Review Article

The novel epithelial-mesenchymal transition-related proteins and their therapeutic targets in cholangiocarcinoma: a narrative review

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Background and Objective: Cholangiocarcinoma (CCA), a liver cancer of bile duct epithelial cells, is a severe health issue in northeastern Thailand. The epithelial-mesenchymal transition (EMT) is a crucial process in the development of CCA. To comprehend oncogenic EMT in CCA, several newly found EMT factors are being explored in these underlying pathways. This narrative review explained the latest *in vitro* and *in vivo* findings on the molecular mechanisms of 21 new EMT-related proteins that affect CCA progression.

Methods: We evaluated the PubMed database for relevant articles that fulfilled our criteria for investigating the molecular pathways of the novel EMT markers involved in oncogenic EMT and how they contribute to CCA development, including cell proliferation, apoptosis, invasion, migration, and chemoresistance.

Key Content and Findings: We discuss the potential of these new EMT markers as diagnostic, prognostic, and therapeutic indicators for CCA and describe their underlying mechanisms in the development of the disease. The discovery of several oncogenic EMT proteins and their key signaling pathways and downstream targets will also broaden novel paths of investigation into the diagnosis and targeted treatment of CCA.

Conclusions: The EMT-related proteins that were found are good sources of knowledge and interesting information for future research. The possible ways to treat CCA that could be tested in clinical trials were also discussed.

Keywords: Cholangiocarcinoma (CCA); epithelial-mesenchymal transition (EMT); biomarkers

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Introduction

Cholangiocarcinoma (CCA), a liver cancer caused by bile duct epithelial cells, is a severe health issue in northeastern Thailand (1). The liver fluke *Opisthorchis viverrini* (*O. viverrini*) causes chronic inflammation and progressive periductal fibrosis, which increases the risk of CCA, especially in Thailand. Otherwise, periductal fibrosis appears to be the main cause of CCA (2). The main issue with this malignancy is that it has a very poor prognosis and is difficult to identify until the disease has progressed to an advanced stage (3). There is an immediate need for the discovery and validation of biomarkers that can be employed in the screening, prognosis, and diagnosis of CCA in clinical settings.

Epithelial-mesenchymal transition (EMT), a biological process that loses the epithelial phenotype and becomes mesenchymal, is a critical factor in many cancer types. Briefly, mesenchymal cells improve extracellular matrix (ECM) formation, invasiveness, and apoptosis resistance (4). EMT leads to metastatic cancer cells by acquiring mesenchymal markers and losing epithelial cell adhesion molecules (5). According to several studies, these protein expressions in human tissues or sera can be biomarkers for EMT in many diseases, including cancer (6,7).

This review summarized the 5-year updated literature on PubMed (https://www.ncbi.nlm.nih.gov/pubmed) using the search terms "cholangiocarcinoma AND epithelial-mesenchymal transition AND biomarker AND diagnosis AND prognosis". All these studies have examined the pathways and possible targeted therapies of the EMT mechanism caused by EMT-related molecules on CCA. Furthermore, the common findings as well as the controversial conclusion regarding the effects of EMT-related factors induced on CCA progression were presented and evaluated comprehensively. We present this article in accordance with the Narrative Review reporting checklist (available at https://jgo.amegroups.com/article/view/10.21037/jgo-22-1126/rc).

Methods

Following discussions among the authors, we conducted a search of the PubMed database for articles on EMT-related proteins and molecular markers of CCA published in the last five years. We then selected, categorized, and summarized the relevant articles. The specific search method is described in *Table 1*.

Effects of the novel EMT-related proteins on CCA progression

Many EMT-related molecules have been studied for their potential roles in driving or restraining cancer growth, but few studies have looked at their prognostic activities in CCA, which may help establish a medical approach to cancer diagnosis.

Alpha7 nicotinic acetylcholine receptor (α7-nAChR)

In normal physiological conditions, α7-nAChR modulates synaptic transmission in every portion of the brain and affects learning and memory (8). Pathophysiological conditions, including immunological and oncological disorders, decrease α7-nAChR regulation, making it a therapeutic target. One study recently found the mechanism of α7-nAChR in EMT, stimulating CCA generation (9). In that study, 50 human CCA tissue samples showed that most patients had high α7-nAChR expression, which was associated with poorly differentiated histological grade, severe tumor stage, lymphatic and distant metastasis, a shorter survival time, and a poor prognosis. In RBE cells, shRNA-α7-nAChR decreased cancer cell growth, migratory and invasive effects, and early apoptosis. After α7-nAChR knockdown, E-cadherin was down-regulated and vimentin, mesenchymal protein, and snail transcription factor were up-regulated. These findings show that α7-nAChR activates EMT to advance CCA. In addition, α7-nAChR inhibition was found to be an effective anti-CCA strategy *in vitro*. *In vivo* studies showed that α7-nAChR gene knockdown reduced tumor volume in BALB/c nude mice with CCA xenografts (9). This is the first study to reveal that α7-nAChR inhibits apoptosis and promotes EMT, causing CCA progression. It may be a useful molecular diagnostic and prognostic factor for CCA.

Annexin A10 (ANXA10)

The biggest class of calcium and phospholipid-binding eukaryotic proteins, the annexin family, is crucial for many physiological activities, including cell division and proliferation (10). ANXA10, a novel member of the Annexin family, is involved in cancer formation but needs more investigation regarding its expression and role in CCA. Sun and colleagues found that elevated ANXA10 expression was an independent biomarker of perihilar CCA (pCCA) and distal CCA (dCCA) using exome and transcriptome sequencing (11). This study found that ANXA10 regulated PLA2G4A, an enzyme that cleaves phospholipids to liberate

Table 1 The search strategy summary

Items	Specification
Date of search	June 1st, 2022
Databases and other sources searched	PubMed database
Search terms used	"Cholangiocarcinoma"; "Epithelial-mesenchymal transition"; "Biomarker"; "Diagnosis"; "Prognosis"
Timeframe	September 1, 2016 to January 1, 2022
Inclusion and exclusion criteria	Inclusion criteria—study type: fundamental and clinical medical research. The articles could only be found in full-text English publications. No particular exclusion criteria
Selection process	The articles were selected individually by the first author and included in the review after extensive consultations and discussions with the corresponding author

free fatty acids (FFAs), and that it was necessary for CCA proliferation, invasion, and EMT. EMT, which gives cancer cells increased aggressivity, resistance to apoptosis, and stem-like traits, is what ANXA10-mediated extrahepatic CCA (eCCA) metastasis depends on. EMT and PLA2G4A/COX-2/PGE2 may work together in eCCA, indicating that the inflammation-EMT axis is important for CCA metastasis. This discovery might identify high-risk pCCA and dCCA patients and offer personalised treatment. Finally, inhibiting the ANXA10/PLA2G4A/PGE2 pathway could alleviate pCCA and dCCA patients' symptoms.

Cell migration inducing hyaluronidase 1 (KIAA1199)

The transmembrane protein KIAA1199 is expressed in many non-cancerous and malignant cells. KIAA1199 overexpression increases cancer metastasis and proliferation. Gastric cancer, breast cancer, and hepatocellular carcinoma (HCC) studies have supported similar effects (12-14). Zhai *et al.* revealed that CCA overexpresses KIAA1199 in cancer databases (15). The testing serum and validation cohorts showed decreased overall and disease-free survival with higher KIAA1199 expression. KIAA1199 and E-cadherin had a very adverse correlation in CCA patients. KIAA1199 also correlated positively with N-cadherin, vimentin, tumor-node-metastasis (TNM) stages, histopathological grade, and lymph node metastases. KIAA1199 increases HuCCT1 and QBC-939 CCA cell proliferation, migration, and invasion. TGF-β-PI3K-AKT signaling via KIAA119 induces EMT. Overexpression and silencing of KIAA1199 influenced downstream target expression in the EMT-related TGF-β pathway. KIAA1199 enhances subcutaneous tumor xenograft CCA cell proliferation in naked mice. This study is the first to reveal that KIAA1199 is linked to EMT and maybe a new CCA biomarker.

Cluster of differentiation 90 (CD90)

Many normal cells contain CD90, a 25–37 kDa glycosylphosphatidylinositol-anchored glycoprotein that interacts with other cells. Many malignancies have a poor prognosis and are affected by CD90 expression (16). CD90 promotes HCC tumor development and metastasis (17). Yamaoka and colleagues examined CD90 expression in intrahepatic CCA (iCCA) clinically (18). CD90 expression has been associated to clinicopathological features and prognosis in human iCCA surgical tissues. In 25 iCCA patients, CD90 expression was strong positive, related with lymph node metastases, and an independent prognostic factor. *In vitro*, $CD90^+$ cells were more migratory and expressed more EMT-related genes like CXCR4 and MMP7 than $CD90^-$ cells. $CD90^+$ cells enhanced EMT-related Wnt/β-catenin signaling. Using these findings, Wnt/β-catenin pathway activation in $CD90^+$ cells induce EMT via overexpressing MMP7 and CXCR4. Hence, CD90 expression may predict iCCA prognosis.

Farnesoid X receptor (FXR)

FXR, a nuclear receptor family member and ligand-activated transcription factor encoded by the *Nr1h4* gene, can initiate EMT in CCA. It is extensively expressed in the kidney, stomach, duodenum, colon, and liver (19). Bile acids (BAs) can activate FXR gene transcription and enhance BA production, transmission, and metabolism (20). Recent research found that most iCCA tumor tissues had reduced FXR expression, which correlated with aggressive tumor characteristics and poor prognosis (21). Moreover, FXR demonstrated predictive relevance in carbohydrate antigen

19-9 (CA19-9)-negative iCCA patients and FXR deficiency increased IL-6 level in iCCA patients. After studying FXR in CCA genesis, they investigated CCA cell lines (RBE, HCCC-9810, HuCCT1, and CCLP1) with obeticholic acid (OCA), an agonist-mediated FXR activation, and FXR-shRNA *in vitro*. FXR suppressed IL-6, elevated E-cadherin, and ZO-1, decreased N-cadherin, snail, and vimentin, and inhibited β-catenin to inhibit CCA cell proliferation, migration, invasion, and EMT. *In vivo*, non-obese diabetic severe combined immunodeficiency (NOD-SCID) mice tumor xenograft model received RBE-cells and OCA and showed that RBE-cell-derived tumors had larger tumor volume, size, and lung metastasis than OCA-fed tumors. *In vivo*, OCA reduced tumor development and lung metastasis. In conclusion, FXR suppresses IL-6 and reduces iCCA tumor development and metastasis, making it a promising biomarker for iCCA prognosis, especially in CA19-9-negative patients.

