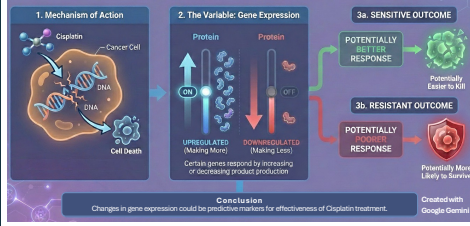


## Background

- 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]
  - > 1/3 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



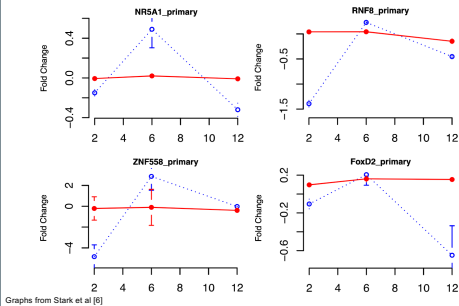
## Research Question

- 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

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]"

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

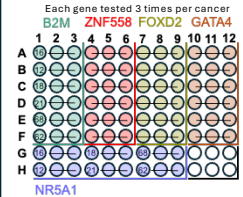
# Cisplatin Effects on Gene Expression in Colon and Lung Cancer Cells

Eleanor Niemier

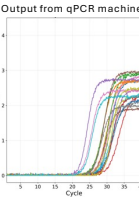
## Materials

- 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



### Amplification Plot

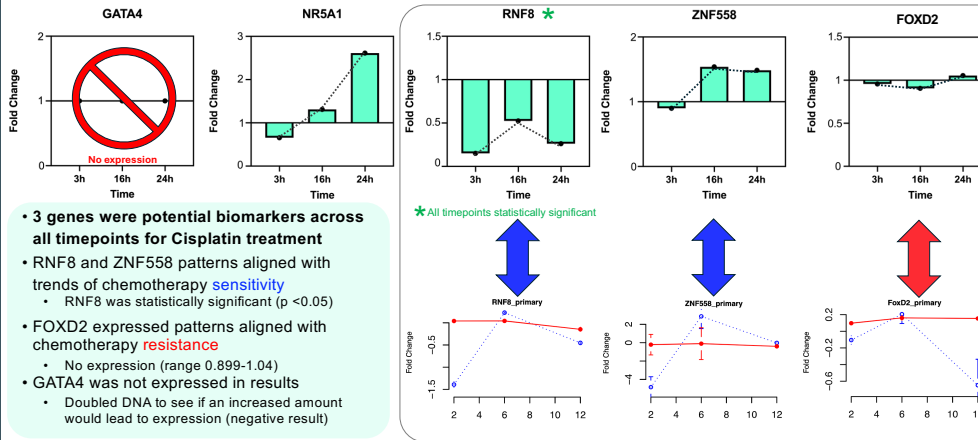


## Procedure

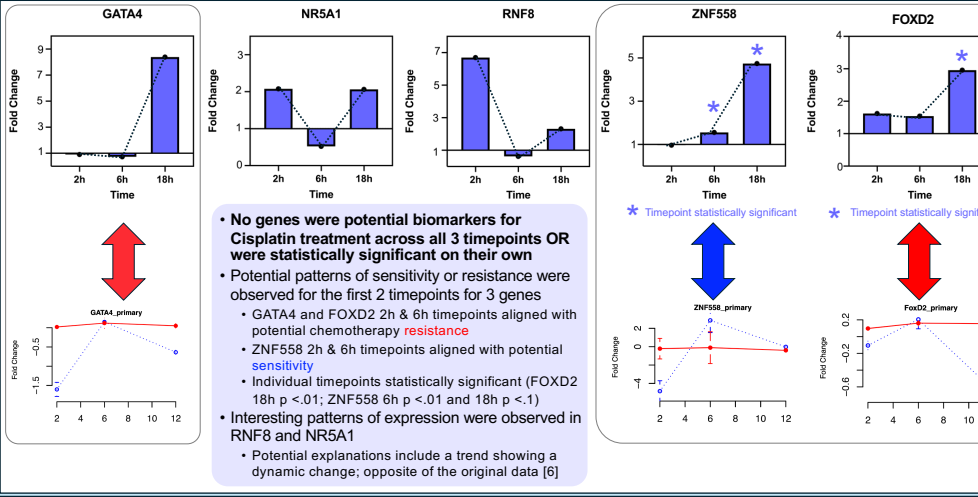
- Thawed cDNA for each cancer cell (Cisplatin & control) for each timestamp; timepoints included:
  - Lung: 2, 6, 18 hour
  - Colon: 3, 16, 24 hour
- Diluted cDNA (1µl) and water (39µl), 12.5ng/µl
  - Added 9 µL into each well
- Combined Primer (19.3µl) and Master mix (193µl)
  - Added 11µL into each well
- Tested each gene 3 times per cancer
- Placed 96-well tray in qPCR machine for amplification & measurement
- To determine fold change, used  $\Delta\Delta Ct$  formula:
  - $[(Ct_{M_{Col}} - Ct_{M_{HCC}}) - (Ct_{M_{Col}} - Ct_{M_{HCC}})_{Control}] \rightarrow 2^{-x}$  (% 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

Variables	
Independent	Gene of interest
Dependent	Gene regulation (up, down, no change) & expression

## Colon Cancer Data and Analysis



## Lung Cancer Data and Analysis



## 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

- Rosen, Andrea MP "Cisplatin in cancer treatment." *Biochemical pharmacology* 206 (2022): 115323. American Cancer Society (2026, January 14). [Colon cancer statistics: How common is colorectal cancer?](https://www.cancer.org/cancer/colon-cancer-statistics/about-colon-cancer-statistics/)
- Stark, et al. "Upregulation of FOXD2-AS was detected [8]" <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6111111/>
- Li, Xuechao, Yifei Ren, Shinghua Pei, Kai Zhao, Guanyu Chen, and Zhonglin He. "Downing the role of epigenetic regulation in oral squamous cell carcinoma microenvironment: mechanisms and therapeutic." *Frontiers in Immunology* 7 (2016): 1750333.
- Qin, Li, et al. "Lung cancer deficient in the tumor suppressor GATA4 is sensitive to TGFβ1 inhibition." *Nature communications* 10, 1 (2019): 1665.
- Kuang, Jingyu, et al. "RNF8 promotes epithelial-mesenchymal transition in lung cancer cells via stabilization of slug." *Molecular Cancer Research* 16, 11 (2008): 1658-1666.
- J. Proteome Res. Nov 3;16(11):4227-4236, 2017.
- Yang, Bin, et al. "Targeting RNF8 effectively overcomes cisplatin and docetaxel resistance in endometrial cancer." *Biochemical and Biophysical Research Communications* 445 (2014): 39-47.
- Li, Xuechao, Yifei Ren, Shinghua Pei, Kai Zhao, Guanyu Chen, and Zhonglin He. "Downing the role of epigenetic regulation in oral squamous cell carcinoma microenvironment: mechanisms and therapeutic." *Frontiers in Immunology* 7 (2016): 1750333.
- Qin, Li, et al. "Lung cancer deficient in the tumor suppressor GATA4 is sensitive to TGFβ1 inhibition." *Nature communications* 10, 1 (2019): 1665.
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