

Review Article

Primary Cholangiocellular Carcinoma Cell Lines

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Abstract

Cholangiocellular Cancer (CCC) is a deadly disease and 5-year overall survival still is below 10%. To improve prognosis of patients with CCC, basic science is absolutely dependent on cell lines to elaborate new insights into pathological, diagnostic and therapeutic options. This is a systematic review about CCC cell lines described in literature to date. Different cell lines were established and characterized in the last 30 years. CCC cell lines can generally be classified into cell lines derived from intrahepatic CCC (iCCC), extrahepatic CCC (eCCC), perihilar CCC (Klatskin) and metastasis. The aim of this review is to give insights in the availability of cell lines and the possibilities that these cell lines might give to researchers focused on CCC.

Introduction

Cholangiocellular Carcinoma (CCC) is a malignancy arising from epithelial cells of the biliary tree. The first case of common bile duct cancer was described by Durand-Fardel in 1840 [1]. Depending on tumor location CCC are differentiated as intrahepatic or extrahepatic. The latter can be further classified as distal and perihilar. 60-70% of CCC arise at the hepatic bifurcation (perihilar) and were first reported by Klatskin in 1965 [2]. Approximately 20-30% accounts for tumors in the distal common bile duct and 5-10% for the intrahepatic type [3]. Known risk factors for cholangiocarcinoma include primary sclerosing cholangitis [4], fibrocystic liver diseases (e.g. Caroli's syndrome) [5], parasitic infection (e.g. *Opisthorcis viverrini*) [6], viral hepatitis and cirrhosis [7], intrahepatic biliary stones [8] and chemical carcinogen exposure (e.g. thorotrast [9], smoking and alcohol [10,11]).

The incidence rate of CCC varies widely, e.g. 1-2/100,000 in the USA and 96/100,000 in Thailand [12]. In general, in Western countries and Japan the incidence of extrahepatic CCC is higher than intrahepatic CCC, whereas in Eastern countries the opposite can be seen [13]. Epidemiologic studies indicate a global increase of incidence and mortality rate for iCCC with stable or decreasing rates for eCCC [14,15].

However, the current treatment options remain still insufficient with a 5-year overall survival rate of 5-15%. The only curative approach is surgical therapy with radical resection, but up to 80% of patients present with advanced disease initially [16].

To elaborate diagnostic and therapeutic options and improve the prognosis of patients basic science is absolutely fundamental. For this purpose, tumor cell lines are essential. Tumor cell lines are an important source of material for "omic" studies, and they allow functional studies that help to better understand tumor biology, especially molecular pathogenesis and pathophysiology [17,18].

However, the amount of cell lines derived from CCC is limited. The majority of research in CCC has been done with only two cell lines EGI-1 and TFK [19-21]. A disadvantage of a limited number of cell lines' use is that these cells cannot sufficiently represent tumor heterogeneity of CCC. Cell lines are prone to genetic drift and shift that

might alter the tumor genome in the course of time. Additionally, cell lines that are cultivated long-term are prone to be contaminated with rapidly growing cell lines like HeLa cells. Due to this the establishment of new cell lines is beneficial [22]. The aim of the present review gives an overview on CCC cell lines available for researchers that focus on this tumor entity. We will discuss characteristics of these cell lines as well as origin of the cell lines, techniques and media that were used to establish these CCC cell lines.

Overview on cholangiocarcinoma cell lines

In the 19th century, the concept of tissue culture was introduced by Wilhelm Roux. Since the first successful isolation of cell lines in the 60ties, several CCC cell lines were described in literature (Table 1).

Yamaguchi established the first CCC cell line from a specimen of an intrahepatic CCC from a patient's autopsy in 1984 [23]. The specimen was minced in small pieces about 1mm diameter and then dispersed in a culture flask with Ham's F12 medium containing 0.1% FBS (fetal bovine serum). The cell line was later known as *HChol-Y1*.

