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TOPIC HIGHLIGHT

New insights on cholangiocarcinoma

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Abstract

Cholangiocarcinoma (CCA) is a devastating cancer arising from the neoplastic transformation of the biliary epithelium. It is characterized by a progressive increase in incidence and prevalence. The only curative therapy is radical surgery or liver transplantation but, unfortunately, the majority of patients present with advanced stage disease, which is not amenable to surgical therapies. Recently, proposed serum and bile biomarkers could help in the screening and surveillance of categories at risk and in diagnosing CCA at an early stage. The molecular mechanisms triggering neoplastic transformation and growth of biliary epithelium are still undefined, but significant progress has been achieved in the last few years. This review deals with the most recent advances on epidemiology, biology, and clinical management of CCA.

Key words: Cholangiocarcinoma; Cholangiocytes; Growth factors; Proliferation; Apoptosis

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INTRODUCTION

Cholangiocarcinoma (CCA) is a malignant tumor arising from the malignant transformation of cholangiocytes, the epithelial cells lining the biliary tree. CCA is the second most common primary hepatic malignancy, with recent epidemiologic studies suggesting a progressive increasing incidence in Western countries[1]. CCA is characterized by a bad prognosis, with a median survival of less than 24 mo[2-4] and a scarce response to chemotherapy[5-9]. From the anatomical point of view, CCA is classified as intrahepatic (IH-CCA) or extra-hepatic (EH-CCA), the latter being further divided into proximal or perihilar and distal, depending on the location of the cancer within the extra-hepatic biliary system. Perihilar CCA is also known as Klatskin tumor. Three different growth patterns of EH-CCA can be observed: (1) periductal infiltrating; (2) papillary or intraductal; and (3) mass forming[10]. Intrahepatic CCA typically presents as an intrahepatic mass. The only curative therapy is surgical resection or liver transplantation but, unfortunately, the majority of patients are diagnosed at advanced stage, when surgical therapies are excluded. This should stimulate research on the identification of effective surveillance strategies that would permit detection of early CCA or, better yet, premalignant lesions in patients at increased risk, particularly patients with primary sclerosing cholangitis (PSC). Serum and bile tumor markers, non invasive and endoscopic-based imaging modalities, and histology and cytology have been attempted with varying success[11].

The manuscript deals with the most recent advances on the epidemiology, biology and clinical management of CCA.

EPIDEMIOLOGY

Epidemiological studies in different geographical areas give information on the incidence, prevalence, and mortality time trends and, therefore, are important as they might yield clues to potential etiological factors[12-16]. Hepatobiliary malignancies account for 13% of the 7.6 million annual cancer-related deaths worldwide and for 3% of the 560 000 annual cancer-related deaths in the United States. CCA accounts for 10% to 20% of the deaths from hepatobiliary malignancies. The prevalence of CCA shows a wide geographical variability, with the highest rates in Asia and the lowest in Australia[14]. In the United States, the incidence of CCA has been reported to be 0.95/100 000 for IH-CCA and 0.82/100 000 for EH-CCA[14,15]. CCA prevalence in different racial and ethnic groups is heterogeneously distributed, with the highest age-adjusted prevalence in Hispanics (1.22/100 000) and the lowest in African Americans (0.17-0.50/100 000)[14-18]. A number of recent studies have highlighted a progressive increase, in the past three decades, in the mortality for IH-CCA[15] , while EH-CCA mortality is stable or slightly decreasing[12,15]. With the exception of Denmark[19,20], this scenario has been reported worldwide[1,15-18]. The significant increase in age-adjusted incidence of IH-CCA was confirmed even after correction for a prior misclassification of hilar CCA as IH-CCA[1]. In Europe, the increase in the IH-CCA mortality was higher in Western Europe than in Central or Northern Europe. In contrast, mortality rates for EH-CCA showed a decreasing trend in most countries[1,15-17]. Very recently, data on the mortality and incidence trend for CCA have also been reported in Italy, where a 40-fold increase in mortality for IH-CCA has been documented from 1980 to 2003. For EH-CCA, in contrast, mortality rates were stable or slightly decreasing in the last 10 years. Thus, as described in most countries, in Italy the increased mortality for CCA mainly involves the intrahepatic form, suggesting different etiology and risk factors for IH- and EH-CCA[14-16]. Interestingly, in all epidemiological studies concerning primary liver malignancies, a high percentage (about 40%) of primitive liver cancers are classified as adenocarcinoma and are therefore excluded from the group of either CCA or hepatocellular carcinoma. This probably accounts for a significant underscoring of IH-CCA incidence and mortality because it is a common clinical opinion that most primary liver adenocarcinomas are indeed CCA[21]. Biological, immunohistochemical, or genetic markers[4,21] could definitively permit an exact diagnosis and classification of primary liver cancers and avoid these classification biases.

