Cisplatin Effects on Gene Expression in Colon and Lung Cancer Cells

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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 Cisplatin 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

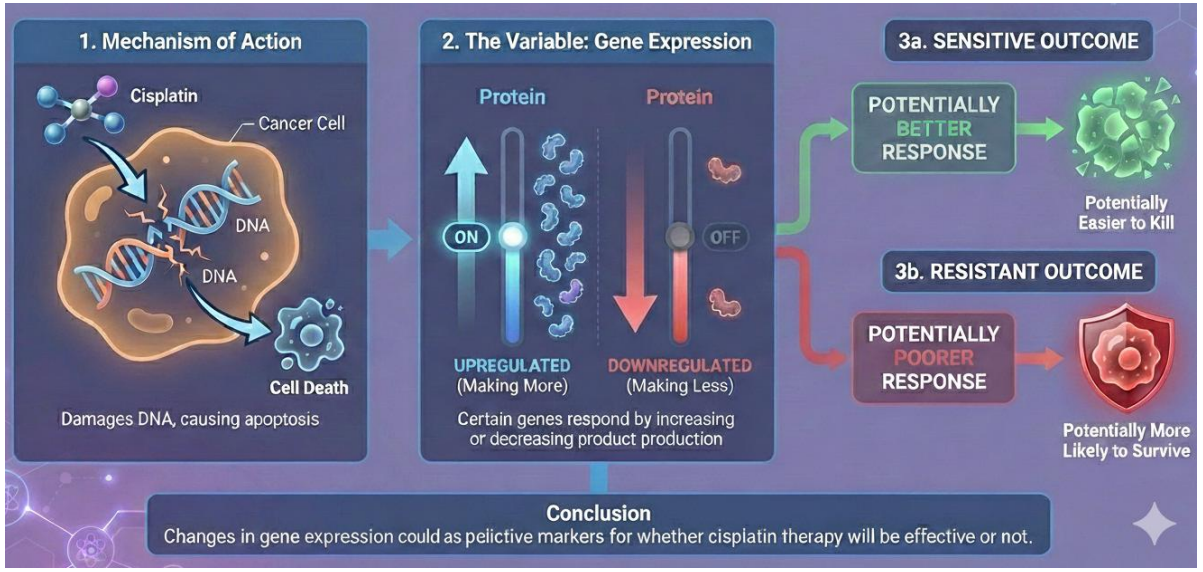


Chart created with Google Gemini

[1] Romani, Andrea MP. "Cisplatin in cancer treatment." *Biochemical pharmacology* 206 (2022): 115323 ■ **[2]** American Cancer Society. (2026, January 13). *Lung cancer statistics: How common is lung cancer?*. Lung Cancer Statistics | How Common Is Lung Cancer? | American Cancer Society. https://www.cancer.org/cancer/types/lung-cancer/about/key-statistics.html ■ **[3]** Lung Cancer Research Foundation. (2026, February 12). *Lung cancer facts*. Lung Cancer Research Foundation. https://www.lungcancerresearchfoundation.org/for-patients/lung-cancer-facts/ ■ **[4]** American Cancer Society. (2026, January 14). *Colorectal cancer statistics: How common is colorectal cancer?*. Colorectal Cancer Statistics | How Common Is ColorectalCancer? AmericanCancerSociety. https://www.cancer.org/cancer/types/colon-rectal-cancer/about/key-statistics.html ■ **[5]** 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

