# Adenocarcinoma of the Gallbladder: Biology of Disease, Prognosticators, and Staging

148

## Contents




**Abstract**

Biology of disease and progression of ordinary gallbladder carcinoma depends on several factors. For local spread, perineural and intraneural invasions are important risk factors. Gallbladder carcinoma often extends beyond the organ boundaries and invades the gallbladder bed, liver, and adjacent soft tissues. Lymph node metastasis is a characteristic feature of gallbladder carcinoma and a significant prognostic factor. Pericholedochal nodes are a common metastatic site, followed by the cystic node. There is a relationship between T stage and the incidence of locoregional lymph node metastases. The prognostically significant patterns of gallbladder cancer spread to the liver include hepatic bed type, hepatic hilum type, bed and hilum type, lymph node type, cystic duct type, and localized type. Gallbladder carcinoma can spread through intraluminal bile duct invasion. It produces a distinct pattern of distant metastases, although extraabdominal metastasis is overall uncommon in gallbladder carcinoma. Important prognosticators for tumor progression are tumor stage, lymph node stage, radicality of resection, perineural invasion, and the histologic grade.




A. Zimmermann, *Tumors and Tumor-Like Lesions of the Hepatobiliary Tract*,
DOI 10.1007/978-3-319-26956-6_148

## Biology of Disease

The natural course of CG is characterized by a high tendency for invasion, spread, and metastasis (Fahim et al. 1962). Most of the patients with CG present with advanced disease at the time point of diagnosis, precluding curative resection resulting in dismal prognosis (Dutta 2012). In some series, almost half of patients showed metastatic disease at the time point of diagnosis (Hamrick et al. 1982). The overall 5-year survival for patients with CG having undergone R0 curative resection was reported to range from 21 % to 69 % in diverse studies (review: Lai and Lau 2008).

## Local Invasion

Perineural and intraneural invasions are important modes of local spread in CG. The incidence ranged from 22 % to more than 50 % (Vadheim et al. 1944; Kuwayti et al. 1957; Fahim et al. 1962; Ouchi et al. 1987; Nagakawa et al. 1993). Perineural cancer invasion has to be distinguished from perineural invasion observed in benign conditions, such as adenomyomatous hyperplasia (Albores-Saavedra et al. 2007) and pyloric gland metaplasia (Albores-Saavedra and Henson 1999). There is a correlation between the prevalence of perineural invasion and higher histologic grade (Fahim et al. 1962). CG often extends beyond the organ's boundaries and invades the gallbladder bed, the liver, soft tissues surrounding the bile ducts, and the hepatoduodenal ligament. In bile ducts located to the hepatoduodenal ligament, perineural invasion in the setting of bile duct infiltration is a sign of aggressive tumor disease (Kaneoka et al. 2003). Pathogenetically, expression of the neural cell adhesion molecule/NCAM by carcinoma cells seems to play an important role as a neurotrophic mechanism (Seki et al. 1995). The direct extension of CG tumor cells from the primary tumor to adjacent organs and structures is only partially elucidated. One mechanism involves mucosal and submucosal lymphatic vessel networks that connect neighboring organs. In patients with CG, cancer cells spread through lymphatic vessels in the submucosal layer of the common bile duct (Chikamoto et al. 2009). CG frequently invades blood vessels, in particular veins. The anatomy of the venous drainage of the gallbladder has been studied in detail (Habighorst et al. 1965; Halvorsen and Myking, 1971 9). The first authoritative investigation based on injection methods dates from 1932 (Karlmark 1932). This author demonstrated that the venules and small veins of the gallbladder form a continuous plexus localized to the adventitia of the organ. This plexus is, via venous bridges, connected with a similar plexus of the extrahepatic bile ducts. The plexus system is in turn drained by a variable number of cholecystic veins showing diverse types of arrangement. On the hepatic side of the gallbladder, the number of cholecystic veins ranges from 2 to 20, and these veins terminate in the quadrate lobe of the liver adjacent to the gallbladder bed. In contrast, the peritoneal side of the gallbladder shows usually only one cholecystic vein (rarely two). This vein drains either into the liver or into the periductal venous plexus, which in turn is connected to the veins of the quadrate lobe. In part of cases, this vein drains into the portal vein (review: Fahim et al. 1962). The significance of portal vein drainage is underlined by the observation of dilated gallbladder veins or gallbladder varices in the setting of portal hypertension (Marchal et al. 1985; Ralls et al. 1988; Kainberger et al. 1990). Veins that enter the quadrate lobe form a plexus of small vessel that finally drain into the hepatic vein of this lobe (Petrén 1932). Small veins of the cystic duct join at the neck of the gallbladder to form either single or double cystic veins which accompany the cystic duct to connect to the periductal veins (review: Fine 1997). This anatomical substrate suggests an important pathway for CG spread both into the region of the extrahepatic bile ducts and the hepatoduodenal ligament and the right-sided liver. In a study of 155 cases of CG, involvement of gallbladder vessels was identified in 13.9 %, invasion involving venules having a diameter of 500–1,200 μm to larger veins with a thick muscular wall. In large veins, traffic of cancer cells through the wall seems to take place via transmural invasion and/or extension along vasa

vasorum (Fahim et al. 1962). CG cancer cells can spread through lymphatic vessels in the submucosa of the common bile duct (Chikamoto et al. 2009).

## Lymph Node Metastasis

Lymph node metastasis is a characteristic feature of CG that represents a significant prognostic factor. The incidence of lymph node metastasis in CG varies from 35 % to 75 % in diverse studies (Cooper 1937; Vadheim et al. 1944; Jones 1950; Willis 1953). Lymphatic spread may show a distinct distribution pattern, with the pericholedochal nodes being the most commonly affected, followed by the cystic node. Lymph node metastasis by CG cab develops in the absence of involvement of the liver (Cappell and Tudhope 1934). This distinct pattern results from the specific anatomy of the lymphatic drainage of the gallbladder. By means of Prussian blue injection into human fetuses 8–9 months old, Clermont (1909) had studied the lymphatic vessels of the gallbladder and gallbladder-associated lymph nodes in detail. The gallbladder wall harbors lymphatic plexus that traverses the entire wall and enters lymphatic collecting trunks that are mainly located on the inferior surface of the gallbladder. They are arranged like the letter "N," as they are present on both the left and the right borders and diagonally toward the left side of the gallbladder neck (Fahim et al. 1962). The left-sided collecting trunks empty into the cystic node which is situated in the acute angle formed between the cystic duct and the common hepatic duct, while the right-sided collecting trunks pass along the right border of the gallbladder neck and extend uninterrupted to empty into the pericholedochal nodes (reviewed by Fahim et al. 1962). Also the lymph from the cystic node ends in the pericholedochal nodes. The latter are usually two lymph nodes, termed the node of the hiatus and the superior pancreaticoduodenal node. The node of the hiatus was a constant finding in Clermont's investigation. It is situated to the right of the common duct, its lower pole being at the level of the attachment of the lesser omentum behind the superior portion of the duodenum. The node of the hiatus also receives lymph from the extrahepatic bile ducts and from the right liver lobe. Three to four efferent lymphatic vessels of the node of the hiatus continue to reach the superior pancreaticoduodenal node, which is located along the superior surface of the pancreas to the right of the common duct (summarized in Fahim et al. 1962). Efferent lymphatics of this node continue either to the preaortic peri-celiac nodes or to the three to four posterior pancreaticoduodenal nodes which are located around the vessel arcade in the posterior pancreaticoduodenal groove. From here, the lymph flows to the nodes around the superior mesenteric artery. Clermont (1909) found lymph nodes along the hepatic pedicle in relation to the hepatic artery, draining the left liver lobe only, but he did not find any Quénu-type hepatic hilar lymph nodes (proposed to exist by Edouard André Victor Alfred Quénu, 1852–1944, French surgeon active in anatomy and surgical pathology). Clermont's studies referred to human fetuses and infants and the situation in adults may differ from findings obtained in the prenatal and pediatric age group. The lymphatic system of the gallbladder was subject to more recent investigations on adult individuals. Ito and coworkers (1991) divided gallbladder lymphatic drainage into three pathways, i.e., the cholecystoretropancreatic pathway (the main pathway), having two routes, namely, one running spirally and posteriorly from the anterior surface of the common bile duct to the right and the other one running almost straight down from the posterior surface of the common bile duct. These routes end at a retroportal lymph node, termed by the authors the principal retroportal node. A second route connected the gallbladder with the celiac nodes, and a third ran in front of the portal vein to the superior mesenteric nodes.

There is a relationship between the T stage and the incidence of locoregional lymph node metastases. In one study, none of 15 patients with stage pT1 tumors had lymph node metastasis, while 60 of 96 patients with pT2-4 tumor disease had lymph node metastases. pT3-4 tumors displayed more lymph node involvement and significantly higher N2:N1 ratios (2.5) than pT2 tumors.

Patients with N2 disease had a worse prognosis than those with N1 (Tsukada et al. 1997). Overexpression of microRNA-155, associated with inflammation-induced carcinogenesis, in CG is closely correlated with the emergence of lymphatic metastasis and poor prognosis. In vitro assays revealed that aberrant expression of miRNA-155 enhanced CG cell proliferation and invasion (Kono et al. 2013).

## Invasion of the Liver and Intrahepatic Metastasis

Among early sites of metastasis, the liver is often involved. In fact, hepatic metastasis is the most frequent mode of recurrence of advanced CG after radical resection, and microscopic liver metastases in CG are an independent prognostic factor (Endo et al. 2004). Infiltration and/or metastatic involvement of the liver was detected in up to 89 % of cases (Lam 1940; Vadheim et al. 1944; Glenn and Hays 1954; Burdette 1957; Sons et al. 1985). It may, however, sometimes be difficult to distinguish hepatic metastases in segments close to the gallbladder from direct invasion of the liver substance. The pathways leading to true hepatic metastases have been discussed, emphasis being placed on the anatomic relationships between gallbladder veins and the anterior portal vein (Shirai et al. 1995; Yoshimitsu et al. 1997, 2001; Ohtsuka et al. 1998; Lin et al. 2005; Table 1). The extent of microscopic angiolymphatic portal tract invasion correlated with the gross depth of direct invasion of the liver (Shirai et al. 1995).

**Table 1** Patterns of gallbladder carcinoma spread to the liver

| ***Kondo system* (2002)** |
|---|
| Hepatic bed type |
| Hepatic hilum type |
| Bed and hilum type |
| Lymph node type |
| Cystic duct type |
| Localized type |
| ***Wakai system* (2010)** |
| Direct invasion through the gallbladder bed |
| Portal tract invasion |
| True hepatic metastatic nodules |

Kondo and coworkers (2002) identified six types of CG spread. In the hepatic bed type, a large mass in the fundus and body of the gallbladder invaded the liver substance through the gallbladder bed. In the hepatic hilum type, CG of the gallbladder neck infiltrates the hepatic hilum causing obstructive jaundice. In the bed and hilum type, huge masses occupying the entire gallbladder involve both the gallbladder bed and the hepatic hilum. In the lymph node type, enlarged metastatic lymph nodes are the most prominent feature. In the cystic duct type, a small mass arising from the cystic duct involves the common bile duct. In the localized type, tumor spread is localized to the gallbladder and presents at the earliest stage of any type (Kondo et al. 2002). In another study, the mode of hepatic spread was classified into three patterns, i.e., direct invasion through the gallbladder bed, portal tract invasion, and true hepatic metastatic nodules. A significant proportion of cases with portal tract invasion revealed invasion of hepatic lymphatic vessels as shown by D2-40/podoplanin immunohistochemistry (Wakai et al. 2010).

## Intraductal Spread of Gallbladder Carcinoma

CG can spread through intraluminal invasion of the extrahepatic biliary tract. It was found in up to 4 % of cases (Fahim et al. 1962). Rarely, CG showed intraluminal implantation into bile ducts (so-called implantation metastasis or formation of intraluminal tumor "thrombi" or "emboli"; Hollings 1963; Al-Qudah 1994; Evans 1994; Midorikawa et al. 2000). In case intraluminal CG metastasis involves the confluence or the common bile duct, obstructive jaundice ensues (Hollings 1963; Midorikawa et al. 2000).

## Distant Metastasis

CG produces a distinct pattern of distant metastases, although extraabdominal metastases are

overall uncommon in CG. The most common metastatic site is the lung (Tokunaga et al. 2005; Jeyaraj et al. 2013), followed by the central nervous system (Takano et al. 1991; Tayo et al. 2005), ovaries (Singh et al. 2010), soft tissues, and breast (Khangembam et al. 2013). In rare instances, pulmonary metastasis of CG presents with a lepidic growth patterns, i.e., cancer cells lining alveolar spaces (Tokunaga et al. 2005). Similar to carcinomas of the extrahepatic bile ducts, CG can produce ovarian metastases that sometimes present as Krukenberg tumors (Andrieux et al. 1972; Chicos et al. 2007). Solitary metastases to the skeleton occur, but are rather uncommon (Rominger et al. 1961; Prakash et al. 2010; Chaudhari et al. 2014; Puranik et al. 2014). Multiple osseous metastases are even less common (Commandre et al. 1965; Misra et al. 1997). Subcutaneous metastasis is also rare in CG, but may present as a rapidly progressive cancer disease (Heavey et al. 2014). Other rare sites comprise the orbita (Puglisi et al. 2005), heart (Gunjiganvi et al. 2013), adrenal gland (Sahoo et al. 2014), and uterus (Martinez-Roman et al. 2005; Kefeli et al. 2009).

