The role of extracellular vesicles in cholangiocarcinoma

Mingzhen Bai, Wenkang Fu, Gang Su, Jie Cao, Long Gao, Chongfei Huang, Haidong Ma, Jinduo Zhang, Ping Yue, Bing Bai, Yanyan Lin, Wenbo Meng, and Xun Li

Abstract
Cholangiocarcinoma (CCA) is a rare tumor that arises from cholangiocytes, the epithelial cells of the bile duct. The tumor is characterized by insidious onset, high degree of malignancy, poor prognosis and high recurrence rate. Due to the lack of specific biomarkers, it is difficult to diagnose CCA early and evaluate prognosis. Extracellular vesicles (EVs), which include apoptotic bodies, microvesicles and exosomes, have emerged as having important roles in cell-to-cell communication in both normal physiology and pathological conditions. Some research has found that EVs play a crucial role in the occurrence and development of CCA. EVs can carry specific molecular substances such as nucleic acids and proteins, which have potential for the diagnosis and therapy of CCA. This article reviews the current knowledge on the role of EVs in CCA. We highlight EVs and their functions in the physiology and pathophysiology of CCA, and discuss their therapeutic potential and their role as biomarkers.

Keywords: Cholangiocarcinoma, Extracellular vesicles, Exosomes, Biomarkers, Therapy

Introduction
Cholangiocarcinoma (CCA) is the most common malignancy of the biliary tree, accounting for approximately 3% of all gastrointestinal tumors and is the second most common primary liver tumor after hepatocellular carcinoma (HCC) [1, 2]. The incidence of CCA varies geographically and demographically, and the overall incidence is still on the rise worldwide [3]. Although the 1-year survival has improved over time, the 5-year survival is less than 10% [4]. Most patients with CCA do not possess exact risk factors and the clinical manifestation may be nonspecific, even in the late-stage of the disease [5]. As such, early detection could improve survival, and this highlights the requirements for novel methods to diagnose and treat CCA.

Recently, the emerging role of extracellular vesicles (EVs) in cholangiocarcinoma progression has attracted extensive attention. To date, the role of EVs has changed from being nonfunctional discards of cellular components to the current research focus [6, 7]. EVs are nanosized, membrane-bound vesicles released from cells that can transport cargo, including DNA, RNA, and proteins, between cells as a form of intercellular communication [8, 9]. With so many contents, the nascent field of EVs has evolved to have a sharper focus, especially in oncology [10]. Therefore, EVs and their derived cargos have emerged as new biomarkers for tumor diagnosis. Tumor-derived EVs play a key role in modulating intercellular communication between tumor and stromal cells in local and distant microenvironments [11, 12]. In addition, EVs can still be used for therapeutic purposes as targets, immunomodulators and delivery vehicles [13]. In this review, we highlight and discuss about the relationship between EVs and CCA, with a special focus on their roles and potential clinical application values as biomarkers and therapeutic targets in CCA.
Epidemiology, risk factors, diagnosis and treatment of CCA

Cholangiocarcinoma is a devastating tumor, currently classified as intrahepatic (iCCA), perihilar (pCCA), or distal (dCCA), characterized by varying degrees of desmoplastic reaction and increasing morbidity and mortality worldwide [14, 15]. While it is more common in Asia, its incidence has risen significantly in Europe and North America in recent decades [16]. Approximately 35,660 patients with iCCA are diagnosed each year in the United States, and the 5-year survival rate is about 10% [17, 18]. The incidence of CCA is highest in Thailand, with 113 per 100,000 men and 50 per 100,000 women per year [19]. In most cases of CCA, the etiology is unknown, but chronic inflammation and cell injury in the bile duct shown a high risk of occurrence of CCA [14]. Pathologically, the release of inflammatory cytokines, increased cell death and proliferation, as well as changes in the liver in fibrosis contribute to the occurrence of tumor [20]. Primary sclerosing cholangitis is considered as the main risk factor for CCA [21]. The diagnostic basis mainly includes imaging methods (ultrasound, computed tomography, magnetic resonance imaging and fluorodeoxyglucose positron emission tomography), histological analysis of a tumor biopsy and serum nonspecific tumor biomarkers, such as carbohydrate antigen 19-9 (CA19-9) and carcinoembryonic antigen (CEA) [22–24]. However, the sensitivity of these tests in the diagnosis of CCA is limited, especially in the early stages of the disease [25]. Management strategies include multispecialty treatments, with consideration of surgical resection, targeted radiation therapy, and systemic chemotherapy [24, 26, 27]. Surgical resection is the only potentially curative treatment, but the majority of patients present with advanced cancer and recurrence after resection is common [16]. Lymph node metastasis is a prominent feature of CCA [28]. Diagnosed CCA is usually advanced and often inoperable, leading to a poor prognosis [29].

Details about EVs

Classification of EVs

According to the current knowledge of their biogenesis, EVs can be broadly divided into two main categories: microvesicles and exosomes [30, 31]. Microvesicles (MVs, 100–1000 nm in diameter) are secreted by the shedding or outward budding of the plasma membrane [32]. The formation of microvesicles is the result of the dynamic interaction of phospholipid redistribution and cytoskeletal protein contraction [33]. Since little information about biogenesis and MV release is understood, we focused on the function of exosomes in CCA.

Characteristics of exosomes

In 1983, Johnstone et al. first isolated exosomes from the supernatant of sheep reticulocyte culture medium [34]. However, due to the role of exosomes being unclear at that time, it is thought to be a nonfunctional particle in the cell. Whereas exosomes are 40–150 nm, endosome-derived, small EVs which are secreted by most cells [35]. RNA, DNA and proteins are reported to be actively and selectively incorporated into intraluminal vesicles, which reside within multivesicular endosomes and are the precursor of exosomes [36, 37]. The release of exosomes into the extracellular space is facilitated by the fusion of multivesicular bodies (MVBs) limiting the membrane with the plasma membrane [35, 38]. Then, exosomes can be carried away by extracellular fluid, such as saliva, urine, blood, semen, amniotic fluid, ascites, alveolar lavage fluid, milk, synovial fluid and cerebrospinal fluid, and taken up by other cells [39–41]. Besides, bile included [42]. The function of exosomes depends on the type and contents of their parent cells. Exosomes from normal cells play a role in maintaining stability in vivo, while tumor cell-derived exosomes are associated with tumor progression [40, 43].

In addition, we need to identify exosomes that can distinguish them from other EVs by their size and proteins markers. For instance, MVB-associated proteins (tumor susceptibility gene 101 (TSG101) and ALIX) [44, 45], fusion proteins, membrane transport proteins (flotillin-1) [46], tetraspanins (CD63, CD81 and CD9) [36], and heat shock proteins (HSP70.1 and HSP20) [42, 47] are often used as protein markers to recognize exosomes in scientific research. Currently, with the identification of a large number of cargo molecules in exosomes, their functions, including regulating immune function, enhancing metastasis, and modulating intercellular communication, have also been explored in tumor cells [48–50]. Some research shows that exosomes from highly metastatic breast cancer cells (4T1 and EO771 cells) can modulate the favorable microenvironment for lung and liver metastasis colonization [51]. In addition, various immune cell-derived exosomes, such as natural killer cells, dendritic cells, macrophages, neutrophils, mast cells and myeloid-derived suppressors, can act on tumor cells, modulating the growth, metastasis and response to chemotherapy [48, 52]. Next, we mainly elucidate the role of exosomes in CCA.

