Dysplasia and Early Carcinoma of the Gallbladder and Bile Ducts

Terminology, Classification, and Significance

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KEYWORDS

• Gallbladder • Bile duct • Dysplasia • Intracholecystic • Intraductal • ICPN • ICTN • IPN

KEY POINTS

- Tumoral intraepithelial neoplasms (intracholecystic neoplasms of gallbladder and intraductal neoplasms of the bile ducts) present as clinically detectable (papillary/polypoid) masses and account for 5% to 10% of the invasive cancers in this region.
- Flat (non-tumoral) type dysplasia are clinically unapparent incidental lesions; high-grade examples are commonly associated with invasive carcinoma, whereas low-grade ones seem to be clinically insignificant.
- Inflammation/injury–precancer–cancer sequence is well established in the biliary tract (with gallstones, parasites, primary sclerosing cholangitis, and hyalinizing cholecystitis as known risk factors). However, anatomic/chemical carcinogenesis model is also being increasingly appreciated (manifested in choledochal cysts, pancreatobiliary maljunction, and low-union of common hepatic duct with the cystic duct).
- Early (ie, in-situ and minimally invasive pTis/T1) gallbladder cancers have a very good prognosis with the 10-year survival above 90%, provided that a pT2 carcinoma has been ruled out with complete sampling. However, some cases develop biliary cancers many years after the diagnosis, attributable to the field-effect phenomenon.
- Field-effect phenomenon appears to be a significant concern for multifocal carcinogenesis in the biliary tract especially in patients with risk conditions.

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INTRODUCTION

Incidence and Significance

Premalignant and cancerous lesions of gallbladder (GB) and bile ducts are relatively rare but present a major clinical challenge as they are highly prone to be missed or misdiagnosed because they are commonly mimicked and hidden by the inflammatory/injurious conditions such as stones, parasites, and sclerosing cholangitis, which, paradoxically, are also their main instigators.

The majority of the pre-malignant lesions encountered in these organs is of the "flat" type; that is, do not form clinically, radiologically, and even grossly detectable masses.[1–4] Since these do not form tumoral lesions by themselves, they are typically discovered incidentally next to established cancers, or in procedures performed for other conditions such as gallstones, cholecystitis, or choledochal cysts. Because cholecystectomy is 1 of the most frequently performed operations, and since premalignant processes are incidentally found in about 1% to 5% of the GBs,[5] they are in fact encountered with respectable regularity in daily practice. In contrast, bile ducts are seldom removed unless there is compelling concern for cancer, and therefore these lesions are far less commonly detected in the bile ducts. Pathologic diagnosis of these non-tumoral lesions is highly challenging, especially because mucosal injury in this region is notorious for generating remarkable atypical changes that are very difficult (and at times impossible) to distinguish from true dysplastic/neoplastic alterations.[1,6–10] Moreover, as cancerous transformation often develops in regenerative processes in these sites, it becomes very difficult to determine, where simple regeneration ends and true carcinomatous changes begin. This also leads to variable impressions about the true frequency of dysplastic lesions, especially in the lesser end of the spectrum.[11,12]

The other category of pre-malignant lesions is the tumoral type, that is, mass-forming preinvasive intra-epithelial/intra-mucosal neoplasia ("adenoma-carcinoma sequence"). They can be viewed as counterparts of pancreatic intraductal papillary mucinal neoplasms (IPMNs). These are less common, and manifest as radiologically, clinically, and grossly recognizable lesions, even when they are not invasive. They reveal various cell types, architectures with different biologic connotations, and spectrum of cancerous transformation. It is important to recognize this group because they are often curable if removed completely. They also offer a fascinating model of cancerous transformation for cancer researchers to analyze, with potential implications in carcinogenesis of other organs as well.

CLINICAL FEATURES

Clinical Presentation

As is the case for invasive cancers of most mucosal/epithelial organs, preinvasive lesions of these sites are also seen predominantly in elderly patients. However, at the same time, in most studies, the patients are almost a decade younger than the patients with invasive cancers, supporting the progression phenomenon.[12,13] Not surprisingly, in patients with risk factors such as choledochal cyst,[14] primary sclerosing cholangitis, and pancreatobiliary maljunction,[15–17] both the cancers as well as precancerous lesions occur in significantly younger patients.[11,12] In the populations with gallstones as the main risk factor such as parts of South America and India, GB carcinoma (GBC) shows striking predilection for women. However, this does not seem to hold as true for Far East,[18] for reasons that are not clearly understood.

"Flat" (non-tumoral) forms of dysplasia are by definition microscopic forms of dysplasia and therefore they do not by themselves cause any signs or symptoms if

unaccompanied by invasive cancer.[1,10,13] As such, they are detected incidentally in specimens removed for other causes.[13] In contrast, tumoral forms of dysplasia, that is, "intracholecystic neoplasms" (in the GB)[19–21] or "intraductal neoplasms" (in the biliary tract) form clinically/radiologically visible masses (**Fig. 1**). Naturally there are overlaps between the non-tumoral and tumoral forms of dysplasia, and for their distinction a rule of thumb arbitrary criterion of 1 cm size is used.[19] As such, the latter often present with obstruction-related signs and symptoms.[20,22–26] Typically, they appear as filling defects in the lumen of the respective site and at times, they can be mistaken as stones. They can be multifocal; the entity previously known as papillomatosis, which can extensively involve biliary system, is also included in this category.[19] In fact, multifocality and the field-effect phenomenon creates a major issue for the long term management of these patients, in particular, when there is no or minimal invasive carcinoma and long term survival is expected.[19,26–29]

Terminology

The terms "dysplasia" and "preinvasive" (which is synonymous with intraepithelial neoplasia) are probably the best and most accurate to describe these lesions. Premalignant is also a commonly employed name. The term pre-neoplastic is inaccurate since they are fundamentally neoplastic lesions; this term can perhaps be reserved for metaplastic/hyperplastic changes that precede the dysplastic ones.[16]

It is important to acknowledge that in the uppermost end of the spectrum of these lesions is in-situ carcinoma, which is composed of cells that have molecularly and genetically undergone full "malignant transformation" at the cytologic level. However, they technically do not have the ability to exhibit malignant behavior such as metastasis due to their location and confinement by the histologic boundaries such as basement membrane and are thus still included in the "pre"-malignant category. Nevertheless, these lesions are classified as "pTis" within the cancer spectrum.

