Objective & Hypotheses

Programmed cell “suicide”—apoptosis—occurs in normal cells that turn cancerous (Böttger et al., 2008). The primary mechanism of apoptosis occurs in the nucleus using a special protein: p53, but secondary action may occur in the mitochondria, mediated by a certain enzyme: HAUSP (Figure 4). (Böttger et al., 2008; Vaseva & Moll, 2008). Understanding this pathway can further the development of cancer treatments in diseases such as human neuroblastoma, and can enhance our current knowledge of the cell cycle.

- **Hypothesis 1**: Treating cells with cancer drug—etoposide—will induce “cell suicide” via the nucleus
- **Hypothesis 2**: Preventing p53 from entering the nucleus and inhibiting the HAUSP enzyme will prevent apoptosis

Materials & Methods

Induce Cellular Stress

IMR-32 Human Neuroblastoma cells were cultured to a concentration of 1.4 x 10^5 cells/ml and were exposed to 1.0 mM/L of the cancer drug, etoposide, for 0, 6, 12 & 24 hours.

Detecting p53 and Apoptosis

1) Morphology stain: Romanovski stain
2) Immunocytochemistry: Vectastain
3) Fluorometric Analysis: Fluorometric TUNEL assay

Results

- **Figure 1**: Morphology stain: visible membrane disruption at time 6, and 24 hrs compared to 0 hrs.
- **Figure 2**: Immunocytochemistry: Localization of p53 at the nucleus at time 12 hrs compared to time 0 hrs.
- **Figure 3**: Fluorometric Analysis: tagged fragments of DNA at t = 24 hrs.
- **Figure 4**: p53 entry into the mitochondria (Vaseva & Moll, 2009)

Discussion & Conclusion

- Visible “blebbing” in morphology stain with increased time of etoposide exposure (Figure 1).
- Localization of p53 to the nucleus indicated cellular stress and efficacy of etoposide (Figure 2).
- Fluorescent tagging of the 3’ ends of the DNA fragments confirmed apoptosis (Figure 3).
- This evidence supports etoposide’s efficacy in inducing apoptosis in IMR-32 cells.
- At present, experimentation is being done to evaluate hypothesis #2

References


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