Characterization of EVs associated with CCA

As mentioned above, EVs can be produced in both cells and body fluids, and their characteristics are similar to each other. Although EVs can be extracted by many methods, differential centrifugation is the most widely
used method. Accumulated evidence has reported that EVs can be extracted from the serum, bile and CCA cells of patients with bile duct carcinoma and identified by specific markers, as shown in Table 1. The morphological and molecular characteristics of these EVs indicate that they are mainly exosomes. However, the contents of EVs are numerous, and their functions are different, and these need to be further studied.

### EVs regulate the progression of CCA

With the further development of EVs, increasing evidence has been presented to demonstrate the role of EVs in the progression of CCA (Fig. 1). Bile duct carcinoma usually has a dense stroma that contains immune cells (including neutrophils, tumor associated macrophages, natural killer cells, and T and B lymphocytes) and an extracellular matrix that promotes connective tissue proliferation [53, 54]. In particular, cancer-associated fibroblasts (CAFs), which communicate not only with tumor cells, but also with stromal cells, play a central role in the progression of CCA [55, 56]. CAFs influence the behavior of CCA by releasing various metabolites and soluble factors, such as vascular endothelial growth factor (VEGF)-A and VEGF-C, which may lead to dilation of the lymphatic vasculature and tumor cell intravasation [57]. Therefore, it also has the property of easy transfer [18]. In this process, EVs play a crucial role in facilitating the communication of various signals (Table 2).

### The function of EVs in tumor microenvironment

Cholangiocytes can be actively involved in the development of bile duct disease by stimulating the recruitment and activation of inflammatory cells in the bile duct microenvironment [58]. Katsumi et al. found that activated bile duct cells are involved in activating the proinflammatory polarization of damage-associated molecular patterns (DAMPs) through the receptor for advanced glycation end products (RAGE) signaling pathway by releasing DAMPs as EV cargo [59]. Prior to this, Masyuk et al. verified exosomes in rat bile [60]. Meanwhile, in the process of studying the cilium interaction of bile duct cells, exosomes were found to induce intracellular signals and functional responses, verifying that bile exosomes participate in intercellular signal communication [60]. This laid a foundation for the influence of bile duct carcinoma exosomes on tumor progression.

In the study of the tumor microenvironment of CCA, Haga et al. exposed marrow mesenchymal stem cells (MSCs) to CCA cell-derived EVs, which enhanced expression of alpha-smooth muscle actin mRNA and release of cytokines/chemokines such as IL-6, thus regulating the tumor microenvironment and promoting the growth of CCA. In addition, CCA cell-derived EVs can contribute to the formation of the tumor stroma through the fibroblast differentiation of MSCs [61]. This further revealed the effect of tumor cell-derived EVs on the local microenvironment. However, they did not identify the specific contents of the EVs. Moreover, proteins in exosomes are essential agents for tumor growth [62, 63]. Another study demonstrated the protein spectrum of CCA-derived exosomes and their potential roles. These researchers isolated exosomes from CCA cell lines (KKU-M213 and KKU-100) and incubated them with normal bile duct cells (H69). After

### Table 1 Characterization of EVs in bile, serum, cholangiocytes and CCA cells

| The source of EVs (TEM)/ (NTA) | Biomarkers | References |
|-------------------------------|------------|-----------|
| CCA bile Rounded, cup-shaped, double-membrane-bound vesicles, 50–750 nm in diameter | TSG101, ALIX, CD9, HSP70.1 | [42] |
| Spherical structures of vesicles, 30–110 nm in diameter, the mode of exosomes sizes is 72.2 nm | CD63, CD81 | [99] |
| Spherical structures, the mode of EVs sizes is 84 nm, 30–110 nm in diameter, 3 x 10^{11} EVs/ml bile | TSG101, CD63 | [98] |
| CCA serum Round morphology, \textasciitilde 180 nm in diameter | CD9, CD63, CD81 | [104] |
| 50–750 nm in diameter | TSG101, ALIX, CD9, HSP70.1 | [42] |
| KKKU-M213 Crescent shaped membrane invagination, 40–100 nm in diameter | Flotillin-1, TSG101, CD81, CD63 | [64] |
| KKU-100 50–150 nm in diameter | Flotillin-1, TSG101, CD81 | [73] |
| KMBC Spherical structures of vesicles, a mean size of 137 \pm 960 nm | ALIX, CD9, CD81 | [61] |
| KKKU-M213D5 40–100 nm in diameter | ALIX, TSG101 | [105] |
| RBE 30–120 nm in diameter | ALIX, TSG101, CD63 | [65] |
| HuCCA-1 50–150 nm in diameter | Flotillin-1, CD81, TSG101 | [73] |
| H69 50–150 nm in diameter | Flotillin-1, CD81, TSG101 | [73] |

CCA cell lines: KKKU-M213, KKU-100, KKKU-M213D5, KMBC, RBE and HuCCA-1. Normal human cholangiocyte cell line: H69

TSG101 tumor susceptibility gene 101, TEM transmission electron microscopy, NTA nanoparticle tracking analysis
proteomics analysis, exosomes were found to be internalized into H69 cells, resulting in migration and invasion of H69 cells, but failing to induce proliferation. In addition, the exosomes of KKU-M213 cells induced the expression of β-catenin and decreased the expression of E-cadherin, suggesting that exosomes might induce the migration and invasion of bile duct cells through the direct transfer of oncogene proteins between cells, thus affecting the specific intracellular mechanism related to CCA carcinogenesis [64]. The proteomics analysis of normal bile duct cells and CCA cells confirmed the differences between these two cell types, and these differences need to be further studied. Moreover, exosomes from another CCA cell line (RBE) could inhibit the antitumor activity of cytokine-induced killer (CIK) cells by downregulating the populations of CD3⁺, CD8⁺,

**Table 2** Summarize the function of EVs contents in CCA

| EVs contents | Source | Downstream target or recipient cells | Function | References |
|--------------|--------|--------------------------------------|----------|------------|
| DAMPs        | Actived cholangiocytes | Macrophage | Upregulate proinflammatory cytokines and profibrogenic factors | [59] |
| Exosomes     | HUCCT1 and KMBC | MSCs | Contribute to tumor cell growth and stromal development | [61] |
| Exosomes     | KRU-M213 | H69 cell | Induce the expression of β-catenin and reduce the expression of E-cadherin | [64] |
| Exosomes     | RBE | CIK cell | Inhibit the antitumor activity of CIK cells | [65] |
| Mir-205-5p   | KRU-M213 | CCA cell | Down-regulation of mir-205-5p can inhibit invasion and migration of CCA cells | [73] |
| Mir-195      | LX2 | A rat model of CCA | Inhibit CCA growth and improve survival in a rat model of CCA | [77] |
| Mir-30e      | HuCCT1 | CCA cell | Inhibit CCA cell invasion and migration via inhibit EMT | [81] |
| Circ-0000284 | HuCCT1 and RBE | Gene LY6E | Enhancing the migration, invasion and proliferation of CCA cells | [86] |

CCA cell lines: KKU-M213, KMBC, RBE and HuCCT-1. Normal human cholangiocyte cell line: H69. A human liver stellate cell line: LX2.
NK (CD56⁺) and CD3⁺CD56⁺ cells and secreting TNF-α and perforin [65]. According to some research, mutations in the IDH1 gene is common in a variety of tumors, including iCCA [66, 67]. Recent studies have shown that the R132C mutation is the most common type of IDH1 mutation in iCCA. The IDH1R132C mutation leads to the downregulation of P2RX7 expression, which further affects the exosome secretion of tumor cells, ultimately affecting the progression of CCA [68]. However, more functional experiments on P2RX7 are needed.

