Abbreviations:

ADCC	Antibody-dependent cellular cytotoxicity
AFP	Alpha-fetoprotein
AG-120	oncometabolite D-2-hydroxyglutarate
AIB1	Amplified in breast cancer 1
Alarmar Blue assay	Cell proliferation assay
ANGPTL4	Angiopoietin-like 4
Annexin V/PI	Annexin V/PI apoptosis assay
aPKC-i	atypical protein kinase C-isoform
ASPH	Aspartate β-hydroxylase
ATF4	Activating transcription factor 4
AZA	5-aza-2′-deoxycytidine
Bcl-2	B-cell lymphoma 2
BICC1	BicC Family RNA Binding Protein 1
BRAF	B-Raf Proto-Oncogene, Serine/Threonine Kinase
Brg1	Brahma-related gene 1
caspase-Glo® 3/7 assay	Apoptosis assay
CCA	Cholangiocarcinoma
CCK-8	Cell Counting Kit-8
CCL2	Chemokine ligand 2
ChIP	Chromatin immunoprecipitation
CK	Compound K
CMA	chaperone-mediated autophagy
c-MET	Mesenchymal-epithelial Transition factor

c-MYC	c-myelocytomatosis
Co-IP	Co-immunoprecipitation
COX2	Cyclooxygenase -2
COX-2	Cyclooxygenase-2
D-2-HG	Oncometabolite D-2-hydroxyglutarate
DAPI	4′,6-diamidino-2-phenylindole
Dcca	Distal Cholangiocarcinoma
DCFH-DA	Dichloro-dihydro-fluorescein diacetate assay
DcR3	Decoy receptor 3
DHMEQ	Dehydroxy methylepoxyquinomicin
E-cadherin	Epithelial cadherins
Edu	Edu proliferation assay
EGFR	Epidermal Growth Factor Receptor
EGFR- TK	Epidermal growth factor receptor (EGFR) tyrosine kinase (TK)
EHCCA	Extracellular Cholangiocarcinoma
eIF2a	Eukaryotic initiation factor alpha
ELISA	Enzyme-linked immunosorbent assay
EMSA	Electrophoretic gel mobility shift assay
EMT	Epithelial–mesenchymal transition
EMT inhibitor	Epithelial–mesenchymal transition inhibitor
ErbB	Erythroblastic leukemia viral oncogene homolog
ErbB1	Epidermal growth factor receptor
ERK1/2	Mitogen-activated protein kinase-1/2
ERKs	Extracellular signal-regulated kinase

ERRFI	ERBB receptor feedback inhibitor
FAP	Fibroblast activation protein
FC	Flow cytometry
FGF	Fibroblast growth factors
FGFR	Fibroblast growth factor receptor
FISH	Fluorescent In Situ Hybridization assay,
FISH	Fluorescence in situ hybridization
FM	Fluorescence Microscope
FoxM1	Fork head box M1
FOXO1	Fork head box protein O1
FXR	Farnesoid X receptor
Gas	Genetic Aberrations
GATA6	GATA -binding protein 6 (GATA6)
GDH	Glutamate dehydrogenase
GeoMx DSP	NanoString's GeoMx Digital Spatial Profiler
GRB2	Growth Factor Receptor-bound protein 2
HCA	Hilar cholangiocarcinoma
HDAC	Histone Deacetylase Inhibitor Trichostatin A
HER2	Human epidermal growth factor receptor 2
HES	Hairy and enhancer-of-split
HEY	Hes-related repressor Herp, Hesr, Hrt, CHF, gridlock)
HGF	Hepatocyte Growth Factor
HIF1a	Hypoxia-inducible factor 1-alpha
HPC	Hepatic progenitor cell

HSP90	Heat Shock protein 90
HSP90 inhibitors	Heat shock protein (Hsp) 90 inhibitors
hUC-MSCs	Human umbilical cord-derived mesenchymal stem cells
HUVECs	Human umbilical vein endothelial cells
HUVECs	Human umbilical vein endothelial cells
IB	Immunoblotting
ICCCAFs	Intrahepatic cholangiocarcinoma -Cancer associated fibroblast
IDH1 and IDH2	Isocitrate dehydrogenase 1 and 2
IF	Immunofluorescence
IGF1R	Type 1 insulin-like growth factor
IHC	Immunohistochemistry
IHCCA	Intrahepatic Cholangiocarcinoma
IL-6	Interleukin 6
IL-6R	Interleukin 6 receptor
IMC	Immunocytochemistry
iNOS	inducible nitric oxide synthase
IP	Immunoprecipitation
iTRAQ	The isobaric tag for relative and absolute quantitation
Jab1	Jun activating domain-binding protein 1
JNK	c-Jun N-terminal kinase
KRAS	Kirsten rat sarcoma gene
LC–MS	Liquid chromatography–Mass Spectrometry
LOXL2	Lysyl oxidase-like 2
MACC1	Metastasis-associated in colon cancer 1

MALT1	Mucosa-associated lymphoid tissue protein 1
MAPK	Mitogen-activated protein kinases
MCL1	Myeloid cell leukemia1
MDR-1	Multidrug resistance gene
MFAP5	microfibrillar-associated protein 5
MFAP5	Proangiogenic microfibrillar-associated protein 5
MGEA5	Meningioma-Expressed Antigen 5
mitoSOX	Red mitochondrial superoxide Indicator
MMP-9	Matrix metallopeptidase 9
MSCs	Mesenchymal stem cells
MST1/2–LATS1/2	serine/threonine kinase relay module
mTOR	Mammalian target of rapamycin
mTOR	Mammalian target of rapamycin
MTT	3-(4,5-dimethylthiazol2-yl)-2,5-diphenyltetrazolium bromide
MVD	Microvessel density
NF-κB	Nuclear Factor-kappaB
NGS	Next Generation Sequencing
NQO1	NAD(P) H Quinone oxidoreductase 1
Ov	Opisthorchis viverrini
p53	53-kilodalton (kDa) protein
PARP	Poly ADP ribose polymerase
pCCA	Perihilar Cholangiocarcinoma
PDX	Patient-derived xenograft
PDX	Patient-derived xenograft

PFS	Progression-free survival.
PGE2	Prostaglandin E2
PI3K	Phosphoinositide 3-kinases
p-p44/42 MAPK	Phosphorylated p44/42 MAPK
PRC2	Epigenetic modification complexes
PTEN	Phosphatase and TENsin homolog deleted on chromosome 10
PTPN6 gene	Protein Tyrosine Phosphatase Non-Receptor Type 6
PVT1	Plasmacytoma variant translocation 1
pY1068	Phosphorylated EGFR
qPCR	Quantitative real-time polymerase chain reaction
RA	Radix Astragali
RBPJ	Recombinant Signal Binding Protein for Immunoglobulin Kappa J Region
ROS	Reactive Oxygen Species
RTCA	Real-Time cell analysis
RTKs	Receptor tyrosine kinases
RT-PCR	Reverse Transcription Polymerase Chain Reaction
S1P	Sphingosine-1-phosphate
SFK	Sarcoma family kinase
SHP	Small Heterodimer Partner
SHP-1	Src homology phosphatase 1
SIC	Sporadic Intrahepatic Cholangiocarcinoma
SICC	sarcomatous intrahepatic Cholangiocarcinoma
SK1-I	SPHK1 inhibitor
SK1-I	SPHK1 inhibitor

SMAD	Mothers Against decapentaplegic homolog
SMIs	Second generation small molecule inhibitors of β-hydroxylase
SNAIL1	Zinc finger protein SNAI1
Sp1	Specificity protein 1
SPHK1	Sphingosine kinase 1
SRB	Sulforhodamine B assay
STAT3	Signal transducer and activator of transcription 3
STR	Short Tandem Repeat
STRPCR	Short Tandem Repeating Sequence PCR
SWI/SNF	SWltch/Sucrose Non-Fermentable
TACC3	Transforming Acidic Coiled-Coil Containing Protein 3
T-DM1	Trastuzumab Emtansine
TGF	Transforming growth factor
TGF-β	Transforming Growth Factor beta
TMA	Tissue Microarray
TNF	Tumor Necrosis Factor
TNF-α	Tumor Necrosis Factor-α
TP7p3-AS1	TP73 antisense RNA 1
TUNEL	Terminal deoxynucleotide transferase-mediated dUTP nick-end labeling
VEGF	Vascular Endothelial Growth Factor
VEGF-A	Vascular Endothelial Growth Factor A
WB	Western blotting
WWOX	WW Domain Containing Oxidoreductase
XIAP	X-linked Inhibitor of apoptosis protein

YAP	Yes-Associated Protein
α7-nAChR	Alpha7-Nicotinic Acetylcholine Receptor

Supplement 1 Investigation of signaling molecules/pathways as biomarkers for CCA pathogenesis, disease progression and prognosis, as well as targets for CCA chemotherapeutics.

