Stem-like cell characteristics of cancer spheroids grown in a microfabricated cell array three-dimensional culture system Cell-able™ Oncology

Koichi Yokota, Tomoko Jomura, Emiko Ozeki, Takeshi Ikeya, Transparent Inc., 4-2-1, Wakahagi, Inzai 270-1609, Japan.

Cell-able™ TOYO GOSEI

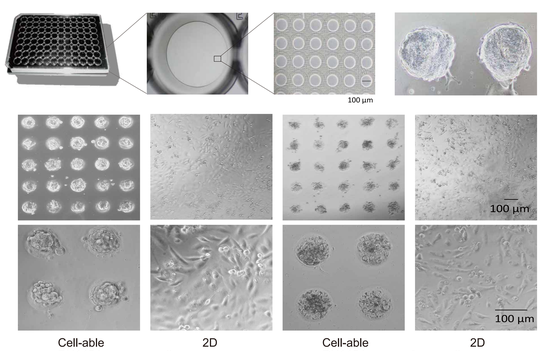
Abstract

Recently, the cell biology field has come to appreciate the dissimilarity between two- (2D) and three-dimensional (3D) environments in which cells routinely operate in vivo. Efficacy of most anti-cancer drugs have been evaluated in 2D environment but it must be desirable to evaluate it in 3D environment. We developed a novel 3D cell culture system named Cell-able™ Oncology utilizing photo-sensitive materials that change to hydro-gel after UV irradiation and the optimized molecular design not to allow the cells adhere to the hydro-gel. In this study, we demonstrated that the cancer spheroids formed on the Cell-able™ Oncology in serum-free and serum-containing media showed chemoresistant, high expression of stem cell markers and drug transporters compared to that on the 2D monolayer plate. Thus, the spheroids grown on the Cell-able™ Oncology could provide novel insight to the pharmacodynamics and pharmacokinetics in the anti-cancer drug research and development.

Materials and Methods

Cell culture

A549 human lung cancer and DU145 human prostate cancer cells (ATCC™) were cultured in RPMI-1640 supplemented with 10% FCS, 1% penicillin and streptomycin (serum media). The cells were trypsinated and resuspended in the serum media or the serum-free media supplemented with 10 ng/ml EGF (Upstate), 20 ng/ml basic FGF (Wako) and human insulin (5 μg/ml) and seeded on Cell-able™ (Transparent) or collagen-coated 2D plates (BD Biosciences). The Cell-able™ plates have arrayed type I collagen-coated cell attachment areas of circle 100 μm in diameter (see below) on each well of the culture plate. The cells were cultured in the serum or serum-free media to form spheroids for several days after seeding on Cell-able™. Patient-derived endometrial cancer cells were prepared from cancer tissues surgically resected from patients. The cancer tissues were digested with collagenase (Roche) and passed through 70 μm Cell Strainer (BD Biosciences). The cells were cultured on Cell-able™ in serum-free media. The typical morphology of DU145 cultured on Cell-able™ was quite different from that on 2D plates as shown below;



Cell viability assays

DU145 human prostate or A594 human lung cancer or human endometrial primary cells cultured in the serum media or the serum-free media were treated with anti-cancer drugs or vehicle (<0.1% DMSO) for 72 hours. The DU145 and primary cells were stained with Click-iT® EdU Cell Proliferation Assay and DAPI (Invitrogen). The high content analysis was performed by ImageXpress MICRO™ system (Molecular Devices). The cell viability was measured by CellTiter-Glo® Cell Viability Assay (Promega) or the nuclear DAPI intensity.