**Exosomal noncoding RNAs in CCA**

In addition, noncoding RNA also play a crucial role in tumor development [69]. As a functional regulatory molecule, noncoding RNAs, such as microRNAs (miRNAs) and circular RNAs (circRNAs), could mediate cellular processes, including chromatin, transcriptional, posttranscriptional modification and signal transduction, and, of course, predict prognosis [69–71].

**Exosomal microRNAs in CCA**

MiRNAs are small noncoding RNAs composed of 19–24 nucleotides [72]. Kitdumrongthum et al. found different miRNA expression profiles in the exosomes released by CCA cells and cholangiocytes, and many miRNAs with abnormal regulation had functions related to a variety of oncogenes [73]. For example, miR-205-5p, the most upregulated miRNA, down-regulation of miR-205-5p can inhibit invasion and migration of CCA cells [73]. Moreover, miR-205-5p has also been reported in other cancers, such as breast cancer and gastric cancer [74, 75]. However, in contrast to CCA, the expression of miR-205-5p is downregulated in breast cancer, and miR-205-5p has an antitumor effect in breast cancer [76]. Li and colleagues reported that EVs could transport miRNA species between human CCA cells and CAFs. They used LX2-derived EVs carrying miR-195 to inhibit CCA growth and improve survival in a mouse model of CCA, demonstrating the communication between the tumor and microenvironment [77]. Epithelial–mesenchymal transition (EMT) is a biological process in which epithelial cells gradually change and lose epithelial characteristics and differentiate into mesenchymal phenotypes, and it is closely related to the invasiveness and motility of tumor cells [78–80]. CCA-derived EVs could transfer miR-30e and inhibit EMT by directly targeting the Snail in receptor cells, thus inhibiting the invasion and migration of bile duct cancer cells [81]. In addition, miR-200a/c-3p in serum exosomes was significantly positively correlated with the CCA stage, and mainly involved in lymphatic metastasis of tumors [82].

**Exosomal circRNAs in CCA**

Recent research has demonstrated that circRNAs also have a biological role in CCA [83, 84]. Wang et al. found that the level of circ-0000284 was increased in CCA cell lines, tumor tissues and plasma exosomes, thus enhancing the migration, invasion and proliferation of CCA cells in vivo and in vitro. The circ-0000284/miR-637/LY6E regulatory axis was involved in this process [85, 86]. In addition, exosome-mediated circ-0000284 could stimulate the malignant behavior of surrounding normal cells and ultimately promote the progression of CCA [86].

**EVs as novel biomarkers for CCA**

A major reason for the poor prognosis of CCA is the lack of early detection. Late diagnosis delays optimal treatment and leads to lower survival. Therefore, it is necessary to develop new methods for the diagnosis of CCA. In recent years, studies have shown that EVs have huge potential in the diagnosis of diseases due to their unique properties and great progress has been made in studying of EVs as tumor diagnostic markers [41, 87, 88]. EVs in bile and blood have opened up new ideas for the early diagnosis of CCA. Compared with traditional CA-199 and CEA, EVs have higher diagnostic value in CCA, which is summarized in Fig. 2.

**The isolation of EVs**

With the development of technology, many exosome separation and purification techniques, including ultracentrifugation, ultrafiltration, immunoaffinity capture, size-exclusion chromatography, microfluidic techniques and charge neutralization-based polymer precipitation, have been exploded [89–91]. Among them, the most common application is ultracentrifugation, which is the gold standard for exosome separation, even though it has some limitations, such as having a low efficiency and being time consuming [92–94]. In the CCA research process, the EVs in the culture media of CCA were separated mainly by ultracentrifugation [61, 64, 65]. Similarly, EVs are extracted from the serum by ultracentrifugation, and polymer-based precipitation kit [95, 96]. However, there is a study that supports some commercial kits, such as ExoQuick and miR-CURY, as being better than ultracentrifugation, even with a limited quantity of EVs [92]. Bile is a lipid-rich fluid, that is secreted primarily by hepatocytes, and contains almost all body components: lipids, proteins, carbohydrates, vitamins, mineral salts and trace elements [96, 97]. Because of the characteristics and complexity of bile extraction, we mainly summarize
the isolation of bile here. Bile samples of CCA are usually collected by endoscopic retrograde cholangiopancreatography (ERCP), percutaneous transhepatic biliary drainage (PTBD) and surgery, and then bile is immediately centrifuged at 4 °C to remove the cell debris and filtered through a 200 nm filter. Finally, the supernatant is collected and added to an ultracentrifuge tube, diluted with PBS for further purification, mainly
including ultracentrifugation, PEG precipitation, membrane filtration and affinity purification [98–100]. Some research, however, initially collected bile and then rapidly diluted with PBS for further centrifuge [42]. The isolation of EVs were used immediately or stored at −80 °C until use (Fig. 3). However, research in this area is limited, and there is still much room for improvement in the separation and purification of exosomes.

Bile EVs
MiRNA in EVs is protected the membrane from degrading enzymes, and this determines its highly stable characteristics in the extracellular environment, thus, miRNA is used for the diagnosis of a variety of cancers, including CCA [101, 102]. Li et al. identified and characterized EVs in human bile for the first time, and miRNA-laden EVs in human bile could be used for the diagnosis of CCA. They defined a new biliary vesicle miR-based panel (miR-191, miR-486-3p, miR-1274b, miR-16 and miR-484) for the diagnosis of CCA with a sensitivity of 67% and specificity of 96% [98]. This research initiates studying EVs in bile. Han and coworkers found that the expression levels of miR-30d-5p and miR-92a-3p in the bile of CCA patients were specifically upregulated compared with those in the bile of patients with benign biliary disease [103]. Compared with CEA and CA19-9, miR-30d-5p had the best effect in differentiating CCA and common bile duct disease, with a sensitivity of 81.1%, specificity of 60.5% and area under the curve (AUC) value of 0.730. In addition, the identification of CCA using the combination of the two bile miRNAs and serum CA19-9 levels was weaker than that of miR-30d-5p alone [103].