RISK FACTORS

A number of different risk factors have been definitively identified. PSC is the most commonly recognized risk factor[22-25]. The prevalence of CCA in PSC is 5%-15%, and the annual incidence rate is 0.6% to 1.5%[22-25]. The majority of PSC patients will develop CCA within the first 2.5 years after the diagnosis of PSC[22-25]. Thus, the symptomatic patient who presents with their first diagnosis of PSC should be carefully screened for CCA. Infectious etiologies, such as parasitic[26,27] and bacterial infections (i.e. Opisthorchis Viverrini, Clonorchis Sinensis, Schistosomiasis Japonica and Salmonella Tiphi), lead to an increased risk of CCA in endemic regions of Asia.

Exposures to certain xenobiotics might lead to an increased risk of CCA. Multiple case-control studies have reported an association between CCA and alcohol use[26-29]. Iatrogenic exposure to thorotrast (thorium dioxide), a radiocontrast agent used in the 1950s and 1960s, first led to reports of CCA in the 1970s[30,31]. Since that time, hundreds of cases of CCA (as well as other primary hepatic malignancies) attributed to thorotrast exposure have been described. Caroli disease, congenital choledocal cist[32-35], bilio-enteric surgical drainage, abnormal biliary-pancreatic junction, and intra-hepatic lithiasis[36-38] are other risk factors for CCA. In contrast to patients submitted to bilio-enteric surgical drainage for benign diseases that represent a well recognized category at risk, patients submitted to endoscopic sphincterotomy during endoscopic retrograde cholangiopancreatography (ERCP) failed to express increased risk of CCA, and this has been definitively demonstrated in three different studies performed in a large series of patients with long follow-up[39-41]. Several case-control studies have described an increased risk of CCA in patients with chronic hepatitis C virus (HCV) infection[42,43], and HCV-RNA has been detected in some cases of resected CCA[44].

A prospective study of 600 HCV-infected individuals in Japan between 1980 and 1997 (median follow-up 7.2 years), detected a 2.3% incidence of CCA, which is well above the baseline population incidence[45]. Although hepatitis B virus nucleic acids have been detected in selected cases of CCA[46,47], an association between hepatitis B virus infection and CCA is less well established[45-48]. More recently, obesity, diabetes[37,49] and smoking have been taken into consideration, especially for IH-CCA, but further confirmation is need. In PSC patients[22-25], smoking and alcohol further increases the risk of CCA development. Whether concomitant ulcerative colitis and its duration could increase CCA risk remains to be definitively established[22-25].

MOLECULAR AND CELLULAR PATHOGENESIS

The molecular mechanisms underlying the development, growth, and metastatic diffusion of biliary tract cancers are still undefined. Recent attention has been paid to the origin of CCA from the neoplastic transformation of resident hepatic stem cells[50,51]. However, evidence for a role of resident stem cells exists, so far, only for primitive hepatic cancers characterized by mixed hepatocellular carcinoma/CCA phenotypes[50,51], which are currently classified as hepato-CCA.

The recognized risk factors for CCA share, as a common basis, a condition of chronic inflammation of the biliary epithelium together with a partial biliary obstruction[52,53]. As a consequence, most studies deal with pathways

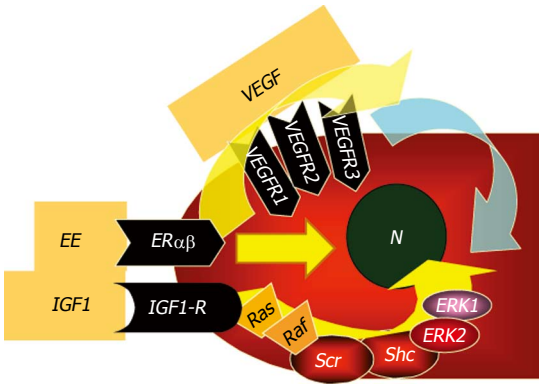

Figure 1 Proposed mechanisms of estrogen-induced proliferation of cholangiocarcinoma (CCA) cells. Cross-talk between IGF1 and estrogens has been recently demonstrated to modulate cholangiocarcinoma proliferation, where estrogens act at several points of the IGF1 signal transduction pathway (see ref.# 93,94). In addition, it has been shown that the estrogen proliferative effect on CCA cells is also due to the stimulation of vascular endothelial growth factor (VEGF) synthesis and secretion (see ref.# 93,94).

