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Review Article

Application and Progress of Cultured Models of Gallbladder Carcinoma

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Abstract

Gallbladder carcinoma (GBC) is a malignant tumor of the biliary system that is aggressive, difficult to detect early, and has a low surgical resection rate and poor prognosis. Appropriate *in vitro* growth models are expected to focus on the study of the biological behavior and assess treatment effects. Nonetheless, cancer initiation, progression, and invasion include spatiotemporal changes and changes in the cell microenvironment intracellular communication, and intracellular molecules, making the development of *in vitro* growth models very challenging. Recent advances in biomaterial methods and tissue engineering, particularly advances in bioprinting procedures, have paved the way for advances in the creative phase of *in vitro* cancer research. To date, an increasing number of cultured models of gallbladder disease have emerged, such as two-dimensional (2D) GBC growth cell cultures, three-dimensional (3D) GBC growth cell cultures, xenograft models, and 3D bioprinting methods. These models can serve as stronger platforms, focusing on tumor growth initiation, the association with the microenvironment, angiogenesis, motility, aggression, and infiltration. Bioprinted growth models can also be used for high-throughput drug screening and validation, as well as translational opportunities for individual cancer therapy. This study focused on the exploration, progress, and significance of the development of GBC cultural models. We present our views on the shortcomings of existing models, investigate new innovations, and plan future improvements and application possibilities for cancer models.

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Introduction

Gallbladder carcinoma (GBC) is one of the most life-threatening diseases worldwide. Its morbidity and mortality are increasing, with 70–80% of patients diagnosed at an advanced stage.[1] Surgery remains the only possible treatment for GBC.[1] However, because of the atypical clinical symptoms of GBC, lymphatic metastasis, and distant metastasis are prone to occur, so only 10% of patients are in a state suitable for surgical resection at the time of diagnosis.[2] For patients with advanced GBC, three classes of chemotherapy drugs are available, gemcitabine, fluoropyrimidines, and platinum-based compounds. Monotherapy has limited efficacy.[3] A combination of gemcitabine and cisplatin (CDDP) improves survival in such patients.[4] Radiation therapy has limited efficacy in patients with advanced disease. Targeted therapy against the epidermal growth factor receptor (EGFR) inhibits proliferation *in vitro* and may provide an effective therapeutic modality in the future.[5] However, comprehensive treatment is still ineffective and the prognosis is very poor. The 5-year survival rate is only 5–15%.[6] Strengthening basic research in the occurrence and progression of GBC is the key to improve the prognosis of patients.

GBC appears to result from a combination of genetic susceptibility and environmental factors.[7] A hypothetical oncogenic process is gallstone-induced inflammation → p53 mutation (↓) → K-ras mutation (↑).[8] Several diseases and chronic inflammation are considered risk factors for GBC, including cholelithiasis, porcelain gallbladder, gallbladder polyps, chronic salmonella infection, and congenital bile duct cysts.[9] The mechanism of cancer occurrence and development has not been fully elucidated, which seriously

Keywords: Gallbladder cancer; 2D gallbladder cancer cell culture; 3D gallbladder cancer cell culture; Xenograft model; 3D bioprinting technology.

Abbreviations: 2D, two-dimensional; 3D, three-dimensional; 5-FU, 5-fluorouracil; AFP, alpha-fetoprotein; CAF, cancer-associated fibroblasts; CDDP, cisplatin; CDX, cell-derived xenograft; CEA, carcinoembryonic antigen; CSF, colony-stimulating factor; CT, computed tomography; E-cad, epithelial cadherin; ECM, extracellular matrix; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; EMT, epithelial-mesenchymal transition; FGF, fibroblast growth factor; GBC, gallbladder carcinoma; GFP, green fluorescent protein; HGF, hepatocyte growth factor; IGF, insulin-like growth factor; JSH, Japan Society of Hepatology; LCSGJ, Liver Cancer Study Group of Japan; MMP, matrix metalloproteinase 1; MRI, magnetic resonance imaging; PDX, patient-derived xenograft; TACE, transarterial chemoembolization; VM, vasculogenic mimicry; VP, verteporfin; YAP1, yes-associated protein 1.

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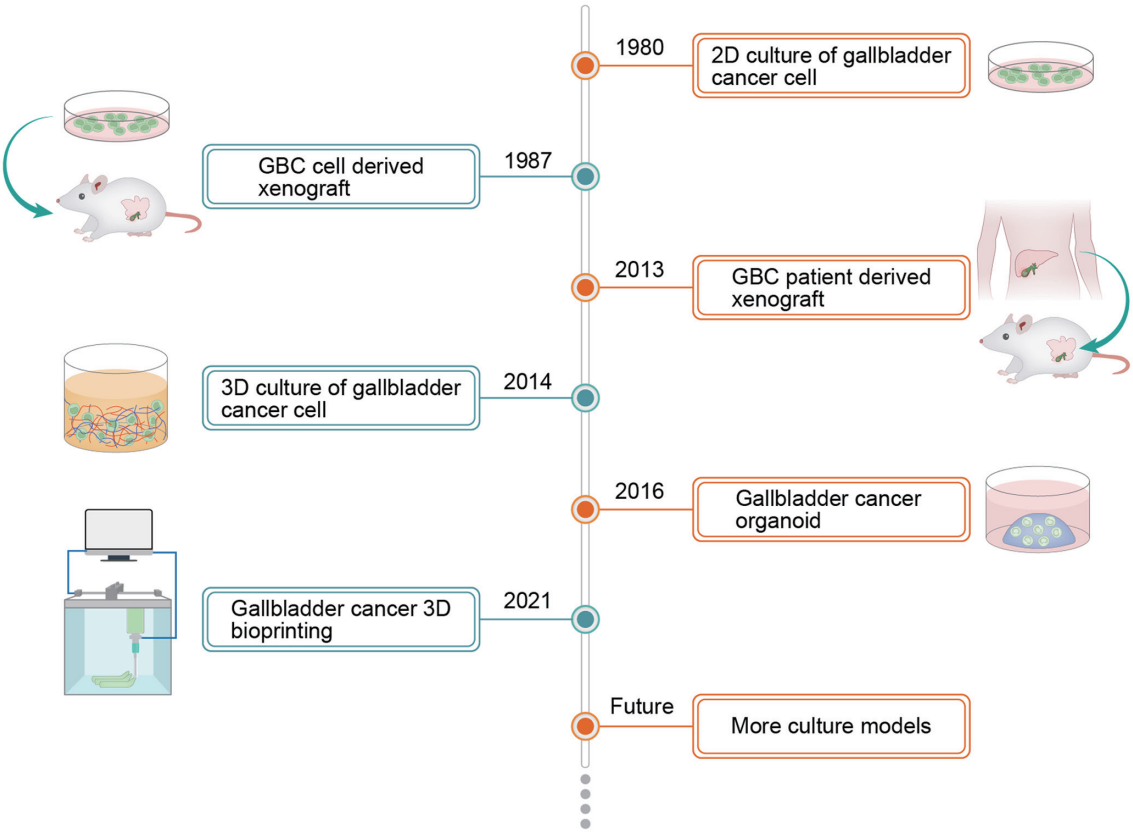

Fig. 1. Timeline of the development of different models of gallbladder cancer cell culture. The number of gallbladder cancer culture models is increasing, and now includes two-dimensional gallbladder cancer cell culture, three-dimensional gallbladder cancer cell culture, xenograft models, and three-dimensional bioprinting technology. GBC, gallbladder carcinoma; 2D, two-dimensional; 3D, three-dimensional.

affects the prognosis and treatment of the disease. Little is known about the biological characteristics of GBC, mainly because a complete *in vitro* culture system is lacking. *In vitro* tumor cell culture is one of the basic conditions for tumor research and is central to many fields such as tumor occurrence, development, invasion, metastasis mechanism, early diagnosis, and treatment evaluation.

Traditional tumor models are mainly used in *in vitro* 2D and biological models, while emerging ones include three-dimensional (3D) spheroids and organoid models. Existing 2D models have discovered and identified tumor invasive growth, including genetic and epigenetic modifications. Nonetheless, 2D models cannot recapitulate key elements of the *in vivo* microenvironment. Animal models including xenografts, chemical induction, and genetic manipulation have often been used in preclinical exploration and in the study of individualized treatment of cancer.[10] 3D cancer spheroids are growth groups formed *in vitro* from 3D cell cultures. These clusters reflect the morphological structure of human tumors, and are useful for precise examination of drug efficacy.[11] A tumor organoid model is a new method of tumor research emerging in recent years.[12] Organoids are derived from stem cells and can recapitulate certain aspects of organ structure and function, including differentiation into multiple cell types.[13]

A "perfect" *in vitro* tumor model should (1) mirror the three-layer developmental environment of growing cells in vivo, (2) mimic the cooperation between cancer and stromal cells, (3) replicate the relevant pathological features of patients, and (4) have an adaptable and time-saving development cycle.[14] It is feasible to tune various parts of the cancer microenvironment and subsequently control cellular capabilities. Given the difficulties, new developments in state-of-the-art 3D bioprinting innovations have been significantly adopted and used in cancer fields by developing dynamic biomolecular/cytokine perspective models, invasive proliferation, and cell-cell communication models.[15,16] The main purpose of this review is to describe the application and progress of GBC culture models, and to provide ideas for the basic and clinical translational research of GBC (Fig. 1) .

