EVALUATING AMPK ACTIVATORS AS POTENTIAL CHEMOTHERAPEUTICS IN A QUANTITATIVE 3D MICROTUMOR MODEL

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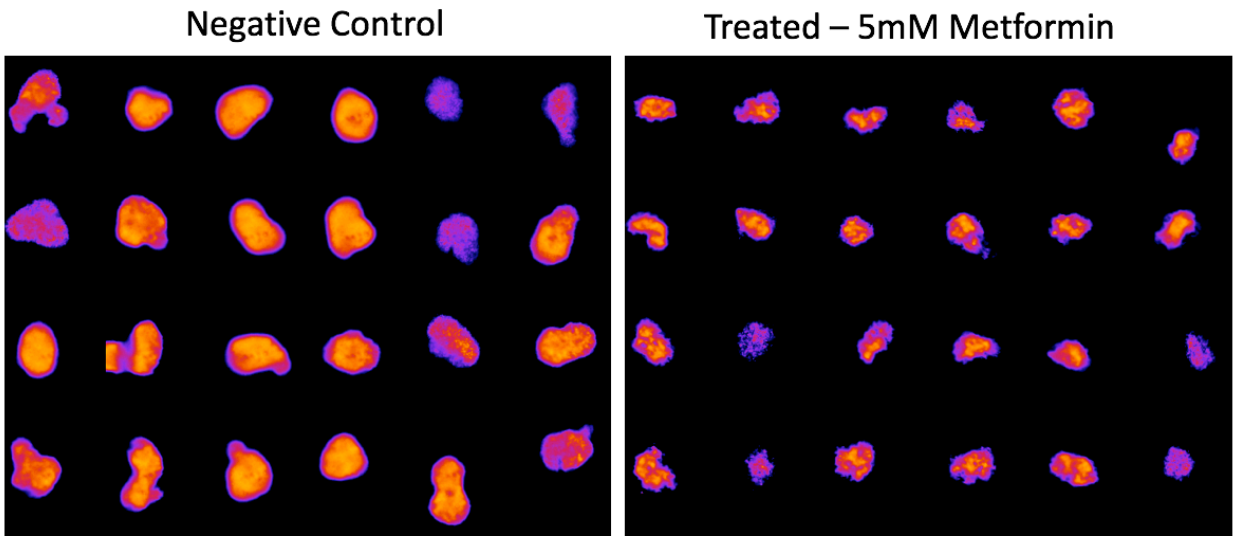


INTRODUCTION

This exercise will allow students to learn how image analysis can be applied to screening chemicals for potential chemotherapeutics in a 3-dimensional microtumor model of hepatocellular carcinoma (HCC). In this exercise, we will identify compounds that substantially alter the normal microtumor progression/growth/size/shape as indicators of efficacy for a potential chemotherapeutic agent against HCC. AMP-activated protein kinase (AMPK), a master energy sensor and metabolic regulator, inhibits energy consumption while increasing energy availability to maintain energetic homeostasis. AMPK has been a widely proposed drug target for T2D, metabolic syndrome and cancer. Small molecules that activate AMPK will initiate a signaling cascade indicating energetic stress to conserve energy by inhibiting pathways that consume ATP and activating pathways that generate ATP. AMPK activators can halt tumor progression by enforcing metabolic checkpoints and by inhibiting cell growth.

In this exercise, you will have access to the following materials:

- Background information on AMPK as a poatential cancer target and its role in maintaining cellular energy homeostasis.

- Open-source and freely available analysis software for use in quantifying biological features in image data.
- Images of standardized human liver 3D microtumors from a high-content, high-throughput drug screen for AMPK activators in dose-response format.

GOALS

The exercise is written to familiarize the student with the following concepts:

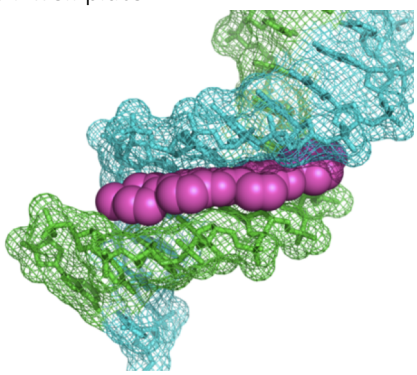
- Basic principles behind screening for drug discovery and the use of 3-dimensional microtissues as a more physiologically relevant model system for human tumors.
- Application and challenges of 3D-imaging and quantitation of biological features as a readout of tumor progression.
- Use of software for quantifying biological features and making investigative decisions based on the measurements obtained.

