UC San Diego
Independent Study Projects

Title

Identification of gestational age-dependent changes in ExRNA expression across normal human pregnancy

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Independent Study Project – Written Description

Title: Identification of gestational age-dependent changes in ExRNA expression across normal human pregnancy

Introduction:
-Extracellular RNAs (exRNAs) are RNA molecules located outside of the cellular environment, and have been found in all studied biofluids
-ExRNAs have been the focus of research as indicators of normal biological processes and disease biomarkers due to their role in intercellular signaling
-This study aims to better understand the role and variability of exRNAs throughout pregnancy

Objectives:
-To develop an atlas of the serum profile of miRNA in normal human pregnancy, and compare variability across gestation, day-to-day variability, and diurnal variability
-To explore whether the ability to detect gestational-age specific changes is dependent on methodology

Hypotheses:
-We expect to observe systematic changes in the relative abundance of subtypes of ExRNAs; some of them should change according to GA
-We expect to see similar results between the miRNeasy and Norgen protocols, as they both utilize total RNA isolation (as opposed to exoRNeasy, which utilizes exosomal RNA isolation)

Methods:
-Maternal serum samples and clinical data were selected and compiled from 43 pregnant women from 3 cohorts at UCSD
-<u>Exosomal</u> RNA isolation performed using exoRNeasy Serum/Plasma Kit (Qiagen)
-<u>Total</u> RNA isolation performed using miRNeasy Micro Kit (Qiagen) or Plasma/Serum Circulating and Exosomal RNA Kit - Slurry Format (Norgen Biotek)
    --Also had miRNeasy Drydown set - 3X concentration of RNA used
-Quality of isolated RNA assessed using the Agilent 2100 Bioanalyzer system (RNA 6000 Pico Kit), which utilizes capillary electrophoresis
-Small RNA libraries were prepared from the isolated ExRNAs using the NEBNext Small RNA Library Kit (New England Biolabs)
    --Purified by Zymo DNA Clean and Concentrator-5 Kit
    --Quality assessed using BioA
-Libraries were then size selected using the Pippin HT/Prep to remove unwanted adapter dimers and other products
-High-throughput, short-read sequencing using an Illumina HiSeq 4000
    --For Norgen samples - MiSeq nanorun was used prior to HiSeq 4000
-Sequencing data was trimmed and mapped using the ExceRpt pipeline

-Resultant data were filtered and normalized, and co-expressed miRNA clusters were identified using affinity propagation

Discussion:
-Norgen protocol gives us the highest percentage of miRNAs compared to the other two techniques (miRNeasy and exoRNeasy)
-Norgen protocol gives us the most uniform complexity out of all of our samples
-We observed a decreased % miRNA biotype across gestation for Norgen and exoRNeasy protocols
-Samples primarily cluster by exRNA isolation kit; miR and miRDry cluster together
    --Norgen is more efficient at RNA isolation and may be considered a less biased method

Conclusion:
-Gestational age-specific miRNA expression patterns in serum can be used for development of a pregnancy "clock" that can aid in dating of pregnancies, where standard clinical gestational age dating criteria are unavailable
-For many miRNAs, including placenta-specific miRNAs, the expression patterns differed markedly between the two datasets, perhaps due to selective secretion of placental miRNAs from different placental cell types
-Variation of results between different RNA isolation methods emphasizes the importance of standardization and proper reporting in this field