MINI-REVIEW

Cholangiocarcinoma: New Insights

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Abstract

Cholangiocarcinoma is a malignant neoplasm originating from biliary epithelial cells. The incidence and mortality of this cancer are rising in the world. Currently, cholangiocarcinoma is accepted as a stem cell disease with many risk factors. Diagnosis is relatively simple but therapy is extremely difficult. Surgery is the mainstay of treatment for early stage patients. Endobiliary approaches, chemotherapy and radiotherapy are other therapeutic approaches.

Keywords: Cholangiocarcinoma- stem cell disease- pathogenesis

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Bile duct tumors

Bile duct tumors are being frequently diagnosed in recent years and are divided into two main groups by WHO as intrahepatic bile duct tumors (IBDT) and extrahepatic bile duct (EHBD) tumors (Khan et al., 2012).

A) IBDT tumors
a) Benign tumors
Bile duct adenoma
Microcystic adenoma
Biliary adenofibroma
b) Premalignan tumors
Biliary intraepithelial neoplasm
Intraductal papillary neoplasm
Mucinous cystic neoplasm
c) Malign tumors
Intrahepatic CC
Intraductal papillary neoplasm with invasive neoplasm
Mucinous cystic neoplasm with invasive neoplasm

B) EHBD tumors (Extrahepatic bile duct tumors)
a. Premalign tumors
Adenoma
Intracystic (GB) or intraductal papillary neoplasm
Mucinous cystic neoplasm
b. Carcinoma
Adenocarcinoma
Adenosquamous carcinoma
Intracystic or intraductal papillary neoplasm + invasive neoplasm
Mucinous cystic neoplasm + invasive neoplasm
Squamous cell carcinoma
Undifferentiated carcinoma.

Cholangiocarcinoma

Cholangiocarcinoma (CC) is a malignant tumor of adenocarcinoma nature originating from the epithelial cells of bile ducts (intrahepatic, hilar and extrahepatic). It has a prevalence of 0.5-1.2/100.000 people and is more common in men than women. CC incidence is gradually increasing especially in patients with intrahepatic cholangiocarcinoma. The rate of 5-year survival is about 5-10% including newly-diagnosed cases and 5-year chance of survival following potential surgery is 25-30%. In metastatic cases, median survival is no longer than 8-12 months even under a pharmaceutical or combined therapy. Japan, Chile, Eastern Asia and India are countries with highest CC incidence (Khan et al., 2014; Patel ., 2014; Gatto et al., 2010).

Cholangiocarcinomas are divided into two groups as intrahepatic CC and extrahepatic CC. Extrahepatic cholangiocarcinomas are also divided into two subgroups as perihilar CC (bifurcation of the main duct) and distal CC. In perihilar CC, Bismuth-Corlette classification is frequently used in daily practice (Figure 1). Intrahepatic CC is divided in 3 forms, i.e. mass form, periductal-infiltrative type and intraductal type (Khan et al., 2014).

Intrahepatic CC (IHCC), perihilar CC and distal CC have different epidemiologic, pathogenic and treatment features (Khan et al., 2014; Patel ., 2014; Fan et al., 2012; Francis et al., 2010; Wise et al., 2008; Andersen et al., 2012). Studies have shown that IHCC and hepatocellular carcinoma (HCC) originates from the same stem cell (Sirica et al., 2013; Zabron et al., 2013; Sia et al., 2013; DeMinics et al., 2013). Particularly IHCC originates from multipotent stem cell population. In IHCC, the origin cell may be differentiated hepatocyte, dysplastic or immature cholangiocyte, hepatic stem/progenitor cell or peribiliary glands.