2D gallbladder cancer cell culture

More than a dozen GBC cell lines are currently available, including G-415, GB-d1, GBK-1, and KMG-A (as shown in Table 1).[17-38] The first established GBC cell line, G415, expresses epithelial cadherin (E-cad) and forms cystic or spherical 3D structures. And a plasmid containing murine E-cad cDNA was transduced into G415 cells to stop E-cad expression.[39] The E-cad-transduced cell line had a typical epithelial shape and forms multicellular cyst-like structures. Shimura *et al*.[17] found that removal of fibroblasts from GB-d1 cell cultures resulted in a decrease of tumor cell invasiveness and that the hepatocyte growth factor (HGF) secreted by fibroblasts could induce the invasive growth of GB-d1. GBC-SD, the first domestic GBC cell line is mainly composed of polygonal, spindle, and square cells.[18] SGC-996 is another domestic GBC cell line. It has a typical epithelial cell morphology, and

Table 1. Reports of human gallbladder cancer cell lines

Author	Cell line	Report year	Characteristic	Doubling time (h)
Koyama[25]	G-415	1980	Differentiated gallbladder cancer cell line	–
Morgan[26]	COLO 346	1981	polygonal cells	36
Knuth[27]	Mz-ChA-1.2	1985	Secretes C3C4C5; express AFP or ferritin	72
Egami[28]	r51 GBK-1	1986	polygonal cells; express CSF 43	43
Maruiwa[29]	KMG-A	1987	Expresses AFP	–
Homma[30]	NOZ	1988	Polymorphism; expresses CEA or ferritin; K-RAS mutation	48
Johzaki[31]	FU-GBC-1	1989	Expresses CEA, CA199, EMA	120
Tsubono[32]	GBC-GN	1992	Well-differentiated adenocarcinoma; cisplatin-sensitive	7
Nakano[33]	HAG-1	1994	Moderately differentiated adenocarcinoma of the gallbladder; no mutation or amplification of H-, K-, or N-ras genes detected	26
Shimura[17]	GB-d1,2	1995	Secretes IL-31	–
Nishida[34]	FU-GBC-2	1997	Signet ring cell; express CEA, CA199, EMA	43
Yamada[35]	OCUG-1	1997	Poorly differentiated adenocarcinoma; secreting TA-4	47.1
Park[36]	KMCH-1	1999	secrete IL-6	39
Liu[18]	GBC-SD	2000	Polymorphism, rapid proliferation, congenital drug resistance	21.4
Ebinuma[37]	PTHrP-GBK	2002	Expresses PTHrP 48	48
Yang[19]	SGC-996	2003	Typical epithelial cell morphology	46
Ghosh[38]	TGBC	2004	Expresses CEA, CA19-9, MUC-1, and c-erbB2	30–96
Li[23]	EH-GB1	2010	Gallbladder carcinoma liver metastases cell line, typical features of malignant epithelial cells, the tumorigenic rate of mice is 100%, and CA19-9 has strong positive expression	24
Wang[22]	EH-GB2	2011	Gallbladder carcinoma liver metastases cell line, EMT, highly expressed Vimentin, Snail, Twist, MMP-1, and MMP-2 mRNA	48
Shinichi[24]	TYGBK-1	2012	p53 missense mutation, showing sensitivity to gemcitabine	48
Liu[20]	TJ-GBC2	2017	Abnormal chromosome structure and number, hypertetraploidy; coexistence of polygonal, fusiform, and irregularly shaped cells	-
Zhou[21]	ZJU-0430	2019	Variety of cell morphologies and characteristic epithelial morphologies	19.81

AFP, alpha-fetoprotein; CEA, carcinoembryonic antigen; CSF, colony-stimulating factor; EMA, epithelial membrane antigen; EMT, epithelial-mesenchymal transition; MMP, matrix metalloproteinase.

neither the morphology nor the chromosome number change significantly in long-term culture. Liu *et al*.[20] successfully established a new cell line, TJ-GBC2, which has a characteristic epithelial tumor morphology and phenotypes consistent with primary GBCs, such as polygonal and irregular cell shapes, CA19-9 and AFP levels were elevated, and E-cadherin was expressed. The cell line is also more invasive and the highest migratory ability compared with other GBC cell lines. Zhou *et al*.[21] established a novel ZJU-0430 cell line from a primary GBC. It had various cell morphologies and characteristic epithelial morphologies, with a population doubling time of 19.81 h. ZJU-0430 cells have greater migration, invasiveness, and proliferative ability than GBC-SD cells *in vitro*. In addition to the primary GBC cell line, Wang *et al*.[22] established the EH-GB2 cell line from primary culture of a liver metastases from a 65 year-old patient cell line. It has a doubling time of approximately 48 h in cell culture and elevated expression of vimentin, Snail, Twist, matrix metalloproteinase 1 (MMP-1), and MMP-2 mRNAs, which are characteristic of epithelial-mesenchymal transition. Another metastatic gallbladder disease cell line, EH-GB1, reported by Li *et al*.[23] was passaged over 20 generations, had an aggressive epithelial morphology and a stable developmental pattern for 24 h. EH-GB1 cells grew in mice after subcutaneous inoculation and were positive for the cancer marker CA19-9. The cell line consistently expressed green fluorescent protein (GFP) and Red 2. In terms of pathological types of GBC, Shinichi *et al*.[24] established a human GBC cell line TYGBK-1 from papillary tubular adenocarcinoma patients. It had p53 missense mutations, produced CA19-9, and was sensitive to gemcitabine.

In vitro drug screening in 2D culture has the advantages of high-throughput and convenient screening, and is widely used in preclinical trials of drugs. An *in vitro* culture model of GBC cell lines has been successfully used in the study of the mechanism of genetic and molecular changes of GBC and drug sensitivity experiments, especially the abnormality of GBC suppressor genes, proto-oncogenes, and DNA repair genes, microsatellite instability, and epigenetic changes. Qin *et al*.[40] used v-src- or cH-ras-transfected HAG-1 human GBC cell lines to find that activated Ras and Src induced resistance to gefitinib by activating one or both of the Akt and Ark signaling pathways. It has also been reported that KRAS, CDKN2A/B and other gene mutations exist in GBC, and are closely related to the invasiveness and metastasis.[6,41] However, studies have shown that epigenetic changes and tumor suppressor gene methylation in GBC are abnormally

increased and closely related to patient survival.[42] Gao *et al*.[43] examined the high expression of NSUN2 in GBC tissues and GBC-SD cell lines and found that NOP2/SunRNA methyltransferase 2 promoted GBC progression through the interaction partner RPL6. Additionally, the role of noncoding RNAs in the occurrence and development of GBC has recently received attention. In the NOZ and SGC-996 cell lines, miR-122 inhibited the proliferation of GBC by interfering with the expression of PKM2, MALAT1 promoted the proliferation and metastasis of GBC by activating the ERK/MAPK pathway.[44] The GBC cell line has a high xenograft tumor formation rate, which is also an important criterion in the identification of cell lines. Tsubono *et al*.[32] used the well-differentiated cell line GBC-GN to establish a nude mouse tumor-bearing model to test the antitumor anticancer effects drugs such as CDDP and 5-fluorouracil (5-FU), alone or in combination. Only CDDP was effective in GBC-GN model, and CDDP+5-FU had an inhibitory effect on both malignant tumors.

However, in no case can 2D models retain the key qualities of the *in vivo* microenvironment. Some studies have suggested cancer cells derived from patients under 2D conditions experience a reduction or complete loss of certain receptors and marker molecules.[45] Comparison of 2D and 3D models shows huge differences in phenotype, gene and protein expression, and drug sensitivity.[46] These elements make 2D an unacceptable technique for accurate drug screening. Due to factors such as genetic drift, loss of heterogeneity, and cell line contamination, the experimental outcomes of cell lines are not very robust in the final animal experiments. That may be because the cell culture process in a 2D environment *in vitro* cannot truly reflect the interactions of the tumor microenvironment surrounding the tumor cells *in vivo*. Primary tumor cells can better retain the characteristics of host cells, but the current *in vitro* culture of primary tumor cells is limited due to difficulties in cell culture technology and cycle. Compared with the widespread application of GBC cell lines, the culture of primary GBC cells has also been further developed and applied. Bo *et al*.[47] collected resected lesions from patients with gallbladder adenoma and GBC, and successfully cultured primary gallbladder tumor cells *in vitro*. To improve the efficiency of primary cell culture, Noggin, epidermal growth factor (EGF), fibroblast growth factor 10 (FGF10), and insulin-like growth factor (IGF) were added to the cell culture medium, and Wnt3a, Rspo1, and prostaglandin E2 were added to the gallbladder adenoma medium at the same time. The researchers then successfully established organoids from primary GBC cells, which were expanded for at least 10 passages and stably cultured for more than 3 months. Feng *et al*.[48] successfully constructed 38 patient-derived primary cancer cell lines with multiregional sampling of tissue samples from seven patients with GBC surgery, and studied the heterogeneity of GBC. In addition to the culture of primary GBC cells, Chen *et al*.[49] cultured primary GBC-related fibroblasts, using enzymatic digestion and tissue blocks for the primary culture of human GBC-related fibroblasts and purification by differential adherence.