linking inflammation and carcinogenesis[54-57]. In general, chronic inflammation is thought to promote carcinogenesis by causing damage in DNA mismatch repair genes/proteins, protooncogenes, and tumor suppressor genes and, by creating a local environment enriched with cytokines and other growth factors capable to accelerating the cell cycle, to favor accumulation of somatic mutations[58,59].

Current evidence supports a primary role played by nitric oxide (NO) induced by pro-inflammatory cytokines (TNF-α, IL-6 *etc*.)[54-57]. These cytokines are able to activate inducible nitric oxyde synthase (iNOS), which, at the immunohistochemical level, is overexpressed in more than 70% of CCA. Increased iNOS activity results in the generation of NO and reactive oxygen species known to interact with cellular DNA and to inhibit DNA repair mechanisms, thus triggering oncogenetic mutations[55,56]. Dysregulation of the proto-oncogene k-ras[60], nuclear accumulation of p53[61-63], and up-regulation of the related proteins mdm-2 and WAF-1 have been observed in CCA[64-66]. Other inactivated suppressor genes include $p16^{INK4a}$[67], DPC4/Smad4, and APC[68-70]. The majority of these genetic changes were described in IH-CCA. NO, together with different cytokines, can also inhibit cholangiocytes apoptosis by nytrosilation of caspase 9 and might induce proliferation, thus favouring accumulation of somatic mutations.

Interleukin-6 (IL-6) also plays a key role in the pathogenesis of CCA and, especially, in cholangiocyte evasion from apoptosis. IL-6 is produced at high levels by CCA cells, and elevated IL-6 serum concentrations have been reported in CCA patients[71,72]. Autocrine and paracrine IL-6 stimulation activates the prosurvival p38 mitogen activated protein kinase[73,74]. In addition, IL-6 upregulated the expression of myeloid cell leukemia-1 (Mcl-1), through STAT3 and AKT related signaling pathways[75,76]. Mcl-1 is an anti-apoptotic protein of the Bcl-2 family of apoptotic proteins.

Cycloxygenase 2 (COX-2), the rate-limiting enzyme in prostaglandins biosynthesis from arachidonic acid, which is activated by inflammatory cytokines and NO, further accelerates cell cycle via PgE2 and inhibits different apoptotic cascades[77]. Indeed, increased COX-2 immunohistochemical expression has been documented in more than 70% of CCA. Oxysterols, oxygenated cholesterol derivatives formed in bile of patients with inflammatory diseases of biliary tree, together with bile acids[78-80], are also able to activate COX-2[81-85]. Other COX-2–inducing molecules include the tyrosine kinase ErbB-2[86], which is overexpressed in CCA and involved in CCA carcinogenesis and progression[87,88]. ErbB-2 is an epidermal growth factor receptor (EGFR) homolog and is able to homodimerize or heterodimerize with other members of the EGF superfamily, resulting in activation of the Raf/MAPK-pathway[89,90]. Hydrophobic bile salts, such as deoxycholate, might play a carcinogenetic role through transactivation of EGFR and impairment of Mcl-1 functions[78]. Constitutive overexpression of ErbB2 and/or ErbB1 in malignant cholangiocytes has been documented in more than 50% of IH-CCA. In addition, rodent models of intrahepatic cholangiocarcinogenes have been developed and are associated with constitutive ErbB2 overexpression[87]. ErbB2 and ErbB1 interact with different molecular signalling pathways associated with IH-CCA development and progression, including bile acids, IL-6/gp130, transmembrane mucins, hepatocyte growth factor/Met[91], and vascular endothelial growth factor (VEGF) signalling. The relevance of ErbB2 or ErbB1 related pathways in CCA have raised interest in the possibility that agents that selectively target these receptors could potentially be effective in CCA therapy[86,89]. However, current experience with such ErbB targeted therapies produced only modest responses in patients with biliary tract cancers. Another recently proposed mechanism linking chronic inflammation with CCA development is related to activation-induced cytidine deaminase (AID), a member of the DNA/RNA editing enzyme family, implicated in human carcinogenesis via its mutagenic activity[92]. AID was found to be increased in biopsies from patients with PSC or CCA, whereas only trace amounts of AID were detected in the normal liver. Very recently, a relevant role in modulating CCA growth and proliferation has been attributed to estrogens, insulin like growth factor 1 (IGF1), leptin, opioid receptor modulators, endothelin, and serotonin[54,93-96]. As far as estrogens are concerned, recent studies suggest their synergistic action with growth factors (IGF1, VEGF) in sustaining the cholangiocyte proliferative machinery and in depressing apoptosis (Figure 1). Indeed, cross-talk between IGF1 and estrogens has been demonstrated to modulate CCA proliferation, where estrogens act at several points of the IGF1 signal transduction pathway (Figure 1)[93,94]. In addition, it has been shown that the estrogen proliferative effect on CCA cells is also due to the stimulation of VEGF synthesis and secretion (Figure 1)[93,94].

