Methylation Homework

Purpose: What are the differences between metastatic and non-metastatic BRCA across the epigenome?

Exploration of Methylation Patterns: Perform each analysis. For each, write a 3-4 sentence description analyzing the plot and answering the following question: What conclusions can you and can you not draw about differences between metastatic and non-metastatic TCGA BRCA patients? Why?

1. Naive differential methylation between non-metastatic and metastatic patients (Volcano plot)

The volcano plot shows many more hypermethylated CpG sites in older compared to younger BRCA patients. These age-related methylation changes could represent normal aging processes rather than cancer-specific alterations, making it difficult to conclude whether they contribute to disease progression or are simply consequences of biological aging. The naive analysis doesn't account for potential confounders like tumor stage or molecular subtype, which limits our ability to draw causal conclusions about age-related methylation in BRCA.

2. Direct comparison of methylation status to transcriptional activity across non-metastatic vs metastatic patients

We identified 3517 genes in this comparison between older and younger BRCA patients. Interestingly, all 3517 genes show the decreased expression, while none of those same genes show any hypomethylation. This means that there are no genes that are both hypomethylated and decreased in expression. This suggests a complex regulatory mechanism beyond simple promoter methylation. The non-existent overlap between differentially methylated and differentially expressed genes indicates that age-related methylation changes may not directly drive most transcriptional differences.

3. Visualization of CpG sites and protein domains for 3 genes (use UCSC genome browser) for a few genes. Describe at least one academic article (research or review) that either supports or doesn't support your final conclusion for one of the genes. If previously published work doesn't support your analysis, explain why this might be the case.

KCNA3 shows 1 CpG island with 199 repeats on chromosome 1 while DNMT3L has only 1 CpG island as well with a small number of 24 repeats on chromosome 21. The SCTR gene on chromosome 2 displays two CpG islands: one in the promoter region and another at the 3' end, with the gene spanning approximately 75 kb. The 3' end has 66 repeats of CpGs. The analysis found that SCTR shows both hypomethylation and downregulation in older BRCA patients, which presents an inverse relationship to the typical expectation that decreased methylation leads to increased expression, suggesting complex regulatory mechanisms beyond simple promoter methylation control. Research by Kang et al. (2015) reported that SCTR has

hypermethylation and downregulation in breast cancer tumors compared to normal tissue, since promoter methylation suppresses this gene's tumor-suppression. The difference between our age-based findings and their tumor-specific findings can be explained by the fact that age-related methylation changes represent different biological processes instead of cancer-specific alterations, with studies showing that breast tissue undergoes general hypomethylation patterns during normal aging that are distinct from the targeted hypermethylation events that occur during carcinogenesis.