Recent progress of the *in vitro* culture of primary tumor cells is reflected by the improvement of cell culture media, cell-separation methods, and conditional reprogramming of cultured cells.[50–53] In terms of the tumor cell culture model, a 3D culture model is gradually replacing 2D culture model.

3D gallbladder cancer cell culture

In recent decades, various methods for culturing 3D spheroids have been developed. 3D culture methods can be divided into scaffold-containing and scaffold-free cell culture methods. Scaffolding materials can be divided into natural scaffolds using matrigel, collagen, gelatin, laminin, chitosan, hyaluronic acid, and synthetic scaffold materials by polyester degradable polymer.[54–57] Scaffold-free culture models mainly refer to the application of various physical methods to prevent tumor cells from adhering to the wall, to suspend tumor cells in the medium, and to promote cell aggregation and growth to form tumor cell spheres. It includes ultra-low adhesion culture plates, dynamic rotation culture, hanging drop methods, and microfluidic chips and acoustic manipulation, which have appeared in recent years.[58–62]

The 3D cell simulates the *in vivo* microenvironment of tumor cell growth. Under 3D culture conditions, almost 100% of the surface area of the cells is in contact with other cells or substrates, and the cultured cells have characteristic biological signal transduction.[63,64] In the 3D culture environment, cells have a scattered distribution in clusters, forming homogeneous scattered microcell spheres. In the microspheres, there may be cell junctions and paracrine communication that cannot be realized in 2D culture, and act to maintain the tumor characteristics.[46]

Currently, 3D culture of GBC uses 3D gels. Wan *et al*.[39] systematically compared the cell phenotype, morphogenesis, and ultrastructure of G-415 GBC cells containing an expression plasmid of mouse E-cad cDNA in 2D and 3D gel culture conditions. In 3D gels, the GBC cells formed round multicellular cyst-like structures that secreted mucous material from the apical surface into the cyst cavity and had the potential for hyperproliferation, which was in contrast to 2D culture.

The main applications of 3D culture model are in drug sensitivity studies and the mechanism of the GBC microenvironment. Jiang *et al*.[65] established a 3D cell culture model of GBC using preparations of cell-containing 3D collagen to study the killing effectiveness and mechanism of a novel nanodrug delivery system in GBC cells. Experiments found that the interaction between cells in 3D culture was significantly enhanced, and the biological characteristics of multicellular spheres were mainly characterized by cell cycle arrest and early apoptosis reduction, which may have been related to multicellular drug resistance. In terms of the occurrence mechanism of GBC, cancer-associated fibroblasts (CAFs) and vasculogenic mimicry (VM) have important roles in the malignant growth of GBC. Pan *et al*.[66] used a 3D collagen gel coculture of human GBC cells and fibroblasts or HUVECs, which showed that CAFs upregulated NOX4 expression to promote VM formation and cancer development, possibly by paracrine secretion of IL6 to complete the IL-6-JAK-STAT3 signaling pathway. NOX4 is a critical gene for VM development in GBC. It is aberrantly expressed in the cancer cells and stroma and is associated with poor prognosis in GBC patients.

New 3D culture methods incorporating acoustic manipulation and microfluidics that avoid limitations of the use of other 3D culture methods for GBC, have been used in studies of bladder cancer, breast cancer, and other malignant tumors (Fig. 2). Gong *et al*.[61] printed tumor organoids with acoustic droplets that mimicked the immune microenvironment of bladder tumors. Their platform facilitated rapid formation of tumor organoids that preserved the original tumor immune microenvironment and the establishment of a personalized bladder cancer immunotherapy model. Using a droplet microfluidic platform, Chen *et al*.[67] prepared delicate structures with fibroblasts in the outer layer and hepatoma cells in the inner layer and achieved homogenization to generate heterogeneous pseudospheres that were used to study interactions between tumors and stromal cells.

However, 3D culture methods have downsides. Scaffold-based 3D cell culture involves conventional or mixed poly-

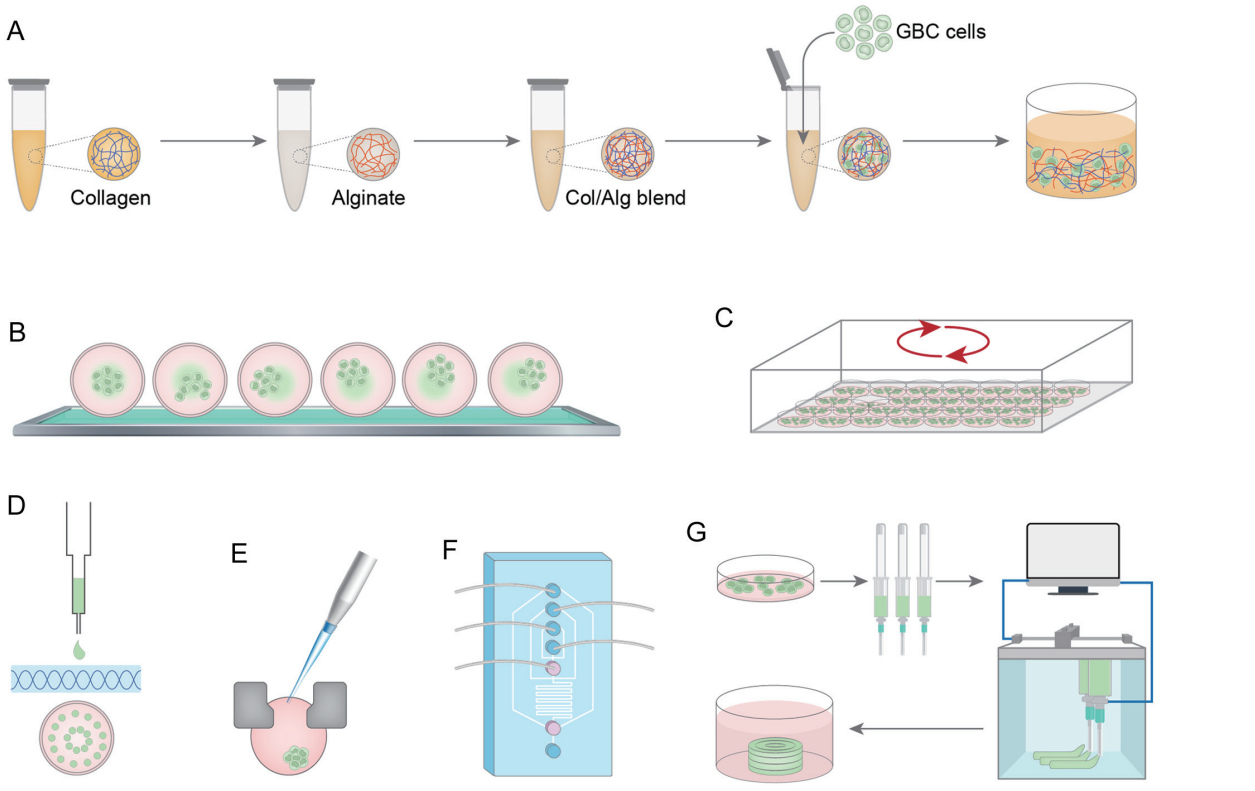

Fig. 2. Different types of Three-dimensional culture models include scaffold-containing and scaffold-free cell methods. (A) Three-dimensional gel cell culture model; (B) Low-adhesion cell culture; (C) Dynamic spin cell culture; (D) Acoustic control of cell culture; (E) Suspension cell culture; (F) microfluidic cell culture; (G) 3D bioprinting cell culture.

mers as the extracellular matrix (ECM), thereby supporting cell development. Commonly used techniques include matrix coverage/embedding culture and microcarriers. Robust cell-ECM interactions can understood by the appearance of ECM material. Nonetheless, the outer materials are toxic and antigenic, separating the spheres from the platform material is difficult, and the platform materials may affect drug diffusion and responses, which results in low reproducibility of drug testing results.[68] Frameless 3D cell culture does not require suspended external materials or power. Rotating and spinner flasks, hanging drops, ultra-low attachment plates, and microporous plates are devices that are routinely used in 3D culture. Rotating and spinner flasks can create stress that is strong enough to damage cells during culture, which makes it challenging to control the consistency of spheroids. Hanging drop assays require intensive work and long-term culture. They are unstable, due to the evaporation of media from the droplets. Ultra-low linker plates have low efficacy, and there is only one spheroid in a culture well, making it difficult to control spheroid uniformity. Microporous plates use specially created microwells through micropatterning. To keep up with suspending cells, the well surface should be covered to reduce attachment, which may hinder the effects of drug molecules.[69]

Gallbladder cancer organoids

Organoids are a novel 3D cell culture technology that can grow primary tissue plants or single cells into self-organizing tissues. Organoids can retain histological features, expression profiles, unique markers, and many other features of the original tissue.[70] The extraordinary strength of this model is that organoids can be cultured over a long period of time (especially for patient-specific tumor cells) and passaged in 3D over a long time. Additionally, organoids can preserve the histological, immunohistochemical, and genetic heterogeneity of the original tumor, making them suitable for high-throughput drug screening.

Lugli *et al*.[71] isolated gallbladders from 2 month-old mice, collected them and incubated them with PBS/EDTA for 2 h. Small clusters of cells were isolated and implanted into matrigel. The cells were incubated in a medium containing nicotinamide and various growth factors including EGF, fibroblast growth factor 10, and noggin. The gallbladder organoids that developed reproduced stably for more than a year and expressed the stem cell marker Prom1 and the transcription factor Sox17.