Long noncoding RNAs (lncRNAs) in bile also play the same role in CCA as miRNAs. Ge et al. conducted a series of experiments on bile exosomes, and showed that the expression of the lncRNAs ENST00000588480.1 and ENST00000517758.1 in the CCA group was significantly increased compared with that of the control group. The AUC of the combined detection of the two IncRNAs was 0.709, and the sensitivity and specificity were 82.9% and 58.9%, respectively [99]. Moreover, with the increase in the tumor TNM stage, the expression levels of ENST00000588480.1 and ENST00000517758.1 were significantly increased, and they could be potential diagnostic markers [99]. The content of EVs in bile can be used as a diagnostic marker, and the concentration of EVs also has potential diagnostic value. Severino et al. found that the accuracy of the bile EV count in the diagnosis of CCA was 100% with a threshold value of 9.46 × 10¹⁴ nanoparticles, with an AUC of 1, and this could correctly distinguish malignant and nonmalignant common bile duct stenoses in this research [42].

Summary of the role of EVs in diagnosing CCA
The studies above indicate that miRNAs, lncRNAs and proteins in blood and bile could be used as diagnostic indicators of CCA (summarized in Table 3). It can be concluded that although there are many substances in EVs, exosomes are the main diagnostic agents. Exosomes coated with lipid bilayers are more stable and more suitable as diagnostic markers [8]. The concentration of EVs in bile may increase as a result of bile flow disorder caused by bile duct stenosis or obstruction in CCA patients [106]. In addition, potential tumor-derived biomarkers may be secreted directly into bile by adjacent CCA cells, and local sampling may be more likely to detect candidate biomarkers directly related to the tumor [106]. Therefore, compared with circulating serum sampling, bile sampling can improve diagnostic performance [103]. However, the study of the EVs in CCA is still not complete. For example, the mechanism of EVs in bile duct cancer is
Application of EVs in CCA therapy

To date, due to the difficulty in the early diagnosis of CCA, there are few treatment options, and radical surgical resection is the only effective treatment method [107, 108]. Postoperative adjuvant chemotherapy can improve the survival and cure rates, but the effect of chemotherapy is not enough [18]. With the development of research on EVs, the treatment of CCA has a new direction. The characteristics of tumor-secreted EVs in regulating the immune microenvironment illustrate their clinical potential in immunotherapy, therapeutic targeting and drug delivery [11, 109].

Table 3 EVs as diagnostic biomarkers for CCA

| Biomarkers | AUC  | Sensitivity | Specificity | Reference |
|------------|------|-------------|-------------|-----------|
| CCA vs. PSC BBO | Null | Null | Null | [98] |
| Bile: miR-based panel | Null | 67% | 96% | [103] |
| CCA vs. BBD | | | | |
| Bile: miR-30d-5p | 0.730 | 81.1 | 60.5 | |
| miR-92a-3p | 0.652 | 65.7 | 66.7 | |
| Serum: CA19-9 | 0.675 | 70.3 | 64.6 | |
| CEA | 0.603 | 64.9 | 60.4 | |
| CCA vs. BBO | | | | |
| Bile IncRNAs: ENST00000588480.1 and ENST00000517758.1 | 0.709 | 82.9 | 58.9 | [99] |
| Serum: CA19-9 | 0.729 | 74.3 | 71.4 | |
| CCA: TNMs I, II vs. TNMs III, IV | | | | [105] |
| Serum: HSP90B1-s255 | 0.936 | 87.27 | 97.62 | |
| MCBDs vs. NMCBDs | | | | [42] |
| Concentration of EVs | 1.000 | Null | Null | |
| Serum: CA19-9 | 0.733 | Null | Null | |
| CCA vs. HC | | | | [82] |
| Serum: miR-200c-3p | 0.930 | Null | Null | |
| Serum: CA19-9 | 0.780 | Null | Null | |
| CCA vs. PSC | | | | [104] |
| FIBG | 0.796 | Null | Null | |
| A1AG1 | 0.794 | Null | Null | |
| S10A8 | 0.759 | Null | Null | |
| Serum: CA19-9 | 0.819 | Null | Null | |
| CCA vs. HC | | | | |
| AMPN | 0.878 | Null | Null | |
| VNN1 | 0.876 | Null | Null | |
| PIGR | 0.844 | Null | Null | |
| Serum: CA19-9 | 0.907 | Null | Null | |

Therapeutic targets

Since the tumor microenvironment promotes the progression and invasion of CCA, targeting the microenvironment and related cells is a strategy for the treatment of CCA [110, 111]. Chen et al. found that RBE-derived exosomes could inhibit the antitumor activity of CIK cells [65]. This suggests that the effect of CIK cell-based immunotherapy is related to EVs, which may be a potential therapeutic target. In addition, circ-0000284 may be a therapeutic target for CCA. Wang et al. proved that the knockdown of exosomal circ-0000284 inhibited CCA growth and metastasis in vivo through animal experiments [86]. Besides, according to Zhang’s research, mutations in the IDH1 gene alter the function of IDH1 and affect the development of CCA by promoting exosome release [68]. Interestingly, IDH1 inhibitors have been reported. For example, the safety and clinical efficacy of mutant IDH1 inhibitor ivosidenib in the recurrence or refractory IDH1-mutated acute myeloid leukemia were demonstrated [112]. At present, inhibitors of IDH1 (AG120 and IDH305) are being tested in iCCA patients [113]. This provides a potential treatment of CCA. Furthermore, one study reported that inhibition of miR-205-5p in the exosomes of CCA could reduce the invasion and migration of CCA, and the miR-200 family was associated with drug resistance [73, 114].

Drug delivery

EVs are natural membrane vesicles involved in intercellular communication; accumulating evidence has revealed that EVs have the characteristics of stability and low immune reactivity, and exosomes in EVs can effectively transport a variety of different types of cargo to target cells [109, 115]. As a result, EVs can also be selected as a therapeutic tool to modulate the function of CCA cells by delivering cargo media [35, 116]. Stromal derived EVs are suitable for the delivery of materials to CCA cells, and this property can be exploited for delivering antitumor therapy to CCA cells [117]. Li et al. proved that EVs could transport miR-195 from fibroblasts to cancer cells. In addition, fibroblasts-derived EVs loaded with miR-195, play a key role in the CCA rat model, by reducing the size of tumors and improving survival in the treated rats [77]. Moreover, after incubating the miR-30e-enriched EVs with CCA cells, Zhang found that the expression of miR-30e in receptor CCA cells increased, which ultimately regulated the invasion and migration of cells. This demonstrates that miR-30e-enriched EVs can be ingested by recipient cells as a means of transferring miR-30e [81]. A recent study showed that methotrexate-loaded tumor-cell-derived microvesicles were injected into the bile duct lumen of patients with extrahepatic CCA, which
mobilized and activated neutrophils and alleviated biliary obstruction [118]. These studies suggest that EVs carrying designed cargo can be used as media carriers to manage the progression of CCA.

