REVIEW

Molecular Targets and Signaling Pathways in Cholangiocarcinoma: A Systematic Review

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Abstract

   Background: Cholangiocarcinoma (CCA) is the second most frequent hepatobiliary cancer after hepatocellular carcinoma with a poor prognosis and limited treatment options. This study aimed to review existing knowledge on the genetic basis of CCA, molecular targets/signaling pathways involved in the pathogenesis, disease progression and prognosis, including potential targets for targeted therapies of CCA. **Methods:** The systematic review was performed in compliance with PRISMA guidelines. A systematic search in PubMed and Science Direct databases was performed using the following keywords: "cholangiocarcinoma", AND "molecular target" AND/OR "signaling pathway", AND/OR "targeted therapy", AND/OR "cancer chemotherapy." The eligibility criteria included: i) full-text articles published in English, ii) articles with in vitro and/or in vivo and/or clinical studies of molecular targets/signaling pathwanys related to CCA pathogenesis/disease progression/prognosis and/or targeted therapy. Seventy-three studies that fulfilled the eligibility criteria were finally included in the final data synthesis. **Results:** A total of 833 relevant articles published up to April 2022 were identified and 73 sttudies that fulfilled the eligibility criteria were finally included in the analysis. The molecular biomarkers and drugs targeting signalling pathways were reported. Recent research has been focused on targeting the apoptotic and cell proliferation pathways, and in addition, the angiogenesis and metastasis pathway. More effort focused on testing the efficacy of combination therapies against the cancer cell and specifically CCA. The *PI3K* (Phosphoinositide 3-kinases)/ERK/Akt (AKT serine/threonine kinase 1)/mTOR (mammalian target of rapamycin) signaling pathway and HER2 (Human epidermal growth factor receptor 2) and *EGFR* (Epidermal Growth Factor Receptor) pathways are the most potential targets for CCA therapy. **Conclusion:** The information obtained could be exploited for further development of diagnostic tools for early diagnosis of CCA, as well as effective CCA-targeted therapies.

   Keywords: PI3K/ERK/Akt/mTOR- HER2- EGFR

Introduction

   Cholangiocarcinoma (CCA) is a rare and highly aggressive cancer that accounts for 20% of all hepatobiliary cancers (Ji et al., 2020). It originates from the epithelium lining the biliary tree and is divided into three subtypes, i.e., intrahepatic (IHCCA), extrahepatic (EHCCA), and hilar (HCCA) tumors. Cirrhosis of the liver, biliary stones, age over 65 years, and chronic infection have all been reported as possible risk factors for the development of CCA (Huang et al., 2016; Ji et al., 2020). The prevalence and mortality rates of CCA are increasing worldwide. IHCCA is the second most common form of liver cancer, accounting for 10-15% of all liver cancers (Chen et al.,

2015). Several recent epidemiological studies reported that its mortality and prevalence are rising, mainly in the western world (Churi et al., 2014) and northeastern Thailand (Clawson et al., 2010; Dokduang et al., 2013; Dokduang et al., 2016). CCA is characterized by a low early diagnosis rate, marked aggressiveness, and a high rate of metastasis and recurrence (Huang et al., 2015; Chen et al., 2019). The majority of CCA cases have a short median survival time of less than 24 months, which could partially result from the resistance of CCA to currently available treatment (Chan-On et al., 2015; Guest et al., 2016; Hu et al., 2019).

   Managing CCA remains challenging due to the limited number of chemotherapy or targeted therapy options

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(Dokduang et al., 2016; Ji et al., 2020). Identification of tumor-associated signaling molecules/pathways will help developing diagnostic tools or targeted CCA therapies or effective chemotherapeutics. A range of signaling molecules and pathways has been identified as putative mechanistic determinants of CCA pathology, disease progression and prognosis (Hong et al., 2013; Chen et al., 2019; Hu et al., 2019). Clinical trials of targeted therapies, such as erlotinib, lapatinib, cetuximab, sorafenib, and bevacizumab, have been conducted to evaluate their clinical effectiveness for the treatment of CCA (Barad et al., 2012; Hu et al., 2019). Molecular-targeted therapy is superior to traditional chemotherapy due to its ability to suppress cancer cells selectively (Hong et al., 2013). Gene overexpression, amplification and mutation have all been associated with the tumorigenesis and progression of CCA (Gui et al., 2012; Hu et al., 2019). A thorough understanding of the molecular basis of CCA pathogenesis, disease progression and prognosis is required for the development of sensitive and specific diagnostic tools and effective chemotherapeutics for CCA.

In this study, we review existing knowledge on the genetic basis of CCA, molecular targets/signaling pathways involved in the pathogenesis, disease progression and prognosis, including potential targets for CCA targeted therapies.

Materials and Methods

Search strategy and eligibility criteria
The systematic review was performed in compliance with PRISMA guidelines. A systematic search in PubMed and Science Direct databases was performed using the following keywords: "cholangiocarcinoma", AND "molecular target" AND/OR "signaling pathway", AND/OR "targeted therapy", AND/OR "cancer chemotherapy." The literature search and data extraction were performed by two independent researchers, and by consensus, the final data, including any discrepancies, were resolved.

There was no publication year restriction; the search was last updated until April 2022. All articles published from various journals were retrieved and downloaded in EndNote X9 for further analysis. The inclusion criteria for article selection were i) full-text articles published in English, ii) articles with in vitro and/or in vivo and/or clinical studies of molecular targets/signaling pathways related to CCA pathogenesis/disease progression/ prognosis and/or targeted therapy. Duplicates, articles with unclear methodology, articles with unclear data, and articles related to molecular signaling pathways of other types of cancer were excluded from data analysis.

After the removal of duplicated articles, an initial review of titles and abstracts was performed to identify articles of potential interest. They were then retrieved for full-text analysis and independent data extraction by the authors. An additional manual search was performed for further relevant references.