GBC organoids have been successfully used to explore the mechanism of occurrence and development of GBC and drug sensitivity (Fig. 3). One study obtained GBC organoids from mice in which the TP53 gene was inactivated and cultured them after infection with salmonella.[72] Organoids with a history of infection lost cohesion and polarity, had enlarged nuclei, irregular and prominent nucleoli, and exhibited neoplastic transformation. Organoids without infection had normal epithelial tissue. Therefore, salmonella has a susceptible genetic background and can promote cellular tumorigenesis. The current first-line chemotherapy regimen for GBC, gemcitabine combined with CDDP, has a median survival of less than 1 year.[73] García *et al*.[74] found that Yes-associated protein 1(YAP1) was highly expressed in 60% of patients with chronic cholecystitis and advanced GBC. High nuclear expression of YAP1 was associated with poor overall surviv-

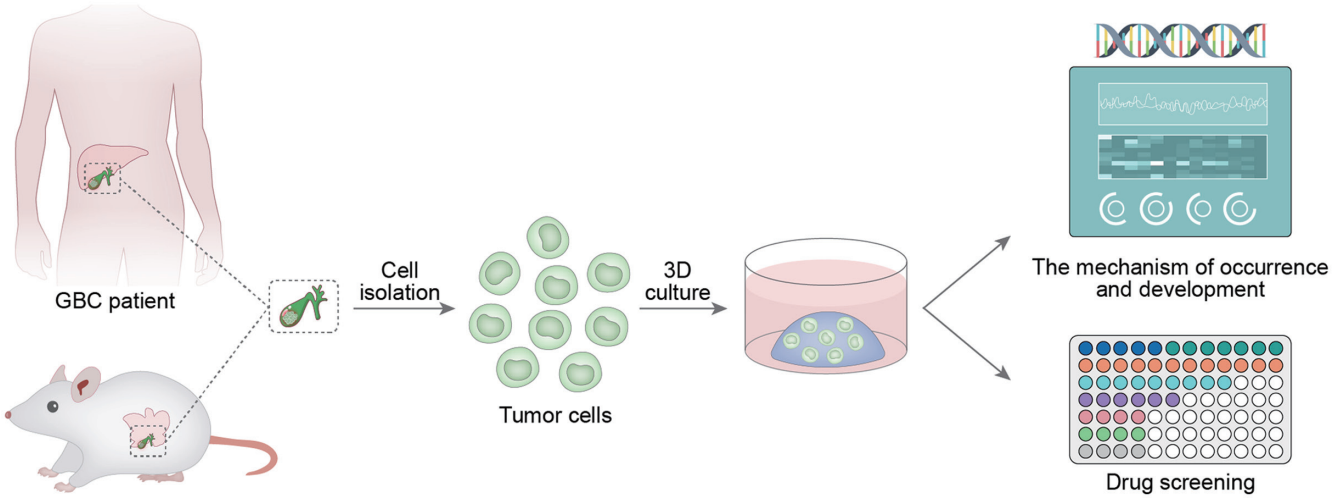

Fig. 3. Applications and steps of three-dimensional gallbladder cancer cell organoids. Gallbladder cancer organoids can be constructed by three-dimensional culture of tumor cells isolated from patients or mice and have been successfully used to explore the mechanism of occurrence and development of gallbladder cancer and studies of drug sensitivity. 3D, three-dimensional.

al in patients with subserous GBC. They also demonstrated that inhibition of YAP1 by verteporfin (VP) reduced migration and invasion of GBC cell lines. To overcome the lack of an intact immune system, Shingo *et al*.[75] recently established a new model using lentiviral Cre transduction and CRISPR/Cas9 gene editing to generate mutant Kras and Trp53-loss gallbladder organoids *in vitro*. Sections of organoid-defined subcutaneous tissue that were sutured to the outer surface of the gallbladder in syngeneic mice produced carcinomas *in situ* that resembled human GBCs histology and gene transcription. The model revealed the penetration of similar immune cell subsets in subcutaneous and orthotopic tumors, confirming a suitable immune environment during carcinogenesis, demonstrated the *in vivo* efficacy of gemcitabine.

There are many limitations of the use of GBC organoid technology. First, many complex and expensive cytokines need to be added to maintain the tumor microenvironment of the organoid. Second, the success rate of *in vitro* tumor organoid construction depends on the tumor's own proliferative capacity. GBC and other low-proliferation tumors, have a low success rate in constructing organoids *in vitro*, which makes the use of tumor organoid technology less extensive than that of patient-derived xenograft (PDX) models.[76] Third, its deficiency in interstitial cell components and the absence of cell-to-cell interactions make it impossible to reconstruct the tumor microenvironment.

Gallbladder cancer xenograft models

Human tumor xenograft models involve transplantation of human primary tumor tissue into immunodeficient animals. At present, there are two types of xenograft models of GBC, subcutaneous and orthotopic (Fig. 4).[77] Subcutaneous tumor models can be prepared by the cell suspension injection or tissue block methods. A cell suspension can be injected subcutaneously or injected intraperitoneally into the lateral femoral side of nude mice. Subcutaneous tumors that have grown into blocks with a diameter of 1.0 cm can be removed and cut into pieces of about 1 mm in size and reinoculated. Subcutaneous tumors prepared by the cell suspension method can be inoculated under the serosa of the gallbladder by surgery.

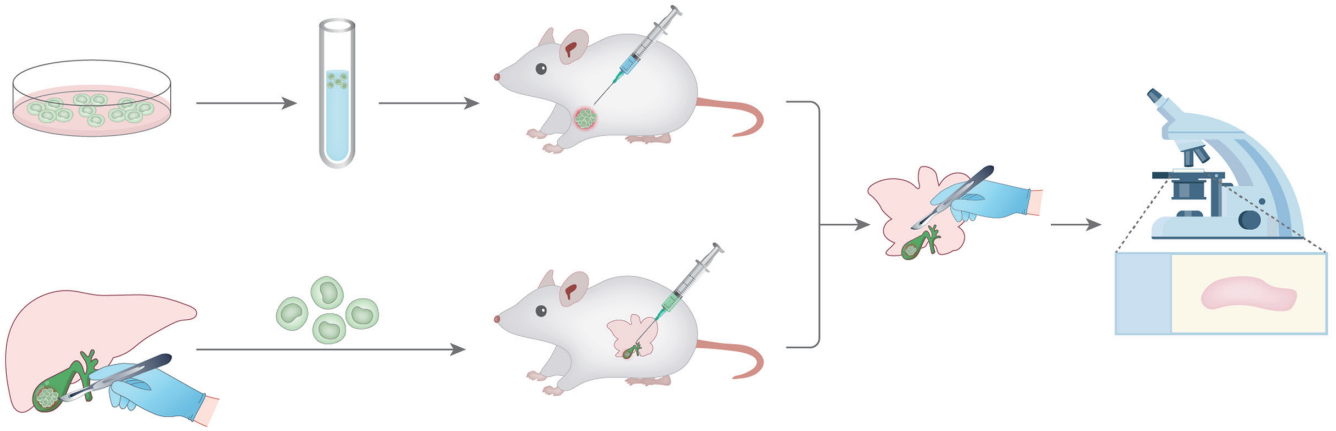

Fig. 4. Classification and steps of preparing gallbladder cancer cell xenograft models. Xenograft models of gallbladder cancer include subcutaneous and orthotopic tumor models. Subcutaneous tumor models can be constructed from injection of cell suspensions or tissue blocks. The injection is either subcutaneous or intraperitoneal into the lateral femoral side of nude mice.

Patient-derived xenografts propagated in immune-compromised mice at ectopic or orthotopic sites replicate the pathological features of patients to a certain extent. Compared with the cell transplantation model (i.e. cell-derived xenograft, CDX) that directly transplants the cell line into nude mice because the PDX model comes directly from the patient, it will not lose heterogeneity and undergo genetic drift like the cell line, so it can better maintain the original tumor morphology, metastasis characteristics, mutation spectrum, and drug response.[69]