FACS analysis

The cells were detouched with Accutase® (Innovative Cell Technologies) to obtain cell suspension and fixed with 3.8% formaldehyde. After blocking, the cells were stained with FITC-labeled anti-human CD24 antibody (Invitrogen) and APC-labeled anti-human CD44 antibody (BD Biosciences). The fluorescence intensities were analyzed by MACSQuant® Analyzer (Miltenyi Biotec).

qRT-PCR

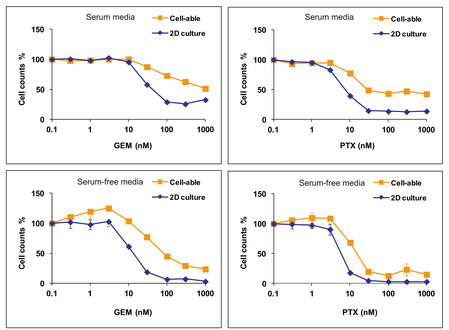
Quantitative real time polymerase chain reaction (qRT-PCR) analysis was performed using TaqMan® Fast Universal PCR Master Mix and probes (Applied Biosystems). The probes: NANOG (Hs04260_366_g1); ABCG2 (Hs0105370_m1).

Fluorogenic substrate

Hypoxic condition and aldehyde dehydrogenase (ALDH) activities in the cells were stained with Lox-1 (Scivax) and ALDEFLUOR™ (Stemcell Technologies).

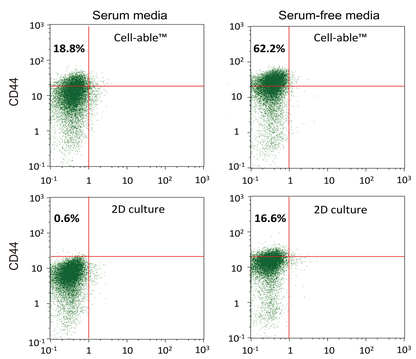
Results

Chemoresistance of spheroids on Cell-able™



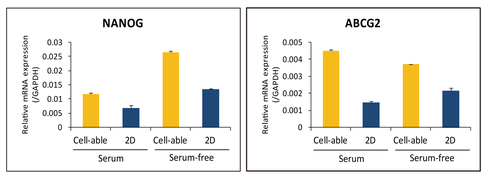
Chemoresistance of the spheroids grown on the Cell-able™ against gemcitabine (GEM) (left) and paclitaxel (PTX) (right) in the serum and the serum-free media compared to cells in 2D culture. The cells were treated with each drug for 72 hours and the cell viabilities were determined by ATP luciferase method (for serum media) or DAPI-staining nuclear intensity quantified by high content screening assay (for serum-free media).

Increased CD44⁺/CD24⁻ cells in spheroids on Cell-able™



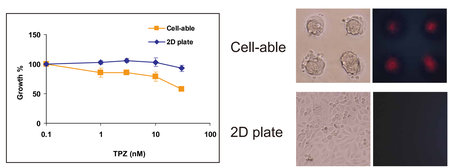
The presence of CD44 and CD24 surface markers of DU145 spheroid cells on Cell-able™ and cells on 2D plates were measured by fluorescence-activated cell sorting (FACS) analysis. Cells cultured in the serum and the serum-free media were labeled with anti-human CD24-FITC antibody (Invitrogen) and anti-human CD44-APC antibody (BD Biosciences). The cells were analyzed by MACSQuant® Analyzer (Miltenyi Biotec).

Increased expression of NANOG and ABCG2 mRNA in spheroids on Cell-able™



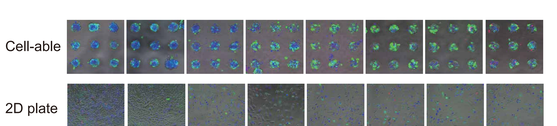
High expression of mRNA for stem cell markers, NANOG and ABCG2 in spheroid cells grown on Cell-able™ compared with cells on the 2D plates. Total RNA was extracted from the cultured cells using NucleoSpin® RNAII (Takara Bio). Complementary DNA was synthesized from total RNA using PrimeScript® RT Maser Mix (Takara Bio). Quantitative real time polymerase chain reaction (qRT-PCR) analysis was performed using TaqMan® Fast Universal PCR Master Mix and probes (Applied Biosystems). The probes: NANOG (Hs04260_366_g1); ABCG2 (Hs0105370_m1).