The mechanisms leading to early and widespread metastasis in CG are only partially known. CG shows an upregulation of the prometastatic microRNA-20, closely associated with local cancer invasion and distant metastasis. A markedly increased level of TGF-beta1 is responsible for the elevation of microRNA-20a, which in turn promotes epithelial-mesenchymal transition thought to promote metastasis (Chang et al. 2013).

## Implant Metastasis

Implant metastasis of CG can develop in cholecystectomy scars (Merz et al. 1993). In some patients, metastases of CG have been found at the port site (Karayannakis and Knight 1997; Reber et al. 1998; Ohmura et al. 1999; Winston et al. 1999; Nakagawa et al. 2000), trocar site (Copher et al. 1995), or umbilicus (Clair et al. 1993; Kessler and Mihaljevic 1994; Jacobi et al. 1995; Jeon et al. 1999) following laparoscopic cholecystectomy. The incidence of recurrence of incidental CG at the port site following laparoscopic cholecystectomy was 14 % in one study and was similar whether the primary tumor was confined to the gallbladder (T1/T2) or locally advanced (T3/T4; Z'graggen et al. 1998). In an international survey, it was found port site recurrence was identified in 17.1 % of 409 patients with a median of 180 days following laparoscopic cholecystectomy for nonapparent CG (Paolucci et al. 1999). Laparoscopic cholecystectomy with the finding of unsuspected CG may be followed by cutaneous seeding (Kim and Roy 1994), and laparoscopic removal of CG may cause peritoneal cancer dissemination (Pezet et al. 1992; Sailer et al. 1995; Marmorale et al. 1998; Ohtani et al. 1998; Shirai et al. 1998). Cancer spread to the peritoneum in the setting of laparoscopic cholecystectomy was mainly found in the presence of T3 tumors (Wysocki and Krzywon 2000). Rarely, CG gave rise to umbilical metastasis, alias Sister Mary Joseph's nodule, in the absence of laparoscopic surgery (Rousselot et al. 1964; Bork et al. 2002; Renner and Sticherling 2007). Sister Mary Joseph's nodule refers to a palpable tumor nodule bulging into the umbilicus due to a metastasis from an abdominal or pelvic malignancy. The eponym is related to Sister Mary Joseph Dempsey (1856–1939), surgical assistant of Dr. William J. Mayo at St. Mary's Hospital in Rochester, who drew Dr. Mayo's attention to the phenomenon of umbilical metastasis, the metastatic pathway probably involving lymphatics along the obliterated umbilical vein. Laparoscopic cholecystectomy can also be complicated by parietal seeding of carcinoma (Barsoum and Windsor 1992) or by seeding into the gallbladder fossa (Evrard et al. 1996). Seeding of CG along the intervention tract was observed following percutaneous transhepatic choledochoscopy (Yamakawa et al. 1983).

## Prognosticators

A growing list of factors affecting prognosis of CG have been identified, relating to growth factors and their receptors, factors regulating

proliferation and apoptosis, adhesion molecules, factors regulating cell motility and locomotion, enzymes involved in tissue invasion, and angiogenic factors (review: Gomez-Roel et al. 2007).

## Stage

Tumor stage (T stage) and lymph node metastases (N stage) are important prognosticators in CG. The T category is a decisive factor for predicting outcome (Kayahara and Nagakawa 2007; Cho et al. 2012). Early CG, limited in its extension to the intramucosal compartment (pT1a), is usually an incidental finding, but this stage is associated with good prognosis (Albores-Saavedra et al. 2011). The presence of lymph node metastases is an important predictor of outcome, whereby the number in involved lymph nodes independently determines prognosis after tumor resection (Sakata et al. 2010; Shirai et al. 2012). Distant positive lymph nodes have a particularly adverse effect on outcome (Meng et al. 2011). There is increasing evidence that the lymph node ratio (LNR) is an important prognostic factor for CG patients. LNR was a powerful predictor of disease-free survival in curatively resected patients, specifically in stage IIIB disease (Negi et al. 2011; Choi et al. 2013). In another investigation, however, the number of positive lymph nodes better predicted patient outcome after resection than LNR or location of positive nodes (Shirai et al. 2012). The presence of lymph node metastases in CG is associated with expression of the high molecular weight glycoprotein, DF3 (MUC 1). Lymph node metastasis was frequently found in the cytoplasmic DF3- and stromal DF3-positive CG, suggesting that DF3 expression plays important roles in cancer cell growth and metastasis of CG (Kashiwagi et al. 2000). The prognostic significance of nodal micrometastases in CG is not yet known in detail, but was shown to have an impact on survival (Sasaki et al. 2006) or even be an independent prognosticator for poor outcome (Nagakura et al. 2001). In one investigation, nodal micrometastases were found in 2.3 % of lymph nodes that were macrometastasis negative at conventional examination. Micrometastases correlated with non-micro-lymph node metastases on conventional tissue sections and were a significant prognostic factor, although they may reflect cancer cell spread to the whole body rather than an initial event of cancer spread (Tanabe et al. 2012). Distant metastases are a decisive factor for prognosis in patients with CG. Hepatic metastasis is the most common mode of recurrence of advanced CG after radical resection. Both macroscopic and microscopic liver metastases are involved in progressive disease and dismal prognosis.

## Radicality of Resection

The presence or absence of carcinoma in the resection margin significantly affects outcome (Chakravarty et al. 2009). Carcinoma spread to the cystic duct is a prognosticator which indicates poor prognosis in CG patients, probably also linked to a high proportion of associated perineural invasion and lymph node metastasis (Nakata et al. 2007). It should be mentioned that high-grade dysplasia may rarely occur in the cystic duct margin in the absence of invasive CG (Bickenbach et al. 2011). Intraoperative frozen section is a common method to evaluate resection margins in the setting of cholecystectomies performed for CG, but has some interpretational drawbacks due to the presence of gallbladder structures that may be confounded with cancer, including glands and (atypical) Rokitansky-Aschoff sinuses. It has been demonstrated that frozen section and permanent diagnoses of the bile duct margin in CG may be inconsistent in up to 25 % of cases due to overdiagnosis in frozen sections and the “appearance” of carcinoma in permanent histology slides (Yamaguchi et al. 2005).

## Incidental Versus Non-incidental CG

A significant fraction of CGs are incidental tumors detected in cholecystectomy specimens obtained via resection of suspected benign disease. Patients with incidental CG (IGC) had a longer survival rate compared to patients with

non-incidental CG (NIGC). In one study, the majority of patients with potentially curable disease had IGC (D'Hondt et al. 2013).

## Histologic Type

With few exceptions, the histologic type of CG did not affect biology of disease (Glenn and Hays 1954). In a comparative study, well-differentiated or moderately differentiated tubular adenocarcinomas were associated with a longer survival time than poorly differentiated carcinomas, the latter also having a higher incidence of hematogenous metastases (Egawa et al. 2004). Most CG shows the histology of adenocarcinoma, and the relatively few neoplasms with distinct morphologies different from ordinary adenocarcinoma mainly comprise papillary and colloid carcinomas. In comparison with all other histologic types, papillary CG had the most favorable prognosis, in some series found in patients with long survival (Fahim et al. 1963; Frank and Spjut 1967; Hart et al. 1972; Ouchi et al. 1986; Henson et al. 1992).

## Histologic Grade

Dedifferentiation is often observed in CG and is associated with poor prognosis. In an ECOG study on 30 patients with advanced CG divided into either low-grade or high-grade lesions, the 13-week low-grade CG patient survival was significantly longer than the 7-week high-grade CG patient survival (Johnson et al. 1987). In part of CG, less differentiated tumor cells form isolated single cells or clusters of fewer than five cancer cells at the invasive front. Similar to the respective situation in colorectal carcinomas, this phenomenon is termed tumor budding, a lesion that reflects prognosis, particularly for T2 tumors (Kai et al. 2011).

## Invasion Patterns

The presence of perineural invasion is a negative prognosticator in patients with CG (Nagakawa et al. 1993; Yamaguchi et al. 2002; Noshiro et al. 2003; Nakata et al. 2007). In one study of 68 patients with CG, perineural invasion was demonstrated in 71 % of patients. The incidence of invasion was correlated with invasion of extrahepatic bile ducts, which was the only independent factor associated with perineural involvement. The 5-year survival rate of patients with perineural invasion was 7 % in comparison with 71 % of patients without detectable perineural invasion (Yamaguchi et al. 2002). Bile duct involvement portends poor prognosis in resected CG (Chan et al. 2005; Eil et al. 2013). In early CG, intraepithelial extension into Rokitansky-Aschoff sinus is an adverse prognostic factor (Roa et al. 2013). As in other carcinomas, vascular invasion is a negative prognostic factor in CG. Expression of vascular endothelial growth factor-C/VEGF-C was expressed in 63 % of CG, and this expression was associated with lymphatic vessel invasion and lymph node metastasis, suggesting that VEGF-C is involved in tumor progression through lymphangiogenesis and facilitation of lymphatic vessel invasion (Nakashima et al. 2003). Invasion of the hepatic artery is an important prognostic factor in patients with CG, as infiltration of this artery confers high risk and a poorer prognosis (Kobayashi et al. 2012).

## Oncogene and Tumor Suppressor Gene Expression and Factors Involved in Chromatin and DNA Metabolism

A significant proportion of CG expresses p53. Fifty-eight percent of these cancers were immunoreactive for p53 in one analysis, seen more often in moderately or poorly differentiated neoplasms (Washington and Gottfried 1996). No significant correlation was found between p53 overexpression and T stage, lymph node metastasis, prognosis, or recurrence in patients with CG, but p53 overexpression was correlated with aneuploidy (Ajiki et al. 1996). p53 nuclear overexpressions in CG were not related to cancer differentiation, depth of gallbladder wall invasion, or patient survival (Hidalgo Grau et al. 2004).

Lack of correlation between p53 expression in CG and survival was found in another investigation (Washington and Gottfried 1996). On the other hand, other investigations reported a role of p53 gene mutation in the transition from premalignancy to malignancy (Ajiki et al. 1996; Moreno et al. 2005) and a correlation between p53 expression in CG and poor survival (Takagawa et al. 2005). An important regulator of chromatin, involved in silencing tumor suppressor genes, is the histone deacetylase (HDAC) system. The Myc oncogene pathway seems to be involved in carcinogenesis and progression of CG. N-myc downstream-regulated gene 1 (NDRG1), a member of the N-myc downstream-regulated gene family, is associated with inhibition of tumor metastasis and tumor suppression. In CG, NDRG1 was expressed in 63.8 % of cases, specifically at the invasive front of the tumors, but not in normal gallbladder epithelium. This expression was significantly associated with high histologic grade, advanced T stage, positive nodal metastasis, venous/lymphatic invasion, and poor survival (Zhang et al. 2012). In CG, overexpression of HDAC 2 in tumor cell nuclei is correlated with higher histologic grade, and predicts unfavorable prognosis (Du et al. 2013a). There is evidence that factors involved in chromatin replication and chromatin remodeling are altered in CG and affect biology of disease. Rsf-1/HBXAP, a nuclear protein with histone chaperon function and member of the remodeling and spacing factor complex which mediates ATPase-dependent chromatin remodeling, is expressed in CG, and its overexpression confers aggressiveness and is associated with disease-specific survival (Chen et al. 2011a). Decreased expression of the chromodomain helicase DNA-binding protein 5 in CG cells is correlated with a higher lymph node metastasis rate and shorter disease-free survival and overall survival (Du et al. 2013b). Dicer and Drosha, two enzymes critically involved in the processing of microRNA, are significantly less expressed in CG than in dysplastic gallbladder epithelia and were less expressed in poorly differentiated carcinomas with lymph node metastases in comparison with well-differentiated adenocarcinomas. Loss of expression of Dicer and Drosha was associated with decreased overall survival (Shu et al. 2012).

## Transcription Factors and Associated Protein Signaling Networks

SOX4, a member of the SRY-related HMG-box (SOX) transcription factor family, plays a significant role in carcinogenic pathways of various malignancies. SOX4 was expressed in 75 % of CG, but in normal gallbladder epithelium. SOX4 expression in CG was significantly associated with low histologic grade, low pathological T stage, early clinical stage, and better disease-free and overall survival (Wang et al. 2012a).

Sonic hedgehog, its receptor, patched, and its downstream transcription factor, Gli1 protein, are overexpressed in CG. The expression of these factors was significantly correlated with stage, lymph node metastasis, venous and lymphatic invasion, liver invasion, and lower survival rates (Li et al. 2012). Expression of the N-myc downstream-regulated gene 2 (NDRG2), a protein that may reduce the metastatic potential of cancers, was detected in 37.6 % of CG. Tumors with downregulation of NDRG2 more often had lymph node metastases and lymphovascular invasion and tended to have higher TNM stage; the patients also had poor prognosis (Song et al. 2012). Caudal-related homeobox protein (CDX2), a homeodomain protein which plays an important role in the regulation of cell proliferation and differentiation of glandular epithelia and which is a regulator of intestinal metaplasia, is absent from the normal gallbladder, but is expressed in part of CG, mostly in well-differentiated tubular adenocarcinomas of the intestinal type (Wu et al. 2005).

