BBA - Molecular Basis of Disease xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

BBA - Molecular Basis of Disease

journal homepage: www.elsevier.com/locate/bbadis

The search for novel diagnostic and prognostic biomarkers in cholangiocarcinoma☆

Rocio I.R. Macias[a,g,*], Jesus M. Banales[b,c,g], Bruno Sangro[d,g], Jordi Muntané[e,g], Matias A. Avila[f,g], Elisa Lozano[a,g], Maria J. Perugorria[b,c,g], Francisco J. Padillo[e,g], Luis Bujanda[b,g], Jose J.G. Marin[a,g]

[a] Experimental Hepatology and Drug Targeting (HEVEFARM), University of Salamanca, IBSAL, Salamanca, Spain
[b] Department of Liver and Gastrointestinal Diseases, Biodonostia Research Institute, Donostia University Hospital, University of the Basque Country (UPV/EHU), San Sebastian, Spain
[c] IKERBASQUE, Basque Foundation for Science, Bilbao, Spain
[d] Liver Unit, Clínica Universidad de Navarra, IDISNA, Pamplona, Spain
[e] Department of General Surgery, "Virgen del Rocío" University Hospital, IBiS/CSIC/University of Sevilla, Spain
[f] Division of Hepatology, Center for Applied Medical Research (CIMA), University of Navarra, IDISNA, Pamplona, Spain
[g] Center for the Study of Liver and Gastrointestinal Diseases (CIBERehd), Carlos III National Institute of Health, Madrid, Spain

ARTICLE INFO

Keywords:
Biliary cancer
Biomarker
Differential diagnosis
Liver cancer
Omics

ABSTRACT

The poor prognosis of cholangiocarcinoma (CCA) is in part due to late diagnosis, which is currently achieved by a combination of clinical, radiological and histological approaches. Available biomarkers determined in serum and biopsy samples to assist in CCA diagnosis are not sufficiently sensitive and specific. Therefore, the identification of new biomarkers, preferably those obtained by minimally invasive methods, such as liquid biopsy, is important. The development of innovative technologies has permitted to identify a significant number of genetic, epigenetic, proteomic and metabolomic CCA features with potential clinical usefulness in early diagnosis, prognosis or prediction of treatment response. Potential new candidates must be rigorously evaluated prior to entering routine clinical application. Unfortunately, to date, no such biomarker has achieved validation for these purposes. This review is an up-to-date of currently used biomarkers and the candidates with promising characteristics that could be included in the clinical practice in the next future. This article is part of a Special Issue entitled: Cholangiocytes in Health and Disease edited by Jesus Banales, Marco Marzioni, Nicholas LaRusso and Peter Jansen.

1. Introduction

After hepatocellular carcinoma (HCC), cholangiocarcinoma (CCA) is the second most common primary tumor of the liver, accounting for approximately 15% of all primary liver cancers [1] and 2% of cancer-related deaths worldwide per year. The term CCA refers to a heterogeneous group of malignancies affecting the biliary epithelium that are classified into three entities depending on the anatomical location: i) intrahepatic CCA (iCCA), arising from the small bile ducts within the liver, and two types of extrahepatic CCAs, both arising from the ductal epithelium of the extrahepatic biliary tree; ii) distal CCA (dCCA) and iii) perihilar CCA (pCCA). Several other classifications have been proposed based on additional aspects of these tumors [2].

The silent evolution of the disease and the fact that its clinical manifestations are nonspecific and mainly related to the biliary obstruction caused by the tumor [3] justify the late diagnosis of patients with CCA, when the tumor is already at an advanced stage. These circumstances partly accounts for the poor prognosis and the high mortality rate of these patients. The overall 5-year survival after resection is usually lower than 40%, while in non-operable CCAs the overall 5-year survival is less than 5%. Diagnosis of CCA is based on a combination of clinical, radiological, biochemical and histological approaches, but none of the currently available biomarkers determined in fluids or in biopsy samples to assist in the diagnosis of this disease are sufficiently sensitive and specific. Extensive research is being carried out to identify biomarkers that can contribute to a better understanding of the

Abbreviations: 5-FU, 5-fluorouracil; AUC, area under the receiver operating characteristic curve; CA, carbohydrate antigen; CCA, cholangiocarcinoma; CTCs, circulating tumor cells; dCCA, distal cholangiocarcinoma; EMT, epithelial to mesenchymal transition; EVs, extracellular vesicles; HCC, hepatocellular carcinoma; iCCA, intrahepatic cholangiocarcinoma; MOC, mechanism of chemoresistance; MS, mass spectrometry; NMR, nuclear magnetic resonance; pCCA, perihilar cholangiocarcinoma; PSC, primary sclerosing cholangitis

☆ This article is part of a Special Issue entitled: Cholangiocytes in Health and Disease edited by Jesus Banales, Marco Marzioni, Nicholas LaRusso and Peter Jansen.

* Corresponding author at: Department of Physiology and Pharmacology, Campus Miguel de Unamuno E.D. B-17, 37007 Salamanca, Spain.
E-mail address: rociorm@usal.es (R.I.R. Macias).

http://dx.doi.org/10.1016/j.bbadis.2017.08.002
Received 12 May 2017; Received in revised form 1 August 2017; Accepted 2 August 2017

molecular basis of the disease, as well as to assist in the diagnosis and prognosis of CCA.

2. Clinical usefulness and limitations of available biomarkers in patients with CCA

The usefulness of tumor biomarkers lies in their ability to provide an early diagnosis of the disease, to establish individual prognosis and risk of relapse, or to help in the choice of the best treatment option (surgical, locoregional or systemic). The ideal biomarker for the diagnosis of CCA, as for any other cancer, should be simple and affordable (widely available), highly sensitive (to detect the disease at an early stage, preferably when curative therapy can be provided), and specific (to distinguish CCA from other malignant or benign diseases). Unfortunately, for CCA, this type of biomarker has yet not been identified.

At present, the carcinoembryonic antigen (CEA) and the carbohydrate antigens 19-9 (CA 19-9) and 125 (CA 125) are used clinically as serum markers for CCA, but they have low sensitivity and specificity and are not adequate for early detection. For diagnostic purposes, serum CA 19-9 is neither highly sensitive nor specific, as moderately elevated le-vels of this biomarker can be observed in situations of benign bile duct obstruction. Thus, the sensitivity and specificity of CA 19-9 for iCCA are 62% and 63%, respectively.

Other serum biomarkers, such as cytokeratin-19 fragment (CYFRA 21-1) and CA-242, have been reported to have higher specificity than CA 19-9 for iCCA in a limited number of studies [4], but are not in routine clinical use. Despite important efforts in past years to identify novel and more specific and sensitive markers for CCA, not only in serum (mucin 5AC, trypsinogen, interleukin 6, platelet-lymphocyte ratio, progranulin, etc.), but also in bile (insulin-like growth factor type 1, lipocalin-2, microRNA-laden vesicles, pancreatic elastase/amylase ratio, proteomic profile, etc) and urine (volatile organic compounds, proteomic profiles), none of the candidates evaluated have been considered suitable for clinical use [5].

Diagnosis of CCA usually relies on a combination of imaging techniques, including ultrasonography, computed tomography (CT), positron emission tomography (PET), magnetic resonance imaging (MRI), or advanced endoscopic biliary imaging techniques (Spyglass Spyscope). Approaches based on brush cytology can be truly challenging in the presence of chronic damage of the biliary tract, even when using molecular techniques to identify genetic and molecular alterations by means of fluorescence *in-situ* hybridization (FISH). Immunostaining to detect markers of HCC (e.g., GPC3, HSP70, and glutamine synthetase) or progenitor cell features (e.g., K19, EpCAM) is recommended to distinguish iCCA from mixed HCC-CCA tumors if this information can influence patient management [6].

The first step to evaluate the usefulness of any novel biomarker in the early diagnosis of CCA is to establish which high-risk population should be screened. The two groups with the highest risk of developing CCA are patients with primary sclerosing cholangitis (PSC) and patients with choledocal cysts. Population-based studies suggest that the annual risk for CCA in PSC patients is approximately 2% with a 10- and 30-year cumulative incidence of 6–11% and 20%, respectively [7]. For this reason, performing liver function tests every 3-6 months and an annual MRI and CA 19-9 analysis is usually recommended among young adults over the age of 20 with large duct PSC, although no formal prospective validation of this follow-up strategy has ever been attempted. Biomarkers are likely more useful among individuals with suspicious imaging features (a new dominant stricture or the development of focal bile duct thickening, irregularity or enhancement), symptoms that suggest biliary obstruction or laboratory tests that worsen over time. In these situations, elevated levels of CA 19-9, greater than 100 IU/ml in the absence of cholangitis, are highly suggestive of CCA. Among patients with choledocal cysts, the risk of CCA varies from 2.5% to 26% [8]. This risk increases notably in patients older than 20 years of age, and it is considered a clear risk factor when symptom onset occurs in patients older than 60. However, in Western countries most CCAs, and particularly iCCA, are diagnosed in the absence of any known risk factor, which makes screening strategies unfeasible.

Once the diagnosis of CCA has been established, a relevant question that biomarkers could help responding is whether the tumors should be resected or not. Currently, there are three commonly used pCCA classification systems: i) the Bismuth-Corlette classification, ii) the Memorial Sloan-Kettering Cancer Center staging system, and iii) the American Joint Committee on Cancer/Union for International Cancer Control (AJCC/UICC) system. None of these systems are perfect and none can be used to predict the survival time of patients with unresectable disease. The AJCC/UICC 7th tumor-node-metastasis (TNM) staging system is the most widely used. Several immunohistochemical markers are associated with worse outcomes after resection, including LIM and SH3 protein 1, suppressor of cytokine signaling 3, interleukin-8, matrix metalloproteinase-9, or CD15 expression. Among the serum biomarkers, high levels of CA 19-9 or GGT are usually associated with larger tumor size, lymph node metastasis or vascular invasion. Patients with unresectable CCA typically have higher CA 19-9 levels than patients with resectable tumors. Some studies have reported that preoperative CA 19-9 levels > 100 U/ml are associated with worse recurrence-free survival post-resection [9]. However, there is no cutoff value that can be used to contraindicate surgery. As mentioned, bile duct obstruction or acute cholangitis may increase CA 19-9 levels. Accordingly, in the case of bile duct obstruction, CA 19-9 levels should be reassessed after biliary drainage, taking into account that the half-life of CA 19-9 is up to three days.

Another important aspect in the usefulness of biomarkers is their ability to discriminate between HCC and iCCA. Serum levels of CA 19-9, sialic acid, and CA 242 tend to be higher in CCA, while alpha-fetoprotein and glypican-3 tend to be higher in HCC; however, there is no combination of biomarkers that specifically distinguishes both tumors [10,11]. In addition, it must be taken into account that tumors with mixed differentiation are not unusual. A recent study has described the usefulness of tumor-associated microparticles in serum for diagnosis of liver tumors. In particular, higher serum concentration of microparticles positive for AnnexinV, EpCAM and ASGPR1 were found in patients with liver tumors (i.e., HCC or CCA) compared to patients with cirrhosis [12]. Differential diagnosis by histology of iCCA *vs* HCC or metastasis remains a challenge and no specific markers have been validated. A panel of immunohistochemistry markers (hepatocyte-specific antigen, MOC-31, pCEA, CD10, and CD34) permits to exclude metastasis, and the cytokeratin profile (CK7 +, CK19 +, CK20-) in combination with the hepatocytic marker HepPar1- permits to exclude HCC [2].

Since no targeted therapy with proved effectiveness against CCA is yet available, the use of biomarkers to customize CCA chemotherapy/immunotherapy makes less sense. However, several agents currently being used in clinical trials with patients with CCA have specific targets expressed in these tumors.