Ref No.	Signaling pathway/molecule	Signaling molecules/Chemotherapeutics which targets the pathways/molecules	Type Of CCA	Type of study	Biochemical tool/Animal /Human	Genes	Proteins	Analytical Techniques	Key results/Conclusion
Aguado-Frail et al., 2021	AKT pathway	Ivosidenib (AG-120) (Mutant IDH1 (mIDH1) inhibitor	IHCCA	Clinical trial phase I	73 patients: tumor biopsy before and after treatment	MYC, MAP2K1, JAG2	-	RNA NGS, GeoMx DSP, PureCN algorithm	- Suppression of production of the oncometabolite D-2-hydroxyglutarate, promoting disease stabilization & improving progression-free survival; stimulation of hepatocyte differentiation program in mIDH1 IHCCA; induction of expression of liver-specific genes. -mIDH1 inhibition: decrease of cytoplasm and expression of hepatocyte lineage markers in patients with prolonged PFS.
Zhou et al., 2021	Wnt/β-catenin signaling	-	IHCCA	*In vivo*	Brg1lox/lox& CK19-creERT mice	Brg1, BRM	AFP, CA 19-9, YAP, P-YAP, NOTCH1, T-B-catenin, NCID, Hes1	IHC, TMA, IMB	-Brg1: unclear role in expansion of IHCCA; binding to TCF4 transcription complex, suggesting a possible novel approach for regulation of Wnt/β-catenin signaling; overexpression in IHCCA and indicating poor prognosis. -PFI-3: functioning through Brg1.
Kendre et al., 2021	MAPK signaling	Infigratinib (FGFR inhibitors)	IHCCA	*In vivo* *In vitro*	Autochtho-nous murine model of ICC Cell lines: FGFR2PPHLN1, FGFR2-AHCYL1	FGFR2, KRAS	pERK,pMEK, pSHP2, pFRS2	shRNA transduction & depletion assays, FC, PCR, WB	-Co-mutational spectrum: accelerator of tumor development, and modification of the response to targeted FGFR inhibitors. -Combination therapy: potential to overcome primary resistance and to sensitize tumors to FGFR inhibition. -FGFR2 fusion-positive tumor cell lines harbouring a mutant Kras allele: resistant to FGFR inhibitors despite efficient interruption of downstream signaling.

Tan et al., 2020	PI3K/ AKT pathway	Copanlisib, Gemcitabine, Cisplatin	IHCCA EHCCA	Clinical trial Phase II	24 patients	PTEN, IDH1 TP53, KRAS/NR, CDKN2A, PBRM, CCND1, PIK3CA	PTEN	IHC, NGS	-Partial response in 6 cases (31.6%); stable disease in 11 cases (57.9%). -Most common grade 3/4 adverse events: decreased neutrophil count (45.83%), anemia (25%), increased lipase (25%) & hypertension (20.8%). -PFS for low PTEN epression (n = 9) = 8.5 months, high PTEN expression (n = 11) = 4.6 months. -Addition of copanlisib to gemcitabine and cisplatin: no improvement of PFS at 6 months. -No PTEN mutations observed.
Song et al., 2020	NF-κB	Anlotinib (tyrosine kinase inhibitor)	IHCCA	*In vivo* *In vitro*	ICC PDX models Human IHCC cell lines: HCCC9810, RBE	VEGRF2	N-cadherin, Vimentin, Asma, CK19, VEGRF2, E-cadherin	CCK-8 assay, Flow cytometry, RT-PCR, Western blot, IMF	-Significant effects on proliferation inhibition, migration & invasion restraint & cell-cycle arrestment; induction of apoptosis and the mesenchymal–epithelial transition; inhibition of IHCCA cell migration and invasion v*ia* EMT inhibition.excellent antitumor activity in IHCCA.
Wang et al., 2019	Tumorigenesis, YAP/Notch signalling	FBXW7 (WD repeat domain-containing 7) promote degradation of numerous oncogenic target proteins)	IHCCA	*In vivo* *In vitro*	Mouse model -20 tissue biopsy from IHCC patients -Human IHCC cell lines: RBE, KMCH, SNU1196 -Normal hepatocyte cell line: AML-12	FBXW7	Yap, Notch2, c-Myc	Western blotting, IHC	-Only c-Myc confirmed as a FBXW7 target in human CCA cell lines. -Selected ablation of c-Myc completely impaired iCCA formation in AKT/Fbxw7 F mice. -Deletion of Yap or Notch2 only delayed tumorigenesis. -Down-regulation of FBXW7 is ubiquitous in human iCCA and cooperates with AKT to induce CCA in mice v*ia* c-Myc-dependent mechanisms. -Targeting cMyc might represent an innovative therapy against iCCA exhibiting low FBXW7 expression.
Chen et al., 2019	Apoptosis, EMT process	α7-nAChR	IHCCA	*In vitro* *In vivo*	-70 human CCA tissue samples - Human CCA cell lines: QBC939, RBE (PLVT7 and PLVE1640–1643) Xenografts in nude mice	α7-nAChR	α7-nAChR, Bcl-2, Caspase-3, E-cadherin, Vimentin, P-Akt, Snail, GAPDH	IHC, Western blotting, qRT- PCR, CCK- 8 assay, Flow cytometry	-High expression of α7-nAChR in CCA tissues, closely related to a shorter survival time. -α7-nAChR knockdown: decreased cell proliferation ability, increased early apoptosis, and weakened cell migration and invasion. -Apoptosis-related proteins and components of the EMT process: altered after α7-nAChR knockdown. -Overexpression of α7-nAChR: induction of CCA progression by blocking apoptosis and promoting the EMT process.