Risk Factors

In CC patients especially in western countries, risk factors are not known in 90% of the cases and chronic

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inflammation and biliary irritation are held responsible (Kokuryo et al., 2012; Wadsworth et al., 2011)). In 10% of the cases, primary sclerosing cholangitis (PSC), obesity, hepatolithiasis, bile stasis-associated cholangitis, hepatitis B and C (C>B), HIV, parasitic infections (endemic in southeastern Asia, such as Thailand in Southeast Asia have the highest incidence of CC, with 14-27-fold increase in CC risk) may result in this disease. Parasitic infestations increase CC risk by leading to chronic inflammation (especially opisthorchis viverrini, clonorchis sinensis, shistosoma Japonica). Besides, diabetes mellitus, smoking, advanced age (65% is above 65 years of age), post-biliary surgery, biliary-enteric anastomosis, chronic inflammatory diseases, chronic typhoid carriers (6 times higher risk) and cryptosporidiosis cases, hepatic cirrhosis, congenital causes (choledochal cysts, Caroli's disease, congenital hepatic fibrosis), chemical agents (thorotrast, dioxin, nitrosamines, asbestos), some medicines (oral contraceptives, isoniazide) with prolonged use may present risk.

Primary Sclerosing Cholangitis and CC association

In PSC; CC, HCC, colorectal, gastric, pancreatic cancer, gallbladder malignant polyp may be seen together. 10% of PSC patients also has CC. In PSC, the prevalence of developing CC is about 30-42% with no correlation between PSC duration and CC incidence. Compared to general population, PSC patients are at 1500-fold higher risk for developing CC. More than 50% of CC develops within 2 years following diagnosis in patients who developed PSC. CC screening in PSC is generally not useful. If screening is to be performed, KCFT, tumor markers and clinical examination should be performed with 6 month intervals and USG and MRCP in 6-12 month intervals for screening. EASL and AASLD do not provide a definitive advice on reducing the risk of developing CC of UDCA use in PSC patients.

The likelihood of developing CC in PSC increases with advanced age, smoking and drinking habit, concurrent prolonged IBH, presence of colorectal cancer, dysplasia in ulcerative colitis, high bilirubin level, prior proctocolectomy, biliary calculi and presence of specific gene polymorphism in NKG2D (Natural killer group 2, member D).

Pathogenesis

Generally, chronic inflammation, high cell turnover, cytokines and growth factor release leads to proliferation and mutation of mutated cells (Diagram 1). CC develops also in the absence of inflammation. Stimulation of angiogenesis (cell migration, interaction in stromal tissues, exposure to bile acid, cell cycle and apoptosis) plays a role here. Angiogenesis is also involved in CC development and VEGF (vascular epidermal growth factor) is expressed at high amounts by CC cells (KMC-1, KMC-2, KMBC and KMG-C) and tumor tissue. VEGF increases malignant cell proliferation. Endothelia 1 (ET-1) generally inhibits, VEGF and VEGF-C expression or release. Therefore, it reduces cell proliferation in tumor tissue and decreases apoptosis and fibrosis. Therefore, boseptan (ET-1 receptor antagonist) may be the curative agent in these patients.

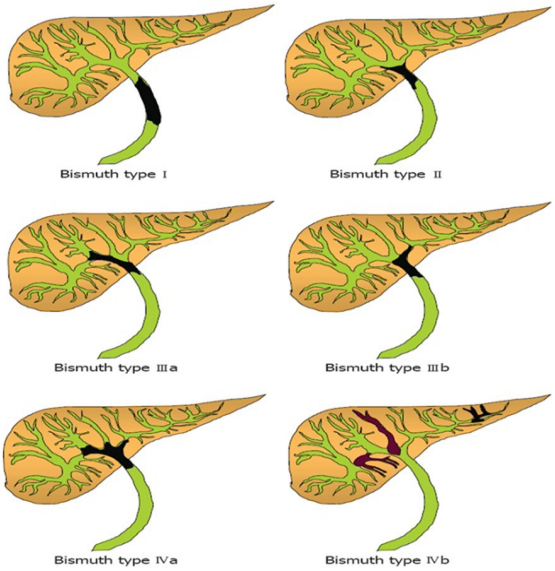

Figure 1. Bismuth- Corlette Classification in Perihilar Cholangiocarcinoma (From: Razumilava N. 2012).