Hypoxic condition inside spheroids on Cell-able™



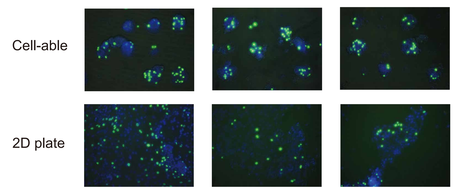
Inhibitory effects of tirapazamine (TPZ) on growth of DU145 human prostate cancer cell line on Cell-able™ and 2D collagen-coated plate. CellTiter-Glo™ was added to each well 72 hours after drug treatment and measured the luminescence intensity. TPZ is an experimental anticancer drug that is activated to a toxic radical only at very low levels of oxygen (hypoxia). Thus, it seems to be hypoxic condition inside the spheroids in Cell-able™ 3D culture. Hypoxic levels were examined on growth of DU145 human prostate cancer cell line on Cell-able™ and 2D collagen-coated plate. Lox-1, phosphorescent hypoxia probe, was added to each well 24 hours before observation.

Chemoresistance of ALDH^{high} cells in spheroids on Cell-able™



Chemoresistance of A549 human lung cancer cells highly expressing aldehyde dehydrogenase (ALDH^{high}) to PTX treatment. The A549 cells were grown on Cell-able™ or collagen-coated 2D plates and PTX (1-1000 nM) was treated for 72 hours. Then the nuclear, dead cells and ALDH^{high} cells were stained with DAPI, ethidium homodimer (Invitrogen) and ALDEFLUOR™ (Stemcell Technologies). The population of ALDH^{high} cells on Cell-able™ was higher than that on the 2D plate. The treatment of PTX decreased the cell viability in a concentration-dependent manner but ALDH^{high} cells showed resistance to the PTX treatment.

Chemoresistance of spheroids from endometrial cancer patients



Chemoresistance of patient-derived endometrial cancer cell spheroids to PTX exposure. Endometrial cancer tissues were surgically resected from patients and immediately digested with collagenase. The cells were seeded on Cell-able™ and cultured for 2-3 days and then PTX was treated for 72 hours. Then the nuclear and proliferating cells were detected by DAPI and Click-iT® EdU Cell Proliferation Assay (green) (Invitrogen), respectively. The treatment of PTX decreased the cell proliferation in a concentration-dependent manner but the cells in spheroids showed more resistant to the PTX treatment than 2D monolayer cells.

Summary

- Cell-able™ has type I collagen-coated cell attachment area (circle 100 μm in diameter) and non attachment area on the bottom of each well, which has been optimized for long-term 3D culture of various cells.
- Most tumor cell lines and primary tumor cells were able to proliferate to form spheroids on Cell-able™.
- The spheroids grown on Cell-able™ were resistant to chemotherapy such as GEM or PTX in DU145 and patient-derived endometrial cancer cells compared to the respective 2D monolayer cells.
- The spheroids grown on Cell-able™ were high stem-like cell population (CD44^{high}/CD24^{low} and ALDH^{high}) and showed high expression of mRNA for stem cell markers, such as NANOG and ABCG2 compared to 2D monolayer cells.
- The stem-like cell population (CD44^{high}/CD24^{low}) and expression of NANOG mRNA were higher in the serum-free media than in the serum media.
- The core of spheroids grown on Cell-able™ was hypoxic condition, which may be associated with higher stem-like cell population.
- The ALDH^{high} cells were resistant to PTX treatment in A549 cells.
- The pharmacodynamic and pharmacokinetic studies using the cancer stem-like cell-rich spheroids could provide meaningful information relevant to the clinical conditions.

Conclusion

The results from our studies indicate that the spheroid grown on Cell-able™ could be relevant to in vivo tumor model and useful for anti-cancer drug screening and testing.