Hu et al., 2019	Wnt/β- ca tenin signaling pathway	MIR22HG	IHCCA	*In vitro* *In vivo*	-64 pairs of CCA tumor and matched non- tumor tissues - Human CCA cell lines: RBE, CCLP1, HuCCT1,QBC 939, HIBEC Male BABL/c nude mice	MIR22HG	β- catenin, cyclin D1, c- myc	RT- PCR, CCK- 8 assay,Western blotting, IF, IHC	-Negatively regulated mRNA and the expression levels of proteins in the Wnt/β- catenin signaling pathway (β- catenin, cyclin D1, c-myc. -Suppresion of CCA cell proliferation, migration and invasion by negatively regulating the Wnt/β- catenin signaling pathway. -MIR22HG expression: significantly down-regulated in CCA tissues and cell lines. -Overexpression: inhibition of CCA cell proliferation, migration and invasion; knockdown: opposite result.
Lin et aal, 2019	STAT3 pathway, Non-T cell related immune mechanisms	Fibroblastic FAP	IHCCA	*In vivo* *In vitro*	Nude mice -6 tissues biopsy of ICC patients -Blood samples -CCA cell lines**:** QBC939, HCCC9810, RBE	fap	CCL2	Western blotting, CoIP, RT-PCR, ELISA, IHC, Flow cytometry	-Fibroblastic FAP expression: critical for STAT3 activation & CCL2 production. -ICCCAFs: primary source of CCL2 in human IHCCA. -Fibroblastic knockdown of FAP: significant impairment of the ability of ICCCAFs to promote ICC growth, MDSCs infiltration & angiogenesis. -Gene knockdown: no effect on IHCCA cell proliferation and apoptotic resistance.
Ahn et al., 2019	Activation of cancer, cellular growth and inflammation-related pathways predicted in subclass B - Metabolism related pathways predicted by activation in subclass A	-	IHCCA	*In vitro*	- 30 tumors tissue samples of ICCA from Korean patients - **3**2 iCCA tissue saples from US patients	95 genes for subgroup B, 53 genes for subgroup A -FGFR2 fusions (FGFR2BICC1, FGFR2-CDYL, FGFR2-WAC), KRAS, PMS2, TP53, ERBB2	-	RNA sequencing, Bioinformatics, Sanger sequencing	- Frequent viral hepatitis and cholangiolar-type pathology in subclass A (better prognosis). - Higher CEA and CA 19-9 levels, enriched inflammation related proteins & TP53 pathways with more frequent KRAS mutations in subclass B (poor prognosis); sensitive to gemcitabine in cell lines with similar gene expression patterns of subclass A.
Yamashita-Kashima et al.,	HER2	Trastuzuma, Emtansine (T-DM1, an antibody–	IHCCA	*In vivo*	Male or female BALB/c-nu/nu mice	-	ADCC HER2, EGFR, HER3,	Microarray, Flow cytometry, Western blotting, IHC, FISH	-Higher anti-proliferative activity of T-DM1 in CCA cell lines. -Antitumor activity in HER2-overexpressing BTC, associated with HER2 expression level or gene amplification.

2019		cytotoxic drug conjugate)		*In vitro*	Human CCA cell lines: KKU-055, KKU-100, KKU-213, KMBC, KMCH-1, SK-ChA-1		AKT, ERK		
Peng et al., 2019	VEGFA signaling	TA6/LOXL2	IHCCA	*In vitro* *In vivo*	- Tissue's specimens - CCA cell lines: QBC939, RBE PDX BALB/c nude mice	VEGFA	GATA6, LOXL2, VEGFA, MVD	RT-PCR, Western blotting, ELISA, TMA, IF, IHC	-GATA6 & LOXL2 expression: regulation of VEGFA expression, angiogenesis & tumor growth. -Drugs targeting this complex: great therapeutic value in the treatment of CCA. -Combination of both markers: independent prognostic indicator of CCA. -Interaction of LOXL2 with GATA6: regulation of VEGFA transcription, promoteion of VEGFA secretion & angiogenesis.
Khophai et al., 2018	Mitochondrial apoptotic pathway	Zileuton (leukotriene synthesis inhibitors…)	IHCCA	*In vitro*	-Tissue samples of CCA - Human CCA cell lines: MMNK, KKU-023, KKU-213, KKU-100	ALOX5, ALOX15	5-LOX, 15-LOX-1	IHC, IF staining, Western blotting, SRB assay Wound-healing assay	-5-LOX: correlation with CCA recurrent status. -15-LOX-1: significantly association with a longer survival time in CCA patients. -Zileuton: no inhibitory effect on CCA cell migration.
Zhao et al., 2018	Akt/Mtor	Compound C (inhibitor of AMP-activated protein kinase -AMPK)	IHCCA	*In vitro*	Human CCA cell lines: QBC939 and RBE	-	LDH, PARP, GAPDH, LC3, PAKT, AKT, P70S6K, ULK1, p38 MAPK, p53	RNA interference, Western blotting, RT–PCR, IMF, FM, CCK-8 assay	-Compound C: a potent inducer of CCA cell death & autophagy; increase of phosphorylated Akt, phosphorylated p70S6K; decrease of mTORregulatedp-ULK1(ser757). -p38MAPK inhibition: promotion of compound C-induced autophagy through JNK activation in human CCA cells.
Lampis et al., 2018	Mitochondrial oxidative metabolism	HSP90 inhibitors	HCA	*In vivo* *In vitro*	Xenografted mice Human CCA cell lines: EGI-1, TFK-1, SNU-1196, SW1, CCLP, SNU-1079	MIR21, IDH1, PBRM1, DNAJB5, HSP70	MIR21	Fluorimetric assay, PDO Targeting Sequencing, PDO, NanoString analysis	- Highest level of sensitivity to histone deacetylase inhibitors in CCA cells with IDH1 & PBRM1 mutations. -miRNA21: mediation of resistance of CCA cells to HSP90 inhibitors by reducing levels of DNAJB5 -HSP90 inhibitors: potetial as anti-CCA. -miRNA21: potential biomarker for sensitivity of CCA to HSP90 inhibitors.

Yu et al., 2018	Silencing ANGPTL4 expression	Long Non-coding RNA PVT1	IHCCA	*In vitro*	-17 paired CCA tumors tissue samples and normal tissues - Human CCA cell lines: HuCCT1, RBE	lncRNA, PVT1, ANGPTL4, PRC2, ETV5, EREG, ENC1, ANGPTL4 SPRY4, GDF15	-	RNA-sequencing, RNA-RIP, TMA, RT-PCR, Flow cytometry, CCK-8 assay, RNAFISH, IMF	-Long non-coding RNAs (lncRNAs): significant regulatory functions in many types of human cancers. -lncRNA PVT1 expression: significant elevation in CCA. -PVT1 knockdown: influence on target genes associated with cell angiogenesis, cell proliferation, and the apoptotic process; inhibition of CCA cell tumorigenesis *in vivo.* -PVT1: binding with EZH2 in the nucleus and epigenetic silencing ANGPTL4, and inhibiting proliferation & migration in CCA cells. -ANGPTL4: a target gene of PVT1.
Yakoi et al., 2018	AKT/mTOR & RAF/MEK/ ERK signaling pathways	Sorafenib (multikinase inhibitor of RAF/MEK/ERK pathway)	IHCCA	*In vitro*	Human CCA cell lines: RBE, YSCCC, Huh28	mTORC2	AKT, ERK, Ser473, mTORC1, mTORC2	Western blotting, MTT assay	-Enhancement of phosphorylation of AKT Ser473 & mTORC2 in CCA cells; enhancement of apoptosis presumably *via* increasing FOXO1.
Xu et al., 2018	Apoptosis	DcR3 (DcR3- siRN) , a protein with anti- apoptotic effect that belongs to the tumor necrosis factor receptor	IHCCA	*In vitro*	Human CCA cell lines: TFK- 1, RBE, HuCCT- 1	DcR3	DcR3	Western blotting, RT-PCR	-Silenced/knocked down by transfection with DcR3- siRNA; affected the growth and apoptosis of CCA; a predictive marker for malignant tumor a potential target for cancer gene therapy.
Srijiwangsa et al., 2018	STAT3	β-eudesmol	HCA	*In vitro*	Human CCA cell lines: KKU-100	-	NQO1, Bax/Bcl-2	Enzymatic assay, Western blotting, SRB assay, Flow cytometry, Fluorescence microscopy	-May serve as a potential anti-CCA candidate particularly when used in combination with conventional chemotherapeutics: significant suppression of NQO1 enzyme activity and protein expression in a concentration-dependent manner; potent cytotoxic activity; potentiation of the cytotoxic & inhibitory activities of 5-FU and doxorubicin on cell migration through induction of cell apoptosis and activation of caspase 3/7; enhancement of chemosensitivity associated with the suppression of NQO1 protein and activation of Bax/Bcl-2 protein expression ratio.
Sugihara et al.,	YAP/Hippo pathway	Dasatinib (SFK	IHCCA	*In vivo*	NOD/SCID mice	SRC, YES, LCK, FYN	SOX9, YAP, P-YAP,	IMP, IB, IMF, IHC, RT-PCR, Genome	-YAP: phosphorylation on tyrosine 357 (Y357) in CCA cell. -SFK inhibition with dasatinib: loss of YAPY357 phosphorylation,