Other factors responsible for CC development: EGFR (epidermal growth factor receptor) and ErbB2, IL-6, apoptosis and cell life, mechanisms associated with inflammation, NO and COX2, leptin, microRNA (miR-141, miR-200, miR-370, miR29b), epigenetic changes (hypermethylation etc.), growth factors (NGF-β 57.1%, VEGF-C 46.4%), GIS hormones, neuroendocrine regulation are involved in CC development. CC is accompanied by tumor suppressor genes' (p53, SMAD-4, bcl-2 and p16) inactivation. It activates carcinogenesis through mutation in oncogenes, chromosomal aneuploidy (PSC-associated CC) via ErbB2, p42/44 mitogen activated protein kinase (MAPK) (Khan et al., 2014; Patel ., 2014; Fan et al., 2012; Francis et al., 2010; Wise et al., 2008; Andersen et al., 2012).

EMT (Epidermal Mesenchymal Transition) plays a key role in development of many cancers. The effect of EMT has been demonstrated in IHCC. In CC cells, E-cadherin, α- and β-catenin were down regulated and N-cadherin, S100A4 and Vimentin were up regulated (Kokuryo et al., 2012; Boris et al 2008). These changes lead to invasion,

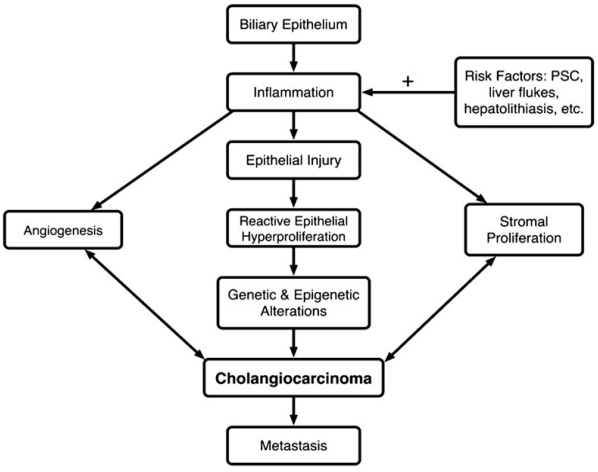

Diagram 1. Etiopathogenetic Mechanism in CC, Etiologic Agents and Cholangiocyte Response to Damage (From: Dalbir S. Liver Int 2008).

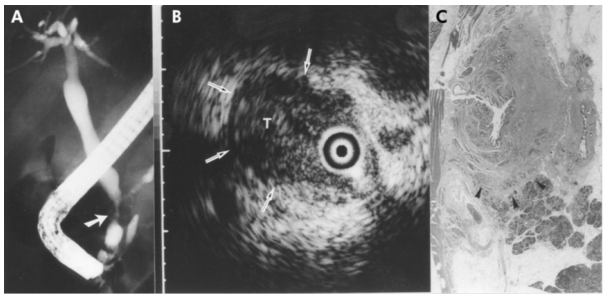

Figure 2 . a) Choledocal Narrowing in ERCP b) Mass in Intraductal USG c) Malign Cytology (From: Weber A. 2008).

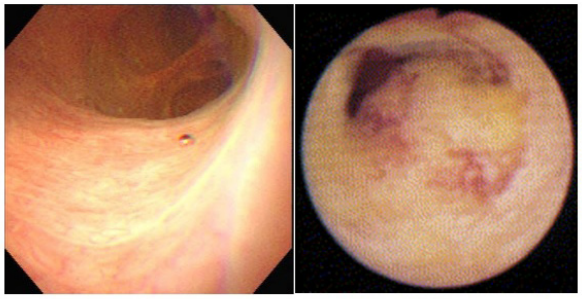

Figure 3. In Cholangioscopy a) Normal Bile Duct b) Mass in Bile Duct (From:Wakai T. 2005)

poor prognosis and metastasis.