Data extraction and collection
The following study characteristics were extracted from each article that fulfilled the inclusion criteria and had none of the exclusion criteria: molecular target or signaling pathway, targeted therapy or biomarker of CCA, the analytical method applied, type of study, animal or human used, and gene and protein investigated. A final eligibility check of the full-text articles was performed; all articles that were relevant to the review question and keywords were obtained and processed for final analysis.

Results

In the initial search, a total of 833 relevant articles published up to April 2021 were identified. Three hundred forty-nine duplicate articles, 210 review articles and 97 non-full-text articles were excluded. Of the remaining 177 articles, 115 articles were available as full-texts for eligibility screening. Irrelevant 42 articles were excluded following abstracts screening. Seventy-three studies that fulfilled the eligibility criteria were finally included in the final data synthesis (Figure 1).

The signaling molecules/pathways relevant to CCA pathogenesis, progression, and prognosis, as well as potential targets for CCA-targeted therapies are summarized in Table 1 and Supplement 1. Genetic research shows that polymorphisms in genes encoding enzymes involved in carcinogen metabolism, DNA repair, and inflammation have been found to be either pro-carcinogenic or anticarcinogenic. Recent advances in genome-wide technology have identified several molecular targets, signaling pathways as biomarkers of CCA diagnosis, disease progression, prognosis, as well as targets for CCA-targeted therapies. These include: cell proliferation and apoptosis signaling pathways (*PI3K / ERK/Akt/mTOR, HER2* and *EGFR* signaling, *EGFR/ FGFR* signaling pathway, *RAS/RAF/MEK/ERK, STAT3, S1P, YAP/Hippo*, and *Wnt/β*-catenin signaling pathways); cell autophagy signaling pathway; inflammation signaling pathway; angiogenesis signaling pathway; and metastasis signaling pathway. Promising drugs/candidate molecules have been identified for most pathways, except *PI3K / ERK/Akt /Mtor, RAS/RAF/MEK/ERK, STAT3, S1P* and *Wnt/β*-catenin signaling pathways (Figure 2, 3).

Discussion

Cell proliferation and apoptosis signaling pathway
Apoptosis is a form of programmed cell death that occurs in multicellular organisms. There are two pathways of apoptosis (i) the intrinsic pathway (Bcl2 inhibitable) activated by cell stress and acts through activation of caspase 9 via cytochrome c and Apaf1, and (ii) the extrinsic pathway (caspase 8/10-dependent) initiated by the death receptors at the cell surface which is promoted by the TNF ligands. Caspases stimulate cell death by activating specific proteins in the cytoplasm and nucleus. Several signalling pathways are active caspases that lead to apoptosis induction, such as the death receptor pathway, the inflammasome pathway, and the kinases pathways, etc.

PI3K /ERK/Akt /mTOR pathway
The *PI3K* (Phosphoinositide 3-kinases)/ERK/Akt (AKT serine/threonine kinase 1)/mTOR (mammalian

Table 1. Summary of Signaling Molecules/Pathways Identified as Biomarkers for CCA Pathogenesis, Disease Progression and Prognosis, as Well as Targeted Therapies for CCA.

Molecular signaling pathway	Signaling molecule	Chemotherapeutics which targets the pathways/ molecules	Type of CCA	Type of gene alteration	References
1) Cell proliferation and apoptosis signaling pathway					
	WWOX	-	IHCCA	Suppression	Huang et al., 2015
	EMT	Anlotinib	IHCCA	Suppression	Song et al., 2020
	Akt-NFκB	Xanthohumol (XN)	IHCCA	-	Dokduang et al., 2016; Walden et al., 2017
PI3K /ERK/ AKT /mTOR	D-2-hydroxyglutarate	Ivosidenib	IHCCA	Suppression	Aguado-Frail et al., 2021
	AIB1	-	IHCCA	Over expression Akt / Nrf2 pathways	Chen et al., 2012
	Integrin α6	-	IHCCA	overexpression	Ding et al., 2013
		Sorafenib, Sunitinib	IHCCA	Suppression	Andersen et al., 2012. Dokduang et al., 2013
		Rapamycin	IHCCA	Suppression	Hong et al., 2013; Zhao et al., 2016
		Nimotuzumab	IHCCA	Suppression	Padthaosong et al., 2017
		Copanlisib, Gemcitabine, Cisplatin	IHCCA EHCCA		Tan et al., 2020
		Salubrinal, Rapamycin	IHCCA		Zhao et al., 2016
		Zileuton	IHCCA	Suppression	Phophai et al., 2018
	NOTCH3	-	pCCA IHCCA		Guest et al., 2016
HER2 and *EGFR*	EGFR	Trastuzumab	IHCCA	Point mutation (KRAS mutations)	Andersen et al., 2012; Yamashita-Kashima et al., 2019
	EGFR	Sorafenib	IHCCA	Point mutation	Andersen et al., 2012
	EGFR	Erlotinib	IHCCA	Point mutation	Andersen et al., 2012; Borad et al., 2014
	HER2 and EGFR	Lapatinib	IHCCA	Point mutation	Andersen et al., 2012
EGFR/ FGFR	EGFR	Ponatinib	SIC	-	Borad et al., 2014
	FGFR2	Erlotinib		Point mutation	
	FGFR2	Pazopanib		Point mutation	
	EGF	-	IHCCA	Suppression	Gui et al., 2012
	FGFR	Ponatinib, Dovitinib, BGJ398	IHCCA	-	Wang et al., 2016
	EGFR	Vandetanib (ZD6474),	IHCCA	-	Yoshikawa et al., 2009
RAS/RAF/MEK/ ERK	EGFR	-	IHCCA EHCCA	Point mutation	Chang et al., 2014
	MALT1	-	IHCCA, EHCCA	Suppression	Yeh et al., 2017
STAT3	let-7c, miR-99a, miR-125b	-	EHCCA	Activation	Lin et al., 2016
	Fibroblastic FAP	-	IHCCA	-	Lin et al., 2019
	caspase 3/7	β-eudesmol	EHCCA	-	Srijiwangsa et al., 2018
(S1P) pathway	SPHK1	-	IHCCA		Chen et l., 2015
YAP/Hippo pathway	YAP	-	IHCCA	Regulation of cell proliferation	Marti et al., 2015
	FGFR	BGJ398	IHCCA	Suppression	Rizvi et al., 2016
	YAP, SFK	Dasatinib	IHCCA	Suppression	Sugihara et al., 2018
Wnt/β-catenin signaling	hUC-MSCs	-	EHCCA	-	Wang et al., 2015
	Brg1	-	IHCCA	-	Zhou et al., 2021
FoxM1	FoxM1	Clioquinol (CQ)	IHCCA	Suppression	Chan-On et al., 2015
		nitroxoline NQ)		Suppression	Chan-On et al., 2015

