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Research Article

Potentiation of Bio Repositories In Personalized Medicine: Tumor Cells Establishment

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Abstract

The introduction of three-dimensional (3D) tumor cultures has revolutionized anticancer drug research as these cultures allow for the study of drug resistance mechanisms that cannot be explored in traditional two dimensional (2D) monolayer cultures. Discoveries in the 3D tumor culture field suggest that individualized drug sensitivity testing of solid tumor specimens through the establishment and use of 3D tumor cell cultures following tissue collection will become a routine service offered by modern tissue repositories as they expand from their traditional research role to active participation in personalized medicine. Unfortunately, most information related to 3D tumor cultures comes from studies using established tumor cell lines rather than primary tumor cultures. However, accumulation of genetic aberrations in cancer cell lines occurs with increasing number of passages severely limiting their usefulness for personalized medicine. There is only very limited information available concerning technologies and standard operating procedures for the efficient and routine isolation and processing of primary tumor cells for the establishment of 3D tumor cultures from solid tumor specimens. The purpose of this work was to review experimental data from the literature that may provide relevant information concerning the isolation and processing of primary tumor cells for the establishment of 3D tumor cultures. Information reviewed here may help bio repositories in the development and standardization of technologies and standard operating procedures related to the use of 3D tumor cultures.

Introduction

Accumulation of genetic aberrations in cancer cell lines occurs with increasing number of passages severely limiting their usefulness for personalized medicine [1,2]. In contrast, the critical importance of technologies related to the derivation, short term culture, and testing of primary tumor cells from solid tumors is increasingly recognized (for a recent review see 2). Short-term primary cultures of tumor cells derived from pieces of solid tumors have been used for predicting anticancer drug responses [3,4]. Observations suggest that individualized drug sensitivity testing of solid tumors through the isolation and culturing primary tumor cells in 3D may soon become routine and modern tissue repositories will need to be ready to support these activities as they expand from their traditional research role to active participation in personalized medicine [5].

Cancer cells grown in 3D cultures in a polymeric ECM closely mimic the biology of tumor development in vivo and numerous studies indicate that 3D cultures are superior to traditional 2D monolayer cultures for studies of key cellular behaviors like differentiation, proliferation, invasion and apoptosis [6-8]. Cancer cells grown in 3D culture are more resistant to chemotherapeutic agents, radiation and oncolytic virotherapy than cells in 2D culture and 3D tumor cell cultures are useful for preclinical evaluation of the cytotoxic effect of anticancer agents [6, 9-16].

Unfortunately, most information related to 3D tumor cultures comes from studies using established tumor cell lines rather than primary tumor cultures and there is only very limited information available concerning technologies and SOPs for the efficient and routine isolation and processing of primary tumor cells for the establishment of 3D tumor cultures from solid tumor specimens.

The purpose of this work was to review experimental data from the literature that may provide relevant information concerning the isolation and processing of primary tumor cells for the establishment of 3D tumor cultures.

Materials and Methods

A review of the English language literature related to the isolation and processing of primary tumor cells for the establishment of 3D tumor cultures from solid tumor specimens was performed.

Results

Only a limited number of reports are available related to the isolation and processing of primary tumor cells for the establishment of 3d tumor cultures from solid tumor specimens. However, these relate to colorectal, lung and brain tumors indicating that isolation and growth of primary tumors cells in 3D cultures is possible for a variety of solid tumors. The protocols have an initial step of mechanical mincing of tissues followed by at least some disruption of the original extracellular matrix by enzymatic dissociation. Isolated tumor cells or fragments can be grown and analyzed in a wide variety of 3D culture systems. Four prominent examples are reviewed below.

Example 1. Kondo J et al. 2011 [17].

Kondo et al found that retaining cell–cell contact enables preparation and culture of spheroids composed of pure primary cancer cells from colorectal cancer specimens that retain the characteristics of the parental tumors, grow in vitro in 3D cultures and could be used for personalized diagnostic applications, including chemosensitivity assays.

To successfully maintain the cell–cell contact, they used Liberase DH (Roche Diagnostics) as a blend of digestion enzymes. They optimized the enzyme concentration and the digestion time to avoid overdigestion of colorectal tissues. Cancer specimens from colorectal cancer patients were mechanically and enzymatically digested and separated into two fractions using a cell strainer: the organoid fraction (ORG), which was retained in a 40-?m strainer, and the flow-through fraction (FT), which passed through the strainer. The FT fraction mainly contained single cells and many of the cells with epithelial markers were dead. In contrast, the ORG contained irregular sheet- or tube-like structures, which were termed organoids. After overnight culture, the organoids became spherical and bright with a smooth surface and were termed cancer tissue–originated spheroids (CTOSs). During formation, the CTOSs were draped with cellular debris that easily detached with pipetting. The diameter of the CTOSs was?40–500 ?m, depending on the cell strainer size. A CTOS with a diameter of 100 ?m consisted of ?100 cells. The authors were able to prepare CTOSs in most of the colorectal cancer specimens regardless of the disease stage and histology. CTOSs were highly viable with only a few dead cells on the outside. CTOSs could be cultured and expanded in vitro. Extracellular matrix was critical for favorable growth of CTOSs. Excellent proliferation was accomplished with the use of a 3D type I collagen-based culture system and serum-free medium designed for embryonic stem cells. The success rate for growth was 73.5%. CTOSs were composed of highly purified and viable cancer cells, that retained the characteristics of the parental tumors, grew in vitro and could be used for personalized diagnostic applications, including chemosensitivity assays.

