Biomarker Study of Cholangiocarcinoma

Qinyi Cai
Medical Laboratory Technology, Chengdu University of TCM, Chengdu, 611137, China

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Abstract: Cholangiocarcinoma is a malignant tumor originating from the epithelial cells of the bile duct mucosa in the biliary tract. The incidence of cholangiocarcinoma is increasing day by day. The current diagnostic technology cannot meet the needs of early diagnosis and the prognosis is poor, resulting in high mortality. Therefore, cholangiocarcinoma is known as the "king of cancer". How to effectively diagnose cholangiocarcinoma as soon as possible is of great significance for prolonging the life of patients with cholangiocarcinoma. Based on the diagnosis and treatment of cholangiocarcinoma, this paper summarizes the existing research results and clinical methods, reviews the current research status of biomarkers for cholangiocarcinoma, and looks into the future development of biomarkers.

1 INTRODUCTION

Cholangiocarcinoma is a kind of malignant tumor originating from epithelial cells, which can be divided into intrahepatic cholangiocarcinoma and extrahepatic cholangiocarcinoma according to the site of the disease. Extrahepatic cholangiocarcinoma can be divided into hilar cholangiocarcinoma and distal cholangiocarcinoma. Cholangiocarcinoma patient inchoate does not have special symptom commonly, abdominal unwell ache, icteric is this disease the most common symptom, as the development of the illness, may appear temperature rises, nausea and vomiting, weak and weak, serious when the patient is immersed in coma.

Carbohydrate antigen 19-9 (CAI9-9) and carcinoembryonic antigen (CEA) is the most widely studied of CCA molecular markers, but its diagnostic value is not high. The current diagnostic methods for cholangiocarcinoma include imaging examination, endoscopy, histological examination and pathological examination, and serum marker examination, but these examinations cannot meet the diagnostic needs. There is still no fully effective biomarker for early detection of cholangiocarcinoma. For these reasons, cholangiocarcinoma is usually diagnosed at advanced stage. And the survival rate of patients with advanced bile duct carcinoma is less than 5%.

Now, the treatment methods for cholangiocarcinoma mainly include surgical resection of tumor focus, liver transplantation and adjuvant therapy (radiotherapy and chemotherapy). Currently, targeted therapy and immunotherapy in cancer treatment cannot be effectively promoted due to the research dilemma of biomarkers for bile duct cancer. Based on the current research status of cholangiocarcinoma, we clearly know that the discovery of biomarkers that can be used for screening diagnosis is of great significance for the treatment of cholangiocarcinoma. Effective biomarkers can undoubtedly save the lives of more patients with cholangiocarcinoma.

2 EPIDEMIOLOGY

Cholangiocarcinoma accounts for about 3% of all digestive tract tumors, with a peak age of 70 years and slightly more men than women. ICC and eCC have different epidemiological characteristics. The incidence and mortality of CCA are on the rise in western countries. The incidence and mortality of ICC showed significant gender and ethnic differences. The incidence of ICC is 15 times higher in men than in women. The incidence of yellow people is 20 times higher than that of white and black people. The three subtypes of CCA also have different epidemiological trends. Over the past few decades, the age-standardized incidence of iCC has steadily increased in most parts of the world, while the age-standardized incidence of eCC has declined.

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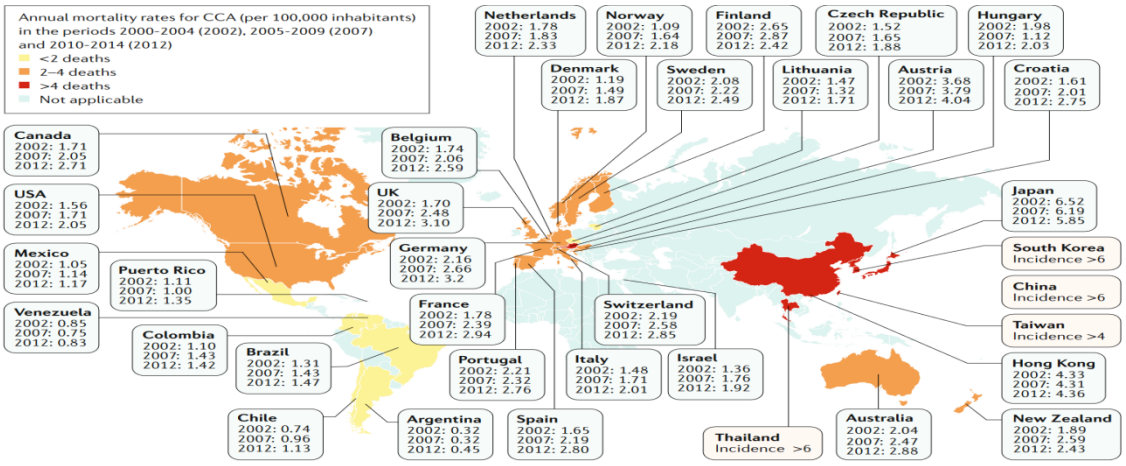

Figure 1: Mortality of cholangiocarcinoma worldwide.