Fascin (FSCN1)

Recent study has shown fascin, a cytoskeletal protein that promotes cells adhere, plays a mechanical role in CCA progression (22). In that study, fascin affected CCA tumorigenicity *in vitro* and *in vivo*. In fascin-shRNA knockdown experiments, QBC-939 cells showed that silenced fascin expression decreased cancer cell proliferation, migration, and invasion. Nude mice were given subcutaneous tumors to determine if fascin affects tumor formation *in vivo*. After 42 days after inoculation, fascin-shRNA-transfected mice had less tumors than the control group, supporting *in vitro* findings. E-cadherin was up-regulated while vimentin was down-regulated in fascin-induced EMT. In QBC-939 cells, fascin knockdown activated GSK3β, increased phosphorylated β-catenin, and decreased nuclear localization. Their findings revealed that fascin knockdown greatly suppressed Wnt/β-catenin signaling triggering EMT. Hence, fascin regulates Wnt/β-catenin pathway in CCA to increase invasion and metastasis in EMT.

Free fatty acid receptor 4 (FFAR4)

The next novel EMT-related molecule is connected to cancer lipid metabolism. FFAs provide energy and food as extracellular signaling molecules by attaching to its receptor (FFA receptors; FFARs), a transmembrane receptor that couples with G-protein-26 (GPCRs). Most articles stated that FFAR4, also known as GPR120, is a novel member of the GPCR family that has been observed as a receptor for FFA in diabetes mellitus. FFAR4 is increasingly implicated in carcinogenesis in breast and prostate cancer (23-26). Meng *et al.* examined FFAR4 expression in 98 human CCA tissues and examined its correlation with clinical characteristics in CCA patients (27). CCA overexpress FFAR4, and statistical analysis showed that severe clinicopathological data corresponded with increased FFAR4 expression. In that retrospective investigation, FFAR4 was adversely correlated with CCA E-cadherin expression. FFAR4 was favorably related with Snail-1, vimentin, CK7, and CK19 in CCA. Overexpression of FFAR4 may highly correlate with EMT-related protein expression. These findings suggest that FFAR4 may participate in EMT via PI3K/Akt signaling pathway to alter CCA invasion and metastasis. Finally, FFAR4, a novel therapeutic and diagnostic target for CCA, appears to be promising.

GATA-binding protein 6 (GATA6)

GATA6 interacts with the GATA motif in the promoter region to affect gene expression (28). It is uncertain what molecular processes GATA6 uses to promote CCA formation. According to recent research, GATA6 induces EMT via a novel mechanism and has the potential to be employed as a predictive biomarker for CCA patients (29). In 91 CCA samples, GATA6 expression was inversely correlated with E-cadherin, negatively correlated with vimentin, and positively correlated with N-cadherin. GATA6 knockdown and overexpression accelerated CCA cell EMT and metastasis *in vitro* and *in vivo*. ChIP-sequencing showed that GATA6 targets MUC1 downstream. N-cadherin and vimentin were favorably correlated with MUC1 expression, while E-cadherin was negatively correlated. In CCA cells, GATA6-induced EMT was markedly reduced by MUC1 knockdown. Furthermore, in CCA tissues, nuclear β-catenin expression exhibited a high correlation with MUC1 expression. In CCA cells, MUC1 binds to β-catenin and increases its expression in the nucleus (29). In conclusion, GATA6 plays a crucial role in inducing EMT in CCA via the MUC1/β-catenin pathway, which may have significance for anti-metastatic therapy strategies in CCA clinical trials.

H2A histone family member Z (H2A.Z)

The following notable unique EMT-factor is H2A.Z. This molecule is essential for DNA replication, chromosomal segregation, and heterochromatic state maintenance (30). A recent study found that H2A.Z is overexpressed, and that

H2A.Z expression is associated with a worse outcome and shorter overall survival time in iCCA patients (31). Cell proliferation was influenced by H2A.Z/S-phase kinase-associated protein 2/p27/p21 signaling *in vitro* and *in vivo*. Moreover, H2A.Z inhibition decreased cell growth and promoted apoptosis in CCLP-1 and HCCC-9810 cell lines. Inhibiting H2A.Z decreased tumor metastasis by inhibiting EMT process and improved the anticancer effects of cisplatin, a chemotherapy drug, in the treatment of iCCA (31). As a whole, H2A.Z increased cell proliferation and EMT in iCCA, suggesting it could be a useful biomarker and therapeutic target for this cancer.

High mobility group A1 (HMGA1)

Small nuclear protein known as HMGA1 serves as a structural transcription factor (32). It is uncommon to observe HMGA1 in mature differentiated tissues, but oncogenic transcription factors, epigenetic modifications, and chromosomal translocation can up-regulate it (33). HMGA1 was expressed in iCCA and has been shown to increase tumorigenicity (34,35). However, the clinical importance of HMGA1 in pCCA remains unknown. Li and colleagues used transcriptome sequencing to identify putative pCCA biomarkers and assessed the predictive importance of HMGA1 in an extensive pCCA cohort (36). Bioinformatics and *in vitro/vivo* study revealed that HMGA1 promoted thyroid hormone receptor interactor 13 (TRIP13) transcription, which increased tumor cell stemness, EMT, proliferation, migration, and invasion. TRIP13 was a poor biomarker for pCCA, however double high expression of HMGA1/TRIP13 may better predict prognosis. By decreasing FBXW7 transcription and stabilizing c-Myc, TRIP13 helped pCCA cell lines (QBC-939 and FRH-0201) progress. The HMGA-TRIP13 axis boosted pCCA stemness and EMT in a positive feedback process, as evidenced by the fact that c-Myc improved the transcription and expression of both HMGA1 and TRIP13. Interestingly, the positive feedback from the c-Myc/Wnt-β-catenin pathway enabled the HMGA-TRIP13 axis to increase pCCA stemness and EMT (36). This revealed that disrupting the HMGA1-TRIP13-c-Myc nexus could be a very promising strategy for treating pCCA, and that detecting HMGA1 and TRIP13 after surgery could assist stratify high-risk patients, directing individual treatments and facilitating the development of customized therapeutics.

Kidney-type glutaminase (GLS1)

Glutaminase initiates glutamine to glutamate conversion. GLS1 and GLS2, kidney and liver glutaminases, were initially identified. GLS1 promotes tumor metabolism (37). Some studies found that GLS1 abnormally expressed and enhanced tumor progression in HCC (38). Nevertheless, the functions of GLS1 in iCCA are mainly unclear. Recently, Cao and coworkers aimed to assess expression and clinical importance of GLS1 in iCCA (39). In many digestive system malignancies, including iCCA, GLS1 expression was higher than in peritumoral tissue. GLS1 overexpression in RBE cells promoted metastasis and invasion. E-cadherin and vimentin were also regulated by GLS1 in iCCA cells. In QBC-939 cells, GLS1 knockdown had the opposite effect. GLS1 expression in iCCA samples was negatively connected with E-cadherin and positively correlated with vimentin in clinical studies. Tumor differentiation and lymphatic metastasis were substantially associated with GLS1 protein expression (P=0.001 and 0.029, respectively). Patients with high GLS1 expression had shorter overall survival and recurrence rates than those with low expression. Independent predictive markers included GLS1 expression (39). Overall, the results of this investigation showed that GLS1 is an independent predictive biomarker of iCCA. GLS1 promotes iCCA development via EMT and might thus be a therapeutic target in iCCA.

Mitochondrial pyruvate carrier 1 (MPC1)

Apparently, cancer cells have a distinct metabolism to help them survive. This metabolic shift increases aggressive cancer cell aggressiveness (40). Consequently, therapeutic targets for cancer metabolism molecules may exist. A recently identified transporter in the mitochondrial inner membrane is called the mitochondrial pyruvate carrier (MPC) (41). MPC consists of two subunits, but MPC1 is linked to poor prognoses in a number of malignancies (42,43). MPC1's effect on iCCA's malignancy was investigated in a recent study (44). iCCA tumor invasion and distant metastases were also associated with decreased MPC1 expression. These events are clearly connected to EMT. Hence, they studied the effect of altering *MPC1* gene expression on cancer cell aggressiveness *in vitro* using CCA cell lines (TFK-1 and CCLP-1). MPC1 expression was downregulated in EMT cells treated with TGF-β. Inhibiting MPC1 expression promoted EMT-related cancer cell, but overexpressing it decreased tumor cell migration (44). In conclusion, MPC1 regulates the generation of EMT through the TGF-β signaling pathway and assists to the cancerous potential of iCCA. MPC1 is downregulated in a wide range of solid tumors, these data suggest that MPC1

may be a potential therapeutic target in iCCA.

Mortalin

Mortalin is a heat shock protein (HSP) 70 family member, a highly conserved molecular chaperone protein essential in many pathological and physiological circumstances (45). As reported by Kang *et al.* (46), 125 iCCA patients had high mortalin expression in cancer tissues, which was associated with shorter overall survival time, increased event to recurrence, metastasis to lymphatic organs, and aggressive tumor differentiation. Knocking down of mortalin mRNA expression on QBC-939 and RBE cell lines suppressed iCCA cell growth, increased apoptosis, reduced cancer cell invasion, and delayed wound closure. Mortalin knockdown cells express more E-cadherin and less snail and vimentin. High mortalin expression linked with snail and vimentin and negatively with E-cadherin. These findings suggest that mortalin may activate EMT and progress iCCA.

Nardilysin (NRDC)

NRDC, a metalloendopeptidase of the M16 family (N-arginine dibasic convertase, NRD-convertase), has been shown to trigger EMT and play important roles in many cancers and inflammation (47). NRDC is highly expressed in many malignancies and promotes tumor growth and poor prognosis proving its importance in cancer biology. Yoh *et al*. first investigated whether NRDC can cause EMT and CCA progression (48). Ninety-eight iCCA patients had elevated serum NRDC levels, which correlated with shorter overall and disease-free survival and tumor severity. The diagnostic capability of NRDC showed that serum NRDC levels [area under the curve (AUC) =0.689] were comparable to carcinoembryonic antigen (CEA) and CA19-9 (AUC =0.569 and 0.671, respectively). NRDC and CA19-9 had the highest AUC value in the three-marker combination diagnostic analysis (0.756). In surgical iCCA specimens, serum NRDC levels associated positively with EMT-inducer (SNAI1 and ZEB1) mRNA levels. NRDC knockdown in HuCCT-1 and SSP-25 cell lines inhibited iCCA cell growth and migration *in vitro*. NRDC knockdown-iCCA cells down-regulated EMT-related genes (vimentin, ZEB1, SNAI1, and TWIST) for EMT-induced NRDC mechanisms. Furthermore down-regulated were cancer stem cell marker SOX2 and EMT pathway trigger hypoxia-inducible factor-1 (HIF-1). *In vivo*, male nude mice were subcutaneously injected with HuCCT-1 negative control and NRDC-knockdown cells for tumor-xenograft. NRDC-knockdown mice had significantly less tumor growth than controls. These findings suggest that NRDC knockdown can inhibit CCA progression, including cancer proliferation and migration.

