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Understanding m6A's role in the myometrium during gestation

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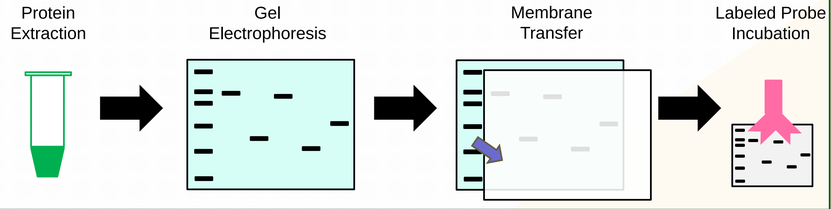
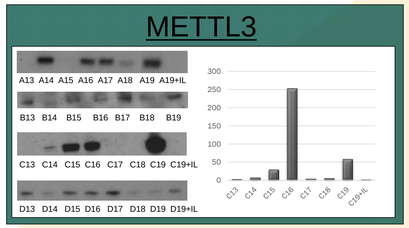
INTRODUCTION

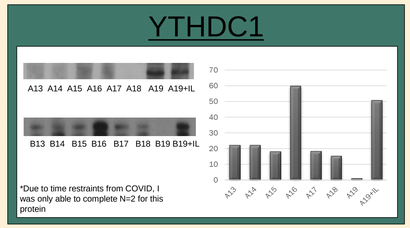
In the United States, 10% of all births are delivered preterm leading to circumstances that can harm both the mother and child. In order to understand the underlying cause, we must investigate the mechanisms involved in the stages of delivery, particularly quiescence. One such mechanism is endoplasmic reticulum stress response (ERSR) conditioning throughout pregnancy. In response to cellular stress, ERSR prompts activation of unfolded protein response (UPR) signaling, which enhances the ability of a cell to cope with increased burden. Within myometrial cells, this process helps prepare the uterus for the stresses of labor and delivery. Preterm labor may occur as a result of inadequate preparation of the uterus because of abnormalities in ERSR pathway. Our lab has shown there is robust activation of the ERSR in association with uterine quiescence in a mouse model and in human myometrium. We hypothesize that one mechanism underlying ERSR in uterine myocytes is alternative splicing and translational regulation. In many circumstances, protein synthesis is regulated by eIF4F complex binding to mRNA via 7-methylguanylate cap-dependent mechanism. This mechanism prevents translation of certain proteins during times of stress, including pregnancy, heat shock, and oxygen deprivation. This allows for moderate buildup of unfolded/misfolded proteins contributing to the ERSR and preconditioning. Many of these silenced proteins are still needed for normal cell function, leading to the necessity of alternate pathways to allow translation. A major post-transcriptional modification that allows for alternative splicing is the addition of 6 methyl-adenosine (m^6A). Coots et. al has established that m^6A facilitates mRNA translation that is independent of eIF4F binding. M^6A methylation leads to variation in splicing and protein function. Previous analysis has demonstrated that alternative splicing of estrogen receptor α has tocolytic effects in myometrial cells. Our lab has determined that an isoform of estrogen receptor α, ERΔ7, specifically limits myometrial estrogen action by suppressing expression of the contractile associated protein (CAP) connexin 43 early in pregnancy, allowing for the maintenance of quiescence. We believe that splicing directed by m6A methylation plays a significant role in regulating the physiology of the pregnant myometrium through alternative splicing of pre-mRNAs, facilitating rapid expansion and plasticity of the genome, producing an altered active proteome allowing for the transition from a quiescent to laboring myometrial compartment. The regulation of m6A modification is dynamic and reversible and is established by m6A methyltransferases ("writers"), such as methyltransferase-like protein 3 (METTL3). It is removed by m6A demethylases ("erasers"), such as α-ketoglutarate-dependent dioxygenase alkB homolog 5 (ALKBH5). The effects of m6A modification on RNA metabolism depend on the recognition by different m6A -binding proteins ("readers"), including the YT521-B homology (YTH) domain family and the heterogeneous nuclear ribonucleoproteins (HNRNPs). By investigating the expression of these writers, readers, and erasers in the pregnant myometrium, we can gain a better understanding of m^6A's function and how it changes throughout gestation.

METHODS: WESTERN BLOT

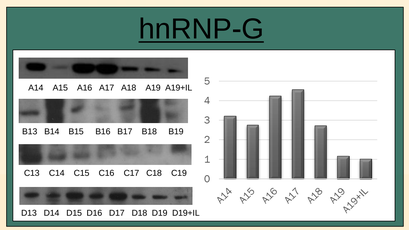


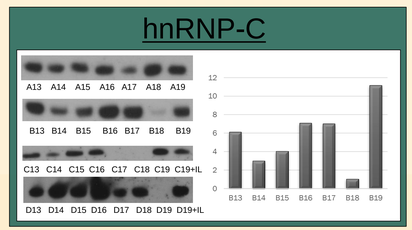
RESULTS

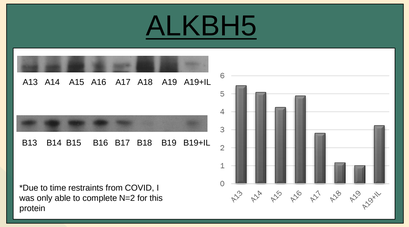




*Due to time restraints from COVID, I was only able to complete N=2 for this protein







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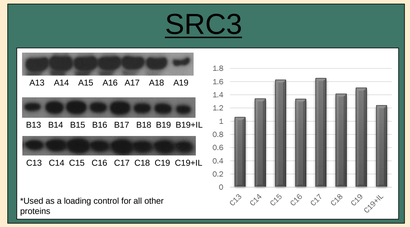
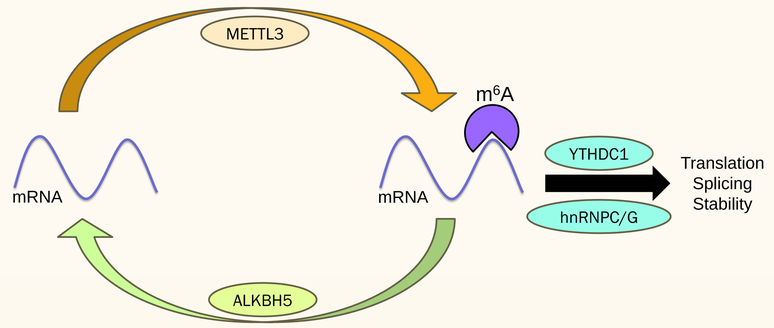


*Used as a loading control for all other proteins

DISCUSSION

For this project, timed pregnant mouse uteri that was previously frozen was used for protein extraction. A bicinchoninic acid assay (BCA) was performed to identify the concentration of protein in each sample to identify loading amount for western blot analysis. Samples were resolved by gel electrophoresis and blotted to membranes. The membranes were blocked and probed with primary antibodies. Immunoreactivity was detected using secondary antibodies and the bands were visualized using an ECL detection system. SRC-3 was used as a loading control.

Western blot analysis of pregnant mouse uteri from mid gestation to term in labor revealed distinct and corollary gestational modifications in the reader and writers METTL3, hnRNPC and YTHDC1, which spiked at mid gestation and again at term correlating with m6A labeling and previously observed increases in myometrial splicing events. In contrast as expected the eraser ALKBH5 levels declined towards term. Future analysis will examine if these gestational changes are hormonally regulated. We believe that alternative splicing and changes in the epitranscriptome through m6A regulation are an underlying mechanism involved in normal term labor and understanding these phenomena may lead to development of an effective therapeutic for preterm labor



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