A Novel Spheroid-based Three-dimensional Invasion Model for Evaluating Potentially Anti-tumor Compounds

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Introduction

In pathology, carcinoma invasion is defined as the penetration of tissue barriers such as basement membrane, and infiltration into the underlying interstitial tissues by malignant tumor cells. Cancer cells that become invasive may disseminate to secondary sites and form metastases.

Traditionally, cell invasion assays are conducted utilizing two-dimensional (2D) cultures that may not accurately reflect the *in vivo* microenvironment. *In vivo*, tumor cells invade the surrounding tissue from cancer cell clusters which are three dimensional (3D) structures. Multicellular spheroids formed *in vitro* can mimic these tumor clusters. Cells grown as multicellular spheroids closely recapitulate the *in vivo* environment of solid cancers.

Here, we developed a spheroid-based 3D invasion model. Multicellular spheroids were formed in a Corning spheroid microplate, embedded in Corning® Matrigel® matrix, and laid on Transwell® permeable supports or Corning FluoroBlok™ inserts. Multiple cells detached from the spheroid, invaded the Corning Matrigel matrix, and passed through the membrane pores when treated with a chemoattractant. The invaded cells were analyzed using imaging analysis, and the anti-invasive activity of compounds on 3D invasion was evaluated.

Materials and Methods

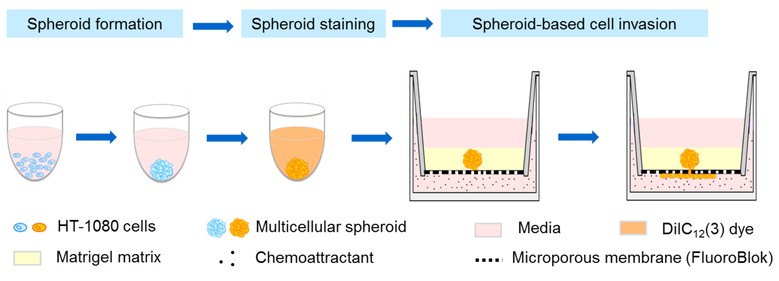
1.Spheroid formation: HT-1080 (ATCC Cat. No. CCL-121) cells were cultured in Minimum Essential Medium (MEM) (Corning Cat. No. 10-009-CV) containing 10% fetal bovine serum (FBS, Corning Cat. No. 35-076-CV). Cells were seeded into 96-well spheroid microplates (Corning Cat. No. 4520) at 10,000 cells per well in 100 µL culture medium and cultured for 24 hours to form the multicellular spheroid.

2.Spheroid-based migration: The culture media of HT-1080 spheroids was discarded, and replaced with 50 µL serum-free medium. The cell spheroid, together with the medium was transferred to the upper chamber of a Transwell (8 µm PET membrane, Corning Cat. No. 3464) with an Axygen® wide bore tip (Corning Cat. No. T-205-WB-C-R-S). Then, 100 µL serum-free media was added to the upper chamber of the Transwell, and 800 µL culture medium with 10% FBS as a chemoattractant was added to the lower chamber. The cells were cultured overnight to allow migration and then stained with crystal violet.

3.Spheroid-based invasion: Corning Matrigel basement membrane matrix (Corning Cat. No. 356234) was diluted with ice-cold serum-free media to 5 mg/mL. The cell spheroid was stained with DiIC$_{12}$(3) fluorescent dye (Corning Cat. No. 354218), then replaced with 50 µL of 5 mg/mL Corning Matrigel matrix on ice, and immediately transferred into Corning FluoroBlok cell culture inserts (Corning Cat. No.351152) together with the Corning Matrigel matrix (one spheroid per insert). The Corning Matrigel matrix was allowed to gel at 37ºC for 30 min. Then, serum-free media was added to the upper chambers, and culture medium with 10% FBS was added to the lower chambers to allow cell invasion towards FBS-containing media. Spheroid-based invasion was monitored using a BioTek Cytation™ 5 Cell Imaging Multi-Mode Reader.

