Green light; Red light: Assessing KillerRed as an Anticancer Therapy
Joshua Malerich and Dr. Jeffrey Thompson
Department of Biological Sciences, York College of Pennsylvania, York, PA 17403

Introduction

- Glioblastoma multiforme (GBM) are cancers of the brain. 5 – year survival rates are dismal, with the median life expectancy post-diagnosis amounting to 12 months (Krex et al. 2007).
- Therapies are limited and include broad spectrum antiproliferative drugs, but a specific target has been desired for diagnostic and therapeutic procedures.
- Debinski et al. (1999) found that GBM’s stably overexpress human cytokine 13α2, a receptor not found in the surrounding neural tissue.
  - Using a fusion protein specific to this receptor, a linked cytotoxin could be delivered to kill tumor cells directly.
- KillerRed, a red fluorescent protein, produces reactive oxygen species (ROS) only when excited (480 – 585 nm).
  - Proposed mechanism of cell death via membrane oxidation (Shirmanova et al. 2012).

Objective

- Evaluate KillerRed in a proof-of-concept study.
  - Assess the efficacy of membrane targeting KillerRed in transfected U251 Cells

Hypothesis: Excited membrane-bound KillerRed will cause cell death.

Results

- Results of Cell Titer 96 Assay were inconclusive (media contamination).

Methods

1.) Culture U251 Cells

- 37° C, > 95% Humidity, 5% CO₂
- Modified DMEM (FBS – 8.7%, Glutamax – 0.87%, Pen-Strep – 0.87%, NEAA – 0.87%, Sodium-pyruvate – 0.87%).

2.) Creation of KillerRed expressing cell lines based on location

- Fugene 6 transfection to create U251 line with membrane target KillerRed
- Alter plasmid to induce KillerRed expression in the cytosol
  1. Endonuclease (HindII, NOTI) to remove KillerRed sequence (Fig. 1)
  2. “Touchdown” PCR with custom primers, ligate into existing plasmid.
  3. Transform E. coli with cKR plasmid
  - Confirm presence of plasmid with 1% Agarose gel (Fig 2.)
  4. Fugene 6 Transfection (Fig. 3)

3.) Excite with Florescence (525 and 585 - 595 nm)

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Future Studies

- Quantitatively assess rates of cellular metabolism.
- Investigate other fluorescent proteins without KillerRed’s limitations.
  - Supernova
  - miniSOG

Conclusions

The hypothesis that KillerRed would cause cell death was not supported under these testing conditions.

- Studies have suggested that KillerRed’s tendency to dimerize reduces its production of ROS and overall functionality as either a membrane-bound or fusion protein (Bulina et al. 2006).

Literature Cited


Acknowledgements

Special thanks to Dr. Thompson for his expertise in the lab as well as his patience and character which extend beyond it.

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