The spectrum of intraepithelial *neoplastic* transformation ranges from minimal alterations that can be difficult to distinguish from metaplasia/hyperplasia to all the way to

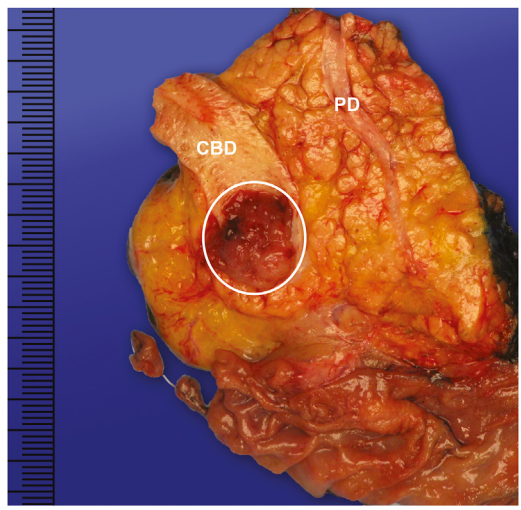

Fig. 1. Biliary IPN. Extra hepatic intraductal papillary neoplasm of the bile ducts, with friable papillary projections (circled), arising from the distal common bile duct (CBD). Main pancreatic duct (PD) is unremarkable.

those that can be qualified as intramucosal or "papillary" adenocarcinoma. Cases previously regarded as "papillomatosis" are also regarded in this spectrum in the tumoral intraepithelial neoplasm category (see later).[7,19,30]

In the World Health Organization (WHO) classification, these lesions are discussed under the heading of biliary intraepithelial neoplasm (BilIN)[31] although the term "dysplasia" is still the one used more widely, especially in the GB.[1] It is important to note here that the term "carcinoma in-situ (CIS)" is mostly abandoned in the WHO classification for the gastrointestinal (GI) tract and replaced by "high-grade dysplasia (HGD)" due to the fear of unwarranted over-interpretation and over-treatment caused by the term "carcinoma".[32] However, in many parts of the world (in particular Far East and South America) the terms CIS and intramucosal adenocarcinoma are widely and liberally used for the uppermost end of the spectrum. This causes controversies in diagnosis and management of these lesions and challenges in analyzing the literature. The authors here also agree that the most aggressive end of the spectrum indeed represents an intramucosal cancerous transformation, and thus should be recognized as such. As a result, bridging the conceptual gap between the East and the West, we use the term "HGD/CIS" together or parenthetically with a commentary for such cases. In fact, for the complex adenocarcinomatous changes confined to the mucosa, experts from Chile where GB cancer incidence is 1 of the highest in the world, the term early GBC (EGBC) is employed for both pTis and pT1 lesions.[27] See later management issues section for further discussion on this issue.

Traditionally, in the guidelines and main texts,[33,34] the proliferations included in this spectrum have been graded, based on the degree of microscopic cytoarchitectural atypia, into 3 tiers as low-grade, intermediate-grade, high-grade, which was later named as BilIN 1, 2, and 3.[35] However, in real life practice a 2-tiered approach have been more widely employed.[1,23] More recently, extrapolating from the modifications in the pancreas as well as in other organs, the 2-tiered approach as low-grade and high-grade has become more official, with the refined criteria that "low-grade" group encompasses the wide spectrum ranging from changes that are metaplasia/hyperplasia-like to convincing low/intermediate-grade dysplasia (ie, corresponding to BilINs 1 and 2), and the "high-grade" terminology is reserved essentially for only frank CIS type lesions.[23] Defined as such, low-grade cases detected incidentally in a resection specimen appear to be clinically insignificant whereas those HGD/CIS cases warrant careful attention because they are often in accompaniment of invasive cancer, or have a high risk of progressing into frank cancer if not treated.

As happened in the pancreas, the mass-forming preinvasive neoplasms (adenoma-carcinoma sequence) are now collected under the conceptual category of tumoral intraepithelial neoplasm and designated as "intracholecystic neoplasms" in the GB, and as "intraductal neoplasms" in the bile ducts. Included in this broad group are a spectrum of lesions including innocuous-appearing polypoid nodules that used to be called "pyloric gland adenomas" to all the way to "papillary adenocarcinoma" or "papillomatosis". Unfortunately, the term papillary adenocarcinoma is still used in some publications as a subset of cholangiocarcinoma, leading to confusion in classification and prognosis. In this broad conceptual group of tumoral intraepithelial neoplasms, distinct entities with different clinicopathologic, behavioral characteristics are being recognized. These include intracholecystic tubular non-mucinous neoplasms and adenomyoma-associated intracholecystic neoplasms in the GB and intraductal papillary neoplasms, intraductal oncocytic papillary neoplasms, and intraductal tubulopapillary neoplasms in the bile ducts.

Risk Factors

Etio-pathogenetically, there are 2 distinct pathways of carcinogenesis in the biliary tract. One is the inflammation-injury associated, which is the predominant one.[36] The other is the anatomic/chemical carcinogenesis pathway.

Inflammation-injury pathway

There are several important conditions that signify risk to develop GB/extrahepatic bile duct (EHBD) cancers through causing inflammation-injury. The common denominator to all these is that more or less they cause local injury that initiates the neoplastic transformation, which starts with regeneration/metaplasia/hyperplasia, and proceeds with dysplasia of various grades, and finally to frank carcinoma.[1,10,35] Some of these (such as gallstone-associated) seem to be more mechanically driven and the transformation takes place in the immediate area of the instigation.

Gallstones are found in association with a significant proportion of the dysplasia of the GB. In high-incidence regions like Chile, about 3% of cholecystectomies removed for gallstones reveal HGD/CIS and significantly higher percentage with precursor metaplastic changes and low-grade dysplasia.[5] While this figure is lower in the Western population, it is still about 1%, which makes up a respectable proportion of millions of cholecystectomies performed every year.[5,37] Of note, about 15% of cholecystectomies reveal epithelial atypia that falls in the differential diagnosis of dysplasia.[5–9,37] These present a major challenge for pathologists.

Parasites, established as risk factor for cancer, are also risk for dysplastic lesions.[38–40] There are various parasites implicated in the process, *Clonorchis sinensis* being the most famous.[41,42] Another one that is worth special mention is *Opistorchis viverrine*, which has been shown to cause intraductal neoplasms in some parts of parts of Thailand.[40] The exact risk of cancerous changes in patients with biliary flukes; however, is difficult to determine.

