A Multicellular 3D Heterospheroid Liver Tumor Models for Anti-Cancer Drug Testing

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Statement of Purpose: Cancer is a complicated disease and before there can be any hope of curing/treating the disease, each aspect of cancer must be understood. However, current 2D and sandwich models used to understand the disease fail to recapitulate many aspects of cancer and therefore, the results of these models may only be pertinent to the model itself and not the disease, especially during anti-cancer drug testing. A more relevant model would incorporate various aspects of cancer including 1) 3 dimensions, 2) multiple cell types, and 3) an extracellular matrix barrier. Spheroids are agglomerations of cells shaped like a sphere. These spheroids are 3 dimensional constructs that resemble the dimensionality and physiology of liver cancer *in vivo* [1]. Cancer is composed of multiple cell types; the cancer itself and various stromal cells. Spheroids can be composed of a singular cell type (homospheroid) or in this case multiple cell types (heterospheroid). Finally, cancer contains a thick barrier of extracellular matrix surrounding it which aids in liver cancer progression [2]. This aspect of cancer can be recapitulated by encapsulating the spheroids in collagen gel. In this study, the hanging drop method and co-culture of liver carcinoma with stromal cells were used to form heterospheroids made from HepG2 liver carcinoma and 3T3 fibroblast cells. These heterospheroids were then encapsulated in collagen in order to create a model of liver cancer that would act more similarly to in vivo conditions. The 3D heterospheroid model was tested with anti-cancer drug to determine how each of the above aspects affects drug resistance.

Methods: Human HepG2 cancer cells were cultured in DMEM, 10% FBS, 1% penicillin/streptomycin, 1% glutamax. Murine 3T3-J2 fibroblasts were cultured in similar media except 10% horse serum and culture was treated with mitomyocin C to arrest cell growth. The hanging drop technique was used to agglomerate these cells into spheroids. For homospheroids, a density of 1,000 HepG2 cells per 30 µL was obtained. For heterospheroids a density of 1,000 HepG2 cells and 1,000 growth arrested 3T3-J2 fibroblasts per 30 µL was obtained. This cell media was then pipetted as 30 µL drops onto a Petri dish lid. Petri dish lid was flipped to form hanging drops which are allowed to incubate for 3 days in an incubator at 37°C, 10% CO_2. The spheroids were collected either to media or to collagen gel. For collagen gel formation, 10:1 ratio of collagen I (1.2 mg/mL) was mixed with 10x DMEM, and then spheroids are mixed. Collagen gel/media/spheroids were allowed to gel in incubator for 60 min, before media is added. When treated with anti-cancer drug, the condition was incubated with 10 µM doxorubicin. To determine the viability of spheroids, the LIVE/DEAD cell assay was performed. To determine cell viability/growth with drug treatment, alamar blue assay was used. Spheroid diameter was measured using optical microscope and imaging software. Alamar blue assay was performed by incubating spheroids in 10 µM resazurin at 37°C, 10% CO_2 for 1 hour. Collected samples were measured with a fluorometer. To determine cytochrome p450 activity of HepG2, EROD assay was performed. Collected samples were measured with a fluorometer.

Results: LIVE/DEAD assay reveals that most cells exhibit green fluorescence and therefore can survive as a spheroid (Fig.1A). Alamar blue assay reveals that 3D spheroid cultures showed significantly higher drug resistance to 2D cell cultures after 4 days of drug treatment (Fig.1B). Homospheroids in collagen gel showed more drug resistance to homospheroids not in collagen gel. Similar results were observed in heterospheroid cultures. Among 3D spheroids cultures without collagen gel, heterospheroids showed significantly higher drug resistance than homospheroids but no significant difference in collagen gel cultures. EROD assay reveals that heterospheroids in gel showed significantly higher drug metabolism function than those without gel, indicating more drug resistance (Fig.2).

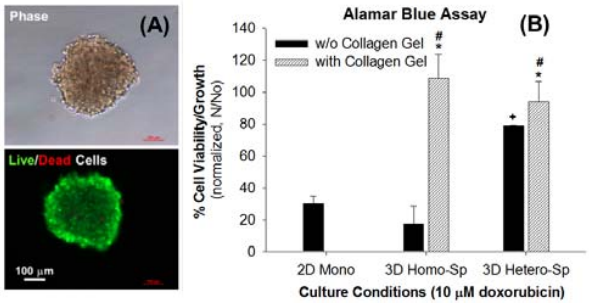

Figure 1. (A) Live/dead cell assay of HepG2 spheroids formed by hanging drop method (day 4). (B) Effect of anti-cancer drug (doxorubicin) on cell viability in 2D and 3D cultures after 4 days of drug treatment. (n=3, P<0.001, * 3D gel vs. no gel, # 3D gel vs. 2D, + 3D no gel heterospheroids vs. homospheroids).

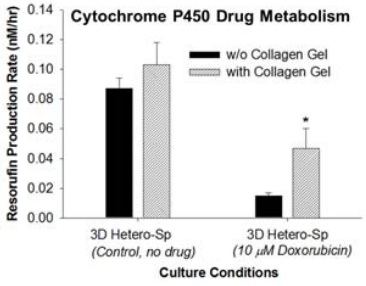

Fig.2. Cytochrome P450 assay (EROD) in 3D heterospheroid cultures with or w/o drug treatment (Day 7). (n=3, * P<0.001, w/o gel vs with gel)

Conclusions: In this study, we developed a new 3D heterospheroid tumor model to mimic in vivo conditions. Results indicate the 3D heterospheroid model is more resistance than 2D and 3D homospheroid model. This study will provide useful information toward the development of biomimetic tumor models in vitro for anti-cancer drug testing.

References:

1. Van Zijl, F. World J. of Hepatology. 2010; 2:1-7
2. Schrader, J. Hepatology. 2011; 53:1192-1205