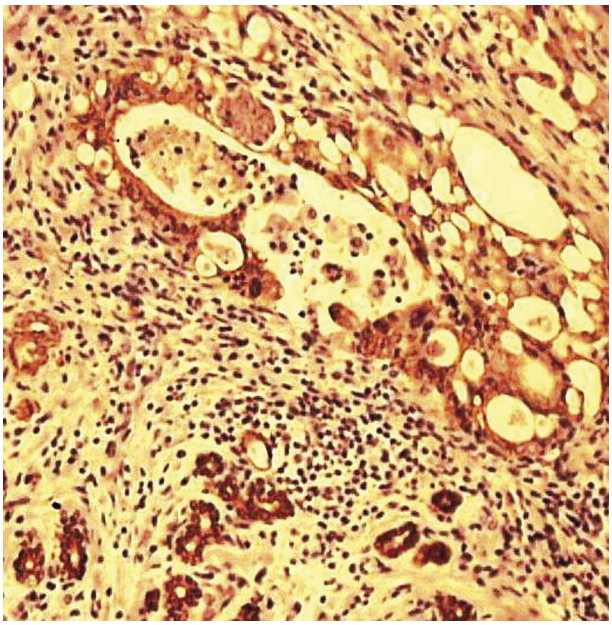

Fig. 2. Malignant epithelial cells within the epithelium of the PBG ducts. CA 19-9, magnification 200×

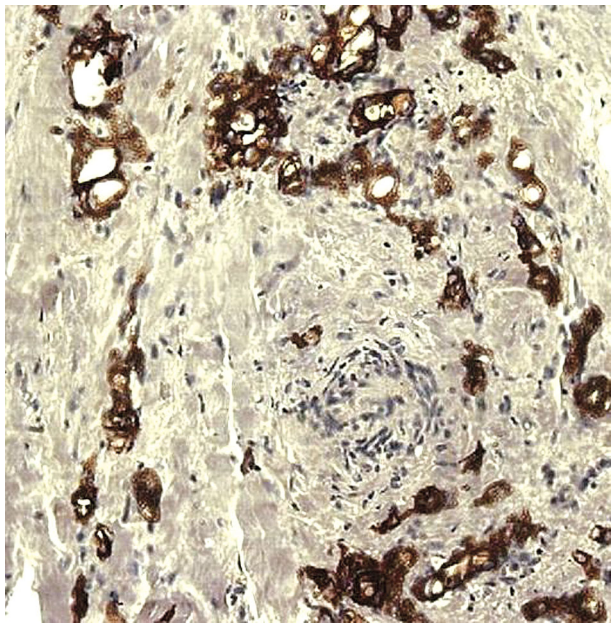

Fig. 3. Individual malignant mucinous cells. HE, magnification 200×

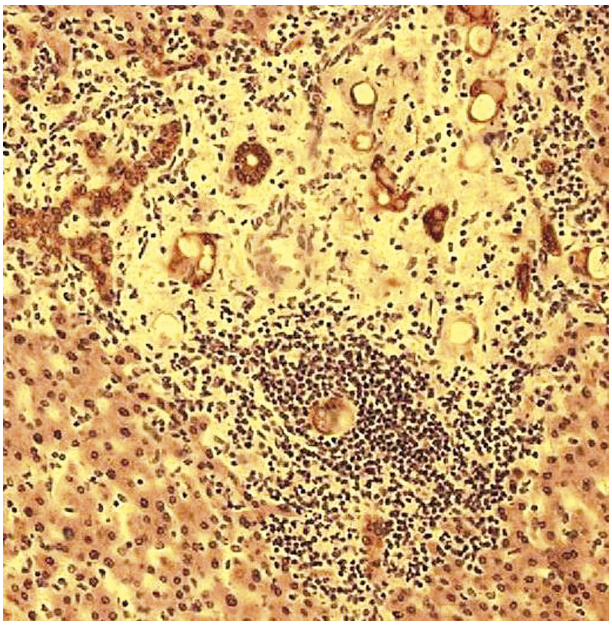

Fig. 4. Tumor tissue infiltrating liver. CK7, magnification 200×

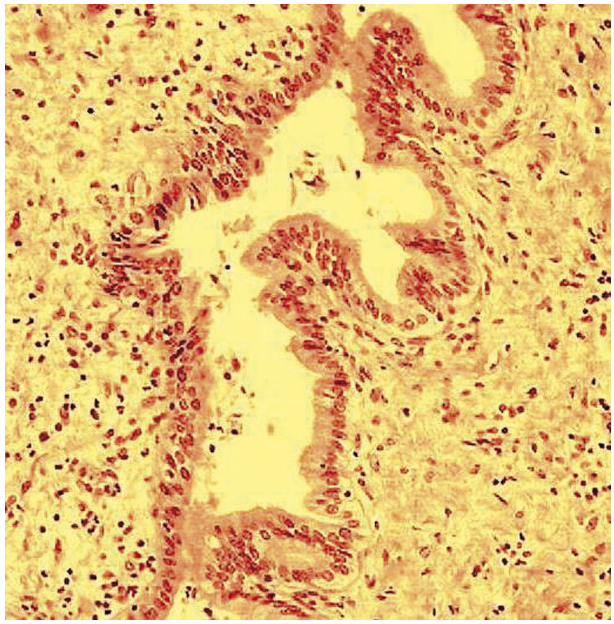

Fig. 5. Pseudostratification and papillary hyperplasia of the epithelium lining the left hepatic duct. HE, magnification 200×