High-throughput, large-scale analytical methods to quantify gene expression, proteins and metabolites in tissue, blood, bile and urine are now available (Fig. 1). The so-called “omics” technologies aim to identify biomarkers that permit to understand tumor biology and use this information to identify novel targets for future therapeutic strategies, define different clinical entities and pathological processes and estimate the severity of the disease and predict long-term outcome for patients. “Omics” approaches include genomics for DNA variations, transcriptomics for all RNA molecules - including messenger RNA (mRNA), ribosomal RNA (rRNA), and other noncoding RNA such as microRNAs (miRNA) -, proteomics for peptides and proteins and metabolomics for intermediate products of metabolism. It has been demonstrated that some chromosomal abnormalities and specific molecular prognostic markers can be used as tools for the stratification of patients into risk groups. Epigenetic analysis refers to heritable

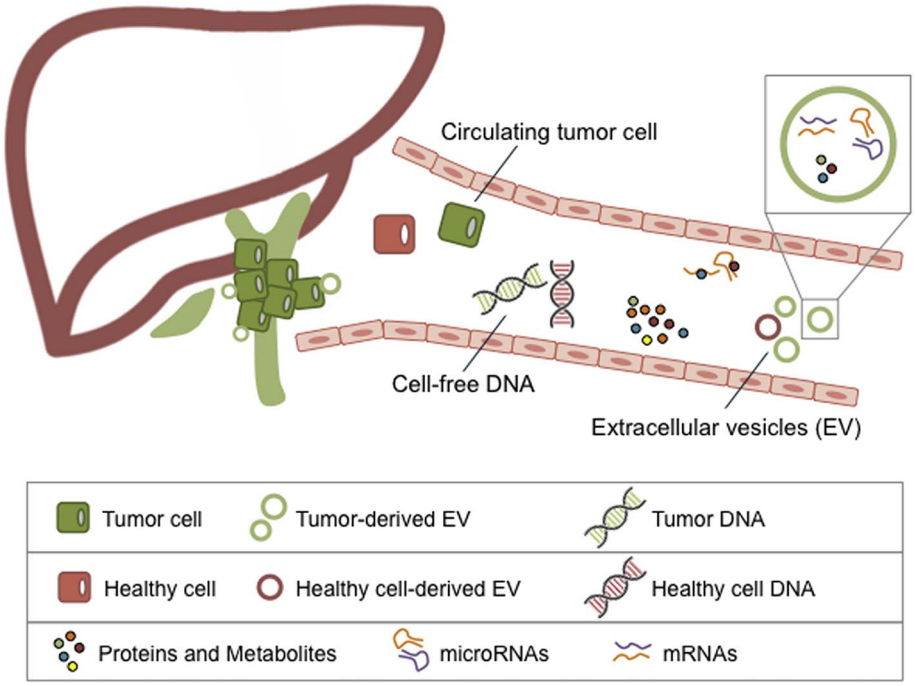

Fig. 1. Circulating biomarkers in cholangiocarcinoma. Different types of biomarkers can be analyzed in peripheral circulation: circulating tumor cells (CTCs) escaping from primary sites, extracellular vesicles (EV) containing nucleic acids and proteins, cell-free DNA and RNA released from tumor cells, and proteins and metabolites secreted by tumor cells.

modifications of the genome that do not affect the DNA sequence but directly affect the pattern of gene expression. These include DNA methylation, histone post-translational modifications, chromatin remodeling and non-coding RNAs (miRNAs). These changes to the genome may be reversible and it has been shown that they affect the epigenome and contribute to the pathogenesis of different diseases, including cancer.

Proteomic analyses complement DNA- and RNA-based studies, as there is a close correlation between the proteome or changes in protein glycosylation and the biological activity of the cell or system. Proteins are directly responsible for different functions in the cell, thus abnormal protein expression or an altered pattern of proteins can be an indication of a pathological condition. The identification of a protein expression profiling in CCA could be a valuable diagnostic and prognostic tool, where a particular protein signature is expressed in a distinct pattern that can be tied to a specific clinical feature or can be used for a differential diagnosis between CCA and HCC. The analysis of the whole metabolome, or molecules with low molecular weight present in a biological compartment (tissue or biological fluid), which are the result of multiple enzymatic reactions, can provide useful information on the biochemical activity and physiological state of an organism. Of note, the use of appropriate statistical analysis and bioinformatics tools is necessary for handling big and complex data volumes. Last but not least, circulating tumor cells (CTCs) are cells that reach the bloodstream from a primary tumor and may be responsible of subsequent growth of additional tumors in distant organs (metastasis) (Fig. 1). They have been detected in various metastatic carcinomas such as breast, lung, and colorectal cancer, but are extremely rare in healthy subjects and patients with nonmalignant diseases.

The search for biomarkers using “omics” technologies has permitted to identify alterations in cellular signaling pathways and biochemical processes, providing an insight into the mechanisms that underlie tumorigenesis. Some proteins currently under investigation as potential either biomarkers or targets for cancer therapy are signaling proteins involved in cell proliferation, motility, or survival pathways, as well as mutated enzymes responsible for the appearance or increased levels of rare metabolites.

It is important to highlight that pre-clinical studies, carried out in cultured cells and animal CCA models, complement the results obtained in clinical setting and the combined used of both can help to increase our understanding of CCA biology. This has been useful, for instance to assess the role in CCA development and progression of altered expression of genes or dysregulated signaling pathways.

In the next sections of the present review, new advances in research fields related to the investigation of novel diagnostic and prognostic biomarkers in CCA will be described in detail. More detailed information regarding targeted and individualized therapeutic approaches to hepatobiliary neoplasia can be found in other chapters of this special issue.

3. Use of OMICs approaches in search of novel biomarkers for CCA

3.1. Genomic features

Several studies have pointed out the genomic heterogeneity and the most prevalent genomic alterations present in CCAs (Fig. 2). These may affect DNA repair (*TP53*) [13–17], growth pathways (*KRAS*, *BRAF*, *SMAD4*, *FGFR2*, *PTPN3*) [13,16–22], chromatin-remodeling (*KMT2C*, *ARID1A*, *PBRM1* and *BAP1*) [14–19] and developmental pathways, such as the Notch and Wnt signaling pathways, which play important roles in the growth of these cancers [23–25]. The *FGFR2* fusion genes are of special interest, as they are not detected in other liver malignancies and are pharmacologically targetable and have diagnostic value [17,18,20,26,27]. Other mutations in *IDH1* and *IDH2* alter the methylation status of CCA cells [15,17–19,28,29]. The genomic variability of CCAs may be the reflection of different etiologies and risk factors and/or the result of the biological selection of the mutations that allow the tumor to grow [15,19,21,24,26]. These genomic mutations result in the expression of different molecular profiles that may allow the stratification of CCAs and a more precise therapeutic strategy. Furthermore, a recent study has demonstrated that targeted therapies may show beneficial effects when CCAs are genetically selected [18].

3.2. Epigenetic features

Little is still known about the epigenetic modifications affecting CCAs (Fig. 2). Mutations in *IDH* genes are known to produce a genome-wide DNA hypermethylation that may change the expression pattern of

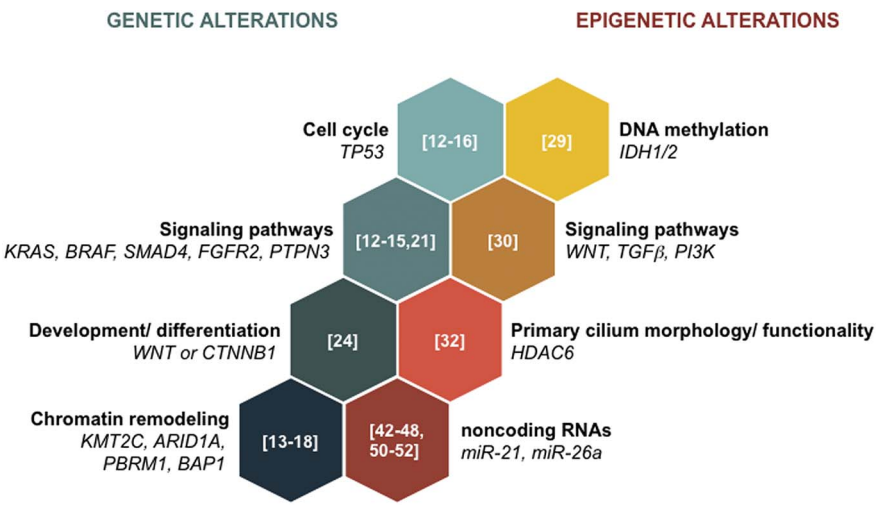

Fig. 2. Most frequent genomic and epigenetic alterations with potential interest in diagnosis of cholangiocarcinoma. Relevant references are indicated in square brackets.
ARID1A, AT-rich interaction domain 1A; *BAP1*, BRCA1-associated protein 1; *CTNNB1*, catenin beta 1; *FGFR2*, fibroblast growth factor receptor 2; *HDAC6*, histone deacetylase 6; *IDH1/2*, isocitrate dehydrogenase 1 and 2; *KMT2C*, lysine methyltransferase 2C; *PI3K*, phosphatidylinositol 3-kinase; *PBRM1*, polybromo 1; *PTPN3*, protein tyrosine phosphatase non-receptor type 3; TGFβ, transforming growth factor beta. Other tumor suppressor genes and oncogenes: TP53, KRAS, BRAF, SMAD4.

several genes [30]. Epigenetic alterations, similarly to genetic mutations, affect important pathways involved in the onset of the disease. Some examples include: *i*) HNF4α silencing, which blocks hepatocyte differentiation, promoting biliary tract cancers [29]; *ii*) signaling pathways such as Wnt/β-catenin, transforming growth factor beta (TGFβ) and PI3K signaling [31]; *iii)* HOX genes [32]; and *iv)* histone deacetylase 6 (HDAC6) overexpression, which causes cholangiocyte primary cilium shortening and promotes cell growth [33]. Epigenetic alterations may also be important for diagnostic and prognostic purposes. CCAs exhibit altered DNA methylation and hidroxymethylation patterns [34]. Interestingly, a DNA methylation gene biomarker profile from cells of biliary brushes has been postulated as being highly sensitive and specific for CCA diagnosis [35]. Moreover, the expression of histone deacetylases, such as HDAC1, may have prognostic value in CCAs [36].

3.3. Transcriptomic biomarkers

Genetic and epigenetic modifications finally result in transcriptomic changes that encompass the different RNA species (mRNAs, miRNAs and other non-coding RNAs). Several studies have shown that CCAs present particular signatures of gene expression when compared with both non-tumor liver tissue and HCC [37–39]. Thus, two different types of iCCA have been described according to their gene expression patterns: the “inflammation class”, characterized by the activation of inflammatory signaling pathways, and the “proliferation class”, related to the activation of classical oncogenic pathways, which is associated with a worse outcome [21].

