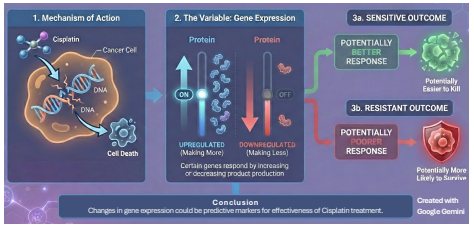
Cisplatin Effects on Gene Expression in Colon and Lung Cancer Cells

Eleanor Niemier

Background

- Cisplatin is platinum-based chemotherapy that forms adducts in DNA [1] ■ used for over 40 years ■ Cisplatin resistance is major treatment limitation
- Biomarkers can predict outcomes, target personal treatments
  - Identifying *more* biomarkers can make chemotherapy and other treatments more effective, and ultimately increase survival rates
- Over 154,000 Americans develop colon cancer annually [2,3]
  - > ⅓ of United States' cancer cases diagnosed in people under 50
  - Rising numbers in young people
- Over 226,000 Americans develop lung cancer each year [4,5]
  - Most common cancer diagnosed worldwide AND most deadly
  - Increasing numbers in non-smokers and young adults

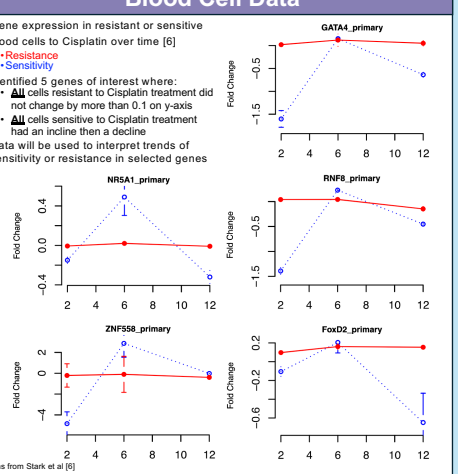


Research Question

- Are genes GATA4, NR5A1, ZNF558, RNF8, FOXD2 potential biomarkers for Cisplatin treatment in colon or lung cancer?

Blood Cell Data

- Gene expression in resistant or sensitive blood cells to Cisplatin over time [6]
  - Resistance
  - Sensitivity
- Identified 5 genes of interest where:
  - **All** cells resistant to Cisplatin treatment did not change by more than 0.1 on y-axis
  - **All** cells sensitive to Cisplatin treatment had an incline then a decline
- Data will be used to interpret trends of sensitivity or resistance in selected genes



Hypotheses

- Minimal or no research about how selected genes can affect lung and colon cancer Cisplatin treatments
- **This research can identify potential new biomarkers**

Colon

Gene of Interest	Predicted Outcome (up/down/no change)	Reasoning/Evidence
GATA4	Down	No studies on effects on colon cancer
NR5A1	No change	No studies on effects on colon cancer
RNF8	Up	"RNF8 expression is positively correlated with Cisplatin [7]"
ZNF558	Down	No studies on effects on colon cancer
FOXD2	Up	"Upregulation of FOXD2-AS was detected [8]"

Lung

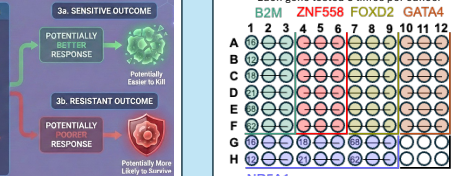
Gene of Interest	Predicted Outcome (up/down/no change)	Reasoning/Evidence
GATA4	Down	"GATA4 functions as an essential tumor suppressor [9]"
NR5A1	No change	No studies on effects on lung cancer
RNF8	Up	"RNF8 expression levels are markedly increased [10]"
ZNF558	Down	No studies on effects on lung cancer
FOXD2	Down	No studies on effects on lung cancer

Materials

- cDNA for cancer cells (Cisplatin & control)
- Primer (for each gene)
- Master mix
- qPCR Step one plus machine
- 96 well plate
- Clear seal
- Pipette & tips

