Isolating Molecular Probes for Obesity During Pregnancy

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Introduction

Maternal Health
-Preeclampsia
-Miscarriage
-Gestational diabetes

Fetal Health
-Macrosomia
-Childhood obesity
-Neural tube defects

Figure 1: Obesity during pregnancy increases the incidence of adverse outcomes in the mother, child and future generations[1-3].

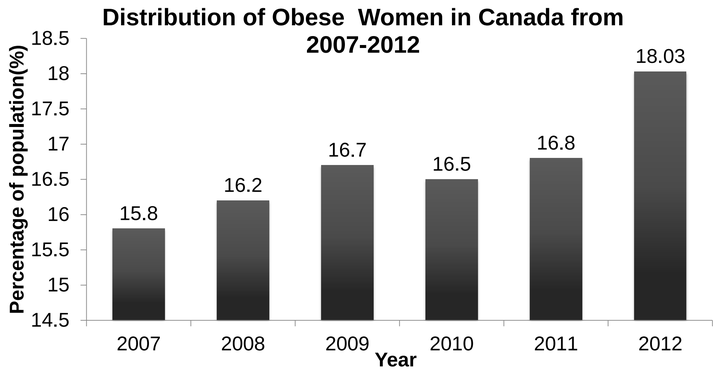

Figure 2: Percentage of obese Canadian women of reproductive age (18 years and older)[4].

Objective: Develop a reliable, non-invasive method to identify diagnostic markers for obesity related complications in women.

A Novel Approach

Platform Technology: Nucleic Acid Enzymes

NAEs are selective, inexpensive and easily modifiable biosensors.

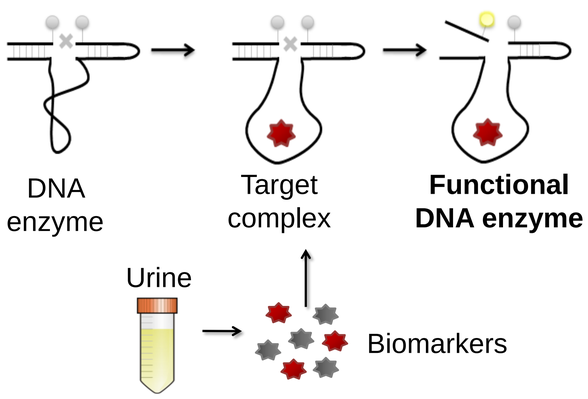

Figure 3: Schematic of fluorogenic DNA enzymes that becomes catalytically active by an unknown target molecule present in urine. The X represents an RNA site which is cleaved by the DNA enzyme upon activation by the target molecule.

Proof of Concept: Characterizing isolation of NAEs in biological samples such as urine. Using NAEs to distinguish between diseased (obese, pregnant) and healthy (pregnant) state.

Methodology

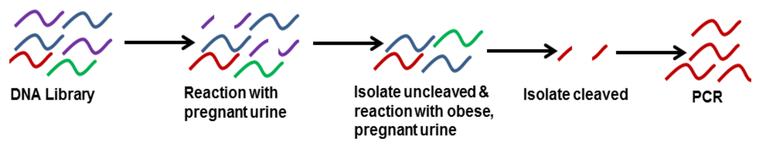

Figure 4: Schematic of *in vitro* selection to isolate DNA enzymes. The steps are repeated until the pool is dominated with catalytically active sequences which is measured with cleavage percentage[5].

