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Alpert Medical School

Blebs of Hope: Searching for markers of preeclampsia in placental exosomes

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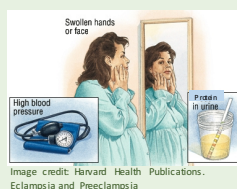


Project goals: To carry out molecular profiling of severe preeclampsia to determine if insights provide clues to treatment, prediction and prevention.

What is preeclampsia?

- Life-threatening increase in maternal blood pressure after 20 weeks gestation
- Leading cause of maternal and fetal mortality worldwideⁱ
- 2-8% of US deliveries affectedⁱⁱ
- Long-term health consequences for mother and child (stroke, heart disease, diabetes)^{iii, iv}

Clinical Symptoms of PEC:
-systolic BP >140mmHg, diastolic BP >90mmHg
-proteinuria >0.3g/24hr
Severe PEC requires at least one of the following:
-systolic BP >160mmHg, diastolic BP >110mmHg
-thrombocytopenia
-impaired liverfunction
-progressive renal insufficiency
-pulmonary edema
-new-onset cerebral/vision disturbances



What are exosomes?

- Tiny extracellular vesicles (30-150 nm), distinct from apoptotic bodies and microvesicles
- Vehicles for RNA, miRNA, cell receptors
- Secreted by all tissue types
- Distinguished by surface markers (CD63)^v

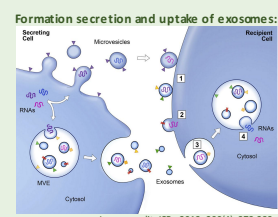


Image credit: JCB. 2013;200(4): 373-383

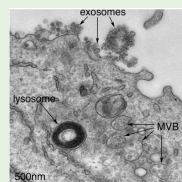


Image credit: BMC Biology. 2016; 14(46).

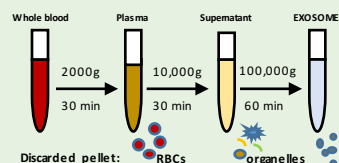
Study design

Enrollment:

- 20 patients with early onset (<34wk) severe pre-eclampsia
- 20 gestational-matched control patients

Sample preparation:

- Differential centrifugation of 8ml whole blood



- Confirm exosome isolation with Western Blot (CD63) and Nanosight Bioanalyzer
- RNA extraction, library construction and high-throughput sequencing

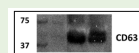


Figure 1a. Western blot for exosome marker CD63

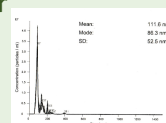


Figure 1b. Nanosight size analysis for representative exosome sample

Preliminary results

Exosome sample quality assessment:

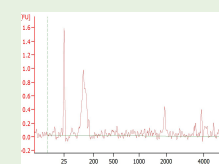


Figure 2a. Electropherogram. Exosome RNA from Agilent Bioanalyzer. Horizontal axis is size in bp.

Sample Name	Clon Passes	Clon bases	Q95(%)
Cord_Blood_F	51,834,362	5,183,436,200	98.94%
Cord_Blood_R	54,920,030	5,492,003,000	98.95%
ExoPE_1214	54,558,016	5,455,801,600	98.75%
ExoPE_1299	53,108,528	5,310,852,800	98.92%
ExoPE_1317	60,002,054	6,000,205,400	98.90%
ExoPE_1317_R	55,782,700	5,578,270,000	98.87%
ExoPE_1812	58,179,582	5,817,958,200	98.49%

Figure 2b. Quality data from initial submission to BGI bioinformatics (7 representative samples)

Exosome RNA analysis:

Description	Sample Handling	Overall read mapping rate (%)
Cord blood control sample	Frozen	68.40
Cord blood control sample	Refrigerated o/n	70.70
Severe PEC 29 ³ wk	Frozen	60.10
33wk Control PROM	Frozen	69.50
Severe PEC 33 ⁵ wk	Frozen	70.00
Severe PEC 33 ⁵ wk	Refrigerated o/n	70.40
Control PROM 33wk	Frozen	60.80

Figure 3. Read mapping of representative samples to human genome

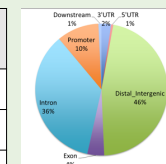
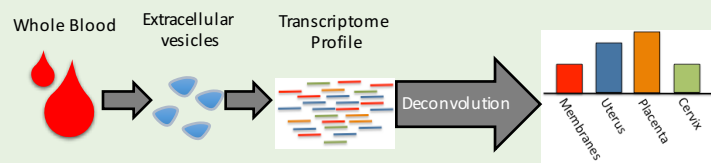


Figure 4. Annotation of genomic reads from exosome sequence data in a representative pregnant patient

Deconvolution (Negative Matrix Factorization)

- An unsupervised machine learning technique that will allow us to determine relative contribution of different tissues to gene expression
- High specificity about tissue type



Current analysis of case and control samples show expression differences in >400 genes

References:

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