Evaluating the Sensitivity of the NanoStrong nCounter® Analysis System to Determine Gene Expression Changes Associated with Chemotherapy Treatment

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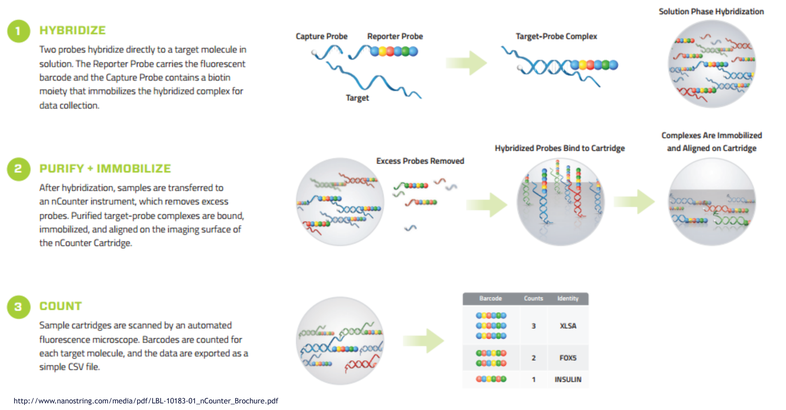
Introduction

- Pancreatic ductal adenocarcinoma (PDA) remains a deadly disease with a 5-year survival of 8% for all stages combined (Siegel RL *et al.*). Currently, it's the third leading cause of cancer-related deaths in the United States and by 2020 it is projected to become the second leading cause (Rahib L, *et al*).
- The poor prognosis in PDA is in part due to the limited therapies that are currently available.
- This highlights the importance of high-throughput technologies for gene expression analyses and drug screens.
- A recently developed NanoString Technologies nCounter® analysis system, an example of high-throughput technology, utilizes a color-coded barcode to directly measure multiple mRNA transcripts simultaneously.
- Our ability to study RNA expression has classically been limited by low detection rate (microarrays) or high cost (RNA-Seq).
- We utilized the nCounter® analysis system to measure changes in cancer-associated gene expression in PDA cell lines caused by different drug treatment.
- We then validated these results using traditional assays to evaluate the sensitivity of the nCounter® analysis system.

Nanostring nCounter® Analysis System



http://www.nanostring.com/media/pdf/LBL-10183-01_nCounter_Brochure.pdf

Methods

- MiaPaca2 and PANC-1 PDA cell lines were used.
- Cells were treated with vehicle, 5-fluorouracil, gemcitabine, or oxaliplatin for 12 hours.
- RNA extraction per manufacturer specifications.
- Nanostring nCounter® PanCancer Pathways, a 770-gene panel was used to simultaneously quantify gene expression changes with each treatment
- Genes that were found to be increased, decreased, or remain unchanged with treatment were identified.
- Validation of identified genes was performed using qRT-PCR and immunoblotting.

Results

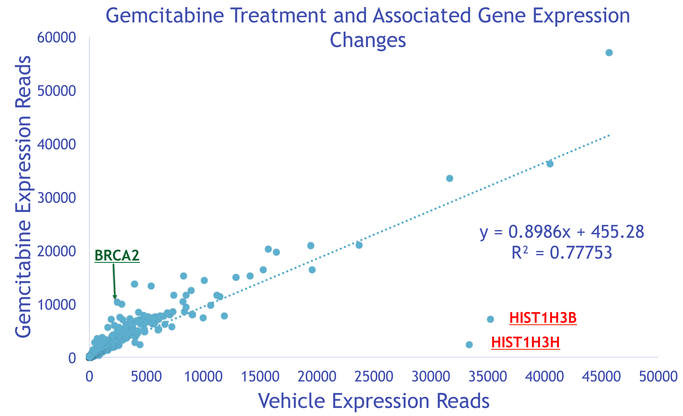


Figure 1: Treatment with gemcitabine resulted in changes of gene expression when compared to vehicle in PANC-1. Experiment performed in triplicate.