Primary sclerosing cholangitis is a well-established risk factor for preinvasive and invasive lesions of the biliary tract with substantial field-effect phenomenon.[43,44] In fact, in resections from these patients, dysplastic lesions and sub-clinical early cancers are not uncommonly discovered, even away from the strictures. This is also true for GBs removed with explants. These patients tend to be relatively younger.[45]

Hyalinizing Cholecystitis, a distinctive variant of chronic cholecystitis characterized by diffuse effacement of the GB wall by a thin band of paucicellular, fibrous tissue with a peculiar clefting pattern and minimal or no calcifications (ie, incomplete porcelain GB, **Fig. 2**) has a strong association with carcinoma.[36] Carcinomas that arise in this setting often have a subtle appearance. Extensive sampling is crucial to reveal the presence and extent of carcinoma.[36]

Anatomic/chemical carcinogenesis pathway

Choledochal cysts have now been well established to have a risk for carcinomatous transformation.[14] In fact, more than 15% of resected choledochal cysts are found to harbor HGD/CIS and half of these also have associated invasive carcinoma.[14] A subset of choledochal cysts appear to be closely related to pancreatobiliary maljunction discussed later, and in fact, may be a result of the latter condition, to an extent that Japanese classifications recognize this group as “dilated pancreatobiliary maljunction.[46,47]

Pancreatobiliary maljunction (also known as anomalous union of pancreatobiliary ducts), is in essence supra-Oddi conjunction of Wirsung and common bile duct that is typically associated with “long common channel” in the ampulla.[15–17] This anomaly allows the reflux of pancreatic enzymes into the biliary tract as confirmed by chemical

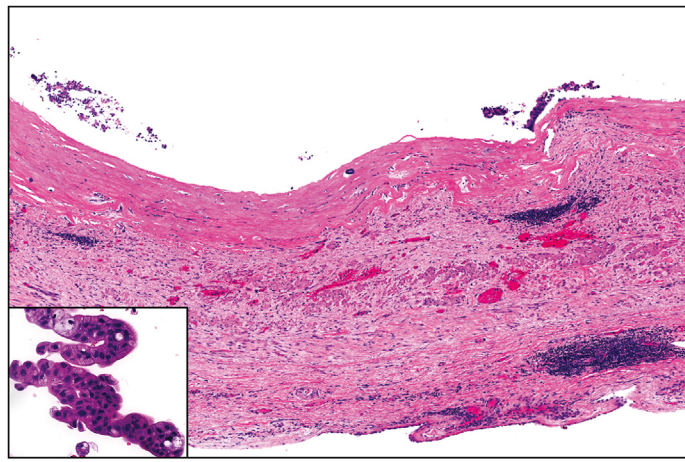

Fig. 2. Hyalinizing cholecystitis is characterized with at least partial hyaline sclerosis of the gallbladder wall. The surface epithelium is extensively, if not completely, denuded. Minimal and often mucosa-associated calcification may be present. Since hyalinizing cholecystitis is typically devoid of epithelium, any epithelial elements on the surface or within the wall should be regarded as a suspect for dysplasia or carcinoma. Hyalinizing cholecystitis with high-grade dysplasia (inset) is depicted here.

analysis of GB bile in these patients. This reflux is believed to be the cause of the very high incidence of GB and bile duct cancers seen in these patients, most proceeding through dysplastic lesions. Pancreatobiliary maljunction is a relatively rare condition in general population, but it accounts for about 8% of GBCs as well as a score of bile duct cancers.[15–17,48] Previously thought to be an Asian disorder, recent studies have shown that pancreatobiliary maljunction also accounts for about 8% of GBCs also in the United States.[16] Patients with pancreatobiliary maljunction exhibit substantial thickening of GB mucosa, which pathologically corresponds to a distinctive mucosal hyperplasia that has been termed reflux cholecystopathy (**Fig. 3**).[15] This

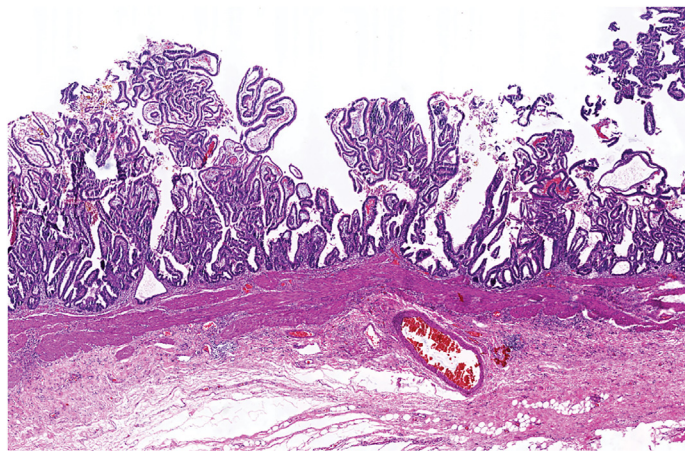

Fig. 3. Reflux cholecystopathy. The distinctive mucosal hyperplasia of the gallbladder seen in pancreatobiliary maljunction. The thick hyperplastic mucosa is continuously pushing into the tunica muscularis. Mucosal folds reveal characteristic bulbous dilatation of the tips.

hyperplasia can then undergo further dysplastic/carcinomatous transformation.[15] Guidelines published by the Japanese Study Group on pancreatobiliary maljunction, established 3 decades ago, recommend abdominal ultrasounds (mandated by the government as a part of general healthcare check-up) to include measurement of GB mucosal thickness. If thickened mucosa is discovered, then further studies are performed to investigate for pancreatobiliary maljunction. If pancreatobiliary maljunction is discovered, patients are taken to cholecystectomy and surveillance of biliary system. Numerous cancer patients have been discovered and many more presumably prevented by this approach.[46,47] Of note, about 25% of pancreatobiliary maljunction-associated cancers develop through intracholecystic neoplasms (and intraductal neoplasms of the bile ducts) whereas these tumoral intraepithelial neoplasms account for 5% to 10% of GBCs otherwise.[15–17] Also the frequency of unusual cancer types such as adenosquamous and neuroendocrine appears to be higher in this group.[49–51] As such, pancreatobiliary maljunction offers a fascinating model of carcinogenesis. It also establishes that reflux-associated chemical induction of carcinoma does occur in this region.[15]

Low-union of common hepatic duct with the cystic duct (within or immediately adjacent to the pancreas) (**Fig. 4**) is an anatomic variation that is seen in less than 15% of the general population but was recently found to occur in more than 40% of periampullary cancers, and as high as 70% of upper EHBD cancers.[17] These figures seem to be beyond coincidence and bring the question of whether this anatomic variation (short common bile duct) leads to chemical milieu alteration in the biliary system