MMP (Matrix metalloproteinase protein) level is increased in patients with CC. MMP breaks down extracellular matrix and results in tumor spread. Expression of MMP-7 (76%) and MMP-9 (48%) was detected in malignant cholangiocytes. Plasma MMP-7 is increased in obstructive perihilar and intrahepatic CC. High tumor MMP-7 levels is a sign of poor postoperative prognosis (Wandswort et al., 2011, Malaguarnera et al., 2013; Rizviet al., 2013; Razumilava et al., 2012). MMP-9 indicates an increase in malignant pancreato-biliary narrowness.

Stromal factors (CAFs); In intrahepatic CC, increase in alpha-SMA positive CAFs (Cancer associated fibroblasts) levels indicate large tumor or poor prognosis. CAFs results in release of many factors and therefore tumor progression. Therefore, it leads to trombospondin-1 over-expression, hypovascularity and metastasis. Periostin shows poor prognosis in IHCC. Tenascin expression emphasizes poor prognosis in IHCC. Galectin-1 expression is together with dedifferentiation. Other: factors such as stromal cell derived factor 1, WISPI (WNT1 inducible pathway protein 1) affect tumor prognosis negatively.

Bile acids; In cases of cholestasis, a number of anomalies occur in bile acids. Deoxycholic acid, a bile acid component, activates EGRF via TGFα and induces COX-2 expression via p42/44 and p38MAPK. Deoxycholic acid shows carcinogenic effect in long-term cholestasis cases while tauroursodeoxicolate inhibits cell growth in CC in vitro.

Exposure to asbestos is also involved in IHCC development especially liver cirrhosis (Patel et al.,2014; Sia et al., 2013). Intrahepatic CC is divided into 8 subgroups according to pathology results in biopsy (A) Wide channel papillary ICC. (B) Well differentiated small channel IHCC. (C) Well differentiated wide channel tubular IHCC (D) Goblet cell metaplasia with wide channel ‘‘intestinal-tip’’ IHCC (E) Cholangiocellular type IHCC (F) mesenchymal/ovarian stroma with biliary cyst adenocarcinoma. (G) HCC-CC. (H) Poorly differentiated IHCC. Larger clinical studies are needed on the characteristics, treatment response rates and prognosis of these subgroups.

Symptoms and Diagnostic methods

Primary symptoms detected in these patients vary from weakness, pain, nausea-vomiting to obstructive jaundice and sepsis and there is currently no specific diagnostic laboratory blood test. AST, ALT are generally normal but may be high in acute obstruction or cholangitis. Prolonged obstruction disturbs fat-soluble vitamin absorption and PTZ is prolonged. Albumin, sedimentation, CRP and Hb values are variable. Urinary proteomic analysis, which recently came to foreground may be beneficial in diagnosis (in differentiating from cholangitis).

Tumor markers; CA 19-9 and CA 125 are the most commonly used tests (Malaguarnera et al., 2013). CA 19-9 is a weak diagnostic marker with a sensitivity of 40-70%, specificity of 50-80% and PPV of 16-40%. CA 19-9 is also increased in PSC and non-malignant obstructive events. CA-125 may be increased in 65% of these patients. In addition, CEA, serum total sialic acid, CYFRA 21-1, TGF-β, TUM2-PK, serotonin levels may also be studied. As new markers, it may sometimes be necessary to look at Mac-2BP, MMP-7, insulin-like growth factor 1, IL-6, trypsinogen and MUCIN-5AC levels.

