Effects of microtubule inhibitors on cancer cell metabolism

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Biolog's Phenotype MicroArrays:
A system for nutrient metabolism and chemosensitivity profiling

e.g., α-D-glucose

one nutrient per well

kinetic readout per nutrient
Timeline for preparing Biolog Phenotype MicroArray assays

Culture cells to confluence in flask

Seed 96-well Biolog plate (minimal media)

Add metabolic dye
A549 cells cultured in 5mM glucose media have higher rates of simple sugar metabolism (vs. 25mM glucose)
High-throughput screening for mitochondrial effects in muscle revealed a role for microtubule modulators

Mitochondrial assays
- viability
- electron transport
- membrane potential
- ATP production
- reactive oxygen species
- gene expression

In muscle cells, they:
- increased OXPHOS gene expression
- decreased cellular ROS

Wagner et al. (2008) Nat Biotech
Do microtubule modulators have an effect on mitochondrial biology in cancer cells?
Selecting appropriate concentrations of microtubule inhibitors

A549 lung cancer cells treated 48h

Paclitaxel = microtubule stabilizer

Vinblastine = microtubule destabilizer
Chemosensitivity plates reveal cases of increased resistance to rotenone

- 25mM glucose (glu)
- 5mM glucose
- 25mM glucose + paclitaxel (pac)
- 25mM glucose + vinblastine (vin)

Cell death

Metabolism

Rotenone

Chemical structure and metabolic pathway diagram.
Cell viability assays (ATP) confirm chemosensitivity results

25nM pac or vin

48h → rotenone

24h → measure ATP

Fold ATP relative to untreated

Rotenone (μM)
Alternate energy metabolism pathways with measurable factors

**Glycolysis (cancer Warburg effect)**

- Glucose → Pyruvic Acid → Lactate
- 2 ATP

**GLYCOLYTIC PHENOTYPE**

- + 6O₂
- Glycolysis

**Krebs Cycle (Respiration)**

- Pyruvic Acid → 36 ATP

**RESPIRING PHENOTYPE**
Lactate production in response to glucose content and microtubule modulator treatment
Oxygen consumption in response to glucose content and microtubule modulator treatment
• **Hypothesis:** Do microtubule modulators have an effect on mitochondrial biology in cancer cells?

• **Conclusions:**
  - A549 cells in 25mM glucose appear more glycolytic.
  - A549 cells in 5mM glucose appear to be respiring.
  - Paclitaxel and vinblastine increase resistance to rotenone.
  - Further experimentation is required to clarify the role of paclitaxel and vinblastine in mitochondrial metabolism.
Future directions

- Do mitochondria-targeting drugs sensitize A549 cells to microtubule modulators?
- Do microtubule modulators induce gene expression changes of interest?
- Can we recapitulate these results with other cancer drugs?
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