Table 1. Continued

Molecular signaling pathway	Signaling molecule	Chemotherapeutics which targets the pathways/ molecules	Type of CCA	Type of gene alteration	References
2) Autophagy signaling pathway.					
		Compound C	IHCCA		Zhao et al., 2018
3) Inflammation signaling pathway					
	DcR3	DcR3, (DcR3-siRNA)	EHCCA	-	Xu et al., 2018
	α7-nAChR	-	IHCCA	Overexpression	Chen et al., 2019
	PGE2, Akt	Celecoxib and Rofecoxib	CCA	Suppression	Zhang et al., 2004
4) Angiogenesis signaling pathway					
	GATA6/LOXL2	-	IHCCA	Regulation	Peng et al., 2019
5) Metastasis signaling pathway					
c-MET	MET	LY2801653	IHCCA		Barat et al., 2016
	MACC1		IHCCA	Overexpression	Lederer et al., 2015

target of rapamycin) is essential kinases that control critical cellular activities, including gene transcription and translation, cell proliferation, growth, and survival. PI3K pathway is activated via down regulation of PTEN (Phosphatase and TENsin homolog deleted on chromosome 10), which is an essential multi-functional tumor suppressor, leading to inhibition of apoptosis process. Activation of the PI3K/Akt pathway has been linked to various human cancers, making it an important target for the development of new anticancer drugs.

Amplified in breast cancer 1 (AIB1) is an important transcriptional coactivator involved in the carcinogenesis of several cancers. AIB1 protein is overexpressed in CCA. AIB1 induced the chemoresistance of CCA cells by
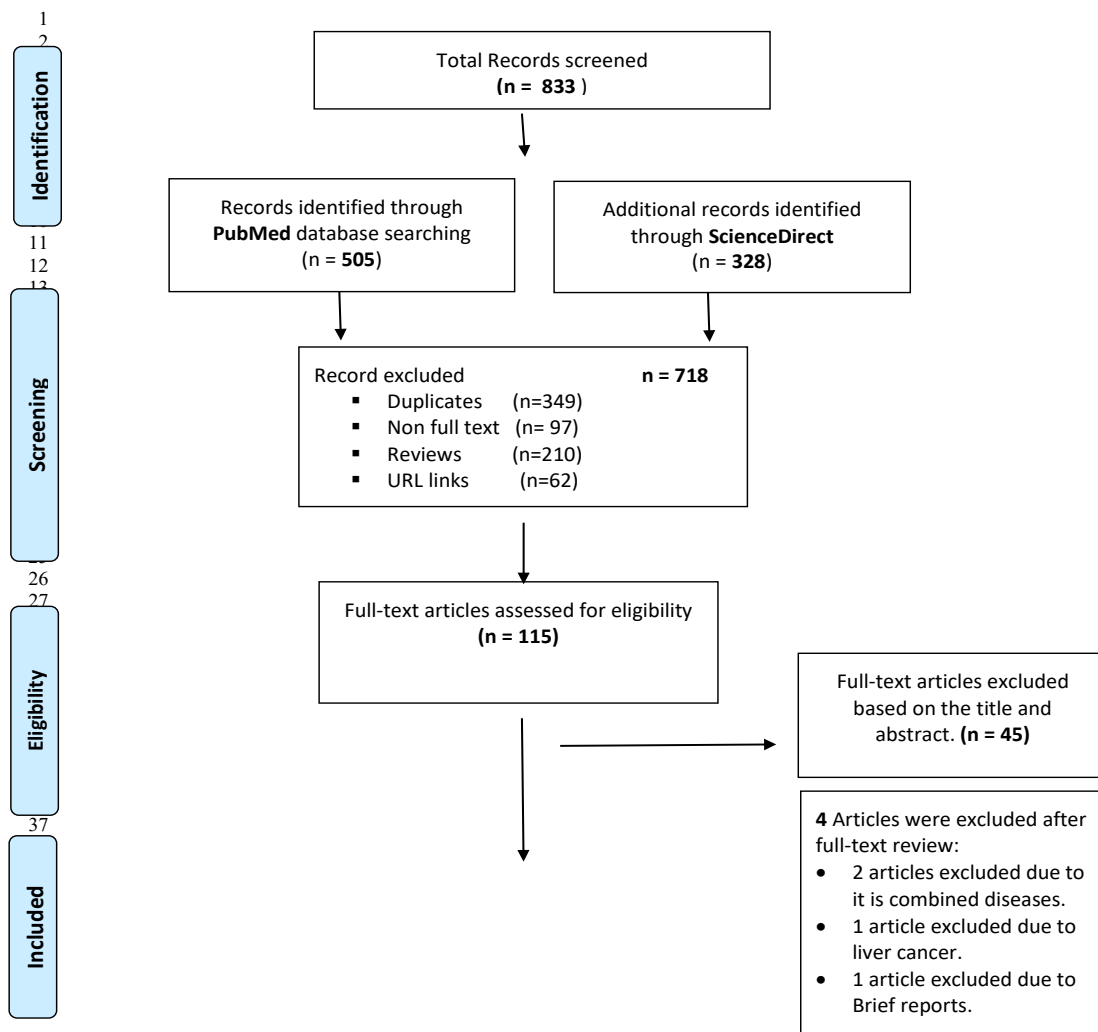

