Clinics in Oncology

Editorial
Published: 09 May, 2016

Simple Primary Culture Method for Hepatocellular Carcinoma: Source for Researches and Possibility of Personalized Treatment

Zu-Yau Lin[1,2]*

[1]Division of Hepatobiliary Medicine, Kaohsiung Medical University Hospital, Taiwan

[2]Department of Internal Medicine, Faculty of Medicine, College of Medicine, Kaohsiung Medical University, Taiwan

Editorial

Hepatocellular carcinoma (HCC) is the most common primary liver cancer. Invasion and metastasis of HCC are determined by the characteristics of cancer cells and the interactions between the cancer cells and the tumor microenvironment. Cancer-associated fibroblast (CAF) is one of the most crucial components of the tumor microenvironment to promote the growth and invasion of cancer cells [1-6]. Several staging systems, such as the Barcelona Clinic Liver Cancer (BCLC) guideline, American Joint Committee on Cancer (AJCC) TNM staging system, and biomarker-combined Japanese Integrated Staging (JIS) score, have been applied clinically as treatment guidelines and tools for prediction of therapeutic outcomes [7,8]. Several molecular markers, such as hepatocyte growth factor, vascular endothelial growth factor, and transforming growth factor β1, have also been applied to predict the outcomes of HCC patients [7,9-11]. However, all currently applied HCC staging systems and biomarkers are unable to predict the proliferative speeds of both cancer cells and CAFs in individual patient. Therefore, patients with same stage of HCC may have quite different outcomes because the characteristics of cancer cells and tumor microenvironment may vary from patients to patients. On the other hand, purchased cancer cell lines are frequently applied in researches. These cells lines usually have uniform characteristics due to homogeneity of cells caused by long term repeated *in vitro* cultures. The experimental results obtained from purchased cell lines can not actually represent the situations in patients as demonstrated in our previous study [12]. Establishment of a simple primary culture method to collect both HCC cells and CAFs not only can reveal their characteristics in individual patient for the possibility of personalized treatment but also can provide us plenty of cells from different patients for further HCC studies. The traditional methods for plating of cells to initiate the primary culture are to mince or dissociate the specimens obtained from biopsy or surgical resection of the tumor. These methods usually need to apply agents such as trypsin-EDTA for dissociation of cell blocks. The use of trypsin-EDTA solution can reduce the antioxidant defense of the cells thus impair the successful rate of plating due to loss of cellular glutathione, which participates in many critical cellular functions, including antioxidant defense and cell growth [13]. The susceptibilities of HCC cells and CAFs obtained from different patients to oxidative stress may be different. The use of trypsin-EDTA solution may cause unpredictably different degrees of damage to both HCC cells and CAFs obtained from different patients, and thus alter our interpretation the results of the primary culture. Moreover, biopsy and surgical resection of the tumor to obtain specimens for culture are hard to be widely applied in clinical practice. Ultrasound-guided fine-needle aspiration (FNA) cytology or biopsy of hepatic tumor is frequently applied in the collection of specimens for diagnosis because it is considered safe, efficacious, accurate, and cost-effective [14,15]. The specimens obtained from FNA are < 0.7 mm in width and are usually < 1–2 mm in length when 22G Chiba needle is applied. According to personal experience, specimens obtained from FNA are not necessary to be further minced or dissociated for plating and the successful rate for primary culture can reach to more than 97% [16]. FNA of the tumor can become a clinically acceptable method for the collection of specimens for primary culture. The detail procedures for primary culture using specimens obtained from FNA were described in our previous papers [17,18]. The key point for the successful primary culture is adequate serum-containing culture medium applied in the initial plating. The specimens should not float in excessive amount of medium and 1.2-1.3 mL medium for a 25-cm^2 flask is an ideal amount for initiation of plating. The first time replacement of medium should be delayed to the 3rd to 5th day after plating. Outgrowth of cells occurs within the 3rd to 7th day of culture and the cells usually will

OPEN ACCESS

Correspondence:
Zu-Yau Lin, Division of Hepatobiliary Medicine, Kaohsiung Medical University Hospital and Department of Internal Medicine, Faculty of Medicine, College of Medicine, Kaohsiung Medical University, No. 100 Tzyou 1st Road, Kaohsiung 807, Taiwan, Tel: 007-3121101;
E-mail: linzuyau@yahoo.com.tw

Received Date: *26 Apr 2016*
Accepted Date: *03 May 2016*
Published Date: *09 May 2016*

Citation:
Zu-Yau Lin. Simple Primary Culture Method for Hepatocellular Carcinoma: Source for Researches and Possibility of Personalized Treatment. Clin Oncol. 2016; 1: 1001.