Imaging methods

The mainstay of diagnostic imaging of cholangiocarcinoma is crossectional imaging (i.e. CT, MRI). A CAT scan is a computerized axial tomography scan The CT scan works by taking multiple x-ray images which are generated by a computer into cross-sectional pictures and structures of the liver and biliary tract. CC diagnosis, the used methods are a) USG b) High resolution /spiral CT c) MRI d) Cholangiography (MRCP, ERCP, PTC) e) EUS, miniprobe USG f) PET (IHCC>1 cm, 85-90%) and PET-BT g) Cholangioscopy (percutaneous, transpapillary), endomicroscopy h) histology and cytology (Rizvi et al., 2013; Razumilava et al., 2012; Vasilieva et al., 2012, Kathyrn et al., 2013; Vasilieva et al., 2013; Weber et al., 2008; Blechaz et al.,2011; Wakai et al.,2005).

Presence of CC is investigated with upper abdominal USG. With intraductal USG, small-diameter intraductal ultrasound miniprobe is used (Wakai et al., 2005). It features better resolution compared to standard EUS and is very helpful with regards to proximal biliary system and surrounding periductal tissue and in differentiating benignant and malignant strictures. Transpapillary cholangioscopy (Spyglass) directly visualizes the bile ducts and allows biopsy from 4 quadrants. EUS-FNA (fine needle aspiration) are very useful methods in diagnosis and biopsy.

Methods such as MRCP, MR and CT are now

commonly used to show tumor in CC. PET is beneficial in demonstrating tumor involvement and metastasis (Weber et al., 2008; Blechaz et al., 2011.

ERCP is quite useful in displaying bile ducts, taking biopsy and bile samples and establishing bile drainage by placing plastic or metallic stents (Figure 2). In cases where ERCP is not feasible, percutaneous transhepatic cholangiography (PTC) is used in an attempt to visualize and ensure bile drainage. Brushing cytology in ERCP fails in 50% of the cases in demonstrating the disease. Cholangioscopy and target biopsy have a success rate of 4-70%. In PSC stricture, presence of polysomy in CC with FISH (Fluorescence in situ hybridization) is around 88%. FISH is a molecular technique that easily detects small genetic changes and when conventional cytological investigation with bile sample taken with brushing cytology with ERCP is negative, hyperdiploidy and structural aberrations in these can be detected with FISH method. Flow cytometry or DIA (digital image analysis) are other diagnosed methods used in diagnosing CC.

It is possible with transpapillar or percutaneous cholangioscopy to visualize bile ducts and perform biopsy in suitable cases (Figure 3). It may also enable performing endoscopic radiofrequency treatment in these patients.

Treatment

Treatment is usually divided in 3 main groups: 1) endobiliary treatments 2) limited pharmacotherapy and 3) surgical treatment.

Endobiliary treatments are performed in inoperable patients (Lau and Lau, 2012; Vasilieva et al.,2012; Rizvi et al., 2013). ERCP-guided bile drainage, plastic or metal stent placement are performed. However, this method has some complications such as sepsis, tumor ingrowth and obstruction. it may sometimes become necessary to repeat the procedures. Stenting with endobiliary RFA is a more reliable method. Preoperative biliary stenting is disputable and may cause bacterobilia, fungal colonization and high postoperative sepsis. Preoperative biliary drainage (PTC: percutaneous transhepatic cholangiography and drainage) and direct surgery do not differ with respect to mortality, morbidity and complications. Generally ≥10 Fr plastic stent is placed. In patients who develop strictures, attempt is made to place stent with percutaneous interventions. The longer life of metallic stents is an advantage. They are associated with lesser hospitalizations, mortality and ERCP need. However, the difficultly in removing them is a disadvantage.

Findings of poor prognosis in CC are a) tumor being perihilar b) margin status c) vascular invasion d) lymph node metastasis e) transmural extension to gallbladder f) hepatic venous invasion g) histology (papillary type with better prognosis) h) gender (female, better) ı) albumin level < 3 g/dl j) bilirubin level > 10 mg/dl indicate poor prognosis.