## Cell Cycle Regulation

High proliferative activity is generally associated with an aggressive phenotype of CG (Hui et al. 2002). CG expresses various factors involved in cell cycle regulation, including cyclin E (Mishra et al. 2009), cyclin D1, and p16 (Xuan

et al. 2005; Srivastava et al. 2013). The expression rate of cyclin D1 (68.3 %) in CG was significantly higher than in nonneoplastic disorders of the gallbladder, whereas the expression rates of p16 and retinoblastoma/Rb protein were significantly lower in CG compared to those in cholecystitis and adenoma (Ma et al. 2005). Cyclin D1 overexpression is a critical event in CG carcinogenesis and independently predicts decreased patient survival (Hui et al. 2000; Itoi et al. 2000). Loss of p16 in CG and bile duct cancers is associated with reduced survival of patients (Karamitopoulou et al. 2008), whereby loss of p16INK4 protein is correlated with overexpression of the retinoblastoma/Rb protein (Shi et al. 2000). In contrast, overexpression of cyclin E was not associated with depth of tumor invasion, tumor stage, or patient prognosis (Eguchi et al. 1999). p27 is a tumor suppressor protein which inhibits both Cdk2/cyclin E and Cdk2/cyclin A complexes and prevents transition to the S phase of the cell division cycle. Expression of p27 is expressed in CG and was correlated with clinical stage of CG (Xuan et al. 2005; Alsheyab et al. 2007), and low p27Kip1 expression in CG is an independent prognostic factor associated with poor prognosis (Filipits et al. 2003). The expression pattern of p27 is critically modulated by two SCF(Skp2) ubiquitin ligase-related proteins, Skp2 and cyclin-dependent kinase subunit 1/Cks1, which are involved in the posttranscriptional degradation of the p27 tumor suppressor. Reduced p21(WAF1/CIP1) expression is an early event in CG carcinogenesis and is of prognostic significance (Li et al. 2001; Puhalla et al. 2007). Expression of Skp2 was found to be an important and independent adverse prognosticator in CG (Li et al. 2007). Cyclooxygenase-2 (COX-2) plays a role in promoting cell proliferation, growth, and metastasis of carcinoma cells. Intense immunostaining of COX-2 was found in hyperplastic gallbladder lesions (65 %), pT2 CG (76 %), and advanced stage CG (64 %), including the associated stroma. In pT2 CG, expression of COX-2 in the stroma adjacent to the carcinoma cells in the subserosal layer correlated with aggressiveness of disease, including the tendency for distant metastasis (Kawamoto et al. 2002). The mammalian target of rapamycin (mTOR), a serine/threonine kinase, plays an essential role in the regulation of cell growth and is frequently deregulated in cancers. In CG, phospho-mTOR is an overexpression in early phases of cancer evolution, associated with poor prognosis (Leal et al. 2013). The sphingosine-1-phosphate receptor 1 (S1P1) is overexpressed in CG, and higher levels of S1P1 were significantly correlated with tumor differentiation, tumor mass, lymph node metastasis, invasion, and decreased survival time (Yuan et al. 2013). S1Ps are a class of G protein-coupled receptors that are a signaling target of sphingosine-1-phosphate and that are involved in the regulation of cell proliferation, cytoskeletal organization, and cell migration.

## Other Factors Involved in Proliferation and Differentiation of Cells

Astrocyte elevated gene-1 is overexpressed in CG and is strongly correlated with the differentiation degree, stage, Ki-67 proliferation index, and liver invasion. Expression of this protein in CG is an independent prognostic marker for CG progression (Sun et al. 2011). Expression of connective tissue growth factor/CTGF in CG was correlated with better survival in two studies (Alvarez et al. 2008; Garcia et al. 2013), while in another investigation a role of CTGF expression in CG progression was found (Garcia et al. 2013). Part of CGs produce several classes of mucins (see below). Univariate analysis showed that MUC4 expression was significantly associated with poor survival, while expression of MUC1 and MUC2 was not correlated to survival. Backward stepwise multivariate analysis exhibited that MUC4 expression was a significant risk factor for poor outcome (Lee et al. 2012a). Expression of the multifunctional redox protein, human thioredoxin-1 (TRX-1), and its reducing enzyme, thioredoxin reductase (TRX-R), in CG affects tumor biology. Specifically, nuclear TRX-1 expression in cancer cells at the invasion front of CG is a significant prognostic marker (Nagano et al. 2012). Estrogen receptors (ERs) are

expressed in part of CG cells (Gupta et al. 2012). ERbeta isoform was expressed in most specimens, but preferentially in central tumor parts. Absent ERbeta expression at the invasive front was significantly associated with lymph node metastasis, advanced stage, lymphatic invasion, and poor prognosis (Sumi et al. 2004).

## Chemokines, Cytokines, and Other Factors Involved in Immune Responses

Chemokine (C-X-C motif) ligand 12 (CXCL12), important for the progression of various malignancies, was differentially expressed in CG tissue, together with its receptor CXCR4, and the magnitude of expression was associated with high histologic grade and nodal metastasis, and expression was a significant risk factor for survival. CXCL12 increased anchorage-dependent and anchorage-independent growth, adhesiveness, and migration and invasive properties of CG cells (Lee et al. 2012b). Toll-like receptors (TLRs), which play a crucial role in inflammatory reactions, including chronic cholecystitis, are also operational in various pathways involved in carcinogenesis. TLR4 is mainly expressed in the glandular and luminal epithelia of the gallbladder. TLR4 expression was lower in CG in comparison with chronic cholecystitis and normal gallbladder (Huan et al. 2012).

## Mechanisms of Apoptosis

There are intimate relationships between CG and apoptosis (Zhang et al. 2003). Positive rates of Fas were not significantly different among CG, dysplasia, and gallbladder adenoma, but positive rates of Fas ligand/FasL in carcinoma were significantly higher than that in chronic cholecystitis, suggesting that FasL expression in CG permits tumor cells to escape from immune surveillance by inducing apoptosis in lymphocytes infiltrating CG tissue (Xu et al. 2005). Higher expression of Bcl-2 in CG is considered to affect growth and progression of the tumors via inhibition of apoptosis (Mikami et al. 1999; Karamitoupoulou et al. 2008). Expression of the cellular Fas-associated death domain-like interleukin-1-converting enzyme inhibitory protein (c-FLIP), an antiapoptotic protein, is upregulated in CG, potentially conferring a growth advantage (Zong et al. 2009). Expression of p53-upregulated modulator of apoptosis (PUMA) was significantly higher in adenocarcinoma of the gallbladder than in adenoma and chronic cholecystitis, whereby PUMA expression was lower in small (less than 2-cm diameter) and well-differentiated CG with N0 than in poorly differentiated larger (>2 cm) CG with lymph node metastasis and invasion of surrounding tissues (Cai et al. 2013). Receptor-binding cancer antigen expressed on SiSo cells (RCAS1), a protein that induces apoptosis in immune effector cells expressing the RCAS1 receptor, was overexpressed in 70 % of CG, but not in precursor lesions. RCAS1 expression was associated with depth of tumor invasion, venous involvement, perineural invasion, tumor stage, and the presence of metastases (Oshikiri et al. 2001). Expression of heat shock protein 70-interacting protein (CHIP), a U-box-type E3 ubiquitin ligase, in CG is associated with poor prognosis (Liang et al. 2013).

## Cell Adhesion and Motility

Deranged cell-to-cell or cell-to-matrix contact mediated by adhesion molecules plays a crucial role within the invasion cascade. Several factors involved in adhesion are altered in CG. Epithelial cell adhesion molecule (Ep-CAM) is overexpressed in almost two thirds of CG, and overexpression of this factor is related to decreased overall survival and is an independent prognostic factor (Varga et al. 2004; Prince et al. 2008). The L1 adhesion molecule, associated with prognosis in several malignancies, is not expressed in normal gallbladder epithelium, but was detectable in 63.8 % of CG, specifically in cells located to the invasive front of the neoplasm. Expression of L1 in CG cells was correlated with high histologic grade, advanced T stage, and venous and lymphatic invasion and was an independent negative prognosticator in multivariate

analysis (Choi et al. 2011). Expression of nectin-2, an adhesion molecule involved in calcium-independent cell adhesion, in CG is correlated to high T stage and poor prognosis (Miao et al. 2013). Thrombospondin-1 (TSP1), an extracellular glycoprotein that affects cell adhesion, motility, and growth, is mainly expressed in CG stromal cells and less so in cancer cells. Stromal expression of TSP1 increased as a function of increasing stage and was associated with lymph node metastasis and venous involvement, suggesting that TSP1 plays a role in CG spread and metastasis (Ohtani et al. 1999). Fascin, a protein interacting with thrombospondin-1, shows a marked overexpression in advanced CG, associated with aggressive clinical features and poor overall survival. Fascin, thrombospondin-1, and syndecan-1 interact to mediate this aggressive phenotype of CG (Roh et al. 2009). Syndecan-1 expression itself was detected in 58.1 % of CG, and syndecan-1-positive neoplasms more frequently showed lymph node metastasis and tended to have a deeper invasion depth and significantly shorter survival (Roh et al. 2008). Significant differences of E-cadherin and beta-catenin expression were detected between normal, inflamed, and cancerous gallbladder tissues (Puhalla et al. 2005). Loss of E-cadherin expression is high in CG (Priya et al. 2010), an alteration that may contribute to failure of intercellular adhesion, individualization of cells, and promotion of an invasive phenotype. N-cadherin and P-cadherin are biomarkers for invasion and metastasis in CG (Yi et al. 2014). Alterations of beta-catenin signaling are frequent events in CG, but these alterations seem to be minor contributors to CG carcinogenesis, but may be related to progression via loss of E-cadherin function (Kimura et al. 2003). Expression of the integrin-linked kinase (ILK) was found to be an independent poor prognostic predictor in CG (Li et al. 2013). CD97, a member of the EGF-TM7 adhesin family binding to its cellular receptor, CD55, plays an important role in invasiveness and aggressiveness of malignancies. It is a complement regulatory protein expressed by cells to protect them from bystander complement attack. CD97 and CD55 are absent or weakly expressed in normal gallbladder epithelium, but are overexpression CG, this expression being associated with high histologic grade, advanced T stage, angioinvasion, and reduced overall survival (Wu et al. 2012). CD44, a molecule involved in the metastatic pathway of cancers, exists in several variants or isoforms, including CD44s (standard variant), variant 3 (CD44v3), and variant 6 (CD44v6). CD44s is present in normal gallbladder epithelium and exhibits membranous immunostaining. In CG, CD44s is stained as strongly as normal epithelium, but reactivity for CD44v3 and CD44v6 was also found. CD44s is significantly less expressed in well-differentiated CG in the invasive component of the tumor than in the intramucosal part, and both CD44v3 and CD44v6 are more strongly expressed in poorly differentiated CG than in well-differentiated CG, but these expression patterns were not associated with outcome (Yanagisawa et al. 2001). Trefoil factor family protein 1/TFF1 (pS2), a factor that interacts with mucins to protect gastrointestinal epithelial cells and which promotes epithelial cell migration, is expressed in low-stage CG, and expression decreases as a function of increased grade and stage. Patients with TFF1-positive CG revealed a more favorable outcome compared with TFF1-negative neoplasms (Kornprat et al. 2005). As other carcinomas, CG displays alterations of the cytoskeleton and cytoskeletal proteins, changes that affect cell motility and cancer cell migration as a component of the invasion cascade. Cofilin-1, an actin-modulating protein that depolymerizes filamentous F actin and inhibits the polymerization of monomeric G actin, is expressed in CG, whereby expression was significantly associated with large tumor size, high T stage, lymph node metastasis, and decreased overall survival (Yang et al. 2013).

## Mechanisms of Invasion and Spread

CG cells express several members of matrix metalloproteinases (MMPs), enzymes that have a central role in the tumor invasion cascade, as they facilitate the egress of cancer cells from the primary tumor and the locomotion and migration of

tumor cells in invaded tissues (Fan et al. 2002). MMP-9 and MMP-14 play a role in CG tumorigenesis (Karadag et al. 2008). MMP-1 and protease-activated receptor-1 are expressed in approximately 70 % of CG. CG expressing these two proteins more often showed lymph node metastasis, a deeper invasion depth, and more frequently lymphovascular invasion (Du et al. 2011). Expression of MMP-2 negatively affected the survival rate (Wu et al. 2009). In mucinous CG, which has a biology different from that of ordinary CG, MMP expression is lower (Karadag et al. 2008). The transcription and expression of MMPs in CG modulated by the neural precursor cell expressed developmentally downregulated 4-lik (Nedd4L) protein. Overexpression of Nedd4L in CG regulates the expression of MMP1 and MMP13 (Takeuchi et al. 2011). ADAM proteins, a multifunctional gene family of membrane proteins having a disintegrin and metalloprotease domain and involved in the metastatic cascade, are overexpressed in CG, this feature being associated with poor prognosis (Wu et al. 2011).

## Expression of Differentiation Antigens and Factors Related to Cancer Cell Metabolism

Carcinoembryonic antigen (CEA), which may function as a metastatic potentiator via modulation of intercellular adhesion and cell migration, is expressed in CG cells and CG stromal cells. Lymph node metastasis was frequently found in cytoplasmic and stromal cell CEA-positive CG (Dowaki et al. 2000). Stromal expression of the mucin MUC1 in CG is associated with tumor aggressiveness and a tendency to form distant organ metastases (Kashiwagi et al. 2001; Kawamoto et al. 2004), while expression of MUC2 was not significantly related to lymphatic invasion, lymph node metastasis, or prognosis (Kashiwagi et al. 2001). Tumor-associated glycoprotein 72 is expressed more frequently in CG of larger size, with lymph node metastasis, with higher stage, and with poor differentiation, suggesting that expression of this antigen is a marker of CG aggressiveness (Ouyang et al. 2010). CD24, a small surface protein, is a marker of malignancy and poor prognosis in CG (Liu et al. 2011). Part of CGs express the hepatocyte antigen (Hep). Expression of Hep was negatively correlated to the grade of differentiation, tumor size, and lymph node metastasis, and elevated Hep expression was associated with increased overall survival, Hep expression being an independent prognostic predictor (Li et al. 2011). Forced expression of LAPTM4B in a CG cell line increased the invasive potential (Zhou et al. 2007, 2010). CG expresses neurotrophin, nerve growth factor, and the receptor TrkA (Artico et al. 2010), but the biologic significance of this expression has not yet been elucidated. Glucose uptake is generally augmented in cancer cells. The glucose transporter, GLUT1, is expressed in CG, and this elevated expression is strongly associated with cancer progression (Kim et al. 2002).