continue to proliferate till around the 14th day of culture. After then, the proliferative speeds of cultured cells can be divided into slow and rapid proliferation two types. The cultured cells can be applied for (1) researches [6,12,18,19] and (2) prediction of outcomes. However, they are three potential pitfalls in the prediction the outcomes of patients using this culture method. (1) Since patient may have multiple tumors with different characteristics, the result of primary culture from one of these tumors theoretically do not represent the characteristics of all tumors in this patient. To overcome this potential pitfall, the largest tumor in each patient that theoretically can represent the tumor with the most malignant characteristic in this patient should be selected for aspiration. (2) HCC tumor may have polyclonal cancer cells with different malignant characteristics. The specimens obtained from one session of FNA have the potential to miss some clones of cancer cells. This pitfall is unable to be completely prevented. However, our previous study showed that the predominant clones of HCC cells in patients with invasive HCC were those with rapid proliferation [16]. Random aspirations from these tumors thus have high probability to obtain these rapidly proliferative cells for culture. (3) The *in vitro* and *in vivo* growth patterns of cells may be different due to different environments for cell growth. Although this primary culture method have above potential pitfalls to represent the characteristic of HCC in each patient, significant associations of the proliferative speeds of cultured cells with HCC invasiveness and 1-year survivals were demonstrated in our previous study [16]. The *in vivo* proliferative pattern of cells may be adequately represented in our primary culture. Co-culture of both cancer cells and CAFs in this method which may represent the similarity of HCC *in vivo* is an important explanation. Patients with rapidly proliferative HCC cells in primary culture have highly malignant phenotype. Moreover, cells with rapidly proliferative character can be easily collected to sufficient amount for further anti-cancer drug sensitivity tests. This makes us have possibility to design a personalized HCC treatment stratagem. The culture equipments required for this method are not expensive and can be seen in almost all hospitals with basic laboratory facility. The technique is also very easy. This method can be applied in clinical practice to provide cells from individual patient for researches and the possibility of personalized HCC treatment.

References

1. Xing F, Saidou J, Watabe K. Cancer associated fibroblasts (CAFs) in tumor microenvironment. Front. Biosci. 2010; 15: 166–179.
2. Shimoda M, Mellody KT, Orimo A. Carcinoma-associated fibroblasts are a rate-limiting determinant for tumour progression. Semin Cell Dev Biol. 2010; 21: 19–25.
3. Mishra P, Banerjee D, Ben-Baruch A. Chemokines at the crossroads of tumor-fibroblast interactions that promote malignancy. J Leukoc Biol. 2011; 89: 31–39.
4. Yang JD, Nakamura I, Roberts LR. The tumor microenvironment in hepatocellular carcinoma: current status and therapeutic targets. Semin Cancer Biol. 2011; 21: 35–43.
5. Mazzocca A, Dituri F, Lupo L, Quaranta M, Antonaci S, Giannelli G. Tumor-secreted lysophostatidic acid accelerates hepatocellular carcinoma progression by promoting differentiation of peritumoral fibroblasts in myofibroblasts. Hepatology. 2011; 54: 920–930.
6. Lin ZY, Chuang YH, Chuang WL. Cancer-associated fibroblasts up-regulate CCL2, CCL26, IL6 and LOXL2 genes related to promotion of cancer progression in hepatocellular carcinoma cells. Biomed Pharmacother. 2012; 66: 525–529.
7. Marrero JA, Kudo M, Bronowicki JP. The challenge of prognosis and staging for hepatocellular carcinoma. Oncologist. 2010; 15: 23–33.
8. Han KH, Kudo M, Ye SL, Choi JY, Poon RT, Seong J, et al. Asian consensus workshop report: expert consensus guideline for the management of intermediate and advanced hepatocellular carcinoma in Asia. Oncology. 2011; 81 (Suppl. 1): 158–164 carcinoma in Asia. Oncology. 2011; 81: 158-164.
9. Choi SB, Lee JG, Kim KS, Yoon DS, Choi JS, Lee WJ, et al. The prognosis and survival analysis according to seven staging systems of hepatocellular carcinoma following curative resection. Hepatogastroenterology. 2008; 55: 2140–2145.
10. Gomaa AI, Khan SA, Leen EL, Waked I, Taylor-Robinson SD. Diagnosis of hepatocellular carcinoma. World J Gastroenterol. 2009; 15: 1301-1314.
11. Hsieh MY, Lin ZY, Chuang WL. Serial serum VEGF-A, angiopoietin-2, and endostatin measurements in cirrhotic patients with hepatocellular carcinoma treated by transcatheter arterial chemoembolization. Kaohsiung J Med Sci. 2011; 27: 314–322.
12. Lin ZY, Chuang WL. Genes responsible for the characteristics of primary cultured invasive phenotype hepatocellular carcinoma cells. Biomed. Pharmacother. 2012; 66: 454–458.
13. Reiners JJ Jr, Mathieu P, Okafor C, Putt DA, Lash LH. Depletion of cellular glutathione by conditions used for the passaging of adherent cultured cells. Toxicol Lett. 2000; 115: 153–163.
14. Wang CW, Lin ZY, Chuang WL, Wang LY, Yu ML, Chen SC, et al. Safety of fine-needle aspiration in patients with small hepatocellular carcinoma. Hepatol Res. 2005; 31: 31–35.
15. Wee A. Fine needle aspiration biopsy of hepatocellular carcinoma and hepatocellular nodular lesions: role, controversies and approach to diagnosis. Cytopathology. 2011; 22: 287–305.
16. Lin ZY, Wu CC, Chuang YH, Chuang WL. Clinical utility of a simple primary culture method in hepatocellular carcinoma patients. J Gastroenterol Hepatol. 2015; 30: 352–357.
17. Lin ZY, Chuang WL, Chuang YH, Yu ML, Hsieh MY, Wang LY, et al. Discordant influence of amphotericin B on epirubicin cytotoxicity in primary hepatic malignant cells collected by a new primary culture technique. J. Gastroenterol. Hepatol. 2006; 21: 398–405.
18. Lin ZY, Chuang WL. Hepatocellular carcinoma cells cause different responses in expressions of cancer-promoting genes in different cancer-associated fibroblasts. Kaohsiung J Med Sci. 2013; 29: 312–318.
19. Lin ZY, Wu CC, Chuang YH, Chuang WL. Anti-cancer mechanisms of clinically acceptable colchicine concentrations on hepatocellular carcinoma. Life Sci. 2013; 93: 323–328.