2018		inhibitor)		*In vitro*	-Human CCA cell line: HuCCT-1 -Mouse CCA cell line: SB1 -Normal human cell line: cholangiocyte	Y, AP, LYN	LCK, SRC	analysis, RNA sequencing, Bioinformatics	promotion of its translocation from the nucleus to the cytoplasm. -LCK & SFK: most potent mediation of YAPY357 phosphorylation. -LCK expression: association with early tumor recurrence. -Tyrosine phosphorylation: regulation of YAP nuclear retention independent of the serine/threonine cytosolic retention pathway.
Kabashima et al., 2018	Non-apoptotic cell death	LY2874455 (Pan-FGFR inhibitor)	IHCCA	*In vivo*	Patient-derived xenograft mice	BCL2	MCL1: N-terminus truncated MCL1, full length MCL1	Western blotting, CRISPR/Cas9 genome editing, PCR, Extracellular flux analysis, IHC	-Induction of non-apoptotic cell death in the CCA cells; depletion of both outer mitochondrial membrane and matrix MCL1 species in CCA cells; causing necrotic cell death.
				In vitro	Human CCA cell lines: KMCH-1, KMBC-1				
Khoontawad et al, 2018	NF-κB signaling pathway	Curcumin	CCA	*In vivo*	Ov-induced CCA-hamsters	CYP2A6	S100A6, lumican, plastin-2, 14-3-3, zeta/delta, vimentin, clusterin	Isobaric labelling, Mass spectrometry, Western blotting, IHC, STITCH software, Soft clustering analysis,	-CCA and co-infection with *O. viverrine*: significantly affected the expression of proteins involved in adhesion, fibrolysis & extracellular matrix degradation. -S100A6: activation of the p38/MAPK pathway, leading to an increase ofCCA cell proliferation; opposite effect when silencing of S100A6. -Clusterin: activation of oncogenic transcription factor NF-κB. -NF-κB: important key player in Ov-induced CCA. -CYP4A14: down-regulation in CCA-hamsters; restoration to normal in CCA+Cur hamsters, suggesting a role for CYP4A14 in NDMA detoxification and anti-CCA mediated by curcumin treatment.
Lin et al., 2018	TGF-β pathway	Heteronemin	IHCCA	*In vitro*	Human CCA cell lines: HuccT1, SSP-25	p53, TGF-β, 105 genes		MTS Assay, RTCA, qRT-PCR, NanoString	-Suppression of mRNA expression of TGF-β, SMAD & Myc (regulation of cell growth, angiogenesis & metastasis); potent cytotoxic effects against CCA cells; alteration of abilities of cell adhesion & migration; modulation of cell adhesion, expression of ECM receptors, TGF-β pathway, cell motility, membrane integration, metastasis response, MMP remodeling; regulation of metabolism, sprouting angiogenesis, transcription factors & vasculogenesis in CCA cell lines

Su et al., 2017	TGF-β signaling pathway	GDH	EHCCA IHCCA	*In vitro*	-155 tissue biopsy from EHCC patients -Human CCA cell lines: QBC, RBE, FRN, FRH0201, 9810, HuH-28	-	GDH, Smad2, Smad3, Smad4, Smad 7, CA19-9, CEA	IHC, MTT assay, Flow cytometry, Western blotting RT-PCR	-Expression level: correlation with CD34 expression, cellular differentiation, the presence or absence of capsular and vascular invasion, lymph node metastasis, neural invasion & patient age. -Promotion of cell proliferation and metastasis through Smad-mediat ed induction of TGF-β signaling pathway.
Yeh et al., 2017	Raf/Erk/Elk-1 pathway	Regorafenib	IHCCA HCA	*In vivo*	Male or female BALB/c-nu/nu mice	ECH1, MALT1, ALAS1, IL-1α, IL-8	Elk-1, Ets-1 PARP, Caspase 9, Caspase 3	MTT assay, RT-PCR, Western blotting	-MALT1: potential target of regorafenib by activation of NF-κB, potential new therapeutic target for CCA. -Overexpression of Elk-1, but not Ets-1 in HuCCT1: marked reduction of sensitivity to regorafenib. -Regorafenib: inhibition of the growth of CCA cells and induction of apoptosis *via* down-regulation of MALT1 expression through suppression of the Raf/Erk/ Elk-1 pathway; confirmed activity in animal model.
				In vitro	Human CCA cell lines: HuCCT, KKU-100, SNU-1079, SNU-1196				
Walden et al., 2017	NF-kB	Xanthohumol	IHCCA	*In vitro*	Human CCA cell lines: CCLP1, SG-231, CC-SW-1	Notch1	cyclin D3, cyclin D1, cyclinE1, CDK4, p21, PARP, Surviving, XIAP	MTT asssy, Western blotting, Luminescence assay	- Significant reduction of CCA growth through the Notch1/AKT signaling; suppression of cell proliferation, colony formation & cell confluency; induction of cell cycle arrest & apoptosis through reduction of cell cycle regulatory proteins as well as an increase in pro-apoptotic markers (cleavage of poly ADP ribose polymerase & caspase 3) & a decrease in anti-apoptotic markers (X-linked inhibitor of apoptosis and survivin); reduction of Notch1 & AKT expression.
				In vivo	Xenograft study using CCLP-1 and SG231 cell lines in athymic mouse model				
Qian et al., 2017	aPKC-i/P-Sp1/Snail signaling pathway	aPKC-I	EHCCA	*In vivo*	Balb/C nude mice	aPKC-i	aPKC-i, P-aPKC-I, Snail, CD4, CD8, CD25, Foxp3, Sp1, P-Sp1	LC–MS,iTRAQ, co-IP, CCK-8 assay, IHC, RT-PCR, Western blotting, Flow cytometry	-Induction of EMT & immunosuppression by regulating Snail. -Sp1: direct phosphorylation by PKC-i on Ser59 (P-Sp1). -Sp1 & P-Sp1: upregulation in CCA tumor tissues; association with clinicopathological features & poor prognosis. -P-Sp1: regulation of Snail expression by increasing Sp1 binding to the Snail promoter; regulation of aPKC-i/Snail-induced EMT-like changes and immunosuppression in CCA cells. -aPKC-i: promotion of EMT & induction of immunosuppression; potential therapeutic target for CCA.
				In vitro	- Paired human EH CCA tissues and nontumor tissues -Human CCA cell lines: TFK-1, HuCCT-1				