Phospholipase C beta 1 (PLCB1)

PLCB1, a phospholipase, hydrolyzes phospholipids and is elevated in colorectal cancer, breast cancer, and small cell lung carcinoma (49,50). The biological relevance of PLCB1 in CCA is unknown. PLCB1 promotes human CCA, and Liang *et al.* studied its role in CCA progression (51). PLCB1 was abundant in human CCA tissues and cell lines. E-cadherin levels were higher in CCA samples with low PLCB1 expression than high expression. PLCB1 and EMT characteristics were related with inverted N-cadherin and vimentin expression patterns. PLCB1 increased tumor growth in CCA animal models, including transposons-based carcinogenesis models. PLCB1 also induced CCA cell EMT via PI3K/AKT signaling. PABPC1, a functional polyadenylate-binding protein conserved gene member, increased PLCB1-mediated EMT via PI3K/AKT/GSK3b/Snail signaling. The AKT inhibitor MK2206 can reverse gemcitabine plus cisplatin resistance caused by ectopic PLCB1. This study also showed for the first time that miR-26b-5p may suppress CCA by targeting PLCB1. These data suggest that a PLCB1-PI3K-AKT signaling axis is crucial for CCA growth and EMT, suggesting that AKT may be a therapeutic target for overcoming chemotherapy resistance in CCA patients with elevated PLCB1. Importance PLCB1, an oncogenic driver of EMT-related CCA, makes AKT inhibition therapeutic.

Protein tyrosine phosphatase type IVA 1 (PTP4A1)

EMT in CCA can also be driven on by PTP4A1, a member of a small class of protein tyrosine phosphatases (PTPs) that removes phosphate groups from phosphorylated tyrosine residues on proteins. PTP4A1's biological significance in CCA was first explored by Liu and colleagues (52). Three hundred and twenty-two paraffin-embedded tumor samples from iCCA patients showed that PTP4A1 was often overexpressed and correlated with aggressive and severe cancer characteristics like lymph node metastasis, advanced TNM stages, poorer survival, and higher recurrence rates. HCCC-9810, HuCCT-1, and RBE cells were knocked down and overexpressed to show that PTP4A1 encourages the growth, colonization, and invasion of cancer cells. In PTP4A1-silenced iCCA cells, E-cadherin was increased whereas N-cadherin, Zeb1, and Snail were downregulated. E-cadherin downregulation and

Zeb1, Snail, and N-cadherin upregulation occurred later than PTP4A1 overexpression in iCCA cells. PTP4A1 may trigger EMT in iCCA, according to their findings. PI3K/AKT signaling pathway enhances metastatic and aggressive tumor microenvironment through EMT process (53). They then used PTP4A1-overexpressed HCCC-9810 cells, PTP4A1-knockdown RBE cells, and their controls to create subcutaneous xenograft tumor animal models. After two weeks of injection, all animals except those injected with PTP4A1-knockdown tumor cells had solid tumors, and the overexpressed mice had faster tumor growth than the silenced mice. *In vivo* data showed that PTP4A1 promotes iCCA development.

Tripartite motif-containing protein 44 (TRIM44)

An essential member of the TRIM family, TRIM44, possessed a zinc-finger domain that was associated with ubiquitin-specific proteases (USPs). TRIM44 is a cancer-promoting gene that has been linked to multiple different types of the disease, including head and neck squamous cell carcinomas and esophagogastric cancer. TRIM44 is responsible for activating the PI3K/AKT/mTOR pathway, which in turn promotes the EMT of cancer cells as well as the initiation and growth of tumors (54,55). TRIM44 expression and function in human iCCA were examined by Peng *et al.* (56). TRIM44 mRNA and protein expressions in iCCA and corresponding peritumoral tissues were evaluated using the public Oncomine database. Second, TRIM44 interference and cDNA transfection were utilized to study its roles and mechanisms in iCCA cells (QBC-939 and RBE). Finally, TRIM44's prognostic effect on CCA progression was examined. TRIM44 expression was higher in iCCA tissues, supporting the public cancer database findings. TRIM44 knockdown reduced iCCA cell invasion and migration and increased apoptosis. High TRIM44 levels also trigger EMT in iCCA cells. AZD6244 prevented cell EMT and death caused by TRIM44 overexpression, which increased MAPK signaling. Clinically, TRIM44 expression was significantly associated with substantial tumor development, lymphatic metastasis, and poor tumor differentiation. In comparison to the TRIM44low group, the TRIM44high group exhibited a poorer overall survival rate and a higher cumulative incidence of recurrence. These findings indicate that TRIM44 is a potential predictive biomarker and therapeutic target for iCCA patients because it promotes iCCA formation by causing cancer cell EMT and apoptosis resistance.

Ubiquitin-conjugating enzyme E2T (UBE2T)

A ubiquitin-conjugating enzyme called UBE2T is overexpressed in bladder and lung malignancies and promotes prostate and breast cancer (57,58). Its role in CCA advancement is neglected. UBE2T expression in CCA was examined recently (59). The results demonstrated that UBE2T is critical to CCA genesis. UBE2T was highly expressed in both *in vitro* and *in vivo* human CCA models. The research showed that UBE2T overexpression increased mesenchymal markers vimentin and N-cadherin, and decreased epithelial markers β-catenin and E-cadherin (59). Overexpression of UBE2T promoted EMT, invasion, migration, and proliferation of CCA cells, while suppressing it had the reverse effect. The mTOR inhibitor rapamycin inhibits UBE2T activity, indicating that it operates through the selected target of mTOR pathway. According to this research, UBE2T might act as a carcinogenic driver of CCA formation via boosting EMT and mTOR pathway. These discoveries have helped uncover underlying processes of EMT and CCA progression and identify novel therapeutic targets for CCA treatment.

V-set domain containing T-cell activation inhibitor 1 (VTCN1/B7x/B7S1/B7 homolog 4) (B7-H4)

B7-H4, a novel member of the B7 family (also known as VTCN1). B7-H4 boosts the synthesis of cytokines and T cell proliferation, allowing tumors to evade immune detection (60). B7-H4 is generally absent in normal human tissues, apart from lung, kidney, and pancreatic epithelial cells (61). B7-H4 is highly expressed in lung, breast, and pancreatic ductal adenocarcinoma, according to recent studies (61-63). The role and mechanism of B7-H4 in iCCA, however, remain unknown. The study of the B7-H4 expression and its therapeutic importance in iCCA progression were recently explored by Xie and colleagues (64). The findings showed that both at the mRNA and protein levels, B7-H4 expression in iCCA was significantly greater than in peritumoral tissues. The elevated B7-H4 in iCCA cells induced EMT and increased tumor cell invasion and metastasis via ERK1/2 signaling. Tumor samples with high B7-H4 expression had correlated with poorer differentiation, advanced tumor stage, and lymph node metastases. B7-H4-expressing iCCA patients had lower overall and disease-free survival. B7-H4 expression also independently predicted survival and tumor recurrence in iCCA patients after surgery (64). In conclusion, enhanced iCCA tumor aggressiveness via EMT is associated with increased B7-H4 expression,

suggesting that this protein may be an interesting target for therapy for iCCA patients.

WD repeat domain 5 (WDR5)

The histone methyltransferase complex SET1/MLL, which largely catalyzes histone 3 lysine 4 methylation (H3K4me), contains WDR5 as a vital component (65). It promotes tumors and poor prognoses in colorectal, breast, bladder, and prostate cancers (66). Moreover, the recruitment of c-Myc at particular chromosomal locations is promoted by WDR5, a critical regulatory element (67), although the way WDR5 and c-Myc interact in CCA was not previously known. The clinical significance of WDR5 and c-Myc expression in all CCA subtypes was recently studied by Chen *et al.* (68). Co-expression of WDR5 and c-Myc was a more accurate prognostic indicator, and WDR5 was highly related with poor CCA prognosis. WDR5's impact on EMT-specific proteins was also examined. WDR5 overexpression had the opposite impact on the expression of EMT biomarkers as compared to WDR5 knockdown, which boosted E-cadherin while decreasing N-cadherin and vimentin. WDR5 increased Myc-induced HIF1A transcription by interacting with the box IIIb (MBIIIb) motif on c-Myc, which promoted CCA EMT, invasion, and metastasis. WDR5 decreased chromatin opening and PHD2 expression while increasing the accumulation of the HIF-1α and perhaps stabilizing and accumulating HIF-1α (68). These findings imply that stratification of high-risk CCA patients and treatment planning can be aided by WDR5, c-Myc, and HIF-1α. CCA patients may benefit from inhibiting the WDR5-Myc interface and HIF-1α accumulation.

Targeting protein for Xenopus kinesin-like protein 2 (TPX2)

Although TPX2 promotes oncogenesis in numerous malignancies, its role in EMT-induced CCA has been challenging to understand. Recently, Zou *et al.*'s article found increased TPX2 expression in CCA tissues (69). TPX2 upregulation linked with severe TNM stage, lymph node metastases, shorter survival, and poorer prognosis. TPX2 suppression by siRNA lentivirus-infection in CCA cell lines has a significant effect on tumor cell biology (HCCC-9810 and RBE). After TPX2-silencing transfection, CCA cells apoptosed, proliferated, and invaded less. TPX2 knockdown enhanced E-cadherin and decreased N-cadherin, β-catenin, MMP-2, and MMP-9 in TPX2 induced-EMT. Zou and colleagues revealed that TPX2 inhibition down-regulated Slug and Twist1, EMT transcription factors. TPX2 modulates EMT via an unknown mechanism. The sole investigation on TPX2 function in CCA revealed that it may be a predictive indicator and therapeutic target.

Finally, *Tables 2-4* summarize the available experimental evidence on the most recent EMT-related proteins that possess the molecular pathways to initiate the EMT process during CCA development.

