Assay of Cell Growth, Stasis, or Death Under Different Nutritional Conditions

Summary

Biolog Phenotype MicroArray™ Technology was used to illustrate a simple cost-effective method for simultaneously measuring multiple energy-producing pathways and effects on cell growth, stasis and death under different nutritional conditions using the PM-M1 MicroPlate (Figure 1).

Assay

HepG2/C3A cells were suspended at 50,000 cells per ml in serum-free RPMI-1640 medium that lacked phenol red and glucose, but contained Pen/Strep and 4 mM glutamine (Figure 2). Cells were dispensed into 6 replicate PM-M1 MicroPlates at 50µl per well (2,500 cells per well) and incubated at 37°C under 5% CO_2-95% air. On six consecutive days, starting on day 0 when the cells were plated, 10µl of Redox Dye Mix MA containing 30 mM glucose (a 6-fold concentrate) was added to one MicroPlate, the plate was sealed with tape to prevent CO_2 loss, and incubated at 37°C in an OmniLog instrument for 18 hours to kinetically record formation of purple formazan in the wells on days 0 (red), 1 (yellow), 2 (green), 3 (blue), 4 (purple), and 5 (black).

Substrates resulting in distinguishable responses are indicated: cell proliferation (green boxes), cell stasis (blue boxes), slow cell death (red boxes), rapid cell death (black boxes). In these kinetic graphs, the X-axis is time and the Y-axis is OmniLog color density units. Figure 3 depicts wells selected for illustration.

Conclusions

In this multiplexed assay of the survival responses of cells under different conditions of substrate supply (Figures 2 and 3), four qualitatively distinguishable responses were observed: proliferation (green boxes, including wells containing D-glucose, D-mannose, D-fructose, D-galactose, uridine and xylitol; stasis (blue boxes), including wells containing D-maltose, D-glucose-6-phosphate, and inosine; slow death (red boxes), including wells containing dextrin, D-sorbitol, and pectin; and rapid death (black boxes), including wells with no substrate, D-raffinose, and butyrate. The set of substrates that are positive for energy production corresponds with substrates supporting prolongation of survival, as was corroborated by imaging of healthy morphology and by increased cell numbers in these wells (not shown).

From: Bochner BR, Siri M, Huang RH, Noble S, Lei X-H, Clemons, P.A., Wagner, B.K., (2011) Assay of the Multiple Energy-ProducingPathways of Mammalian Cells. PLoS ONE 6(3):e18147. doi:10.1371/journal.pone.0018147

