PHOTODYNAMIC THERAPY OF MEDULLOBLASTOMA IN VITRO EXPLORED USING FLUORESCENCE MICROSCOPY
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Abstract
There is very little application of photodynamic therapy to pediatric brain tumors, thus it is important to explore the localization of the commonly used photosensitizer Photofrin in a novel cell line in vitro. To date there are no studies quantifying the optimal concentrations and uptake of Photofrin by medulloblastoma cell lines. Various concentrations of Photofrin and incubation times will be explored to determine the optimal concentrations and incubation period for Photofrin in vitro and where the Photofrin is sequestered within the cells. Cellular localization will be assessed using fluorescence microscopy and the relative fluorescence intensity of the cells will be quantified.

Materials and Methods

- Dacy cells (ATCC) cultured in Eagle’s Minimum Essential Medium (EMEM) supplemented with 10% Fetal Bovine Serum (FBS)
- 1x10^6 to 1x10^8 cells plated to each well of a chambered coverglass
- PF-doped medium added to the cells (0 – 20 μg/mL) for various incubation times (0 – 48 hours)
- Zeiss LSM 5 live line-scanning confocal microscope (λem = 405 nm, λex = 630 nm, power = 150 μW, 333 ms duration, long-pass filter)
- MetaMorph software to quantify fluorescence
- Controls: cell control (no PF), medium control (no cells or PF), PF control (no cells)

Results

- PDT: FDA-approved to treat some skin and esophageal cancers
- Photosensitizer administered to a patient, tumor irradiated with light of a certain wavelength
- Tumor cells retain the photosensitizer longer than normal tissue, thus PDT selectively kills cancer cells
- Mechanism of cell death: superoxide mediated, leading to apoptosis
- The application of PDT to a variety of brain cancers is a promising field in need of further exploration and refinement in order to allow clinical applications of these treatments

- Photofrin: photosensitizer that has been approved for clinical PDT use in certain cancers
- Photofrin: mixture of porphyrin oligomers, activated at 408 nm, fluoresces at 630 nm
- Depending on the hydrophobicity and charge of the photosensitizer, the localization of a photosensitizer may vary
- Photofrin diffusely localizes to the cytoplasm in other cell lines
- Diffuse cytoplasmic localization correlates with the most effective PDT in vitro

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- Photofrin exhibits perinuclear localization
- Future work with other cell lines (vascular endothelial cells, astrocytes) and in vivo studies with nude mice
- PDT shows promise to detect and treat medulloblastoma tumors

Conclusions

- Medulloblastoma cells are able to take up Photofrin in culture (no lymph support cells required), thus the cells are intrinsically capable of absorbing Photofrin
- Photofrin exhibits perinuclear localization
- Future work with other cell lines (vascular endothelial cells, astrocytes) and in vivo studies with nude mice
- PDT shows promise to detect and treat medulloblastoma tumors

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