Discussion

Organ development and cancer are only two examples of the many processes in which EMT plays a role. By understanding about the key regulatory mechanisms and EMT-mediators involved in this process, novel therapeutics can be established. There is no doubt that EMT plays a vital role in CCA development given the massive amount of data presented in this analysis. EMT must be studied extensively because of its potential to induce advanced tumor metastasis and chemoresistance characteristics. New therapy approaches that can halt this cellular transition during CCA progression must be developed, and it is imperative that researchers focus on identifying and targeting the key EMT pathways involved in this cancer.

This narrative review covers seven proteins involved in EMT, most of which can activate this process through the PI3K/AKT/mTOR signaling cascade. These proteins include α7-nAChR, FFAR4, mortalin, PLCB1, PTP4A1, TRIM44, and UBE2T. The PI3K/AKT/mTOR pathway appears to be the most important mechanism for regulating EMT-induced CCA formation. In addition, the Serine/Threonine kinase AKT plays a major role in regulating a wide variety of cellular functions, including proliferation, survival, glucose metabolism, and neovascularization (70,71). Growth factor receptors activate PI3K, a signal transducer enzyme, which in turn phosphorylates AKT (72). Several studies demonstrate that EMT, angiogenesis, and metastasis can be induced by AKT, which is overexpressed in many human malignancies and promotes cancer cell proliferation, metabolism, and survival (73). Furthermore, CCA cells are resistant to radiation therapy and chemotherapy when the PI3K/AKT pathway is activated, but can be made more sensitive to these treatments when the pathway is inhibited (74). In recent studies, a new dual PI3K/mTOR inhibitor known as NVPBEZ235 was shown to drastically limit CCA cell proliferation and migration by inhibiting AKT. Moreover, it significantly triggered G1 arrest

Table 2 The expressions of EMT-related proteins and its correlation in CCA

EMT-related proteins	Study model in CCA			Expression	Correlation with clinical data	Ref
	Sample types	Sample size	Methods			
Alpha7 nicotinic acetylcholine receptor (α7-nAChR)	Tissue	50	IHC	High	Shorter survival time in patients	(9)
Annexin A10 (ANXA10)	Tissue	91 iCCA, 128 pCCA, 84 dCCA	IHC	High in pCCA and dCCA	Poorer differentiation of cancer and confirmed as independent prognostic factor in eCCA patients	(11)
	Cell lines	QBC-939	qRT-PCR, WB	High	ND	
Cell migration inducing hyaluronidase 1 (KIAA1199)	Serum	177	ELISA	High	Shorter overall survival and disease-free survival times, LN metastasis, and TNM stage	(15)
	Tissue	177	IHC	High	Negative relationship with E-cadherin and positively connected with N-cadherin, vimentin, histological grade, LN metastases, TNM stage, and CA19-9 level	
	Cell lines	HuCCT1, RBE, HCCC-9810	qRT-PCR, WB	High	ND	
Cluster of differentiation 90 (CD90)	Tissue	77 iCCA	IHC	25 positive cases	Lymph node metastasis, an independent prognostic factor	(18)
	Cell lines	RBE, SSP-25	qRT-PCR, WB	High	ND	
Farnesoid X receptor (FXR)	Tissue	332	IHC	Low	Shorter TTR, decreased overall survival, lymph node metastases, vascular invasion, tumor number, tumor differentiation, TNM stage	(21)
Fascin (FSCN1)	Tissue	142	IHC	High	Vascular invasion, lymph node or distant metastases, and prognosis of the tumor	(22)
Free fatty acid receptor 4 (FFAR4)	Tissue	98	IHC	High	Positively correlated with that of CK7, CK19, Snail-1 and vimentin but negatively correlated with E-cadherin	(27)
					Histological grade, perineural invasion, LNM, advanced TNM stage, shorter survival time, and preoperative serum CA19-9	
GATA-binding protein 6 (GATA6)	Tissue	51	IHC	High	Lymph node metastasis, decreased overall survival, and early recurrence, with a positive connection with N-cadherin and vimentin expression but a negative correlation with E-cadherin expression	(29)
	Cell lines	QBC-939	qRT-PCR, WB	High	ND	
H2A histone family member Z (H2A.Z)	Tissue	28 iCCA	IHC, WB	High	TNM stage and decreased overall survival	(31)
	Cell lines	CCLP-1, HuCCT-1, RBE, HCCC-9810	qRT-PCR, WB	High	ND	

Table 2 (*continued*)

Table 2 (continued)

EMT-related proteins	Study model in CCA			Expression	Correlation with clinical data	Ref
	Sample types	Sample size	Methods			
High mobility group A1 (HMGA1)	Tissue	106 pCCA	IHC	High	Unfavorable prognosis because to lymphatic infiltration and advanced TNM stage	(36)
	Cell lines	QBC-939, FRH-0201	qRT-PCR, WB	High	ND	
Kidney-type glutaminase (GLS1)	Tissue	138 iCCA	IHC	High	Lymphatic metastasis and tumor differentiation, shorter time to recurrence and worse overall survival, negatively linked with the expression of E-cadherin and favorably connected with the expression of vimentin	(39)
	Cell lines	QBC-939	qRT-PCR, WB	High	ND	
Mitochondrial pyruvate carrier 1 (MPC1)	Tissue	64 iCCA	IHC	Low	Poor prognosis, CA19-9 levels, vascular invasion, and distant metastasis	(44)
	Cell lines	CCLP-1	qRT-PCR, WB	Low	ND	
Mortalin (MOT)	Tissue	125	IHC	High	Lymphatic metastasis, tumor metastasis stage, tumor differentiation, and recurrence	(46)
	Tissue	10	WB	High	ND	
Nardilysin (NRDC)	Tissue	43	IHC	High	Shorter overall and disease-free survival, and the presence of numerous tumors	(48)
	Serum	98	ELISA	High	Positively correlated with SNAI1, ZEB1 and HIF-1α mRNA levels	
					Preoperative serum NRDC levels (AUC =0.689) had prognostic value comparable to serum CEA (0.569) and CA19-9 (0.671) values	
Phospholipase C beta 1 (PLCB1)	Tissue	60	IHC	High	Severe cancer stage, lymph node status, metastasis, shorter overall, and disease-free survival time. Correlated with high level of tumor proliferation markers (Ki-67) and EMT markers	(51)
	Cell lines	CCLP1, RBE, KMBC, QBC-939, HCCC-9810, HuCCT1	qRT-PCR, WB	High	ND	
Protein tyrosine phosphatase type IVA 1 (PTP4A1)	Tissue	60	qRT-PCR	High	ND	(52)
	Tissue	322	IHC	High	Larger tumor size, lymph node metastases, and advanced tumor stage are examples of aggressive tumor features	
					A lower chance of survival and a higher chance of postoperative recurrence	

Table 2 (*continued*)

Table 2 (*continued*)

EMT-related proteins	Study model in CCA			Expression	Correlation with clinical data	Ref
	Sample types	Sample size	Methods			
Tripartite motif-containing protein 44 (TRIM44)	Tissue	22	qRT-PCR, WB	High	Large tumor size, lymphatic metastasis, poor tumor differentiation, shorter overall survival, and higher cumulative rate of recurrence	(56)
		71 iCCA	IHC	High		
	Cell lines	QBC-939	qRT-PCR, WB	High	ND	
Ubiquitin-conjugating enzyme E2T (UBE2T)	Tissue	10	IHC, qRT-PCR	High	Positively correlated with TNM stage	(59)
	Cell lines	HuCCT1, QBC-939, RBE	qRT-PCR, WB	High	ND	
V-set domain containing T-cell activation inhibitor 1 (VTCN1/B7x/B7S1/B7 homolog 4) (B7-H4)	Tissue	35 iCCA	qRT-PCR, WB,	High	ND	(64)
		144 iCCA	IHC	High	Early recurrence, metastasis to lymph nodes, a high TNM stage, poor tumor differentiation, a shorter overall survival time, and a greater cumulative recurrence rate. Vimentin and Snail are upregulated, while E-cadherin is downregulated	
	Cell lines	QBC-939, RBE	qRT-PCR, WB	High	ND	
WD repeat domain 5 (WDR5)	Tissue	78 iCCA, 141 pCCA, 88 dCCA	IHC, tissue microarray	High	The co-expression of c-Myc and WDR5, in particular, was an independent predictor of poorer prognosis for CCA	(68)
	Cell lines	QBC-939	qRT-PCR, WB	High	ND	
Targeting protein for Xenopus kinesin-like protein 2 (TPX2)	Tissue	70	IHC	High	TNM stage and lymph node metastasis	(69)

EMT, epithelial-mesenchymal transition; CCA, cholangiocarcinoma; iCCA, intrahepatic CCA; pCCA, perihilar CCA; dCCA, distal CCA; qRT-PCR, quantitative reverse transcription polymerase chain reaction; WB, western blot; IHC, immunohistochemistry; ELISA, enzyme-linked immunosorbent assay; ND, no data; TTR, time to recurrence; TNM, tumor-node-metastasis; LNM, lymph node metastasis; CA19-9, carbohydrate antigen 19-9; AUC, area under the curve; CEA, carcinoembryonic antigen.