Example 2. Endo H. et al. 2013 [18].

This study demonstrated the usefulness of cancer tissue–originated spheroid (CTOS) method for the primary culture of lung cancer cells. Surgical specimens and pleural effusion samples from lung cancer patients were minced with a scalpel into approximately one square mm pieces, and washed with Hank's balanced salt solution. Specimens were then digested in DMEM supplemented with Liberase DH, at 37°C for 1 to 2 hours with gentle stirring by a magnetic bar. Digested tissue suspensions were passed through 500-?m and 250-?m metal mesh filters to remove large masses of undigested fragments. Suspensions were further filtered through 100-?m and 40-?m cell strainers. Fragments on the cell strainer and cells in the flow-through fractions were collected separately, and were each washed with HBSS and cultured in StemPro hESC medium in a nontreated dish. Pleural effusions were transferred to 50-ml tubes and centrifuged at 200 g. Pellets were resuspended in HBSS, filtered through 40-?m cell strainers, and collected and cultured in the same manner as surgical specimens. CTOSs were embedded in Matrigel GFR and cultured in 100 ?l of basal medium containing growth factors (NRG1, Long-IGF1, bFGF , Activin A, or EGF). Basal medium consisted of DMEM/F12, 2% BSA fraction V, nonessential amino acids, penicillin, streptomycin, ascorbic acid, human transferrin, ?-mercaptoethanol, and trace elements. Lung carcinoma CTOSs grew in vitro and could be used for personalized chemosensitivity assays.

Example 3. Jiquet Jiglaire et al. 2014 [19].

Glioblastoma tumor samples were automatically sectioned using a tissue-chopper. Cell suspensions were obtained after enzymatic dissociation with both 5mg/mL of trypsin and 200U/mL of DNAse for 10 min at 37 1C. Cell suspension filtration was done on a 0.40 mm filter and suspension was centrifuged at 1300 rpm for 5min. Cells were resuspended in PBS and were grown in a commercially available 3D hydrogel (Extracel™, Tebu- bio, LePerray-en Yvelines,France).

Example 4. Xu Z et al. 2013 [16].

Cells from fresh lung cancer tissues were cultured and tested in 3D using a microfluidic chip-based, three-dimensional (3D) co-culture drug sensitivity test platform. Immediately after removal, lung cancer specimens were immersed in culture medium and kept cold while being transported to the lab. The necrotic areas, fatty tissue, blood clots, and connective tissue were removed. The tumors were finely minced then digested with collagenase I (0.3 mg/mL) for 2 h at 37C. The culture medium was collected and centrifuged (200 g, 5 min). The sediments were prepared as single cell suspensions in serum-free DMEM and separated into different fractions using Percoll discontinuous gradients centrifugation (400 g, 20 min; 30% and 70% Percoll). The cells at the interface of 30% and 70% Percoll were collected and washed twice with PBS to remove the gradient medium and obtain the cancer cells. Then, the primary celleBME mixtures were introduced into the chambers of a microfluidic chip-based, three-dimensional (3D) co-culture system.

Discussion

Accumulation of genetic aberrations in cancer cell lines occurs with increasing number of passages severely limiting their usefulness for personalized medicine [1,2]. Research reviewed here indicates that isolation and culturing of primary tumor cells in 3D is possible and it is likely that primary 3D tumor cultures will soon be routinely used for individualized solid tumor testing. Modern tissue repositories will need to be ready to support these activities as they expand from their traditional research role to active participation in personalized medicine.

Published protocols for the isolation and culturing of primary tumor cells in 3D consist of an initial step of mincing of solid tumor specimens followed by at least some disruption of the original extracellular matrix by enzymatic dissociation. Isolated tumor cells or fragments can be grown and analyzed in a wide variety of 3D culture systems. Research indicates that methods for isolation and processing of primary tumor cells for the establishment of 3D tumor cultures need to be tumor type specific. As more research findings become available in this clinically highly relevant field, it will be important to standardize tissue and tumor-specific protocols related to the establishment of primary 3D tumor cell cultures. An introduction of 3D primary tumor cultures in routine bio repository activities will also require an adjustment of standard operating procedures, consent procedures and institutional review board (IRB) protocols to include routine isolation of primary tumor cells at the time of tumor tissue collection.

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