Figure 1. Flow Diagram Summarizing Steps for Exclusion and Inclusion of the Research Articles Included in the Analysis.
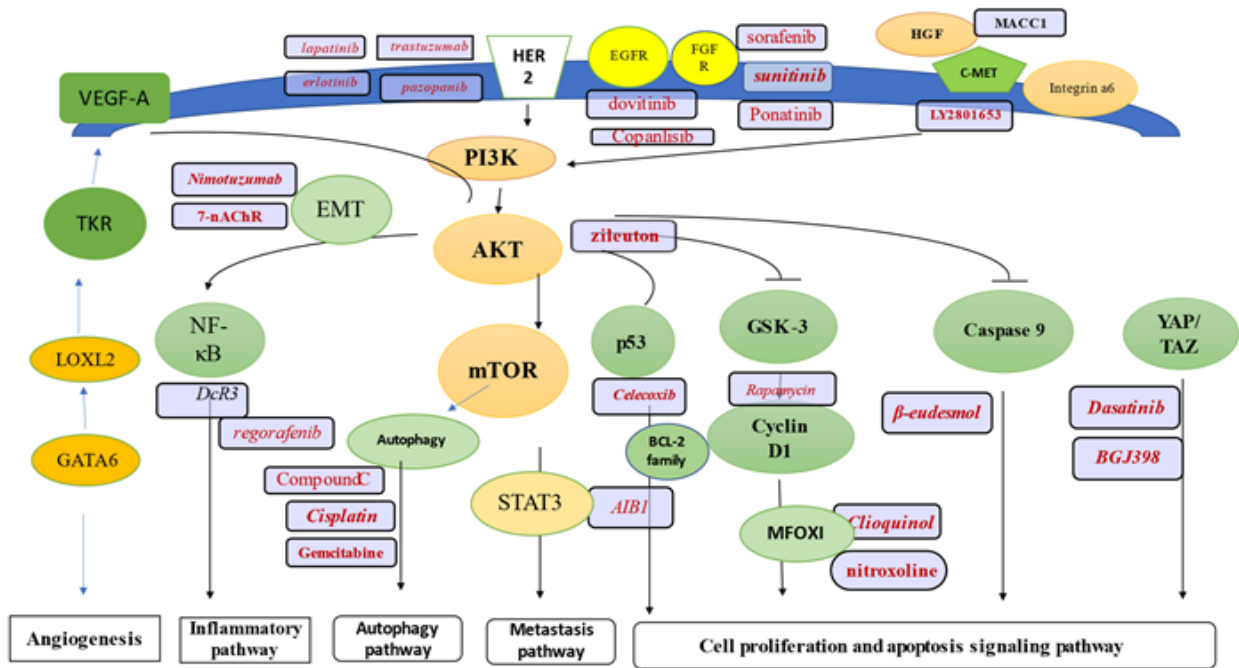
Figure 2. Descriptive Diagram Summarizing Signaling Pathways and Its Molecular Targets

regulating the expression of the antiapoptotic protein Bcl2. Down-regulation of AIB1 suppressed the Akt pathway, resulting in inhibition of CCA cell proliferation (Chen et al., 2012). Notch is a potential driver of gastric epithelial cell proliferation forced exogenous overexpression of Notch 1 in hepatocytes resulted in biliary tumors. Notch is targeted by toxins that are known to be RBPJ (recombinant signal binding protein for immunoglobulin kappa J region)-dependent. Notch 3 is a key player in EHCCA and IHCCA, as it controls cell survival without the involvement of RBPJ (Guest et al., 2016). After Notch

3 deletion, tumor development was dramatically slowed, and Notch 3 silencing resulted in lower HES (hairy and enhancer-of-split) and HEY (Hes-related repressor Herp, Hesr, Hrt, CHF, gridlock) expression (Guest et al., 2016).