Table 3 Summary of *in vitro* studies on the effects of EMT-related proteins in CCA

EMT-related proteins	Study model	Interventions	Major findings in CCA (*In vitro*)					Interpretation	Ref
			Proliferation	Migration and invasion	EMT	Apoptosis	Others		
Alpha7 nicotinic acetylcholine receptor (α7-nAChR)	QBC-939, RBE	Knockdown	Decreased	Decreased	Decreased vimentin, p-Akt, and snail and increased E-cadherin	Increased early apoptosis by increased caspase-3 and decreased Bcl-2	ND	α7-nAChR induces CCA progression by blocking apoptosis and promoting EMT	(9)
Annexin A10 (ANXA10)	QBC-939	Knockdown	Decreased	Decreased	Increased expression of E-cadherin and decreased expression of Snail and Vimentin	No remarkable difference on cell cycle, apoptosis, and necrosis between the scrambled and siANXA10 groups	The phospholipase metabolic pathway was significantly downregulated. PLA2G4A and ANXA10 also exhibited a strong correlation and decreased the STAT3 phosphorylation	ANXA10 played an essential role in the EMT, invasion and metastasis of pCCA	(11)
	QBC-939	Overexpression	ND	ND	Increased Snail and Vimentin and decreased E-cadherin levels	ND	Increased the phosphorylation of STAT3	PLA2G4A was the key effector in ANXA10-mediated invasion and metastasis according to EMT	
	QBC-939	Overexpression + PLA2G4A inhibitor (AACOCT3)	ND	Decreased	Abolished these EMT changes	ND	ND		
	QBC-939	Overexpression + COX-2 inhibitor (celecoxib)	ND	Decreased	Inhibited the EMT process	ND	Celecoxib inhibited STAT3 phosphorylation	STAT3 phosphorylation was required for ANXA10/PLA2G4A-induced EMT and metastasis	
Cell migration inducing hyaluronidase 1 (KIAA1199)	HuCCT1	Knockdown	Decreased	Decreased	E-cadherin was increased. N-cadherin and vimentin were decreased	ND	TGF-β was dramatically downregulated, and the TGF-β-regulating proteins PI3k, AKT and mTOR were significantly downregulated	KIAA1199 promotes CCA cell proliferation, cell migration and invasion. KIAA119 upregulates the TGF-β-PI3K-AKT mediated EMT signaling pathway	(15)
	QBC-939	Overexpression	Increased	Increased	E-cadherin was decreased, while N-cadherin and vimentin were increased	ND	TGF-β, PI3k, AKT and mTOR expression were high		
	QBC-939	Overexpression + TGF-β inhibitor (SB431542) and PI3K inhibitor (LY294002)	ND	Decreased	ND	ND	ND		
Cluster of differentiation 90 (CD90)	RBE, SSP-25	CD90+ cell sorting	ND	Increased	CXCR4 and MMP7 expressions are higher	ND	Positively for active nuclear β-catenin	CD90+ cells were involved in the EMT via CXCR4 and MMP7 by activating Wnt/β-catenin pathway	(18)
		CD90 knockdown by siRNA in CD90+ cells	ND	Decreased	Decreased CXCR4 and MMP7 expression	ND	ND		
		CD90+ cell treated with Wnt/β-catenin inhibitor (ICG-001)	ND	ND	Decreased CXCR4 and MMP7 expression	ND	ND		
Farnesoid X receptor (FXR)	RBE, CCLP1	Knockdown	Increased	Increased	Increased IL-6, E-cadherin and ZO-1, while decreased N-cadherin, Snail and Vimentin	ND	ND	FXR inhibited the proliferation, migration, invasion and EMT of iCCA cells via suppression of IL-6 act as metastasis suppressor	(21)
	HuCCT-1,	Obeticholic acid, an agonist of FXR	Decreased	Decreased	Decreased IL-6, E-cadherin, ZO-1, while increased N-cadherin, Snail and Vimentin	ND	ND		
Fascin (FSCN1)	QBC-939	Knockdown	Decreased	Decreased	Decreased vimentin, while increased E-cadherin, GSK-3β and phosphorylated β-catenin	ND	ND	Fascin promotes cell proliferation, migration, and invasion, EMT of CCA cells, through regulating Wnt/β-catenin signaling	(22)
GATA-binding protein 6 (GATA6)	QBC-939	Knockdown	ND	Decreased	E-cadherin was upregulated, while N-cadherin, vimentin, and β-catenin were downregulated	ND	MUC1 mRNA were significantly downregulated	Through the upregulation of MUC1 in CCA cells, GATA6 induces EMT	(29)
	RBE	Overexpression	ND	Increased	N-cadherin, vimentin, and β-catenin were elevated whereas E-cadherin was decreased	ND	MUC1 mRNA were significantly upregulated		

Table 3 (*continued*)

Table 3 (*continued*)

EMT-related proteins	Study model	Interventions	Major findings in CCA (*In vitro*)					Interpretation	Ref
			Proliferation	Migration and invasion	EMT	Apoptosis	Others		
H2A histone family member Z (H2A.Z)	CCLP-1 and HCCC-9810	Knockdown	Decreased	Decreased	N-cadherin, Slug, and Snail expression was inhibited whereas E-cadherin expression was increased	Induced cell cycle arrest	ND	By inhibiting EMT, H2A.Z down-regulation decreased tumor metastasis and improved the anticancer effects of cisplatin in the treatment of iCCA	(31)
	CCLP-1	Knockdown and treatment with cisplatin	Decreased	ND	ND	Increased apoptosis and induced the expression of apoptotic markers	ND		
High mobility group A1 (HMGA1)	QBC-939	HMGA1- knockdown	Decreased	Decreased	E-cadherin expression was decreased, and N-cadherin, Vimentin, Snail, Twist-1, and Claudin-1 were additional EMT indicators that were increased	ND	ND	By promoting TRIP13 expression, decreasing FBXW7 expression, and stabilizing c-Myc, HMGA1 improved pCCA proliferation, migration, invasion, stemness, and EMT	(36)
	FRH-0201	HMGA1-overexpression	Increased	Increased		ND	ND		
	QBC-939 and FRH-0201	HMGA1-overexpression and TRIP13-knockdown	Decreased	Decreased	Impaired HMGA1-induced cell stemness and the EMT	ND	ND		
	QBC-939 and FRH-0201	TRIP13-knockdown	Decreased	Decreased	Attenuated stemness and EMT	ND	Increased the transcription and expression of FBXW7. Reduced c-Myc expression		
	QBC-939 and FRH-0201	FBXW7-knockdown	Increased	Increased	Reversed stemness and EMT	ND	c-Myc expression was elevated		
Kidney-type glutaminase (GLS1)	QBC-939	Knockdown	ND	Decreased	Reduced vimentin expression, whereas increased E-cadherin expression	ND	ND	GLS1 positively regulates the migratory and invasive abilities and EMT of iCCA cells	(39)
	RBE	Overexpression	ND	Increased	Increased vimentin expression, whereas reduced E-cadherin expression	ND	ND		
Mitochondrial pyruvate carrier 1 (MPC1)	TFK-1	Human recombinant TGF-β1	ND	ND	Decreased E-cadherin	ND	A morphological change from a valvate-like shape to a spindle-like shape	MPC1 functions as a key modulator of EMT induction in the same way as TGF-β	(44)
		Knockdown	ND	ND	Decreased E-cadherin	ND			
	CCLP-1	Overexpression	Not affected	Decreased	Low levels of E-cadherin	ND	ND		
Mortalin (MOT)	QBC-939, RBE	Knockdown	Decreased	Decreased	Decreased vimentin and Snail, while increased E-cadherin	Increased apoptosis rate	ND	Mortalin may promote cell proliferation and invasion via induction of EMT of iCCA cells	(46)
Nardilysin (NRDC)	HuCCT-1, SSP-25	Knockdown	Decreased	Decreased	Decreased vimentin, ZEB1, SOX2, HIF-1α, SNAI1 and TWIST1, increased E-cadherin	ND	Increased sensitivity to gemcitabine	Knockdown of NRDC can inhibit on the proliferation, migration, EMT, and promote chemosensitivity of iCCA cells	(48)
Phospholipase C beta 1 (PLCB1)	HuCCT1, KMBC	Knockdown	Decreased by inhibited the G1–S transition	Decreased	Increased E-cadherin and snail and decreased N-cadherin and vimentin	ND	ND	PLCB1 activated AKT signaling to induce CCA cells proliferation, migration, and invasion to undergo EMT	(51)
	RBE, CCLP1	Overexpression	Increased by accelerated the G1–S transition	Increased	Reduced E-cadherin and snail and increased N-cadherin and vimentin	ND	ND		
		Overexpression + MK2206, inhibitor of AKT	ND	ND	E-cadherin downregulation was reversed, but N-cadherin and vimentin were upregulated	ND	PLCB1-induced chemotherapeutic resistance to gemcitabine/cisplatin can be reversed by MK2206		

Table 3 (*continued*)

Table 3 (*continued*)

EMT-related proteins	Study model	Interventions	Major findings in CCA (*In vitro*)					Interpretation	Ref
			Proliferation	Migration and invasion	EMT	Apoptosis	Others		
Protein tyrosine phosphatase type IVA 1 (PTP4A1)	RBE, HuCCT-1	Knockdown	Decreased	Decreased	Decreased AKT (Thr308, Ser473) and GSK3β (Ser9). Increased E-cadherin and decreased N-cadherin, Zeb1 and Snail	ND	Decreased CyclinD1	PTP4A1 induced iCCA cells invasion was through activating PI3K/AKT signaling controlled EMT process by up-regulating Zeb1 and Snail	(52)
	HCCC-9810	Overexpression	Increased	Increased	Vice versa	ND	Increased CyclinD1		
Tripartite motif-containing protein 44 (TRIM44)	QBC-939	Knockdown	Decreased	Decreased	Increased E-cadherin, while decreased vimentin, β-catenin and snail	Increased the rate of apoptosis. Upregulation of Bax and several caspase family proteins and downregulation of Bcl-2	p-AKT was repressed	TRIM44 serves as a promoter of iCCA cells aggressiveness and induces EMT and apoptosis inhibition via MAPK pathway	(56)
	RBE	Overexpression	Increased	Increased	Decreased E-cadherin level. Increased vimentin, β-catenin and snail	Decreased apoptosis cells. Downregulation of Bax and several caspase proteins and upregulation of Bcl-2	p-AKT expression was up-regulated. Up-regulate phosphorylation of MEK and phosphorylation of ERK1/2		
		Overexpression + AZD6244 (MEK inhibitor)	ND	Decreased	Upregulation of E-cadherin, β-catenin, and Bax, but also downregulated vimentin, snail and Bcl-2	ND	ND		
Ubiquitin-conjugating enzyme E2T (UBE2T)	HuCCT1, QBC-939	Knockdown	Decreased	Decreased	Higher levels of E-cadherin and α-catenin and lower levels of N-cadherin and vimentin	Enhance cell cycle arrest at G2/M phase	Lower expression levels of total mTOR and phosphorylated mTOR (p-mTOR)	UBE2T regulates proliferation, EMT process, migration and invasion of human CCA cells via mTOR pathway	(59)
		Overexpression	Increased	Increased	Vice versa	Reduced the percentage of cells in G2/M phase	Increased total mTOR and p-mTOR levels		
		Overexpression + rapamycin (RAPA; mTOR inhibitor)	Decreased	Decreased	Elevated E-cadherin and α-catenin and decreased N-cadherin and vimentin, 4E-BP, phosphorylated 4E-BP (p-4E-BP), S6K1 and phosphorylated S6K1 (p-S6K1)	ND	ND		
V-set domain containing T-cell activation inhibitor 1 (VTCN1/B7x/B7S1/B7 homolog 4) (B7-H4)	QBC-939, RBE	Knockdown	Decreased	Decreased	Downregulated of Snail, vimentin, and N-cadherin, and upregulated of E-cadherin	Increased apoptosis rate. Increased Bax mRNA and a decreased expression of Bcl-2 mRNA	Decreased expression of ERK1/2 phosphorylation	B7-H4 promote tumor progression of iCCA cells through induction of EMT, inhibition of apoptosis, and activation of ERK1/2 signal	(64)
	HCCC-9810	Overexpression	Increased	Increased	Vice versa	Vice versa	Vice versa		
WD repeat domain 5 (WDR5)	QBC-939	Knockdown	No obvious effect	Decreased	Increased E-cadherin and decreased N-cadherin and vimentin	ND	WDR5 interacted with the Myc box IIIb (MBIIIb) motif of c-Myc and facilitated Myc-induced HIF1A transcription. WDR5 enhanced HIF-1α accumulation	WDR5 facilitated EMT and metastasis of CCA by increasing HIF-1α accumulation in a Myc-dependent pathway to promote HIF-1α transcription and a Myc-independent pathway	(68)
	RBE	Overexpression		Increased	Vice versa	ND			
Targeting protein for Xenopus kinesin-like protein 2 (TPX2)	HCCC-9810, RBE	Knockdown	Decreased	Decreased	Decreased N-cadherin, β-cadherin, MMP-2, MMP-9, Slug, and Twist1. Increased E-cadherin	Increased apoptosis by upregulation p53, Bax and downregulation Bcl-2	G2-M arrest by increased cyclin A1 and cyclin B1, decreased cyclin D1 and CDK2	TPX2 in human CCA cells promoted cell proliferation, cell cycle, invasion, migration, EMT and decreased apoptosis	(69)