In the last decade, an increasing number of studies have highlighted the implication of different microRNAs in the pathogenesis of CCA, as well as their potential value for diagnosis and prognosis (Table 1) [40,41]. Thus, deregulated microRNA expression has been linked to different molecular aspects of cholangiocarcinogenesis, such as proliferation, invasion and migration, epithelial to mesenchymal transition (EMT), epigenetic modifications, chemoresistance and/or apoptosis evasion [42]. In this regard, abnormal microRNA profiles in CCA tumor samples and cell lines have been reported (Table 1). Particularly, miR-21 overexpression in CCA tissue specimens, regardless of its etiology, seems to be a potential candidate biomarker, with 95% sensitivity and 100% specificity in discriminating between CCA and normal tissues [43,44]. Tissue miR-21 expression significantly correlates with the clinical stage at diagnosis and the differentiation status, and high miR-21 expression in iCCA tissues is associated with poor overall and progression-free survival [45,46]. Likewise, miR-21 expression is highly upregulated in CCA cell lines compared with nonmalignant cholangiocytes [47]. Experimental inhibition of miR-21 significantly reduces cell proliferation, anchorage-independent growth and invasion *in vitro* as well as tumor growth *in vivo* [46,48]. MiR-21 target genes include tumor suppressor-genes such as phosphatase and tensin homolog (*PTEN*) and protein tyrosine phosphatase non-receptor type 14 (*PTPN14*), tissue inhibitor of metalloproteinase-3 (*TIMP-3*), *PDCD4*, and *RECK*, among others [43–48]. MicroRNA signatures in tissue samples could also provide insight into the different histological grades and clinical subtypes of iCCA. A recent study, for instance, proposes a specific panel of miRNAs linked to the different subtypes of iCCA induced by *O. viverrini* infection [49]. MicroRNA profiling in tissue specimens can also serve to specifically differentiate tumors with similar clinical presentations such as CCA and pancreatic adenocarcinoma [50]. One interesting feature of miRNAs as biomarkers is their presence and stability in biofluids, which enables non-invasive approaches for CCA diagnosis. In this regard, some of the previous results obtained in tissue specimens are consistent with determinations carried out in plasma and serum samples. Thus, circulating levels of miR-21 have shown a high capacity to discriminate between patients with iCCA and healthy controls, with an 87.8% sensitivity and 90.5% specificity when determined in serum and with an area under the receiver operating characteristic curve (AUC) of 0.9081 in serum and 0.94 in plasma [46,51]. Similarly, the expression of miR-26a not only is increased in CCA tissues and cell lines promoting CCA growth, but also is elevated in serum of CCA patients with 84.8% sensitivity and 81.8% specificity in distinguishing CCA from healthy controls and an AUC value of 0.899 [52,53]. Interestingly, miR-26a expression could also serve as a potential prognostic tool in CCA, since its levels significantly correlate with TNM stage and specifically fall down after potentially curative surgeries [53]. The expression of other miRNAs such as miR-106a is downregulated in serum of CCA and may function as a predictor of poor prognosis [54]. Although miR-150 expression levels are significantly lower in iCCA compared with healthy tissue, the levels of this miRNA are elevated in plasma of iCCA patients, with a sensitivity and specificity of 80.6% and 58.1%, respectively. Furthermore, the use of miR-150 in combination with CA 19-9 further improves the power of screening iCCA [55]. Concerning liver fluke-associated iCCA, serum miR-192 was reported as an interesting candidate for *O. viverrini*-related CCA diagnosis (with 74% sensitivity and 72% specificity and 0.803 AUC value), and high serum levels of this miRNA correlated with shorter survival and lymph-node metastasis [56]. Of note, urinary miR-192 and miR-21 levels are also potential biomarkers for CCA, and their combined levels hold stronger diagnostic power differentiating CCA patients from healthy subjects than a single miRNA [57]. Moreover, the analysis of iCCA and controls tissue samples permitted the identification of miRNA profiles associated with increasing histological differentiation. Also seven miRNAs (miR-16-2, miR-320a, miR-193a, miR-378a, miR-320b,

Table 1
Diagnostic value of miRNAs as biomarkers in CCA.

Name	Change in CCA	Clinical sample	Sensitivity (%)	Specificity (%)	AUC value	Reference
miR-21	CCA *vs* normal tissue	Tissue	95	100	0.995	[42]
miR-21	CCA *vs* normal tissue	Tissue	95	100	0.995	[43]
miR-21	Upregulation iCCA *vs* Healthy Controls	Serum	87.8	90.5	0.908	[45]
miR-21	Upregulation iCCA *vs* Healthy Controls	Plasma	–	–	0.94	[50]
miR-26a	Upregulation CCA *vs* Healthy Controls	Serum	84.8	81.8	0.899	[52]
miR-106a	Downregulation CCA *vs* Healthy	Serum	81.6	85	0.89	[53]
miR-150	Upregulation iCCA *vs* Healthy Controls	Plasma	80.6	58.1	0.764	[54]
miR-192	Upregulation *O. viverrini* CCA *vs* Healthy Controls	Serum	74	72	0.803	[55]
miR-9	Upregulation CCA/GBC *vs* choledocholitiasis	Bile	88.9	100	0.975	[58]
miR-145	Upregulation CCA/GBC *vs* choledocholitiasis	Bile	77.8	100	0.975	[58]
miR-105	Upregulation CCA/GBC *vs* choledocholitiasis	Bile	77.8	100	–	[58]
miR-147b	Upregulation CCA/GBC *vs* choledocholitiasis	Bile	66.7	100	–	[58]
miR-302	Upregulation CCA/GBC *vs* choledocholitiasis	Bile	88.9	100	–	[58]
miR-199-3p	Upregulation CCA/GBC *vs* choledocholitiasis	Bile	88.9	100	–	[58]
miR-222	Upregulation CCA/GBC *vs* choledocholitiasis	Bile	88.9	100	–	[58]
miR-942	Upregulation CCA/GBC *vs* choledocholitiasis	Bile	77.8	100	–	[58]
miR-26a	Downregulation CCA *vs* PSC	Serum	52	93	0.78	[59]
miR-30b	Downregulation CCA *vs* PSC	Serum	52	88	0.78	[59]
miR-122	Downregulation CCA *vs* PSC	Serum	32	90	0.65	[59]
miR-126	Downregulation CCA *vs* PSC	Serum	68	93	0.87	[59]
miR-1281	Downregulation CCA *vs* PSC	Serum	55	90	0.83	[59]
miR-222	Upregulation CCA *vs* PSC	Serum	–	–	0.71	[60]
miR-483-5p	Upregulation CCA *vs* PSC	Serum	–	–	0.70	[60]
miR-412	Upregulation PSC/CCA *vs* PSC	Bile	50	89	0.81	[59]
miR-640	Upregulation PSC/CCA *vs* PSC	Bile	50	92	0.81	[59]
miR-1537	Upregulation PSC/CCA *vs* PSC	Bile	67	90	0.78	[59]
miR-3189	Upregulation PSC/CCA *vs* PSC	Bile	67	89	0.8	[59]
miR-21	Upregulation *O. viverrini* CCA *vs* Healthy Controls	Urine	63.6	71.4	0.682	[56]
miR-192	Upregulation *O. viverrini* CCA *vs* Healthy Controls	Urine	63.6	66.7	0.682	[56]
miR-21, miR-192	Upregulation *O. viverrini* CCA *vs* Healthy Controls	Urine	81.8	71.4	0.849	[56]
miR-191, 486-3p, 1274b, 16, 484	CCA *vs* Control group	EV in Bile	67	96	–	[63]

AUC, area under the receiver operating characteristic curve; CCA, cholangiocarcinoma; EV, extracellular vesicles; GBC, gallbladder carcinoma; PSC, primary sclerosing cholangitis.

miR-505 and miR-483) were found dysregulated in matched plasma of these patients [58]. In addition to these examples regarding plasma, serum and urine, owing to its direct contact with the tumor, bile seems to be also a suitable biofluid for miRNA-based CCA diagnosis. High-throughput PCR-based miRNA expression profiling performed in bile samples revealed miR-9 as a candidate for CCA diagnosis (with 88.9% sensitivity and 100% specificity and an AUC of 0.975). Moreover, miR-145* could also be considered another potential candidate (with 77.8% sensitivity and 100% specificity and an AUC of 0.975) [59]. Given that CCA has a poor prognosis, diagnosis at an early stage, when the disease is still amenable to cure, is crucial. Since PSC is a well-established risk factor for CCA development, various studies have analyzed the miRNA profile in bile and serum of patients with PSC and/or CCA [60,61]. One of these studies identified a significant difference in serum miR-222 and miR-483-5p in CCA compared with PSC [61], and in another study, the expression of a panel of 5 miRNAs (miR-1281, miR-126, miR-26a, miR-30b and miR-122) was significantly different between patients with CCA *vs* PSC [60]. In bile, miR-412, miR-640, miR-1537 and miR-3189 were identified as differentially expressed when comparing patients with PSC and PSC/CCA [60].

Recently, to assess the overall diagnostic value of miRNAs as biomarkers for CCA, some meta-analyses have been carried out recently [62,63]. The pooled analysis of the available data after carrying out a systematic literature search has demonstrated that miRNAs are promising tools for the diagnosis of CCA patients. Two independent meta-analyses have shown a pooled sensitivity of 0.83 and 0.75, and specificity of 0.79 and 0.914 and a pooled AUC of 0.88 and 0.90 [62,63]. Regarding the specimen types, bile exhibited the highest diagnostic efficiency (AUC value of 0.9572), followed by serum (0.9132), tissue (0.8465) and urine (0.7448) [62].

Finally, extracellular vesicles (EVs) are attracting great interest as potential containers of biomarkers for diseases (Fig. 1). Recently, microRNAs contained in bile-extracted extracellular vesicle have been postulated as an option for CCA diagnosis [64]. Here, a novel bile-based 5-miRNA panel and a mathematical model with a sensitivity and specificity of 67% and 96%, respectively, were suggested. However, further studies are needed to validate these data as well as to evaluate the miRNA profile of serum and urine EVs from CCA patients.

3.4. Proteomic biomarkers

Mass spectrometry (MS) technology allows the massive identification and relative quantification of proteins present in different biological samples [65]. With the clinical demand of minimal or non-invasive biomarkers, MS-based proteomics has become a useful tool for the analysis of different biofluids to find accurate and specific proteins biomarkers for diagnosis, prognosis, patient stratification or response to therapy [66]. Several proteomic studies have identified potential diagnostic biomarkers in bile and urine with a better diagnostic value than the general non-specific tumor markers used in serum. A specific peptide pattern accurately discriminated between benign and malignant biliary diseases, showing 78% specificity, 84% sensitivity and an AUC of 0.87 in CCA patients compared to patients with cholangitis [67]. Additionally, the SSP411 protein has been identified as a promising potential bile biomarker for CCA diagnosis, with 85.7% of sensitivity and 76.9% of specificity and 0.836 AUC for CCA patients compared with patients with PSC [68]. However, since invasive procedures are needed for the collection of bile samples, urine-based proteomics has been proposed as a feasible alternative to overcome this limitation. A peptide marker panel in the urine of patients with CCA, PSC or benign biliary disorder showed high diagnostic potential for CCA in comparison to PSC and benign biliary disorder with 83% sensitivity, 73% specificity and AUC of 0.87 [69]. Serum is also another accessible biofluid that requires a non-invasive method of collection. However, MS proteomics are hard to implement in these samples due to the high abundance of some proteins, such as albumin or

immunoglobulins, which present a high dynamic range, making the identification of less abundant proteins difficult [65,67]. Advances in the MS technology or new methodologies for serum protein fractionation would be necessary for biomarker discovery in this fluid.

Regarding EVs, a recent study has described differentially expressed proteins in serum EVs of CCA, PSC, HCC or healthy individuals. Several of these proteins showed high sensitivity and specificity for the differential diagnosis and have been suggested as novel potential biomarkers [70].

