**Introduction**

Breast Cancer and Available Treatments

- 2nd leading cause of cancer death in American women.
- Traditional chemotherapy treatment includes Cyclophosphamide (CP) and Doxorubicin.
- Growing research focus on immunotherapy to make the immune system the body’s greatest weapon against cancer.
- CP can induce immunogenic cell death (ICD) when administered in metronomic schedule (MC).

**Research Objective**

- Analyze how breast cancer cells stimulate the immune system when treated with 4HC, active component of CP.
- Evaluate if murine E0771 breast cancer cells can induce interferon-stimulated gene expression and potentially be used in vivo.

**Methods**

**Cell Line:** E0771 Cells

**Drug Used:** 4HC, active component of CP

**General Experimental Design**

- **Time Point:**
  - 24 hours: untreated - 1
  - 24 hours: untreated - 2
  - 24 hours: 1.5µM 4HC - 1
  - 24 hours: 1.5µM 4HC - 2
  - 3µM 4HC - 1
  - 3µM 4HC - 2

**RNA Yield (µg)**

- 5.96
- 4.90
- 4.35
- 4.13
- 4.82
- 3.39
- 4.20

**Cell Processing**

- **Time Point:**
  - 48 hours: untreated - 1
  - 48 hours: untreated - 2
  - 48 hours: 1.5µM 4HC - 1
  - 48 hours: 1.5µM 4HC - 2
  - 3µM 4HC - 1
  - 3µM 4HC - 2

**Fold expression**

- **Gene Probed:** Cxcl10 and Igtp (ISGs)

**Time and concentration-dependent induction of Cxcl10 and Igtp mRNA levels in 4HC treated E0771 cells**

- **Time Point:**
  - 24 hours: untreated - 1
  - 24 hours: untreated - 2
  - 24 hours: 1.5µM 4HC
  - 24 hours: 3µM 4HC

**Conclusion**

**Findings**

- 4HC shows a concentration-dependent response in E0771 cells.
- RNA isolation had minimal protein contamination as seen by 260:280 ratios for all samples.
- 4HC shows a time and concentration-dependent response in ISG induction in E0771 cells.
- At 24 hours: no induction of Cxcl10 or Igtp for 1.5µM and 3µM 4HC treated E0771 cells.
- At 48 hours: statistically significant induction of Cxcl10 and Igtp for 3µM 4HC treated E0771 cells compared to untreated cells.

**Future Steps**

- Study additional ISGs activated by 4HC in E0771 cells.
- Validate role of IFN signaling in 4HC-induced ISG induction.
- Analyze potency of cytokines secreted by 4HC-treated E0771 cells in activating paracrine INF signaling (conditioned media experiment).
- Evaluate effect of CP in anti-tumor immune system activation in syngeneic mouse tumor model with E0771 tumor in C57BL/6 mouse.

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**References**

5. Image by Marios, Retrieved under a CC BY 2.0 license.