Xenograft models are widely used in drug screening and individualized treatment of various tumors, such as melanoma,[78] non-small cell lung cancer,[79] breast cancer,[80] ovarian cancer,[81] and hepatocellular carcinoma.[82] Many studies using subcutaneous transplantation models have been carried out by researchers after the successful cultivation of cell lines, and have mainly focused on anticancer drugs and early diagnosis of tumors. Pan *et al*.[83] used the NOZ cell line to establish a nude mouse subcutaneous tumor and GBC orthotopic models in nude mice, in which they analyzed and compared the mechanisms of GBC metastasis. The nude mice in the orthotopic tumor transplant group showed signs of spontaneous metastasis. The tumors showed strong invasiveness, but there was no obvious spontaneous metastasis occurred in the subcutaneous tumor group. The results reflect the relationship of the invasiveness of tumor cells to the surrounding environment of the organ. In a study of the antitumor effectiveness of gemcitabine, Mita *et al*.[84] also inoculated NOZ cells into the gallbladder, founding that lymph nodes, liver, and lung metastasis occurred in nude mice. To determine whether the trauma caused by the puncture of the abdominal wall could promote the implantation of tumors at the puncture site, some researchers injected the GBC cell line GB-d1,2 cells into the abdominal cavity of nude mice undergoing different surgical operations. They found that most of the patients with peritoneal injury, laparoscopic operation, peritoneal burns, or liver-surface burns experienced tumor implantation and metastasis at the operation site, and that implantation was greatly reduced by repairing the peritoneum.[85] Animal-induced models of GBC were reported as early as 1974.[86] Some people believe that azotoluidine nitrosamines, artificial cholesterol stones, and other materials implanted in the gallbladder, can lead to the occurrence of GBC, but successful cases have been very rare. Talima *et al*.[87] successfully established a GBC model in female Syrian mice. First, distal common bile duct ligation and cholecystoduodenal anastomosis were performed, and nitrosamine feeding was administered 4 weeks after the operation. The GBC showed papillary growth, and a morphology similar to that of human GBC. Bromodeoxyuridine and anti-bromodeoxyuridine monoclonal antibody immunohistochemical staining confirmed that gallbladder duodenal anastomosis significantly accelerated the activity of gallbladder epithelial cells, which may play a major role in the carcinogenesis of gallbladder epithelial cells. Reflux and stasis of pancreatic juice can lead to chronic inflammation of the gallbladder, intestinal metaplasia, and finally to the occurrence of differentiated GBC. A study by Ikemaltsu *et al*.[88] confirmed the above perspective, and found that nitrosamines induced GBC in mice, and oral administration of cholestyramine inhibited carcinogenesis. It was inferred that bile acid is involved in gallbladder carcinogenesis.

A major limitation of xenograft models is the loss of human tumor stroma, which is completely replaced by murine stroma in the second generation.[89] To overcome it, co-implantation of human stromal cells with primary tumor tissue optimizes traditional xenograft models. In addition, the long cycle of xenograft model establishment limits its application in guiding the treatment of the original patient. The time gap was bridged by the rapid *in vivo* drug sensitivity assay of mini-xenograft model, which can test the response to antitumor drugs within 7 days.[90] To establish a mini-xenograft model, cancer cell suspensions were transferred to a modified microcapsule and hollow-fiber culture system. The capsules were subcutaneously transplanted into immunodeficient mice, and the mice were subsequently treated with antitumor drugs. Treatment response was assessed by cell proliferation. However, xenograft models established in immunocompromised mice are ineffective in studying the tumor microenvironment, including the infiltration of immune cells and crosstalk between the tumor and the immune system. To overcome those challenges, several humanized mice have recently been developed, including genetically engineered humanized mice and immune humanized mice.[91]

Emergence of 3D bioprinting technology

3D bioprinting is essentially the same as organoids. and it is also an *in vitro* 3D culture technology based on matrigel, but with the help of precision engineering instruments, precise models can be constructed with millimeter-level errors. 3D printing is an innovative process using rapid prototyping materials, layer-by-layer superposition, and point-by-point printing to produce 3D graphics. This rapid and lowest-cost innovation has some control over the development of multicellular, multimaterial, and multibiomolecular structures in a multilevel space. It can also reproduce the growth microenvironment and mimic the complex organic structure of cancer tissue.[92] Additionally, different cell components can be added. The multichannel printing has the advantages of controlling the 3D position of each cell component, restoring the different distribution characteristics of different cells in the tissue and achieving the purpose of accurately simulating the *in vivo* microenvironment. That is sufficient to overcome the randomness of organoid technology and the defects of homogenization of cell distribution.[93]

The use of 3D bioprinting has been explored in personalized treatment schemes and construction of a model tumor microenvironment consistent with the *in vivo* parental tumor tissue with respect to gene sequences, gene expression levels. We have used 3D bioprinting technology to successfully construct human normal liver tissue,[94] liver cancer tissue,[95] and intrahepatic cholangiocarcinoma tissue,[96] confirming that 3D printing has the advantages of a high success rate and stability for study of drugs and tumor pathogenesis (Fig. 2G). Recently, our experimental team used 3D bioprinting to construct an *in vitro* multicellular GBC model that accurately simulated the tumor microenvironment of GBC and can be used to explore the mechanism of occurrence and development of GBC and to conduct drug sensitivity experiments.

3D biological printing has disadvantages compared with 3D culture. Whether it is an ordinary mechanical extrusion printer or a newer type such as light curing printer, building a specific model of cells requires more preliminary work, such as the preparation of bigels, and takes a long time for completion. Long-term exposure of cells to biological ink leaving the culture medium also affects their function.[14] Additionally, the mechanical compression of the extrusion printer can also cause cell damage that can result in the death of some fragile primary cells soon after printing, making it impossible to successfully build a model. Further development of biomaterials and mechanical engineering technology is needed.[14]

Table 2. Comparison of common *in vitro* gallbladder cancer culture models

Feature	2D cell line	3D cell culture	Organoids	Xenograft model	3D bioprinting
Method	Cell culture dish	Collagen-based scaffold	Thermosensitive basement membrane extract; hydrosol	Implanted	Bioink;3D printer
In vivo/vitro	Vitro	Vitro	Vitro	Vivo	Vitro
Success rate	High	High	Medium	Low	High
Advantages	Low maintenance cost; high reproducibility; simple and feasible operation; real-time assessment of cell survival and proliferation	Easy to build; high reproducibility	Explore the histological and biological characteristics of the original tumor; simulate the complex tumor microenvironment	Study tumor biological interactions in vivo; simulate tumor microenvironment; long-term culture;	Retain the biological and genetic characteristics of the original tumor; simple and convenient operation; long-term survival and passage
Defects	Easy to accumulate mutations, tumor cells lack interaction with other surrounding cells	Lack of histological and morphological features	Highly heterogeneous; depending on the inherent proliferation ability of the original tumor	Tumor environment different from the human body; interference of animal's own immune system; high cost of facilities and skill training; ethical controversy	Expensive instruments; mechanical scissors during printing
Number of papers published	778	2	14	238	–

2D, two-dimensional; 3D, three-dimensional.

Outlook

Although GBC still faces many problems such as a high degree of malignancy, a single treatment option, and a low 5-year survival rate, they will gradually be solved with the in-depth understanding of the pathogenesis, clinical diagnosis, and treatment. By comparing the advantages and shortcoming of above models, it contributes to provide the future directions (as shown in Table 2). With the continuous updating of primary tumor cell culture technology, long-term *in vitro* culture of primary GBC cells obtained from patients will facilitate the realization of individualized and precise treatment. In view of the increasingly important role of 3D tumor cell culture, additional models will be used to explore the mechanism of GBC occurrence and development in the future. 3D bioprinting techniques have become the focus of current research because they can model cell-to-cell interactions. More importantly, 3D bioprinting technology can print multiple cells to simulate the tumor microenvironment and better recapitulate the complexity of tumors, which is of great significance for clinical diagnosis and personalized medicine of tumors. Currently, research on 3D bioprint culture models of GBC are in their infancy. The construction success rate, application, and prospects need more research. However, that does not affect the research value and bright prospects of 3D bioprinting technology for GBC.

In addition to GBC and malignant tumors, current research in the causes, occurrence, development, and treatment of benign diseases of the gallbladder, such as gallbladder stones, adenomyosis of the gallbladder, and others are mainly carried out in animal models and mathematical exercises.[97,98] The main experimental animal models of gallstones are implanting human gallstones into animal gallbladders and observing the changes and the influence of Chinese and Western medicines, the other models are induced by food.[98] The former is suitable for the experimental study of promoting gallstones and expelling stones but not for research on the cause of gallstones and stone prevention. The latter can be used to study stone prevention, but has a long observation time of at least 2 months. The use of *in vitro* culture models for the study of benign gallbladder disease is currently very limited. More models of gallbladder disease are expected to appear in the future.

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Conflict of interest

The authors have no conflict of interest related to this publication.

Author contributions

Study conception and design (JX, GX, XW), drafted the first version of the manuscript (JX, GX, XW, YM, XS), edited and revised the manuscript (PD, SD, HY). All authors have approved the final version of the article, including the authorship list.

References

[1] Vega EA, Mellado S, Salehi O, Freeman R, Conrad C. Treatment of Resectable Gallbladder Cancer. Cancers (Basel) 2022;14(6):1413. doi:10.3390/cancers14061413, PMID:35326566.