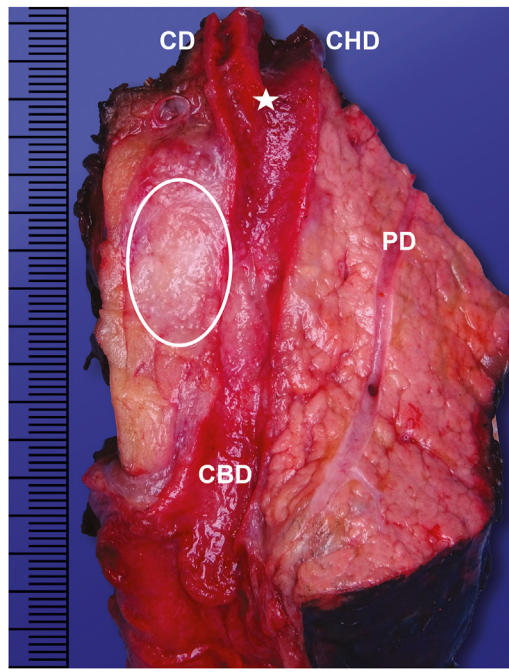

Fig. 4. Low-union. Insertion (star) of the gallbladder's cystic duct (CD) into the common hepatic duct (CHD) within or immediately above (within 5 mm of the pancreas border), known as low-union, is a rare anatomic variation that has been identified in a substantial subset of pancreatic, bile duct and ampullary cancers. A pancreatic ductal adenocarcinoma (circled) is depicted here.

and thus plays a role in carcinogenesis similar to reflux-associated gastroesophageal cancers or pancreatobiliary maljunction-associated cancers discussed earlier. In many cases diagnosed with "pancreatic cancer", the lesion is in the region of this low insertion. Pre-malignant lesions occurring in low-union patients require further scrutiny.

Field-Effect. As commonly observed in the patients with the risk factors discussed earlier, multifocality with synchronous and metachronous dysplastic lesions in different compartments of the biliary tract (including GB and any compartment of EHBD) is a substantial issue. Field-effect (field-defect), a concept well known for other mucosal organs such as the oral cavity and urothelium, seems to be as valid if not more so for the biliary tract.[52] In the literature, it is best documented for patients with primary sclerosing cholangitis,[43,45] although the phenomenon may be even more significant (though less well studied and appreciated) in pancreatobiliary maljunction cases and its associate choledochal cysts.[15–17] This field-effect phenomenon is also an important consideration when precancerous lesions are discovered incidentally. In particular, when an HGD/CIS is found in a cholecystectomy specimen, regardless of whether it is flat (non-tumoral) or tumoral type, there appears to be risk for the biliary tract, with some patients developing cancer in the bile ducts several years after the cholecystectomy.[2,3,19] If the same patient also has (or subsequently found to have) primary sclerosing cholangitis or pancreatobiliary maljunction, then this concern becomes much bigger.

Therefore, in a patient who is discovered to have a precancerous lesion in the GB or bile ducts, it is crucial to investigate the patient for these risk diseases and if found, then the patient should be placed under even-closer surveillance. Along those lines, if a patient with dysplasia or carcinoma in the biliary tract is undergoing a second operation of the region, we advocate to perform bile duct brushing of the remaining system to determine whether there are sub-clinical carcinomatous changes.

PATHOLOGY

Gallbladder

Flat (non-tumoral) dysplasia of gallbladder

These are typically detected incidentally in cholecystectomy specimens. (Pseudo)pyloric gland metaplasia occurs commonly in injured GBs and do not seem to have any recognizable association with dysplasia-carcinoma process, and does not even need to be reported.[1,35,53] Whereas, intestinal metaplasia is observed more commonly in the in background of carcinomatous changes and thus warrant more careful attention and additional examination.[53,54] A form of metaplasia-dysplasia sequence that is being increasingly recognized in the GI tract as "hypermucinous" and "foveolar", is also being characterized also in the GB.[35,37,55] This appears to occur more frequently in the high-incidence regions.[5,55] Transitioning with these metaplastic changes render low-grade dysplasia (LGD) difficult to define (**Fig. 5**). Moreover, LGD has substantial overlaps with atypical regenerative changes. As a result, the diagnosis of LGD is highly subjective and it is difficult to define widely-applicable criteria for it.[1,10,35] However, since LGD does not seem to have any clinical significance by itself, its recognition and accurate diagnosis seems to be of no clinical consequence.[1]

HGD/CIS of GB is detected in 1% to 3% of cholecystectomies depending on the population.[5,37] Importantly, HGD/CIS is seldom caught as a focal finding in otherwise normal mucosa. But rather, when it is diagnosed, it typically involves most of the preserved mucosa. This indicates when carcinomatous transformation takes place in the epithelium, it rapidly spreads to the remainder of the mucosa like a wildfire. HGD/CIS

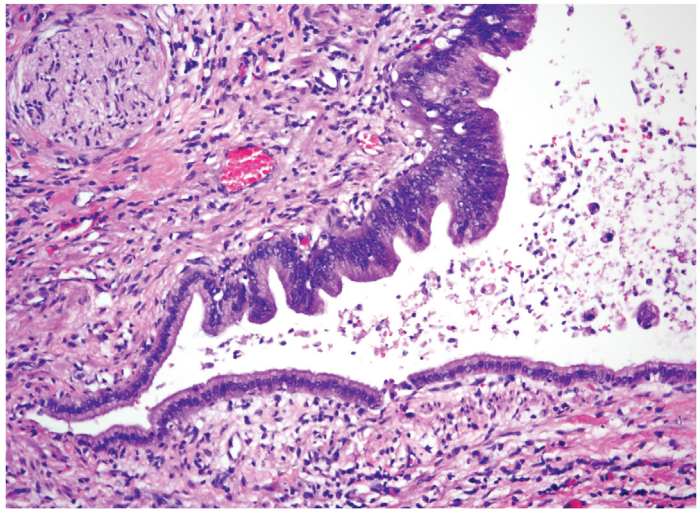

Fig. 5. Low-grade dysplasia (LGD) is difficult to distinguish from reactive atypia. However, pseudostratification of relatively uniform elongated nuclei involving the surface epithelium in the absence of any congestion, active inflammation, or stromal fibrosis is regarded as LGD by most authors.

is defined by diffuse and substantial cytologic atypia showing virtually all the attributes of cancers but by definition are still confined to the epithelium/mucosa (**Fig. 6**).[1,7,10] HGD/CIS display various architectural patterns as well as different cell types.[2–4,55] The significance of these patterns and cell types is still under investigation. Of note, foveolar/hypermucinous cell type, akin to their GI counterparts, is only beginning to be recognized as a form of dysplasia. While foveolar type dysplasia, like its GI kindreds, appears innocuous and is difficult to distinguish from metaplasia; it may be more sinister in biology than the other types.[5,37,55]