## Angiogenesis and Lymphangiogenesis

Angiogenesis of tumors, induced and controlled by complex signaling pathways involving VEGF, plays a significant role in tumor progression, including CG (Giatromanolaki et al. 2002; Harino et al. 2008). Increased tumor vascularization caused by angiogenic mechanisms plays a significant role in cancer progression. A high microvessel density in CG determined by Chalkley counting was associated with worse prognosis (da Rocha et al. 2009). In CG, microvessel density was found to increase as a function of depth of invasion, suggesting a role of angiogenesis in the invasive process (Kalekou and Miliaras 2011). Microvessel density correlated with tumor stage and liver metastasis (Chen et al. 2011b). However, microvessel density was not an independent prognosticator in multivariate analysis (Sugawara et al. 1999). Average microvessel counts were lower in cases of well-differentiated carcinoma, small tumor size (less than 2-cm diameter), and negative nodes,

while higher microvessel counts and expression of CD146 in CG were associated with poor survival (Wang et al. 2012b). In a fraction of CG showing augmented angiogenesis, vascular endothelial growth factor/VEGF is expressed (Yamamoto et al. 1998; Giatromanolaki et al. 2003). The presence and expression level of VEGF were associated with tumor size, lymphatic invasion, and advanced disease stage (Okita et al. 1998; Letelier et al. 2014). Expression of platelet-derived endothelial growth factor/thymidine phosphorylase was detected in 63 % of CG, while hyperplastic epithelia or adenomas did not show significant expression. However, the magnitude of expression did not correlate with angiogenesis, but with depth of invasion, lymph node metastasis, and tumor stage (Yamamoto et al. 2000). Cyclooxygenase-2, which affects the expression of VEGF, induces angiogenesis in CG and is associated with poor prognosis (Zhi et al. 2005). A similar effect was found for the expression of inducible nitric oxide synthase (Niu et al. 2004). Tumor endothelial marker 8 (TEM8) protein is highly specific to angiogenesis in malignant neoplasms and is not required for normal adult angiogenesis. TEM8 is expressed in endothelial cells of CG, and its expression increased significantly with increasing CG stage (Maurya et al. 2011). TGF-beta, a protein with multiple functions, also promotes angiogenesis and is expressed in CG, where it augments angiogenesis and is associated with tumor progression (Kitamura et al. 2003).

CG shows vigorous lymphangiogenesis mediated by VEGF-C. TNF-alpha promotes formation of lymphatic vessels in CG through NF-kappaB-mediated upregulation of VEGF-C (Qiang et al. 2014). Expression of vascular endothelial growth factor-D in CG promotes growth, lymphangiogenesis, and lymphatic metastasis in CG and plays a role in CG progression (Lin et al. 2012).

Vasculogenic mimicry, a phenomenon associated with increased tumor-related mortality in several cancers, was observed in CG cell lines cultured in a 3D matrix, and vasculogenic mimicry in these cells was stimulated by HIF-1alpha. In CG analyzed in vivo, vasculogenic mimicry was also observed, and HIF-1alpha expression and vasculogenic mimicry were associated with poorer overall survival (Sun et al. 2012). Vasculogenic mimicry in CG is mediated by signaling pathways that involve PI3K/MMPs/Ln-5gamma2 and EphA2/FAK/paxillin networks (Lu et al. 2013).

## Tumor Ploidy

Abnormal DNA contents were observed in 51 % of CG, whereby 44.4 % of tumors were aneuploid. In comparison, aneuploidy was detected in 81.3 % of metastatic lesions (Roa et al. 1993), suggesting effects of progressive genomic instability. Aneuploidy of CG was significantly associated with poorly differentiated adenocarcinoma, higher T stage, and a high mitotic index. A significant advantage in terms of 5-year survival was found in patients with diploid neoplasms in comparison with those having aneuploid tumors (Sato et al. 1993). In contrast, cytometric determination of DNA ploidy provided no prognostic information in CG as compared to conventional tumor staging in other studies (Yamamoto et al. 1990; Baretton et al. 1994).

## MicroRNAs

MicroRNA-34 is associated with poor prognosis in CG through the regulation of telomere length in stem cells (Jin et al. 2014). An increased expression of microRNA-335 in CG predicts a favorable prognosis, but this miRNA is downregulated in the majority of CG, being one factor that may determine the aggressive biology of this cancer (Peng et al. 2013). Upregulation of the prometastatic microRNA-20a was closely associated with local invasion, distant metastasis, and poor prognosis (Chang et al. 2013). MicroRNA-155 was overexpressed in CG in comparison with nonneoplastic gallbladder, associated with the presence of nodal metastases and poor prognosis (Kono et al. 2013).

## Staging

Determination of tumor stage is a decisive factor in prognostication of CG. However, up to 40 % of CG cases are diagnosed at an advance stage of disease (Henson et al. 1992), limiting the differential estimation of outcome as a function of stage. The current staging system formulated by the AJCC is shown in Table 2. It corresponds to the UICC system (Fong et al. 2006; Gore and Shelhamer 2007; Edge et al. 2010).

The advantages of pitfalls of the AJCC/UICC have recently been discussed (Adsay et al. 2012). The gallbladder does not have the distinct layering as other gastrointestinal organs do. Therefore, it was stated that the definitions of Tis/T1a/T1b may lack practicability, and therefore, the "early gallbladder carcinoma" category proposed in high regions may have to be recognized instead. Furthermore, it was proposed that documentation of hepatic versus serosal involvement should be performed in cases of advanced tumors (Adsay et al. 2012).

In addition to T stage, which describes depth of invasion and extension into adjacent organs and/or structures, other modes of invasion have been proposed as prognostically relevant parameters. T2 carcinomas with subserosal invasion form a distinct group of gallbladder cancers that may profit from radical surgery including resection of the gallbladder bed (Chijiiwa et al. 2001). In T2 tumors, free resection margins and the absence of perineural invasion and of lymph node metastasis are related to good prognosis. The depth of subserosal invasion in pT2 tumors has an impact on outcome in patients with pT2 neoplasms (Wakai et al. 2003). Depth of invasion of the subserosal layer was divided into three categories, i.e., ss1, ss2, and ss3, representing invasion of the upper, middle, or lower thirds of the subserosal layer, respectively (Sasaki et al. 2005). Expression of N-acetylglucosaminyl-transferase V, an enzyme that catalyzes the beta1-6 branching of N-acetyl-glucosamine on asparagine-like oligosaccharides of cellular proteins and enhances the malignant features of cancer cells, is expressed in CG located to the subserosal layer and correlates with postsurgical survival in pT2 tumors (Onuki et al. 2014). Expression of MUC1 mucins in the subserosal layer correlated with postsurgical prognosis (Kawamoto et al. 2001). A further prognostic feature related to stage is the mural invasion

**Table 2** AJCC staging of gallbladder carcinoma (7th edition; Edge et al. 2010)

| **T** | | | |
|---|---|---|---|
| TX | Primary tumor cannot be assessed | | |
| T0 | No evidence of primary tumor | | |
| Tis | Carcinoma in situ | | |
| T1 | Tumor invades the lamina propria or muscular layer | | |
| T1a | Tumor invades the lamina propria | | |
| T1b | Tumor invades the muscular layer | | |
| T2 | Tumor invades perimuscular connective tissue; no extension beyond the serosa or into the liver | | |
| T3 | Tumor perforates the serosa (visceral peritoneum) and/or directly invades the liver and/or one other adjacent organ or structure, such as the stomach, duodenum, colon, pancreas, omentum, or extrahepatic bile ducts | | |
| T4 | Tumor invades the main portal vein or hepatic artery or invades at least two extrahepatic organs or structures | | |
| **N** | | | |
| NX | Regional lymph nodes cannot be assessed | | |
| N0 | No regional lymph node metastasis | | |
| N1 | Metastases to nodes along the cystic duct, common bile duct, hepatic artery, and/or portal vein | | |
| N2 | Metastases to periaortic, pericaval, superior mesenteric artery, and/or celiac artery lymph nodes | | |
| **M** | | | |
| M0 | No distant metastasis | | |
| M1 | Distant metastasis | | |
| **Anatomic stage/prognostic groups** | | | |
| *Stage* | *T* | *N* | *M* |
| 0 | Tis | N0 | M0 |
| I | T1 | N0 | M0 |
| II | T2 | N0 | M0 |
| IIIA | T3 | N0 | M0 |
| IIIB | T1-3 | N1 | M0 |
| IVA | T4 | N0-1 | M0 |
| IVB | Any T | N2 | M0 |
| | Any T | Any N | M1 |

Generally, CGs of stages T1 and T2 are classified as early CG

There are some notable differences between the UICC staging system and the staging system of the Japanese Society of Biliary Surgery (JSBS) staging system (Japanese Society of Biliary Surgery 2001; Table 3)

**Table 3** Gallbladder cancer extension classified according to the 2001 version of the classification of biliary tract carcinoma of JSBS (2001)

| | |
|---|---|
| **T** (tumor categories) | |
| T1 | S0, Hinf0, Binf0, PV0, A0 |
| T2 | S1, Hinf1, Binf1, PV0, A0 |
| T3 | S2, Hinf2, Binf2, PV1, A1 |
| T4 | S3, any Hinf, Binf3, PV2,3, A2,3 |
| **S** (grade of serosal invasion) | |
| S0 | No invasion of the serosa |
| S1 | Doubtful invasion of the serosa |
| S2 | Definite invasion of the serosa |
| S3 | Invasion of other organs or structures |
| **Hinf** (grade of hepatic invasion) | |
| Hinf0 | No direct invasion of the liver |
| Hinf1 | Doubtful direct invasion of the liver |
| Hinf2 | Definite direct invasion of the liver and invasion around the gallbladder bed |
| Hinf3 | Mass formation because of direct invasion of the liver |
| **Binf** (grade of hepatoduodenal ligament (bile duct) invasion | |
| Binf0 | No invasion of the hepatoduodenal ligament |
| Binf1 | Doubtful invasion of the hepatoduodenal ligament |
| Binf2 | Definite invasion of the hepatoduodenal ligament |
| Binf3 | Severe invasion of the hepatoduodenal ligament |
| **PV** (grade of portal vein invasion) | |
| PV0 | No invasion of any of portal veins |
| PV1 | Doubtful invasion of portal veins |
| PV2 | Definite invasion of portal veins |
| PV3 | Severe invasion of portal veins (narrowing or constriction) |
| **A** (grade of hepatic artery invasion) | |
| A0 | No invasion of any of hepatic arteries |
| A1 | Doubtful invasion of hepatic arteries |
| A2 | Definite invasion of hepatic arteries |
| A3 | Severe invasion of hepatic arteries (narrowing or constriction) |
| **N** (lymph node involvement) | |
| N0 | No evidence of lymph node metastasis |
| N1 | Lymph node involvement in a primary lymph node group close to tumor |
| N2 | Lymph node involvement in a secondary lymph node group |
| N3 | Lymph node involvement in a tertiary lymph node group |
| N4 | Lymph node involvement in the fourth lymph node group |

(*continued*)

**Table 3** (continued)

| **Staging system** | | | |
|---|---|---|---|
| Stage I | T1 | N0 | M0 |
| Stage II | T1 | N1 | M0 |
| | T2 | N0, N1 | M0 |
| Stage III | T1, T2 | N2 | M0 |
| | T3 | N0, N1, N2 | M0 |
| Stage IVA | T4 | N0, N1, N2 | M0 |
| | Any T | N3 | M0 |
| Stage IVB | Any T | N4 | M0 |
| | Any T | Any N | M1 |

pattern of CG. Two intramural invasion patterns were defined as the infiltrative growth type (IG, infiltrative growth in the muscle layer without destruction) and a destructive growth type (DG, massive growth with destruction of the muscle layer; Okada et al. 2009a). Scirrhous growth was found more often in DG lesions, and the overall survival rate of patients with DG tumors was significantly lower than that of patients with IG tumors (Okada et al. 2009a). A negative effect of the DG pattern was also found in pT2 CG with a subserosa-invasive growth pattern (Okada et al. 2012). Tumors with a DG growth pattern show a higher proliferative activity, a poorer differentiation, a stromal laminin-5gamma2 chain expression, and a distant lymph node metastasis (Okada et al. 2009b).