### Conclusion and future prospects

EVs are present through the occurrence, development and metastasis of tumors, providing new clues for the diagnosis and treatment of CCA. Many substances in EVs, such as miRNAs, circRNAs, proteins and even EV concentrations, can be used as new biomarkers. It is also important to improve the early diagnosis of CCA. Moreover, according to the role of EVs in the tumor microenvironment and immunity, corresponding targeted drugs and immunotherapy can be used for the treatment of CCA. Exosomal programmed-death ligand (PD-L1) has been found to contribute to immunosuppression and has been associated with the anti-PD-1 response, although it is mainly involved in melanoma [119]. Moreover, PD-L1 expression in iCCA and perihilar cholangiocarcinoma has also been reported, and is mainly expressed in tumors with a high density of tumor-infiltrating lymphocytes [120]. Immunotherapy for PD-L1 in CCA has entered clinical research, but the efficacy is not clear [121]. EVs have shown great potential in the immunotherapy of tumors, although there is not much data on the immunotherapy of EVs in CCA and therefore, further research is needed. In summary, this review focuses on the current research status of EVs in CCA. First, the characteristics of EVs in CCA were described. Second, the mechanism of EVs in tumor growth and metastasis was discussed. Finally, we demonstrated that the contents of EVs could be used for the clinical diagnosis and treatment of CCA. Although the existing studies have partially uncovered the mechanism of EVs in CCA, there are still a few challenging problems to solve. Firstly, the detailed mechanism to describe the role of EVs in CCA needs further clarification. In addition, standardized methods for the separation, purification and analysis of EVs in body fluids are needed. Last but not least, most of the pathophysiological studies are conducted through in vitro analysis, and there are few in vivo experiments based on EVs in animal models. Follow-up studies should be conducted to better apply EVs to the clinical diagnosis and treatment of CCA in the future. Therefore, more efforts are needed to study the role of EVs in CCA.

### Abbreviations

CCA: Cholangiocarcinoma; HCC: Hepatocellular carcinoma; EVs: Extracellular vesicles; MVs: Microvesicles; MVs: Multivesicular bodies; TSG101: Tumor susceptibility gene 101; HSP70.1: Heat shock proteins 70.1; HSP20: Heat shock proteins 20; TEM: Transmission electron microscopy; NTA: Nanoparticle tracking analysis; CAFs: Cancer-associated fibroblasts; VEGF-A: Vascular endothelial growth factor-A; VEGF-C: Vascular endothelial growth factor-C; RAGE: Receptor for advanced glycation end products; DAMPs: Damage-associated molecular patterns; MSCs: Marrow mesenchymal stem cells; iCCA: Intrahepatic cholangiocarcinoma; miRNAs: MicroRNAs; circRNAs: Circular RNAs; EMT: Epithelial–mesenchymal transition; CA19-9: Carbohydrate antigen 19-9; CEA: Carcinoembryonic antigen; pCCA: Perihilar cholangiocarcinoma; dCCA: Distal cholangiocarcinoma; FIBG: Fibrinogen gamma chain; A1AG1: Alpha-1-acid glycoprotein 1; S10A8: S100A8; AMPN: Aminopeptidase N; VNN1: Pantetheinase; PGR: Polymeric immunoglobulin receptor; ERCP: Endoscopic retrograde cholangiopancreatography; PTBD: Percutaneous transhepatic biliary drainage; AUC: Area under the curve; IncRNAs: Long noncoding RNAs; PSC: Primary sclerosing cholangitis; EpCAM: Epithelial cell adhesion molecule; ASGPR1: Asialoglycoprotein receptor 1; BBO: Benign biliary obstruction; BBD: Benign biliary disease; MCBDS: Malignant common bile duct stenoses; NMCBDS: Nonmalignant common bile duct stenoses; HC: Healthy controls; PD-L1: Programmed-death ligand.

### Acknowledgements

Not applicable.

### Authors’ contributions

MB, WF, WM and YL designed the research. MB drafted the manuscript. XL, WM, YL, GS, WF critically revised the manuscript. MB, WF, LG, JC, CH, HM, JZ, PY and BB provided intellectual contribution. All authors read and approved the final manuscript.

### Funding

This research was supported by the National Natural Science Foundation (Grant Number 81872036), and Lanzhou Science and Technology Bureau (Grant Number 2019-4-43), and Science and Technology Project of Chengguan District of Lanzhou City (Grant Number 2019JSCX092 and 2019RCCX0038), and Lanzhou talent innovation project (Grant Number 2018-RC-13).

### Availability of data and materials

The materials supporting the conclusions of this review are included in the article.

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

### Author details

1. The First Clinical Medical College, Lanzhou University, Lanzhou 730000, China. 2. Department of Special Minimally Invasive Surgery, The First hospital of Lanzhou University, Lanzhou 730000, China. 3. Division of Scientific Research and Development Planning, The First Hospital of Lanzhou University, Lanzhou 730000, China. 4. Institute of Genetics, School of Basic Medical Sciences, Lanzhou University, 730000 Lanzhou, China. 5. Gansu Province Institute of Hepatopancreaticobiliary, 730000 Lanzhou, China. 6. Gansu Province Key Laboratory Biotherapy and Regenerative Medicine, 730000 Lanzhou, China. 7. The Second Department of General Surgery, The First Hospital of Lanzhou University, 730000 Lanzhou, China.

### Received

26 June 2020  Accepted: 29 August 2020

Published online: 04 September 2020

### References

1. Rizvi S, Gores GJ. Pathogenesis, diagnosis, and management of cholangiocarcinoma. Gastroenterology. 2013;145(6):1215–29.
2. Pellino A, Loupakis F, Cadamuro M, Dadduzio V, Fassan M, Guido M, et al. Precision medicine in cholangiocarcinoma. Transl Gastroenterol Hepatol. 2018;3:40.
3. Montomoli J, Erichsen R, Norgaard M, Hoyer M, Hansen JB, Jacobsen JB. Survival of patients with primary liver cancer in central and northern Denmark, 1998–2009. Clin Epidemiol. 2011;3(Suppl 1):3–10.

4. Bengquist A, von Seth E. Epidemiology of cholangiocarcinoma. Best Pract Res Clin Gastroenterol. 2015;29(2):221–32.

5. Tyson GL, El-Seraq HB. Risk factors for cholangiocarcinoma. Hepatology. 2011;54(1):173–84.

6. Maas SLN, Breakefield XO, Weaver AM. Extracellular vesicles: unique intercellular delivery vehicles. Trends Cell Biol. 2017;27(3):172–88.

7. Cocucci E, Racchetti G, Meldolesi J. Shedding microvesicles: artefacts no more. Trends Cell Biol. 2009;19(2):43–51.

8. Zaborowski MP, Balaj L, Breakefield XO, Lai CP. Extracellular vesicles: composition, biological relevance, and methods of study. Bioscience. 2015;65(8):783–97.

9. Roma-Rodrigues C, Raposo LR, Cabral R, Paradinha F, Baptista PV, Fernandes AR. Tumor microenvironment modulation via gold nanoparticles targeting malignant exosomes: implications for cancer diagnostics and therapy. Int J Mol Sci. 2017;18(11):162.