The mTOR signalling pathway is proposed to be an attractive target for cancer therapeutics because it is up-regulated in various cancer types, including CCA (Hong et al., 2013). PI3K cell signaling pathway is activated when growth factors such as hepatocyte growth factor (HGF) and fibroblast growth factors (FGF) bind to their corresponding receptor tyrosine kinases. Following
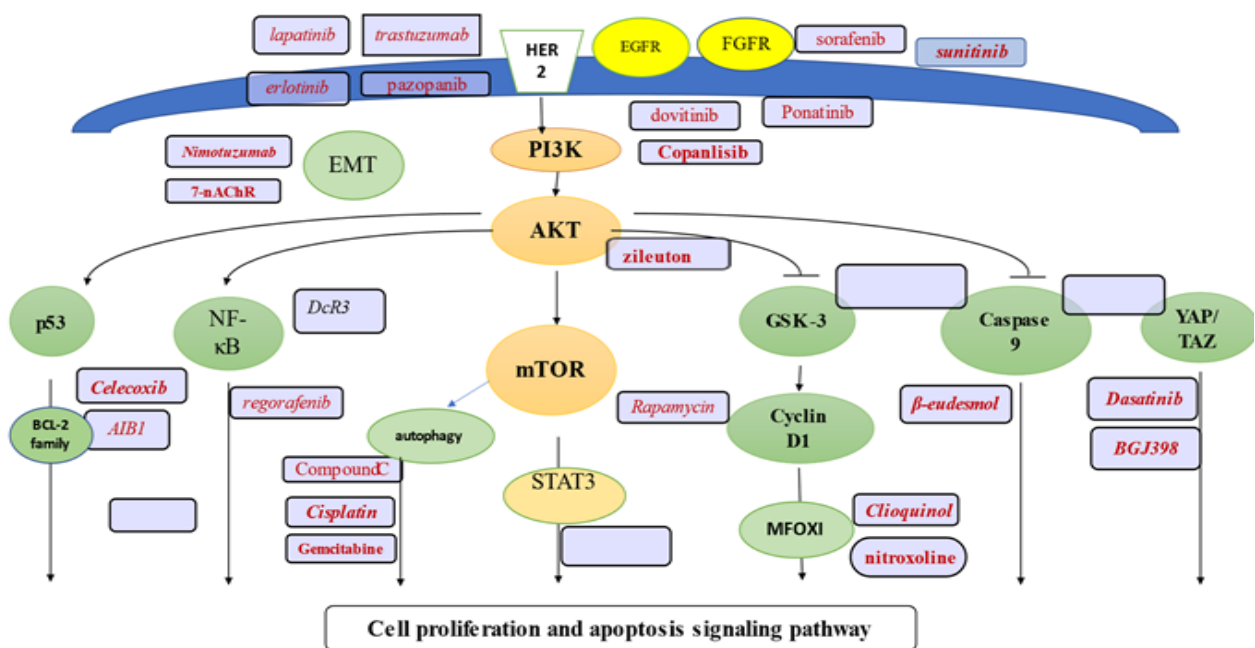

Figure 3. Descriptive Diagram Summarizing Apoptosis Signaling Pathway and Molecular Targets. Reference : KEGG PATHWAY: Apoptosis - Homo sapiens (human) (genome.jp)
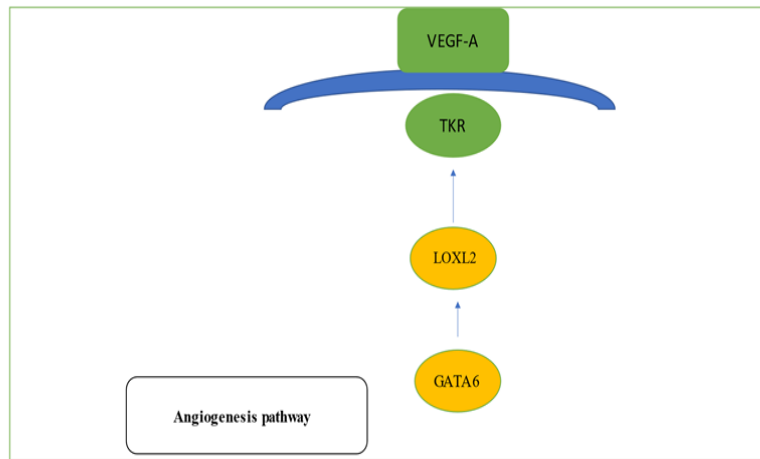
Figure 4. Descriptive Diagram Summarizing Angiogenesis Signaling Pathway and Molecular Targets.

Akt activation, the mTOR pathway is phosphorylated and regulated. The activation of Erk1/2 (extracellular signal-regulated protein kinase) and Akt were higher in CCA tissues than in normal tissues (Dokduang et al., 2013). The use of multi-targeted kinase inhibitors such as sorafenib and sunitinib to suppress kinase activation resulted in considerable cell growth suppression (Chen et al., 2012; Dokduang et al., 2013). Ivosidenib (mIDH1 inhibitors) suppressed AG-120 (oncometabolite D-2-hydroxyglutarate), resulting in disease stability and enhancing progression-free survival (Aguado-Fraile et al., 2021).

Rapamycin, an antitumor and immunosuppressive drug, has been shown to inhibit sarcomatous intrahepatic CCA (SICC) motility by inhibiting mTORC2 assembly and thus, down-regulating p-STAT3 (S727) (Hong et al., 2013; Zhao et al., 2016). Nimotuzumab is a monoclonal antibody that inhibits EGFR (epidermal growth factor receptor) activity. It inhibits CCA cell growth and metastasis through suppression of the EMT (epithelial-mesenchymal transition) process and reducing the expression of MMP9 (matrix metallopeptidase 9), which will also inhibit CCA cell invasion (Pathaisong et al.,

2017). Phase II clinical trial performed on six patients with IHCCA and EHCCA indicated that gemcitabine, cisplatin, and copanlisib, including the combination of copanlisib and gemcitabine or cisplatin did not improve progression-free survival (PFS) at six months, and none of the mutations observed was associated with clinical outcomes (Tan et al., 2020). The leukotriene synthesis inhibitor (zileuton) inhibited CCA cell proliferation and migration by inhibiting the Akt signaling pathway (Khopai et al., 2018).

Up to the present, there has been no report of any therapeutic agent that targets the PI3K /ERK/Akt /mTOR pathway.

HER2 and EGFR signaling pathway
HER2 (human epidermal growth factor receptor 2) and EGFR (epidermal growth factor receptor) are cell surface receptor tyrosine kinases. The *HER2* gene encodes *HER2* protein (*HER2/neu* protein), which is a receptor on the surface of breast cells that regulates cell growth, division, and repair. EGFR is a member of the ErbB family of receptors EGFR gene encoding a protein involved in cell growth and survival. EGFR mutated gene is associated
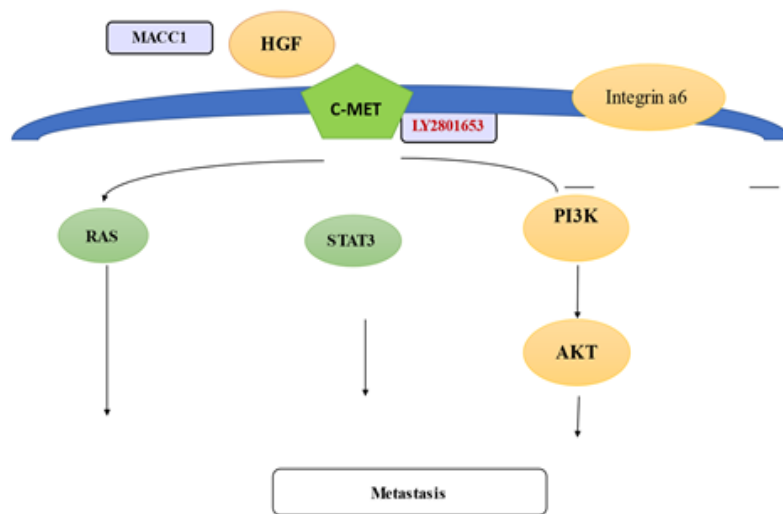