The most frequent used cell line in research, *TFK-1*, was established from a fragmented eCCC tumor specimen of a 63 years old male. After digesting with dispase (1,000 U/ml), it was cultured in 12-well plates using RPMI-1640 medium containing 10% FBS with antibiotic agents. The TFK-1 cells grew in polygonal epithelial monolayers with a cell doubling time of 37 hours. A modal chromosome number of 73 with some structural abnormalities was observed. The tumor markers, CEA and CA-19-9, were not found in successful xenotransplanted SCID mice. Several surface antigens were detected and revealed promising approaches for targeted therapy with e.g. cetuximab. The majority of experimental studies on CCC today is performed with this cell line.

Classification of CCC cell lines

Classification of CCC cell lines can be based on the localization of the native tumor. As already described above, cell lines can be derived from iCCC, eCCC or Klatskin CCC. A sub-classification of the cell lines from primary tumor and metastases can be made. iCCC and eCCC represent 39% and 37%, respectively of the main isolated locations of cell lines in literature. Cell lines of Klatskin tumors account only for 5% of all cell lines and 19% of CCC cell lines are derived from metastases.

Table 1: CCC cell lines described in literature.

Yamaguchi, 1985 [23]	HChol-Y1	Explant, iCCC	Ham F12 + 0.1%FBS	M, DD, HC, Chr, Xeno, TM
Knuth, 1985 [24] Andresen, 2012 ¹	Mz-ChA-1	Explant, abdominal wall mets of GB	CMRL + 15%FBS + AA	M, DD, HC, Chr, Xeno, TM, FA
	Mz-ChA-2	Explant, liver mets of GB	CMRL + 15%FBS + AA	M, DD, HC, Chr, Xeno, TM, FA
	Sk-ChA-1	Ascites, eCCC-mets	CMRL + 15%FBS + AA	M, DD, HC, Chr, Xeno, TM, FA
Murakami, 1987 [28] Andresen, 2012 ¹	KMCH-1	Explant, CCC/HCC	DMEM + 20%FBS + AA	M, DD, HC, Chr, Xeno, TM, FA
Scherdin, 1987 [48] Andresen, 2012 ¹	EGI-1	Explant, eCCC	DMEM + 10%FBS	M, DD, IHC, mut, DNA, Xeno, FA
Homma, 1987 [49]	Oz	Ascites, iCCC-mets	W/E + 10%FBS + AA	M, DD, HC, Chr, Xeno
Kusaka, 1988 [50]	HuH-28	Explant, iCCC	RPMI 1640 + 20%FBS	M, DD, HC, Chr, Xeno, TM
Katoh, 1988 [27]	CHGS	CCC	-	M, DD, HC, Xeno
Miyagiwa, 1989 [30] Andresen, 2012 ¹	HuCC-T1	Ascites, iCCC-mets	RPMI 1640 + 10%FBS + AA	M, DD, HC, IHC, Chr, mut, FA, TM, Xeno
Storto, 1990 [29] Yokomuro 2000 [51]	PCI:SG231	Explant, iCCC	DMEM + 15%FBS + AA	M, IHC; Chr, Xeno, FA
Yoshida, 1990 [52]	MEC	Pleural Effusion, eCCC-mets	-	M, DD, HC, IHC, Chr, Xeno, TM
Sirisinha, 1991 [45]	HuCCA-1	Explant, iCCC	Ham F12 + 10%FBS + AA	M, DD, HC, IHC, Chr, TM
Iemura, 1992 [31]	KMC-1	Xenograft, iCCC	DMEM + 20%FBS	M, DD, HC, Chr, TM
Shimizu, 1992 [42]	CC-SW-1	Explant, iCCC	DMEM + 15%FBS + AA	M, DD, HC, IHC, Chr, Xeno, TM, FA
	CC-LP-1	Explant, iCCC	DMEM + 15%FBS + AA	M, DD, HC, IHC, Chr, Xeno, TM, FA
Yano, 1992 [53] Andresen, 2012 ¹	KMBC	Explant, eCCC	RPMI 1640 + 5%FBS + AA	M, DD, IHC, Chr, Xeno, TM, FA
Purdum, 1993 [39] Oertel, 2003 [54]	BDC	Explant, eCCC	DMEM + 10%FBS + AA	M, DD, HC, FA
Saijyo, 1995 [55] Andresen, 2012 ¹	TFK-1	Explant, eCCC	RPMI 1640 + 10%FBS + AA	M, DD, IHC, Chr, Xeno, TM, FA
Yamada, 1995 [35]	OCUCh-LM1	Explant, eCCC-mets (liver)	DMEM + 10%FBS + AA	M, DD, IHC, Chr, DNA, Xeno, TM
Yano, 1996 [33]	KMCH-2	Explant, iCCC	DMEM + 20%FBS + AA	M, DD, Chr, Xeno, TM
Enjoji 1997 [41,56]	ETK1	Ascites, iCCC-mets	RPMI 1640 + 10%FBS	M, DD, HC, IHC, Chr, Xeno, FA
	RBA	Explant, iCCC	RPMI 1640 + 10%FBS	M, DD, HC, IHC, Chr, Xeno, FA
	SSP-25	Explant, iCCC	RPMI 1640 + 10%FBS	M, DD, HC, IHC, Chr, Xeno, FA