In this experimental background, a number of differ-

ent genetic/epigenetic abnormalities involving inflammation-related genes, as well as genes involved in the control of cell cycle or DNA repair, have been documented with implications in terms of genetic susceptibility of individual/categories at risk[79,80]. These genetic studies, however, carry a number of biases caused by the anatomical and morphological CCA heterogeneity, geographic/racial differences, and different concurrent risk factors. In addition, most studies come from oriental countries that are not necessarily applicable in western populations[79]. In spite of all these difficulties, genetic variants of the Natural Killer Cell Receptor G2D receptor[80], which drives the clearance of damaged cholangiocytes by natural killer lymphocytes, have been recently associated with the development of CCA in PSC patients, suggesting the possibility to identify and submit to strict surveillance a subgroup of PSC patients. Among other inflammation-related genes, specific haplotypes of COX2-coding gene (PTGS2) or IL8RB have been recently associated with a significant risk of CCA development in oriental populations[79].

SERUM AND BILE BIOMARKERS AIDING CCA DIAGNOSIS

In the majority of cases, CCA is clinically silent, with symptoms only developing at advanced stages. Surgical resection is the only effective therapeutic option for CCA, but this is applicable in less than 50% of cases, because this cancer is mostly diagnosed at late stages. In early-cancer, the five-year survival after radical surgery is higher than 80% and therefore, any attempt to facilitate early diagnosis is to be welcomed. For many years, efforts have been made to identify, in serum or biological fluid[97-99], biomarkers with adequate diagnostic accuracy for CCA, which could also be useful for population screening or for the surveillance of pathologies at risk, including PSC[98-101]. Serum tumor markers are attractive because of the ease of obtaining samples and their relatively low cost. Therefore, they have been the objects of extensive investigation to aid CCA diagnosis but, unfortunately, none of these markers has reached adequate specificity for CCA (Table 1)[102-105].

Carcinoembryonic antigen (CEA), which is mainly used for colorectal cancers, is of scarce utility being increased only in approximately 30% of patients with CCA[102-104]. Carbohydrate antigen (CA 19-9) is the most widely used serum marker for CCA but it is also elevated in pancreatic cancer, gastric cancer, and primary biliary cirrhosis. In smokers, it might be transiently increased during cholangitis or cholestasis. The sensitivity and specificity for detection of CCA in PSC are 79% and 98%, respectively, at a cutoff value of 129 U/mL. Other investigators have documented that only a higher cutoff (> 180 U/mL) could achieve this degree of specificity[106]. According to Levy *et al*[107] a change from baseline of > 63 U/L has a sensitivity of 90% and specificity of 98% for CCA detection. In patients without PSC, CA 19-9 sensitivity is 53% at a cutoff of > 100 U/L and its negative predictive value

Table 1 Recently proposed serum and bile biomarkers for the diagnosis of Cholangiocarcinoma

Biomarker	Sensitivity (%)	Specificity (%)
Serum		
CA19-9	53-92	50-98
CEA	33-68	79-100
IL-6	73	92
Trypsinogen-2	AUC = 0.804	
MUC5AC	71.01	90
CYFRA 21-1	74.7	92.2
Bile		
CA19-9	46-61	60-70
CEA	67-84	33-80
IGF1	100	100
Pancreatic elastase/ amylase ratio	82	89
Mcm5	62	67