[2] Hundal R, Shaffer EA. Gallbladder cancer: epidemiology and outcome. Clin Epidemiol 2014;6:99–109. doi:10.2147/clep.s37357, PMID:24634588.
[3] Dutta U. Gallbladder cancer: can newer insights improve the outcome? J Gastroenterol Hepatol 2012;27(4):642–653. doi:10.1111/j.1440-1746.2011.07048.x, PMID:22168580.
[4] Pawlik TM, Gleisner AL, Vigano L, Kooby DA, Bauer TW, Frilling A, *et al*. Incidence of finding residual disease for incidental gallbladder carcinoma: implications for re-resection. J Gastrointest Surg 2007;11(11):1478–1486. doi:10.1007/s11605-007-0309-6, PMID:17846848.
[5] Benson AB 3rd, Abrams TA, Ben-Josef E, Bloomston PM, Botha JF, Clary BM, *et al*. NCCN clinical practice guidelines in oncology: hepatobiliary cancers. J Natl Compr Canc Netw 2009;7(4):350–391. doi:10.6004/jnccn.2009.0027, PMID:19406039.
[6] Song X, Hu Y, Li Y, Shao R, Liu F, Liu Y. Overview of current targeted therapy in gallbladder cancer. Signal Transduct Target Ther 2020;5(1):230. doi:10.1038/s41392-020-00324-2, PMID:33028805.
[7] Dutta U, Nagi B, Garg PK, Sinha SK, Singh K, Tandon RK. Patients with gallstones develop gallbladder cancer at an earlier age. Eur J Cancer Prev 2005;14(4):381–385. doi:10.1097/00008469-200508000-00011, PMID:16030429.
[8] Saetta AA. K-ras, p53 mutations, and microsatellite instability (MSI) in gallbladder cancer. J Surg Oncol 2006;93(8):644–649. doi:10.1002/jso.20532, PMID:16724348.
[9] Sheth S, Bedford A, Chopra S. Primary gallbladder cancer: recognition of risk factors and the role of prophylactic cholecystectomy. Am J Gastroenterol 2000;95(6):1402–1410. doi:10.1111/j.1572-0241.2000.02070.x, PMID:10894571.
[10] Barroca NCB, Della Santa G, Suchecki D, García-Cairasco N, Umeoka EHL. Challenges in the use of animal models and perspectives for a translational view of stress and psychopathologies. Neurosci Biobehav Rev 2022;140:104771. doi:10.1016/j.neubiorev.2022.104771, PMID:35817171.
[11] Nunes AS, Barros AS, Costa EC, Moreira AF, Correia IJ. 3D tumor spheroids as in vitro models to mimic in vivo human solid tumors resistance to therapeutic drugs. Biotechnol Bioeng 2019;116(1):206–226. doi:10.1002/bit.26845, PMID:30367820.
[12] Grassi L, Alfonsi R, Francescangeli F, Signore M, De Angelis ML, Addario A, *et al*. Organoids as a new model for improving regenerative medicine and cancer personalized therapy in renal diseases. Cell Death Dis 2019;10(3):201. doi:10.1038/s41419-019-1453-0, PMID:30814510.
[13] Gao D, Vela I, Sboner A, Iaquinta PJ, Karthaus WR, Gopalan A, *et al*. Organoid cultures derived from patients with advanced prostate cancer. Cell 2014;159(1):176–187. doi:10.1016/j.cell.2014.08.016, PMID:25201530.
[14] Mao S, Pang Y, Liu T, Shao Y, He J, Yang H, *et al*. Bioprinting of in vitro tumor models for personalized cancer treatment: a review. Biofabrication 2020;12(4):042001. doi:10.1088/1758-5090/ab97c0, PMID:32470967.
[15] Zhao Y, Yao R, Ouyang L, Ding H, Zhang T, Zhang K, *et al*. Three-dimensional printing of Hela cells for cervical tumor model in vitro. Biofabrication 2014;6(3):035001. doi:10.1088/1758-5082/6/3/035001, PMID:24722236.
[16] Grolman JM, Zhang D, Smith AM, Moore JS, Kilian KA. Rapid 3D Extrusion of Synthetic Tumor Microenvironments. Adv Mater 2015;27(37):5512–5517. doi:10.1002/adma.201501729, PMID:26283579.
[17] Shimura H, Date K, Matsumoto K, Nakamura T, Tanaka M. Induction of invasive growth in a gallbladder cancer cell line by hepatocyte growth factor in vitro. Jpn J Cancer Res 1995;86(7):662–669. doi:10.1111/j.1349-7006.1995.tb02450.x, PMID:7559084.
[18] Liu B, Wang Z, Wu X, Liu C, Li Z, Liu J. Establishment of human gallbladder cancer cell line GBC-SD. Journal of Shandong Medical University 2000;03:332–333.
[19] Yang Y, Tao H, Yang H. Establishment of human primary gallbladder carcinoma cell line SGC-996. Journal of Tongji University (Medical Edition) 2003;06:457–459.
[20] Liu ZY, Xu GL, Tao HH, Yang YQ, Fan YZ. Establishment and characterization of a novel highly aggressive gallbladder cancer cell line, TJ-GBC2. Cancer Cell Int 2017;17:20. doi:10.1186/s12935-017-0388-8, PMID:28194091.
[21] Zhou F, Zhang Y, Sun J, Yang X. Characteristics of a novel cell line ZJU-0430 established from human gallbladder carcinoma. Cancer Cell Int 2019;19:190. doi:10.1186/s12935-019-0911-1, PMID:31367188.
[22] Wang JH, Li LF, Yu Y, Li B, Jin HJ, Shen DH, *et al*. Establishment and characterization of a cell line, EH-GB2, derived from hepatic metastasis of gallbladder cancer. Oncol Rep 2012;27(3):775–782. doi:10.3892/or.2011.1570, PMID:22134783.
[23] Li LF, Hu HZ, Liu C, Wang JH, Wu HP, Jin HJ, *et al*. [Establishment and characterization of a human gallbladder carcinoma cell line EH-GB1 originated from a metastatic tumor]. Zhonghua Zhong Liu Za Zhi 2010;32(2):84–87. PMID:20403235.
[24] Sekine S, Shimada Y, Nagata T, Moriyama M, Omura T, Yoshioka I, *et al*. Establishment and characterization of a new human gallbladder carcinoma cell line. Anticancer Res 2012;32(8):3211–3218. PMID:22843894.
[25] Koyama S, Yoshioka T, Mizushima A, Kawakita I, Yamagata S, Fukutomi H, *et al*. Establishment of a cell line (G-415) from a human gallbladder carcinoma. Gan 1980;71(4):574–575. PMID:7429092.
[26] Morgan RT, Woods LK, Moore GE, McGavran L, Quinn LA, Semple TU. A human gallbladder adenocarcinoma cell line. In Vitro 1981;17(6):503–510. doi:10.1007/bf02633511, PMID:7262900.
[27] Knuth A, Gabbert H, Dippold W, Klein O, Sachsse W, Bitter-Suermann D, *et al*. Biliary adenocarcinoma. Characterisation of three new human tumor cell lines. J Hepatol 1985;1(6):579–596. doi:10.1016/s0168-8278(85)80002-7, PMID:4056357.
[28] Egami H, Sakamoto K, Yoshimura R, Kikuchi H, Akagi M. Establishment of a cell line of gallbladder carcinoma (GBK-1) producing human colony stimulating factor. Jpn J Cancer Res 1986;77(2):168–176. PMID:3082828.