Wang, 1997 [57] Wu, 2003 [58]	QBC939	-,eCCC	DMEM + 10%FBS + AA	FA
Takiyama, 1998 [59]	ICBD-1	Explant, eCCC	DMEM + 10%FBS	M, DD, Chr, Xeno, TM, FA
Watanabe, 2000 [43]	TK	Ascites, eCCC-mets	RPMI 1640 + 15%FBS	M, DD, HC, IHC, Xeno, TM
Jiao, 2000 [34]	HBDC	Ascites, Klatskin-mets	W/E + 10%FBS	M, DD, HC, IHC, Chr, DNA, Xeno, TM, FA
Kim, 2001 [60]	SCK	Explant, eCCC	DMEM + 10%FBS + AA	M, HC, IHC, Chr, DNA
	JCK	Explant, eCCC	DMEM + 10%FBS + AA	M, HC, IHC, Chr, DNA
	Cho-CK	Explant, eCCC	DMEM + 10%FBS + AA	M, HC, IHC, Chr, DNA
	Choi-CK	Explant, eCCC	DMEM + 10%FBS + AA	M, HC, IHC, Chr, DNA
Steffen, 2001 [61] Moore 1971 [29]	RPMI 7451	Explant, -	DMEM + 15%FBS + AA	M, IHC; Chr, Xeno, FA
Ku, 2002 [18]	SNU-245	Explant, eCCC	RPMI 1640 + 10%FBS	M, DD, mut, DNA
	SNU-308	Explant, gall bladder	RPMI 1640 + 10%FBS	M, DD, mut, DNA
	SNU-478	Explant, ampulla of Vater	RPMI 1640 + 10%FBS	M, DD, mut, DNA
	SNU-869	Explant, ampulla of Vater	RPMI 1640 + 10%FBS	M, DD, mut, DNA
	SNU-1079	Explant, iCCC	RPMI 1640 + 10%FBS	M, DD, mut, DNA
	SNU-1196	Explant, Klatskin	RPMI 1640 + 10%FBS	M, DD, mut, DNA
Emura, 2003 [62]	TGBC-47	Explant, eCCC	-	M, DD, HC, IHC, Chr, TM
Ghosh, 2005 [26]	TGBC-51	Explant, ampulla of Vater	-	M, DD, HC, IHC, Chr, TM
	TBCN6	Explant, eCCC	-	M, DD, HC, IHC, Chr, TM
Sripa, 2005 [40]	KKU-100	Explant, Klatskin	Ham F12 + 20%FBS + AA	M, DD, HC, IHC, Chr, Xeno, TM
Rattanasinganchan, 2006 [44]	RMCCA-1	Explant, eCCC	Ham F12 + 20%FBS + AA	M, DD, HC, IHC, Chr, TM, FA
Ma, 2007 [63]	HKGZ-CC	Explant, iCCC	DMEM + 10%FBS + AA	M, DD, HC; Chr, Xeno
Ojima, 2010 [32]	NCC-BD1	Xenograft/Explant, eCCC	RPMI 1640 + 10%FC + AA	M, HC, IHC, mut, FA
	NCC-BD2	Xenograft/Explant, eCCC	RPMI 1640 + 10%FC + AA	M, HC, IHC, mut, FA
	NCC-CC1	Xenograft/Explant, iCCC	RPMI 1640 + 10%FC + AA	M, HC, IHC, mut, FA
	NCC-CC3-1	Xenograft/Explant, iCCC	RPMI 1640 + 10%FC + AA	M, HC, IHC, mut, FA
	NCC-CC3-2	Xenograft/Explant, iCCC	RPMI 1640 + 10%FC + AA	M, HC, IHC, mut, FA
	NCC-CC4-1	Xenograft/Explant, iCCC	RPMI 1640 + 10%FC + AA	M, HC, IHC, mut, FA
Liu, 2013 [64]	HCCC-9810	Explant, iCCC	DMEM + 10%FBS	M, DD, HC, Xeno, FA