3 SCREENING AND DIAGNOSIS

3.1 Imaging Examination Methods

At present, the commonly used imaging examination methods for the diagnosis of extrahepatic cholangiocarcinoma include ultrasound (US), computed tomography (CT), magnetic resonance imaging (MRI or MRCP), percutaneous transhepatic puncture cholangiography (PTC), endoscopic retrograde cholangiography (ERCP).

3.1.1 Abdominal B-Type Ultrasound

Abdominal B-mode ultrasonography is the first choice in the diagnosis of cholangiocarcinoma because it is noninvasive, simple and economical. However, the accuracy of abdominal ultrasound in the diagnosis of cholangiocarcinoma varies greatly. Hennedige et al. showed that the sensitivity and specificity of B-ultrasound in the diagnosis of extrahepatic cholangiocarcinoma were 89% and 80% ~ 95% respectively, which are mostly used as routine screening for cholangiocarcinoma.

3.1.2 CT Examination

CT is one of the traditional methods for the examination of bile duct diseases, which can show the location, scope and invasion of surrounding organs more comprehensively. The accuracy of multi-slice spiral CT (MDCT) in the diagnosis of extrahepatic cholangiocarcinoma can reach 78.6% ~ 92.3%, and the sensitivity and specificity of the diagnosis of cholangiocarcinoma can reach 84%.

3.1.3 MRI

MRI examination is currently the gold standard for non-invasive examination of hepatobiliary system diseases. It is a safe and effective non-invasive imaging technique. A number of studies have shown that MRCP has a high sensitivity for extrahepatic cholangiocarcinoma and can identify the lesion site.

3.1.4 ERCP and PTC

ERCP and PTC, as direct cholangiography methods, have good spatial resolution and can accurately understand the location and scope of the tumor. ERCP has an accuracy of 75% in diagnosing cholangiocarcinoma with duct obstruction. Yu et al. showed that preoperative PTCD combined with bile reperfusion can improve the resection rate and safety of patients with hilar cholangiocarcinoma.

3.1.5 Other Imaging Examinations

Positron emission computed tomography (PET) is a noninvasive imaging method, which has obvious advantages in early detection of malignant lesions and monitoring of recurrence and metastasis. Park et al. showed that whether distant lymph node metastasis was detected by preoperative PET/CT was positively correlated with the recurrence rate of patients with cholangiocarcinoma one year after surgery. Confocal laser microendoscopy (CLE) was initially used for the diagnosis of gastrointestinal diseases, and its subtype pCLE was used for the examination of diseases of the pancreatic bile duct system due to its good flexibility. Giovannini et al. confirmed the feasibility of pCLE for the diagnosis

of bile duct obstruction with a sensitivity and specificity of 83% and 75%, respectively.

3.2 Endoscopy

3.2.1 Endoscopic Ultrasonography (EUS)

EUS currently plays an important role in diagnosing cholangiocarcinoma. Mohamadnejad et al. found that EUS was relatively more sensitive to the diagnosis of cholangiocarcinoma, with a detection rate up to 94%.

3.2.2 Duodenoscopic Intrabile Duct Ultrasound (IDUS)

IDUS has more advantages than EUS in the diagnosis of cholangiocarcinoma, which can be used to determine the location of cholangiocarcinoma and evaluate the possibility of its resection. Compared with ERCP, IDUS has obvious advantages in sensitivity and accuracy.

3.3 Serum Tumor Markers

3.3.1 A Separate Test

Ca19-9 is the most studied diagnostic and prognostic indicator of cholangiocarcinoma, and its level is related to the development stage of the disease. Coelho et al. showed that CA19-9 level ≥103U/L can be used as a predictor of survival and metastasis in patients with cholangiocarcinoma. Hu et al. showed that serum CA19-9 could be used as an independent risk factor for resectable hilar cholangiocarcinoma. It is believed that CEA can also be used to guide clinical diagnosis of cholangiocarcinoma. Tang et al. proposed that the diagnostic sensitivity of CEA in extrahepatic cholangiocarcinoma was affected by the tumor location, with a high sensitivity to the middle cholangiocarcinoma, while the sensitivity to the distal cholangiocarcinoma was only 15.4%. CEA cannot be used in the diagnosis of cholangiocarcinoma alone, but is usually used as an indicator to observe the clinical effect and postoperative follow-up.