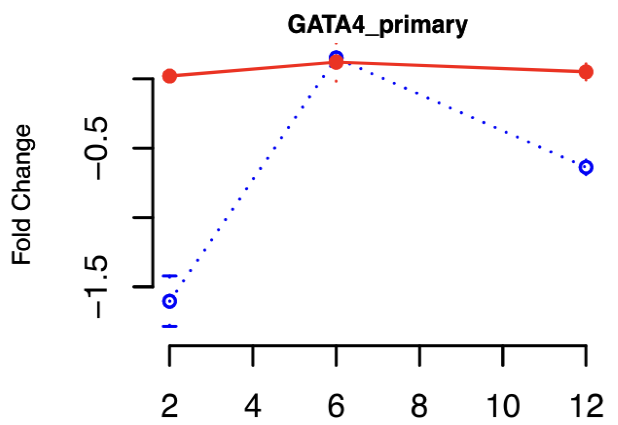
Research Questions

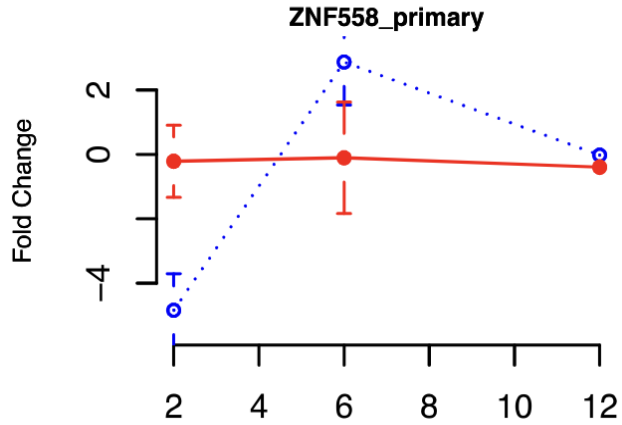
- Are genes GATA4, NR5A1, ZNF558, RNF8, FOXD2 potential biomarkers for Cisplatin treatment in colon or lung cancer?

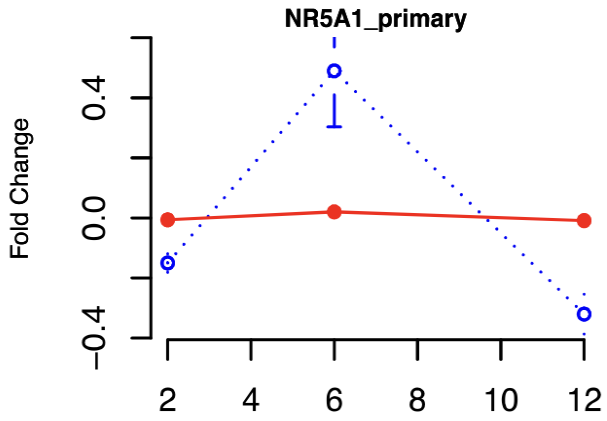
Blood Cell Data

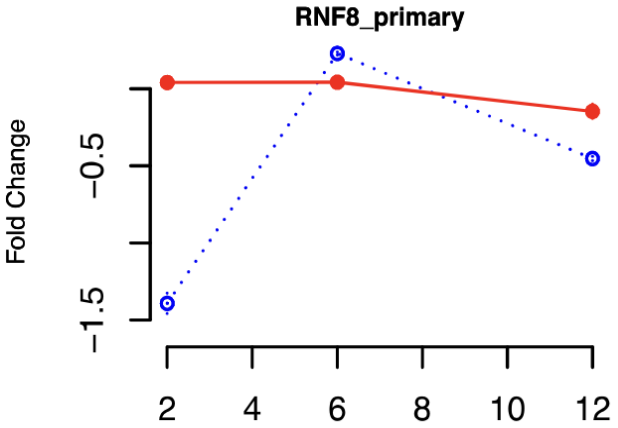
- Gene expression in resistant or sensitive blood cells to Cisplatin over time [6] – Resistance or Sensitivity
- Identified 5 genes of interest where:
  - **All** the cells resistant to Cisplatin treatment did not change by more than 0.1 on y-axis
  - **All** sensitive cells to Cisplatin treatment had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