EMT, epithelial-mesenchymal transition; CCA, cholangiocarcinoma; ICG, inhibitor of β-catenin/TCF mediated transcription; TGF-β1, transforming growth factor-beta; AKT, serine/threonine protein kinase; MEK, mitogen-activated protein kinase; RAPA, rapamycin; mTOR, mammalian target of rapamycin; ND, no data; p-Akt, phosphorylated serine/threonine protein kinase; IL, interleukin; MMP, matrix metalloproteinase; PI3k, phosphatidylinositol 3-kinase; iCCA, intrahepatic CCA; pCCA, perihilar CCA.

Table 4 Summary of *in vivo* studies on the effects of EMT-related proteins in CCA

EMT-related molecules	Study model	Age	Interventions	Duration	Major findings	Interpretation	Ref
Alpha7 nicotinic acetylcholine receptor (α7-nAChR)	Female BALB/c nude mice	5 weeks	shRNA-α7-nAChR cells	30 days	Decreased tumor growth	α7-nAChR promotes growth of subcutaneous CCA xenografts in nude mice	(9)
Annexin A10 (ANXA10)	Female BALB/c nude mice	4–5 weeks	Lentivirus carrying ANXA10 shRNA stable cells	4 weeks	Tumor volumes and weights were both significantly reduced, and the number of metastatic lesions has been reduced	ANXA10 promotes the progression of CCA *in vivo*	(11)
			QBC939 cells with stable ANXA10 overexpression and treatment with or without AACOCF3	Every 2 days for 3 weeks	Mice with AACOCF3 treatment weighed more than mice without AACOCF3 treatment. The use of AACOCF3 significantly reduced the number of metastatic lesions in the liver and lungs	PLA2G4A is required for ANXA10-mediated EMT and metastasis	(11)
			Mice were injected with ANXA10-overexpressing QBC939 cells and administered Celecoxib or not	Every 2 days for 3 weeks	Celecoxib lowered the weight loss caused by ANXA10 overexpression and the number of metastatic lesions in the lungs and livers	Celecoxib, a COX-2 inhibitor, inhibited eCCA invasion and metastasis caused by ANXA10	(11)
Cell migration inducing hyaluronidase 1 (KIAA1199)	Nude mice	ND	Silencing KIAA1199 with shRNA-1 in Hucct-1	15 days	Reduced size and weight of tumors	KIAA1199 promotes CCA growth *in vivo*	(15)
			Overexpressing KIAA1199 in QBC-939 with lentivirus carrying LV-KIAA1199		Increased size and weight of tumors		
Farnesoid X receptor (FXR)	NOD-SCID mice	ND	RBE-RFP cells and received OCA, an agonist of FXR	5 weeks	The tumor size, weight, lung metastasis, and metastatic nodules created by RBE-RFP cells were considerably greater than tumors formed in animals given OCA	In tumor xenograft models, OCA, an FXR agonist, inhibited tumor development and lung metastasis	(21)
Fascin (FSCN1)	BALBc nu/nu nude mice	8 weeks	Fascin-shRNA cells	42 days	Decreased tumor formation	Fascin induces tumor formation	(22)
GATA-binding protein 6 (GATA6)	Male nude mice	4 weeks	GATA6 overexpression	8 weeks	Metastasis of the liver has increased. E-cadherin mRNA and protein levels were lower, but N-cadherin, vimentin, β-catenin, and MUC1 mRNA and protein levels were higher	In CCA cell implanted nude mice, GATA6 upregulates MUC1 and promotes metastasis	(29)
H2A histone family member Z (H2A.Z)	BALB/c male nude mice	8 weeks	H2A.Z-silenced CCLP-1 cells	3 weeks	Tumor growth rate, average volume, and weight were all reduced. Ki67 staining was reduced, however p21 staining was enhanced. Reduced the number of lung metastases. Tumors have a higher percentage of TUNEL-positive cells	*In vivo*, H2A.Z knockdown suppresses tumor development and metastasis	(31)
High mobility group A1 (HMGA1)	Female BALB/c nude mice	5 weeks	Stable QBC-939 cells with HMGA1 knockdown	5 weeks	Decreased the tumor volume and weight	HMGA1 and TRIP13 as prognostic indicators of pCCA *in vivo*	(36)
			Stable QBC-939 cells with HMGA1 overexpression		Increased the tumor volume and weight		
			HMGA1-overexpressing stable cells with TRIP13 knockdown		Reduced tumor volume and weight, reduced the number of metastatic lesions		
Nardilysin (NRDC)	Male nude mice	7–9 weeks	NRDC-knocked down HuCCT-1 cells	ND	Decreased tumor growth	NRDC promotes tumor growth *in vivo*	(48)
Phospholipase C beta 1 (PLCB1)	Male BALB/c athymic nude mice	4–6 weeks	RBE-PLCB1 overexpressing cell	8 weeks	The number of tumors and metastatic nodules in the liver increased, as did the formation of metastatic nodules in the lungs	PLCB1 promotes CCA metastasis and EMT by activating the Snail and AKT pathways *in vivo*	(51)
			HuCCT1-PLCB1-KD3 silencing cell		Reduced the number of tumors and metastatic nodules in the liver, as well as the formation of metastatic nodules in the lungs		
			RBE-PLCB1-overexpressing cell-sh Snail		The liver metastatic nodules were fewer and smaller		
			HuCCT1-PLCB1-KD3 silencing cell-Snail overexpression		Increased in liver metastatic nodules		
			RBE-PLCB1-overexpressing cell + MK2206 (inhibitor of AKT)		Reduced the number of lung metastatic nodules		
	Female C57BL/6 mice and female FVB/N (H2d) mice	7 weeks	Overexpression via pX330 vectors with sgRNAs targeting PTEN/P53 and Cas9, as well as plasmids containing PLCB1	8 weeks	Increased tumor volume and burden, raised key indicators of the G1-S transition in subcutaneous tumor tissues, and significantly increased AKT phosphorylation		
Protein tyrosine phosphatase type IVA 1 (PTP4A1)	NOD/SCID nude mice	4 weeks	HCCC-9810-PTP4A1 cells, shPTP4A1-1 cells	2 weeks	Formed palpable tumors, increased tumor growth rates and volume	PTP4A1 could significantly promote iCCA growth and progression *in vivo*	(52)
			shPTP4A1-1 cells		Decreased tumor growth rates and volume		
V-set domain containing T-cell activation inhibitor 1 (VTCN1/B7x/B7S1/B7 homolog 4) (B7-H4)	Male nude mice	4 weeks	QBC-939-B7-H4 shRNA cells	33 days	Reversed effects from HCCC-9810-B7-H4 cells in a subcutaneous xenograft model	B7-H4 could significantly promote tumor growth and tumor progression of iCCA cells *in vivo*	
			HCCC-9810-B7-H4 cells		Tumors grew faster, larger, and increased the lung metastasis rate		
WD repeat domain 5 (WDR5)	BALB/c nude mice	ND	Stable WDR5-silenced QBC939 cells	ND	Weight gain, reduced liver weight, and fewer metastatic nodules in the livers and lungs	WDR5 facilitated CCA cell metastasis *in vivo*	(68)

EMT, epithelial-mesenchymal transition; CCA, cholangiocarcinoma; BALB, Bagg and Albino; NOD-SCID, non-obese diabetic severe combined immunodeficiency; ND, no data; OCA, obeticholic acid; RBE-RFP, RBE cell line with red fluorescent protein; AKT, serine/threonine protein kinase; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling; eCCA, extrahepatic CCA; pCCA, perihilar CCA; NRDC, nardilysin; iCCA, intrahepatic CCA.

without inducing apoptosis while simultaneously enhancing autophagy response (75).

Both *in vitro* and *in vivo* investigations have indicated that targeting the cooperation that exists amongst EMT signaling pathways is beneficial (76). For instance, the essential cytokine TGF-β1 is involved in a variety of cellular processes, and it is this cytokine that stimulates the mTOR signaling pathway (77). mTORC2 is a critical downstream effector that is part of the TGF-β signaling cascade. It directly phosphorylates Akt, which in turn promotes EMT (78). In addition, KIAA1199, GLS1, and MPC1 are shown to drive EMT in CCA through the TGF- β1 signaling pathway in this narrative review. Treatment strategies that are based on anti-TGF-β have been examined using preclinical models of CCA. Neutralizing monoclonal antibodies against TGF-β were recently tested *in vivo* on mice with an induced liver fibrosis model, and the results showed that these animals had lower levels of fibrosis and CCA formation (79). Additionally, M7824 has a dual anti-tumor action in that it boosts immune defenses against the tumor by inhibiting the immunological checkpoint protein programmed cell death ligand-1 (PD-L1). This is accomplished by trapping the TGF-β ligand binding in the tumor microenvironment and preventing it from occurring (80,81). As a result, patients with second-line biliary tract cancer, including CCA, who have locally progressed or metastatic disease, are participating in a recently begun multicenter phase II clinical research (NCT03833661) that is assessing the efficacy of M7824 monotherapy (82).