APPROACH

To begin this experiment, we grow human hepatocellular carcinoma (HepG2) cells in culture to expand their numbers. We then deposit 500 cells in a specialized non-stick, round bottom multiwell dish with 384 wells. The cells slide down the walls of the round bottom well and begin to adhere to one another forming a dense microtumor. After 3-days of cell attachment and growth, we dispense a different chemical compound in each well and allow an additional 5-days for the chemical to act upon the cells. The microtumors are then stained with Hoechst-33342, a cell permeable fluorescent dye that binds and intercalates nuclear DNA and each well is imaged with a robotically-controlled automated fluorescence microscope.

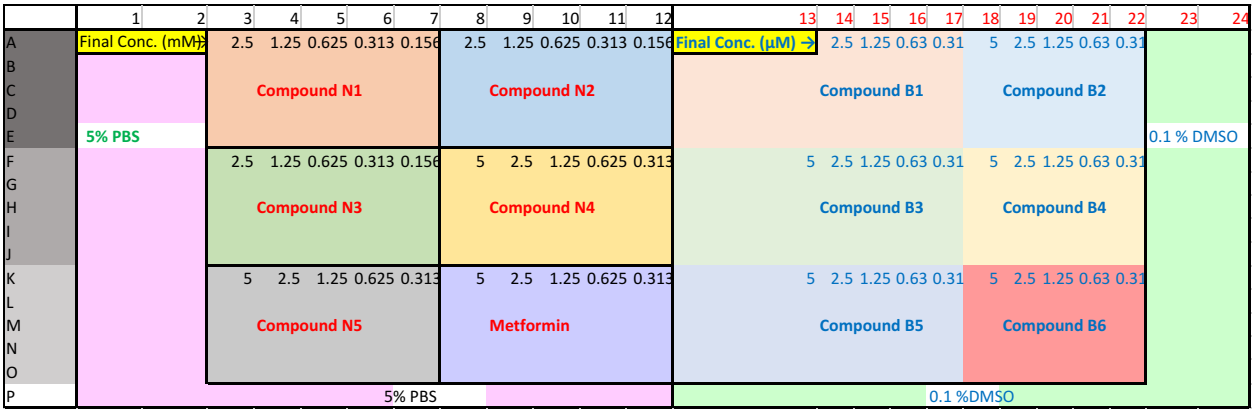
In the data set provided, compounds were tested in quadruplicate and the concentrations shown in the plate map below. All compounds are experimental and are compared to the widely-prescribed diabetes drug Metformin, a known AMPK activator (amongst other pharmacological effects), and the two vehicles – phosphate buffered saline (PBS) and DMSO. The N-series compounds were solubilized in PBS and the B-series

Corning spheroid round-bottom 384-well plate



Hoechst-33342 binding to dsDNA

compounds (right side of plate) were solubilized in DMSO. The plate map is also provided in an excel spreadsheet.



ACTIVITY 1 - VISUALIZE IMAGES IN IMAGEJ

To get a sense of compound effects relative to vehicle controls, we will import a series of images as a stack, crop, apply the "Fire" false-color look-up-table, optimize brightness/contrast and perform some semi-automated object measurements.

Step 1: Download and install ImageJ & the Bioformats Importer

- Download Dataset from QUBES collection – Hoechst.zip
- https://imagej.nih.gov/ij/download.html
- http://downloads.openmicroscopy.org/bio-formats/5.1.10/
- Deposit downloaded bio-formats JAR file into the ImageJ plugins directory and restart.
- Drag one of the C01 image files onto the ImageJ toolbar.
- Open files, and adjust brightness & contrast & apply Look-up-tables (LUTs)
- Threshold the image ->Image -> Adjust -> Threshold
- Measure the particle size ->Analyze -> Analyze Particles
- Get a sense of the normal size and area of negative control microtumors.

Step 2: Automated Analysis of Microtumor size with CellProfiler

- Download and Install CellProfiler - http://cellprofiler.org/releases/
- Download Bioquest_spheroid.cpproj file. This is the CellProfiler "Project File" that contains the instructions for analysis.
- Launch Cell Profiler and Load the project file.
- Drag images into the "Images" window.
- Step through the input modules and Analysis modules to get familiar with the steps.
- Click "Start Test Mode" and "Step" through the analysis modules to see the output.
- Make sure to click "View Output Settings" and choose a convenient location for the output.
- After satisfactory testing, click "Analyze Images" to analyze the entire set.

- After analysis is done, the output CSV files will be in the default output directory.

Data Analysis:

- Open CSV File in Excel and use Pivot Table to summarize by well (Metadata_WellID)
- In your statistics package of choice, Join the compound treatment map to the Cellprofiler output to associate the well-level result with the treatment condition.
- Tabulate responses per compound (Area, or diameter or ???) and indicate which compound has the greatest “efficacy”.