3.5. Metabolomic biomarkers

Metabolomics combines high-throughput analytical methodologies, mainly MS or nuclear magnetic resonance (NMR) spectroscopy with multivariate data analysis, to screen and compare low molecular mass metabolites (< 1.5 kDa) in biological samples (Fig. 1). Investigating the molecular composition of bile can provide important mechanistic information regarding the pathological alterations of biliary epithelia, and may also allow the identification of biomarkers released into bile from nearby tumor cells. Some metabolite profiling studies of human bile have been performed over the past few years to assess bile composition and identify relevant biomarkers for CCA [71] and other hepatopancreatobiliary diseases [72]. Early works using NMR demonstrated that the levels of phosphatidylcholine, bile acids and other biliary lipids could distinguish CCA patients from individuals with benign biliary diseases with high sensitivity and specificity [73]. More recently, metabolic profiling of bile, also using NMR, established that lower phosphatidylcholine, and elevated glycine- and taurine conjugated bile acids levels, were consistently found in CCA patients compared with individuals with benign biliary diseases [74,75]. Although these findings are encouraging there are concerns about the reproducibility of many of these studies. This situation may be due in part to the multiple analytical platforms used and the sample preparation protocols, which need better standardized procedures [76]. Ideally, once biomarkers are identified in bile they could be tested in more accessible and less invasive fluids, such as urine or serum. Alternatively, metabolomics studies can also be performed directly on serum. Remarkably, a recent report using a MS-based profiling platform in serum from fasting patients identified an interesting metabolomic signature based on four metabolites (21-deoxycortisol, bilirubin, lysoPC(14:0) and lysoPC(15:0)) that could be used for diagnosing CCA with high accuracy. Moreover, this signature was able to differentiate between intrahepatic and extrahepatic CCA [77]. The validation of this promising set of metabolites in future prospective studies is eagerly awaited.

4. Potential interest of CTCs in the search of CCA biomarkers

The interest of the liquid biopsy, especially that derived from blood, is based on its capability to facilitate the identification of biomarkers using a less invasive, less dangerous and less expensive but more informative approach than solid biopsy. Liquid biopsy could permit the analysis of DNA, miRNA, and EV, which may, as aforementioned, be useful in the search of biomarkers for CCA. Nevertheless, we will focus this section on the information provided on CTCs, which could help to improve screening protocols and therapy, as well as supply valuable data related to cancer chemoresistance and the risk of relapses [78].

Several strategies based on the identification of epithelial adhesion molecules have been experimentally developed to identify CTCs in different malignancies. However, CellSearch system by Veridex (Janssen/Veridex; Raritan, NJ) is the first automated, standardized, reproducible and FDA approved test for the use in advanced metastatic breast [79], colon [80] and prostate cancer [81]. It is designed to capture cells expressing cancer specific epithelial cell adhesion molecules (EpCAM) using antibody-coated magnetic beads followed by positive identification of intact tumor cells using fluorescent nuclear staining and cytokeratin detection using labeled antibodies. Biliary cancer cells express EpCAM, which allows their identification using CellSearch [82]. A preliminary pilot study including 13 patients with iCCA showed that the presence of 2 or more CTCs, assessed by CellSearch in 7.5 ml of patients' blood, at diagnosis (3/13) was not significantly related to the staging of the diseases and patient survival after a one-year follow-up [82]. Another study including a greater number of patients with CCA (n = 88) showed that CTCs ≥ 2 (HR 8.2; 95% CI 1.8–57.5; $p < 0.01$) and CTCs ≥ 5 (HR 7.7; 95% CI 1.4–42.9; $p = 0.02$) were both associated with shorter survival among patients with metastasis, reaching no significance for non-metastatic CCA [83]. The study also showed that CTCs ≥ 2 (10.5; 95% CI 2.2–40.1; $p < 0.01$) and CTCs ≥ 5 (HR 10.2; 95% CI 1.5–42.3; $p = 0.02$) were both associated with shorter survival among patients with p/dCCA, but only CTC ≥ 5 was associated with shorter survival of patients with iCCA (HR 4.2; 95% CI 1.1–14.1; $p = 0.04$) [83]. Results from another study including 50 untreated patients with histologically or cytologically confirmed advanced biliary cancer, either iCCA (58%), extrahepatic CCA (20%) or gallbladder carcinoma (22%), at inoperable stage III or IV with normal organ and marrow function, revealed that patients without detectable CTCs (< 1 CTC per 7.5 mL blood) correlated with higher overall survival as opposed to those with ≥ 1 CTC per 7.5 ml blood (13.7 *vs* 9.4 months) [84]. However, these results were not statistically significant ($p = 0.29$), which might be due to the small sample size and low power of the study. Further investigation in this field is clearly needed.

5. Biomarkers in the prediction of CCA chemoresistance

The refractoriness of cancer to pharmacological treatment partly depends on the expression of genes involved in different mechanisms of chemoresistance (MOC), which were initially classified into five families from MOC-1 to MOC-5 [85]. This classification, however, has been recently extended to seven by including MOC-6 and MOC-7, taking into consideration the updated advances in the field [86]. Following this classification (Fig. 3), we will comment on the potential interest of MOC genes as biomarkers to predict chemoresistance in CCA [87].

Impaired drug uptake (MOC-1a) can result in a lower response to chemotherapy due to inaccessibility of the drug to target molecules, similar to what occurs with sorafenib, whose uptake is mediated by OCT1 that is dramatically downregulated both in HCC and CCA [88]. Some transporters involved in drug uptake could be used as biomarkers to predict chemoresistance. Another example involves the equilibrative nucleoside transporter 1 (ENT1), whose expression levels have been suggested to predict of response to gemcitabine in CCA patients [89].

Several members of the superfamily of ATP-binding cassette (ABC) proteins are involved in the efflux of a large variety of anticancer drugs from tumor cells. The over-expression of these pumps is one of the major causes of chemoresistance (MOC-1b). Elevated expression and function of Multidrug Resistance Protein 1 (MDR1) has been associated with lower sensitivity to 5-fluorouracil (5-FU) in CCA cells [90]. *In vitro* studies using CCA cells have demonstrated the existence of the relationship between elevated expression of Multidrug Resistance-Associated Protein 1 (MRP1) and resistance to gemcitabine, and between high levels of MRP3 overexpression and the chemoresistance to etoposide and anthracyclines [91]. Moreover, the overexpression of several MRPs has been detected in clinical samples of CCA [37] and has been associated with poor prognosis in CCA patients [92]. The contribution of the Breast Cancer Resistance Protein (BCRP) to the chemoresistance in CCA is not clear. However, it has been reported that 5-FU resistant CCA cells exhibit high expression levels of this export pump [90]. Furthermore, some data indicate that BCRP expression is increased in CCA due to the activation mediated by the transcriptional coactivator amplified in breast cancer 1 (AIB1) that has been found overexpressed in CCA samples [93]. Thymosin β10, the main

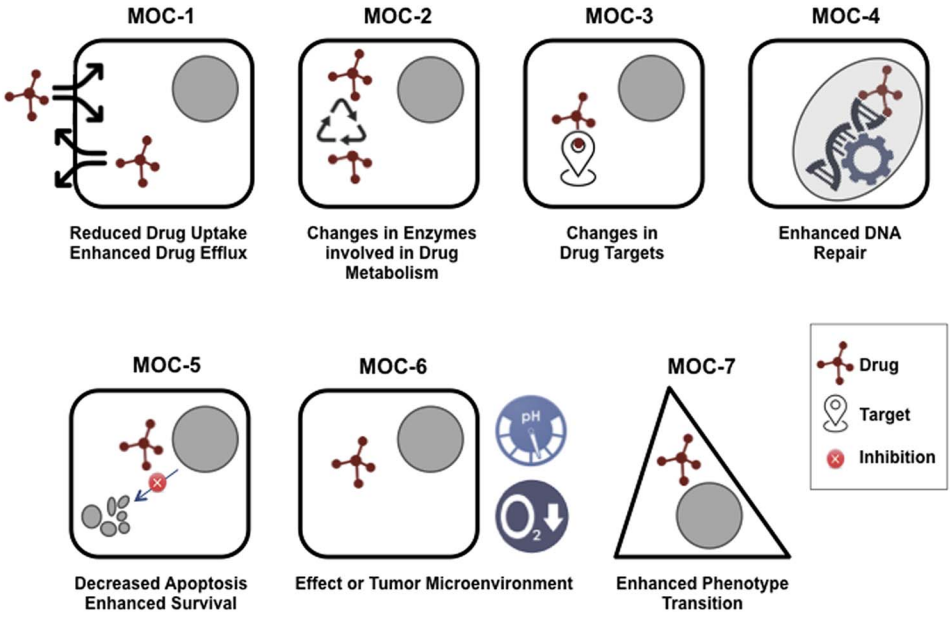

Fig. 3. Schematic representation of different mechanisms of chemoresistance (MOCs) in cholangiocarcinoma cells.

intracellular G-actin-sequestering protein involved in cell motility, has been proposed as a predictive biomarker of response to 5-FU in CCA because this protein is overexpressed in 5-FU resistant cells and its levels reflected the expression of ABC proteins [90].

Changes in the expression of enzymes involved in drug metabolism, either activating pro-drugs or inactivating active agents, can affect the intracellular concentration of active compounds (MOC-2). The orotate phosphoribosyl transferase (OPRT), responsible of the enzymatic transformation of 5-FU to the active compound, has been proposed as predictor of the outcome of CCA patients treated with 5-FU, because significantly higher mRNA OPRT levels were found in 5-FU-CCA responder patients than in non-responders [94]. Glutathione S-transferase-pi (GSTP1) could be a biomarker of resistance to different drugs. More precisely, high expression of this enzyme has been observed in CCA specimens whereas the sensitivity to adriamycin, cisplatin, and alkylating agents was higher when levels of this enzyme were reduced [95].

Changes in the expression/functionality of molecular targets can also account for anticancer drug resistance (MOC-3). As an example, up-regulation of epidermal growth factor receptor (EGFR) has been associated with chemoresistance to erlotinib [96] and cisplatin [97] in CCA cells.

An enhanced ability of tumor cells to repair cytostatic drug-induced alterations in DNA molecule contributes to poor response to chemotherapy with drugs, such as cisplatin, whose mechanism of action is based on damaging replicating DNA (MOC4). The excision repair cross-complementing protein 1 (ERCC1) is an endonuclease that removes a wide variety of bulky DNA adducts, such as those generated by cisplatin and alkylating agents. Overexpression of ERCC1 has significant prognostic value in advanced biliary tract carcinoma patients treated with cisplatin [98]. The uracil-DNA glycosylase 1 (UNG1), which catalyzes the hydrolysis of the N-glycosylic bond between uracil and sugar, has been found up-regulated in 5-FU-resistant CCA cell lines [99], and increased expression of the ribonucleotide reductase p53R2 has been proposed to predict resistance to gemcitabine in CCA cell lines [100].

Changes in the balance between pro- and anti-apoptotic proteins play a major role in the chemoresistance to many antitumor drugs (MOC-5). Bcl-2 upregulation together with Bax downregulation have been observed in 5-FU and cisplatin-CCA resistant cells [101]. BCL2 upregulation in clinical samples of CCA has been also reported [37]. Another example is the coupled appearance of upregulation of the apoptotic inhibitor BIRC5 together with downregulation of the pro-apoptotic factor TP73, which has been associated with development of acquired 5-FU resistance in CCA cell lines [99]. BIRC5 upregulation was also observed in paired samples of CCA and adjacent liver tissue [37]. Upregulation of a mutated isoform of p53 named Δ133p53 has been correlated with poor survival outcome in CCA patients treated with 5-FU [102], and the depletion of the transcription factor FoxO3, considered a tumor suppressor gene has been associated with resistance to cisplatin [103].

The special characteristics of tumor microenvironment affect the response to chemotherapy (MOC-6) and may be responsible for providing cancer cells with several pro-invasive functions [86]. Although some data regarding MOC-6 in CCA have been recently published, the actual importance of this group of MOC in CCA is still poorly understood. Thus, in malignant cholangiocytes, the pro-inflammatory cytokine leukemia inhibitory factor (LIF) has been found overexpressed. This factor protects tumor cells from chemotherapy-induced apoptosis via a STAT3- and MAPK-independent, PI3K/AKT-dependent Mcl-1 activation. Another example is laminin-332, a protein of the extracellular matrix that is overexpressed in hepatic tumors with high levels of CK19 and induces chemoresistance to doxorubicin and sorafenib [104].