Results

System Optimization

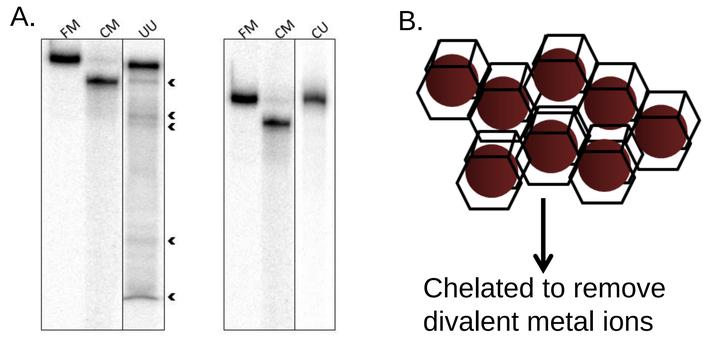

Figure 5: A)Inherent nuclease activity in urine demonstrates cleavage throughout the DNA enzyme. The chelated sample has reduced cleavage. FM; full marker, CM;cleaved marker UU;untreated urine, CU; chelated urine B) Metal ions in urine are sequestered due to chelation.

In vitro Selection

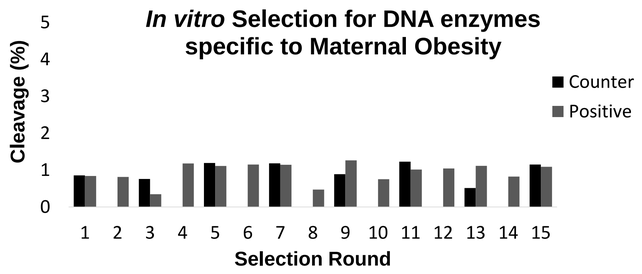

Figure 6: Progress of *in vitro* selection using samples from pregnant obese mice as positive selection and urine from pregnant mice as counter selection. The background activity is 1% for both positive and counter selection.

Structural Analysis and Binding Interaction

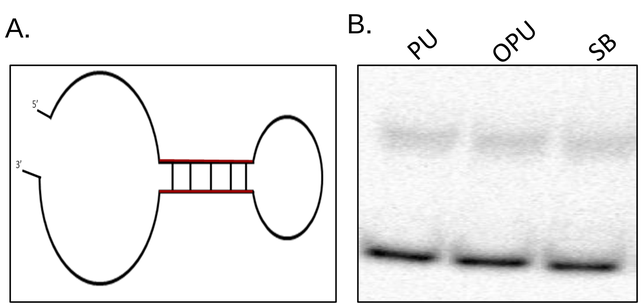

Figure 7: A)The proposed secondary structure of a top ranking sequence. The outlined region represents the conserved duplex[6]. B) Binding interaction is not specific to urine but also seen in the buffer. OPU;obese,pregnant urine, PU; pregnant urine, SB; selection buffer.

Implications and Conclusion

The *in vitro* selection demonstrated low catalytic activity following 15 rounds of selection. Deep sequencing data showed the top ranking sequences had a conserved duplex region. However, this duplex represents a persistent structure which has not been denatured as opposed to a DNA binding sequence for a target molecule in the urine. Therefore, the system requires further optimization to proceed with selection.

Commercialization Potential

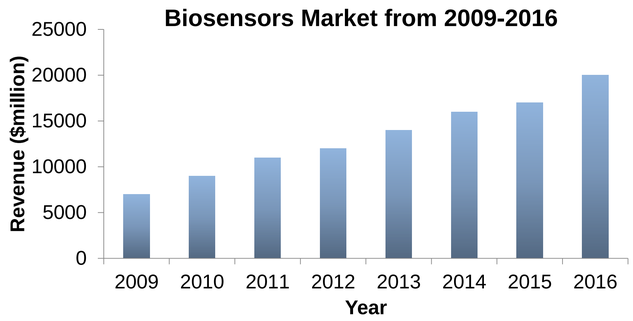

Figure 8: Global revenue of the biosensors market forecasted to 2016[7].

Future Directions

Further Experimentation

Upon identification of a DNA enzyme that is specific to maternal obesity , the kinetics and selectivity are future areas of exploration. The properties and identification of the target molecule which activates the enzyme can be examined as a potential biomarker for maternal obesity.

Utility of Nucleic Acid Enzymes

The validation of this technique will provide support for utilizing DNA enzymes to create diagnostics for adverse pregnancy related outcomes. The potential application can extend beyond pregnancy to multiple diseases with unidentified biomarkers.

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