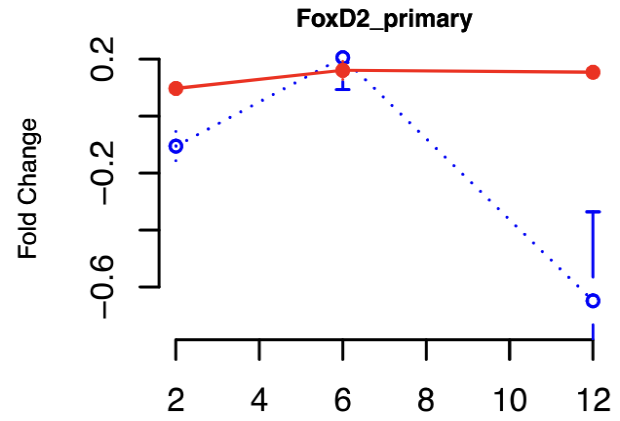
Graphs from Stark et al [6]











[6] Effect of cisplatin on gene expression of blood cells from the study Identification of Novel Protein Expression Changes following Cisplatin Treatment and Application in Combination Therapy by Stark, et al. (2017)

Hypotheses

- Minimal or no research about how selected genes can affect lung and colon cancer Cisplatin treatments
  - **This research can provide 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

[7] Liu, Huasong, et al. "Overexpression of the long noncoding RNA FOXD2-AS1 promotes Cisplatin resistance in esophageal squamous cell carcinoma through the miR-195/Akt/mTOR axis." *Oncology research* 28.1 (2020): 65. ■ **[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): 1638-1649.

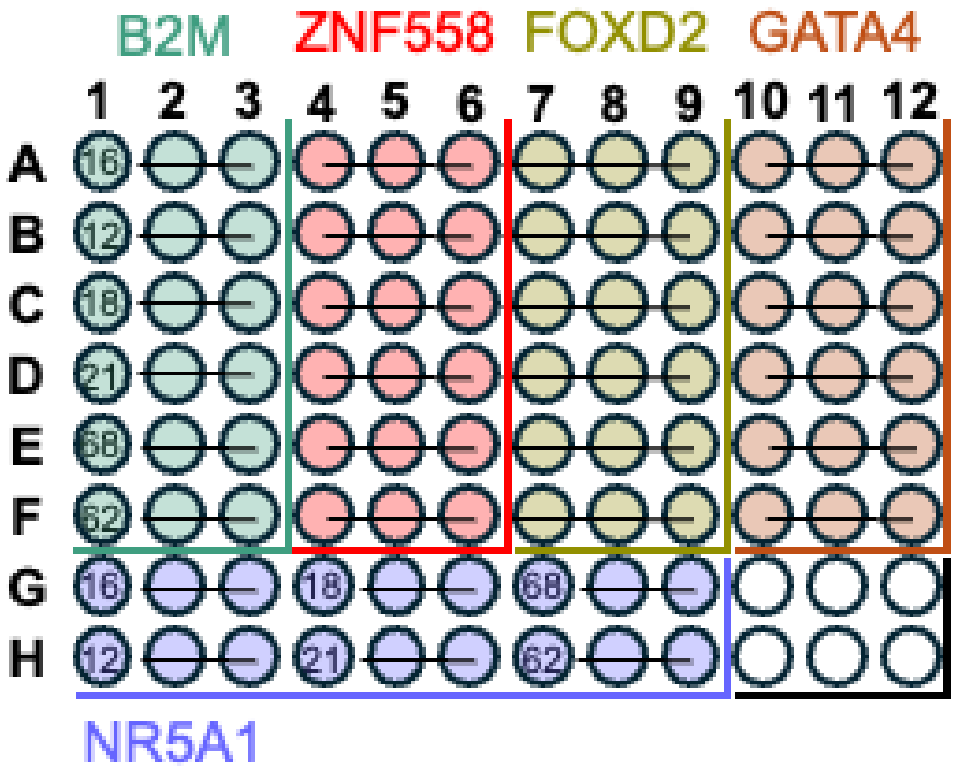
Materials

- cDNA for cancer cells (Cisplatin & control)
- Primer (for each gene)
- Master mix
- qPCR Step one plus machine