The EMT process refers to the transformation of epithelial cells to a mesenchymal phenotype, characterized by enhanced migratory behavior, invasive ability, and resistance to apoptosis activation. Thus, EMT has been associated to poorer response to chemotherapy (MOC-7) and has been described in hepatic tumors, including CCA, where EMT has been associated with changes in the expression of oncogenes and tumor suppressor genes [105]. For instance, the overexpression of lipocalin-2 in CCA cells stimulates EMT progression and downregulates tumor suppressor genes (NDRG1 and NDRG2), which results in enhanced resistance to chemotherapy [106].

The identification of biomarkers associated with chemoresistance can be useful to predict in an individualized manner what pharmacological regimes are likely to be less effective against a precise CCA patient. Alterations in biomarkers in response to different therapies have also been described and this knowledge can be used to select strategies to overcome CCA chemoresistance. These questions have been reviewed in a separate chapter of this special issue [107].

6. Conclusions and perspectives

There is an urgent need for new biomarkers for the diagnosis and prognosis of CCA, especially in those populations at risk of developing

CCA, as well as early biomarkers of recurrence after surgery [2,24]. The development of "omics" approaches offers a broad panel of new ways to obtain large amounts of potentially useful information with respect to the search of novel biomarkers. Although the field of liquid biopsy is in its infancy, this has promising prospects, not only associated with CTC, but also with miRNAs and EVs. In particular, miRNAs are gaining importance in the field of CCA as potential biomarkers and targets for therapy. These molecules could be especially helpful for solving diagnosis and prognosis challenges in CCA. Nevertheless, it should be highlighted that there are many unsolved questions, including the optimal biofluid source of miRNA. Additionally, there are some aspects that will need to be refined and optimized, such as normalization approaches and sample processing. Moreover, in order to standardize the use of miRNAs in clinical practice, the best candidates should be validated in international collaborative studies that include different patient cohorts. In the future, a multidisciplinary approach, including genetic, epigenetic and proteomic analyses, will help to provide a more complete picture of the pathophysiological mechanisms involved in the development of CCAs and the MOC involved in their high refractoriness to available chemotherapy. This is a required step for the identification of biomarkers with diagnostic and prognostic utility, but also specific targeted therapies. Additionally, the search for new markers will need to take into account the marked heterogeneity of these tumors. The selection of a panel of biomarkers and appropriate algorithms will help to unravel the complexity of this disease and, hopefully in the near future, provide oncologists with more sensitive and specific tools to achieve an accurate diagnosis and prognosis of CCA.

Transparency Document

The Transparency Document associated with this article can be found in the online version.

Acknowledgements

The authors thank E. Keck for the English language revision.

Funding

This work was supported by the CIBERehd [EHD15PI05/2016], the Spanish Ministry of Economy, Industry and Competitiveness [SAF2016-75197-R to RIRM], the Fondo de Investigaciones Sanitarias, Instituto de Salud Carlos III [PI16/00598 to JJGM, PI16/01845 to BS, PI16/01126 to MAA, PI15/01132 to JMB, PI14/00399 to MJP], the Miguel Servet Programme [CON14/00129 to JMB], the Ramon y Cajal Programme [RYC-2015-17755 to MJP], the Fundación Mutua Madrileña [Call 2015/OBS], the Diputación Foral de Gipuzkoa [DFG15/010, DFG16/004 to JMB], the Basque Foundation for Innovation and Health Research: EiTB Maratoia [BIO15/CA/011 to MAA, BIO15/CA/016/BD to JMB], the Department of Health of the Basque Country [2013111173 to JMB, 2015111100 to MJP], the Hepacare Project from Fundacion La Caixa to MAA, the Andalusian Government, Consejería de Economía, Innovación, Ciencia y Empleo [CTS-6264 to JM] and the Consejería de Igualdad, Salud y Políticas Sociales [PI13/00025 to FJP].

References

[1] Y. Shaib, H.B. El-Serag, The epidemiology of cholangiocarcinoma, Semin. Liver Dis. 24 (2004) 115–125.

[2] J.M. Banales, V. Cardinale, G. Carpino, M. Marzioni, J.B. Andersen, P. Invernizzi, G.E. Lind, T. Folseraas, S.J. Forbes, L. Fouassier, A. Geier, D.F. Calvisi, J.C. Mertens, M. Trauner, A. Benedetti, L. Maroni, J. Vaquero, R.I. Macias, C. Raggi, M.J. Perugorria, E. Gaudio, K.M. Boberg, J.J. Marin, D. Alvaro, Expert consensus document: cholangiocarcinoma: current knowledge and future perspectives consensus statement from the European Network for the Study of Cholangiocarcinoma (ENS-CCA), Nat. Rev. Gastroenterol. Hepatol. 13 (2016) 261–280.

[3] M.L. DeOliveira, S.C. Cunningham, J.L. Cameron, F. Kamangar, J.M. Winter, K.D. Lillemoe, M.A. Choti, C.J. Yeo, R.D. Schulick, Cholangiocarcinoma: thirty-one-year experience with 564 patients at a single institution, Ann. Surg. 245 (2007) 755–762.

[4] M.H. Chapman, N.S. Sandanayake, F. Andreola, D.K. Dhar, G.J. Webster, J.S. Dooley, S.P. Pereira, Circulating CYFRA 21-1 is a specific diagnostic and prognostic biomarker in biliary tract cancer, J. Clin. Exp. Hepatol. 1 (2011) 6–12.

[5] R.I. Macias, Cholangiocarcinoma: biology, clinical management, and pharmacological perspectives, ISRN Hepatol. 2014 (2014) 828074.

[6] J. Bridgewater, P.R. Galle, S.A. Khan, J.M. Llovet, J.W. Park, T. Patel, T.M. Pawlik, G.J. Gores, Guidelines for the diagnosis and management of intrahepatic cholangiocarcinoma, J. Hepatol. 60 (2014) 1268–1289.

[7] S. Rizvi, J.E. Eaton, G.J. Gores, Primary sclerosing cholangitis as a premalignant biliary tract disease: surveillance and management, Clin. Gastroenterol. Hepatol. 13 (2015) 2152–2165.

[8] X.D. He, L. Wang, W. Liu, Q. Liu, Q. Qu, B.L. Li, T. Hong, The risk of carcinogenesis in congenital choledochal cyst patients: an analysis of 214 cases, Ann. Hepatol. 13 (2014) 819–826.

[9] A.H. Patel, D.M. Harnois, G.G. Klee, N.F. LaRusso, G.J. Gores, The utility of CA 19-9 in the diagnoses of cholangiocarcinoma in patients without primary sclerosing cholangitis, Am. J. Gastroenterol. 95 (2000) 204–207.

[10] L.Y. Tao, L. Cai, X.D. He, W. Liu, Q. Qu, Comparison of serum tumor markers for intrahepatic cholangiocarcinoma and hepatocellular carcinoma, Am. Surg. 76 (2010) 1210–1213.

[11] P. Tangkijvanich, T. Chanmee, S. Komtong, V. Mahachai, N. Wisedopas, P. Pothacharoen, P. Kongtawelert, Diagnostic role of serum glypican-3 in differentiating hepatocellular carcinoma from non-malignant chronic liver disease and other liver cancers, J. Gastroenterol. Hepatol. 25 (2010) 129–137.

[12] H. Julich-Haertel, S.K. Urban, M. Krawczyk, A. Willms, K. Jankowski, W. Patkowski, B. Kruk, M. Krasnodebski, J. Ligocka, R. Schwab, I. Richardsen, S. Schaaf, A. Klein, S. Gehlert, H. Sanger, M. Casper, J.M. Banales, D. Schuppan, P. Milkiewicz, F. Lammert, V. Lukacs-Kornek, M. Kornek, Cancer-associated circulating large extracellular vesicles in cholangiocarcinoma and hepatocellular carcinoma, J. Hepatol. 67 (2017) 282–292.

[13] C.K. Ong, C. Subimerb, C. Pairojkul, S. Wongkham, I. Cutcutache, W. Yu, J.R. McPherson, G.E. Allen, C.C. Ng, B.H. Wong, S.S. Myint, V. Rajasegaran, H.L. Heng, A. Gan, Z.J. Zang, Y. Wu, J. Wu, M.H. Lee, D. Huang, P. Ong, W. Chan-on, Y. Cao, C.N. Qian, K.H. Lim, A. Ooi, K. Dykema, K. Furge, V. Kukongviriyapan, B. Sripa, C. Wongkham, P. Yongvanit, P.A. Futreal, V. Bhudhisawasdi, S. Rozen, P. Tan, B.T. Teh, Exome sequencing of liver fluke-associated cholangiocarcinoma, Nat. Genet. 44 (2012) 690–693.

[14] Y. Jiao, T.M. Pawlik, R.A. Anders, F.M. Selaru, M.M. Streppel, D.J. Lucas, N. Niknafs, V.B. Guthrie, A. Maitra, P. Argani, G.J. Offerhaus, J.C. Roa, L.R. Roberts, G.J. Gores, I. Popescu, S.T. Alexandrescu, S. Dima, M. Fassan, M. Simbolo, A. Mafficini, P. Capelli, R.T. Lawlor, A. Ruzzenente, A. Guglielmi, G. Tortora, F. de Braud, A. Scarpa, W. Jarnagin, D. Klimstra, R. Karchin, V.E. Velculescu, R.H. Hruban, B. Vogelstein, K.W. Kinzler, N. Papadopoulos, L.D. Wood, Exome sequencing identifies frequent inactivating mutations in BAP1, ARID1A and PBRM1 in intrahepatic cholangiocarcinomas, Nat. Genet. 45 (2013) 1470–1473.

[15] W. Chan-On, M.L. Nairismagi, C.K. Ong, W.K. Lim, S. Dima, C. Pairojkul, K.H. Lim, J.R. McPherson, I. Cutcutache, H.L. Heng, L. Ooi, A. Chung, P. Chow, P.C. Cheow, S.Y. Lee, S.P. Choo, I.B. Tan, D. Duda, A. Nastase, S.S. Myint, B.H. Wong, A. Gan, V. Rajasegaran, C.C. Ng, S. Nagarajan, A. Jusakul, S. Zhang, P. Vohra, W. Yu, D. Huang, P. Sithithaworn, P. Yongvanit, S. Wongkham, N. Khuntikeo, V. Bhudhisawasdi, I. Popescu, S.G. Rozen, P. Tan, B.T. Teh, Exome sequencing identifies distinct mutational patterns in liver fluke-related and non-infection-related bile duct cancers, Nat. Genet. 45 (2013) 1474–1478.

[16] S. Zou, J. Li, H. Zhou, C. Frech, X. Jiang, J.S. Chu, X. Zhao, Y. Li, Q. Li, H. Wang, J. Hu, G. Kong, M. Wu, C. Ding, N. Chen, H. Hu, Mutational landscape of intrahepatic cholangiocarcinoma, Nat. Commun. 5 (2014) 5696.

[17] J.S. Ross, K. Wang, L. Gay, R. Al-Rohil, J.V. Rand, D.M. Jones, H.J. Lee, C.E. Sheehan, G.A. Otto, G. Palmer, R. Yelensky, D. Lipson, D. Morosini, M. Hawryluk, D.V. Catenacci, V.A. Miller, C. Churi, S. Ali, P.J. Stephens, New routes to targeted therapy of intrahepatic cholangiocarcinomas revealed by next-generation sequencing, Oncologist 19 (2014) 235–242.

