Differential Gene Expression Analysis in Lean and Obese Mice with Triple Negative Breast Cancer

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Introduction

- Triple-negative breast cancer (TNBC) is one of the four subtypes of breast cancer, characterized by the absence of estrogen receptors (ER), progesterone receptors (PR), and human epidermal growth factor receptor 2 (HER2) expression.
- Compared to the other invasive breast cancers, TNBC generally grows faster and has fewer options for treatments.
- Patients with higher BMI's have been shown to be at a higher risk of TNBC as well as having higher tumor grades and larger tumor sizes.
- The goal of this study is to use a mice model to study differential gene expression between TNBC tumors in lean and obese mice and to determine the molecular signatures of lean and obese TNBC.
- In the future, we will identify if the similar molecular signatures can be observed in TNBC patients to determine actionable biomarkers.

Methods

- Six FVB female mice were included in this study with three mice put on a western diet for four months to induce obesity, while another three mice were put on a control diet.
- Following four months, the mice were injected with mouse TNBC 0321 cells into their mammary fat glands and tracked for three weeks.
- RNA was extracted from the tumor sample and used to construct a sequencing library for the Illumina platform.
- The raw reads were quality controlled and trimmed using bowtie 2 and aligned to mice genome mm10 using STAR, generating non-normalized read counts per gene.
- The non-normalized read counts were then inputted into DESeq2 to identify statistically significant differential gene expression
- Output was visualized in R and significantly differentially expressed genes were entered into gene ontology to identify any potential differentially expressed pathway

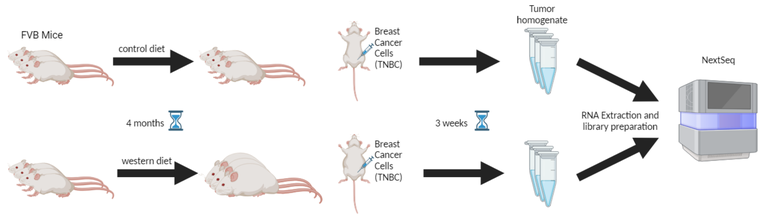

Figure 1. General experimental design schematic

Library Preparation

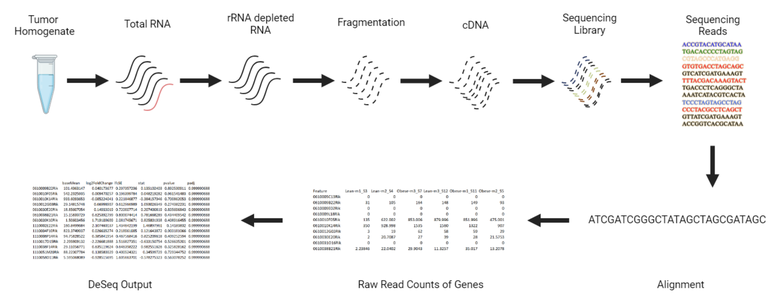

Figure 2. Library preparation and computational analysis schematic

- Library construction from cDNA involves first adenylating the 3' end followed by ligation of anchors with unique sample identifiers.

DESeq Data

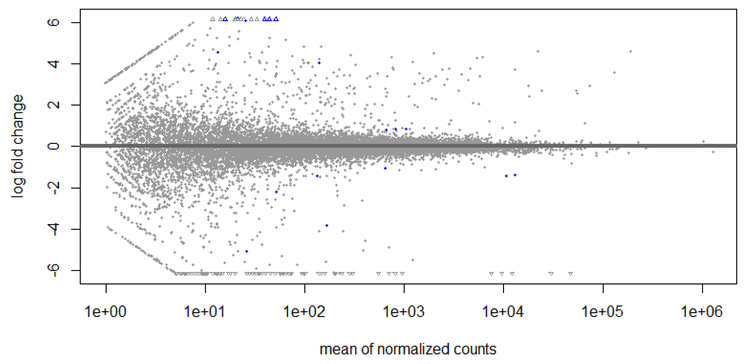

Figure 3. MA plot of fold change of gene expression in obese libraries

- Blue dots of MA plot signify statistically significant fold changes in gene expression in obese mice in comparison to lean mice