10. Bebelman MP, Smit MJ, Pegtel DM, Baglio SR. Biogenesis and function of extracellular vesicles in cancer. Pharmacol Ther. 2018;188:1–11.

11. Becker A, Thakur BK, Weiss JM, Kim HS, Peinado H, Lyden D. Extracellular vesicles: a cell-to-cell mediators of metastasis. Cancer Cell. 2016;30(6):836–49.

12. Sheehan C, D’Souza-Schorey C. Tumor-derived extracellular vesicles: molecular parcels that enable regulation of the immune response in cancer. J Cell Sci. 2019. https://doi.org/10.1242/jcs.235085.

13. Walker S, Busatto S, Pham A, Tian M, Suh A, Carson K, et al. Extracellular vesicle-based drug delivery systems for cancer treatment. Theranostics. 2019;9(28):8001–17.

14. Sirica AE. Cholangiocarcinoma: molecular targeting strategies for chemoprevention and therapy. Hepatology. 2005;41(1):15–15.

15. Alsahli M, Lefflty Z, Barbera TA, Sithithaworn P, Khuntikeo N, Loilome W, et al. Cholangiocarcinoma: a guide for the nonspecialist. Int J Gen Med. 2019;12:13–23.

16. Blechacz B, Cholangiocarcinoma. Current knowledge and new developments. Gut Liver. 2017;11(1):13–26.

17. Zhang H, Yang T, Wu M, Shen F. Intrahepatic cholangiocarcinoma: epidemiology, risk factors, diagnosis and surgical management. Cancer Lett. 2016;379(2):198–205.

18. Sato K, Glaser S, Alvaro D, Meng F, Francis H, Alpini G. Cholangiocarcinoma: clinical aspects. J Hepatobiliary Pancreat Sci. 2015;22(8):783–97.

19. Rizvi S, Khan A, Cartier B, Gores GJ. Cholangiocarcinoma: clinical implications and evaluation of the potential prognostic biomarkers. Cancer Lett. 2016;379(2):198–205.

20. Tsen A, Barbara M, Rosenkranz L. Dilemma of elevated CA 19-9 in cholangiocarcinoma. J Cancer. 2019. https://doi.org/10.2188/jca.235085.

21. Kirstein MM, Vogel A. Epidemiology and risk factors of cholangiocarcinoma. J Gastroenterol Hepatol. 2011;26(Suppl 1):3–10.

22. Seydoux C, D’Souza-Schorey C. Tumor-derived extracellular vesicles: molecular parcels that enable regulation of the immune response in cancer. J Cell Sci. 2019. https://doi.org/10.1242/jcs.235085.

23. Tsen A, Barbara M, Rosenkranz L. Dilemma of elevated CA 19-9 in cholangiocarcinoma. J Cancer. 2019. https://doi.org/10.2188/jca.235085.

24. Wang Z, Gao X, Dong Y, Zhao Y, Chen X, Yang G, et al. Exosome-mediated cell-cell communication in tumor progression. Am J Cancer Res. 2018;8(9):1661–73.

25. Abels ER, Breakefield XO. Introduction to extracellular vesicles: biogenesis, RNA cargo selection, content, release, and uptake. Cell Mol Neurobiol. 2016;36(3):301–12.

26. Street JM, Kortzinsky EH, Glispie DM, Star RA, Yuen PS. Urine exosomes: an emerging trove of biomarkers. Adv Clin Chem. 2017;78:103–22.

27. Kaliri R, LeBlou E. The biology, function, and biomedical applications of exosomes. Science. 2020;367(6478):eaau6977.

28. Li Y, Zhao J, Yu S, Wang Z, He X, Su Y, et al. Extracellular vesicles long RNA sequencing reveals abundant mRNA, circRNA, and IncRNA in human blood as potential biomarkers for cancer diagnosis. Clin Chem. 2019;65(6):798–808.

29. Akers JC, Gonda D, Kim R, Carter BS, Chen CC. Biogenesis of extracellular vesicles in cancer. Dis Model Mech. 2018;11(10):1197–216.

30. Kowal J, Tkach M, Thery C. Biogenesis and secretion of exosomes. Curr Opin Cell Biol. 2014;29:116–25.

31. Zhang H, Yang T, Wu M, Shen F. Intrahepatic cholangiocarcinoma: epidemiology, risk factors, diagnosis and surgical management. Cancer Lett. 2016;379(2):198–205.

32. Sato K, Glaser S, Alvaro D, Meng F, Francis H, Alpini G. Cholangiocarcinoma: novel therapeutic targets. Expert Opin Ther Targets. 2020;24(4):345–57.

33. Rizvi S, Khan SA, Hallemeyer CL, Kelley RK, Gores GJ. Cholangiocarcinoma—evolving concepts and therapeutic strategies. Nat Rev Clin Oncol. 2018;15(2):95–111.

34. Zevalinova N, Gores GJ. Cholangiocarcinoma. The Lancet. 2014;383(9935):2168–79.

35. Kowal J, Tkach M, Thery C. Biogenesis and secretion of exosomes. Curr Opin Cell Biol. 2014;29:116–25.

36. Tischer G, Dorji PW, Chajjareouk W, Na-Bangchang K. Biomarkers for the diagnosis of cholangiocarcinoma: a systematic review. Am J Trop Med Hyg. 2018;98(6):1788–97.

37. Tsen A, Barbara M, Rosenkranz L. Dilemma of elevated CA 19-9 in biliary pathology. Pancreatology. 2018;18(8):862–7.

38. Doherty B, Nambudiri VE, Palmer WC. Update on the diagnosis and treatment of cholangiocarcinoma. Curr Gastroenterol Rep. 2017;19(1):2.

39. Tang Z, Yang Y, Zhang J, Fu W, Lin Y, Su G, et al. Quantitative proteomic analysis and evaluation of the potential prognostic biomarkers in cholangiocarcinoma. J Cancer. 2019;10(17):3985–99.

40. Edeline J, Benabdallah M, Bertrant A, Watelt J, Hammel P, Joly JP, et al. Gmctabine and Oxalplatin Chemotherapy or Surveillance in Repeated Biliary Tract Cancer (PRODIGE 12-ACCORD 18-UNICANCER GivA: A Randomized Phase III Study). J Clin Oncol. 2019;37(8):658–67.

41. Dover LL, Oster RA, McDonald AM, DuBay DA, Wang TN, Jacob R. Impact of adjuvant chemoradiation on survival in patients with resectable cholangiocarcinoma. HPB (Oxford). 2016;18(10):843–50.

42. Ji GW, Zhu Y, Zhang YD, Liu XS, Wu PY, Wang K, et al. A radiomics approach to predict lymph node metastasis and clinical outcome of intrahepatic cholangiocarcinoma. Eur Radiol. 2019;29(7):3725–35.

43. Street JM, Koritzinsky EH, Glispie DM, Star RA, Yuen PS. Urine exosomes: an emerging trove of biomarkers. Adv Clin Chem. 2017;78:103–22.