[18] M.J. Borad, M.D. Champion, J.B. Egan, W.S. Liang, R. Fonseca, A.H. Bryce, A.E. McCullough, M.T. Barrett, K. Hunt, M.D. Patel, S.W. Young, J.M. Collins, A.C. Silva, R.M. Condjella, M. Block, R.R. McWilliams, K.N. Lazaridis, E.W. Klee, K.C. Bible, P. Harris, G.R. Oliver, J.D. Bhavsar, A.A. Nair, S. Middha, Y. Asmann, J.P. Kocher, K. Schahl, B.R. Kipp, E.G. Barr Fritcher, A. Baker, J. Aldrich, A. Kurdoglu, T. Izatt, A. Christoforides, I. Cherni, S. Nasser, R. Reiman, L. Phillips, J. McDonald, J. Adkins, S.D. Mastrian, P. Placek, A.T. Watanabe, J. Lobello, H. Han, D. Von Hoff, D.W. Craig, A.K. Stewart, J.D. Carpten, Integrated genomic characterization reveals novel, therapeutically relevant drug targets in FGFR and EGFR pathways in sporadic intrahepatic cholangiocarcinoma, PLoS Genet. 10 (2014) e1004135.

[19] A. Fujimoto, M. Furuta, Y. Shiraishi, K. Gotoh, Y. Kawakami, K. Arihiro, T. Nakamura, M. Ueno, S. Ariizumi, H.H. Nguyen, D. Shigemizu, T. Abe, K.A. Boroevich, K. Nakano, A. Sasaki, R. Kitada, K. Maejima, Y. Yamamoto, H. Tanaka, T. Shibuya, T. Shibata, H. Ojima, K. Shimada, S. Hayami, Y. Shigekawa, H. Aikata, H. Ohdan, S. Marubashi, T. Yamada, M. Kubo, S. Hirano, O. Ishikawa, M. Yamamoto, H. Yamaue, K. Chayama, S. Miyano, T. Tsunoda, H. Nakagawa, Whole-genome mutational landscape of liver cancers displaying biliary phenotype reveals hepatitis impact and molecular diversity, Nat. Commun. 6 (2015) 6120.

[20] H. Nakamura, Y. Arai, Y. Totoki, T. Shirota, A. Elzawahry, M. Kato, N. Hama,

F. Hosoda, T. Urushidate, S. Ohashi, N. Hiraoka, H. Ojima, K. Shimada, T. Okusaka, T. Kosuge, S. Miyagawa, T. Shibata, Genomic spectra of biliary tract cancer, Nat. Genet. 47 (2015) 1003–1010.

[21] D. Sia, Y. Hoshida, A. Villanueva, S. Roayaie, J. Ferrer, B. Tabak, J. Peix, M. Sole, V. Tovar, C. Alsinet, H. Cornella, B. Klotzle, J.B. Fan, C. Cotsoglou, S.N. Thung, J. Fuster, S. Waxman, J.C. Garcia-Valdecasas, J. Bruix, M.E. Schwartz, R. Beroukhim, V. Mazzaferro, J.M. Llovet, Integrative molecular analysis of intrahepatic cholangiocarcinoma reveals 2 classes that have different outcomes, Gastroenterology 144 (2013) 829–840.

[22] Q. Gao, Y.J. Zhao, X.Y. Wang, W.J. Guo, S. Gao, L. Wei, J.Y. Shi, G.M. Shi, Z.C. Wang, Y.N. Zhang, Y.H. Shi, J. Ding, Z.B. Ding, A.W. Ke, Z. Dai, F.Z. Wu, H. Wang, Z.P. Qiu, Z.A. Chen, Z.F. Zhang, S.J. Qiu, J. Zhou, X.H. He, J. Fan, Activating mutations in PTPN3 promote cholangiocarcinoma cell proliferation and migration and are associated with tumor recurrence in patients, Gastroenterology 146 (2014) 1397–1407.

[23] S. Zender, I. Nickeleit, T. Wuestefeld, I. Sorensen, D. Dauch, P. Bozko, M. El-Khatib, R. Geffers, H. Bektas, M.P. Manns, A. Gossler, L. Wilkens, R. Plentz, L. Zender, N.P. Malek, A critical role for notch signaling in the formation of cholangiocellular carcinomas, Cancer Cell 23 (2013) 784–795.

[24] N. Razumilava, G.J. Gores, Notch-driven carcinogenesis: the merging of hepatocellular cancer and cholangiocarcinoma into a common molecular liver cancer subtype, J. Hepatol. 58 (2013) 1244–1245.

[25] L. Boulter, R.V. Guest, T.J. Kendall, D.H. Wilson, D. Wojtacha, A.J. Robson, R.A. Ridgway, K. Samuel, N. Van Rooijen, S.T. Barry, S.J. Wigmore, O.J. Sansom, S.J. Forbes, WNT signaling drives cholangiocarcinoma growth and can be pharmacologically inhibited, J. Clin. Invest. 125 (2015) 1269–1285.

[26] Y. Arai, Y. Totoki, F. Hosoda, T. Shirota, N. Hama, H. Nakamura, H. Ojima, K. Furuta, K. Shimada, T. Okusaka, T. Kosuge, T. Shibata, Fibroblast growth factor receptor 2 tyrosine kinase fusions define a unique molecular subtype of cholangiocarcinoma, Hepatology 59 (2014) 1427–1434.

[27] D. Sia, B. Losic, A. Moeini, L. Cabellos, K. Hao, K. Revill, D. Bonal, O. Miltiadous, Z. Zhang, Y. Hoshida, H. Cornella, M. Castillo-Martin, R. Pinyol, Y. Kasai, S. Roayaie, S.N. Thung, J. Fuster, M.E. Schwartz, S. Waxman, C. Cordon-Cardo, E. Schadt, V. Mazzaferro, J.M. Llovet, Massive parallel sequencing uncovers actionable FGFR2-PPHLN1 fusion and ARAF mutations in intrahepatic cholangiocarcinoma, Nat. Commun. 6 (2015) 6087.

[28] D.R. Borger, K.K. Tanabe, K.C. Fan, H.U. Lopez, V.R. Fantin, K.S. Straley, D.P. Schenkein, A.F. Hezel, M. Ancukiewicz, H.M. Liebman, E.L. Kwak, J.W. Clark, D.P. Ryan, V. Deshpande, D. Dias-Santagata, L.W. Ellisen, A.X. Zhu, A.J. Iafrate, Frequent mutation of isocitrate dehydrogenase (IDH)1 and IDH2 in cholangiocarcinoma identified through broad-based tumor genotyping, Oncologist 17 (2012) 72–79.

[29] S.K. Saha, C.A. Parachoniak, K.S. Ghanta, J. Fitamant, K.N. Ross, M.S. Najem, S. Gurumurthy, E.A. Akbay, D. Sia, H. Cornella, O. Miltiadous, C. Walesky, V. Deshpande, A.X. Zhu, A.F. Hezel, K.E. Yen, K.S. Straley, J. Travins, J. Popovici-Muller, C. Gliser, C.R. Ferrone, U. Apte, J.M. Llovet, K.K. Wong, S. Ramaswamy, N. Bardeesy, Mutant IDH inhibits HNF-4alpha to block hepatocyte differentiation and promote biliary cancer, Nature 513 (2014) 110–114.

[30] P. Wang, Q. Dong, C. Zhang, P.F. Kuan, Y. Liu, W.R. Jeck, J.B. Andersen, W. Jiang, G.L. Savich, T.X. Tan, J.T. Auman, J.M. Hoskins, A.D. Misher, C.D. Moser, S.M. Yourstone, J.W. Kim, K. Cibulskis, G. Getz, H.V. Hunt, S.S. Thorgeirsson, L.R. Roberts, D. Ye, K.L. Guan, Y. Xiong, L.X. Qin, D.Y. Chiang, Mutations in isocitrate dehydrogenase 1 and 2 occur frequently in intrahepatic cholangiocarcinomas and share hypermethylation targets with glioblastomas, Oncogene 32 (2013) 3091–3100.

[31] B. Goeppert, C. Konermann, C.R. Schmidt, O. Bogatyrova, L. Geiselhart, C. Ernst, L. Gu, N. Becker, M. Zucknick, A. Mehrabi, M. Hafezi, F. Klauschen, A. Stenzinger, A. Warth, K. Breuhahn, M. Renner, W. Weichert, P. Schirmacher, C. Plass, D. Weichenhan, Global alterations of DNA methylation in cholangiocarcinoma target the Wnt signaling pathway, Hepatology 59 (2014) 544–554.

[32] Y. Shu, B. Wang, J. Wang, J.M. Wang, S.Q. Zou, Identification of methylation profile of HOX genes in extrahepatic cholangiocarcinoma, World J. Gastroenterol. 17 (2011) 3407–3419.

[33] S.A. Gradilone, B.N. Radtke, P.S. Bogert, B.Q. Huang, G.B. Gajdos, N.F. LaRusso, HDAC6 inhibition restores ciliary expression and decreases tumor growth, Cancer Res. 73 (2013) 2259–2270.

[34] S. Udali, P. Guarini, S. Moruzzi, A. Ruzzenente, S.A. Tammen, A. Guglielmi, S. Conci, P. Pattini, O. Olivieri, R. Corrocher, S.W. Choi, S. Friso, Global DNA methylation and hydroxymethylation differ in hepatocellular carcinoma and cholangiocarcinoma and relate to survival rate, Hepatology 62 (2015) 496–504.

[35] K. Andresen, K.M. Boberg, H.M. Vedeld, H. Honne, P. Jebsen, M. Hektoen, C.A. Wadsworth, O.P. Clausen, K.E. Lundin, V. Paulsen, A. Foss, O. Mathisen, L. Aabakken, E. Schrumpf, R.A. Lothe, G.E. Lind, Four DNA methylation biomarkers in biliary brush samples accurately identify the presence of cholangiocarcinoma, Hepatology 61 (2015) 1651–1659.

[36] Y. Morine, M. Shimada, S. Iwahashi, T. Utsunomiya, S. Imura, T. Ikemoto, H. Mori, J. Hanaoka, H. Miyake, Role of histone deacetylase expression in intrahepatic cholangiocarcinoma, Surgery 151 (2012) 412–419.

[37] P. Martinez-Becerra, J. Vaquero, M.R. Romero, E. Lozano, C. Anadon, R.I. Macias, M.A. Serrano, N. Grane-Boladeras, L. Munoz-Bellvis, L. Alvarez, B. Sangro, M. Pastor-Anglada, J.J. Marin, No correlation between the expression of FXR and genes involved in multidrug resistance phenotype of primary liver tumors, Mol. Pharm. 9 (2012) 1693–1704.

[38] Y. Murakami, S. Kubo, A. Tamori, S. Itami, E. Kawamura, K. Iwaisako, K. Ikeda, N. Kawada, T. Ochiya, Y.H. Taguchi, Comprehensive analysis of transcriptome and metabolome analysis in intrahepatic cholangiocarcinoma and hepatocellular carcinoma, Sci. Rep. 5 (2015) 16294.

[39] S. Likhitrattanapisal, J. Tipanee, T. Janvilisri, Meta-analysis of gene expression profiles identifies differential biomarkers for hepatocellular carcinoma and cholangiocarcinoma, Tumour Biol. 37 (2016) 12755–12766.

[40] H. Haga, I. Yan, K. Takahashi, J. Wood, T. Patel, Emerging insights into the role of microRNAs in the pathogenesis of cholangiocarcinoma, Gene Expr. 16 (2014) 93–99.

[41] K. Piontek, F.M. Selaru, MicroRNAs in the biology and diagnosis of cholangiocarcinoma, Semin. Liver Dis. 35 (2015) 55–62.

[42] A. Esparza-Baquer, I. Labiano, L. Bujanda, M.J. Perugorria, J.M. Banales, MicroRNAs in cholangiopathies: potential diagnostic and therapeutic tools, Clin. Res. Hepatol. Gastroenterol. 40 (2016) 15–27.