Figure 5. Descriptive Diagram Summarizing Metastasis Signaling Pathway and Molecular Targets. Reference: www.genome.jp

with some types of cancer.

Aberrant *HER2* expression has been found in various malignancies such as ovarian and gastric cancer, but most notably in breast cancer, where it plays a crucial role in malignant transformation and therapy selection (Andersen et al., 2012). *HER2* up-regulation was found only in tumors from patients with poor prognosis, who were also characterized by a frequent coactivation of ERBB3 (erythroblastic leukemia viral oncogene homolog) and EGFR (Andersen et al., 2012; Yamashita-Kashima et al., 2019).

Sorafenib and erlotinib are receptor tyrosine kinase (RTK) inhibitors which are the first-line therapy for IHCCA. Treatment of CCA cell lines with trastuzumab and lapatinib, a dual-target TKI (*HER2* and EGFR) which activate EGFR and *HER2*, showed therapeutic potential of these drugs in the subgroup of individuals with *HER2* and EGFR signaling activation (Andersen et al., 2012; Yamashita-Kashima et al., 2019). Lapatinib was reported to be more effective in suppressing the growth of CCA cells than trastuzumab (Andersen et al., 2012).

EGFR/ FGFR signaling pathway

EGFR (epidermal growth factor receptor)/FGFR (Fibroblast growth factor receptor 2) signaling pathway is an essential pathway that regulates cellular growth, survival, proliferation, and differentiation. FGFR2 fusions and ERRFI (ERBB receptor feedback inhibitor) mutations may represent novel targets in SIC. Transcriptome sequence analyses and integrated genome-wide were performed on tissue samples from patients with SIC revealed two novel targeted therapies. The anticancer activity of pazopanib was observed in a patient with FGFR2-TACC3 fusion. Erlotinib, an EGFR kinase inhibitor, has rapid and potent efficacy on ERRFI1 inactivated tumor (Borad et al., 2014). EGFR protein expression was markedly downregulated under sustained EGF stimulation (Gui et al., 2012). Recent genomic analysis of CCA patients revealed the presence of FGFR2 fusion proteins in up to 13% of IHCCA.

FGFR inhibitors suppress cell proliferation and induce apoptosis in CCA tumors harboring FGFR2 fusions (expressed only in IHCCA). Antitumor effect of FGFR inhibitors on derived xenograft (PDX) mouse model using an FGFR2-CCDC6 fusion protein from an IHCCA patient was evaluated. Ponatinib, dovitinib, and BGJ398 (most potent) inhibited cell proliferation and induced apoptosis in IHCCA (Wang et al., 2016). Vandetanib (ZD6474), a VEGFR and EGFR inhibitor, is another promising therapeutic agent for CCA; it has the potential to suppress tumor growth at a lower dose. The presence of EGFR amplification and absence of KRAS (Kirsten rat sarcoma gene) mutation may possibly be a predictive molecular indicator of the effectiveness of EGFR-targeted therapy (Yoshikawa et al., 2009).

RAS/RAF/MEK/ERK signaling pathway

The mutation of RAS (small GTPase) B-Raf proto-oncogene, serine/threonine kinase (BRAF) is rare in CCA, while that of EGFR and KRAS are common. EGFR mutation was an independent prognostic marker in CCA in addition to tumor stage and differentiation. No simultaneous EGFR and KRAS mutations in EHCCA and gallbladder carcinoma were found. It was proposed that EGFR and KRAS mutations should be evaluated when tailoring molecular-targeted therapy to patients (Chang et al., 2014). MALT1 (mucosa-associated lymphoid tissue protein 1) is essential for activating NF-κB, the potential target of regorafenib. Regorafenib suppressed CCA growth via down-regulating MALT1 expression through suppression of the Raf/Erk/ Elk1 pathway (Yeh et al., 2017).

Up to the present, there has been no report of any therapeutic agent that targets the RAS/RAF/MEK/ERK signaling pathway.

STAT3 signaling pathway

STAT3 (signal transducer and activator of transcription 3) is one of the seven proteins that transmit signals from active cytokine and growth factor receptors in the plasma membrane to the nucleus, where they control gene transcription. STAT3 plays a critical role in proliferation, survival, apoptosis, angiogenesis, and metastasis.

Let-7c, miR-99a and miR-125b, which are three miRNAs of the same cluster, were down-regulated in CCA. All targeted IL6 (interleukin 6), IL6R and IGF1R (Type 1 insulin-like growth factor) are essential cytokines and receptors of the IL6/STAT3 pathway and have critical roles in inflammation and CCA initiation. Expression of let-7c, miR-99a or miR-125b reduced the activity of STAT3 and suppressed CCA cell migration and tumorigenicity in vivo (Lin et al., 2016).