44. Kowal J, Tkach M, Thery C. Biogenesis and secretion of exosomes. Curr Opin Cell Biol. 2014;29:116–25.

45. Zhang H, Yang T, Wu M, Shen F. Intrahepatic cholangiocarcinoma: epidemiology, risk factors, diagnosis and surgical management. Cancer Lett. 2016;379(2):198–205.

46. Schoor RM. Cholangiocarcinoma: clinical implications and therapeutic targeting. Hepatology. 2014;59(6):2397–402.

47. Zheng H, Personeni N, Aghemo A, Lleo A. The immune milieu of cholangiocarcinoma: challenges and opportunities. Autoimmun Rev. 2019;18(11):17–26.

48. Seo N, Akiyoshi K, Shiku H. Exosome-mediated metastasis: communication from a distance. Dev Cell. 2019;49(3):347–60.

49. Wartziel I, Dörer S, Kenific CM, Lyden D. Exosome-mediated metastasis: communication from a distance. Dev Cell. 2019;49(3):347–60.

50. Seo N, Akioyoshi K, Shiku H. Exosome-mediated metastasis: communication from a distance. Dev Cell. 2019;49(3):347–60.
Bai et al. Cancer Cell Int. (2020) 20:435

55. Chuyasri C, Thuwajit P, Pauparajai A, Chau-In S, Suthiphongchai T, Thuwajit C. Alpha-smooth muscle actin-positive fibroblasts promote biliary cell proliferation and correlate with poor survival in cholangiocarcinoma. Oncol Rep. 2009;21(4):597–69.

56. Vaquerio J, Aoudjehane L, Fouassier L. Cancer-associated fibroblasts in cholangiocarcinoma. Curr Opin Gastroenterol. 2020;36(2):63–9.

57. Cadamuro M, Brivio S, Mertens J, Vismara M, Monseck A, Milani C, et al. Platelet-derived growth factor-D enables liver myofibroblasts to promote tumor lymphangiogenesis in cholangiocarcinoma. J Hepatol. 2019;70(4):700–9.

58. Pinto C, Giordano DM, Masoni L, Marzioni M. Role of inflammation and proinflammatory cytokines in cholangiocyte pathophysiology. Biochim Biophys Acta Mol Basis Dis. 2018;1864(4 Pt B):1270–8.

59. Katsami T, Guicciardi ME, Azad A, Bronk SF, Krishnan A, Gores GJ. Biliary exosomes influence cholangiocyte regulatory mechanisms and proliferation through interaction with primary cilia. Am J Physiol Gastrointest Liver Physiol. 2010;299(4):G990–9.

60. Hagia H, Yan C, Irik, Takahashi K, Wood J, Zubair A, Patel T. Tumour cell-derived extracellular vesicles interact with mesenchymal stem cells to modulate the microenvironment and enhance cholangiocarcinoma growth. J Extracell Vesicles. 2015;4:24900.

61. Blomme A, Fahmy K, Peulens O, Costanza B, Fontaine M, Strumian I, et al. Myofibron is a novel exosomal protein and functional regulator of cancer-derived exosomes. Oncotarget. 2016;7(50):85669–83.

62. Kim H, Kim DW, Cho JY. Exploring the key communicator role of activated cholangiocytes release macrophage-polarizing extracellular vesicles bearing the DAMP S100A11. Am J Physiol Cell Physiol. 2019;317(4):C788-C99.

63. Dutta S, Reamtong O, Panvongsa W, Kitdumrongthum S, Janpipatkul K, Sangvanich P, et al. Proteomics profiling of cholangiocarcinoma cells by targeting miR-551b-3p/CCND1 axis. Clin Exp Pathol. 2020;133(18):1935–53.

64. Jiang XM, Li ZL, Li X, Xu Y, Leng KM, Cui YF, et al. A novel prognostic biomarker for cholangiocarcinoma: circRNA Cdr1as. Eur Rev Med Pharmacol Sci. 2018;22(2):365–71.

65. Louis C, Despoteux M, Couluarn C. Exosomal circRNAs: new players in the field of cholangiocarcinoma. Clin Sci. 2019;133(21):2329–44.

66. Yang D, Zhang W, Zhang H, Zhang C, Chen L, Ma L, et al. Progress, opportunity, and perspective on exosome isolation - efforts for efficient exosome-based theranostics. Theranostics. 2020;10(8):3684–707.

67. Helwa I, Cai J, Dreyry MD, Zimmerman A, Dinkins MB, Khaled ML, et al. A comparative study of serum exosome isolation using differential ultracentrifugation and three commercial reagents. PLos ONE. 2017;12(1):e0170628.

68. Tang YT, Huang YY, Zheng L, Qin SH, Xu XP, An TX, et al. Comparison of isolation methods of exosomes and exosomal RNA from cell culture medium and serum. Int J Mol Med. 2017;40(3):834–44.

69. Peng Q, Zhang J, Zhou G. Comparison of plasma exosomes by differential ultracentrifugation and solvent precipitation methods. Clin Lab. 2018;64(6):991–8.

70. Helwa I, Cai J, Dreyry MD, Zimmerman A, Dinkins MB, Khaled ML, et al. Cancer-associated circulating large extracellular vesicles in the field of cholangiocarcinoma. Biomed Pharmacother. 2019;113:108774.

71. Xu Y, Humphries B, Yang C, Wang Z. miR-205 dysregulations in breast cancer: the complexity and opportunities. Noncoding RNA. 2019;5(4):53.

72. Li L, Piontek K, Ishida M, Fausther M, Dranoff JA, Fu R, et al. Extracellular vesicles carry microRNA-195 to intrahepatic cholangiocarcinoma and improve survival in a rat model. Hepatology. 2017;65(2):501–14.

73. Blackwell RH, Foreman KE, Gupta GN. The role of cancer-derived exosomes in tumorigenicity & epithelial-to-mesenchymal transition. Cancers. 2017;9(8):105.

74. Lamouille S, Xu J, Derynck R. Molecular mechanisms of epithelial–mesenchymal transition. Nat Rev Mol Cell Biol. 2014;15(3):178–96.

75. Yu J, Zhang B, Zhang H, Qi Y, Wang Y, Wang W, et al. E2F1-induced perforin. J Zhejiang Univ Sci B. 2016;17(7):537–44.

76. Xiao Y, Humphries B, Yang C, Wang Z. MiR-205 dysregulations in gastric cancer and targets phosphatase and tensin homolog (PTEN) in SGC-7901 human gastric cancer cells. Med Sci Monit. 2019;25:6367–77.

77. Blackwell RH, Foreman KE, Gupta GN. The role of cancer-derived exosomes in tumorigenicity & epithelial-to-mesenchymal transition. Cancers. 2017;9(8):105.

78. Boukouris S, Mathivanan S. Exosomes in bodily fluids are a highly stable resource of disease biomarkers. Proteomics Clin Appl. 2015;9(3–4):358–67.

79. Rahbarghazi R, Jabbari N, Sani NA, Asghari R, Salimi L, Kalashani SA, et al. Tumor-derived extracellular vesicles: reliable tools for Cancer diagnosis and clinical applications. Cell Commun Signal. 2019;17(1):73.