CEA: Carcinoembryonic antigen; IL-6: Interleukin-6; IGF1: Insulin like growth factor 1

is 76%-92%[108]. CA 19-9 can also be elevated in bacterial cholangitis and other gastrointestinal and gynecologic neoplasias; patients lacking the blood type Lewis antigen (10% of individuals) do not produce this tumor marker[109-112]. In general, as extensively discussed in recent reviews[102-104], high sensitivity and specificity have been reported for CA 19-9 in patients with CCA, depending on the study population and cutoff values, and this is also true for CCA complicating PSC. In addition, CA 19-9 usually allows CCA diagnosis in advanced stages when radical treatments are not allowed. Therefore, current efforts aim to identify novel serum markers that can be substituted for CA19-9 or can improve, when measured together, the diagnostic accuracy of CA 19-9. The serum level of interleukin 6[71], at a 25.8 pg/mL cutoff, provides a diagnostic sensitivity of 73% and a specificity of 92%. Serum levels of IL-6 have been correlated with tumor burden in CCA patients and, interestingly, one month after treatment with photodynamic therapy, the mean IL-6 level decreased significantly. Although these findings are encouraging, it should be considered that serum IL-6 is also elevated in many patients with hepatocellular carcinoma, benign biliary disease, and metastatic lesions[105]. Other biomarkers such as trypsinogen-2[113], platelet–lymphocyte ratio (PLR), mucin-5AC[114,115], soluble fragment of cytokeratin 19 (CYFRA21-1)[116] have been recently shown to help in the diagnosis of CCA with, in some cases, a prognostic value[117,118]. In particular mucin 1 (MUC1) and MUC5AC are not expressed by hepatocellular carcinoma, suggesting a possible role in the differential diagnosis[105].

As far as bile is concerned, the ratio of pancreatic elastase/amylase, mucin-4, Mcm5 (minichromosome maintenance replication protein) and IGF1 have been explored with the IGF1 biliary concentration capable of completely discriminating CCA from benign biliary pathologies and pancreatic cancer (Table 1). Specifically, we measured IGF1 and VEGF in the bile of patients undergoing ERCP for biliary obstruction and evaluated the

performance of these markers in differentiating EH-CCA from pancreatic cancer or benign biliary abnormalities[99]. The biliary IGF1 concentration was 15-20 fold higher in EH-CCA than in the other two groups. In contrast, biliary VEGF concentration was similar in the three groups. In substance, this study indicated a marker (bile IGF1), which could be used, with absolute diagnostic accuracy, to differentiate EH-CCA from either pancreatic cancer or benign biliary disorders, in patients undergoing ERCP for biliary obstruction.

Proteomic analysis of serum and bile are under investigation but definitive findings are currently unavailable. Another strategy is to evaluate the diagnostic performance of serum CA 19-9 together with imaging techniques[119-121]. It has been demonstrated that the combination of serum CA 19-9 with CT, MRI, MRCP or ERCP shows the best sensitivity (about 100%) but a low specificity (about 40%) in diagnosing CCA occurring in PSC patients. In contrast, the combination of serum CA19-9 and US had intermediate specificity (62%) with a good sensitivity (91%) for detecting CCA. On the basis of test properties, cost and availability, combination of serum CA 19-9 (cut-off value of 20 U/mL) and abdominal US at 12-mo intervals was proposed as a useful strategy for the screening/surveillance of CCA in PSC[10,100].

TREATMENT

There is no current effective medical therapy for CCA, and the median survival after treatment of unresectable disease is 9-12 mo[122,123]. Complete operative resection might be curative, but local extension of the disease often precludes complete resection[124,125].