- 96 well plate
- Clear seal
- Pipette & tips

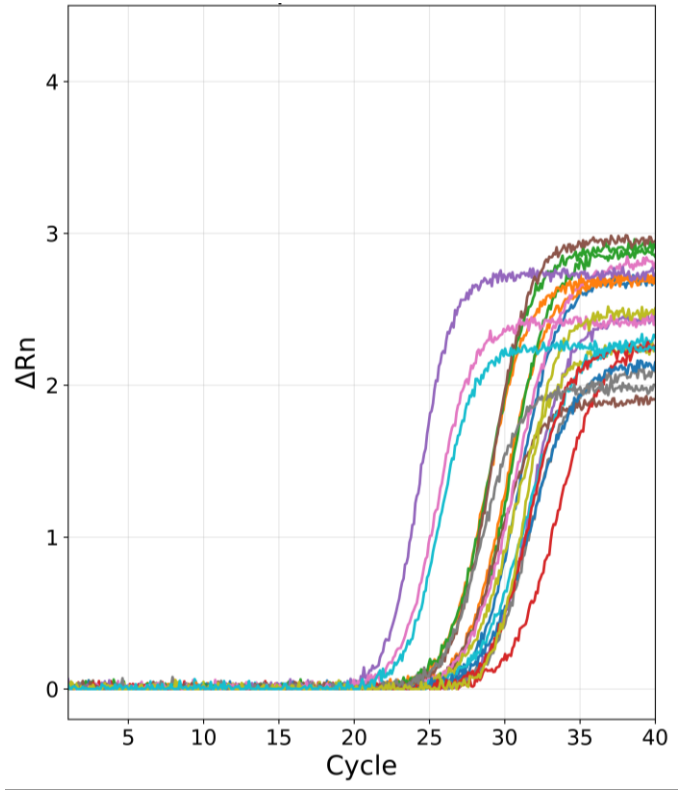
Plate map

Each gene tested 3 times per cancer



Amplification Plot

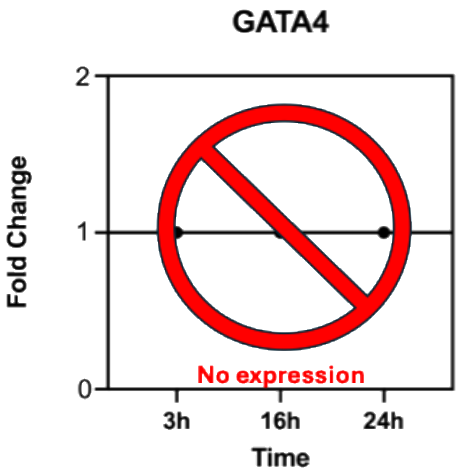
Output from qPCR machine

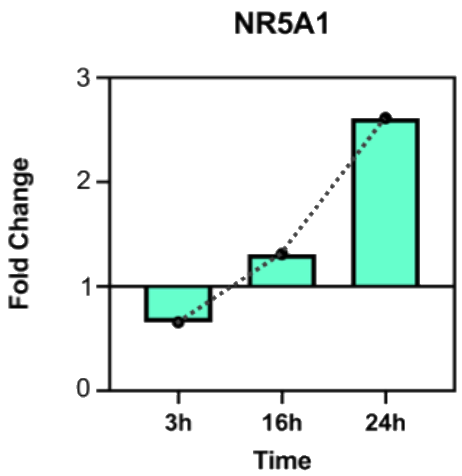


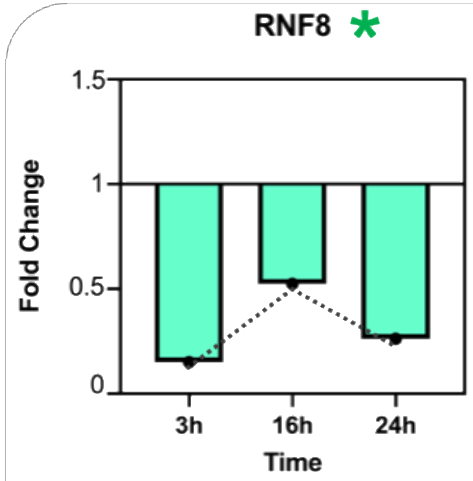
Procedure

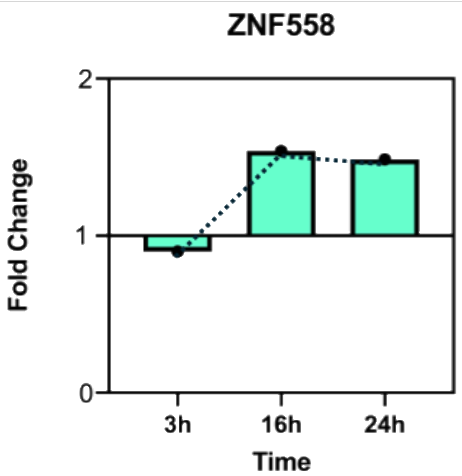
- Thawed cDNA for each cancer cell (Cisplatin & control) for each timestamp; timepoints included:
  - Lung: 2, 6, 18 hour
  - Colon: 3, 16, 24 hour
- Diluted cDNA (1µl) and water (39µl), 12.5ng/µl
  - Added 9 µL into each well
- Combined Primer (19.3µl) and Master mix (193µl)
  - Added 11µL into each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