A1 Negative Control	A2 Negative Control	A3 Negative Control	A4 α-Cyclodextrin	A5 Dextrin	A6 Glycogen	A7 Mallitol	A8 Maltotriose	A9 D-Maltose	A10 D-Trehalose	A11 D-Cellobiose	A12 β-Gentiobiose
B1 D-Glucose-6-Phosphate	B2 α-D-Glucose-1-Phosphate	B3 L-Glucose	B4 α-D-Glucose	B5 α-D-Glucose	B6 α-D-Glucose	B7 3-O-Methyl-D-Glucose	B8 α-Methyl-D-Glucoside	B9 β-Methyl-D-Glucoside	B10 D-Salicin	B11 D-Sorbitol	B12 N-Acetyl-D-Glucosamine
C1 D-Glucosaminic Acid	C2 D-Glucuronic Acid	C3 Chondroitin-6-Sulfate	C4 Mannan	C5 D-Mannose	C6 α-Methyl-D-Mannoside	C7 D-Mannitol	C8 N-Acetyl-β-D-Mannosamine	C9 D-Melezitose	C10 Sucrose	C11 Palatinose	C12 D-Turanose
D1 D-Tagatose	D2 L-Sorbose	D3 L-Rhamnose	D4 L-Fucose	D5 D-Fucose	D6 D-Fructose-6-Phosphate	D7 D-Fructose	D8 Stachyose	D9 D-Raffinose	D10 D-Lactitol	D11 Lactulose	D12 α-D-Lactose
E1 Melibionic Acid	E2 D-Melibiose	E3 D-Galactose	E4 α-Methyl-D-Galactoside	E5 β-Methyl-D-Galactoside	E6 N-Acetyl-Neuraminic Acid	E7 Pectin	E8 Sedoheptulosan	E9 Thymidine	E10 Uridine	E11 Adenosine	E12 Inosine
F1 Adonitol	F2 L- Arabinose	F3 D-Arabinose	F4 β-Methyl-D-Xylopyranoside	F5 Xylitol	F6 Myo-Inositol	F7 Meso-Erythritol	F8 Propylene glycol	F9 Ethanolamine	F10 D,L- α-Glycerol-Phosphate	F11 Glycerol	F12 Citric Acid
G1 Tricarballylic Acid	G2 D,L-Lactic Acid	G3 Methyl D-lactate	G4 Methyl pyruvate	G5 Pyruvic Acid	G6 α-Keto-Gluturic Acid	G7 Succinamic Acid	G8 Succinic Acid	G9 Mono-Methyl Succinate	G10 L-Malic Acid	G11 D-Malic Acid	G12 Meso-Tartaric Acid
H1 Acetoacetic Acid (a)	H2 γ-Amino-N-Butyric Acid	H3 α-Keto-Butyric Acid	H4 α-Hydroxy-Butyric Acid	H5 D,L-β-Hydroxy-Butyric Acid	H6 γ-Hydroxy-Butyric Acid	H7 Butyric Acid	H8 2,3-Butanediol	H9 3-Hydroxy-2-Butanone	H10 Propionic Acid	H11 Acetic Acid	H12 Hexanoic Acid

Figure 1: Plate Map of Phenotype MicroArray MicroPlate PM-M1

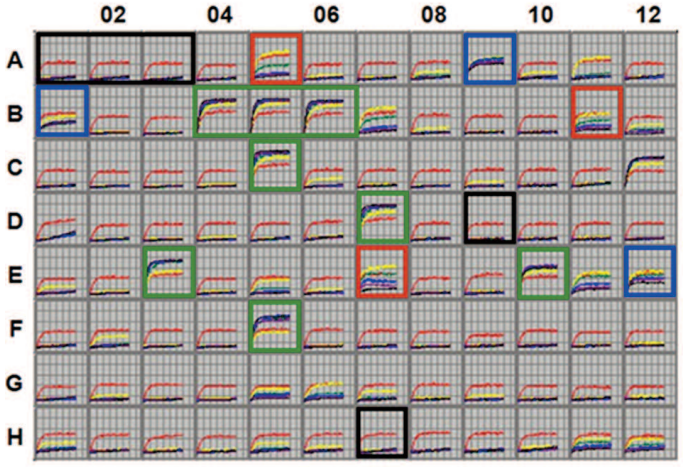

Figure 2: Survival responses of cells under different nutritional conditions

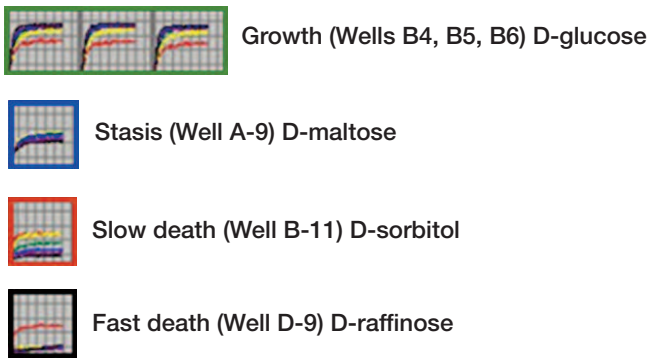

Figure 3: Expanded view of selected cells illustrating growth, stasis, slow death and fast death