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Establishment of various biliary tract carcinoma cell lines and xenograft models for appropriate preclinical studies

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Abstract

We recently reported several driver genes of biliary tract carcinoma (BTC) that are known to play important roles in oncogenesis and disease progression. Although the need for developing novel therapeutic strategies is increasing, there are very few BTC cell lines and xenograft models currently available for conducting preclinical studies. Using a total of 88 surgical BTC specimens and 536 immunodeficient mice, 28 xenograft models and 13 new BTC cell lines, including subtypes, were established. Some of our cell lines were found to be resistant to gemcitabine, which is currently the first choice of treatment, thereby allowing highly practical preclinical studies to be conducted. Using the aforementioned cell lines and xenograft models and a clinical pathological database of patients undergoing BTC resection, we can establish a preclinical study system and appropriate parameters for drug efficacy studies to explore new biomarkers for practical applications in the future studies.

Key words: Biliary tract carcinoma; Cell line; Xenograft model; Preclinical study

Core tip: Although the need for developing novel

therapeutic strategies for biliary tract carcinoma (BTC) is increasing, there are only few xenograft models and cell lines available for *in vivo* and *in vitro* studies, respectively. To conduct appropriate preclinical studies, we established 28 xenograft models and 13 new BTC cell lines using several surgical BTC specimens and immunodeficient mice. Using the aforementioned cell lines and xenograft models and a clinical pathological database of patients undergoing BTC resection, we can establish appropriate parameters for drug efficacy studies to explore new biomarkers for practical applications in the future studies.

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TO THE EDITOR

Biliary tract carcinoma (BTC) is an extremely malignant tumor. The incidence and mortality rates of BTC are currently rising and are particularly high in Asian countries. Surgical resection is the only curative treatment; however, most cases are diagnosed to be at advanced and inoperable stages by the time patients visit a hospital. The most serious problem is that there are no efficient chemotherapeutic regimens for patients with inoperable or recurrent BTC. Worldwide, gemcitabine-cisplatin combination therapy is the first choice, but clinicians are not satisfied with its efficacy. New drugs are needed for BTC patients.

Recently, we conducted genomic analyses of clinical specimens from 260 patients, which is the largest study till date, wherein we identified genomic abnormalities, which could be potential therapeutic targets, in 32 driver genes that play important roles in oncogenesis and disease progression in approximately 40% of BTC patients[1]. Although the need for developing novel therapeutic strategies is increasing, there are very few BTC-related resources currently available for conducting preclinical studies. The main reasons are as follows: the number of surgical BTC patients is not high at a single institute, and there is no large clinicopathological database. It is difficult to obtain surgical specimens for basic research. Therefore, there are only few xenograft models and cell lines available for *in vivo* and *in vitro* studies.

To conduct appropriate preclinical studies, surgical BTC specimens (collected from Japanese patients at the National Cancer Center Hospital, Tokyo, Japan since 2005 in an appropriate manner without any interference to pathological diagnosis) were directly transplanted into immunodeficient mice and subjected to cell culture medium to establish xenograft models and cell lines, respectively, as reported in 2010[2]. From a total of 88 BTC specimens and 536 immunodeficient mice during the period 2005-2013, we established 28 xenograft models (18 intrahepatic cholangiocarcinoma, four perihilar, and six distal BTC) and 13 new BTC cell lines, including subtypes (eight intrahepatic cholangiocarcinoma, two perihilar, and three distal BTC) (Table 1). Some of our established cell lines were found to be resistant to gemcitabine (Table 2), thereby allowing highly practical preclinical studies to be conducted. In addition, we conducted molecular pathology analyses of cell lines and constructed a clinical pathological database of patients undergoing BTC resection to establish appropriate parameters for drug efficacy studies to explore new biomarkers for practical applications (Figure 1)[2-5]. All experiments were approved by the Animal Care and Ethics Committee of the National Cancer Center (ID: T05-046). This study was approved by the Ethical Committee of the National Cancer Center (ID: 2007-022).

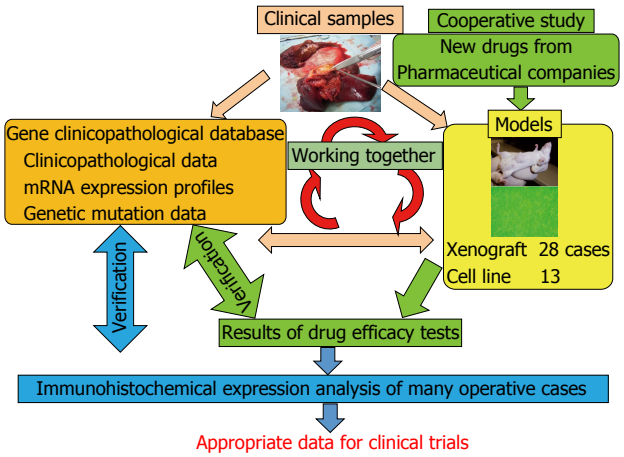

Figure 1 Relationship between our materials and databases. There are three key factors: clinical samples, databases, and biliary tract carcinoma (BTC) models. Both the models and the databases are derived from the clinical samples. These databases comprise "clinicopathological data", "mRNA expression profiles", and "genetic mutation data". BTC models are "xenograft models" and "cell lines". These models are used for cooperative studies with pharmaceutical companies for translational research. For example, they provide us with new anti-cancer drugs, and we can perform drug efficacy tests. If necessary, we can also perform an immunohistochemical expression analysis. Then, we can compare the results of the analysis with those in the databases and validate them. After these steps, we can provide appropriate data to clinicians. Together, these databases and materials make translational research far more detailed and suitable for clinical trials.