## References

Adsay NV, Bagci P, Tajiri T, Oliva I, Ohike N, Balci S, Ganzalez RS, Basturk O, Jang KT et al (2012) Pathologic staging of pancreatic, ampullary, biliary, and gallbladder cancers: pitfalls and practical limitations of the current AJCC/UICC TNM staging system and opportunities for improvement. Semin Diagn Pathol 29:127–141

Ajiki T, Onoyama H, Yamamoto M, Asaka K, Fujimori T, Maeda S, Saitoh Y (1996) p53 protein expression and prognosis in gallbladder carcinoma and premalignant lesions. Hepatogastroenterology 43:521–526

Albores-Saavedra J, Henson DE (1999) Pyloric gland metaplasia with perineural invasion of the gallbladder: a lesion that can be confused with adenocarcinoma. Cancer 86:2625–2631

Albores-Saavedra J, Keenportz B, Bejarano PA, Alexander AA, Henson DE (2007) Adenomyomatous hyperplasia

of the gallbladder with perineural invasion: revisited. Am J Surg Pathol 31:1598–1604
Albores-Saavedra J, Chable-Montero F, Angeles-Albores-D, Schwartz A, Klimstra DS et al (2011) Early gallbladder carcinoma: a clinicopathologic study of 13 cases of intramucosal carcinoma. Am J Clin Pathol 135:637–642
Al-Qudah MS (1994) Intraluminal implantation of gallbladder cancer into the bile ducts. Br J Surg 81:590
Alsheyab FM, Ziadeh MT, Bani-Hani KE (2007) Expression of p21 and p27 in gallbladder cancer. Saudi Med J 28:683–687
Alvarez H, Corvalan A, Roa JC, Argani P, Murillo F, Edwards J, Beaty R, Feldmann G et al (2008) Serial analysis of gene expression identifies connective tissue growth factor expression as a prognostic biomarker in gallbladder cancer. Clin Cancer Res 14:2631–2638
Andrieux J, Parache RM, Fery M (1972) A further case of Krukenberg's tumor manifesting a vesiculocholedochal cancer (in French). Chirurgie 98:219–223
Artico M, Bronzetti E, Alicino V, Ionta B, Bosco S, Grande C, Bruno M, Transquili Leali FM et al (2010) Human gallbladder carcinoma: role of neurotrophins, MIB-1, CD34 and CA15-3. Eur J Histochem 54:e10
Baretton G, Blasenbreu S, Vogt T, Löhrs U, Rau H, Schmidt M (1994) DNA ploidy in carcinoma of the gallbladder. Prognostic significance and comparison of flow and image cytometry on archival tumor material. Pathol Res Pract 190:584–592
Barsoum and Windsor 1992. http://www.ncbi.nlm.nih.gov/pubmed/1393498
Bickenbach KA, Shia J, Klimstra DS, DeMatteo RP, Fong Y, Kingham TP, Allen PJ et al (2011) High-grade dysplasia of the cystic duct margin in the absence of malignancy after cholecystectomy. HPB (Oxford) 13:865–868
Bork K, Schreiber J, Bräuninger W (2002) Umbilical metastasis of a gallbladder carcinoma: "Sister Mary Joseph's nodule" (in German). Dtsch Med Wochenschr 127:553–556
Burdette WJ (1957) Carcinoma of the gallbladder. Ann Surg 145:832–844
Cai W, Li Q, Yang Z, Miao X, Wen Y, Huang S, Ouyang J (2013) Expression of p53 upregulated modulator of apoptosis (PUMA) and C-myb in gallbladder adenocarcinoma and their pathological significance. Clin Transl Oncol 15:818–824
Cappell DF, Tudhope GR (1934) Unrevealed primary carcinoma of the gall-bladder. Glasgow Med J 122:185
Chakravarty KD, Yeh CN, Jan YY, Chen MF (2009) Factors influencing long-term survival in patients with T3 gallbladder adenocarcinoma. Digestion 79:151–157
Chan KM, Yeh TS, Yu MC, Jan YY, Hwang TL, Chen MF (2005) Gallbladder carcinoma with biliary invasion: clinical analysis of the differences from nonbiliary invasion. World J Surg 29:72–75
Chang Y, Liu C, Yang J, Liu G, Feng F, Tang J, Hu L, Li L, Jiang F, Chen C, Wang R et al (2013) MiR-20a triggers metastasis of gallbladder carcinoma. J Hepatol 59:518–527
Chaudhari S, Hatwal D, Bhat P (2014) A rare presentation of gallbladder carcinoma metastasis. J Clin Diagn Res 8:FD19–FD20
Chen TJ, Huang SC, Huang HY, Wei YC, Li CF (2011a) Rsf-1/HBXAP overexpression is associated with disease-specific survival of patients with gallbladder carcinoma. APMIS 119:808–814
Chen Y, Chen Y, Yu G, Ding H (2011b) Lymphangiogenic and angiogenic microvessel density in gallbladder carcinoma. Hepatogastroenterology 58:20–25
Chicos SC, Beznea A, Chebac GR, Ceausu M, Ceausu M (2007) The Krukenberg tumor caused by an adenocarcinoma of the gallbladder (in Romanian). Chirurgia (Bucur) 102:481–485
Chijiiwa K, Nakano K, Ueda J, Noshiro H, Nagai E, Yamaguchi K, Tanaka M (2001) Surgical treatment of patients with T2 gallbladder carcinoma invading the subserosal layer. J Am Coll Surg 192:600–607
Chikamoto A, Tsuji T, Nakahara O, Sakamoto Y, Ikuta Y, Tanaka H, Takamori H, Hirota M et al (2009) Cancer cells spread through lymph vessels in the submucosal layer of the common bile duct in gallbladder carcinoma. J Hepatobiliary Pancreat Surg 16:557–561
Cho SY, Han SS, Park SJ, Kim YK, Kim SH, Woo SM, Lee WJ, Kim TH, Hong EK (2012) T-category reflects the histopathologic characteristics of gallbladder cancer. Eur J Surg Oncol 38:537–542
Choi SY, Jo YS, Huang SM, Liang ZL, Min JK, Hong HJ, Kim JM (2011) L1 cell adhesion molecule as a novel independent poor prognostic factor in gallbladder carcinoma. Hum Pathol 42:1476–1483
Choi BG, Kim CY, Cho SH, Kim HJ, Koh YS, Kim JC, Cho CK, Kim HJ, Hur YH (2013) Impact of lymph node ratio as a valuable prognostic factor in gallbladder carcinoma, focusing on stage IIIB gallbladder carcinoma. J Korean Surg Soc 84:168–177
Clair DG, Lautz DB, Brooks DC (1993) Rapid development of umbilical metastases after laparoscopic cholecystectomy for unsuspected gallbladder carcinoma. Surgery 113:355–358
Clermont D (1909) Lymphatiques des voies biliaires: cancer de la vésicule (anatomie pathologique et traitement chirurgical). Thesis, University of Toulouse, France
Commandre F, Dalbera-Gayraud L, Besson R, Berato J, Gilly R (1965) A case of cancer of the gallbladder with multiple osseous metastases (in French). Arch Mal Appar Dig Mal Nutr 54:1174–1176
Cooper WA (1937) Carcinoma of the gallbladder. Arch Surg 35:431–448
Copher JC, Rogers JJ, Dalton ML (1995) Trocar-site metastasis following laparoscopic cholecystectomy for unsuspected carcinoma of the gallbladder. Case report and review of the literature. Surg Endosc 9:348–350
D'Hondt M, Lapointe R, Benamira Z, Pottel H, Plasse M, Letourneau R, Roy A, Dagenais M (2013) Carcinoma of the gallbladder: patterns of presentation, prognostic

factors and survival rate. An 11-year single centre experience. Eur J Surg Oncol 39:548–553
da Rocha AO, Coutinho LM, Schall JG (2009) The prognostic value of angiogenesis by Chalkley counting in gallbladder carcinoma. Hepatogastroenterology 56:34–38
Dowaki S, Kijima H, Kashiwagi H, Ohtani Y, Tobita K, Tsukui M, Tanaka Y, Tazawa K et al (2000) CEA immunohistochemical localization is correlated with growth and metastasis of human gallbladder carcinoma. Int J Oncol 16:49–53
Du X, Wang S, Lu J, Cao Y, Song N, Yang T, Dong R, Zang L, Yang Y, Wu T, Li J (2011) Correlation between MMP1-PAR1 axis and clinical outcome of primary gallbladder carcinoma. Jpn J Clin Oncol 41:1086–1093
Du X, Zhao H, Zang L, Song N, Yang T, Dong R, Yin J, Wang C, Lu J (2013a) Overexpression of histone deacetylase 2 predicts unfavorable prognosis in human gallbladder carcinoma. Pathol Oncol Res 19:397–403
Du X, Wu T, Lu J, Zang L, Song N, Yang T, Zhao H, Wang S (2013b) Decreased expression of chromodomain helicase DNA-binding protein 5 is an unfavorable prognostic marker in patients with primary gallbladder carcinoma. Clin Transl Oncol 15:198–204
Dutta U (2012) Gallbladder cancer: can newer insights improve the outcome ? J Gastroenterol Hepatol 27:642–653
Edge SB, Byrd DR, Compton CC et al (eds) (2010) AJCC cancer staging manual, 7th edn. Springer, New York, pp 211–217
Egawa N, Tu Y, Kamisawa T, Amemiya K, Tsuruta K, Okamoto A, Funata N (2004) A comparative clinical study of gallbladder tubular adenocarcinoma according to the grade of differentiation. J Gastroenterol 39:975–980
Eguchi N, Fujii K, Tsuchida A, Yamamoto S, Sasaki T, Kajiyama G (1999) Cyclin E overexpression in human gallbladder carcinomas. Oncol Rep 6:93–96
Eil R, Hansen PD, Cassera M, Orloff SL, Sheppard BC, Diggs B, Billingsley KG (2013) Bile duct involvement portends poor prognosis in resected gallbladder carcinoma. Gastrointest Cancer Res 6:101–105
Endo I, Shimada H, Takimoto A, Fujii Y, Miura Y, Sugita M, Morioka D, Masunari H et al (2004) Microscopic liver metastasis: prognostic factor for patients with pT2 gallbladder carcinoma. World J Surg 28:692–696
Evans HJ (1994) Intraluminal implantation of gallbladder cancer into the bile ducts. Br J Surg 81:1393–1394
Evrard S, Mutter D, Bui A, Marescaux J (1996) Neoplastic seeding in the gallbladder fossa after laparoscopic cholecystectomy. Surgery 119:357
Fahim RB, McDonald JR, Richards JC, Ferris DO (1962) Carcinoma of the gallbladder: a study of its modes of spread. Ann Surg 156:114–124
Fahim RB, Ferris DO, McDonald JR (1963) Carcinoma of the gallbladder: a appraisal of its surgical treatment. Arch Surg 86:334
Fan YZ, Zhang JT, Yang HC, Yang YQ (2002) Expression of MMP-2, TIMP-2 protein and the ratio of MMP-2/TIMP-2 in gallbladder carcinoma and their significance. World J Gastroenterol 8:1138–1143
Filipits M, Puhalla H, Wrba F (2003) Low p27Kip1 expression is an independent prognostic factor in gallbladder carcinoma. Anticancer Res 23:675–679
Fine A (1997) The cystic vein: the significance of a forgotten anatomic landmark. JSLS 1:263–266
Fong Y, Wagman L, Gonen M, Crawford J, Reed W, Swanson R, Pan C, Ritchey J et al (2006) Evidence-based gallbladder cancer staging. Changing cancer staging by analysis of data from the National Cancer database. Ann Surg 243:767–774
Frank SA, Spjut HJ (1967) Inapparent carcinoma of the gallbladder. Am Surg 33:367–372
Garcia P, Leal P, Alvarez H, Brebi P, Ili C, Tapia O, Roa JC (2013) Connective tissue growth factor immunohistochemical expression is associated with gallbladder cancer progression. Arch Pathol Lab Med 137:245–250
Giatromanolaki A, Sivridis E, Koukourakis MI, Polychronidis A, Simopoulos C (2002) Prognostic role of angiogenesis in operable carcinoma of the gallbladder. Am J Clin Oncol 25:38–41
Giatromanolaki A, Koukourakis MI, Simopoulos C, Polychronidis A, Sivridis E (2003) Vascular endothelial growth (VEGF) expression in operable gallbladder carcinomas. Eur J Surg Oncol 29:879–883
Glenn F, Hays DM (1954) The scope of radical surgery in the treatment of malignant tumors of the extrahepatic biliary tract. Surg Gynecol Obstet 99:529–541
Gomez-Roel X, Arrieta O, Leon-Rodriguez E (2007) Prognostic factors in gallbladder and biliary tract cancer. Med Oncol 24:77–83
Gore RM, Shelhamer RP (2007) Biliary tract neoplasms: diagnosis and staging. Cancer Imaging 7:S15–S23
Gunjiganvi M, Singh KK, Harsha HS, Bipin T (2013) Cardiac metastasis from gallbladder carcinoma. Int J Surg Case Rep 4:1156–1158
Gupta P, Agarwal A, Gupta V, Singh PK, Pantola C, Amit S (2012) Expression and clinicopathological significance of estrogen and progesterone receptors in gallbladder cancer. Gastrointest Cancer Res 5:41–47
Habighorst LV, Albers P, Zeitler E (1965) Vessels of the gallbladder in postmortem angiography (in German). Fortschr Geb Rontgenstr Nuklearmed 103:63–70
Hamrick RE, Liner J, Hastings PR, Cohn I (1982) Primary carcinoma of the gallbladder. Ann Surg 195:270–273
Harino Y, Imura S, Kanemura H, Morine Y, Fujii M, Ikegami T, Uehara H, Shimada M (2008) Role of tumor angiogenesis in gallbladder carcinoma: with special reference to thymidine phosphorylase. Int J Clin Oncol 13:452–457
Hart J, Modan B, Hashomer T (1972) Factors affecting survival of patients with gallbladder neoplasms. Arch Intern Med 129:931–934
Heavey SF, Roeland EJ, Tipps AM, Datnow B, Sicklick JK (2014) Rapidly progressive subcutaneous metastases from gallbladder cancer: insight into a rare presentation