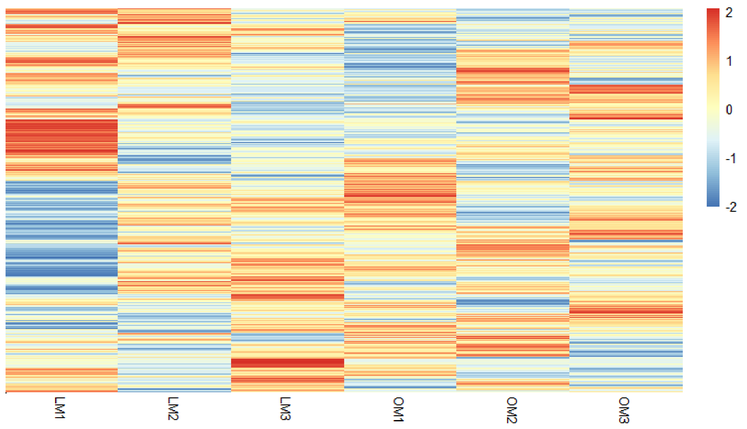

Figure 4. Gene expression heatmap for all mice genes

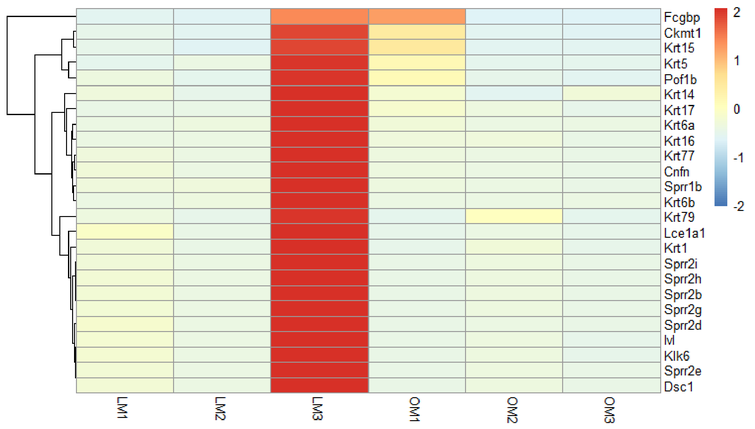

Figure 5. Gene expression heatmap for top 25 expressed genes

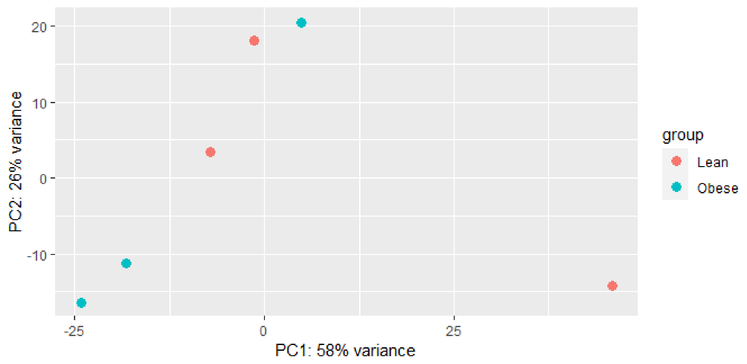

Figure 6. PCA plot of all mice

Significant DE Genes

Sample ID	Total Mapped Reads
Lean Mice 1	6,194,709
Lean Mice 2	20,033,683
Lean Mice 3	33,941,308
Obese Mice 1	33,519,187
Obese Mice 2	16,002,529
Obese Mice 3	38,329,827

Table 1. Total mapped reads of libraries

- From the DeSeq output, there were 7 genes with statistically significant differential expression in obese mice in comparison to lean mice
- Gm7030
  - Predicted to be involved with antigen processing and positive regulation of T cell mediated cytotoxicity
  - Expected human orthologs of this gene are associated with autoimmune disease
- Hspa1a (heat shock protein 1A)
  - Associated with protein chaperone function
  - Involved in lysosomal transport, DNA repair and telomere maintenance
- Hspa1b (heat shock protein 1B)
  - Associated with protein chaperone function
  - Human ortholog associated with obesity
- Shisa4
  - Poorly described function
  - Homozygous knockout mice are phenotypically normal
- Slc2a4 (solute carrier family 2, member 4)
  - Associated with glucose transmembrane transporter activity
  - Involved in brown fat cell differentiation and cellular response to tumor necrosis factor
  - Human ortholog implicated in type 2 diabetes mellitus
- Slc38a3
  - Associated with amino acid transport and intracellular amino acid homeostasis
  - Homozygous knockout leads to premature death and altered amino acid levels as well as decreased ammonia excretion
- Vsig8 (V-set and immunoglobin domain containing 8)
  - Poorly described function
  - Predicted to localize in the membrane of the kidney and ovary among other organs

Discussion

- The obese mice had larger tumors than their lean counterparts, reflecting what was is already reported in literature.
- While multiple gene candidates have been identified, further experimentation is required to illuminate if these genes aid in the increased risk of TNBC in patients with higher BMI.
- gene expression of the tumor in lean mice had larger variation in expression than the obese mice.
- This may be indicative of higher heterogeneity but needs more investigation for any definitive conclusions.

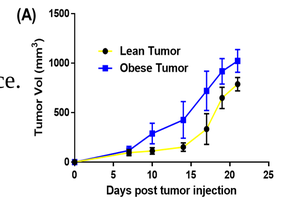

Figure 8. Tumor volume over time

Acknowledgements

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