Preclinical studies have found very little evidence regarding the combined effects of prospective anticancer combination therapies, including gemcitabine. Therefore, we continue to examine the combined effects of the utility of the Bliss method and combination index to assess the prognosis of BTC. Moreover, we are going to release some of our resources and data in the near future. We believe that our materials and data will not only aid in conducting appropriate preclinical studies but

Table 1 Clinicopathological features of original biliary tract tumors

Xenograft	Pathological diagnosis of original tumor	Age/sex	Histologic type	Prognosis (survival days)	Chemotherapy	Clinical evaluation of chemotherapy effect (effective days)	Established cell line
1	CCC	70/F	Adeno, mod	Death (402)	Non		NCC-CC1
2	CCC	71/F	Adeno, mod	Death (175)	Non		NCC-CC3-1 NCC-CC3-2
3	CCC	59/M	Adeno, mod	Alive (2172)	Non		NCC-CC4-1 NCC-CC4-2 NCC-CC4-3(NCC-CC5)
4	CCC	31/M	Adeno, mod + PSC	Death (386)	GEM + TS1	SD (84 d)	NCC-CC6-1 NCC-CC6-2
5	Distal BDCa	58/F	Adeno, mod	Death (299)	GEM	PD	NCC-BD1
6	Distal BDCa	77/F	Adeno, mod	Death (393)	GEM	PD	NCC-BD2[1]
7	Distal BDCa	80/M	Adeno, mod	Death (212)	Non		NCC-BD3
8	Hilar BDCa	74/M	Adeno, mod	Death (172)	Non		NCC-BD4-1 NCC-BD4-2
9	Hilar BDCa	48/M	Adeno, well	Alive (500)	GEM	PD	NA
10	Hilar BDCa	43/M	Adeno, mod	Alive (1422)	Non		NA
11	CCC	69/M	Adeno, mod	Death (174)	Non		NA
12	CCC	54/F	Adeno, mod	Death (181)	Non		NA
13	CCC	56/M	Adeno, mod	Death (319)	GEM	PD	NA
14	CCC	73/M	Adeno, mod	Death (53)	Non		NA
15	CCC	54/M	Adeno, mod	Alive (2608)	Non		NA
16	CCC	45/F	Adeno, mod	Alive (882)	GEM + CDDP	Unknown	NA
17	CCC	72/M	Muc	Death (749)	GEM/GEM + TS1	Unknown	NA
18	CCC	78/M	Adeno, mod	Death (382)	GEM	Unknown	NA
19	CCC	66/M	Adeno, mod	Death (168)	Non		NA
20	CCC	65/M	CoCC	Alive (1604)	Non		NA
21	CCC	70/M	Adeno, por	Death (851)	GEM	SD (49 d)	NA
22	CCC	63/F	Adeno, mod	Alive (363)	Unknown	Unknown	NA
23	CCC	72/M	Adeno, mod	Death (394)	GEM	PD	NA
24	CCC	77/F	Adeno, mod	Death (445)	GEM	SD (105 d)	NA
25	Hilar BDCa	66/M	Adeno, mod	Alive (102)	GEM + TS1	Unknown	NA
26	Distal BDCa	54/M	Adeno, mod	Alive (2096)	Non		NA
27	Distal BDCa	67/M	Adeno, mod	Death (672)	GEM + TS1	PD	NA
28	Distal BDCa	80/M	Adeno, mod	Alive (2024)	GEM	PR-CR (548 d)	NA

[1]BD2 was obtained from the direct culture of patient specimens. CCC: Cholangiocellular carcinoma; BDCa: Bile duct carcinoma; Adeno: Adenocarcinoma; mod: Moderately differentiated; PSC: Primary sclerosing cholangitis; Muc: Mucinous carcinoma; CoCC: Cholangiolocellular carcinoma; por: Poorly differentiated; non: No chemotherapy received; GEM: Gemcitabine; CDDP: Cisplatin; SD: Stable disease; PD: Progressive disease; PR: Partial response; CR: Complete response.

Table 2 Sensitivity to gemcitabine in each cell line

Cell line	Sensitivity to gemcitabine in cell line[1]			
	IC_{50} (μmol/L)	IC_{60} (μmol/L)	IC_{70} (μmol/L)	IC_{80} (μmol/L)
NCC-CC1	86.78	N.A	N.A	N.A
NCC-CC3-1	0.04	1.82	9.31	85.21
NCC-CC3-2	0.10	1.92	43.83	N.A
NCC-CC4-1	0.05	4.08	N.A	N.A
NCC-CC4-2	0.03	11.53	N.A	N.A
NCC-CC4-3 (NCC-CC5)	0.06	4.92	95.10	N.A
NCC-CC6-1	0.01	0.02	0.06	3.76
NCC-CC6-2	10.98	35.67	N.A	N.A
NCC-BD1	7.66	58.00	N.A	N.A
NCC-BD2	N.A	N.A	N.A	N.A
NCC-BD3	N.A	N.A	N.A	N.A
NCC-BD4-1	0.04	0.06	0.09	2.93
NCC-BD4-2	0.06	0.07	0.19	5.37

[1]The cytotoxicity of gemcitabine for each cell line was assessed by a modified 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt assay with CellTiter 96 Aqueous One Solution Reagent (Promega, Madison, WI, United States). Tumor cells (3000 cells/well) in the exponential growth phase were grown in 96-well plates. IC: Inhibitory concentration.

also accelerate basic research of BTC.

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