80. Kalluri R, Weinberg RA. The basics of epithelial-mesenchymal transition. Nat Rev Cancer. 2018;18(1):5–18.

81. Ota Y, Takahashi K, Otake S, Tamaki Y, Okada M, Aso K, et al. Extracellular vesicle-encapsulated miR-30e suppresses cholangiocarcinoma cell invasion and migration via inhibiting epithelial-mesenchymal transition. Oncotarget. 2018;9(23):16400–17.

82. Waitkus MS, Diplas BH, Yan H. Biological role and therapeutic potential of IDH mutations in cancer. Cancer Cell. 2018;34(2):186–95.

83. Jiang XM, Li ZL, Li X, Xu Y, Leng KM, Cui YF, et al. A novel prognostic biomarker for cholangiocarcinoma: circRNA Cdr1as. Eur Rev Med Pharmacol Sci. 2018;22(2):365–71.

84. Li L, Masica D, Ishida M, Tomuleasa C, Umegaki S, Kalloo AN, et al. Human bile contains microRNA-Laden extracellular vesicles that can be used for cholangiocarcinoma diagnosis. Hepatology. 2014;60(3):896–907.

85. Li L, Masica D, Ishida M, Tomuleasa C, Umegaki S, Kalloo AN, et al. Human bile contains microRNA-Laden extracellular vesicles that can be used for cholangiocarcinoma diagnosis. Hepatology. 2014;60(3):896–907.
99. Ge X, Wang Y, Nie J, Li Q, Tang L, Deng X, et al. The diagnostic/prognostic potential and molecular functions of long non-coding RNAs in the exosomes derived from the bile of human cholangiocarcinoma. Oncotarget. 2017;8(41):69995–70005.

100. Yan IK, Berdah VX, Patel T. Isolation of extracellular RNA from bile. Methods Mol Biol. 2018;1740:59–67.

101. Cortez MA, Bueso-Ramos C, Ferdin J, Lopez-Berestein G, Sood AK, Calin GA. MicroRNAs in body fluids—the mix of hormones and biomarkers. Nat Rev Clin Oncol. 2011;8(8):467–77.

102. Olazola P, Lee-Law PY, Arbelaiz A, Lapitz A, Perugorria MJ, Bujanda L, et al. MicroRNAs and extracellular vesicles in cholangiopathies. Biochim Biophys Acta. 2018;1864(4 Pt B):1293–307.

103. Han HS, Kim MJ, Han JH, Yun J, Kim HK, Yang Y, et al. Bile-derived circulating extracellular miR-30d-5p and miR-92a-3p as potential biomarkers for cholangiocarcinoma. Hepatobiliary Pancreat Dis Int. 2020;19(1):41–50.

104. Arbelaiz A, Azkargorta M, Krawczyk M, Santos-Laso A, Lapitz A, Perugorria MJ, et al. Serum extracellular vesicles contain protein biomarkers for primary sclerosing cholangitis and cholangiocarcinoma. Hepatology. 2017;66(4):1125–43.

105. Weeraphan C, Phongdara A, Chaiyawat P, Diskul-Na-Ayudthaya P, Chokchaichamnankit D, Verathamjamras C, et al. Phosphoproteome profiling of isogenic cancer cell-derived exosome reveals HSP90 as a potential marker for human cholangiocarcinoma. Proteomics. 2019;19(12):e1800159.

106. Mayr C, Beyreis M, Wagner A, Pichler M, Neureiter D, Kiesslich T. Deregulated microRNAs in biliary tract cancer: functional targets and potential biomarkers. Biomed Res Int. 2016;2016:4805270.

107. Cillo U, Fondevila C, Donadon M, Gringeri E, Mocchegiani F, Schlitt HJ, et al. Surgery for cholangiocarcinoma. Liver Int. 2019;39(Suppl 1):143–55.

108. Rahmen-Azaz AA, Weisbrod AB, Dillhoff M, Schmidt C, Pawlik TM. Intrahepatic cholangiocarcinoma: current management and emerging therapies. Expert Rev Gastroenterol Hepatol. 2017;11(S5):439–49.

109. Vader P, Mol EA, Pasterkamp G, Schifflers RM. Extracellular vesicles for drug delivery. Adv Drug Deliv Rev. 2016;106:148–56.

110. Zhou G, Sprengers D, Mancham S, Erkens R, Boor PPC, van Beek AA, et al. Reduction of immunosuppressive tumor microenvironment in cholangiocarcinoma by ex vivo targeting immune checkpoint molecules. J Hepatol. 2019;71(4):753–62.

111. Gentilini A, Pastore M, Marra F, Raggi C. The role of stoma in cholangiocarcinoma: the intriguing interplay between fibroblastic component, immune cell subsets and tumor epithelium. Int J Mol Sci. 2018;19(10):2885.

112. Golub D, Iyengar N, Dogra S, Wong T, Bready D, Tang K, et al. Mutant isocitrate dehydrogenase inhibitors as targeted cancer therapeutics. Front Oncol. 2019;9:417.

113. Massironi S, Pilla L, Elvevi A, Longarini R, Rossi RE, Bidoli P, et al. New and emerging systemic therapeutic options for advanced cholangiocarcinoma. Cells. 2020;9(3):688.

114. Okamoto K, Miyoshi K, Murawaki Y. miR-29b, miR-205 and miR-221 enhance chemosensitivity to gemcitabine in HuH28 human cholangiocarcinoma cells. PLoS ONE. 2013;8(10):e77623.

115. Barile L, Vassalli G. Exosomes. Therapy delivery tools and biomarkers of diseases. Pharmacol Ther. 2017;174:63–78.

116. Bungulawala EJ, Wang W, Yin T, Wang N, Durkan C, Wang Y, et al. Recent advancements in the use of exosomes as drug delivery systems. J Nanobiotechnol. 2018;16(1):81.

117. Mendt M, Rezvani K, Shpall E. Mesenchymal stem cell-derived exosomes for clinical use. Bone Marrow Transplant. 2019;54(S2):789–92.

118. Gao Y, Zhang H, Zhou N, Xu P, Wang J, Gao Y, et al. Methotrexate-loaded tumour-cell-derived microvesicles can relieve biliary obstruction in patients with extrahepatic cholangiocarcinoma. Nat Biomed Eng. 2020;4(7):743–53.

119. Chen G, Huang AC, Zhang W, Zhang G, Wu M, Xu W, et al. Exosomal PD-L1 contributes to immunosuppression and is associated with anti-PD-1 response. Nature. 2018;560(7718):382–6.

120. Fontugne J, Augustin J, Pujals A, Compagnon P, Rousseau B, Luciani A, et al. PD-L1 expression in perihilar and intrahepatic cholangiocarcinoma. Oncotarget. 2017;8(15):24644–51.

121. Jakubowski CD, Azad NS. Immune checkpoint inhibitor therapy in biliary tract cancer (cholangiocarcinoma). Chin Clin Oncol. 2020;9(1):2.

Publisher's Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.