advanced stage (III and IV) at the time of diagnosis [4], diagnostic imaging problems and the difficulty to completely excise the tumor because of its high propensity to spread in the liver or within the duct system [5, 6]. Clinical signs depend on the tumor location. The intrahepatic CCs more often present with late symptoms [2, 6], as in our case, when the patient had been free of symptoms for two years. The elevated serum CA19-9 and CEA in our patient, together with the high rates of liver enzymes, indicated the pathological process of hepatobiliary origin, although it was not diagnosed using MRCP on two occasions (in 2008 and 2010). In the detection of biliary malignancy, MR along with MRCP is comparable to the endoscopic retrograde cholangiopancreatography (ERCP) [2], but only ERCP clearly detected the obstruction of the left hepatic duct. The ERCP is a powerful tool in the diagnosis, with its ability to define the anatomy, determine the extent of

the bile duct involvement and tissue specimen sampling. In this case, another advantage of ERCP was applied – the stent placement in order to relieve jaundice [2].

The hilar CC has an invasive growth pattern and an extensive subepithelial spread which emphasizes the importance of confirmation of negative margins by a frozen section during the resection. The mean distance of the microscopic invasion beyond the gross margin toward the liver was found to be 16.8 mm, which underlines the importance of wider resections [1]. The patient had left hepatectomy in order to achieve the R0 excision, and negativity of resection margins of the right hepatic duct was confirmed during the operation. In our case, the tumor had a very prominent direct extension to the liver, following the interlobular and perilobular septa. Cholangiocarcinoma cells exhibited the typical immunophenotype (CK 7 positive, CA 19-9 strongly positive, CK 20 negative) like normal secretory units of the peribiliary glands, which were, to a smaller extent, CA 19-9 positive. Several centers specialized in hepato-pancreato-biliary surgeries have recently reported improved 5-year survival results (20-35%) after extensive resections [7]. However, these results have been burdened by high rates of operative morbidity and mortality due to liver failure and/or postoperative complications [7]. The latter was a reason for the lethal outcome in our patient. The survival rates for Klatskin tumor (1-year period) have been 23% [4]. Patients with Klatskin tumor had poorer quality of life (global decrease of quality of life in the first 3 months) and more frequent post-therapeutic complications [4].

The liver, extrahepatic biliary system and ventral pancreas appear in the 3rd gestation week as an outgrowth of the ventral endoderm of the foregut [15, 20]. At about 9-10 weeks, primitive hepatocytes near the liver hilum form a ductal plate composed of multipotent and bipotent hepatic stem cells, which later on differentiate into either hepatocytes (Wnt pathway) or cholangiocytes (Notch pathway) [19, 20]. The PBGs also derive from the ductal plate [20] during the 40th week of gestation with full maturation at the age of 15 [11].

The ductal plates in fetal liver and the canals of Hering in adult liver are intrahepatic stem cell niches, and PBGs are extrahepatic stem cell niches of the biliary tree. At the same time, PBGs around the large intrahepatic bile ducts may be additional intrahepatic stem cell niches [13]. These relatively quiescent stem cells in adults have a low proliferation rate and the ability to migrate and differentiate, contributing to the renewal/repair of the biliary epithelium, liver or pancreas, as well as in the neoplastic processes of the biliary tree [13, 14]. Cardinale *et al.* reported a maturational lineage process from PBGs deep within the bile ducts and ending at the duct lumen [13].

Certain pathological conditions that cause either acute or chronic biliary epithelial injury appear to predispose to CC [1]. Ito *et al.* and Nakanuma *et al.* stated that PBGs, particularly those intramural, are induced by pathological conditions of the biliary tree [5, 11], which confirms their role in the renewal of the biliary epithelium [14]. The injury of the biliary epithelium, often coupled with the inflammation and obstruction of the bile flow, increases the cellular turnover [1] which, together with inhibition of the apoptosis in the biliary epithelia and in PBGs, causes hyperplasia, disturbs the balance of the stem cell proliferation and death, and increases the risk of neoplastic transformation [14]. One can rarely see so obviously as in our case, the neoplastic process limited to PBGs and their ducts, with only hyperplasia in the bile duct epithelium, which may suggest epithelial injury. This clearly shows that the extramural intrahepatic PBGs and their excretory ducts are the area of the origin of Klatskin tumor in this specific case, although the lining biliary epithelium often gives rise to CC. The fact that the tumor had emerged from PBGs indicates that the neoplastic change could have happened at the level of stem cells and/or epithelial cells within PBGs. Our results are in accord with the statements and interpretation of Nakanuma *et al.* [11, 14], and Ito *et al.* [5].

As mentioned above, the biliary system and ventral pancreas share a common origin and mutual stem cells during the fetal life [15]. We found a group of acini of the exocrine pancreas and their excretory ducts in the surrounding connective tissue close to the hilar extrahepatic bile ducts (ectopic or heterotopic pancreas) that could occur in one of the following ways: the differentiation out of the hepatic stem cells in PBGs of adulthood, or as ectopic or heterotopic pancreas during the fetal development [13].

Based on all the obtained data, it is concluded that the tumor tissue of the common hepatic duct confluence and the left hepatic duct in our case originates from the surface epithelium of the excretory ducts of PBGs and their acinar cells, primarily of the extramural intrahepatic PBGs. It has pathohistological characteristics of the mucinous adenocarcinoma, partly with a signet-ring cells form, and corresponds to hilar cholangiocarcinoma – Klatskin tumor (Bismuth-Corlette type IIIb).

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