3.3.2 The Joint Detection

In view of the limitations of single application of CA19-9, CA125 and CEA, clinicians advocate the combination of multiple tumor markers to reduce the rate of missed diagnosis. Combined detection of ≥2 tumor markers can improve the sensitivity and specificity of diagnosis. Franco et al. found that the combined detection of CEA, CA19-9, cytokeratin 19 fragment and matrix metalloproteinase-7 can be used for preliminary screening of cholangiocarcinoma.

3.3.3 microRNAs

In recent years, miRNAs have received extensive attention due to their biostability.

Wang et al. found that serum Mir-26a concentration in patients with cholangiocarcinoma was significantly higher than that in healthy controls ($P < 0.01$), which was associated with clinical stage, distant metastasis, differentiation state and low survival rate of cholangiocarcinoma, and was an independent prognostic indicator of cholangiocarcinoma, and provided a new therapeutic target for cholangiocarcinoma.

Deng et al. also showed that serum Mir-29A level is associated with the progression of cholangiocarcinoma, which is an independent prognostic factor for patients with cholangiocarcinoma and can be used as a new biomarker to evaluate the prognosis of patients with cholangiocarcinoma.

Cheng et al. found that with the decrease of serum Mir-106A concentration, the risk of lymph node metastasis increased, and its expression level could serve as a strong prognostic indicator for patients with cholangiocarcinoma.

3.3.4 Other Tumor Markers

Specific modification of n-glycosylation of glycoprotein is recognized as a key component in cancer progression. Wang et al. found that hematoqing n-glycosylation can be used as a new tumor marker for diagnosis of extrahepatic cholangiocarcinoma. Its diagnostic value was higher than that of CA19-9. Okada et al. found that S-p53-ABS can be used for diagnosis of extrahepatic cholangiocarcinoma. In addition, studies showed that MUC5AC in blood serum could be used as a surrogate indicator for the diagnosis of cholangiocarcinoma.

Histopathological Examination

3.4.1 Cytological Examination

ERCP can be used for bile duct biopsy, brush examination and exfoliated cell examination by endoscopy to obtain pathological data. Korc et al. showed that the specificity of brush cytology was

close to 100%, and the sensitivity was 30%-57%. Endoscopic ultrasound fine needle aspiration (EUS-FNA) is another method for obtaining extrahepatic bile duct cancer cells.

3.4.2 Biopsy Histology

Histological diagnosis is highly specific. Chen et al. showed that the sensitivity and specificity of extrahepatic bile duct biopsy were 53.85% and 100.00%, respectively. In summary, there are many studies on the diagnosis of extrahepatic cholangiocarcinoma in recent years. However, the diagnosis and treatment of extrahepatic cholangiocarcinoma is still a complex problem at the present stage. How to improve its diagnosis and treatment will be the focus of future research.

4 BIOMARKER CLASSIFICATION OF CHOLANGIOCARCINOMA

4.1 Circulating Nucleic Acids

MiRNA is a kind of tiny non-coding RNA that plays an important role in regulating protein expression. MiRNA has been confirmed to be involved in the development and metastasis of many human cancers. Because mirnas are common in the early stage of some cancers, they are often used as diagnostic indicators of some cancers. Studies have shown that the contents of mirnas (Mir-21, Mir-34c, Mir-200b and Mir-221) in serum of iCCA patients are 2.18-3.79 times higher than those in non-tumor cells. In addition, serum Mir-483-p and Mir-222, Mir-26a and Mir-150 have certain diagnostic value for CCA.

Chen et al. found that mir-21, Mir-141 and Mir-200b were highly expressed in malignant bile duct cancer cells, and the sensitivity of cancer cells to chemotherapy drug gemcitabine was increased by inhibiting the expression of Mir-21, and the potential tumor suppressor target gene PTEN of Mir-21 was screened out through experiments. In the study of Mir-21, Selaru et al. found that TMP3 protein is a tumor suppressor gene that promotes apoptosis of cancer cells and inhibits invasion and metastasis of cancer cells, and also found the relationship between Mir-21 and TMP3 protein: inhibition of cancer cell growth means inhibition of Mir-21 and enhancement of negative regulatory relationship of TMP3 protein. You Hao et al. obtained a tumor suppressor gene, Maspin, and found that maspin was related to tumor size and invasion degree in cholangiocarcinoma, and its expression could be increased by inhibiting Mir-21. Zeng et al. found that the overexpression of Mir-124 inhibits the migration and invasion of cancer cells in intrahepatic cholangiocarcinoma.