Possible correlations between miRNAs and inflammation and strategy for miRNA-based therapy for CCA by inhibiting IL6/STAT3 was proposed (Lin et al., 2016). FAP (fibroblast activation protein) expression in fibroblasts was required for STAT3 activation and CCL2 (chemokine ligand 2) production. In IHCCA, the ICCCAFs (intrahepatic cholangiocarcinoma-cancer associated fibroblast) were the primary source of CCL2. The ability of ICCCAFs to promote IHCCA growth, MDSC infiltration, and angiogenesis was significantly reduced when FAP was knocked down in the fibroblasts. IHCCA cell proliferation and apoptotic resistance were unaffected (Lin et al., 2019).

Up to the present, there has been no report of any therapeutic agent that targets the STAT3 signaling pathway.

S1P signaling pathway

S1P (sphingosine-1-phosphate) signaling pathway is an essential intracellular metabolic pathway that plays a role in various biological processes, including cell proliferation, differentiation, apoptosis, and cellular signaling. SPHKs (sphingosine kinases) convert sphingosine to S1P and are essential regulators of cell fate. SPHK1 and SPHK2, the two isoforms of SPHK enzymes with distinct functions, have been discovered. In contrast to ceramide and sphingosine, which induce cell apoptosis and growth arrest, bioactive S1P is now recognized as a critical regulator of cell survival and proliferation. SPHK1 has oncogenic roles in proliferation, angiogenesis, and transformation. It is identified as an independent

marker of poor prognosis. SK1I modulated the balance of S1P and induced CCA apoptosis and produced potent antiproliferative effects on CCA cells that were time- and concentration-dependent (Chen et al., 2015).

Up to the present, there has been no report of any therapeutic agent that targets the S1P signaling pathway.

YAP/Hippo signaling pathway

YAP (Yes-associated protein), the Hippo pathway effector, is a transcriptional coactivator implicated in the pathogenesis of CCA. YAP is regulated by a serine/threonine kinase relay module (MST1/2–LATS1/2) and phosphorylated on tyrosine 357 (Y357) in the CCA cell. Microarray expression profiling of CCA cells with down-regulated or overexpressed YAP reveals that YAP regulates genes involved in apoptosis, proliferation, and angiogenesis. The combination of YAP with TEAD (transcription factors) prevented apoptosis induced by cytotoxic drugs. YAP was shown to be a critical regulator of proliferation and anti-apoptotic processes in CCA. Elevated YAP activity in CCA was correlated with increased pro-angiogenic MFAP5 (microfibrillar-associated protein 5) expression and CD311 vasculature. These findings support the role of YAP in promoting angiogenesis by regulating the expression of secreted pro-angiogenic proteins (Marti et al., 2015).

SFK (Sarcoma family kinase) inhibition with Dasatinib resulted in the loss of YAPY357 phosphorylation and promoted its translocation from the nucleus to the cytoplasm (Sukihara et al., 2018). A significant decrease in YAP activation was observed in YAP-positive CCA cell lines treated with BGJ398, which induced cell death due to Mcl1 depletion. Nuclear YAP expression could be used as a biomarker in FGFR-directed therapy (Rizvi et al., 2016).

Wnt/β-catenin signaling pathway

Wnt/β-catenin signaling is a process that causes the β-catenin protein to accumulate in the cytoplasm, which then translocates to the nucleus, where it acts as a transcriptional coactivator. It regulates cellular functions such as cell proliferation, differentiation, migration, genetic stability, apoptosis, and stem cell. A *Wnt/β*-catenin signaling pathway is involved in the initiation and development of CCA.

MSCs (mesenchymal stem cells) are non-hematopoietic stem cells, which are capable of differentiation at least into bone, cartilage, muscle, and adipose tissues. When combined with human CCA cell lines, hUC-MSCs (human umbilical cord-derived mesenchymal stem cells) significantly increased cancer cell proliferation and migration potency through targeting the *Wnt/β*-catenin signaling. MSCs and their conditioned media (MSC-CM) could improve the drug resistance of tumor when the compound K (CK), a major intestinal bacterial metabolite of panaxoside, was administered. (Wang et al., 2015).

Brg1 (Brahma-related gene 1), an enzyme member of SWI/SNF (SWltch/sucrose non-fermentable) complex that is important in stem cell maintenance and tumor development, is up-regulated in both hepatic progenitor cell (HPC) and IHCCA, although its role is still unclear. The binding of Brg1 with the TCF4 (β-catenin/transcription factor 4) transcription complex is a possible approach for regulating *Wnt/β*-catenin signaling. Overexpression of Brg1 indicates poor prognosis in IHCCA. Therapies targeting Brg1-expressing, like PFI3, are promising for the treatment of liver cirrhosis and IHCCA (Zhou et al., 2021).

FoxM1 (fork head box M1) signalling pathway is a major transcription factor altered in cancer through altered signal transduction pathways. Activation of FOXM1 via RAS/ERK signaling It is found to be elevated in several types of cancer, including CCA.

The quinoline-based compounds, clioquinol and nitroxoline, were shown to inhibit CCA cell proliferation by decreasing the expression of FoxM1 signaling (Chanon et al., 2015).

Up to the present, there has been no report of any therapeutic agent that targets the *Wnt/β*-catenin signaling pathway.

Autophagy signaling pathway

Autophagy is an intracellular apoptotic process triggered by cellular stresses. There are three types of autophagy: (i) macroautophagy, (ii) chaperone-mediated autophagy (CMA), and (iii) late endosomal microautophagy. Autophagy can target proteins, lipids, cell organelles, and pathogens. Autophagy is reported to have both tumor-suppressing and activity-promoting properties.

Compound C, commonly known as dorsomorphin, is a pharmacological AMP-activated protein kinase (AMPK) inhibitor that effectively suppresses AMPK metabolic functions; it inhibits AMPK protective autophagy in CCA. Through the p53 (53-kilodalton (kDa) protein) activation and JNKs (c-Jun N-terminal kinases) pathway, compound C causes protective autophagy in human CCA cells (Zhao et al., 2018).

