RNA-Seq of mammary epithelial organoids from virgin and lactating glands of 15 Holstein-Friesian cows

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Background

Although the process of lactation is vital to the commercial production of milk, normal mammary biology is incompletely understood. Next-generation sequencing studies have enabled unprecedented visibility into the genes that are expressed to produce milk. However, previous RNA sequencing (RNA-Seq) has been based on whole mammary glands or milk cells and only a few animals. In the current study, we investigated gene expression in mammary epithelial organoids isolated from the glands of 15 dairy cows in which biopsies were taken from the same cow before their first pregnancy and then again at peak lactation.

Methods

Mammary gland biopsies were obtained from 25 Holstein-Freisian cows in New Zealand at approximately 14 months of age. Of the 25 cows, 24 were mated and 19 were sampled at 40–60 days lactation. Sequencing-quality RNA was obtained from mammary epithelial organoids of 15 pairs of virgin and lactation samples. Transcripts were aligned to the UMD3.1/BosTau6 genome release with Ensembl 78 annotations. Differential expression analysis was performed with DESeq2.

Results

RNA-Seq yielded an average of 72 million reads per sample. Over 12,000 genes were expressed in the mammary epithelial organoids of these dairy cows. Consistent with previous studies, we find transcripts corresponding to abundant milk proteins which are highly expressed in the lactating mammary gland. Mitochondrial genes are highly expressed during lactation, reflecting the increased energy needs of the lactating state. The high number of animals in this study enabled us to detect significant fold changes as small as 1.15-fold. Principle component analysis (PCA) shows a clear segregation of virgin and lactation samples, but with greater heterogeneity among the lactation samples. PCA results suggest that there are three lactation subsets of 8, 5, and 2 animals, respectively. Given that the animals had similar milk yield and that bovine mammary gland tissue is highly heterogeneous, we suspect that the variance in the samples is due to tissue heterogeneity. Even with

this heterogeneity, over 8,000 genes were differentially expressed between virgin and lactation. A deeper understanding of these vast regulatory changes requires both knowledge of how these changes in expression arise, and the biochemical impact of the resulting quantitative differences. To this end, we are generating ATAC-seq data, which exposes active chromatin regions, and performing pathway enrichment analysis to display the functional consequences of gene expression changes.

Conclusions:
By integrating genomic and transcriptomic data in the context of biological networks we hope to describe the regulatory logic and the functional consequences that lead to the unique and specialized biology of the lactating mammary gland. This information is needed to improve the efficient production of milk for better sustainability.