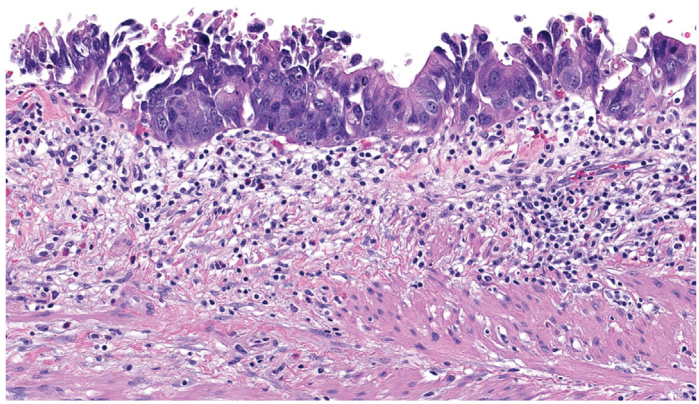

Fig. 6. High-grade dysplasia/carcinoma-in-situ is characterized by diffuse and severe architectural and/or cytologic atypia.

As discussed in the biologic behavior section, perhaps the most important problem regarding the literature on nature of HGD/CIS-only cases (and as an extension of that, of minimally invasive carcinoma cases), is that this diagnosis should not be rendered unless the entire specimen is examined to rule out more deeply invasive carcinomas.[2–4,27] Invasive carcinomas in the GB/EHBD can be extremely subtle as also evidenced by the fact that even close to half of advanced GBCs are diagnosed as "clinically/grossly unapparent".[13] Therefore, evolving guidelines emphasize the importance of total sampling of GB before the diagnosis of HGD/CIS-only is rendered.[1,19]

Tumoral intraepithelial (intracholecystic) neoplasms

These are characterized by papillary/polypoid (grossly and radiologically visible, typically >1 cm) mucosal masses that are distinct from the remaining mucosa and are fundamentally composed of dysplastic cells.[19,22] Essentially, they represent adenoma-carcinoma sequence.

Intracholecystic papillary tubular neoplasms (ICPNs, previously also called intracystic papillary neoplasm) are the prototype and the most common examples of intracholecystic neoplasms.[19] In the earlier literature regarded under 9 different names, they were later collected under 1 heading of ICPNs with the understanding that there are some subsets but there are also striking overlaps.[19,56] There is a spectrum of architectural patterns and spectrum of cell types, often in a mixture (**Fig. 7**). There is also spectrum in the degree of dysplastic transformation. In the lower-most end is the polypoid collection of normal-appearing pyloric type glands that had been dignified as pyloric gland adenoma, with the mean size in largest series being 0.6 and 0.8 cm, most of which are now regarded as polypoid metaplasia unless they form distinct visible polyps (preferably >1 cm).[19,57] The other end are those exuberant papillary tumors with HGD/CIS, which used to be called "papillary adenocarcinomas". Of note, "flat" (non-tumoral) dysplasia can have prominent papillary configuration that forms feathery change in the mucosa but are distinguished from the ICPNs by the lack of a visible tumor/polyp formation. Invasive carcinomas are detected in about 60% of resected ICPNs, and about 5% to 10% of GBCs arise in ICPNs.[19] Invasion can be microscopic and difficult to detect. Therefore, the sampling issues, and

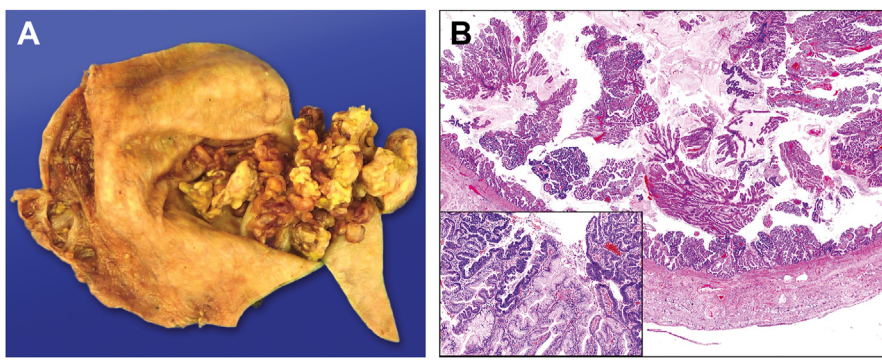

Fig. 7. (*A*) Intracholecystic papillary tubular neoplasms are characterized by a distinct polypoid or papillary mass(es) protruding into the lumen. (*B*) Intracholecystic papillary tubular neoplasms reveal an intraluminal growth of back-to-back papillary and/or tubular units with minimal intervening stroma. Due to their intramucosal nature, the base of the lesions is usually sharply demarcated. However, extension into the Aschoff-Rokitansky sinuses may be seen and mimic invasion. Transition from low-grade to high-grade dysplasia (inset) is evident in most cases. (Courtesy of Dr. Ryan Des Jean)

biologic behavior concepts discussed earlier including the potential concerns for field-effect and margin positivity are even more applicable to ICPNs.

Intracholecystic tubular non-mucinous neoplasm (ICTN) is a highly distinct and invasion-resistant type of intracholecystic neoplasms with specific molecular characteristics. It was previously regarded under the heading of ICPN as its "complex pyloric variant"[19] and also viewed by some in the spectrum of "pyloric gland adenoma".[48,58,59] However, recently the distinctive characteristics of this group became more clearly elucidated.[20,60] These tumors appear to be arising in cholesterol polyps, showing the exact same pedunculated cauliflower-like configuration with very thin stalks. Microscopic examination reveals a complex proliferation with minimal/no mucin (which was recognized as "non-mucinous" in the main paper on this[45]) that readily warrants the diagnosis of "HGD/CIS". In addition to MUC6-positive pyloric differentiation, they also commonly exhibit scattered beta-catenin expressing morules and display Wnt signaling pathway alterations.[61] However, thus-far none of the well-characterized cases reported to have invasive carcinoma. As importantly, just like the cholesterol polyps, they typically occur in GBs without any injury in the GB, and without any dysplastic changes elsewhere. Emerging evidence leads to the conclusion that these most likely arise in cholesterol polyps. Accordingly, the field-effect phenomenon discussed earlier does not at all seem to be applicable to ICTNs.[45]

Intracholecystic neoplasms arising in adenomyomas (or "mural" ICNs) also appear to form a distinct group. By default, they form mural and submucosal-appearing nodules in the fundic region that can be missed. These show several analogies to branch-duct type IPMNs of the pancreas by their localized nature, often multicystic appearance, papillary elements lined by gastric-type epithelium, and carcinomatous changes in about 15%, as well as the lack of dysplastic lesions in the remainder of the luminal GB mucosa.[21] Similar to ICTNs, these intracholecystic neoplasms arising in adenomyomas do not appear to bear the detrimental field-effect that conventional ICPNs present.[19,21]