Long non-coding RNA (lncRNA) and circRNA (circRNA). Xu et al. found that inhibition of SPRY4-IT1 expression could weaken the proliferation, metastasis and invasion ability of HuCCT1 and RBE cells in cholangiocarcinoma. Wang et al. found that overexpression of lncRNADANCR could promote proliferation, metastasis and invasion of bile duct cancer cells. XingLC et al. found through experimental studies that low expression of hsa-circ-0001649 can enhance the ability of cancer cell proliferation, metastasis and invasion. Lu and Fang found that the expression of CIRC-SmarCA5 was negatively correlated with TNM staging of cholangiocarcinoma and abnormal status of CA199.

CfDNA is a section of DNA that can be released into the blood by physiological or pathological mechanisms. In malignant tumors, cfDNA is mainly derived from apoptotic and necrotic tumor cells, which are characterized by genetic and epigenetic abnormalities, such as point mutation, loss of heterozygosis (LOH), microsatellite instability (MSI) and DNA methylation, etc.

Dicer, one of the RNase iii endoribonuccinases, plays an important role in regulating the methylation of CpG islands in mammalian cancer cells. Cheng et al. found through experiments that Dicer promoted DNA methylation of SFRP1 promoter, thus leading to proliferation and invasion of bile duct cancer cells. Liu et al. conducted a comprehensive analysis of tissue samples from 152 patients with cholangiocarcinoma, and found that GATA5 inhibits the proliferation and metastasis of cholangiocarcinoma by inactivating Wnt/β -catenin signal transduction.

4.2 Other Markers

4.2.1 In Serum

IL-6 is a 184-amino acid pleiotropic cytokine that promotes acute inflammatory response. Serum levels of L-6 cannot be measured under normal circumstances, but serum IL-6 is significantly elevated in hepatocellular carcinoma and cholangiocarcinoma. The positive judgment value of IL-6 as a serum marker was 83.3% for

cholangiocarcinoma and 81.3% for hepatocellular carcinoma. The sensitivity and specificity of IL-6 increased in cholangiocarcinoma was 100% and 91.4%. The serum IL-6 value of patients with cholangiocarcinoma was positively correlated with tumor load, and the mean and median value of il-6 activity were significantly higher than those of other tumors. IL-6 is one of the ideal tumor markers for the diagnosis of cholangiocarcinoma.

Frampton et al. found that IL-6 can regulate the activity of ERK1/2 / RSK1 / C/EBP pathway, catalyzing the expression of PGRN, and further promoting cell proliferation. Long-term up-regulation of PGRN can promote cell malignancy. IL-6 can also enhance the methylation level of tumor suppressor factor by modulating DNMT-1 (DNMT-1) and promote cell proliferation. IL-6 induced low cell cycle modulation of egg white P21 (WAF1 / CIP1) by activating p38MAPK, and up-regulated anti-apoptotic egg white Mcl 1 by increasing stat-3 activity.

Carcinoembryonic antigen (CEA) is present in the serum, bile and bile duct epithelium of patients with cholangiocarcinoma. The bile CEA of patients with cholangiocarcinoma [(50.2±5.8)ng/ml] was significantly higher than that of patients with benign biliary stenosis [(1±3.9)ng/ml], suggesting that the determination of CEA in serum and bile is helpful for the early diagnosis of cholangiocarcinoma, the evaluation of residual tumor and the prognosis of cholangiocarcinoma .

Ca19-9, CA50, CA242 and other glycochain group tumor markers were highly sensitive to hilar cholangiocarcinoma. Patel et al. analyzed serum CA19-9 of patients with benign and malignant biliary tract diseases and found that when serum CA19-9>100μ/ mL, the sensitivity of cholangiocarcinoma was 53%, while that of patients with benign liver disease and benign biliary tract stenosis was 24% and 8%, respectively. The serum CA19-9 concentration in patients with tumor resection was significantly lower than that in patients without tumor resection. Therefore, CA19-9 may be an effective tumor marker for the diagnosis of cholangiocarcinoma and the monitoring of efficacy. The sensitivity and specificity of CA50 in the diagnosis of cholangiocarcinoma were 94.5% and 33.3% respectively. Brockmann et al. analyzed the concentrations of CA19-9, CEA, CA72-4, CA125 and AFP in gallbladder bile of patients with pancreatic biliary system malignant tumor, and found that the concentration of CA19-9 was very high. The sensitivity of CA19-9 in cholangiocarcinoma was 100%, and the sensitivity and specificity of CEA in mastoid carcinoma was 100%. Ca19-9 differs between primary carcinoma and lymph node metastasis. Therefore, CA19-9, CEA and CA72-4 are valuable in the early diagnosis and prognosis of cholangiocarcinoma.