Chemotherapy using may be in two forms in these patients: a) chemotherapy in locally advanced or metastatic cases and b) adjuvant therapy. In CC, the benefit of adjuvant therapy has not been established. There are phase II studies on gemcitabine/cisplatin, gemcitabine/ oxaliplatin, gemcitabine/capecitabine, capecitabin/ cisplatin, capecitabine/ oxaliplatin, 5-FU/oxaliplatin, 5-FU/cisplatin and single agent therapy (gemcitabine, cisplatin, and 5-FU). In intrahepatic or extrahepatic CC, postoperative adjuvant treatment is recommended after complete surgery (Croitori et al., 2012).

There are some guidelines (e.g. NCCN, ESMO) for chemotherapy. According to NCCN (The National Comprehensive Cancer Network) guideline:

1) if there is extrahepatic CC a) in margin negative, local lymph node negative cases following complete resection: fluoropyrimidine or gemcitabine -based chemotherapy, fluoropyrimidine-based chemoradiotherapy is recommended. b) in cases of marginal carcinoma in-situ or positive -margin invasive disease; fluoropyrimidine-based chemoradiotherapy is recommended. c) in the presence of positive regional lymph node; fluoropyrimidine or gemcitabine -based chemotherapy is recommended.

2) In intrahepatic CC is present; a) adjuvant therapy is not recommended is there is no residual disease b) in the presence of positive margin, fluoropyrimidine or gemcitabine based chemoradiotherapy or fluoropyrimidine or gemcitabine -based chemotherapy is recommended.

According to ESMO (European Society of Medical Oncology) criteria: In intrahepatic or extrahepatic CC; following non-curative surgical resection, supportive treatment or palliative chemotherapy and/or radiotherapy, or following complete surgical resection, chemoradiotherapy is recommended (Croitori et al., 2012).

Neoadjuvant therapy is currently not recommended in these patients. Benefit may be derived in some selected cases. In locally advanced CC; 50-90% of patients with CC has local unresectable disease. Their prognosis is very poor (few months) and palliative therapy may improve symptoms (e.g. jaundice, itch) and quality life. Chemoradiation is performed as radiotherapy (EBRT; stereotactic therapy and brachytherapy with indium-192). There are a number of treatment protocols in advanced CC. 5-FU, gemcitabine, cisplatin and oxaliplatin are used most commonly.

As new agents, TKI anti-EGFR (erlotinib), other anti-EGFR (cetuximab) and antiangiogenics (bevacizumab) are investigational drugs. However, their effects are currently disputable.

In hilar cholangiocarcinoma; as curative therapy: 1) local excision 2) combined partial hepatectomy 3) ex situ vivo liver resection and autotransplantation 4) orthotropic liver transplantation 5) central lobe resection 6) neoadjuvant/adjuvant therapies may be given. As palliative therapy; 1) palliative surgical treatment 2) endoscopic stent 3) percutaneous stent 4) photodynamic treatment 5) intraluminal brachytherapy 6) external radiation and systemic chemotherapy may be administered (Kuhlman et al., 2013; Ramai et al., 2013; Lau and Lau, 2012).

Radiotherapy: a) external beam radyoterapi and chemoradiation or b) local radiation techniques (intraoperative and intraductal brachytherapy) is performed. -Locoregional treatments as a) -TACE b) Radiofrequency ablation c) - radioembolization may be performed in suitable CC patients.

Endoscopic radiofrequency

Photofrin (porfimer sodium) is used, which is a selective method and accumulates in neoplastic tissue. It is used at 2 mg/kg intravenously 48 hours before laser activation. It is administered percutaneously or via cholangioscopy at the site where stricture is detected. Opens malignant narrowing and provides bile drainage (Wadsworthet al., 2013, Jayant et al.,2010; Rerknimitr et al., 2013).

In conclusion, cholangiocellular carcinoma is a malignant disease which is suggested to be a stem cell disease triggered by inflammation, environmental and genetic factors. It originates from bile ducts and has a poor diagnosis. Future advances in the etiopathogenesis of the disease will contribute to a better understanding of the condition and its better treatment.

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