in gastrointestinal malignancies. J Gastrointest Oncol 5:E58–E64
Henson DE, Albores-Saavedra J, Corle D (1992) Carcinoma of the gallbladder. Histologic types, stage of disease, grade and survival rates. Cancer 170:1493–1497
Hidalgo Grau LA, Badia JM, Salvador CA, Manso TS, Canaleta JF, Nogues JM et al (2004) Gallbladder carcinoma: the role of p53 protein overexpression and Ki-67 antigen expression as prognostic markers. HPB (Oxford) 6:174–180
Hollings RM (1963) Tumour embolism of the common bile duct – a cause of jaundice in carcinoma of the gall-bladder. Med J Aust 2:618–620
Huan P, Maosheng T, Zhigian H, Long C, Xiaojun Y (2012) TLR4 expression in normal gallbladder, chronic cholecystitis and gallbladder carcinoma. Hepatogastroenterology 59:42–46
Hui AM, Li X, Shi YZ, Takayama T, Torzilli G, Makuuchi M (2000) Cyclin D1 overexpression is a critical event in gallbladder carcinogenesis and independently predicts decreased survival for patients with gallbladder carcinoma. Clin Cancer Res 6:4272–4277
Hui AM, Shi YZ, Li X, Sun L, Guido T, Takayama T, Makuuchi M (2002) Proliferative marker Ki-67 in gallbladder carcinomas: high expression level predicts early recurrence after surgical resection. Cancer Lett 176:191–198
Ito M, Mishima Y, Sato T (1991) An anatomical study of the lymphatic drainage of the gallbladder. Surg Radiol Anat 13:89–104
Itoi T, Shinohara Y, Takeda K, Nakamura K, Takei K, Sanada J, Horibe T, Saito T et al (2000) Nuclear cyclin D1 overexpression is a critical event associated with cell proliferation and invasive growth in gallbladder carcinogenesis. J Gastroenterol 35:142–149
Jacobi CA, Keller H, Mönig S, Said S (1995) Implantation metastasis of unsuspected gallbladder carcinoma after laparoscopy. Surg Endosc 9:351–352
Japanese Society of Biliary Surgery (2001) Classification of biliary tract carcinoma, 1st edn. Kanehara, Tokyo
Jeon HM, Kim JS, Lee CD, Kim EK, Kim SN (1999) Late development of umbilical metastasis after laparoscopic cholecystectomy for a gallbladder carcinoma. Oncol Rep 6:283–287
Jeyaraj P, Sio TT, Iott MJ (2013) An unusual case of isolated, serial metastases of gallbladder carcinoma involving the chest wall, axilla, breast and lung parenchyma. Rare Tumor 5:e7
Jin K, Xiang Y, Tang J, Wu G, Li J, Xiao H, Li C, Chen Y, Zhao J (2014) miR-34 is associated with poor prognosis of patients with gallbladder cancer through regulating telomere length in tumor stem cells. Tumour Biol 35:1503–1510
Johnson LA, Lavin PT, Dayal YY, Geller SA, Doos WG, Cooper HS, Gerber JE et al (1987) Gallbladder adenocarcinoma: the prognostic significance of histologic grade. J Surg Oncol 34:16–18
Jones CJ (1950) Carcinoma of the gallbladder: a clinical and pathologic analysis of fifty cases. Ann Surg 132:110–120
Kai K, Kohya N, Kitahara K, Masuda M, Miyoshi A, Ide T, Tokunaga O, Miyazaki K, Noshiro H (2011) Tumor budding and dedifferentiation in gallbladder carcinoma: potential for the prognostic factors in T2 lesions. Virchows Arch 459:449–456
Kainberger FM, Vergesslich KA, Eilenberger M, Poeltner S, Ponhold W (1990) Color-coded Doppler evaluation of cholecystic varices in portal hypertension. Pediatr Radiol 21:71–72
Kalekou H, Miliaras D (2011) Cytokeratin 7 and 20 expression in gallbladder carcinoma. Pol J Pathol 62:25–30
Kaneoka Y, Yamaguchi A, Isogai M, Harada T, Suzuki M (2003) Hepatoduodenal ligament invasion by gallbladder carcinoma: histologic patterns and surgical recommendation. World J Surg 27:260–265
Karadag N, Kirimlioglu H, Isik B, Yilmaz S, Kirimlioglu V (2008) Expression of matrix metalloproteinases in gallbladder carcinoma and their significance in carcinogenesis. Appl Immunohistochem Mol Morphol 16:148–152
Karamitopoulou E, Tornillo L, Zlobec I, Cioccari L, Carafa V, Borner M, Schaffner T et al (2008) Clinical significance of cell-cycle and apoptosis-related markers in biliary tract cancer: a tissue microarray-based approach revealing a distinctive immunophenotype for intrahepatic and extrahepatic cholangiocarcinoma. Am J Clin Pathol 130:780–786
Karayannakis AJ, Knight MJ (1997) Umbilical port metastasis from gallbladder carcinoma after laparoscopic cholecystectomy. Eur J Surg Oncol 23:186–187
Karlmark E (1932) Die Lokalisationstendenz bei Metastasierung durch die Venen in die Leber. Acta Pathol Microbiol Scand 18(Suppl 13):1–203
Kashiwagi H, Kijima H, Dowaki S, Ohtani Y, Tobita K, Tsukui M, Tanaka Y, Matsubayashi H et al (2000) DF3 expression in human gallbladder carcinoma: significance for lymphatic invasion. Int J Oncol 16:455–459
Kashiwagi H, Kijima H, Dowaki S, Ohtani Y, Tobita K, Yamazaki H, Nakamura M et al (2001) MUC1 and MUC2 expression in human gallbladder carcinoma: a clinicopathological study and relationship with prognosis. Oncol Res 8:485–489
Kawamoto T, Shoda J, Asano T, Ueda T, Furukawa M, Kawamoto T, Shoda J, Irimura T, Miyahara N, Furukawa M, Ueda T, Asano T, Kano M et al (2001) Expression of MUC1 mucins in the subserosal layer correlates with postsurgical prognosis of pathological tumor stage 2 carcinoma of the gallbladder. Clin Cancer Res 7:1333–1342
Kawamoto et al. 2002. http://www.ncbi.nlm.nih.gov/pubmed/11920595
Kawamoto T, Shoda J, Miyahara N, Suzuki H, Furukawa M, Todoriki T, Tanaka N et al (2004) Expression of MUC1 recognized by a monoclonal antibody MY.1E12is a useful biomarker for tumor

aggressiveness of carcinoma of the gallbladder. Clin Exp Metastasis 21:353–362
Kayahara M, Nagakawa T (2007) Recent trends of gallbladder cancer in Japan. An analysis of 4770 patients. Cancer 110:572–580
Kefeli M, Gonullu G, Can B, Malatyalioglu E, Kandemir B (2009) Metastasis of adenocarcinoma of the gall bladder to en endometrial polyp detected by endometrial curettage: case report and review of the literature. Int J Gynecol Pathol 28:343–346
Kessler T, Mihaljevic L (1994) Implantation metastasis of a gallbladder cancer at the umbilicus after laparoscopic cholecystectomy (in German). Chirurg 65: 564–565
Khangembam BC, Sharma P, Naswa N, Sahoo MK, Kumar R (2013) Solitary breast metastasis from recurrent gallbladder carcinoma simulating a second primary on 18F-FDG PET/CT. Clin Nucl Med 38:e433–e434
Kim HJ, Roy T (1994) Unexpected gallbladder cancer with cutaneous seeding after laparoscopic cholecystectomy. South Med J 87:817–820
Kim YW, Park YK, Yoon TY, Lee SM (2002) Expression of the GLUT1 glucose transporter in gallbladder carcinomas. Hepatogastroenterology 49:907–911
Kimura Y, Furuhata T, Mukaiya M, Kihara C, Kawakami M, Okita K, Yanai Y, Zenbutsu H et al (2003) Frequent beta-catenin alteration in gallbladder carcinomas. J Exp Clin Cancer Res 22:321–328
Kitamura K, Kasuya K, Tsuchida A, Mimuro A, Inoue K, Aoki T, Aoki T, Koyanagi Y (2003) Immunohistochemical analysis of transforming growth factor beta in gallbladder cancer. Oncol Rep 10:327–332
Kobayashi A, Oda T, Fukunaga K, Sasaki R, Ohkohchi N (2012) Invasion of the hepatic artery is a crucial predictor of poor outcomes in gallbladder carcinoma. World J Surg 36:645–650
Kondo S, Nimura Y, Kamiya J, Nagino M, Kanai M, Uesaka K, Hayakawa N (2002) Mode of tumor spread and surgical strategy in gallbladder carcinoma. Langenbecks Arch Surg 387:222–228
Kono H, Nakamura M, Ohtsuka T, Nagayoshi Y, Mori Y, Takahata S, Aishima S, Tanaka M (2013) High expression of microRNA-155 is associated with the aggressive malignant behavior of gallbladder carcinoma. Oncol Rep 30:17–24
Kornprat P, Rehak P, Lemmerer M, Gogg-Kamerer M, Langner C (2005) Analysis of trefoil factor family protein 1 (TFF1, pS2) expression in chronic cholecystitis and gallbladder carcinoma. Virchows Arch 446:505–510
Kuwayti K, Baggenstoss AH, Stauffer MH, Priestley JT (1957) Carcinoma of the major intrahepatic and extrahepatic bile ducts exclusive of the papilla of vater. Surg Gynec Obstet 104:357–366
Lai CH, Lau WY (2008) Gallbladder cancer – a comprehensive review. Surgeon 6:101–110
Lam CR (1940) The present status of carcinoma of the gallbladder: a study of thirty-four clinical cases. Ann Surg 111:403–410
Leal P, Garcia P, Sandoval A, Letelier P, Brebi P, Ili C, Alvarez H, Tapia O, Roa JC (2013) Immunohistochemical expression of phosphor-mTOR is associated with poor prognosis in patients with gallbladder adenocarcinoma. Arch Pathol Lab Med 137:552–557
Lee HK, Cho MS, Kim TH (2012a) Prognostic significance of muc4 expression in gallbladder carcinoma. World J Surg Oncol 10:224
Lee HJ, Lee K, Lee DG, Bae KH, Kim JS, Liang ZL, Huang SM, Suk OY, Kim HY et al (2012b) Chemokine (C-X-C motif) ligand 12 is associated with gallbladder carcinoma progression and is a novel independent poor prognostic factor. Clin Cancer Res 18:3270–3280
Letelier P, Garcia P, Leal P, Ili C, Buchegger K, Riquelme I, Sandoval A, Tapia O, Roa JC (2014) Immunohistochemical expression of vascular endothelial growth factor A in advanced gallbladder carcinoma. Appl Immunohistochem Mol Morphol 22:530–536
Li X, Hui AM, Shi YZ, Takayama T, Makuuchi M (2001) Reduced p21 (WAF1/CIP1) expression is an early event in gallbladder carcinogenesis and is of prognostic significance for patients with carcinomas of the gallbladder. Hum Pathol 32:771–777
Li SH, Li CF, Sung MT, Eng HL, Hsiung CY, Huang WW, Lin CN, Yu SC, Huang HY (2007) Skp2 is an independent prognosticator of gallbladder carcinoma among p27(Kip1)-interacting cell cycle regulators: an immunohistochemical study of 62 cases by tissue microarray. Mod Pathol 20:497–507
Li QL, Yang ZI, Liu JQ, Miao XY (2011) Expression of CDX2 and hepatocyte antigen in benign and malignant lesions of gallbladder and its correlation with histopathologic type and clinical outcome. Pathol Oncol Res 17:561–568
Li J, Wu T, Lu J, Cao Y, Song N, Yang T, Dong R, Yang Y, Zang L, Du X, Wang S (2012) Immunohistochemical evidence of the prognostic value of hedgehog pathway components in primary gallbladder carcinoma. Surg Today 42:770–775
Li J, Yang ZL, Ren X, Zou Q, Yuan Y, Liang L, Chen M, Chen S (2013) ILK and PRDX1 are prognostic markers in squamous cell/adenosquamous carcinomas and adenocarcinoma of gallbladder. Tumour Biol 34: 359–368
Liang ZL, Kim M, Huang SM, Lee HJ, Kim JM (2013) Expression of carboxyl terminus of Hsp70-interacting protein (CHIP) indicates poor prognosis in human gallbladder carcinoma. Oncol Lett 5:813–818
Lin HT, Liu GJ, Wu D, Lou JY (2005) Metastasis of primary gallbladder carcinoma in lymph node and liver. World J Gastroenterol 11:748–751
Lin W, Jiang L, Chen Y, She F, Han S, Zhu J, Zhou L, Tang N, Wang X, Li X (2012) Vascular endothelial growth factor-D promotes growth, lymphangiogenesis and lymphatic metastasis in gallbladder cancer. Cancer Lett 314:127–136
Liu B, Zhang Y, Liao M, Deng Z, Gong L, Jiang J, Lynn L, Wu K, Miao X (2011) Clinicopathologic and