WNT signaling collaborates with members of the fibroblast growth factor (FGF) and TGF-β families to regulate EMT during gastrulation and neural crest cell delamination (83,84), whereas Notch and TGF-β signaling regulate endocardial cushion formation (85). In cancer cell, there is a synergy between TGF-β signaling and RTK signaling, which is triggered by the epidermal growth factor (EGF)-related TGF-α or FGF. Indeed, TGF-β enhances the epithelial to mesenchymal gene expression shift in cancer cell types by promoting EGF- or FGF-induced EMT (86-88). Similarly, TGF-β induced EMT is promoted by the activation of ERK/MAPK pathway when responding to mutant RAS or growth factors (89). Moreover, CD90, fascin, and HMGA1 in this study can trigger the canonical WNT signaling that binding of WNT ligands to Frizzled receptors inhibits GSK3β, resulting in inhibiting β-catenin phosphorylation, ubiquitylation, and degradation while allowing β-catenin to influence gene expression (90). GSK3β kinase inhibition promotes EMT through increasing SNAIL stability (91). Thus, WNT signaling is important in developmental EMT in cancer. In conclusion, it appears that targeting these signaling pathways might be a promising therapeutic strategy for preventing EMT, metastasis, and invasion in tumor cells.

For CCA, EMT has shown great promise as a therapeutic target. Further work is required to develop combination treatments targeting EMT in CCA due to redundancy and bypasses among the multiple signaling pathways and cell types involved. Even though many studies have failed to find direct evidence that these serum levels predict the prognosis of CCA patients, we believe that the levels of these potential EMT-related proteins may emerge as a novel biomarker in predicting diagnosis and prognosis, as well as a potential therapeutic target for those CCA patients.

Conclusions

This narrative review compiles *in vitro* and *in vivo* findings that support the idea that novel EMT-related proteins can trigger CCA progression. In CCA, the expression of epithelial markers can be induced by some of these molecules, while the expression of mesenchymal markers can be reversed by others. This narrative review summarizes how the molecules shown to mediate EMT in CCA formation can generate molecular pathways that ultimately lead to cell proliferation, migration, and invasion. Both pathways ultimately lead to a poorer prognosis in the treatment of CCA, independently and seriously. Hence, future treatment methods to handle anti-tumor-related difficulties in CCA patients may prioritize addressing these EMT-related molecules' pathways. EMT-related proteins may serve as important molecular markers in the diagnosis, prognosis, and therapy of CCA; however, more study is needed to establish this. Better patient data as well as important and relevant clinical experience are two of these aspects.

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Footnote

Reporting Checklist: The authors have completed the Narrative Review reporting checklist. Available at https://

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Conflicts of Interest: Both authors have completed the ICMJE uniform disclosure form (available at https://jgo.amegroups.com/article/view/10.21037/jgo-22-1126/coif). The authors have no conflicts of interest to declare.

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References

1. Sripa B, Kaewkes S, Sithithaworn P, et al. Liver fluke induces cholangiocarcinoma. PLoS Med 2007;4:e201.
2. Wynn TA. Cellular and molecular mechanisms of fibrosis. J Pathol 2008;214:199-210.
3. Bergquist A, von Seth E. Epidemiology of cholangiocarcinoma. Best Pract Res Clin Gastroenterol 2015;29:221-32.
4. Kalluri R, Weinberg RA. The basics of epithelial-mesenchymal transition. J Clin Invest 2009;119:1420-8.
5. Thiery JP, Acloque H, Huang RY, et al. Epithelial-mesenchymal transitions in development and disease. Cell 2009;139:871-90.
6. Nieto MA, Huang RY, Jackson RA, et al. EMT: 2016. Cell 2016;166:21-45.
7. Brabletz T, Kalluri R, Nieto MA, et al. EMT in cancer. Nat Rev Cancer 2018;18:128-34.
8. Shin SS, Dixon CE. Targeting α7 nicotinic acetylcholine receptors: a future potential for neuroprotection from traumatic brain injury. Neural Regen Res 2015;10:1552-4.
9. Chen S, Kang X, Liu G, et al. α7-Nicotinic Acetylcholine Receptor Promotes Cholangiocarcinoma Progression and Epithelial-Mesenchymal Transition Process. Dig Dis Sci 2019;64:2843-53.
10. Mussunoor S, Murray GI. The role of annexins in tumour development and progression. J Pathol 2008;216:131-40.
11. Sun R, Liu Z, Qiu B, et al. Annexin10 promotes extrahepatic cholangiocarcinoma metastasis by facilitating EMT via PLA2G4A/PGE2/STAT3 pathway. EBioMedicine 2019;47:142-55.
12. Matsuzaki S, Tanaka F, Mimori K, et al. Clinicopathologic significance of KIAA1199 overexpression in human gastric cancer. Ann Surg Oncol 2009;16:2042-51.
13. Evensen NA, Kuscu C, Nguyen HL, et al. Unraveling the role of KIAA1199, a novel endoplasmic reticulum protein, in cancer cell migration. J Natl Cancer Inst 2013;105:1402-16.
14. Jiang Z, Zhai X, Shi B, et al. KIAA1199 overexpression is associated with abnormal expression of EMT markers and is a novel independent prognostic biomarker for hepatocellular carcinoma. Onco Targets Ther 2018;11:8341-8.
15. Zhai X, Wang W, Ma Y, et al. Serum KIAA1199 is an advanced-stage prognostic biomarker and metastatic oncogene in cholangiocarcinoma. Aging (Albany NY) 2020;12:23761-77.
16. Rege TA, Hagood JS. Thy-1 as a regulator of cell-cell and cell-matrix interactions in axon regeneration, apoptosis, adhesion, migration, cancer, and fibrosis. FASEB J 2006;20:1045-54.
17. Cheng BQ, Jiang Y, Li DL, et al. Up-regulation of thy-1 promotes invasion and metastasis of hepatocarcinomas. Asian Pac J Cancer Prev 2012;13:1349-53.
18. Yamaoka R, Ishii T, Kawai T, et al. CD90 expression in human intrahepatic cholangiocarcinoma is associated with lymph node metastasis and poor prognosis. J Surg Oncol 2018;118:664-74.
19. Sun L, Cai J, Gonzalez FJ. The role of farnesoid X receptor in metabolic diseases, and gastrointestinal and liver cancer. Nat Rev Gastroenterol Hepatol 2021;18:335-47.
20. Claudel T, Staels B, Kuipers F. The Farnesoid X receptor: a molecular link between bile acid and lipid and glucose metabolism. Arterioscler Thromb Vasc Biol 2005;25:2020-30.
21. Lv B, Ma L, Tang W, et al. FXR Acts as a Metastasis Suppressor in Intrahepatic Cholangiocarcinoma by Inhibiting IL-6-Induced Epithelial-Mesenchymal Transition. Cell Physiol Biochem 2018;48:158-72.
22. Mao X, Duan X, Jiang B. Fascin Induces Epithelial-Mesenchymal Transition of Cholangiocarcinoma Cells by Regulating Wnt/β-Catenin Signaling. Med Sci Monit 2016;22:3479-85.

23. Chu X, Zhou Q, Xu Y, et al. Aberrant fatty acid profile and FFAR4 signaling confer endocrine resistance in breast cancer. J Exp Clin Cancer Res 2019;38:100.
24. Wang J, Hong Y, Shao S, et al. FFAR1-and FFAR4-dependent activation of Hippo pathway mediates DHA-induced apoptosis of androgen-independent prostate cancer cells. Biochem Biophys Res Commun 2018;506:590-6.
25. Serna-Marquez N, Diaz-Aragon R, Reyes-Uribe E, et al. Linoleic acid induces migration and invasion through FFAR4- and PI3K-/Akt-dependent pathway in MDA-MB-231 breast cancer cells. Med Oncol 2017;34:111.
26. Houthuijzen JM. For Better or Worse: FFAR1 and FFAR4 Signaling in Cancer and Diabetes. Mol Pharmacol 2016;90:738-43.
27. Meng FT, Huang M, Shao F, et al. Upregulated FFAR4 correlates with the epithelial-mesenchymal transition and an unfavorable prognosis in human cholangiocarcinoma. Cancer Biomark 2018;23:353-61.
28. Ayanbule F, Belaguli NS, Berger DH. GATA factors in gastrointestinal malignancy. World J Surg 2011;35:1757-65.
29. Deng X, Jiang P, Chen J, et al. GATA6 promotes epithelial-mesenchymal transition and metastasis through MUC1/β-catenin pathway in cholangiocarcinoma. Cell Death Dis 2020;11:860.
30. Rangasamy D, Greaves I, Tremethick DJ. RNA interference demonstrates a novel role for H2A. Z in chromosome segregation. Nat Struct Mol Biol 2004;11:650-5.
31. Yang B, Tong R, Liu H, et al. H2A.Z regulates tumorigenesis, metastasis and sensitivity to cisplatin in intrahepatic cholangiocarcinoma. Int J Oncol 2018;52:1235-45.
32. Fusco A, Fedele M. Roles of HMGA proteins in cancer. Nat Rev Cancer 2007;7:899-910.
33. Xian L, Georgess D, Huso T, et al. HMGA1 amplifies Wnt signalling and expands the intestinal stem cell compartment and Paneth cell niche. Nat Commun 2017;8:15008.
34. Quintavalle C, Burmeister K, Piscuoglio S, et al. High mobility group A1 enhances tumorigenicity of human cholangiocarcinoma and confers resistance to therapy. Mol Carcinog 2017;56:2146-57.
35. Abe N, Watanabe T, Izumisato Y, et al. High mobility group A1 is expressed in metastatic adenocarcinoma to the liver and intrahepatic cholangiocarcinoma, but not in hepatocellular carcinoma: its potential use in the diagnosis of liver neoplasms. J Gastroenterol 2003;38:1144-9.
36. Li Z, Liu J, Chen T, et al. HMGA1-TRIP13 axis promotes stemness and epithelial mesenchymal transition of perihilar cholangiocarcinoma in a positive feedback loop dependent on c-Myc. J Exp Clin Cancer Res 2021;40:86.
37. Lee SY, Jeon HM, Ju MK, et al. Dlx-2 and glutaminase upregulate epithelial-mesenchymal transition and glycolytic switch. Oncotarget 2016;7:7925-39.
38. Yu D, Shi X, Meng G, et al. Kidney-type glutaminase (GLS1) is a biomarker for pathologic diagnosis and prognosis of hepatocellular carcinoma. Oncotarget 2015;6:7619-31.
39. Cao J, Zhang C, Jiang GQ, et al. Expression of GLS1 in intrahepatic cholangiocarcinoma and its clinical significance. Mol Med Rep 2019;20:1915-24.
40. Pavlova NN, Thompson CB. The Emerging Hallmarks of Cancer Metabolism. Cell Metab 2016;23:27-47.
41. Herzig S, Raemy E, Montessuit S, et al. Identification and functional expression of the mitochondrial pyruvate carrier. Science 2012;337:93-6.
42. Schell JC, Olson KA, Jiang L, et al. A role for the mitochondrial pyruvate carrier as a repressor of the Warburg effect and colon cancer cell growth. Mol Cell 2014;56:400-13.
43. Wang L, Xu M, Qin J, et al. MPC1, a key gene in cancer metabolism, is regulated by COUPTFII in human prostate cancer. Oncotarget 2016;7:14673-83.
44. Ohashi T, Eguchi H, Kawamoto K, et al. Mitochondrial pyruvate carrier modulates the epithelial-mesenchymal transition in cholangiocarcinoma. Oncol Rep 2018;39:1276-82.
45. Na Y, Kaul SC, Ryu J, et al. Stress chaperone mortalin contributes to epithelial-mesenchymal transition and cancer metastasis. Cancer Res 2016;76:2754-65.
46. Kang Q, Cai JB, Dong RZ, et al. Mortalin promotes cell proliferation and epithelial mesenchymal transition of intrahepatic cholangiocarcinoma cells in vitro. J Clin Pathol 2017;70:677-83.
47. Hospital V, Prat A. Nardilysin, a basic residues specific metallopeptidase that mediates cell migration and proliferation. Protein Pept Lett 2004;11:501-8.
48. Yoh T, Hatano E, Kasai Y, et al. Serum Nardilysin, a Surrogate Marker for Epithelial-Mesenchymal Transition, Predicts Prognosis of Intrahepatic Cholangiocarcinoma after Surgical Resection. Clin Cancer Res 2019;25:619-28.
49. Strassheim D, Shafer SH, Phelps SH, et al. Small cell lung carcinoma exhibits greater phospholipase C-beta1 expression and edelfosine resistance compared with non-

