The Clonogenic Assay

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Outline

• Clonogenic Survival
• The Assay
• Methods
• Compiling Results
• Mathematical Model
• Interpretation of Data
Utility of the Assay

- IR induces DNA damage
- Measure of reproductive potential
- Assessment of proliferation ability of cells exposed to radiation, through colony-forming ability

Eriksson D and Stigbrand T et al. Tumor Biol. 2010; 31:363
Assay Endpoint

• Endpoint observed depends directly on reproductive integrity of individual cells

• Important Parameters:

  • Plating Efficiency = \( \frac{\text{# of colonies formed}}{\text{# of cells seeded}} \) \times 100

  • Survival Fraction = \( \frac{\text{# of colonies formed}}{\text{# of cells seeded}} \) / PE \times 100
Clonogenic Survival: Principle

100 Cells Seeded
70 Colonies Formed

2000 Cells Seeded
32 Colonies Formed

Plating Efficiency:
70/100
0.7 or 70%

Survival Fraction:
(32/2000) / 0.7
0.023 or 2.3%

Micro-Colony
Visible-Colony
Colony Formation Under the Microscope
Methods

- Actively growing cells are harvested
- Number of cells counted and appropriate dilutions made
- Cells plated onto 6-well plates or individual 35mm plates
- Incubate for 9-14 days. Monitor regularly
- Wash plates with PBS
- Add fixative. Leave for 2hr at room temp
- Add stain. Leave for 2h-24h depending on stain
- Count colonies and analyze results
Summary of steps:

Faxitron X-Ray Irradiator

Incubate for 9 – 14 days

<table>
<thead>
<tr>
<th>Radiation Dose (Gy)</th>
<th>No. of cells plated</th>
<th>No. of colonies counted</th>
<th>Plating efficiency</th>
<th>Surviving Fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>70</td>
<td>0.7</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>200</td>
<td>72</td>
<td>0.36</td>
<td>0.5</td>
</tr>
<tr>
<td>4</td>
<td>600</td>
<td>70</td>
<td>0.12</td>
<td>0.16</td>
</tr>
<tr>
<td>6</td>
<td>1000</td>
<td>8</td>
<td>0.008</td>
<td>0.1</td>
</tr>
</tbody>
</table>
Counting Methods

• Manual visualization + Manual counting

• Manual visualization + Assisted counting
  • Image print or with software

• Automated detection + Automated counting
  • UVP System, Image J, Other photo-acquisition and thresholding software
## Compiling Results

<table>
<thead>
<tr>
<th>( X )</th>
<th>( Y )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dose (Gy)</strong></td>
<td><strong>Cells Seeded</strong></td>
</tr>
<tr>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>200</td>
</tr>
<tr>
<td>4</td>
<td>400</td>
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<tr>
<td>6</td>
<td>1000</td>
</tr>
<tr>
<td>8</td>
<td>3000</td>
</tr>
<tr>
<td>10</td>
<td>8000</td>
</tr>
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</table>
Graph of Survival Fraction Versus Dose
Cell kill is the result of single lethal hits plus accumulated damage from 2 independent sublethal events

\[ D \] is the dose in Gy,
\[ \alpha \] is the cell kill per Gy of the initial linear component (on a log-linear plot)
\[ \beta \] is the cell kill per Gy\(^2\) of the quadratic component of the survival curve.

\[ \frac{\alpha}{\beta} \] describe repair kinetics
Early (\( \frac{\alpha}{\beta} = 10 \)) -> tumor, skin, GI
Late (\( \frac{\alpha}{\beta} = 1-3 \)) -> brain, kidneys
Survival Curves for Mammalian cells

First *in-vitro* survival curve was reported in 1956

\[ D_q : \text{The quasi-threshold dose for a given population that measures the width of the shoulder} \]

**Figure 3.7.** Survival curve for HeLa cells in culture exposed to x-rays. Characteristically, this cell line has a small initial shoulder. (From Puck TT, Markus PI: Action of x-rays on mammalian cells. J Exp Med 103:653–666, 1956, with permission.)
Comparing Curves

Survival Fraction vs. Dose (Gy)

- A = 0.90
- B = 0.67
- C = 0.38

Radioresistant: SF2 > 0.5
Radiosensitive: SF2 < 0.5
Summary

• Clonogenic cell survival assay is the gold standard in radiobiology for assessing reproductive/proliferation of cells after IR
• Assay completion in 9-14 days
• Need for optimization of seeding cell density
• Analysis of data provides important information on cell radiosensitivity
• Useful tool for intra and inter cell line comparisons involving radiation
Questions
Chemosensitivity testing of human lung cancer cell lines using the MTT assay

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4U.S. Naval Institute for Research on the Health Sciences, Bethesda, Maryland 20889, USA.

Summary: Thirty human lung cancer cell lines were tested for chemosensitivity using the semi-automated, non-isotopic MTT assay. The tumor cell lines came from three major categories of patients: untreated small-cell lung cancer (SCLC), SCLC relapsing or chemotherapy resistant, and non-SCLC predominantly from untreated patients. From these data, IC50 values were derived for each drug in each cell line. With some exceptions, resistant cell lines were identified, the rank order of chemosensitivity within each cell line was within the order of the RCR (relative chemosensitivity) of melphalan. Thus, the IC50 values of the chemotherapeutic agents were similar in SCLC and non-SCLC cell lines. Some drug-resistant non-SCLC cell lines exhibited cross-tolerance to other sensitive cell lines. These results suggest that the MTT assay may prove useful for testing drug combinations and evaluating new anti-cancer agents in vivo.

Materials and methods

Cell lines

Exponentially growing cultures maintained in a humidi- fied atmosphere of 95% air:5% CO2 at 37°C. Adherent cell lines, all established at the NCI-Navy Medical Oncology Branch, or cell lines established for the 10-year period, all cell lines having been in culture for at least 6 months. Passage number at the time of testing were also included.