Padthaisong et al., 2017	Akt signaling pathway	Nimotuzumab (Monoclonal antibody that inhibits EGFR activity)	IHCCA	*In vitro*	- Human CCA tissues - Human CCA Cell lines: KKU-214, KKU-213	EGFR	SNAIL1, Vimentin, MMP9, E-cadherin	IHC,Western blotting, Wound healing assay, Cell proliferative assay	-Inhibition of CCA cell growth and metastasis *via* suppression of the EMT process; inhibition of CCA cell invasion by reducing the expression of MP9.
Wang et al., 2016	EGFR/ FGFR signaling pathway	Ponatinib, Dovitinib, BGJ398 (FGFR inhibitors)	IHCCA	*In vivo*	PDX mouse model	FGFR2, BICC1 , FGFR2, AHCYL1 MMP2, MMP3, MMP9, CCDC6	FGFR2, p-FGFR, p-FRS2, p-AKT, p-ERK, MMP2, MMP3, MMP9	NGS, RT–PCR, FISH, IHC, Western blotting	-Modulation of FGFR signaling; inhibition of cell proliferation & induction of cell apoptosis in CCA tumors harboring FGFR2 fusions. -BGJ398: more potent than ponatinib and dovitinib in inhibiting the growth of CCA cells. -FGFR2-fusions: almost exclusively in the intrahepatic subtype.
Wang et al., 2016	Bcl-xL expression	CDDP + (chenodeoxycholic acid) FXR (farnesoid X receptor)	IHCCA	*In vivo* *In vitro*	Nude mice Human CCA cell lines: GBC-SD, RBE, SGC996, QGC939	SHP, Bcl-Xl, GAPDH	Caspase 3, Bax, BaK, MCL1, Bcl-2, Bcl-Xl, FXR, STAT3, SHP	Flow cytometry, Immunoblotting, MTT assay, RT-PCR, TMA	-FXR agonists GW4064 & CDCA (chenodeoxycholic acid) ▪ cisplatin (CDDP) -Combination treatment: marked enhancement of chemosensitivity of CCA cells. -Activation of FXR: induction off SHP expression, STAT3 phosphorylation, resulting in down-regulation of Bcl-xL expression.
Zhao et al., 2016	eIF2α & mTOR pathways	Salubrinal (eIF2a inhibitor), Rapamycin (mTOR inhibitor)	IHCCA	*In vivo* *In vitro* *In vitro* *In vitro*	QBC939 CCA xenografted nude mice Human CCA cell lines: QBC93, RBE -85 tissue samples of ICC patients - CCA cell lines: HuCCT1 Human CCA cell lines: QBC939, RBE	-	p-p70S6K, p70S6K, p-Akt, p-eIF2α, ATF4, Bcl-2, Bcl-Xl, PARP, Caspase-3	IHC, CCK-8 assay, Western blotting	-Both: significant inhibition of the proliferation of human CCA cells. -Inhibition of Akt by salubrinal: potentiation of the efficacy of rapamycin both *in vitro* and *in vivo*. -Rapamycin: up-regulation of Bcl-xL in a xenograft mouse model. - Rapamycin/salubrinal combination: more potent growth inhibition, antiproliferation effect & apoptosis induction (synergistic effects through regulating Akt signaling).
Rizvi et al., 2016	FGFR, YAP, Hippo	BGJ398 (pan-FGFR inhibitor)	IHCCA	*In vivo*	YAP-associated mouse model	FGFR, FGF, YAP, CTGF, SOX4,	TBX5, MST1, MST2	IHC, IMF, IMC, RT-PCR, ChIP, IP	- BGJ398: decrease in YAP activation in YAP-positive CCA cells and inducing cell death due to Mcl-1 depletion. -YAP & Hippo signaling pathways: Mcl-1-regulation of tumor

				In vitro	-Human CCA cell lines: KMCH (20), KMBC (21), and HuCCT-1 -Normal cell: NHC	MCL1, TBX5			survival pathway. -Nuclear YAP expression: a biomarker of FGFR-targeted therapy.
Lin et al., 2016	IL-6/ STAT3 pathway	miRNA-based therapy *via* IL-6/STAT3 targeting	EHCCA	*In vivo*	Nude mice	let-7c, miR-99a miR-125b	IL-6, IL-6R IGF1R,VEGF,TGF-β	RT-PCR, Sequencing, Western blotting	-let-7c, miR-99a & miR-125b: down-regulation in CCA & targeted IL6, IL6R & IGF1R. -Enforced expression of let-7c, miR-99a or miR-125b: reduction of STAT3 activity and suppression of CCA cell migration & tumorigenicity *in vivo*. let-7c/miR-99a/miR-125b cluster significant decrease of the ability of CCA cells for cancer stem cell-like mammosphere generation by down-regulating CD133 and CD44. -Potential links between miRNAs & inflammation.
				In vitro	-6 CCA tissues samples -Human CCA cell lines:MZ-cha-1, SK-cha-1				
Barat et al. 2016	Tyrosine kinase c-MET signaling	LY2801653	IHCCA	*In vivo*	9 Xenograft nude mice	c-MET, p-MET	HGF, c-MET, p-MET, p-ERK, ERK, p-AKT, AKT, p-STAT3, STAT3, RAC1, HIF-1a	Microarray, IHC, Western blotting, Immunoblotting, Flow cytometry	-Inhibition of c-MET: a possible alternative treatment of CCA. -MET inhibition by LY2801653: through blocking the phosphorylation of MET. -LY2801653: partially down-regulation og HIF-1α expression, especially in TFK-1; effective inhibition over the activation of c-MET & p-MET under hypoxic conditions.
				Invitro	Human CCA cell lines: TFK-1 SZ-1 Huh-7 -breast carcinoma cell line MCF 7				
Dokduang et al., 2016	STAT3, Akt-NFκB	Xanthohumol	IHCCA	*In vivo*	BALB/cAJcl-nu/nu mice		Cyclin D1, CDK4, Bcl-2, BAX, Ki67	Western blotting, IHC, TUNEL	-Inhibition of CCA cell growth & apoptosis through inhibition of STAT3 activation both *in vivo* and *in vitro* due to suppression of the Akt-NFκB signaling pathway.
				In vitro	Human CCA cell lines: M214, M139				

Guest et al., 2016	PI3K/AKT/mTOR and Hes/Hey pathways	NOTCH3	pCCA IHCCA	*In vivo*	8-weeks male Sprague–Dawley rates, Transgenic mice, Mice carrying constitutive deletion of the Notch 3 gene	p53, Notch1, Notch2, Notch4, Fas, Fadd, Rac1	NOTCH3, RBPJ, Ki67	NOTCH PCR array, RT-PCR, IHC, Western blotting, IMF	-Differential overexpression across species; progressive up-regulation with disease development; promotion of tumor cell survival *via* activation of PI3k-Akt. - Notch 3 deletion: significant tumor growth suppression. - Notch 3 silencing: suppression of Hes/Hey expression. - Notch 3: an important driver in CCA, driving cell survival independently of RBPJ.
				In vitro	1-5 tissue samples 2- 48 CC cases compared with control 3- Human CCA cell lines: CC-LP-1				
Huang et al., 2016	AKT/Mtor	SMIs of β-hydroxylase inhibitors	IHCCA	*In vivo*	Male nude mice	HEY1, HES1	ASPH, Notch receptors, Cyclin D1	Immunoblotting, RT-PCR	-Enzymatic activity of ASPH: critical for mediating CCA progression & inhibition of apoptosis. -Apoptosis stimulation: through increasing caspase 3 cleavage, reducing enzymatic activity *via* exposure to a SMI ofASPH's enzymatic activity. -ASPH overexpression: promotion of Notch activation & modulation of CCA progression through Notch1-dependent cyclin D1 pathway. -Targeting ASPH with shRNAs or a SMI: significant suppression of CCA growth *in vivo*.
				In vitro	- Human CCA cell line: ETK1, H1, NEC, RBE, SSP25 ,BDE-Neu, CL24 , BNLT3, Hep3B -HepG2 , Huh7, - SkHep1 -HAK1A -HAK1B -HEK-293T - Normal hepatocyte cell line: OUMS-29				
Chan-On et al., 2015	FoxM1 signaling	Clioquinol (CQ), Nitroxoline (NQ)	IHCCA	*In vitro*	Human CCA cell lines: HuCCT, Huh28	FoxM1, MMP-2, MMP-9	Cyclin D1, p21	RT- PCR, MTS assay, Wound healing assay, Western blotting	-CQ: a dose- & time-dependent decrease in cell viability, with down-regulation of oncogenic FoxM1 & cyclin D1 and up-regulation of p21.