Origin of cell lines is indicated (intrahepatic= iCCC; extra hepatic= eCCC; perihilar= Klatskin; metastasis= suffix "-Mets") (FBS= fetal bovine serum; AA= antibiotic agent; M= morphology; DD= doubling time; HC= histochemistry; IHC= immunohisto chemistry; CHR= chromosomal analysis; TM= tumor/biochemical markers; Xeno= xenograft; mut= mutational analysis; DNA= DNA index; FA= functional analysis; DMEM= Dulbecco's modified Eagle medium; W/E= Williams E medium)

Table 2: Characterization of selected CCC cell lines.

Author, Year	Cell line	Morphology	HC	DD	Chr	DNA index	mut	TM	FA
Purdum, 1993 [39] Oertel, 2003 [54]	BDC	-	-	-	-	-	-	-	Photodynamic therapy
Shimizu, 1992 [42]	CC-SW-1	LM, EM	HE	72h	-	-	-	HLA1, No AFP/ CEA/CA19-9,	Growth by insulin, inhibition by IL1-beta
	CC-LP-1	LM, EM	HE	180h	-	-	-	HLA1, No AFP/ CEA/CA19-9,	Growth by Insulin and EGF, inhibition by IL1-beta
Scherdin, 1987 [48] Andresen, 2012 ¹ Xu [38], 2010	EGI-1	-	-	-	-	-	kras	EGFR, HGFR, IGF1R IGF2R, VEGFR1, VEGFR2	Cetuximab; Genes for promoter methylation
Jiao, 2000 [34]	HBDC	LM,PC	HE, PAS, CEA- 125, CK-19, vimentin	32.3h – 35.8h	76-93	0.85	-	CA19-9,Span- 1,KMO-1, ALP, γGT	-
Yamaguchi, 1985 [23]	HChol-Y1	LM, EM	HE, PAS, anti- CEA, anti-AFP, anti CA 19-9	52h	54-70	-	-	CA 19-9, CEA, No AFP	-
Ma, 2007 [63]	HKGZ-CC	LM, PC	HE	48h	64-74	-	-	-	-
Sirisinha, 1991 [45]	HuCCA-1	LM	HE	55h	-	-	-	CA125, MCA, AFP	-
Miyagiwa, 1989 [30] Andresen, 2012 ¹	HuCC-T1	-	-	-	-	-	-	-	Genes for promoter methylation
Kusaka, 1988 [50]	HuH-28	LM,PC	PAS	80h	59 (modal)	-	-	BMG, Elastase, Ferritin,TPA, ALP, γGT, No AFP/CEA/ CA19-9	-
Yano, 1992 [53] Andresen, 2012 ¹	KMBC	-	-	-	-	-	-	-	Genes for promoter methylation
Knuth, 1985 [24] Andresen, 2012 ¹	Sk-ChA-1	EM, PC	-	48h	61-164	-	-	CEA, NANA, PHI, LDH, HLA-I, C3/C5, α1-anti-Trypsin, α2-macro- globulin	Genes for promoter methylation
Iemura, 1992 [31]	KMC-1	LM, EM, PC	HE, PAS, mucine	54h	73-83	1.76	-	TPA,hCG, CA19-9, CEA, Ferritin	-
Murakami, 1987 [28] Andresen, 2012 ¹	KMCH-1	LM, PC, EM	HE, PAS, mucicarmine	39h	60-98	-	-	No AFP, No CEA	Genes for promoter methylation
Yano, 1996 [33]	KMCH-2	LM,EM	HE, alcian blue, mucicarmine	30h	40-41	-	-	CA 19-9, TPA, CEA, Ferritin	-
Sripa, 2005 [40]	KKU-100	LM, PC	HE	72h	56-92	-	-	Cytokeratin, CEA, CA125, EMA	-
Ojima, 2010 [32]	NCC-BD1	LM	HE	-	-	-	Kras, p53 (3/5, 60%)	MAGEH-1	Gemcitabine sensitivity