Extrahepatic Bile Ducts

Flat (non-tumoral) dysplasia

Flat dysplasia in EHBD is typically discovered as a side in resections performed for cancer or 1 of the risk lesions such as choledochal cyst, primary sclerosing cholangitis or pancreatobiliary maljunction.[13] For LGD, the association with metaplasia and regenerative atypia discussed earlier for the GB is also valid for the EHBD. Similarly, LGD of EHBD is by itself of no known clinical significance but should alert the search for higher grade lesions. HGD/CIS, on the other hand, is rarely discovered in isolation. Most of the cases show invasive carcinoma somewhere in the system, bringing up the question of whether they are true preinvasive lesions or post-invasive retrograde "colonization" (cancerization, a.k.a ductal spread of invasive carcinoma cells). Regardless, they warrant careful analysis and complete removal if possible. If HGD/CIS is discovered at a margin, further resection should be attempted, if clinically feasible.[31]

Tumoral intraepithelial (intraductal) neoplasms

Intraductal papillary neoplasms of the bile ducts (IPNBs) are, for all practical purposes, biliary counterparts of pancreatic IPMNs. The entities previously designated as pyloric gland adenoma, intestinal-type adenoma, papillomatosis, or papillary cholangiocarcinoma (papillary adenocarcinoma) of the bile ducts are now all collected under the heading of IPNB.[60,62] Many of the clinicopathologic and biologic characteristics described earlier for ICPNs of GB are also applicable to IPNBs. This includes spectrum

of patterns, cell types, and dysplasia; high frequency of association with invasive carcinoma; multifocality; and field-effect concerns.[19,60,62] Recently, sub-classification of IPNBs as Type A (less complex) versus Type B (more complex and variegated) has been proposed and appears to correlate with frequency of invasion and progression rates.[63–65] Type B lesion appear to have higher rates of progression and aggression.

Intraductal oncocytic papillary neoplasm (IOPN) was for a while regarded as a "variant" of IPNB but is now regarded as a separate category.[60,66] Unlike ordinary IPNBs, IOPNs typically present as complex multilocular cystic and solid masses that is radiologically classified as "cystadenocarcinoma". The papillae are florid and arborizing. However, there is a certain degree of organization and monotony, which, combined with the oncocytic cytology, imparts the distinctive appearance to these tumors (**Fig. 8**). Although they are highly complex and may even appear infiltrative and un-resectable due to their expansile nature, in fact many of the cases have a long protracted clinical course even if there is invasive carcinoma, with 10-year survival over 90% if completely resected.[60,67–70] In addition to their distinctive morphology and more benevolent behavior, these tumors were also found to carry a fusion of PRKACA and PRKACB genes, not seen in other intraductal neoplasms or cholangiocarcinomas.[71,72] They also lack the classical molecular make up of IPNBs and invasive carcinomas of the biliary system.

Intraductal tubulopapillary neoplasms (ITPNs) are another type of mass-forming intraductal neoplasm of the bile ducts.[24,26,60] Unlike the IPNBs, these have tubular architecture and with minimal or no mucin production by the cells (**Fig. 9**).[26,60,73,74] They are often invasive but even then, they appear to have a more indolent behavior. Due to their tubular configuration, they often receive the diagnosis of an ordinary "adenocarcinoma" (cholangiocarcinoma) in limited specimens. However, they lack the molecular genetic alterations typically present in cholangiocarcinomas, and they are also different from IOPNs and IPNBs at the molecular level.[60,73,74]

Mucinous cystic neoplasms (MCNs), similar to those in the pancreas and liver, characterized by the presence of ovarian-type stroma can also occur in the bile ducts and peri-GB region, and almost exclusively in women of perimenopausal age group.[75–77] They typically form multilocular cystic tumors but some may have an intraductal growth. These tumors also represent "adenoma-carcinoma" sequence with neoplastic transformation

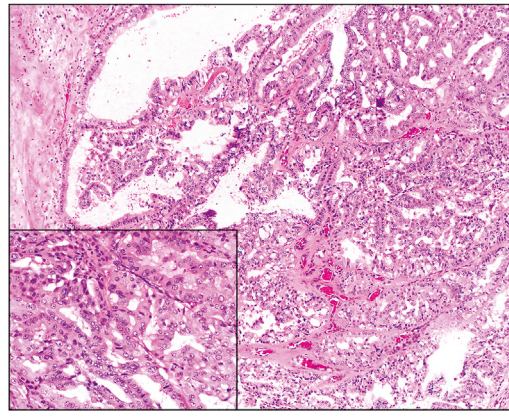

Fig. 8. Intraductal oncocytic papillary neoplasm of bile ducts are characterized with complex papillary projections lined by stratified cells. The cells have abundant eosinophilic granular cytoplasm and nuclei with single, prominent nucleoli (Inset).

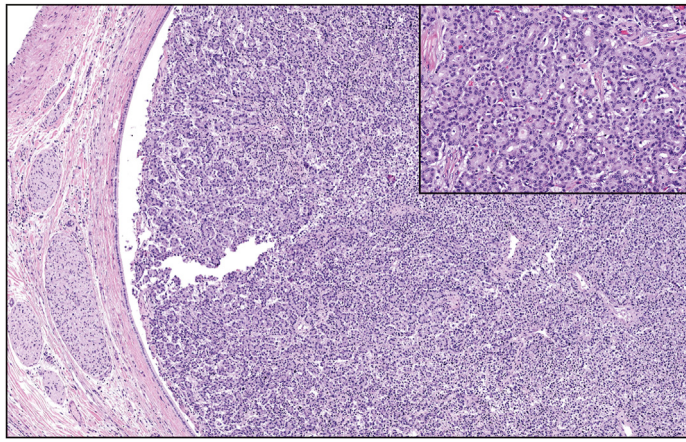

Fig. 9. Intraductal tubulopapillary neoplasm of bile ducts composed of back-to-back tubular glandular structures or punctuated solid areas. The tumor cells have modest amounts of cytoplasm and small and atypical nuclei. There is no obvious intracellular mucin (Inset).

from LGD to HGD to frank carcinoma. However, it appears that carcinomatous transformation is much less common (<5% of the cases) and is often limited in extent and not much clinical consequence as opposed to their pancreatic kindreds where about 15% of the cases show invasive carcinoma and is often mortal.[78]

PATHOLOGIC DIFFERENTIAL DIAGNOSES

Dysplasia Versus Reactive Changes

The differential diagnosis of dysplasia and reactive changes is a well-known problem in GB and EHBD pathology.[79] As discussed previously, pre-malignant lesions often develop in the context of injury and regeneration, and it can be impossible to know where one ends and the other one begins. Molecular studies to document abnormalities in cancer-associated genes (findings that could help to establish the neoplastic nature of lesions) have been limited. Therefore, dysplasia is defined and distinguished from other epithelial lesions based primarily on morphologic principles, drawing in part from experience with early neoplastic changes in the pancreatobiliary tract.