Chen et al., 2015	AKT & ERK signaling pathways (SPHK1)/ (S1P) pathway (SPHK1)/ (S1P) pathway	SK1-I (SPHK1 inhibitor)	IHCCA	*In vitro* *In vivo*	-96 patients followed up regularly until death -Human CCA cell lines: HuCCT, SNU478 12 male BALB/c nude mice	SPHK1	SPHK1, SPHK2, PARP, Caspase 9, Caspase 3	Microarray, Western blotting, Flow cytometry, IHC, Bioinformatics	-Increase of intracellular ceramide and inhibition of ERK & AKT signaling when combined with JTE013; growth arrest associated with cell accumulation in sub-G1 phase; apoptosis induction in CCA cell lines; inhibition of CCA proliferation *in vitro* and *in vivo;* -SPHK1: an independent marker of poor CCA prognosis.
Huang et al., 2015	Apoptotic pathways	WWOX	IHCCA	*In vitro*	-113 ICC patients tissue sample - Human CCA cell lines: RBE, HCCC-9810, HuCCT1, HIBEpiC	WWOX	Bcl-2, Bax, Caspase 9, Caspase 3, Caspase 8	Western blotting, IF, IHC, RT- PCR	-Expression of WWOX in IHCCA tissues: much lower than that in nontumorous. -WWOX expression: suppression of the growth of WWOX-deficient ICC cells through activation of the intrinsic apoptotic signaling pathway. -Restoration of WWOX expression: suppression of the growth of WWOX-deficient ICC cells through activation of the intrinsic apoptotic signaling pathway; no effect on WWOX-sufficient human intrahepatic biliary non-cancer cells. -Down-regulation of WWOX: a result of hypermethylation and implying a poor prognosis in IHCCA. -WWOX re-expression: a potential molecular therapeutic target for IHCCA.
Lederer et al., 2015	HGF / Met/ mitogen-activated protein kinase pathway	MACC1 (regulator of HGF/Met/ mitogen-activated protein kinase pathway)	IHCCA, Klatskin tumor	*In vitro*	- Tissues biopsy: IHCC = 80 Klatskin: 76 normal bile duct tissue :3 -Human CCA cell line EGI-1	-	MACC1, Met, HGF	RT-PCR, IHC	-Klatskin tumor patients with a history of tumor recurrence: significant higher MACC1 expression than those without tumor recurrence. -Klatskin tumor patients with high MACC1: significant shorter overall (OS) & disease-free survival (DFS). -MACC1: a potential prognostic biomarker for OS and DFS in Klatskin tumor patients.
Marti et al., 2015	YAP)/Hippo pathway	YAP, MFAP5	IHCCA	*In vitro*	- Human CCA tissues - Human CCA Cell lines: HuCCT, SNU-478	YAPS127A, YAPS94A, shYAP1/2	MFAP5, CD311	Microarray, Western blotting, RT-PCR	-YAP: regulation of genes involved in proliferation, apoptosis & angiogenesis of CCA; promotion of CCA cell growth by functionally interacting with TEADs; a key regulator of proliferation and antiapoptotic mechanisms in CCA.
Wang et al., 2015	Wnt/β-catenin signaling	hUC-MSCs	EHCCA	*In vivo* *In vitro*	Male BALB/c-nu/nu mice Human CCA cell line: QBC939, Mz-ChA-1	MMPs family, cyclin D1, c-Myc	MMPs, cyclin D1, c-Myc	IHC, IF, Western blotting, Flow cytometry, Transwell assay, Colony-forming assay, Annexin V/PI assay and Edu Assay	-MSCs: modulation of tumor growth & metastasis, increasing CCA cells proliferation & metastatic potency; improvement of drug resistance administration in xenograft tumor mice. -MSCs-CM: stimulation of Wnt activity by promoting the nuclear translocation of β-catenin & up-regulation of Wnt target genes MMPs family, cyclin D1, c-Myc. -MSCs & MSCs-CM: promotion of CCA cell proliferation & migration through targeting the Wnt/β-catenin signaling.

Shirota et al., 2015	Posttranslational folding, Tumorigenesis	NVP-AUY922 (HSP90 inhibitor)	IHCCA, EHCCA	*In vivo*	8-weeks-old female BALB/c-nu/nu mice	-	HSP90, AKT, pAKT EGFR, pEGFR, HER2, pHER2	IHC, Western blotting	-HSP90: a key component of a multichaperone complex involved in the posttranslational folding of several tumorigenic proteins. -HSP90 positive cells: 44.6% in IHCCA, 32.8% in EHCCA. -Potent antiproliferative activity and reduction of growth-associated signaling in human CCA cells *invitro.* -HSP90 overexpression: a prognostic marker for CCA.
				In vitro	-Tissue biopsy from 399 CCA patients: 276 males**,** 123 females**;** 177 cases of IHCC, 222 cases of EHCC -Human CCA cell lines: IHCC cells- NCC-CC1, NCC-CC3-1, NCCCC3-2, NCC-CC4-1, NCC-CC4-2, NCC-CC5, NCC-CC6-1, NCC-CC6-2, TKKK, TGBC24TKB, HuCCT1 EHCC cells- NCCBD1, NCC-BD2, NCC-BD3, NCC-BD4-1, NCC-BD4-2, OZ				
Chang et al., 2014	RAS/RAF/MEK/ERK pathway	Anti-EGFR	IHCCA EHCCA	*In vitro*	Tissue samples of CCA patients: 57 IHCC, 45 EHCC	EGFR, KRAS, BRAF	-	DNA sequencing, PCR, IHC	-Overexpression of EGFR: 8.1-47% of CCA cases in Taiwan & Japan. -EGFR mutation: more common in EHCCA than in IHCCA; an independent prognostic marker in addition to tumor stage and differentiation.
Gao et al., 2014	Neddylation	MLN4924	IHCCA	*In vivo*	QBC939 subcutaneous xenograft Mice	-	E1, E2, NEDD8, pIκB-α, PCNA, Ki-	IHC, Flow cytometry, Microarray	- Inhibition of the growth of CCA cell lines & primary IHCCA cells through induction of G2 cell-cycle arrest, followed by apoptosis or senescence in CCA cells. -E1 (NAE1, UBA3) & E2 (UBC12) enzymes & global NEDD8

				In vitro	- Tissue samples from 322 consecutive ICC patients - Human CCA cell lines: QBC939, RBE		67		conjugation: up-regulation in over 2/3 of human IHCCA. - NAE1: an independent prognosticator for postoperative recurrence; a combination of NEDD8 & NAE1: a better power for predicting patient clinical outcomes. -Up-regulation of neddylation pathway: involves with IHCCA progression.
Borad et al., 2014	EGFR/ FGFR signaling pathways	Ponatinib (pan-FGFR inhibitor)	IHCCA	Clinical trial phase II	1 patient	FGFR2-MGEA5 fusion	-	Genome-wide structural analysis, FISH, IHC, CT	-Anti-tumor activity: aproximately 6 weeks after initiation of therapy; shrinkage of metastatic lymph nodes, stable disease.
		Erlotinib (EGFR kinase inhibitor)	SIC		2 patients	EGFR ERRFI1 mutation	-		-Rapid & robust disease regression in ERRFI1 inactivated tumor; significant tumor shrinkage & decrease in lymph nodes size; significant reduction in metabolic activity
		Pazopanib (FGFR2 anti-tumor)	IHCCA		2 patient	FGFR2-TACC3	-		-Anti-tumor activity on a patient with an FGFR2-TACC3 fusion; significant tumor shrinkage.
		Pazopanib, ponatinib	SIC		1 patient				-Initial response to pazopanib, followed by disease progression followed by resistance and subsequently responded to ponatinib.
		PEGPH20	IHCCA		1 patient	ERRFI			no response to therapy
									-FGFR2 fusions & ERRFI mutations: novel targets in SIC. - Immediate treatment with EGFR and FGFR tyrosine kinase inhibitors: recommended in SIC patients with tumors harboring ERFFI1 or FGFR fusions