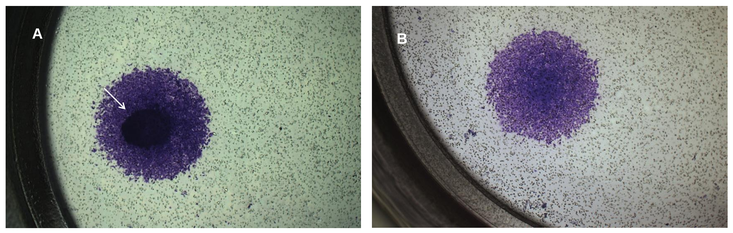
4.Evaluation of compounds activity: Doxorubicin and doxycycline were added respectively to the media in both the upper and lower chambers of Corning FluoroBlok inserts after gel formation. The effect of the compounds on 3D invasion was evaluated by imaging analysis of the invaded cells with the BioTek Gen™ 5 software.

Process Overview



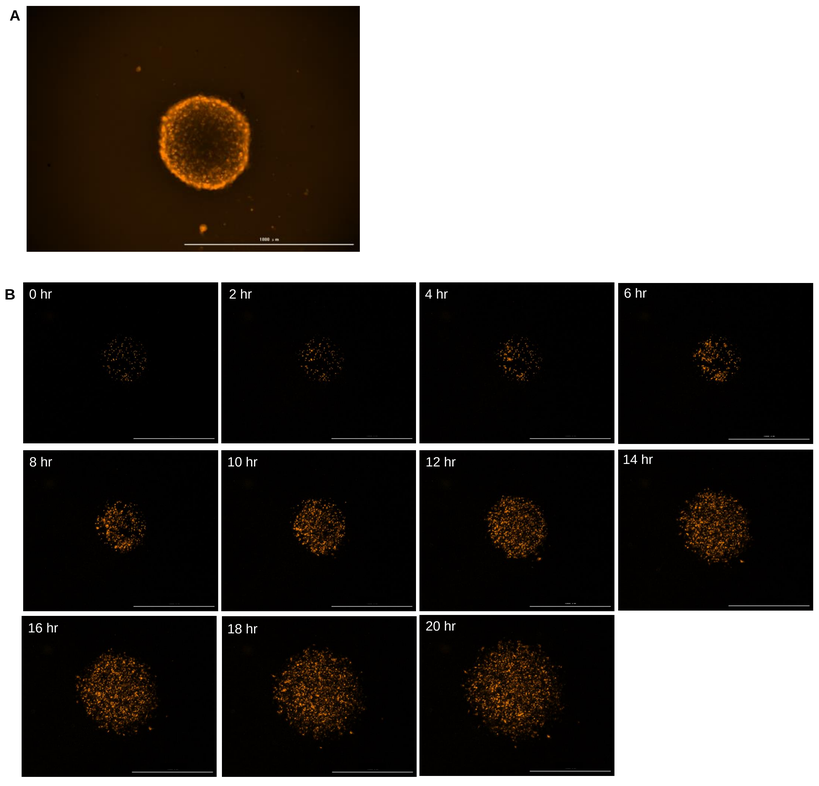
Results

Spheroid-based Migration



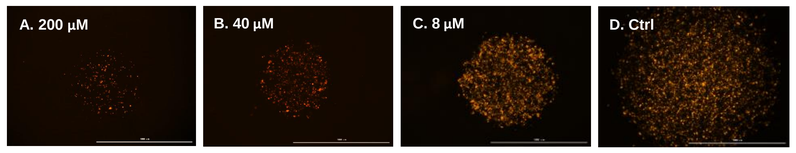
Representative photomicrographs of spheroid migration. HT-1080 multicellular spheroid attached to the microporous membrane of the Transwell permeable support. Then, the cells detached from the spheroid, passed through the micropores, and migrated in a radial pattern. Cells were stained with crystal violet. **(A)** Total cells in both upper and lower chambers after cell migration. The arrow indicates a multicellular spheroid. **(B)** Cells migrated through the Transwell from a spheroid (cells in the upper chamber were removed). This demonstrated that the cells can migrate from multicellular spheroid and pass through the pores towards 10% FBS.