The architectural pattern of growth is helpful in diagnosing dysplasia. Nuclear enlargement and prominent, cherry-red nucleoli are also characteristic features of dysplasia, not seen in reactive lesions.[80] Mitotic figures, including atypical forms, can be prominent in areas of regeneration and are not helpful.

Of note, HGD shows a wildfire phenomenon in the GB, which means it is typically extensive at the time it is detected.[5,80,81] Therefore, focal epithelial atypia in a background of well-preserved non-dysplastic epithelium is more likely to represent reactive changes.

High-Grade Dysplasia Versus Early Invasive Carcinoma

The GB epithelium normally shows undulations and there is no muscularis mucosa to separate the mucosa from submucosa. More importantly, the tunica muscularis is highly irregular and porous.[82] Therefore, dysplastic glands can often be seen lying within or deep to the tunica muscularis. Also, there are no basal or myoepithelial cells that can help distinguish native epithelium from invasive carcinoma. Nevertheless, features that favor true invasive carcinoma include invasion of nerves or blood vessels,

haphazard distribution pattern, lack of luminal bile and lack of a connection to benign epithelium at the surface. In the EHBD, pagetoid extension of HGD/CIS into the peribiliary accessory glands can create a pseudo-invasive appearance. The lobular architecture and even size of units favor the process being non-invasive.

Tumoral Intraepithelial Neoplasms Versus Smaller/Lesser Lesions

Polypoid papillary proliferations, smaller than 1 cm, may occasionally be encountered. If these show clear-cut cytoarchitectural atypia of conventional dysplasia, then they should be acknowledged as pre-malignant. However, especially in the GB, small polypoid collections entirely composed of innocuous pyloric type glands should not be dignified as either "adenoma" or ICPN.[22]

ICPNs also ought to be distinguished from ordinary flat dysplasia, which may reveal epithelial proliferations forming small collections. But these cases should not be regarded as ICPN unless they form a distinct clinically evident and grossly visible mass.

ANCILLARY DIAGNOSTIC TESTS

Immunohistochemical and Molecular Features of Flat (Non-tumoral) Dysplasia

Immunohistochemically, mCEA and MUC1 typically shows staining at the apical border of dysplastic cells. However, intracytoplasmic labeling with mCEA and MUC1 is uncommon. Therefore, dense intracytoplasmic staining favors cancerization over dysplasia. TP53 nuclear staining occurs in more than 30% of cases of dysplasia and tends to be more common in HGD.[83] But it should be kept in mind that it can also be seen in areas of regenerative changes.[84] Similarly, although the Ki67 labeling index is high in cases of dysplasia and increases by grade, it can also be high in areas of regenerative changes.

Oncogenic *KRAS* mutations are uncommon in GB and proximal bile duct dysplasia.[85,86] However, they are identified in about 40% of distal bile duct lesions. *KRAS* mutation represents an early molecular event during the progression of bile duct dysplasia, whereas *TP53* mutation represents a late molecular event.[87,88] *Claudin 18* (*CLDN18*) abnormalities are also common.[89] Alterations in cell cycle proteins, including *CDKN1A*, *cyclin D1* and *SMAD4 (DPC4)* may be detected in some bile duct dysplasia cases.[88]

Immunohistochemical and Molecular Features of Tumoral Intracholecystic Neoplasms

The immunophenotype of ordinary ICPNs[19,90] and IPNBs[60,91] corresponds to their line of differentiation. Most express mucin-related glycoproteins and oncoproteins, including mCEA. The MUC1 is typically confined to HGD. Microsatellite instability can be identified in 10% of IPNBs.[92,93]

Current evidence indicates that molecular alterations of ICPNs are different than those observed in the conventional dysplasia-carcinoma sequence in the GB. They are more similar to those described in intraductal neoplasms of the intrahepatic and extrahepatic bile ducts.[94] Although *KRAS* mutations are common in ICPNs,[83,95] they are uncommon in IPNBs, except for the gastric-type.[60,94] *GNAS* mutation, which is seen in about two-thirds of pancreatic IPMNs[96] is rarely seen in ICPNs[59,62,87] and IPNBs.[62] This disparity is presumably related to the rarity of the intestinal variant in western populations.

Recently, it has been reported that ICTN are associated with the Notch and Wnt/CTNNB1 signaling pathways alterations, harbor mutations in *APC2 and MLL2* (two known regulators of ß-catenin signaling) and reveal aberrant nuclear CTNNB1 protein expression.[61,97]

Immunohistochemical and Molecular Features of Mucinous Cystic Neoplasm

Immunohistochemically, actin, desmin and nuclear progesterone receptor expression is typical. Calretinin, inhibin and CD99 may also be positive. *KRAS* mutations are identified in 20% of MCNs, especially in cases with HGD. However, *GNAS, RNF43* and *PIK3CA* are wild-type in all cases.[75,98]

BIOLOGIC BEHAVIOR AND TREATMENT

Low-grade dysplasia (LGD) does not seem to have any clinical significance in any compartment of the biliary tract. For example, LGD discovered in a cholecystectomy specimen or in a choledochal cyst does not require any further attention provided that there is no other risk factor and the presence of in-situ or invasive carcinoma has been definitively excluded.[11,12,99] It should be acknowledged here that there are substantial subjectivity and reproducibility regarding the diagnosis of LGD and its distinction from regenerative changes. This also emphasizes the importance of second opinions if there is any possibility of a more clinically significant (higher grade) lesion (ie, HGD/CIS) in the differential diagnosis, because HGD/CIS has a very different connotation as discussed later. This also underscores the importance of thorough examination to rule out HGD/CIS in each case.[1,10,99]

Convincing examples of HGD/CIS has been proven to bear a major risk, not only for the same area (as a precursor) but also to the rest of the tract (as a marker, see earlier for field-effect discussion).[2–4,52] This concern of field-risk is higher if the process is extensive. One important aspect in the evaluation of the risk of HGD/CIS is the difficulty of distinguishing them from the "colonization" ("cancerization") phenomenon. Colonization/cancerization refers to the situation in which invasive carcinoma cells invade back into the mucosa retrogradely and mimicking CIS. This process can be impossible to distinguish from a true preinvasive process. They are fundamentally the same cells in different stages, and as of yet, there are no reliable markers to distinguish them.[1,35] These aspects signify the necessity to treat HGD/CIS as a full-blown, albeit curable, form of cancer if an accompanying invasive cancer can be definitively excluded. The presence of underlying risk disease multiplies the concern for progression. Along the same lines, if HGD/CIS is recorded at a margin of resection, the rest of the biliary tract should be regarded as under great risk for cancer development.