[29] Maruiwa M. Establishment of a new human gallbladder carcinoma cell line (KMG-A) from an alpha-fetoprotein-producing gallbladder carcinoma transplanted into nude mice. Kurume Med J 1987;34(3):133–146. doi:10.2739/kurumemedj.34.133, PMID:2450224.
[30] Homma S, Hasumura S, Nagamori S, Kameda H. [Establishment and characterization of a human gall bladder carcinoma cell line NOZ]. Hum Cell 1988;1(1):95–97. PMID:3154020.
[31] Johzaki H, Iwasaki H, Nishida T, Isayama T, Kikuchi M. A human gallbladder adenocarcinoma cell line. Cancer 1989;64(11):2262–2268. doi:10.1002/1097-0142(19891201)64:11<2262::aid-cncr2820641114>3.0.co;2-h, PMID:2680052.
[32] Tsubono M, Nio Y, Tseng CC, Kawabata K, Masai Y, Hayashi H, *et al*. Experimental chemotherapy for xenograft cell lines of human bile duct and gall bladder cancers in nude mice. J Surg Oncol 1992;51(4):274–280. doi:10.1002/jso.2930510415, PMID:1434661.
[33] Nakano S, Tatsumoto T, Esaki T, Nakamura M, Baba E, Kimura A, *et al*. Characterization of a newly established human gallbladder carcinoma cell line. In Vitro Cell Dev Biol Anim 1994;30A(11):729–732. doi:10.1007/bf02631291, PMID:7881622.
[34] Nishida T, Johzaki H, Tanaka S, Shimura H, Iwasaki H, Masuda Y, *et al*. [Two different types of monoclonal antibodies against gallbladder carcinoma cell line]. Nihon Geka Gakkai Zasshi 1991;92(11):1627–1635. PMID:1770935.
[35] Yamada N, Chung Y, Ohtani H, Ikeda T, Onoda N, Sawada T, *et al*. Establishment and characterization of a new human gallbladder carcinoma cell line (OCUG-1) producing TA-4. Int J Oncol 1997;10(6):1251–1255. doi:10.3892/ijo.10.6.1251, PMID:21533512.
[36] Park J, Tadlock L, Gores GJ, Patel T. Inhibition of interleukin 6-mediated mitogen-activated protein kinase activation attenuates growth of a cholangiocarcinoma cell line. Hepatology 1999;30(5):1128–1133. doi:10.1002/hep.510300522, PMID:10534331.
[37] Ebinuma H, Imaeda H, Fukuda Y, Miyaguchi S, Yasui T, Hoshino T, *et al*. A case of parathyroid hormone-related peptide producing gallbladder carcinoma and establishment of a cell line, PTHrP-GBK. Dig Dis Sci 2002;47(1):125–129. doi:10.1023/a:1013279907310, PMID:11837712.
[38] Ghosh M, Koike N, Yanagimoto G, Tsunoda S, Kaul S, Hirano T, *et al*. Establishment and characterization of unique human gallbladder cancer cell lines. Int J Oncol 2004;24(5):1189–1196. PMID:15067341.
[39] Jiao W, Miyazaki K, Kitajima Y. Exogenous expression of E-cadherin in gallbladder carcinoma cell line G-415 restores its cellular polarity and differentiation. Int J Oncol 2001;19(6):1099–1107. doi:10.3892/ijo.19.6.1099, PMID:11713577.
[40] Qin B, Ariyama H, Baba E, Tanaka R, Kusaba H, Harada M, *et al*. Activated Src and Ras induce gefitinib resistance by activation of signaling pathways downstream of epidermal growth factor receptor in human gallbladder adenocarcinoma cells. Cancer Chemother Pharmacol 2006;58(5):577–584. doi:10.1007/s00280-006-0219-4, PMID:16532343.
[41] Li M, Zhang Z, Li X, Ye J, Wu X, Tan Z, *et al*. Whole-exome and targeted gene sequencing of gallbladder carcinoma identifies recurrent mutations in the ErbB pathway. Nat Genet 2014;46(8):872–876. doi:10.1038/ng.3030, PMID:24997986.
[42] House MG, Wistuba II, Argani P, Guo M, Schulick RD, Hruban RH, *et al*. Progression of gene hypermethylation in gallstone disease leading to gallbladder cancer. Ann Surg Oncol 2003;10(8):882–889. doi:10.1245/aso.2003.02.014, PMID:14527906.
[43] Gao Y, Wang Z, Zhu Y, Zhu Q, Yang Y, Jin Y, *et al*. NOP2/Sun RNA methyltransferase 2 promotes tumor progression via its interacting partner RPL6 in gallbladder carcinoma. Cancer Sci 2019;110(11):3510–3519. doi:10.1111/cas.14190, PMID:31487418.
[44] Hu YP, Jin YP, Wu XS, Yang Y, Li YS, Li HF, *et al*. LncRNA-HGBC stabilized by HuR promotes gallbladder cancer progression by regulating miR-502-3p/SET/AKT axis. Mol Cancer 2019;18(1):167. doi:10.1186/s12943-019-1097-9, PMID:31752906.
[45] Imamura Y, Mukohara T, Shimono Y, Funakoshi Y, Chayahara N, Toyoda M, *et al*. Comparison of 2D- and 3D-culture models as drug-testing platforms in breast cancer. Oncol Rep 2015;33(4):1837–1843. doi:10.3892/or.2015.3767, PMID:25634491.
[46] Chitcholtan K, Asselin E, Parent S, Sykes PH, Evans JJ. Differences in growth properties of endometrial cancer in three dimensional (3D) culture and 2D cell monolayer. Exp Cell Res 2013;319(1):75–87. doi:10.1016/j.yexcr.2012.09.012, PMID:23022396.
[47] Yuan B, Zhao X, Wang X, Liu E, Liu C, Zong Y, *et al*. Patient-derived organoids for personalized gallbladder cancer modelling and drug screening. Clin Transl Med 2022;12(1):e678. doi:10.1002/ctm2.678, PMID:35075805.
[48] Feng F, Cheng Q, Li B, Liu C, Wang H, Li B, *et al*. Establishment and characterization of 38 novel patient-derived primary cancer cell lines using multi-region sampling revealing intra-tumor heterogeneity of gallbladder carcinoma. Hum Cell 2021;34(3):918–931. doi:10.1007/s13577-021-00492-5, PMID:33813726.
[49] Chen C, Shen H, Tao J, Song H, Ma L, Wang L, *et al*. Effects of tumor-associated fibroblasts on the growth and invasion of gallbladder cancer cells. Journal of Southern Medical University 2015;35(08):1149–1154.
[50] Wang J, Zhang H, Zhao P, Wang G, Zou C. A preliminary study on a three-dimensional tumor drug screening model based on primary cell culture. Hainan Medicine 2021;32(14):1778–1781.
[51] Hu M, Liu J, Chen X, Xu A, Shu S, Wang C, *et al*. Culture of human malignant meningioma primary cells and establishment of an intracranial orthotopic tumor model in mice. Journal of Southern Medical University 2018;38(03):340–345.
[52] Zhang C, Cai Z, Wang X, Jiang D, Wu Q, Wang J. Improved invitro culture method of primary human ovarian serous adenocarcinoma cells. Journal of Anhui Medical University 2020;55(06):979–983.