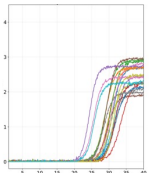
Plate map

Each gene tested 3 times per cancer



Amplification Plot

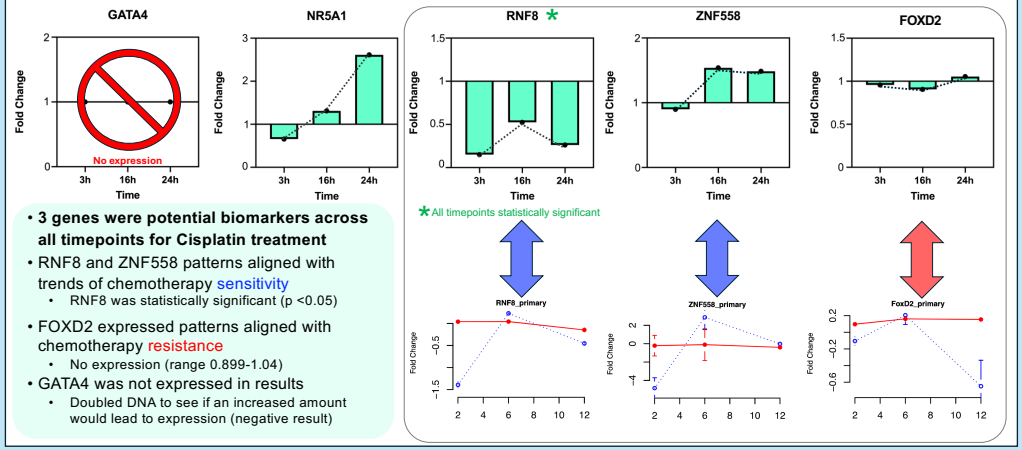
Output from qPCR machine



Procedure

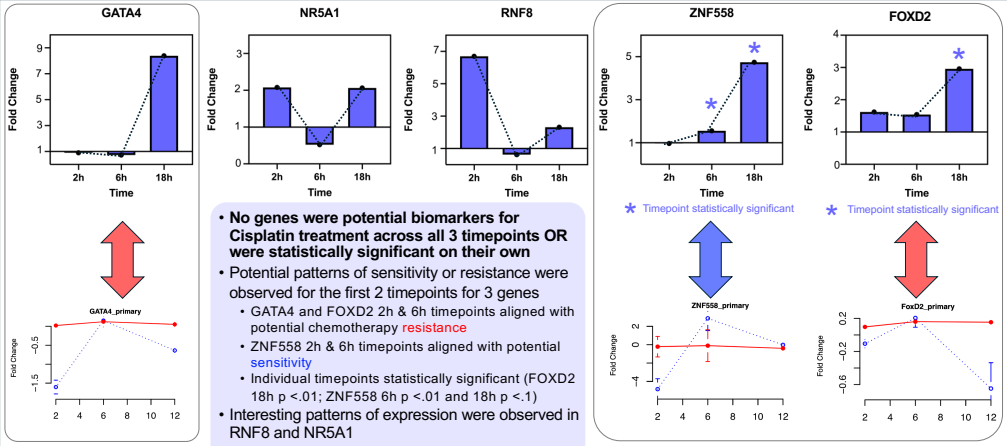
- Thawed cDNA for each cancer cell (Cisplatin & control) for each timestamp; timepoints included:
  - Lung: 2, 6, 18 hour
  - Colon: 3, 16, 24 hour
- Diluted cDNA (1µl) and water (39µl), 12.5ng/µl
  - Added 9 µL into each well
- Combined Primer (19.3µl) and Master mix (193µl)
  - Added 11µL into each well
- Tested each gene 3 times per cancer
- Placed 96-well tray in qPCR machine for amplification & measurement
- To determine fold change, used ΔΔCT formula:
  - $[(Ct_{M_{GOI}} - Ct_{M_{HKG}})_{Cisplati} - (Ct_{M_{GOI}} - Ct_{M_{HKG}})_{Control} = x] \rightarrow 2^{-x}$ (% increase/decrease)
- Analyzed patterns of expression
- Conducted t-test analysis to determine if results statistically significant
- Utilized prior data of gene expression in blood cells to interpret trends of sensitivity or resistance in selected genes

Colon Cancer Data and Analysis



- **3 genes were potential biomarkers across all timepoints for Cisplatin treatment**
- RNF8 and ZNF558 patterns aligned with trends of chemotherapy sensitivity
  - RNF8 was statistically significant (p <0.05)
- FOXD2 expressed patterns aligned with chemotherapy resistance
  - No expression (range 0.899-1.04)
- GATA4 was not expressed in results
  - Doubled DNA to see if an increased amount would lead to expression (negative result)

Lung Cancer Data and Analysis



- **No genes were potential biomarkers for Cisplatin treatment across all 3 timepoints OR were statistically significant on their own**
- Potential patterns of sensitivity or resistance were observed for the first 2 timepoints for 3 genes
  - GATA4 and FOXD2 2h & 6h timepoints aligned with potential chemotherapy resistance
  - ZNF558 2h & 6h timepoints aligned with potential sensitivity
  - Individual timepoints statistically significant (FOXD2 18h p <.01; ZNF558 6h p <.01 and 18h p <.1)
- Interesting patterns of expression were observed in RNF8 and NR5A1
  - Potential explanations include a trend showing a dynamic change; opposite of the original data [6]

