Comparison of the effects of pharmaceutical compounds on tumor cells in 2D and 3D in vitro models using label-free, quantitative 4 dimensional holographic imaging.

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Introduction

Development of in vitro models for the evaluation of drugs represents a useful approach as in vivo studies may be costly and time consuming. Ideal models should take into account the effects of the cellular microenvironment, which includes the extra-cellular matrix, stroma and neighboring cells.

Holographic Imaging Cytometry

Instrumentation:

- Time-lapse holographic imaging cytomter HoloMonitor™ M4 (Phase Holographic Imaging, Lund, Sweden) for label-free long-term kinetic cellular analysis.
- Quantitative phase shift measurements are translated by software algorithms into morphological parameters.

Experimental protocols:

- Experiments were performed on glass-bottom Petri dishes (MatTek, Ashland MA).
- A proprietary dish cover (Phase Holographic imaging) with a water immersion prism was employed to mitigate the effects of condensation and vibrations.
- In some experiments, the collagen and poly(L lysine) treated dishes were used.
- HeLa (human cervical adenocarcinoma) and HT-1080 (human fibrosarcoma) cells were obtained from the ATCC.
- In 2D studies, medium was used; cells would rapidly settle to the bottom surface and adhere and acquire for 24 hours. Cultures were treated with test compounds for 4 hours, the media was changed, and long-term imaging followed.
- In 3D studies, cells were first allowed to adhere to the bottom surface and treated with compounds for 4 hours. The media was removed and replaced with 1 mg/ml collagen containing media.

Pharmacodynamics application

We are developing multifunctional nanoparticles that specifically target tumor cells. The components include:
- CLV Dox Conjugate, which releases Doxorubicin after cleavage by MMP2 enzyme (overexpressed in HT-1080 cells).
- TAT conjugate, which helps in internalization of the particles by the cells.

2D models of non-motile adherent cells

Human HT-1080 fibrosarcoma cells alternate between amoeboid and migratory motion not in 2D culture but in 3D cultures, and offering the possibility of emulating multi-dimensional biological processes and offer the possibility of those in vivo assays.

3D models of motile adherent cells

Human HT-1080 fibrosarcoma cells alternate between amoeboid and mesenchymal phenotypes representing different motility mechanisms.

Summary:

- The HoloMonitor M4 enabled long-term live cellular analysis, tracking cells in a label-free manner with quantitative data linked to images and videos of any cell in the analysis at any time-point.
- By analyzing the HoloMonitor data in Image J we developed a novel 4-D holographic imaging method following XY positions of the cells and changes in the cellular thickness over time. We used this technique to characterize the population dynamics of untreated HeLa cells, and those treated with free Doxorubicin, revealing the morphology of dying cells in 4D plots.
- We introduced a simplified method for creating an extra-cellular matrix - instead of embedding cells within the matrix, we allow the cells to adhere to the substrate followed by matrix overlay.
- We applied the newly developed methodologies to an ongoing study, where the individual components of a complex nanoparticle formulation for Doxorubicin delivery were tested on HT-1080 cells.
- Our example of HT1080 cells treated with dox clearly shows the superiority of the 3D model, an important step in developing assays that better emulate multi-dimensional biological processes and offer the possibility of evaluating effects of drugs at lower cost and experimental complexity than those of in vivo assays.

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