For uppermost end of the spectrum where carcinomatous transformation in the mucosa acquires more complex architecture, both the terminology and management become more problematic. he distinction of whether this is to be qualified as merely HGD, or pTis or even pT1a (intramucosal adenocarcinoma) or pT1b (minimally invasive) can often be quite subjective.[1,29,100] This has been most problematic in the GB where a combination of multiple factors have led to different views. First, lack of a complete and well-defined muscularis mucosa layer, as well as the common occurrence of mucosal invaginations (that are permitted by the porous tunica muscularis) allow CIS type changes to form complex invaginations without being truly invasive. Second, there are significant geographic variations in the way such lesions are evaluated by pathologists and treated by clinicians from different continents. To illustrate the magnitude of the issue, in an international consensus study, GBs that had been classified as HGD-only in the United States by multiple experts were actually classified by Asian and South American pathologists not only as CIS, but often as pT1 and even pT2 in close to half of the cases.[100] This is very similar to the issue in the early cancers of the stomach,[32] and it appears that practice-related cultural differences play a role in this. For example, in the Far East the term "carcinoma" does not carry the same concern because of the way pathologic diagnoses are shared with or explained to the patients, and moreover, in

some countries patients prefer to receive the "carcinoma" designation because then the government takes on the treatment expenses. In contrast, in the West, the term carcinoma generates unnecessary social connotations including the possible loss of insurance, which oppositely drives the preference to avoidance of this term. As a result, WHO classification essentially eliminated the term "carcinoma in-situ (CIS)" in exchange with "high-grade dysplasia (HGD)" going along with the Western approach, although the concept obviously does exist and has various practical uses and is widely employed in Far East. Irrespective of the reasons for these variations, a case that is confidently classified as HGD in the United States may receive the diagnosis of pT1 uniformly in the East or South America, and unfortunately there are widely different views regarding the management of these 2 diagnoses.

For the GB, circumventing all these criterial variations, in Chile where the GBC incidence is 1 of the highest and GBCs are most well studied, the term early GB cancer (EGBC) has been employed for the spectrum of neoplastic transformation from simple HGD to more atypical forms qualifiable as CIS (pTis) to the frank intramucosal adenocarcinoma (pT1a) with demonstrable invasive carcinoma cells within the mucosa but not beyond. Studies on cohorts in which pT2 (perimuscular invasive) carcinoma has been ruled out with total sampling of GB have shown that in fact not only pTis (HGD/CIS) but also even more complex ones (pT1a) have very good prognosis.[27–29,101] Unfortunately, the literature from the Surveillance Epidemiology End Results (SEER) database, which is still commonly used in reference, draws a much more bleak picture for HGD/CIS indicating that 30% or more of cases succumb to cancer.[1,29,56] This is attributed to the fact that most of the cases in the SEER are based on "random sampling" and thus many are believed to represent under-staged pT2.[27–29,101] In fact, recent studies based on well sampled and well characterized cases have disclosed that not only pT1b cancers[29] but even very superficial pT2 carcinomas have a very good prognosis.[102,103] If a true pT2 GBC (with perimuscular invasion) has been ruled out with careful examination, the 10-year survival of HGD/CIS (and even early invasive cases[5,37]) is above 90%.[28] All of these recent observations serve as further assurance that lesser lesions (pTis cases, ie, HGD/CIS) are indeed much more benevolent than implied in the Western literature, which is mostly based on SEER.

In summary, it is becoming increasingly clear that in the GB, HGD/CIS and even its more complex forms have a very good prognosis, incomparably better than what has been indicated in the earlier literature. However, at the same time, about 5% of the cases show progression and dissemination. The early recurrences are believed to be mostly missed invasive carcinomas and emphasize the importance of sampling and exclusion of deeper lesions.[28,101] At the same time, there are late progressors, some 8 to 10 years after the cholecystectomy, and for these the field-effect and metachronous cancers in the remainder of the biliary tract are suspected to be the source. Extensiveness of HGD/CIS, cell type (for example, biliary), degree of papilla formation, margin positivity, suspect foci of invasion, involvement of Rokitansky-Aschoff sinuses, and especially history of a risk disease like pancreatobiliary maljunction are believed to bring higher risk for progression.

CLINICS CARE POINTS

- Low-grade dysplasia discovered in a cholecystectomy specimen or in a choledochal cyst does not require any further attention if there is no other risk factor, however, it is crucial that the presence of in-situ or invasive carcinoma has been definitively excluded by additional sampling.

- If high-grade dysplasia/carcinoma in-situ is recorded in any part of the biliary tract, the rest of the tract should be regarded as under risk for cancer development. Especially in patients with more extensive high-grade dysplasia/carcinoma in-situ, papillary configuration, biliary phenotype, margin positivity and the presence of an underlying risk factor like primary sclerosing cholangitis or pancreatobiliary maljunction, this risk is much greater, and long-term surveillance is warranted.
- Along those lines, if a patient with dysplasia or carcinoma in the biliary tract is undergoing a second operation of the region, we advocate to perform bile duct brushing of the remaining system to determine whether there are sub-clinical carcinomatous changes.
- *Hyalinizing Cholecystitis*, a distinctive variant of chronic cholecystitis, has a strong association with carcinoma. However, carcinomas arising in this setting often have a subtle appearance. Extensive, if not total, sampling is crucial to reveal the presence and extent of carcinoma.
- Patients with *pancreatobiliary maljunction* present an interesting model of carcinogenesis developing from a distinctive mucosal hyperplasia ("reflux cholecystopathy") to dysplasia (often tumoral type) and finally to invasive carcinoma, which occurs in a very significant proportion of the patients if untreated. It also connotes risk for entire biliary tract mucosa.
- SEER database draws a much more aggressive picture for in-situ and minimally invasive cancers, but this is attributable to the undersampled and underdiagnosed cases of more advanced cancers, because, the data are not supported in carefully crafted institutional studies. This underscores the importance of not rendering the diagnosis of high-grade dysplasia/carcinoma in-situ unless total sampling and careful exclusion of a more advanced carcinomatous process is conducted definitively.

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