[53] Alamri AM, Kang K, Groeneveld S, Wang W, Zhong X, Kallakury B, *et al*. Primary cancer cell culture: mammary-optimized vs conditional reprogramming. Endocr Relat Cancer 2016;23(7):535–554. doi:10.1530/erc-16-0071, PMID:27267121.
[54] Yan W, Liu W, Qi J, Fang Q, Fan Z, Sun G, *et al*. A Three-Dimensional Culture System with Matrigel Promotes Purified Spiral Ganglion Neuron Survival and Function In Vitro. Mol Neurobiol 2018;55(3):2070–2084. doi:10.1007/s12035-017-0471-0, PMID:28283883.
[55] Calabrese G, Forte S, Gulino R, Cefalì F, Figallo E, Salvatorelli L, *et al*. Combination of Collagen-Based Scaffold and Bioactive Factors Induces Adipose-Derived Mesenchymal Stem Cells Chondrogenic Differentiation In vitro. Front Physiol 2017;8:50. doi:10.3389/fphys.2017.00050, PMID:28210226.
[56] Zhang H, Mao X, Du Z, Jiang W, Han X, Zhao D, *et al*. Three dimensional printed macroporous polylactic acid/hydroxyapatite composite scaffolds for promoting bone formation in a critical-size rat calvarial defect model. Sci Technol Adv Mater 2016;17(1):136–148. doi:10.1080/14686996.2016.1145532, PMID:27877865.
[57] Zhang K, Fu Q, Yoo J, Chen X, Chandra P, Mo X, *et al*. 3D bioprinting of urethra with PCL/PLCL blend and dual autologous cells in fibrin hydrogel: An in vitro evaluation of biomimetic mechanical property and cell growth environment. Acta Biomater 2017;50:154–164. doi:10.1016/j.actbio.2016.12.008, PMID:27940192.
[58] Selby M, Delosh R, Laudeman J, Ogle C, Reinhart R, Silvers T, *et al*. 3D Models of the NCI60 Cell Lines for Screening Oncology Compounds. SLAS Discov 2017;22(5):473–483. doi:10.1177/2472555217697434, PMID:28288283.
[59] Chiang MC, Lin H, Cheng YC, Yen CH, Huang RN, Lin KH. β-adrenoceptor pathway enhances mitochondrial function in human neural stem cells via rotary cell culture system. J Neurosci Methods 2012;207(2):130–136. doi:10.1016/j.jneumeth.2012.04.005, PMID:22524992.
[60] Lin B, Miao Y, Wang J, Fan Z, Du L, Su Y, *et al*. Surface Tension Guided Hanging-Drop: Producing Controllable 3D Spheroid of High-Passaged Human Dermal Papilla Cells and Forming Inductive Microtissues for Hair-Follicle Regeneration. ACS Appl Mater Interfaces 2016;8(9):5906–5916. doi:10.1021/acsami.6b00202, PMID:26886167.
[61] Gong Z, Huang L, Tang X, Chen K, Wu Z, Zhang L, *et al*. Acoustic Droplet Printing Tumor Organoids for Modeling Bladder Tumor Immune Microenvironment within a Week. Adv Healthc Mater 2021;10(22):e2101312. doi:10.1002/adhm.202101312, PMID:34558233.
[62] Zhang Y, Zhang W, Qin L. Mesenchymal-mode migration assay and anti-metastatic drug screening with high-throughput microfluidic channel networks. Angew Chem Int Ed Engl 2014;53(9):2344–2348. doi:10.1002/anie.201309885, PMID:24478127.
[63] Thoma CR, Zimmermann M, Agarkova I, Kelm JM, Krek W. 3D cell culture systems modeling tumor growth determinants in cancer target discovery. Adv Drug Deliv Rev 2014;69-70:29–41. doi:10.1016/j.addr.2014.03.001, PMID:24636868.
[64] Zhang H, Zhu Y, Shen Y. Microfluidics for Cancer Nanomedicine: From Fabrication to Evaluation. Small 2018;14(28):e1800360. doi:10.1002/smll.201800360, PMID:29806174.
[65] Jiang H, Wang Z, Li Y, Bian R, Shi W. Effect and mechanism of antigallbladder cancer activity of a novel nano-drug delivery system based on 3D cell culture model. Journal of Clinical Hepatobiliary Diseases 2014;30(03):264–268.
[66] Pan MS, Wang H, Ansari KH, Li XP, Sun W, Fan YZ. Gallbladder cancer-associated fibroblasts promote vasculogenic mimicry formation and tumor growth in gallbladder cancer via upregulating the expression of NOX4, a poor prognosis factor, through IL-6-JAK-STAT3 signal pathway. J Exp Clin Cancer Res 2020;39(1):234. doi:10.1186/s13046-020-01742-4, PMID:33153467.
[67] Chen Q, Utech S, Chen D, Prodanovic R, Lin JM, Weitz DA. Controlled assembly of heterotypic cells in a core-shell scaffold: organ in a droplet. Lab Chip 2016;16(8):1346–1349. doi:10.1039/c6lc00231e, PMID:26999495.
[68] Mehta G, Hsiao AY, Ingram M, Luker GD, Takayama S. Opportunities and challenges for use of tumor spheroids as models to test drug delivery and efficacy. J Control Release 2012;164(2):192–204. doi:10.1016/j.jconrel.2012.04.045, PMID:22613880.
[69] Nath S, Devi GR. Three-dimensional culture systems in cancer research: Focus on tumor spheroid model. Pharmacol Ther 2016;163:94–108. doi:10.1016/j.pharmthera.2016.03.013, PMID:27063403.
[70] Clevers H. Modeling Development and Disease with Organoids. Cell 2016;165(7):1586–1597. doi:10.1016/j.cell.2016.05.082, PMID:27315476.
[71] Lugli N, Kamileri I, Keogh A, Malinka T, Sarris ME, Talianidis I, *et al*. R-spondin 1 and noggin facilitate expansion of resident stem cells from non-damaged gallbladders. EMBO Rep 2016;17(5):769–779. doi:10.15252/embr.201642169, PMID:26993089.
[72] Scanu T, Spaapen RM, Bakker JM, Pratap CB, Wu LE, Hofland I, *et al*. Salmonella Manipulation of Host Signaling Pathways Provokes Cellular Transformation Associated with Gallbladder Carcinoma. Cell Host Microbe 2015;17(6):763–774. doi:10.1016/j.chom.2015.05.002, PMID:26028364.
[73] Weigt J, Malfertheiner P. Cisplatin plus gemcitabine versus gemcitabine for biliary tract cancer. Expert Rev Gastroenterol Hepatol 2010;4(4):395–397. doi:10.1586/egh.10.45, PMID:20678012.
[74] García P, Rosa L, Vargas S, Weber H, Espinoza JA, Suárez F, *et al*. Hippo-YAP1 Is a Prognosis Marker and Potentially Targetable Pathway in Advanced Gallbladder Cancer. Cancers (Basel) 2020;12(4):778. doi:10.3390/cancers12040778, PMID:32218280.
[75] Kato S, Fushimi K, Yabuki Y, Maru Y, Hasegawa S, Matsuura T, *et al*. Precision modeling of gall bladder cancer patients in mice based on orthotopic implantation of organoid-derived tumor buds. Oncogenesis 2021;10(4):33. doi:10.1038/s41389-021-00322-1, PMID:33866327.
[76] Weeber F, Ooft SN, Dijkstra KK, Voest EE. Tumor Organoids as a Pre-clinical Cancer Model for Drug Discovery. Cell Chem Biol 2017;24(9):1092–1100. doi:10.1016/j.chembiol.2017.06.012, PMID:28757181.
[77] Ji L, Tu J, Jiang F. Research status of human gallbladder cancer model. Chinese Journal of Hepatobiliary Surgery 2007;13(11):791–792.
[78] Taetle R, Jones OW, Honeysett JM, Abramson I, Bradshaw C, Reid S. Use of nude mouse xenografts as preclinical screens. Characterization of xenograft-derived melanoma cell lines. Cancer 1987;60(8):1836–1841. doi:10.1002/1097-0142(19871015)60:8<1836::aid-cncr2820600827>3.0.co;2-o, PMID:3652009.
[79] Merk J, Rolff J, Becker M, Leschber G, Fichtner I. Patient-derived xenografts of non-small-cell lung cancer: a pre-clinical model to evaluate adjuvant chemotherapy? Eur J Cardiothorac Surg 2009;36(3):454–459. doi:10.1016/j.ejcts.2009.03.054, PMID:19502076.
[80] de Plater L, Laugé A, Guyader C, Poupon MF, Assayag F, de Cremoux P, *et al*. Establishment and characterisation of a new breast cancer xenograft obtained from a woman carrying a germline BRCA2 mutation. Br J Cancer 2010;103(8):1192–1200. doi:10.1038/sj.bjc.6605900, PMID:20877358.
[81] Kolfschoten GM, Pinedo HM, Scheffer PG, Schlüper HM, Erkelens CA, Boven E. Development of a panel of 15 human ovarian cancer xenografts for drug screening and determination of the role of the glutathione detoxification system. Gynecol Oncol 2000;76(3):362–368. doi:10.1006/gyno.1999.5689, PMID:10684711.
[82] Huynh H, Soo KC, Chow PK, Panasci L, Tran E. Xenografts of human hepatocellular carcinoma: a useful model for testing drugs. Clin Cancer Res 2006;12(14 Pt 1):4306–4314. doi:10.1158/1078-0432.ccr-05-2568, PMID:16857806.
[83] Pan W, Chen Y, Du Q, Jiang L. Establishment and discussion of orthotopic transplantation model of gallbladder carcinoma. Fujian Medical Journal 2016;38(06):48–51.
[84] Mita Y, Ajiki T, Kamigaki T, Okazaki T, Hori H, Horiuchi H, *et al*. Antitumor effect of gemcitabine on orthotopically inoculated human gallbladder cancer cells in nude mice. Ann Surg Oncol 2007;14(4):1374–1380. doi:10.1245/s10434-006-9191-9, PMID:17235714.
[85] Aoki Y, Shimura H, Li H, Mizumoto K, Date K, Tanaka M. A model of port-site metastases of gallbladder cancer: the influence of peritoneal injury and its repair on abdominal wall metastases. Surgery 1999;125(5):553–559. PMID:10330945.
[86] Enomoto M, Naoe S, Harada M, Miyata K, Saito M, Noguchi Y. Carcinogenesis in extrahepatic bile duct and gallbladder—carcinogenic effect of N-hydroxy-2-acetamidofluorene in mice fed a "gallstone-inducing" diet. Jpn J Exp Med 1974;44(1):37–54. PMID:4274953.
[87] Tajima Y, Eto T, Tsunoda T, Tomioka T, Inoue K, Fukahori T, *et al*. Induction of extrahepatic biliary carcinoma by N-nitrosobis(2-oxopropyl)amine in hamsters given cholecystoduodenostomy with dissection of the common duct. Jpn J Cancer Res 1994;85(8):780–788. doi:10.1111/j.1349-7006.1994.tb02948.x, PMID:7928622.
[88] Ikematsu Y, Tomioka T, Kitajima T, Inoue K, Tajima Y, Kanematsu T. Tauroursodeoxycholate and cholestyramine enhance biliary carcinogenesis in hamsters. World J Surg 2000;24(1):22–26. doi:10.1007/s002689910005, PMID:10594198.
[89] Blomme A, Van Simaeys G, Doumont G, Costanza B, Bellier J, Otaka Y, *et al*. Murine stroma adopts a human-like metabolic phenotype in the PDX model of colorectal cancer and liver metastases. Oncogene 2018;37(9):1237–1250. doi:10.1038/s41388-017-0018-x, PMID:29242606.
[90] Zhang F, Wang W, Long Y, Liu H, Cheng J, Guo L, *et al*. Characterization of drug responses of mini patient-derived xenografts in mice for predicting cancer patient clinical therapeutic response. Cancer Commun (Lond) 2018;38(1):60. doi:10.1186/s40880-018-0329-5, PMID:30257718.
[91] Brown ZJ, Heinrich B, Greten TF. Mouse models of hepatocellular carcinoma: an overview and highlights for immunotherapy research. Nat Rev Gastroenterol Hepatol 2018;15(9):536–554. doi:10.1038/s41575-018-0033-6, PMID:29904153.
[92] Mak IW, Evaniew N, Ghert M. Lost in translation: animal models and clinical trials in cancer treatment. Am J Transl Res 2014;6(2):114–118. PMID:24489990.
[93] Langer EM, Allen-Petersen BL, King SM, Kendsersky ND, Turnidge MA, Kuziel GM, *et al*. Modeling Tumor Phenotypes In Vitro with Three-Dimensional Bioprinting. Cell Rep 2019;26(3):608–623.e6. doi:10.1016/j.celrep.2018.12.090, PMID:30650355.
[94] Yang H, Sun L, Pang Y, Hu D, Xu H, Mao S, *et al*. Three-dimensional bioprinted hepatorganoids prolong survival of mice with liver failure. Gut 2021;70(3):567–574. doi:10.1136/gutjnl-2019-319960, PMID:32434830.
[95] Xie F, Sun L, Pang Y, Xu G, Jin B, Xu H, *et al*. Three-dimensional bio-printing of primary human hepatocellular carcinoma for personalized medicine. Biomaterials 2021;265:120416. doi:10.1016/j.biomaterials.2020.120416, PMID:33007612.
[96] Mao S, He J, Zhao Y, Liu T, Xie F, Yang H, *et al*. Bioprinting of patient-derived in vitro intrahepatic cholangiocarcinoma tumor model: establishment, evaluation and anti-cancer drug testing. Biofabrication 2020;12(4):045014. doi:10.1088/1758-5090/aba0c3, PMID:32599574.
[97] Li WG, Luo XY, Hill NA, Ogden RW, Smythe A, Majeed A, *et al*. A mechanical model for CCK-induced acalculous gallbladder pain. Ann Biomed Eng 2011;39(2):786–800. doi:10.1007/s10439-010-0205-1, PMID:21108005.
[98] Ren X, Xu H. The formation principle of cholecystitis and establishment of animal model. Huaihai Medicine 2009;27(03):276–277.