Churi et al., 2014	DNA repair genes, MAP/ERK, mTOR, FGFR	IDH1, ERBB2	EHCCA	*In vitro*	55 ihCCA patients, 20 ECCA patients	AKT1, BRAF, FGFR1, GNAS, IDH1, FGFR2, KRAS, NRAS, PIK3CA, MET, RET, EGFR, JAK2, MPL, PDGFRA, PTEN, TP53, FGFR3, FLT3, KIT, ERBB2, ABL1, HNF1A, HRAS, ATM, RB1, CDH1, SMAD4, STK11, ALK, SRC,SMARCB1 , VHL, MLH1, CTNNB1, KDR, FBXW7, APC, CSF1R, NPM1, SMO, ERBB4, CDKN2A, NOTCH1 , JAK3, PTPN11	-	PCR, NGS, DNA sequencing, Immunohistochemistry, Ingenuity pathway analysis, IPA	-IDH1 & DNA repair gene alterations: more frequent in IHCCA; ERBB2 Gas: more frequent in EHCCA. -KRAS, TP53 or MAPK/mTOR Gas: significant association with worse prognosis; FGFR Gas:association with a relatively indolent disease course. -BAP1 & PBRM1: association with bone metastases & worse survival in EHCCA.
			IHCCA, EHCCA	Clinical trials phase I & II	7 Patients treated with erlotinib	KRAS mutation	-	-	-Sustained stable disease
					1 Patient treated with Pazopanib +	KRAS mutation	-	-	-Experienced stable disease after prior progression on first-line chemotherapy

					Trametinib				
					2 Patients treated with BRAF inhibitor	BRAF mutation	-	-	-Partial responses
					1 Patient treated with c-met inhibitor	c-met mutation	-	-	-Experienced a metabolic response
					2 Patients treated with Trastuzumab and Lapatinib	her2/neu mutations	-	-	-No metabolic response
Seubwai et al., 2014	EMT pathway Rho/ROCK2	DHMEQ (NF-kB inhibitor)	IHCCA	*In vivo*	NOD/Scid/Jak 3 deficient (NOJ) male mice	-	p50, p52, p65	MTT assay, Flow cytometry, Western blotting, IHC, IMF	- Significant inhibition of human CCA cell line growth in a dose- and time-dependent manner through nhibitory action on NF-κB; increase of cell apoptosis by decreasing antiapoptotic protein expressions–Bcl-2, XIAP–and activating caspase pathway.
				In vitro	- Tissues specimens - Human CCA cell lines: KKU-M139, KKU-M156, KKU-M213, KKUM214, KKU-100				
Ding et al., 2013	ERK1/2 and AKT signals	Integrin α6	IHCCA	*In vitro*	- 20 tissue samples from ICCA patients & 20 nontumor samples - Human CCA cell lines: RBE, HuH-28, SSP-25, HCCC-9810 -Hepato-cellular carcinoma cell line: Hep2G	ITGA6	AKT, p-AKT, ERK, p-ERK, FAK, p-FAK	IHC, RT-PCR, Western blotting, MTT Assay	-Integrin α 6 expression in ICC tissues: much higher than that in nontumor samples; significant inhibitaion of the ability of IHCCA cells to metastasize; significant inhibition of cell proliferation. -Integrin α6 overexpression: significant association with larger tumors, multiple nodular, microvascular/bile duct invasion & lymphatic metastasis, and a migratory and invasive phenotype of IHCCA.

Dokduang et al., 2013	I3K/Akt, Ras/MAPK, JAK/STAT, Wnt/β-catenin	Sorafenib, Sunitinib (multi-targeted kinase inhibitors)	IHCCA	*In vitro*	-20 tissue samples from ICCA patients and their adjacent tissues - Human CCA cell lines: M156, M214, OCA17, KKU100	-	Protein kinases	Western blotting, Phospho-RTKs & Phospho-kinase arrays, Alarmar Blue assay, caspase-Glo® 3/7 assay	-Erk1/2 7 Akt activation: increased in CCA tissues than in normal tissues. - Significant cell growth inhibition & apoptosis induction *via* suppression of Erk1/2 and Akt activation. - Drugs with specificity to a single kinase showed lower potency. -Multiple kinase proteins in CCA: potential therapeutic targets for anti-CCA.
Hong et al., 2013	Akt-NFκB	Rapamycin	IHCCA	*In vitro* *In vivo*	Human CCA cell lines: SCK JCK, Cho-CK, Choi-CK Six-week-old male nude mice	STAT3	p-AKT (S473), p-mTOR (S2448), p-STAT3 (S727), STAT3	Western blotting, IMP	-Prolonged treatments with rapamycin: disruption of mTORC2 assembly and reduction of STAT3 phosphorylation at Ser 727. - Decrease of both mRNA & protein levels of MMP2 & Twist1. -Suppression of the motility of sarcomatoid CC by down-regulating STAT3 S727 through the impairment of mTORC2 assembly. - Overexpression of STAT3 S727A: lack of phosphorylation site, resulting in significantly low sensitivity to rapamycin than the overexpression of STAT3 WT.
Iwaki et al., 2013	EGF-Dependent Migration	AZA (DNAdemethylating agent 5-aza-29-deoxycytidine (5) + HDAC (histone deacetylase (HDAC) inhibitor trichostatin A= Autophagy Activator)	IHCCA	*In vitro*	Human Cell lines: HuCCT1, HIBEpiC, TKKK, Huh28, IHGGK, TFK-1	miR-376c	IL 1β, MMP-9	Transwell migration assay, Microarrays, Western blotting, RT-PCR, Cell migration assay, Gene expression analysis	-Epigenetic repression of miR-376c: acceleration o EGF-dependent cell migration through its target GRB2. -miR-376c: a tumor suppressor; potential targets for IHCCA.
Zhang et al., 2013	VEGF and COX-2 signaling pathways	miRNAs	CCA	Invitro	CCA cell lines: CCLP1, HuCCT1, andTFK1 noncancerous CC cell: H69	VEGFmRNA	VEGF COX-2 Luciferase PGE2	In situ hybridization qRT-PCR Western blot IHC	▪ Forced overexpression of miR101 significantly inhibited CCA growth in severe combined immunodeficiency mice ▪ decreased capillary densities and decreased levels of VEGF and COX-2 miR-101 inhibits CCA angiogenesis by targeting VEGF directly and indirectly via inhibition of COX2ederived PGE2 signaling

Peng et al., 2013	Lipid mediator pathways (LTs and LXs)	miR-200b/c	pCCA dCCA	*In vivo*	PDX BALB/c nude mice	SUZ12, ROCK2	-	Microarray, RT PCR, Northern & Western blotting	-Ectopic miR-200b/200c: inhibition of the migration and invasion of CCA cells; regulation of cell migration and invasion capacities by directly targeting rho-kinase 2 & regulation of tumorigenic properties by directly targeting SUZ12; a critical role in the regulation of the tumorigenic and metastatic capacity of CCA and reveals the probable underlying mechanisms.
				In vitro	-Human CCA tissues - Human CCA Cell lines: TFK-1, QBC939				
Chen et al., 2012	Akt & Nrf2 pathways	AIB1	IHCCA	*In vitro*	-20 patients: tissue sample -Human CCA cell lines: HCCC9810, QBC939, SKChA-1, Mz-ChA-1	GPx2, GCLC, GCLM	AIB1, Akt Cyclin A, Cyclin B, Cdk1, Bcl-2	Western blotting, Flow cytometry, PCR	-Induction of G2/M arrest & down-regulation of mitosis-promoting factors including cyclin A, cyclin B & Cdk1 through suppressing the Akt pathway, resulting in inhibiting CCA cell proliferation; regulation of the expression of Bcl-2; suppression of intracellular ROS; increase of the expression of ABCC2 & ABCG2; down-regulation of AIB1 expression and enhancing sensitivity of CCA cells to chemotherapeutic drugs.
Andersen et al., 2012	EGFR and HER2 signaling	Trastuzumab, Lapatinib (Receptor tyrosine kinase (RTK) inhibitors)	IHCCA	*In vitro*	-104 tissue samples of CCA patients - Human CCA cell lines: YSCCC, RBE, KMCH, KMBC, SSP25, HuCCT1, WITT	KRAS, EGFR, BRAF, ITGA2, MPRSS4C, A 0CAM6	TNF, TGF-β, ERK1/2	Transcriptomics, Western blotting, IHC	-Lapatinib (an inhibitor of HER2 & EGFR): more potent inhibition of CCA growth than trastuzumab. -Prognostic 36-gene classifier, either alone or in combination with other molecular predictors: improvement of the molecular classification and outcome prediction in CCA. - Limited success in CCA.
Gui et al., 2012	EGFR-signaled pathways	EGF	IHCCA	*In vitro*	-Human CCA cell lines: RBE -Human cell lines: MMNK-1,	EGFR	pY1068, p-p44/42, MAPK, Tyr1045	IF, IP, Confocal fluorescence microscopy, Flow cytometry ,RT-PCR, cDNA sequencing, RNA interference ,Western blotting	-Impaired EGFR degradation: association with hypo-phosphorylation of Tyr1045 and enhancement of recycling of EGFR to the cell membrane. -Gene & protein expression levels of EGFR before EGF stimulation: similar between RBE & MMNK-1 cell lines. -EGFR protein expression: marked down-regulation under sustained EGF stimulation in MMNK-1 cells.