[43] F.M. Selaru, A.V. Olaru, T. Kan, S. David, Y. Cheng, Y. Mori, J. Yang, B. Paun, Z. Jin, R. Agarwal, J.P. Hamilton, J. Abraham, C. Georgiades, H. Alvarez, P. Vivekanandan, W. Yu, A. Maitra, M. Torbenson, P.J. Thuluvath, G.J. Gores, N.F. LaRusso, R. Hruban, S.J. Meltzer, MicroRNA-21 is overexpressed in human cholangiocarcinoma and regulates programmed cell death 4 and tissue inhibitor of metalloproteinase 3, Hepatology 49 (2009) 1595–1601.

[44] Q. He, L. Cai, L. Shuai, D. Li, C. Wang, Y. Liu, X. Li, Z. Li, S. Wang, Ars2 is overexpressed in human cholangiocarcinomas and its depletion increases PTEN and PDCD4 by decreasing microRNA-21, Mol. Carcinog. 52 (2013) 286–296.

[45] P. Chusorn, N. Namwat, W. Loilome, A. Techasen, C. Pairojkul, N. Khuntikeo, A. Dechakhamphu, C. Talabnin, W. Chan-On, C.K. Ong, B.T. Teh, P. Yongvanit, Overexpression of microRNA-21 regulating PDCD4 during tumorigenesis of liver fluke-associated cholangiocarcinoma contributes to tumor growth and metastasis, Tumour Biol. 34 (2013) 1579–1588.

[46] L.J. Wang, C.C. He, X. Sui, M.J. Cai, C.Y. Zhou, J.L. Ma, L. Wu, H. Wang, S.X. Han, Q. Zhu, MiR-21 promotes intrahepatic cholangiocarcinoma proliferation and growth in vitro and in vivo by targeting PTPN14 and PTEN, Oncotarget 6 (2015) 5932–5946.

[47] F. Meng, R. Henson, M. Lang, H. Wehbe, S. Maheshwari, J.T. Mendell, J. Jiang, T.D. Schmittgen, T. Patel, Involvement of human micro-RNA in growth and response to chemotherapy in human cholangiocarcinoma cell lines, Gastroenterology 130 (2006) 2113–2129.

[48] Q. Huang, L. Liu, C.H. Liu, H. You, F. Shao, F. Xie, X.S. Lin, S.Y. Hu, C.H. Zhang, MicroRNA-21 regulates the invasion and metastasis in cholangiocarcinoma and may be a potential biomarker for cancer prognosis, Asian Pac. J. Cancer Prev. 14 (2013) 829–834.

[49] J.L. Plieskatt, G. Rinaldi, Y. Feng, J. Peng, P. Yonglitthipagon, S. Easley, T. Laha, C. Pairojkul, V. Bhudhisawasdi, B. Sripa, P.J. Brindley, J.P. Mulvenna, J.M. Bethony, Distinct miRNA signatures associate with subtypes of cholangiocarcinoma from infection with the tumourigenic liver fluke *Opisthorchis viverrini*, J. Hepatol. 61 (2014) 850–858.

[50] A.L. Collins, S. Wojcik, J. Liu, W.L. Frankel, H. Alder, L. Yu, T.D. Schmittgen, C.M. Croce, M. Bloomston, A differential microRNA profile distinguishes cholangiocarcinoma from pancreatic adenocarcinoma, Ann. Surg. Oncol. 21 (2014) 133–138.

[51] C. Correa-Gallego, D. Maddalo, A. Doussot, N. Kemeny, T.P. Kingham, P.J. Allen, M.I. D'Angelica, R.P. DeMatteo, D. Betel, D. Klimstra, W.R. Jarnagin, A. Ventura, Circulating plasma levels of microRNA-21 and microRNA-221 are potential diagnostic markers for primary intrahepatic cholangiocarcinoma, PLoS One 11 (2016) e0163699.

[52] J. Zhang, C. Han, T. Wu, MicroRNA-26a promotes cholangiocarcinoma growth by activating beta-catenin, Gastroenterology 143 (2012) 246–256 (e248).

[53] L.J. Wang, K.L. Zhang, N. Zhang, X.W. Ma, S.W. Yan, D.H. Cao, S.J. Shi, Serum miR-26a as a diagnostic and prognostic biomarker in cholangiocarcinoma, Oncotarget 6 (2015) 18631–18640.

[54] Q. Cheng, F. Feng, L. Zhu, Y. Zheng, X. Luo, C. Liu, B. Yi, X. Jiang, Circulating miR-106a is a novel prognostic and lymph node metastasis indicator for cholangiocarcinoma, Sci. Rep. 5 (2015) 16103.

[55] S. Wang, J. Yin, T. Li, L. Yuan, D. Wang, J. He, X. Du, J. Lu, Upregulated circulating miR-150 is associated with the risk of intrahepatic cholangiocarcinoma, Oncol. Rep. 33 (2015) 819–825.

[56] R. Silakit, W. Loilome, P. Yongvanit, P. Chusorn, A. Techasen, T. Boonmars, N. Khuntikeo, N. Chamadol, C. Pairojkul, N. Namwat, Circulating miR-192 in liver fluke-associated cholangiocarcinoma patients: a prospective prognostic indicator, J. Hepatobiliary Pancreat. Sci. 21 (2014) 864–872.

[57] R. Silakit, W. Loilome, P. Yongvanit, S. Thongchot, P. Sithithaworn, T. Boonmars, S. Koonmee, A. Titapun, N. Khuntikeo, N. Chamadol, A. Techasen, N. Namwat, Urinary microRNA-192 and microRNA-21 as potential indicators for liver fluke-associated cholangiocarcinoma risk group, Parasitol. Int. 66 (2015) 479–485.

[58] J. Plieskatt, G. Rinaldi, Y. Feng, J. Peng, S. Easley, X. Jia, J. Potriquet, C. Pairojkul, V. Bhudhisawasdi, B. Sripa, P.J. Brindley, J. Bethony, J. Mulvenna, A microRNA profile associated with *Opisthorchis viverrini*-induced cholangiocarcinoma in tissue and plasma, BMC Cancer 15 (2015) 309.

[59] K. Shigehara, S. Yokomuro, O. Ishibashi, Y. Mizuguchi, Y. Arima, Y. Kawahigashi, T. Kanda, I. Akagi, T. Tajiri, H. Yoshida, T. Takizawa, E. Uchida, Real-time PCR-based analysis of the human bile microRNAome identifies miR-9 as a potential diagnostic biomarker for biliary tract cancer, PLoS One 6 (2011) e23584.

[60] T. Voigtlander, S.K. Gupta, S. Thum, J. Fendrich, M.P. Manns, T.O. Lankisch, T. Thum, MicroRNAs in serum and bile of patients with primary sclerosing cholangitis and/or cholangiocarcinoma, PLoS One 10 (2015) e0139305.

[61] F. Bernuzzi, F. Marabita, A. Lleo, M. Carbone, M. Mirolo, M. Marzioni, G. Alpini, D. Alvaro, K.M. Boberg, M. Locati, G. Torzilli, L. Rimassa, F. Piscaglia, X.S. He, C.L. Bowlus, G.X. Yang, M.E. Gershwin, P. Invernizzi, Serum microRNAs as novel biomarkers for primary sclerosing cholangitis and cholangiocarcinoma, Clin. Exp.