Dynamic Monitoring of Spheroid-based 3D Invasion



Time lapse imaging of HT-1080 cell invasion. (A) The HT-1080 multicellular spheroid was stained with DiIC$_{12}$(3) before invasion. **(B)** The dynamic invasion of Corning Matrigel matrix embedded HT-1080 cell spheroid in Corning FluoroBlok inserts was recorded for 20 hours with 2-hour intervals. By bottom-reading with a live cell fluorescence imaging reader, the cells present in the upper chamber were shielded from detection by the fluorescence blocking membrane of the FluoroBlok inserts. The spots at 0 hours were visible fluorescence dots from the spheroid in the upper chamber due to pores on the membrane. Individual cells invading through the pores were clearly visible from 4 hours. Images were obtained using BioTek Cytation 5 Cell Imaging Multi-Mode Reader. Scale bar = 1,000 µm.

Evaluation of the Activity of Compounds with a 3D Model



Representative photomicrographs of the effect of compound on HT-1080 3D invasion. The HT-1080 spheroid was treated with different doses of doxorubicin for 24 hours. Cell invasion was inhibited by doxorubicin in a dose-response manner. Scale bar = 1,000 µm.

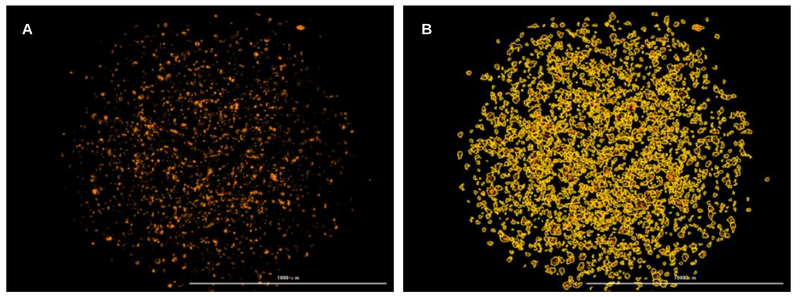
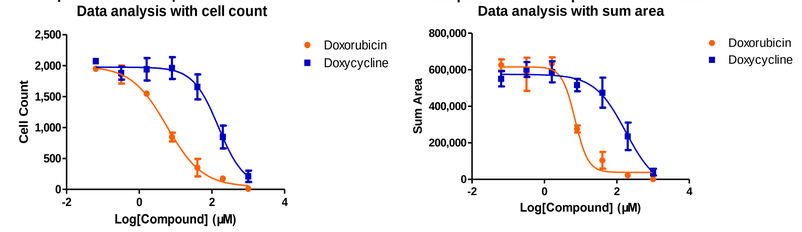


Image-based data analysis development. (A) Raw photomicrograph obtained using BioTek Cytation 5. **(B)** Invaded cells were selected by the BioTek Gen 5 software, and highlighted by a golden object mask. Scale bar = 1,000 µm.



IC_{50} (µM)	Doxorubicin	Doxycycline
Cell Count	5.8	151.3
Sum Area	7.0	176.2

Dose-response relationship of compounds on spheroid-based 3D invasion. Two parameters were analyzed for data analysis: **(A)** cell count; **(B)** sum area. Cell count indicates the total invaded cell number counted by the software. To eliminate the influence of overlapping cells, sum area was developed by determining the total area of the masked cells. Both analytical methods showed similar IC_{50} values. Our result is consistent with previous studies that evaluated the effect of compounds on HT-1080 xenograft *in vivo* models.

Summary/Conclusions

- A 3D invasion model was developed by embedding Corning spheroid microplate-produced multicellular spheroids in Corning Matrigel matrix, then placing on Transwell permeable supports and treated with chemoattractant.
- The invasion process can be dynamically monitored with a bottom-reading live cell fluorescence imaging reader by pre-staining the spheroids with DiIC$_{12}$(3) fluorescence dye. Cells present in the upper chamber of Corning FluoroBlok inserts were shielded from detection by the fluorescence blocking membrane.
- The 3D invasion model with Corning FluoroBlok inserts is automation-friendly, and can be easily used for compound screening in a non-destructive manner. Compound evaluation with 3D invasion model was developed with image-based data analysis.
- Corning 96-well spheroid microplates, Axygen wide bore tips, Corning Matrigel matrix, and Corning FluoroBlok inserts comprise a valuable, novel system for studying spheroid-based 3D invasion and evaluating the potential anti-tumor activity of compounds.

References

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