Inflammation signaling pathway

Inflammation is a process by which the body is protected from harmful outside invaders. Through phagocytosis, macrophages eliminate pathogens and antigens and induce inflammatory responses by generating cytokines and enzymes such as TNF (tumor necrosis factor), IL6 (interleukin 6), iNOS (inducible nitric oxide synthase), and COX2 (cyclooxygenase-2).

TNF and IL6 affect the growth, differentiation, and proliferation of numerous cell types, including CCA. TNF enhances CCA cell motility by increasing the expression of EMT markers. It is suggested that anti-TNF medication might be a viable treatment for CCA

Celecoxib is a COX2 inhibitor that suppresses PGE2 (prostaglandin E2), increases Bax translocation to mitochondria, and increases cytochrome c release into the cytosol. Celecoxib caused CCA cell apoptosis via a mechanism that involved Akt inactivation, Bax translocation, and cytochrome c release (Zhang et al., 2004). DcR3 (Decoy receptor 3) is a protein with the anti-apoptotic effect that belongs to the tumor necrosis factor receptor. Down-regulation of DcR3 in CCA decreased CCA cell viability to 61.87% (Xu et al., 2018). Overexpression of 7-nAChR (7-nicotinic acetylcholine receptor) promoted CCA progression by inhibiting

apoptosis and promoting EMT (epithelial-mesenchymal transition). 7-nAChR is a promising therapeutic target in CCA because it is an effective molecular biomarker and prognostic factor (Chen e al., 2019).

Angiogenesis signaling pathway

Angiogenesis signaling pathway is important for cell growth, development, wound healing, and carcinogenesis. It is regulated by several growth factors, including VEGF (Vascular endothelial growth factor protein family) (Figure 4). VEGFA plays an important role in tumor angiogenesis. GATA6 (GATA-binding protein 6) is essential for the differentiation, proliferation and development of the digestive system, cardiovascular system, and some tissues during embryonic development. GATA6 and LOXL2 (Lysyl oxidase-like 2) expression influence secreted VEGFA expression, angiogenesis, and tumor development. The interaction of LOXL2 with GATA6 was shown to regulate VEGFA transcription, enhance VEGFA secretion and angiogenesis. Drugs targeting this complex pathway may possess great therapeutic value in the treatment of CCA (Peng et al., 2019).

Targeted therapies under investigation for the angiogenesis signaling pathway include lenvatinib (multi-targeted tyrosine kinase inhibitor that inhibits VEGFR1-3, FGFR1-4, PDGFR, KIT, and RET) and pembrolizumab (humanized antibody that inhibits the programmed cell death 1 (PD-1) receptor). Both significantly decreased the population of immunosuppressive tumor-associated macrophages and increased interferon-γ-producing cluster of differentiation 8+ (CD8+) T cells. The addition of PD-L1 (programmed cell death protein 1/programmed death-ligand) inhibitors helps reverse VEGF-mediated immune suppression, restore T cell function, and promotes T cell tumor infiltration. The combination of lenvatinib and pembrolizumab has demonstrated promising activity with manageable adverse events in various solid tumor types (www.ClinicalTrials.gov).

Metastasis signaling pathway

c-MET (Mesenchymal-epithelial Transition factor) signaling pathway, the proto-oncogene encoding the RTK, plays an important role in cell proliferation, survival, motility, invasion, and metastasis through regulating multiple signaling cascades (Figure 5). c-MET is overexpressed in more than 50% of human CCA and 80% of murine CCA (Barat et al., 2016).

MACC1 (metastasis-associated in colon cancer 1) is a recently identified regulator of the hepatocyte growth factor (HGF)/Met/mitogen-activated protein kinase pathway, which stimulates proliferation, migration, invasion, and metastasis of tumors. MACC1 expression was considerably higher in Klatskin HCA tumor patients with history of tumor recurrence than in those without history of recurrence. MACC1 is considered as a highly predictive biomarker for shorter overall (OS) and disease-free survival (DFS) in Klatskin tumor patients. It is, however, not a prognostic marker for the survival of IHCCA (Lederer et al., 2015).

Although the function of c-MET signaling pathway in CCA remains unclear, it appears to be a promising alternative therapeutic strategy (Barat et al., 2016). LY2801653 (small-molecule kinase inhibitor) suppressed the overactivation of c-MET and p-MET under hypoxic circumstances by partially down-regulating HIF1a (Hypoxia-inducible factor 1-alpha) expression in CCA cell line.

Integrin a6, a cell surface receptor, mediates adhesion to the extracellular matrix (ECM) and enhances cell motility and invasion. The expression of integrin a6 in IHCC tissues was higher than that in nontumor samples. Integrin a6 overexpression was found to be significantly correlated with larger tumors, multiple nodular, microvascular/bile duct invasion, and lymphatic metastasis (Ding et al., 2013).

In summary, identification of signaling molecules/pathways involved in CCA pathogenesis, disease progression and prognosis has been a focus of CCA research in recent years. The information obtained has been exploited for further development of diagnostic tools for early detection of CCA, as well as effective CCA-targeted therapies. Despite the significant advances achieved toward molecularly profiling CCA, there are still substantial gaps in our knowledge. The interactions among signaling pathways and components that induce cancer cell phenotypes are still poorly understood. A better understanding of the epigenetic processes underlying biliary carcinogenesis is likely to lead to the discovery of new therapeutic targets. Evidence suggests that combining different targeted drugs can prevent the development of resistance while also improving efficacy. Recent developments in immunotherapy may create additional treatment options for CCA.

Author Contribution Statement

Acknowledgements

Ethics approval

This article does not involved any human or animal study.

Availability of data and material

The datasets generated during and/or analyzed during this study are available from the corresponding author on reasonable request.

Conflict of Interest

The authors declare no competing interests

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