prognostic significance of CD24 in gallbladder carcinoma. Pathol Oncol Res 17:45–50
Lu XS, Sun W, Ge CY, Zhang WZ, Fan YZ (2013) Contribution of the PI3k/MMPs/Ln-5g2 and EphA2/FAK/Paxillin signaling pathways to tumor growth and vasculogenic mimicry of gallbladder carcinomas. Int J Oncol 42:2103–2115
Ma HB, Hu HT, Di ZL, Wang ZR, Shi JS, Wang XJ, Li Y (2005) Association of cyclin D1, p16 and retinoblastoma protein expressions with prognosis and metastasis of gallbladder carcinoma. World J Gastroenterol 11:744–747
Marchal GJ, Van Holsbeeck M, Tshibwabwa-Ntumba E, Goddeeris PG, Fevery J, Oyen RH et al (1985) Dilatation of the cystic veins in portal hypertension: sonographic demonstration. Radiology 154: 187–189
Marmorale C, Scibé R, Siquini W, Massa M, Brunelli A, Landi E (1998) Parietal seeding of unsuspected gallbladder carcinoma after laparoscopic cholecystectomy. Ann Ital Chir 69:613–617
Martinez-Roman S, Frumovitz M, Deavers MT, Ramirez PT (2005) Metastatic carcinoma of the gallbladder mimicking an advanced cervical carcinoma. Gynecol Oncol 97:942–945
Maurya SK, Tewari M, Kumar M, Thakur MK, Shukla HS (2011) Expression pattern of tumor endothelial marker 8 protein in gallbladder carcinomas. Asian Pac J Cancer Prev 12:507–512
Meng H, Wang X, Fong Y, Wang ZH, Wang Y, Zhang ZT (2011) Outcomes of radical surgery for gallbladder cancer patients with lymphatic metastases. Jpn J Clin Oncol 41:992–998
Merz et al. 1993. http://www.ncbi.nlm.nih.gov/pubmed/8356516
Miao X, Yang ZL, Xiong L, Zou Q, Yuan Y, Li J, Liang L, Chen M, Chen S (2013) Nectin-2 and DDX3 are biomarkers for metastasis and poor prognosis of squamous cell/adenosquamous carcinomas and adenocarcinoma of gallbladder. Int J Exp Pathol 6:179–190
Midorikawa Y, Kubota K, Komatsu Y, Hasegawa K, Koike Y, Mori M, Makuuchi M (2000) Gallbladder carcinoma with a tumor thrombus in the common bile duct: an unusual cause of obstructive jaundice. Surgery 127:473–474
Mikami T, Yanagisawa N, Baba H, Koike M, Okayasu I (1999) Association of Bcl-2 protein expression with gallbladder carcinoma differentiation and progression and its relation to apoptosis. Cancer 85:318–325
Mishra PK, Jatawa SK, Raghuram GV, Pathak N, Jain A, Tiwari A, Varshney S, Maudar KK (2009) Correlation of aberrant expression of p53, Rad50, and cyclin-E proteins with microsatellite instability in gallbladder adenocarcinomas. Genet Mol Res 8:1202–1210
Misra S, Chaturvedi A, Misra NC (1997) Carcinoma gallbladder presenting with skeletal metastases. Indian J Gastroenterol 16:74
Moreno M, Pimentel F, Gazdar AF, Wistuba II, Miquel JF (2005) TP53 abnormalities are frequent and early events in the sequential pathogenesis of gallbladder carcinoma. Ann Hepatol 4:192–199
Nagakawa T, Mori K, Nakano T, Kadoya M, Kobayashi H, Akiyama T, Kayahara M et al (1993) Perineural invasion of carcinoma of the pancreas and biliary tract. Br J Surg 80:619–621
Nagakura S, Shirai Y, Yokoyama N, Hatakeyama K (2001) Clinical significance of lymph node micrometastasis in gallbladder carcinoma. Surgery 129:704–713
Nagano M, Hatakeyama K, Kai M, Nakamura H, Yodoi J, Asada Y, Chijiiwa K (2012) Nuclear expression of thioredoxin-1 in the invasion front is associated with outcome in patients with gallbladder carcinoma. HPB (Oxford) 14:573–582
Nakagawa S, Tada T, Furukawa H, Abe M, Hatakeyama K (2000) Late-type recurrence at the port site of unexpected gallbladder carcinoma after a laparoscopic cholecystectomy: report of a case. Surg Today 30:853–855
Nakashima T, Kondoh S, Kitoh H, Ozawa H, Okita S, Harada T, Shiraishi K, Ryozawa S et al (2003) Vascular endothelial growth factor-C expression in human gallbladder cancer and its relationship to lymph node metastasis. Int J Mol Med 11:33–39
Nakata T, Kobayashi A, Miwa S, Soeda J, Miyagawa S (2007) Impact of tumor spread to the cystic duct on the prognosis of patients with gallbladder carcinoma. World J Surg 31:155–161
Negi SS, Singh A, Chaudhary A (2011) Lymph nodal involvement as prognostic factor in gallbladder cancer: location, count or ratio ? J Gastrointest Surg 15:1017–1025
Niu XJ, Wang ZR, Wu SL, Geng ZM, Zhang YF, Qing XL (2004) Relationship between inducible nitric oxide synthase expression and angiogenesis in primary gallbladder carcinoma tissue. World J Gastroenterol 10:725–728
Noshiro H, Chijiiwa K, Yamaguchi K, Shimizu S, Sugitani A, Tanaka M (2003) Factors affecting surgical outcome for gallbladder carcinoma. Hepatogastroenterology 50:939–944
Ohmura Y, Yokoyama N, Tanada M, Takiyama W, Takashima S (1999) Port site recurrence of unexpected gallbladder carcinoma after a laparoscopic cholecystectomy: report of a case. Surg Today 29:71–75
Ohtani T, Takano Y, Shirai Y, Hatakeyama K (1998) Early intraperitoneal dissemination after radical resection of unsuspected gallbladder carcinoma following laparoscopic cholecystectomy. Surg Laparosc Endosc 8:58–62
Ohtani Y, Dowaki S, Kashiwagi H, Tobita K, Tsukui M, Tanaka Y, Tsuchida T, Tokunaga T et al (1999) Stromal expression of thrombospondin-1 is correlated with growth and metastasis of human gallbladder carcinoma. Int J Oncol 15:453–457
Ohtsuka M, Miyazaki M, Itoh H, Nakagawa K, Ambiru S, Shimizu H, Nakajima N, Akikusa B et al (1998) Routes of hepatic metastasis of gallbladder carcinoma. Am J Clin Pathol 109:62–68

Okada K, Kijima H, Imaizumi T, Hirabayashi K, Matsuyama M, Yazawa N, Oida Y et al (2009a) Wall-invasion pattern correlates with survival of patients with gallbladder adenocarcinoma. Anticancer Res 29:685–691

Okada K, Kijima H, Imaizumi T, Hirabayashi K, Matsuyama M, Yazawa N, Oida Y et al (2009b) Stromal laminin-5gamma2 chain expression is associated with the wall-invasion pattern of gallbladder adenocarcinoma. Biomed Res 30:53–62

Okada K, Kijima H, Imaizumi T, Hirabayashi K, Matsuyama M, Yazawa N, Dowaki S et al (2012) Clinical significance of wall invasion pattern of subserosa-invasive gallbladder carcinoma. Oncol Rep 28:1531–1536

Okita S, Kondoh S, Shiraishi K, Kaino S, Hatano S, Okita K (1998) Expression of vascular endothelial growth factor correlates with tumor progression in gallbladder cancer. Int J Oncol 12:1013–1018

Onuki K, Sugiyama H, Ishige K, Kawamoto T, Ota T, Ariizumi S, Yamato M, Kadota S et al (2014) Expression of N-acetylglucosaminyltransferase V in the subserosal layer correlates with postsurgical survival of pathological tumor stage 2 carcinoma of the gallbladder. J Gastroenterol 49:702–714

Oshikiri T, Hida Y, Miyamoto M, Hashida H, Katoh K, Suzuoki M, Nakakubo Y, Hiraoka K et al (2001) RCAS1 as a tumour progression marker: an independent negative prognostic factor In gallbladder cancer. Br J Cancer 85:1922–1927

Ouchi K, Goto H, Owada Y, Sato T (1986) Spreads of gallbladder carcinoma: significance of histological classification of the tumor (in Japanese). Nihon Geka Gakkai Zasshi 87:878–882

Ouchi K, Owada Y, Matsuno S, Sato T (1987) Prognostic factors in the surgical treatment of gallbladder carcinoma. Surgery 101:731–737

Ouyang M, Wu W, Zou Y, Zhou J, Wang Z, Wan X (2010) Immunoreactivity and prognostic value of tumor-associated glycoprotein 72 in primary gallbladder carcinoma. Surg Oncol 19:82–87

Paolucci V, Schaeff B, Schneider M, Gutt C (1999) Tumor seeding following laparoscopy: international survey. World J Surg 23:989–995

Peng HH, Zhang YD, Gong LS, Liu WD, Zhang Y (2013) Increased expression of microRNA-335 predicts a favorable prognosis in primary gallbladder carcinoma. Oncol Targets Ther 6:1625–1630

Petrén T (1932) Die extrahepatischen Gallengangsvenen und ihre pathologisch-anatomische Bedeutung. Verh Anat Ges 41:139–143

Pezet D, Fondrinier E, Rotman N, Guy L, Lemesle P, Lointier P, Chipponi J (1992) Parietal seeding of carcinoma of the gallbladder after laparoscopic cholecystectomy. Br J Surg 79:230

Prakash M, Aiyappan SK, Kumar A, Sreenivasan R, Yadav TD, Khandelwal N (2010) Solitary skeletal metastasis in carcinoma gallbladder: two case reports. Cancer Imaging 10:121–123

Prince S, Zeidman A, Dekel Y, Ram E, Koren R (2008) Expression of epithelial cell adhesion molecule in gallbladder carcinoma and its correlation with clinicopathologic variables. Am J Clin Pathol 129: 424–429

Priya TP, Kapoor VK, Krishnani N, Agrawal V, Agrawal S (2010) Role of E-cadherin gene in gall bladder cancer and its precursor lesions. Virchows Arch 456:507–514

Puglisi F, Capuano P, Gentile A, Lobascio P, Russo S, Martines G, Lograno G, Memeo V (2005) Retrobulbar metastasis from gallbladder carcinoma after laparoscopic cholecystectomy. A case report. Tumori 91:428–431

Puhalla H, Herberger B, Soleiman A, Filipits M, Laengle F, Gruenberger T, Wrba F (2005) E-cadherin and beta-catenin expression in normal, inflamed and cancerous gallbladder tissue. Anticancer Res 25:4249–4254

Puhalla H, Wrba F, Kandioler D, Lehnert M, Huynh A, Gruenberger T, Tamandi D et al (2007) Expression of p21 (Wafl/Cip1), p57 (Kip2) and HER2/neu in patients with gallbladder cancer. Anticancer Res 27:1679–1684

Puranik AD, Purandare N, Agrawal A, Shah S, Rangarajan V (2014) Bone marrow metastases in an otherwise operable gall bladder cancer: rare site of distant metastases detected on FDG PET/CT. Clin Nucl Med 39: e169–e172

Qiang D, Lei J, Xiaoqian W, Meiping W, Feifel S, Yanling C (2014) TNF-a promotes the lymphangiogenesis of gallbladder carcinoma through NF-kB-mediated up-regulation of VEGF-C. Cancer Sci. doi:10.1111/cas.12504

Ralls PW, Mayekawa DS, Lee KP, Colletti PM, Johnson MB, Halls JM (1988) Gallbladder wall varices: diagnosis with color flow Doppler sonography. J Clin Ultrasound 16:595–598

Reber PU, Baer HU, Patel AG, Schmied B, Büchler MW (1998) Port site metastases following laparoscopic cholecystectomy for unsuspected carcinoma of the gallbladder. Z Gastroenterol 36:901–907

Renner R, Sticherling M (2007) Sister Mary Joseph's nodule as a metastasis of gallbladder carcinoma. Int J Dermatol 46:505–507

Roa I, Araya JC, Shiraishi T, Yatani R, Wistuba I, Villaseca M, De Aretxbala X (1993) DNA content in gallbladder carcinoma: a flow cytometric study of 96 cases. Histopathology 23:459–464

Roa JC, Tapia O, Manterola C, Villaseca M, Guzman P, Araya JC, Bagci P, Saka B et al (2013) Early gallbladder carcinoma has a favorable outcome but Rokitansky-Aschoff sinus involvement is an adverse prognostic factor. Virchows Arch 463:651–661

Roh YH, Kim YH, Choi HJ, Lee KE, Roh MS (2008) Syndecan-1 expression in gallbladder cancer and its prognostic significance. Eur Surg Res 41:245–250