Immunol. 185 (2016) 61–71.
[62] Z. Liang, X. Liu, Q. Zhang, C. Wang, Y. Zhao, Diagnostic value of microRNAs as biomarkers for cholangiocarcinoma, Dig. Liver Dis. 48 (2016) 1227–1232.
[63] J. Zhou, Z. Liu, S. Yang, X. Li, Identification of microRNAs as biomarkers for cholangiocarcinoma detection: a diagnostic meta-analysis, Clin. Res. Hepatol. Gastroenterol. 41 (2017) 156–162.
[64] L. Li, D. Masica, M. Ishida, C. Tomuleasa, S. Umegaki, A.N. Kalloo, C. Georgiades, V.K. Singh, M. Khashab, S. Amateau, Z. Li, P. Okolo, A.M. Lennon, P. Saxena, J.F. Geschwind, T. Schlachter, K. Hong, T.M. Pawlik, M. Canto, J. Law, R. Sharaiha, C.R. Weiss, P. Thuluvath, M. Goggins, E.J. Shin, H. Peng, V. Kumbhari, S. Hutfless, L. Zhou, E. Mezey, S.J. Meltzer, R. Karchin, F.M. Selaru, Human bile contains microRNA-laden extracellular vesicles that can be used for cholangiocarcinoma diagnosis, Hepatology 60 (2014) 896–907.
[65] R. Aebersold, M. Mann, Mass spectrometry-based proteomics, Nature 422 (2003) 198–207.
[66] E. Csosz, G. Kallo, B. Markus, E. Deak, A. Csutak, J. Tozser, Quantitative body fluid proteomics in medicine - a focus on minimal invasiveness, J. Proteome 153 (2017) 30–43.
[67] T.O. Lankisch, J. Metzger, A.A. Negm, K. Vosskuhl, E. Schiffer, J. Siwy, T.J. Weismuller, A.S. Schneider, K. Thedieck, R. Baumeister, P. Zurbig, E.M. Weissinger, M.P. Manns, H. Mischak, J. Wedemeyer, Bile proteomic profiles differentiate cholangiocarcinoma from primary sclerosing cholangitis and choledocholithiasis, Hepatology 53 (2011) 875–884.
[68] J. Shen, W. Wang, J. Wu, B. Feng, W. Chen, M. Wang, J. Tang, F. Wang, F. Cheng, L. Pu, Q. Tang, X. Wang, X. Li, Comparative proteomic profiling of human bile reveals SSP411 as a novel biomarker of cholangiocarcinoma, PLoS One 7 (2012) e47476.
[69] J. Metzger, A.A. Negm, R.R. Plentz, T.J. Weismuller, J. Wedemeyer, T.H. Karlsen, M. Dakna, W. Mullen, H. Mischak, M.P. Manns, T.O. Lankisch, Urine proteomic analysis differentiates cholangiocarcinoma from primary sclerosing cholangitis and other benign biliary disorders, Gut 62 (2013) 122–130.
[70] A. Arbelaiz, M. Azkargorta, M. Krawczyk, A. Santos-Laso, A. Lapitz, M.J. Perugorria, O. Erice, E. Gonzalez, R. Jimenez-Aguero, A. Lacasta, C. Ibarra, A. Sanchez-Campos, J.P. Jimeno, F. Lammert, P. Milkiewicz, M. Marzioni, R.I.R. Macias, J.J.G. Marin, T. Patel, G.J. Gores, I. Martinez, F. Elortza, J.M. Falcon-Perez, L. Bujanda, J.M. Banales, Serum extracellular vesicles contain protein biomarkers for primary sclerosing cholangitis and cholangiocarcinoma, Hepatology (2017), http://dx.doi.org/10.1002/hep.29291.
[71] P. Seeree, P. Pearngam, S. Kumkate, T. Janvilisri, An omics perspective on molecular biomarkers for diagnosis, prognosis, and therapeutics of cholangiocarcinoma, Int. J. Genomics 2015 (2015) 179528.
[72] G.A. Gowda, Human bile as a rich source of biomarkers for hepatopancreatobiliary cancers, Biomark. Med. 4 (2010) 299–314.
[73] N. Albiin, I.C. Smith, U. Arnelo, B. Lindberg, A. Bergquist, B. Dolenko, N. Bryksina, T. Bezabeh, Detection of cholangiocarcinoma with magnetic resonance spectroscopy of bile in patients with and without primary sclerosing cholangitis, Acta Radiol. 49 (2008) 855–862.
[74] A.W. Sharif, H.R. Williams, T. Lampejo, S.A. Khan, D.S. Bansi, D. Westaby, A.V. Thillainayagam, H.C. Thomas, I.J. Cox, S.D. Taylor-Robinson, Metabolic profiling of bile in cholangiocarcinoma using in vitro magnetic resonance spectroscopy, HPB (Oxford) 12 (2010) 396–402.
[75] M.S. Hashim Abdalla, S.D. Taylor-Robinson, A.W. Sharif, H.R. Williams, M.M. Crossey, G.A. Badra, A.V. Thillainayagam, D.S. Bansi, H.C. Thomas, I.A. Waked, S.A. Khan, Differences in phosphatidylcholine and bile acids in bile from Egyptian and UK patients with and without cholangiocarcinoma, HPB (Oxford) 13 (2011) 385–390.
[76] D. Beyoglu, J.R. Idle, The metabolomic window into hepatobiliary disease, J. Hepatol. 59 (2013) 842–858.
[77] Q. Liang, H. Liu, T. Zhang, Y. Jiang, H. Xing, H. Zhang, Serum metabolomics uncovering specific metabolite signatures of intra- and extrahepatic cholangiocarcinoma, Mol. BioSyst. 12 (2016) 334–340.
[78] A. Izzotti, S. Carozzo, A. Pulliero, D. Zhabayeva, J.L. Ravetti, R. Bersimbaev, Extracellular microRNA in liquid biopsy: applicability in cancer diagnosis and prevention, Am. J. Cancer Res. 6 (2016) 1461–1493.
[79] M. Cristofanilli, K.R. Broglio, V. Guarneri, S. Jackson, H.A. Fritsche, R. Islam, S. Dawood, J.M. Reuben, S.W. Kau, J.M. Lara, S. Krishnamurthy, N.T. Ueno, G.N. Hortobagyi, V. Valero, Circulating tumor cells in metastatic breast cancer: biologic staging beyond tumor burden, Clin. Breast Cancer 7 (2007) 471–479.
[80] S.J. Cohen, C.J. Punt, N. Iannotti, B.H. Saidman, K.D. Sabbath, N.Y. Gabrail, J. Picus, M. Morse, E. Mitchell, M.C. Miller, G.V. Doyle, H. Tissing, L.W. Terstappen, N.J. Meropol, Relationship of circulating tumor cells to tumor response, progression-free survival, and overall survival in patients with metastatic colorectal cancer, J. Clin. Oncol. 26 (2008) 3213–3221.
[81] J.S. de Bono, H.I. Scher, R.B. Montgomery, C. Parker, M.C. Miller, H. Tissing, G.V. Doyle, L.W. Terstappen, K.J. Pienta, D. Raghavan, Circulating tumor cells predict survival benefit from treatment in metastatic castration-resistant prostate cancer, Clin. Cancer Res. 14 (2008) 6302–6309.
[82] O. Al Ustwani, D. Iancu, R. Yacoub, R. Iyer, Detection of circulating tumor cells in cancers of biliary origin, J. Gastrointest. Oncol. 3 (2012) 97–104.
[83] J.D. Yang, M.B. Campion, M.C. Liu, R. Chaiteerakij, N.H. Giama, H. Ahmed Mohammed, X. Zhang, C. Hu, V.L. Campion, J. Jen, S.K. Venkatesh, K.C. Halling, B.R. Kipp, L.R. Roberts, Circulating tumor cells are associated with poor overall survival in patients with cholangiocarcinoma, Hepatology 63 (2016) 148–158.
[84] R.V. Iyer, V.K. Pokuri, A. Groman, W.W. Ma, U. Malhotra, D.M. Iancu, C. Grande, T.B. Saab, A multicenter phase II study of gemcitabine, capecitabine, and bevacizumab for locally advanced or metastatic biliary tract cancer, Am. J. Clin. Oncol. (2016), http://dx.doi.org/10.1097/COC.0000000000000347.
[85] J.J. Marin, M.R. Romero, O. Briz, Molecular bases of liver cancer refractoriness to pharmacological treatment, Curr. Med. Chem. 17 (2010) 709–740.
[86] O. Briz, M.J. Perez, J.J. Marin, Further understanding of mechanisms involved in liver cancer chemoresistance, Hepatoma Res. 3 (2017) 22–26.
[87] J.J. Marin, E. Lozano, O. Briz, R. Al-Abdulla, M.A. Serrano, R.I. Macias, Molecular bases of chemoresistance in cholangiocarcinoma, Curr. Drug Targets 18 (2017) 889–900.
[88] E. Herraez, E. Lozano, R.I. Macias, J. Vaquero, L. Bujanda, J.M. Banales, J.J. Marin, O. Briz, Expression of SLC22A1 variants may affect the response of hepatocellular carcinoma and cholangiocarcinoma to sorafenib, Hepatology 58 (2013) 1065–1073.
[89] I. Borbath, L. Verbrugghe, R. Lai, J.F. Gigot, Y. Humblet, H. Piessevaux, C. Sempoux, Human equilibrative nucleoside transporter 1 (hENT1) expression is a potential predictive tool for response to gemcitabine in patients with advanced cholangiocarcinoma, Eur. J. Cancer 48 (2012) 990–996.
[90] S. Sribenja, N. Natthasirikul, K. Vaeteewoottacharn, K. Sawanyawisuth, C. Wongkham, P. Jearanaikoon, S. Wongkham, Thymosin beta10 as a predictive biomarker of response to 5-fluorouracil chemotherapy in cholangiocarcinoma, Ann. Hepatol. 15 (2016) 577–585.
[91] N. Tepsiri, L. Chaturat, B. Sripa, W. Namwat, S. Wongkham, V. Bhudhisawasdi, W. Tassaneeyakul, Drug sensitivity and drug resistance profiles of human intrahepatic cholangiocarcinoma cell lines, World J. Gastroenterol. 11 (2005) 2748–2753.
[92] S. Rau, F. Autschbach, H.D. Riedel, J. Konig, H. Kulaksiz, A. Stiehl, J.F. Riemann, D. Rost, Expression of the multidrug resistance proteins MRP2 and MRP3 in human cholangiocellular carcinomas, Eur. J. Clin. Investig. 38 (2008) 134–142.
[93] Q. Chen, W. Li, Y. Wan, X. Xia, Q. Wu, Y. Chen, Z. Lai, C. Yu, Amplified in breast cancer 1 enhances human cholangiocarcinoma growth and chemoresistance by simultaneous activation of Akt and Nrf2 pathways, Hepatology 55 (2012) 1820–1829.
[94] C. Hahnvajanawong, J. Chaiyagool, W. Seubwai, V. Bhudhisawasdi, N. Namwat, N. Khuntikeo, B. Sripa, A. Pugkhem, W. Tassaneeyakul, Orotate phosphoribosyl transferase mRNA expression and the response of cholangiocarcinoma to 5-fluorouracil, World J. Gastroenterol. 18 (2012) 3955–3961.
[95] T. Nakajima, T. Takayama, K. Miyanishi, A. Nobuoka, T. Hayashi, T. Abe, J. Kato, K. Sakon, Y. Naniwa, H. Tanabe, Y. Niitsu, Reversal of multiple drug resistance in cholangiocarcinoma by the glutathione S-transferase-pi-specific inhibitor O1-hexadecyl-gamma-glutamyl-S-benzylcysteinyl-D-phenylglycine ethylester, J. Pharmacol. Exp. Ther. 306 (2003) 861–869.
[96] A. Jimeno, B. Rubio-Viqueira, M.L. Amador, D. Oppenheimer, N. Bouraoud, P. Kulesza, V. Sebastiani, A. Maitra, M. Hidalgo, Epidermal growth factor receptor dynamics influences response to epidermal growth factor receptor targeted agents, Cancer Res. 65 (2005) 3003–3010.
[97] H. Yoon, J.K. Min, D.G. Lee, D.G. Kim, S.S. Koh, H.J. Hong, L1 cell adhesion molecule and epidermal growth factor receptor activation confer cisplatin resistance in intrahepatic cholangiocarcinoma cells, Cancer Lett. 316 (2012) 70–76.
[98] I.G. Hwang, J.S. Jang, J.H. Do, J.H. Kang, G.W. Lee, S.Y. Oh, H.C. Kwon, H.J. Jun, H.Y. Lim, S. Lee, K.C. Chi, S.J. Lee, Different relation between ERCC1 overexpression and treatment outcomes of two platinum agents in advanced biliary tract adenocarcinoma patients, Cancer Chemother. Pharmacol. 68 (2011) 935–944.
[99] N. Namwat, P. Amimanan, W. Loilome, P. Jearanaikoon, B. Sripa, V. Bhudhisawasdi, W. Tassaneeyakul, Characterization of 5-fluorouracil-resistant cholangiocarcinoma cell lines, Chemotherapy 54 (2008) 343–351.
[100] J. Sato, T. Kimura, T. Saito, T. Anazawa, A. Kenjo, Y. Sato, T. Tsuchiya, M. Gotoh, Gene expression analysis for predicting gemcitabine resistance in human cholangiocarcinoma, J. Hepatobiliary Pancreat. Sci. 18 (2011) 700–711.
[101] H. Yoon, J.K. Min, J.W. Lee, D.G. Kim, H.J. Hong, Acquisition of chemoresistance in intrahepatic cholangiocarcinoma cells by activation of AKT and extracellular signal-regulated kinase (ERK)1/2, Biochem. Biophys. Res. Commun. 405 (2011) 333–337.
[102] N. Nutthasirikul, C. Hahnvajanawong, A. Techasen, T. Limpaiboon, C. Leelayuwat, S. Chau-In, P. Jearanaikoon, Targeting the 133p53 isoform can restore chemosensitivity in 5-fluorouracil-resistant cholangiocarcinoma cells, Int. J. Oncol. 47 (2015) 2153–2164.
[103] L. Guan, L. Zhang, Z. Gong, X. Hou, Y. Xu, X. Feng, H. Wang, H. You, FoxO3 inactivation promotes human cholangiocarcinoma tumorigenesis and chemoresistance through Keap1-Nrf2 signaling, Hepatology 63 (2016) 1914–1927.
[104] O. Govaere, J. Wouters, M. Petz, Y.P. Vandewynckel, K. Van den Eynde, A. Van den Broeck, S. Verhulst, L. Dolle, L. Gremeaux, A. Ceulemans, F. Nevens, L.A. van Grunsven, B. Topal, H. Vankelecom, G. Giannelli, H. Van Vlierberghe, W. Mikulits, M. Komuta, T. Roskams, Laminin-332 sustains chemoresistance and quiescence as part of the human hepatic cancer stem cell niche, J. Hepatol. 64 (2016) 609–617.
[105] S. Brivio, M. Cadamuro, L. Fabris, M. Strazzabosco, Epithelial-to-mesenchymal transition and cancer invasiveness: what can we learn from cholangiocarcinoma? J. Clin. Med. 4 (2015) 2028–2041.
[106] K.C. Chiang, T.S. Yeh, R.C. Wu, J.S. Pang, C.T. Cheng, S.Y. Wang, H.H. Juang, C.N. Yeh, Lipocalin 2 (LCN2) is a promising target for cholangiocarcinoma treatment and bile LCN2 level is a potential cholangiocarcinoma diagnostic marker, Sci. Rep. 6 (2016) 36138.
[107] J.J.G. Marin, E. Lozano, E. Herraez, M. Asensio, S. Di Giacomo, M.R. Romero, O. Briz, M.A. Serrano, T. Efferth, R.I.R. Macias, Chemoresistance and chemosensitization in cholangiocarcinoma, Biochim. Biophys. Acta (2017), http://dx.doi.org/10.1016/j.bbadis.2017.06.005.