Cyfra21-1 is released into the blood by malignant epithelial cells and is a molecular biomarker for a variety of malignancies, including non-small cell lung cancer and gastric cancer. Similarly, elevated levels of CyFRA21-1 were found in serum samples from patients with primary liver tumors, including CCA. Huanget al found that the diagnostic effect of CYFRA21-1 was superior to CEA and CA19-9 in iCCA.

EXT1 is one of the five genes encoding the exon globulin family, and its mutation is believed to be the cause of hereditary diseases. Studies have found that the level of EXT1 in the blood of CCA patients is significantly higher than that of healthy subjects.

Mmp-7 is an endopeptidase in the MMP family, which can degrade extracellular matrix proteins. In CCA tumor tissues, it is closely related to tumor invasiveness and poor prognosis. Mmp-7 in serum of CCA patients can also be used as a molecular biomarker for the diagnosis of CCA.

Heat shock protein 70(HSP70) is a stress response protein, and excessive production can enhance the resistance of cancer cells to apoptosis-inducing factors such as tumor necrosis factor α. One study showed that plasma HSP70 antibody titers were highest in patients with CCA compared with cholangitis and healthy controls.

Angpt-2 (ANGPT-2) sophora lectin (SJA) is the antagonistic ligand of tyrosine kinase Tie2 in the Angpt/Tie2 system. Since ANGPT-2 is strongly expressed in the vascular system of many tumors, it is speculated that it promotes tumor development together with other cytokines. Serum ANGPT-2 levels were higher in patients with CCA than in patients with primary sclerosing cholangitis (PSC) and in patients with bile duct stones. Therefore, serum ANGPT-2 is feasible as a molecular biomarker for the diagnosis of CCA.

4.2.2 In the Bile

MUC4, a mucin protein in bile specimens, is considered as a potential diagnostic marker for cholangiocarcinoma. Matull et al. did not use western blots to analyze MUC4 of the biliary tract, and the results showed high specificity in differentiating biliary tract cancer from other malignant tumors, PSC and other benign biliary tract diseases. In addition, it has also been reported that

SSP411 protein is a potential molecular biomarker for the diagnosis of CCA.

4.2.3 In the Urine

Molecular biomarkers related to the diagnosis of CCA in urine mainly include urinary protein and miRNA. Urine contains proteins and peptides derived from ultrafiltration of plasma, and urine protein bodies are highly sensitive to changes in both renal and other non-renal diseases. Urine proteomic analysis (UPA) of the urine peptide-labeled model showed high sensitivity and specificity for the diagnosis of CCA.

5 DISCUSSION AND CONCLUSION

Cholangiocarcinoma is characterized by high incidence and difficult improvement of long-term postoperative survival rate, which makes it a major problem in clinical treatment. Therefore, it is extremely important to find and diagnose the disease as early as possible to save the lives of patients. Based on this, this paper summarized the existing findings through the review of relevant international studies, hoping to be helpful for the study of biomarkers for cholangiocarcinoma. In this review, the current treatment and diagnosis methods are summarized. It is not difficult to see from the current research results that there are still many blank fields in the research of cholangiocarcinoma. Although many biomarkers have been found to have diagnostic value in cholangiocarcinoma, the further mechanism and other studies are not clear. Through the efforts of scientists, the clinical treatment of cholangiocarcinoma has changed significantly in the past few years, improving the survival rate, especially in advanced patients, but the survival rate is still very low, so how to improve the rate of early diagnosis is still the goal of research. At present, biomarkers have become a powerful tool for diagnosis, prognosis and prediction of treatment response, and the development of biomarkers plays an important role in improving the prognosis of these patients. However, biomarker guided liver tumor treatment is currently a closed clinical practice, and current technical methods still cannot fully meet its early diagnosis needs, we still lack accurate non-invasive biomarkers to diagnose and evaluate the prognosis of patients with cholangiocarcinoma. There is still a long way to go to study biomarkers for cholangiocarcinoma.

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