Data and analysis graphs created by Eleanor Niemier. Blood cell graphs from Stark et al. [6]

Results

Colon:

Gene of Interest	Statistically Significant?	Biomarker?	Hypothesis {correct, incorrect}?
GATA4	N/A ✗	✗	Hypothesis incorrect ✗
NR5A1	0.4566 ✗	✗	Hypothesis incorrect ✗
RNF8	0.0249 ✓	Sensitive ✓	Hypothesis incorrect ✗
ZNF558	0.2716 ✗	Sensitive ✓	Hypothesis incorrect ✗
FOXD2	0.2940 ✗	Resistant ✓	Hypothesis incorrect ✗

Lung:

Gene of Interest	Statistically Significant?	Biomarker?	Hypothesis {correct, incorrect}?
GATA4	0.5236 ✗	Potential resistant ○	Hypothesis incorrect ✗
NR5A1	0.3956 ✗	✗	Hypothesis incorrect ✗
RNF8	0.1730 ✗	✗	Partially supported by **2h** & **18h** timepoints ○
ZNF558	**6h** 0.0031 **18h** 0.0786 ○	Potential sensitive ○	Hypothesis incorrect ✗
FOXD2	**18h** 0.0082 ○	Potential resistant ○	Hypothesis incorrect ✗

Conclusions and Implications

Conclusions

- Comparing results to blood cell data identified more potential biomarkers in colon cancer than lung cancer
- In colon cancer cell lines, ZNF558 and RNF8 are potential sensitivity biomarkers, and FOXD2 is a potential resistance biomarker
  - Patterns of expression aligned across all timepoints
- Potential patterns of sensitivity or resistance were observed in lung cancer cell lines across 2 timepoints
  - FOXD2 and GATA4 aligned with potential Cisplatin resistance, and ZNF558 aligned with potential sensitivity

Implications

- Cisplatin chemotherapy is *less likely* to be effective in patients with patterns that follow FOXD2 gene expression
  - Other forms of treatment – e.g., radiation, other chemotherapies, surgery – may be more effective for these patients
- Cisplatin chemotherapy is *more likely* to be effective in patients with patterns that follow the ZNF558 or RNF8 gene expression
- Comparing across various cancers allows for the observation of varying results/patterns

Future Work

- Further investigate potential biomarkers identified in this research
- Evaluate how selected genes are expressed in other cancers
- Research effects of other cancer treatments on gene expression

References

[1] Romani, Andrea MP. "Cisplatin in cancer treatment." *Biochemical pharmacology* 206 (2022): 115323.
[2] American Cancer Society. (2026, January 14). *Colorectal cancer statistics: How common is colorectal cancer?* https://www.cancer.org/cancer/types/colon-rectal-cancer/about/key-statistics.html
[3] Staff, C. (2025, September 23). *Colorectal cancer rates are skyrocketing in young adults-is your lifestyle putting you at risk?* . Cancer Research Institute. https://www.cancerresearch.org/blog/colorectal-cancer-awareness-month
[4] American Cancer Society. (2026, January 13). *Lung cancer statistics: How common is lung cancer?* American Cancer Society. https://www.cancer.org/cancer/types/lung-cancer/about/key-statistics.html
[5] Lung Cancer Research Foundation. (2026, February 12). *Lung cancer facts.* Lung Cancer Research Foundation. https://www.lungcancerresearchfoundation.org/for-patients/early-lung-cancer-facts/
[6] Identification of Novel Protein Expression Changes following Cisplatin Treatment and Application in Combination Therapy by Stark, et al. *J Proteome Res.* Nov 3;16(11):4227-4236, 2017.
[7] Yang, Ben, et al. "Targeting RNF8 effectively reverses cisplatin and doxorubicin resistance in endometrial cancer." *Biochemical and biophysical research communications* 545 (2021): 89-97.
[8] Li, Xuechao, Yifei Ren, Shenghua Pei, Kai Zhao, Guanyu Chen, and Zhenglin He. "Dissecting the role of epigenetic regulation in oral squamous cell carcinoma microenvironment: mechanisms and therapeutics." *Frontiers in Immunology* 17 (2026): 1758433.
[9] Gao, Lei, et al. "Lung cancer deficient in the tumor suppressor GATA4 is sensitive to TGFBR1 inhibition." *Nature communications* 10.1 (2019): 1665.
[10] Kuang, Jingyu, et al. "RNF8 promotes epithelial–mesenchymal transition in lung cancer cells via stabilization of slug." *Molecular Cancer Research* 18.11 (2020): 1636-1649.