PANC-1 Candidate Genes With Gemcitabine Treatment

Gene	G/V Fold change	p value
HIST1H3H	-13.89	≤0.00005
HIST1H3B	-4.95	≤0.00005
PIM1	1.1	0.11
BRCA2	2.84	0.04

Table 1: Candidate genes identified for validation using qRT-PCR.

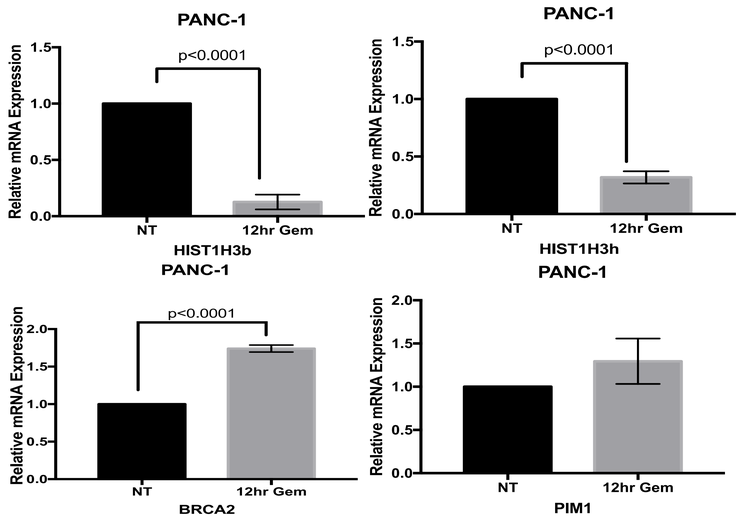


Figure 2: Validation was performed using traditiona qRT-PCR. Genes that were identified to decrease (HIST1H3b and HIST1H3h) reproducibly decreased with 12 hours of gemcitabine treatment. Similarly, BRCA2 was increased after 12 hours of treatment. PIM1 (control) remained unchanged with treatment. Relative mRNA expression was compared to 18s.

Results, cont.

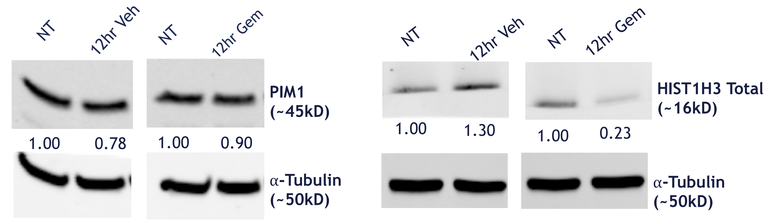


Figure 3: Validation of target genes using immunoblotting. PIM1 protein expression remains unchanged. HIST1H3 total protein expression showed a 77% decreased with 12 hours of gemcitabine treatment when compared to the vehicle.

Conclusion

- We demonstrate that high-throughput technology, such as the NanoString nCounter® analysis system offers several advantages over traditional techniques:
  - Sensitivity
  - Specificity
  - Reproducibility
- This technology can also accurately and reliably detect changes in mRNA expression with drug treatment in PDA cell lines compared to traditional methods.
- These results can be utilized to further study the biological and clinical relevance of the genes that were identified.
- This assay lends itself for high-throughput drug screens in order to identify both new therapeutic targets and treatment options for patients with pancreatic cancer.

Future Directions

- We have curated 300 genes by performing an extensive literature search. These genes were identified to be important in cancer and common human diseases.
- We intend to utilize this technology in a drug screen using 350 FDA-approved drugs in an attempt to evaluate whether alternative therapies for PDA could be identified.

References & Acknowledgements

- Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2017. CA: a cancer journal for clinicians. 2017;67(1):7-30.
- Rahib L, Smith BD, Aizenberg R, Rosenzweig AB, Fleshman JM, Matrisian LM. Projecting cancer incidence and deaths to 2030: the unexpected burden of thyroid, liver, and pancreas cancers in the United States. Cancer research. 2014;74(11):2913-21.

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