small cell lung carcinoma. Cancer Res 2000;60:2730-6.
50. Sengelaub CA, Navrazhina K, Ross JB, et al. PTPRN2 and PLCβ1 promote metastatic breast cancer cell migration through PI(4,5)P2-dependent actin remodeling. EMBO J 2016;35:62-76.
51. Liang S, Guo H, Ma K, et al. A PLCB1-PI3K-AKT Signaling Axis Activates EMT to Promote Cholangiocarcinoma Progression. Cancer Res 2021;81:5889-903.
52. Liu LZ, He YZ, Dong PP, et al. Protein tyrosine phosphatase PTP4A1 promotes proliferation and epithelial-mesenchymal transition in intrahepatic cholangiocarcinoma via the PI3K/AKT pathway. Oncotarget 2016;7:75210-20.
53. Xu W, Yang Z, Lu N. A new role for the PI3K/Akt signaling pathway in the epithelial-mesenchymal transition. Cell Adh Migr 2015;9:317-24.
54. Järvinen AK, Autio R, Kilpinen S, et al. High-resolution copy number and gene expression microarray analyses of head and neck squamous cell carcinoma cell lines of tongue and larynx. Genes Chromosomes Cancer 2008;47:500-9.
55. Peters CJ, Rees JR, Hardwick RH, et al. A 4-gene signature predicts survival of patients with resected adenocarcinoma of the esophagus, junction, and gastric cardia. Gastroenterology 2010;139:1995-2004.e15.
56. Peng R, Zhang PF, Zhang C, et al. Elevated TRIM44 promotes intrahepatic cholangiocarcinoma progression by inducing cell EMT via MAPK signaling. Cancer Med 2018;7:796-808.
57. Wen M, Kwon Y, Wang Y, et al. Elevated expression of UBE2T exhibits oncogenic properties in human prostate cancer. Oncotarget 2015;6:25226-39.
58. Ueki T, Park JH, Nishidate T, et al. Ubiquitination and downregulation of BRCA1 by ubiquitin-conjugating enzyme E2T overexpression in human breast cancer cells. Cancer Res 2009;69:8752-60.
59. Liu F, Zhu C, Gao P, et al. Ubiquitin-conjugating enzyme E2T regulates cell proliferation and migration in cholangiocarcinoma. Anticancer Drugs 2020;31:836-46.
60. Chinnadurai R, Grakoui A. B7-H4 mediates inhibition of T cell responses by activated murine hepatic stellate cells. Hepatology. 2010;52:2177-85.
61. Sun Y, Wang Y, Zhao J, et al. B7-H3 and B7-H4 expression in non-small-cell lung cancer. Lung Cancer 2006;53:143-51.
62. Tringler B, Zhuo S, Pilkington G, et al. B7-h4 is highly expressed in ductal and lobular breast cancer. Clin Cancer Res 2005;11:1842-8.
63. Awadallah NS, Shroyer KR, Langer DA, et al. Detection of B7-H4 and p53 in pancreatic cancer: potential role as a cytological diagnostic adjunct. Pancreas 2008;36:200-6.
64. Xie N, Cai JB, Zhang L, et al. Upregulation of B7-H4 promotes tumor progression of intrahepatic cholangiocarcinoma. Cell Death Dis 2017;8:3205.
65. Guarnaccia AD, Tansey WP. Moonlighting with WDR5: A Cellular Multitasker. J Clin Med 2018;7:21.
66. Tan X, Chen S, Wu J, et al. PI3K/AKT-mediated upregulation of WDR5 promotes colorectal cancer metastasis by directly targeting ZNF407. Cell Death Dis 2017;8:e2686.
67. Thomas LR, Wang Q, Grieb BC, et al. Interaction with WDR5 promotes target gene recognition and tumorigenesis by MYC. Mol Cell 2015;58:440-52.
68. Chen T, Li K, Liu Z, et al. WDR5 facilitates EMT and metastasis of CCA by increasing HIF-1 accumulation in Myc-dependent and independent pathways. Mol Ther 2021;29:2134-50.
69. Zou Z, Zheng B, Li J, et al. TPX2 level correlates with cholangiocarcinoma cell proliferation, apoptosis, and EMT. Biomed Pharmacother 2018;107:1286-93.
70. Altomare DA, Testa JR. Perturbations of the AKT signaling pathway in human cancer. Oncogene 2005;24:7455-64.
71. Manning BD, Cantley LC. AKT/PKB signaling: navigating downstream. Cell 2007;129:1261-74.
72. Manning BD, Toker A. AKT/PKB Signaling: Navigating the Network. Cell 2017;169:381-405.
73. Thiery JP. Epithelial-mesenchymal transitions in tumour progression. Nat Rev Cancer 2002;2:442-54.
74. Karimi Roshan M, Soltani A, Soleimani A, et al. Role of AKT and mTOR signaling pathways in the induction of epithelial-mesenchymal transition (EMT) process. Biochimie 2019;165:229-34.
75. Yothaisong S, Dokduang H, Techasen A, et al. Increased activation of PI3K/AKT signaling pathway is associated with cholangiocarcinoma metastasis and PI3K/mTOR inhibition presents a possible therapeutic strategy. Tumour Biol 2013;34:3637-48.
76. Lamouille S, Xu J, Derynck R. Molecular mechanisms of epithelial-mesenchymal transition. Nat Rev Mol Cell Biol 2014;15:178-96.
77. Seoane J, Gomis RR. TGF-β Family Signaling in Tumor Suppression and Cancer Progression. Cold Spring Harb Perspect Biol 2017;9:a022277.
78. Martelossi Cebinelli GC, Paiva Trugilo K, Badaró Garcia S, et al. TGF-β1 functional polymorphisms: a review. Eur

Cytokine Netw 2016;27:81-9.
79. Ling H, Roux E, Hempel D, et al. Transforming growth factor β neutralization ameliorates pre-existing hepatic fibrosis and reduces cholangiocarcinoma in thioacetamide-treated rats. PLoS One 2013;8:e54499.
80. Batlle E, Massagué J. Transforming Growth Factor-β Signaling in Immunity and Cancer. Immunity 2019;50:924-40.
81. Lan Y, Zhang D, Xu C, et al. Enhanced preclinical antitumor activity of M7824, a bifunctional fusion protein simultaneously targeting PD-L1 and TGF-β. Sci Transl Med 2018;10:eaan5488.
82. Papoutsoglou P, Louis C, Coulouarn C. Transforming Growth Factor-Beta (TGFβ) Signaling Pathway in Cholangiocarcinoma. Cells 2019;8:960.
83. Heisenberg CP, Solnica-Krezel L. Back and forth between cell fate specification and movement during vertebrate gastrulation. Curr Opin Genet Dev 2008;18:311-6.
84. Sauka-Spengler T, Bronner-Fraser M. A gene regulatory network orchestrates neural crest formation. Nat Rev Mol Cell Biol 2008;9:557-68.
85. Timmerman LA, Grego-Bessa J, Raya A, et al. Notch promotes epithelial-mesenchymal transition during cardiac development and oncogenic transformation. Genes Dev 2004;18:99-115.
86. Shirakihara T, Horiguchi K, Miyazawa K, et al. TGF-β regulates isoform switching of FGF receptors and epithelial-mesenchymal transition. EMBO J 2011;30:783-95.
87. Uttamsingh S, Bao X, Nguyen KT, et al. Synergistic effect between EGF and TGF-beta1 in inducing oncogenic properties of intestinal epithelial cells. Oncogene. 2008;27:2626-34.
88. Wendt MK, Smith JA, Schiemann WP. Transforming growth factor-β-induced epithelial-mesenchymal transition facilitates epidermal growth factor-dependent breast cancer progression. Oncogene 2010;29:6485-98.
89. Zavadil J, Böttinger EP. TGF-beta and epithelial-to-mesenchymal transitions. Oncogene 2005;24:5764-74.
90. Niehrs C. The complex world of WNT receptor signalling. Nat Rev Mol Cell Biol 2012;13:767-79.
91. Zhou BP, Deng J, Xia W, et al. Dual regulation of Snail by GSK-3beta-mediated phosphorylation in control of epithelial-mesenchymal transition. Nat Cell Biol 2004;6:931-40.

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