Co-culture spheroids for phenotypic preclinical assessment of direct drug delivery to paediatric brain tumours

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Medulloblastoma

Most common malignant brain tumour in childhood

Therapy – surgery, radiation & systemic chemo; survival 75%

Radiotherapy lowers IQ, causes growth and endocrine problems

Difficulties with education, independence, employment, driving, dating

Drug-loaded nanoparticles
Residual Tumour tissue
Gel/foam carrier formulation
Safety and efficacy in brain cancer therapy

**Safety**
- Neural progenitor cells
- Human fetal brain tissue
- Proliferating part of brain

**Efficacy**
- UW228 Tumour cells:
  - Human medulloblastoma
  - Invading cancer cells
Co-culture models

- Mimics interaction between normal and tumour tissue
- Cells marked with supravital stains (CDCFDA SE, CellTrace)
- Maintains tissue heterogeneity
Experimental setup

Seed marked cell mix
- Green: normal
- Blue: tumour

Etoposide
- Co-culture ready for compound screening

Fresh media
- Early drug effects

Analyse
- Late drug effects

Days in culture:
- Growth: 0-1
- Drug treatment: 3
- Drug-free phase: 5-7
Co-culture analysis

- Co-culture + Etoposide → Drug effect

Intact spheroid microscopy

- Flow cytometry

One-Photon Fluorescence

- Excited State $E=E_1 - E_0 = hf$
- Excitation Photon $E = hf/2$
- Fluorescence Photon
- Ground State $E=E_0$

Two-Photon Fluorescence

- Excited State $E=E_1 - E_0 = hf$
- Excitation Photon $E = hf/2$
- Fluorescence Photon
Flow cytometry analysis

Dissociation

Co-culture

Tumours

Normal cells
Dose response curve computation

Viability %

Etoposide, μM

IC50

Control 0.01 0.1 1 10 100 1000

NSC UW 228-3

10μM Etoposide

Viability %

NSC UW 228

IC50 3 1
Conclusions

Supravital dyes can be used to mark heterogeneous mixes of cells
Protocol is HT compatible and universally applicable
Model is uniquely suited to study tumour/stroma interactions
Internal normal tissue control puts drug effects in perspective

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