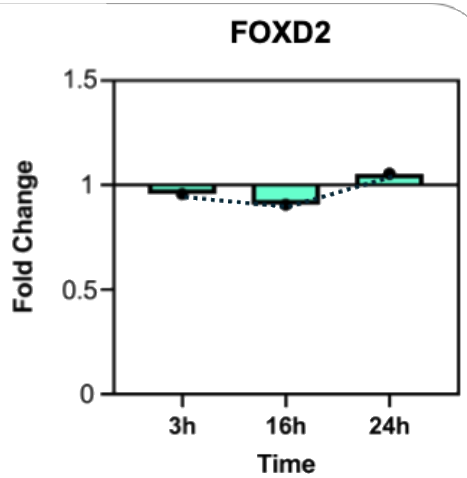
Colon Cancer Data and Analysis







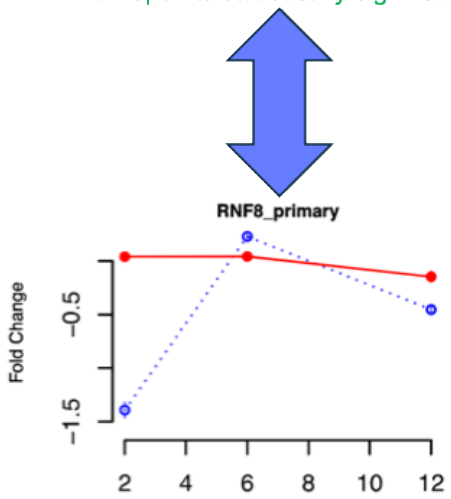


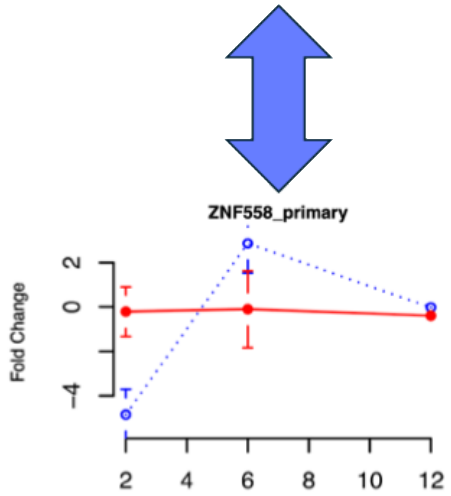


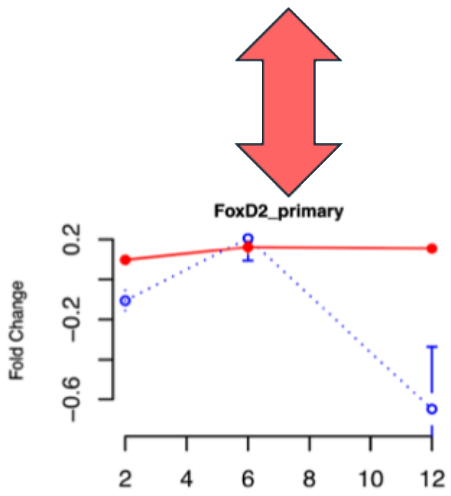
- **3 genes were potential biomarkers across all timepoints for Cisplatin treatment**
- RNF8 and ZNF558 patterns aligned with trends of chemotherapy sensitivity
  - RNF8 was statistically significant ($p < 0.05$)
- FOXD2 expressed patterns aligned with chemotherapy resistance
  - No expression (range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