Roh YH, Kim YH, Choi HJ, Lee KE, Roh MS (2009) Fascin overexpression correlates with positive thrombospondin-1 and syndecan-1 expressions and a more aggressive clinical course in patients with

gallbladder cancer. J Hepatobiliary Pancreat Surg 16:315–321
Rominger CJ, Lockwood DW, Canino CW (1961) Carcinoma of the gallbladder with mandibular metastasis: report of case. J Oral Anesth Hosp Dent Serv 19:425–427
Rousselot R, Labram C, Janvier R (1964) Umbilical tumor, metastasis indicative of gallbladder cancer (in French). Bull Soc Fr Dermatol Syphiligr 71:670–672
Sahoo MK, Sharma P, Suman S, Jain TK, Bal C, Kumar R (2014) Gallbladder carcinoma metastasizing to adrenal gland: a rare site demonstrated with (18)F-FDG PET/CT. Clin Nucl Med 39:184–185
Sailer M, Debus S, Fuchs KH, Thiede A (1995) Peritoneal seeding of gallbladder cancer after laparoscopic cholecystectomy. Surg Endosc 9:1298–1300
Sakata J, Shirai Y, Wakai T, Ajioka Y, Hatakeyama K (2010) Number of positive lymph nodes independently determines the prognosis after resection in patients with gallbladder carcinoma. Ann Surg Oncol 17:1831–1840
Sasaki R, Uesugi N, Itabashi H, Fujita T, Takeda Y, Hoshikawa K, Takahashi M, Funato O et al (2005) Clinicopathological study of depth of subserosal invasion in patients with pT2 gallbladder carcinoma. J Surg Oncol 92:83–88
Sasaki E, Nagino M, Ebata T, Oda K, Arai T, Nishio H, Nimura Y (2006) Immunohistochemically demonstrated lymph node micrometastasis and prognosis in patients with gallbladder carcinoma. Ann Surg 244:99–105
Sato Y, Tanaka J, Koyama K, van Gulik TM, Lygidakis NJ, van der Heyde MN (1993) Tumor DNA content in gallbladder carcinoma. Hepatogastroenterology 40:375–379
Seki H, Koyama K, Tanaka J, Sato Y, Umezawa A (1995) Neural cell adhesion molecule and perineural invasion in gallbladder cancer. J Surg Oncol 58:97–100
Shi YZ, Hui AM, Li X, Takayama T, Makuuchi M (2000) Overexpression of retinoblastoma protein predicts decreased survival and correlates with loss of p16INK4 protein in gallbladder carcinomas. Clin Cancer Res 6:4096–4100
Shirai Y, Tsukada K, Ohtani T, Watanabe H, Hatakeyama K (1995) Hepatic metastases from carcinoma of the gallbladder. Cancer 75:2063–2068
Shirai Y, Ohtani T, Hatakeyama K (1998) Laparoscopic cholecystectomy may disseminate gallbladder carcinoma. Hepatogastroenterology 45:81–82
Shirai Y, Sakata J, Wakai T, Ohashi T, Ajioka Y, Hatakeyama K (2012) Assessment of lymph node status in gallbladder cancer: location, number, or ratio of positive nodes. World J Surg Oncol 10:87
Shu GS, Yang ZL, Liu DC (2012) Immunohistochemical study of Dicer and Drosha expression in the benign and malignant lesions of gallbladder and their clinicopathological significances. Pathol Res Pract 208:392–397
Singh S, Gupta P, Khanna R, Khanna AK (2010) Simultaneous breast and ovarian metastasis from gallbladder carcinoma. Hepatobiliary Pancreat Dis Int 9:553–554
Song SP, Zhang SB, Liu R, Yao L, Hao YQ, Liao MM, Zhang YD, Li ZH (2012) NDRG2 down-regulation and CD24 up-regulation promote tumor aggravation and poor survival in patients with gallbladder carcinoma. Med Oncol 29:1879–1885
Sons HU, Borchard F, Joel BS (1985) Carcinoma of the gallbladder: autopsy findings in 287 cases and review of the literature. J Surg Oncol 28:199–206
Srivastava V, Patel B, Kumar M, Shukla M, Pandey M (2013) Cyclin D1, retinoblastoma and p16 protein expression in carcinoma of the gallbladder. Asian Pac J Cancer Prev 14:2711–2715
Sugawara Y, Makuuchi M, Harihara Y, Noie T, Inoue K, Kubota K, Takayama T (1999) Tumor angiogenesis in gallbladder carcinoma. Hepatogastroenterology 46:1682–1686
Sumi K, Matsuyama S, Kitajima Y, Miyazaki K (2004) Loss of estrogen receptor beta expression at cancer front correlates with tumor progression and poor prognosis of gallbladder cancer. Oncol Rep 12: 979–984
Sun W, Fan YZ, Xi H, Lu XS, Ye C, Zhang JT (2011) Astrocyte elevated gene-1 overexpression in human primary gallbladder carcinomas: an unfavorable and independent prognostic factor. Oncol Rep 26:1133–1142
Sun W, Shen ZY, Zhang H, Fan YZ, Zhang WZ, Zhang JT, Lu XS, Ye C (2012) Overexpression of HIF-1a in primary gallbladder carcinoma and its relation to vasculogenic mimicry and unfavourable prognosis. Oncol Rep 27:1990–2002
Takagawa M, Muguruma N, Oguri K, Imoto Y, Okamoto K, Ii K, Ito S (2005) Prediction of prognosis in gallbladder carcinoma by mucin and p53 immunohistochemistry. Dig Dis Sci 50:1410–1413
Takano S, Yoshii Y, Owada T, Shirai S, Nose T (1991) Central nervous metastasis from gallbladder – case report. Neurol Med Chir (Tokyo) 31:782–786
Takeuchi T, Adachi Y, Nagayama T, Furihata M (2011) Nedd4L modulates the transcription of metalloproteinase-1 and -13 genes to increase the invasive activity of gallbladder cancer. Int J Exp Pathol 92:79–86
Tanabe M, Endo I, Masunari H, Sugita M, Morioka D, Ishikawa T, Ichikawa Y, Shimada H (2012) Is lymph-node micrometastasis in gallbladder cancer a significant prognostic factor? Hepatogastroenterology 59:31–35
Tayo JR, Al-Abdulkarim H, Al-Rayess M (2005) Brain metastasis as an manifestation of a gallbladder carcinoma. Neurosciences (Riyadh) 10:235–237
Tokunaga T, Arakawa H, Kuwashima Y (2005) A case of lepidic pulmonary metastasis from adenocarcinoma of the gallbladder mimicking acute interstitial pneumonia. Clin Radiol 60:1213–1215
Tsukada K, Kurosaki I, Uchida K, Shirai Y, Oohashi Y, Yokoyama N, Watanabe H et al (1997) Lymph node spread from carcinoma of the gallbladder. Cancer 80:661–667

Vadheim JL, Gray HK, Dockerty MB (1944) Carcinoma of the gallbladder; a clinical and pathologic study. Am J Surg 63:173–180

Varga M, Obrist P, Schneeberger S, Mühlmann G, Felgel-Farnholz C, Fong D, Zitt M et al (2004) Overexpression of epithelial cell adhesion molecule antigen in gallbladder carcinoma is an independent marker for poor survival. Clin Cancer Res 10:3131–3136

Wakai T, Shirai Y, Yokoyama N, Ajioka Y, Watanabe H, Hatakeyama K (2003) Depth of subserosal invasion predicts long-term survival after resection in patients with T2 gallbladder carcinoma. Ann Surg 10: 447–454

Wakai T, Shirai Y, Sakata J, Nagahashi M, Ajioka Y, Hatakeyama K (2010) Mode of hepatic spread from gallbladder carcinoma: an immunohistochemical analysis of 42 hepatectomized specimens. Am J Surg Pathol 34:65–74

Wang C, Zhao H, Lu J, Yin J, Zang L, Song N, Dong R, Wu T, Du X (2012a) Clinicopathological significance of SOX4 expression in primary gallbladder carcinoma. Diagn Pathol 7:41

Wang W, Yang ZL, Liu JQ, Jiang S, Miao XY (2012b) Identification of CD146 expression, angiogenesis, and lymphangiogenesis as progression, metastasis, and poor-prognosis related markers for gallbladder adenocarcinoma. Tumour Biol 33:173–182

Washington K, Gottfried MR (1996) Expression of p53 in adenocarcinoma of the gallbladder and bile ducts. Liver 16:99–104

Willis RA (1953) Pathology of tumors, 2nd edn. Mosby, St. Louis, p 997

Winston CB, Chen JW, Fong Y, Schwartz LH, Panicek DM (1999) Recurrent gallbladder carcinoma along laparoscopic cholecystectomy port tracks: CT demonstration. Radiology 212:439–444

Wu XS, Akiyama Y, Igari T, Kawamura T, Hiranuma S, Shibata T, Tsuruta K, Koike M et al (2005) Expression of homeodomain protein CDX2 in gallbladder carcinomas. J Cancer Res Clin Oncol 131:271–278

Wu W, Wang R, Liu H, Peng J, Huang D, Li B, Ruan J (2009) Prediction of prognosis in gallbladder carcinoma by CD147 and MMP-2 immunohistochemistry. Med Oncol 26:117–123

Wu K, Liao M, Liu B, Deng Z (2011) ADAM-17 overexpression in gallbladder carcinoma correlates with poor prognosis of patients. Med Oncol 28: 475–480

Wu J, Lei L, Wang S, Gu D, Zhang J (2012) Immunohistochemical expression and prognostic value of CD97 and its ligand CD55 in primary gallbladder carcinoma. J Biomed Biotechnol 2012:587672

Wysocki A, Krzywon J (2000) Does the laparoscopic cholecystectomy accelerate the intraperitoneal dissemination of previously unrecognized gallbladder cancer. Int J Surg Investig 2:313–317

Xu LN, Zou SQ, Wang JM (2005) Action of mechanism of Fas and Fas ligand in immune escape of gallbladder carcinoma. World J Gastroenterol 11:3719–3723

Xuan YH, Choi YL, Shin YK, Kook MC, Chae SW, Park SM, Chae HB, Kim SH (2005) An immunohistochemical study of the expression of cell-cycle-regulated proteins p53, cyclin D1, RB, p27, Ki67 and MSH2 in gallbladder carcinoma and its precursor lesions. Histol Histopathol 20:59–66

Yamaguchi R, Nagino M, Oda K, Kamiya J, Uesaka K, Nimura Y (2002) Perineural invasion has a negative impact on survival of patients with gallbladder carcinoma. Br J Surg 89:1130–1136

Yamaguchi K, Shirahane K, Nakamura M, Su D, Konomi H, Motoyama K, Sugitani A et al (2005) Frozen section and permanent diagnoses of the bile duct margin in gallbladder and bile duct cancer. HPB (Oxford) 7:135–138

Yamakawa T, Itoh S, Hirosawa K, Miyoshi T, Katoh K, Iizumi S, Kawabata K (1983) Seeding of gallbladder carcinoma along the tract after percutaneous transhepatic choledochoscopy. Am J Gastroenterol 78:649–651

Yamamoto M, Oda N, Tahara E (1990) DNA ploidy patterns in gallbladder adenocarcinoma. Jpn J Clin Oncol 20:83–86

Yamamoto S, Fujii K, Kitadai Y, Yamamoto S, Tsuchida A, Kajiyama G (1998) Expression of vascular endothelial growth factor in human gallbladder lesions. Oncol Rep 5:1065–1069

Yamamoto S, Kitadai Y, Tsuchida A, Saski T, Matsubara K, Kajiyama G (2000) Expression of platelet-derived endothelial cell growth factor/thymidine phosphorylase in human gallbladder lesions. Eur J Cancer 36:257–263

Yanagisawa N, Mikami T, Mitomi H, Saegusa M, Koike M, Okayasu I (2001) CD44 variant overexpression in gallbladder carcinoma associated with tumor dedifferentiation. Cancer 91:408–416

Yang ZL, Miao X, Xiong L, Zou Q, Yuan Y, Li J, Liang L, Chen M, Chen S (2013) CFL1 and Arp3 are biomarkers for metastasis and poor prognosis of squamous cell/adenosquamous carcinomas and adenocarcinomas of gallbladder. Cancer Invest 31:132–139

Yi S, Yang ZL, Miao X, Zou Q, Li J, Liang L, Zeng G, Chen S (2014) N-cadherin and P-cadherin are biomarkers for invasion, metastasis, and poor prognosis of gallbladder carcinomas. Pathol Res Pract 210:363–368

Yoshimitsu K, Honda H, Kaneko K, Kuroiwa T, Irie H, Chijiiwa K, Takenaka K, Masuda K (1997) Anatomy and clinical importance of cholecystic venous drainage: helical CT observations during injection of contrast medium into the cholecystic artery. AJR Am J Roentgenol 169:505–510

Yoshimitsu K, Honda H, Kuroiwa T, Irie H, Aibe H, Tajima T, Chijiiwa K, Shimada M, Masuda K (2001) Liver metastasis from gallbladder carcinoma: anatomic correlation with cholecystic venous drainage demonstrated by helical computed tomography during injection of contrast medium in the cholecystic artery. Cancer 92:340–348

Yuan LW, Liu DC, Yang ZL (2013) Correlation of S1P1 and ERp29 expression to progression, metastasis, and poor prognosis of gallbladder adenocarcinoma. Hepatobiliary Pancreat Dis Int 12:189–195

Z'graggen K, Birrer S, Maurer CA, Wehrli H, Klaiber C, Baer HU (1998) Incidence of port site recurrence after laparoscopic cholecystectomy for preoperatively unsuspected gallbladder carcinoma. Surgery 124:831–838

Zhang M, Pan JW, Ren TR, Zhu YF, Han YJ, Kühnel W (2003) Correlated expression of inducible nitric oxide synthase and P53, Bax in benign and malignant diseased gallbladder. Ann Anat 185:549–554

Zhang SB, Song SP, Li B, Zhou YS, Zhang YD (2012) Expression of N-myc downstream-regulated gene 1 in primary gallbladder carcinoma and its correlation with clinicopathological features and clinical outcome. Med Oncol 29:1866–1872

Zhi YH, Liu RS, Song MM, Tian Y, Long J, Tu W, Guo RX (2005) Cyclooxygenase-2 promotes angiogenesis by increasing vascular endothelial growth factor and predicts prognosis in gallbladder carcinoma. World J Gastroenterol 11:3724–3728

Zhou L, He XD, Chen J, Cui QC, Qu Q, Rui JA, Zhao YP (2007) Overexpression of LAPTM4B-35 closely correlated with clinicopathological features and postresectional survival of gallbladder carcinoma. Eur J Cancer 43:809–815

Zhou L, He XD, Yu JC, Zhou RL, Xiong FX, Qu Q, Rui JA (2010) Expression of LAPTM4B in gallbladder carcinoma cells: the role in invasive potential. Hepatogastroenterology 57:207–211

Zong H, Yin B, Chen J, Ma B, Cai D, He X (2009) Overexpression of c-FLIP confers the resistance to TRAIL-induced apoptosis on gallbladder carcinoma. Tohoku J Exp Med 217:203–208