	NCC-BD2	LM	HE	-	-	-	Kras, p53 (3/5, 60%)	MAGEH-1	Gemcitabine sensitivity
	NCC-CC1	LM	HE	-	-	-	Kras, p53 (3/5, 60%)	MAGEH-1	Gemcitabine sensitivity
	NCC-CC3-1	LM	HE	-	-	-	Kras, p53 (3/5, 60%)	MAGEH-1	Gemcitabine sensitivity
	NCC-CC3-2	LM	HE	-	-	-	Kras, p53 (3/5, 60%)	MAGEH-1	Gemcitabine sensitivity
	NCC-CC4-1	LM	HE	-	-	-	Kras, p53 (3/5, 60%)	MAGEH-1	Gemcitabine sensitivity
Yamada, 1995 [35]	OCUCh-LM1	LM, EM, PC	HE	31h	63 (modal)	1.76		SLX, CA-19-9, SPan, CEA	-
Homma, 1987 [49]	Oz	-	-	-	-	-	No kras mut	EGFR, HGFR, IGF1R IGF2R, VEGFR, VEGFR2	Cetuximab; genes for promoter methylation
Storto, 1990 [29] Yokomuro 2000(51)	PCI:SG231	LM, EM,PC	Anti-keratin, anti-CEA	-	67 (modal)	-	-	Keratin, CEA	-
Rattanasinganchan, 2006 [44]	RMCCA-1	LM, PC	HE	48h	54-61	-	-	CA-19-9, AE1/AE3, MMP	Motility invasion assay
Saijyo, 1995 [55] Andresen, 2012 ¹ Xu [38], 2010	TFK-1	LM, EM	HE	37h	73	-	No kras mut	EGFR, HGFR, IGF1R IGF2R, VEGFR1, VEGFR2	Cetuximab;genes for promoter methylation
Watanabe, 2000 [43]	TK	LM, EM, PC	HE,PAS,anti-CEA, anti-CA-19-9	29h	66-132	-	-	CEA, CA-19-9, No AFP	-

(LM= light microscope;EM= electron microscope; PC= phase contrast microscope; HE= Hematoxylin and Eosin; PAS= Periodic Acid Shift)

In current literature, there are some cell lines derived from the biliary tract that are not particularly from CCC. These are cell lines from gall bladder cancer [24,25] cancer of the ampulla of Vater [26] and not further characterized cholangiocarcinoma cell lines [27], or from combined hepatocellular and cholangiocarcinoma neoplasms (HCC/CCC) [28].