Accurate preoperative staging will determine the treatment approach in these patients. Although a pathological staging system has been developed for ductal CCA, it is of limited value in EH-CCA. TNM classification does not correlate with resectability in patients with EH-CCA. Conversely, the Memorial Sloan-Kettering staging system can evaluate the biliary and vascular involvement of these tumors, which clearly correlates with resectability and survival. In addition, clinical staging is fundamental in pre-surgical evaluation, and this is based on evaluation of the proximal and distal extent of the disease, vascular involvement, and presence of metastasis, which can be done by Doppler ultrasound, MRI, CT, or EUS examinations. FDG-PET scanning changes the surgical management in a third of patients, with an overall sensitivity for metastasis detection of approximately 65%. Solitary IH-CCAs are managed by segmentectomy or lobectomy. Five-year survival rates are 22%-44% and correlate with R0 (negative margin) resection, absence of lymph node metastases, and vascular invasion[126-130]. Even with complete resection, local recurrence is common, with most series reporting five-year survival of 25%-35%. In PSC, outcomes of surgical resection are complicated by advanced liver disease in the majority of these patients, recurrent cholangitis with a biliary –enteric anastomosis, the multi-focal nature of the cancer, and the increased risk for further CCA[131]. Biliary-enteric anastomosis is a risk factor for *de novo* CCA[132,133]; therefore, creating a biliary-enteric anastomosis in a PSC patient should be viewed with caution, and informed consent regarding the potential development of additional CCA should be considered. Patients with PSC plus CCA might be better evaluated as potential liver transplant candidates. In the past, CCA was considered as a contraindication for liver transplantation. Recently, however, the development of new liver transplantation protocols at the Mayo Clinic and the University of Nebraska yielded promising results for EH-CCA[134,135]. Strict selection criteria have been developed, and this protocol includes neoadjuvant therapy with external beam radiation concurrent with 5-fluorouracil (5-FU) chemotherapy, followed by brachytherapy and chemotherapy with capecitabine. The patients, prior to transplantation, underwent explorative laparotomy for restaging. Survival analysis of patients treated according to the Mayo Clinic protocol has yielded one-and five-year survival rates of 91% and 76%, respectively[136,137].

For the majority of CCA patients who are not candidates for surgical resection or transplantation, recent proposals deal with the use of photodynamic therapy (PDT)[138-140]. In these studies, PDT alone, or plus stenting, improved cholestasis and quality of life considerably, and had a favourable side-effect profile. In the light of these findings, recent review articles recommended PDT for patients with not resectable disease[140]. The role of PDT adjuvant treatment before or after surgical resection needs to be assessed. Finally, it is important to reiterate that PDT requires carefully patient management. Radiotherapy (external, intraluminal, or brachytherapy) has received recent attention and positive findings have been reported by different centers for perihilar-CCA[140].

As far as pharmacological therapy is concerned, CCA is characterized by a remarkable resistance to common chemotherapy[122,123]. Several drugs have been tested alone in unresectable CCA and in restricted phase Ⅱ studies (5-FU, methanesulfon-m-anisidide, cisplatin, rifampicin, mitomycin C, paclitaxel, and gemcitabine) with partial response of 0%-9% and average survival of 2-12 mo. Certainly, 5-FU was the drug most used, but with the same disappointing results of the other drugs used in monotherapy[141]. Recently, the Italian Health Care System approved the use of gemcitabine. In 2005, a review went back over 13 single arm phase Ⅱ studies suggesting the role of gemcitabine as an alternative supportive care[142]. However, randomized controlled trials are necessary to assert the real and potential increase of survival determined by the drug which, nevertheless, shows a low toxicity. With regard to combination therapies, one of the most used therapeutic plans is ECF (epirubicin + cisplatin + 5-FU), but with disappointing results[143]. Several phase Ib and Ⅱ studies evaluated the addition to gemcitabine[144], cetuximab[145,146], oxaliplatin/cisplatin[147-149], erlotinib[150], or capecitabine[151]. Among these studies, the effect seems to be more consistent with the combination of capecitabine and addition of oxaliplatin/cisplatin. With regard to bio-

logical therapy, there are ongoing pilot studies, not yet published, considering the use of sorafenib, lapatinib, or bevacizumab in the treatment of unresectable CCA. Studies of cellular and molecular biology have clearly demonstrated, in a high percentage of CCA, the activation of signaling pathways such as PI3-kinase and MEK/ERK, multiple receptor pathways (epidermal growth factor EGF; IGF1; estrogen receptors; VEGF) and COX2/PgE2. On the basis of this evidence, there is a rationale for pilot studies testing biological drugs acting on these targets. Paucity of data makes it impossible to use adjuvant chemotherapy after surgical resection[152]. In particular, chemotherapy with 5-FU and mitomycin C failed to improve the survival of patients undergoing surgical resection, as recently observed in a phase Ⅲ study[153].

The benefit of adjuvant radiotherapy after surgical resection has been recently reviewed[152]. Overall, there are retrospective data which, taken together, show a benefit, especially with regard to dose-scaling radiation[151]. However, the only prospective study[153] denies a real benefit of radiotherapy after surgical resection of peri-hilar CCA in terms of survival. Further prospective studies are needed to reach a definitive conclusion. Although data are poor, there are some studies[152] demonstrating a slight but significant improvement in survival for distal common bile duct cancers undergoing surgical resection and adjuvant radio-chemotherapy[154].

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