Kamigaki et al., 2011	Classical MAPK pathway	Pitavastatin, Atorvastatin (Statins)	IHCCA	*In vitro*	Human CCA cell lines: HuCCT1, YSCCC	-	Caspase-3, p-ERK	FACS, Western blotting, CCK-8 assay	-Statin: reduction of G2M fraction and increase of sub-G1 fraction; increase of cleaved caspase 3 level and decreased of p-ERK; in combination with gemcitabine, cisplatin & 5-FU: additive antiproliferative activity; apoptosis induction *via* suppression of the classical MAPK pathway.
Loilome et al., 2011	PI3K/Akt, JAK/STAT, Wnt/b-catenin	PKA inhibitor, cAMP analogs	IHCAA	*In vitro*	-Tissue's biopsy -Human Cell lines: M156, OCA17, KKU100, M214	PRKAR1A, PRKAR2B	Protein kinase A	RT-PCR, IHC, Western blotting, Flow cytometry, Phospho-kinase array	-PRKAR1A: a potential drug target for CCA therapy. -Silencing PRKAR1A expression: induction of growth inhibition and apoptosis, with an associated decrease in mitogen-activated protein kinases, PI3K/Akt, JAK/STAT & Wnt/b-catenin pathway signaling. -Inhibition of PKA using a PKA inhibitor and cAMP analogs: significant cell growth inhibition.
Shen et al., 2011	Mitochondrial Caspase-dependent pathway	β- Escin	IHCCA	*In vitro*	Human CCA cell lines: QBC939, Sk-ChA- 1, MZ-ChA- 1	-	bcl- 2, bax, p53, cyclin D, cyclin B1, $p21^{Cip1/Waf1}$	MTT assay, Flow cytometry, Fluorescence microscopy, Western blotting	-Inhibition of CCA cell growth in a dose- and time- dependent manner through apoptosis indcution associated with the collapse of the mitochondrial membrane potential and the activation of caspase 3 and the increase in ROS. -Cell cycle arrest of QBC939 & Sk- ChA- 1 cells in the G2/M phase, and MZ- ChA- 1 cells in G1 phase.
Zhang et al., 2010	ErbB1 & ErbB2 signaling	Lapatinib (dual ErbB1/ErbB2 inhibitor)	IHCCA	*In vivo*	Rat model	c-erbB2	Phospho-ErbB2Tyr1248, ErbB1, ErbB2, Caspase-3	IHC, Western blotting, IHC, ELISA, FISH	-Lapatinib: significant suppression of IHCCA when administered early. -Tryphostin AG879 targeting ofErbB1 & ErbB2 signaling: significantly more effective in suppressing the growth of both rat and human CCA cells than individual receptor targeting. -Lapatinib: CCA apoptosis induction regardless of differences in their levels of ErbB1 or ErbB2 protein expression.
		Tryphostin AG1517 (ErbB1 inhibitor) AND/OR Tryphostin AG879 AND Lapatinib	EHCCA	*In vitro*	-Human CCA cell lines: HuCCT1, TFK1 -Rat CCA cell lines: BDEneu, C611B				
Yabuuchi et al., 2009	p27Kip1	ZD1839 (IRESSA®),an EGFR-TK inhibitor	EHCCA	*In vitro*	-Human CCA cell lines: TFK- 1, HuCCT- 1	EGFR	p27Kip1, Jab1, EGFR	Western blotting, TUNEL, DNA sequencing, Immunostaining, MTS assay	-Induction of apoptotic cell death; dose-dependent inhibition of cell proliferation; increase of p27Kip1 stability associated with Jab1 down-regulation; control of cell proliferation & apoptosis; reduction of cell survival after radiation exposure.

Yoshikawa et al., 2009	VEGFR-2 /EGFR pathway	Vandetanib (ZD6474) (inhibitor of VEGFR and EGFR signalling)	IHCCA	*In vivo* *In vitro*	8-week-old female BALB/c-nu/nu athymic mice -Xenografted mice -Human CCA cell lines: OZ, HuCCT1, TGBC24TKB, TKKK	EGFR, VEGF, VEGFR-2	VEGF, Pegfr, MAPK, AKT, pAKT	RT–PCR, Western blot, MTS assay, FISH, IHC *In vivo* tumour imaging	-Inhibition of both VEGFR & EGFR signaling; a promising therapeutic approach for CCA. -Absence of KRAS mutation & presence of EGFR amplification: potential predictive molecular marker of sensitivity to EGFR-targeted therapy.
Jinawath et al., 2007	Hedgehog and ERK1/2 pathways	Cyclopamine and/or U0126	IHCCA	*In vitro*	Human CCA cell lines: HuCCT1, HuH-28, MEC, TFK-1, RBE, TGBC24TK, KKU-M156	KRAS, BRAF	Cycline D1, Cyclin B1	WST-1 assay, Fluorescence-activated cell sorter, Western blotting, Cell proliferation assay, RT-PCR	-Cyclopamine: decrease of cell proliferation & arrest f cell cycle at the G1 phase. -U0126: decrease of CCA cell proliferation with KRAS mutation stronger than with wild-type KRAS. -Combination of both inhibitors: additive antiproliferative effect. -Hedgehog & ERK1/2 pathways: important for CCA cell proliferation; simultaneous inhibition: stronger decrease of cell growth & viability in a subset of CCA cases.
Chen et al., 2006	Fas-mediated apoptosis, AKT Signaling	-3,3†-diindolylmethane (DIM), a vegetable autolysis product AKT & FLIP: molecular targets	EHCCA	*In vivo* *In vitro*	8-week athymic female BALB/c mice 10 human CCA tissue samples	-	Caspase-3, Caspase -8, Caspase -9 NF-KB CaMKII, AKT, pAKT, FLIP	IHC, Western blotting, Flow cytometry	-Fas-resistant CCA cells: increase of AKT phosphorylation compared with Fas-sensitive cells. -DIM: promotion of Fas-mediated apoptosis of CCA cells; inhibition of AKT phosphorylation; activation of FLICE like-inhibitory-protein (FLIP); an effective sensitizer to Fas-mediated apoptosis in CCA cells *via* inhibition of AKT and FLIP activation. -Inhibitors of phosphatidylinositol 3-kinase/Akt signaling: enhancement of Fas-mediated apoptosis in CCA cells.
Zhang et al., 2004	Apoptotic signaling pathways	Celecoxib, Rofecoxib (cyclooxygenase-2 or COX-2 inhibitor)	CCA	*In vivo* *In vitro*	344 Male Fischer rats Rat CCA cell line: C611B	-	PGE2, Bcl-2, Bcl-xL, COX-2, MAPKs, Caspase-9, Caspase-3, PARP	Western blotting, DAPI	-Celecoxib: potent inhibition of PGE2; induction of apoptosis in C611B ChC cells through Akt inactivation, Bax translocation & cytochrome c release; enhancement of translocation of Bax to mitochondria and released cytochrome c into cytosol.