The following table gives an overview about the 52 CCC cell lines described in literature so far (Table 1).

Isolation techniques used for the establishment of CCC cell lines

As shown in Table 1 different techniques are available to establish CCC cell lines. In general, explant culture is the most frequently used technique [29]. Processing malignant body fluids such as pleural effusion or ascites with a culture medium is another frequent method to establish cell cultures [30]. Xenografting technique is the inoculation of tumor specimens into athymic mice and provides a successful source to establish cancer lines [31,32]. Actually one must consider a fast processing time of samples which is essential for the success rate of cell lines. Contaminating cells such as fibroblasts can be mechanically removed by a sterile cannula under a phase contrast microscope or by enzymatic digestion. Typically, a varying amount (0.1% - 20%) of FBS or FC (10%) combined with antibiotic agents to reduce bacterial contamination are used for the propagation process. In literature, Ham’s F12, CMRL, DMEM, Williams E and RPMI 1640 are the basic culture mediums used to establish CCC cell lines.

Characterization of CCC cell lines

The analysis of the genome, transcriptome and proteome can

give interesting insights into the pathophysiology of CCC (table 2). In former times, chromosome analysis was performed by characterization and counting chromosomes in Giemsa staining with a wide range of different chromosome numbers between 40 and 164 [33]. Some authors also describe nuclei DNA content by flow cytometric methods [34,35]. Today, spectral karyotyping (SKY), a technique based on fluorescence in situ hybridization (FISH) [36], enables the painting of each chromosomes in different colors. By this means, chromosomes with unknown origin or structural abnormalities (e.g. complex translocations) can easily be identified by visual interpretation [37]. However, there was no study so far performing this analysis in CCC cell lines.

Mutational analysis is also commonly performed. Kras and p53 mutations constitute the typical genetic fingerprint of intrahepatic cholangiocellular carcinoma and this was studied in some cell lines [25,32].

Investigations of proteins involve the study of proteomes and secretomes. Depending on cell line different structural proteins e.g. receptors (EGFR, HGFR, IGF1R IGF2R, VEGFR1, VEGFR2) [38], intermediate filaments (keratin [39,40], vimentin [41]), antigens (HLA-1 [42], MAGEH-1 [32]) or secreted proteins e.g. typical tumor markers (CEA [23,24,26], CA-19-9 [43,44], AFP [44,45], CA-125 [45]) were detected. There is a heterogeneous variety of protein expression among the cell lines demonstrating the differences in tumor biology [25,32,42].

Conclusion

Basic research in cancer is absolutely dependent on cancer cell lines

to understand tumor biology. In the last three decades, several CCC cell lines could be established by researchers around the world. The establishment of primary CCC cell lines can be done with reasonable expenses and renders interesting insights into the molecular biology of CCC. The success rate of establishing cell lines is very low. The typical ratio that is given in most publications is about 10%. There are several obstacles for the successful isolation. First of all, not every (little) piece of tissue taken from a tumor sample contains tumor cells. The time from sampling to processing plays a major role and should not be too long because cells in a non-physiological environment are prone to necrosis. Even if these two obstacles are considered not every tumor sample will show growth of tumor cells, even in optimal culture conditions. An underlying reason for this might be the genetic make-up of the tumor cells [46]. Even in the rare case of outgrow that is seen in about 20% of cases there might be fungal or bacterial contamination or the cancer cell might be overgrown by fibroblasts [47]. A success rate of 10% seems very low. The effect of genetic versus other factors of influence is hard to assess. An approach to improve isolation techniques might be to test new medias or supplements for the isolation of CCC. This could be achieved by testing established CCC cell lines.

In this review, we listed systematically all established CCC cell lines in the world literature so far. By this, we hope to give researchers interested in CCC a tool to better represent the heterotypic tumor biology of CCC.

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