* All timepoints statistically significant

All data analysis graphs created by Eleanor Niemier







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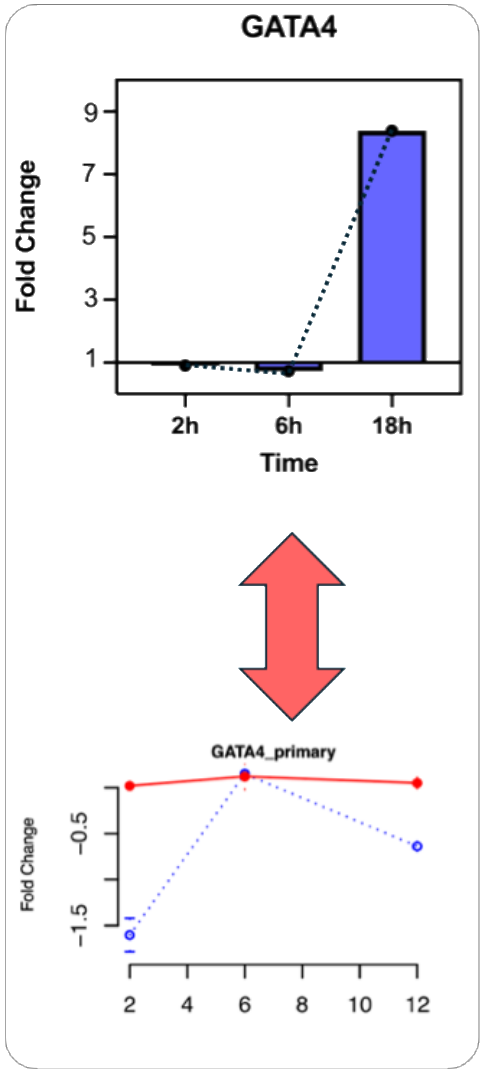
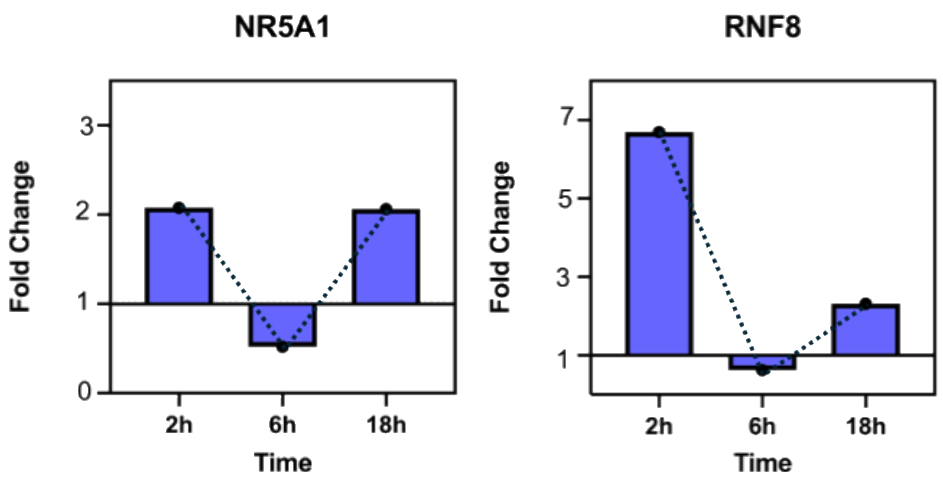
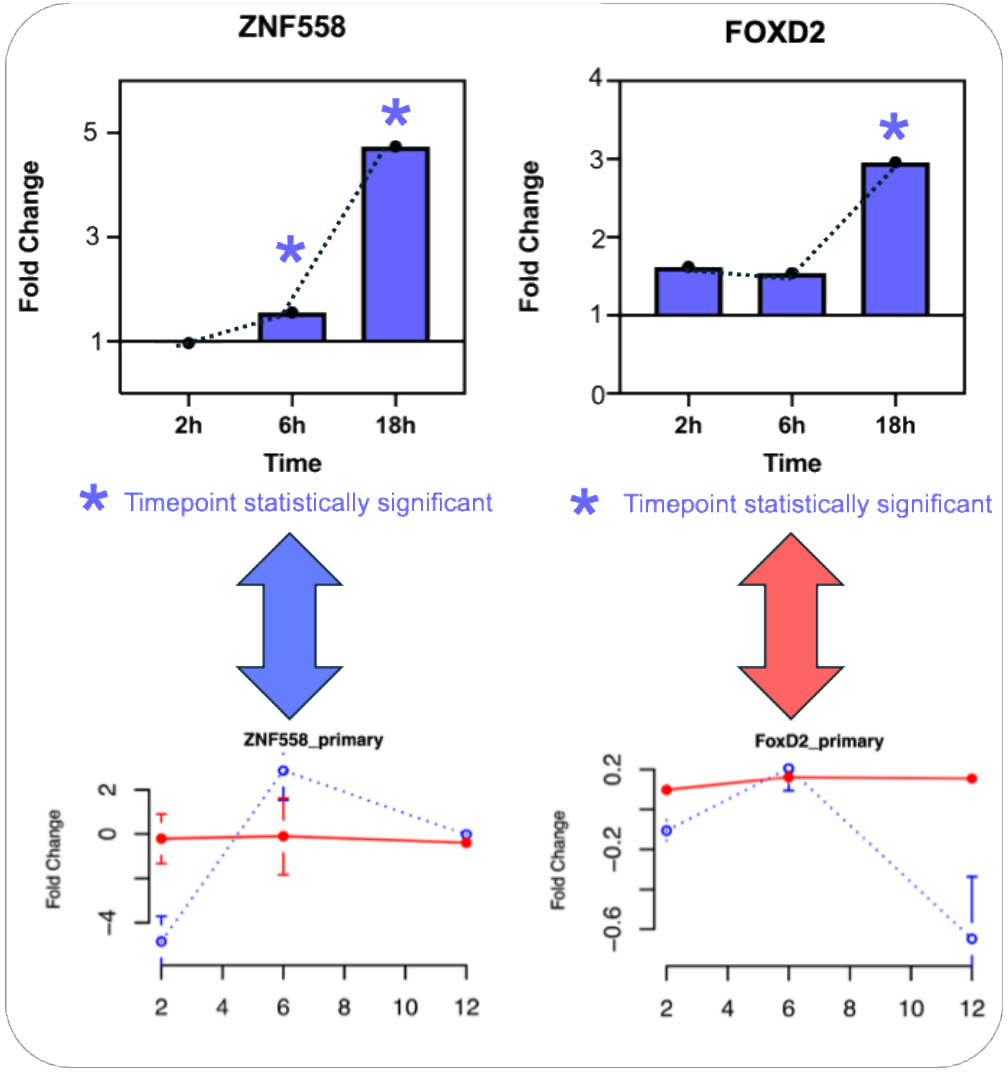
Lung Cancer Data and Analysis





- **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
  - FOXD2 and GATA4 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]



All data analysis graphs created by Eleanor Niemier

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Results and Conclusions

- The selected genes had varying levels of expression and statistical significance
- Comparing results to blood cell data identified more potential biomarkers in colon 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/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

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 ✗

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