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FUNCTIONAL ANALYSIS OF A LONG NON-CODING RNA ASSOCIATED WITH LUPUS PROTECTION. JC Olivas, SA Boackle (MD, MS), Department of Medicine, University of Colorado, Denver, CO.

**Background:** A single-nucleotide polymorphism (SNP) in the complement receptor 2 (CR2) gene was previously identified in a large lupus association study to be associated with decreased risk of lupus and increased expression of complement receptor 1 (CR1), specifically on B cells. The SNP lies within a putative B cell enhancer and is associated with increased levels of a nearby long non-coding RNA (lncRNA). Previous work has also shown a positive correlation between CR1 expression and mTOR pathway activation in B cells, as well as increased mTOR pathway activation in B cells of individuals with the SNP compared to those without.

**Purpose/Hypothesis:** To further define the relationship between the CR2 lncRNA, CR1/CR2, and mTOR, and possibly identify new mechanisms by which the lncRNA may confer protection against lupus and be utilized for future treatment studies. We predict that the CR2 lncRNA confers lupus protection by directly modulating the transcription of CR1 in B cells and promoting antigen-specific B cell tolerance via the mTOR pathway.

**Methods:** Knockdown of mTOR was performed on isolated primary B cells using previously developed siRNAs. RNA transcripts were harvested after a 24-hour incubation period, and expression levels of mTOR, CR1, and  $\beta$ -actin, an endogenous gene control, were assessed using qPCR. Relative expressions of each sample were evaluated using the comparative CT method. We then ran a standard curve qPCR protocol using known amounts of RNA template obtained from primary B cells in order to assess our ability to accurately measure expression levels of CR2 and other lncRNAs of interest in anticipation for knocking down the CR2 lncRNA.

**Results:** Results showed successful knockdown of mTOR for one of the siRNAs at 10nM and 1nM concentrations, with knockdown efficiencies around 50%, which is in line with those seen in previous literature. The other two siRNAs showed no change in mTOR transcription. For CR1, no change was seen in expression levels for all knockdown samples. Additionally, while the CR2 standard curve showed satisfactory results, the assays for the CR2 lncRNA and other lncRNAs displayed high variability and poor efficiencies.

**Conclusions:** The mTOR knockdown results suggest that CR1 expression may not be dependent on mTOR activity, but more subjects are needed to confirm this hypothesis. The poor efficiencies seen in the standard curve qPCR protocol for the various lncRNAs of interest may be due to a low copy number in the samples, resulting in stochastic error when measuring fold-changes. Another possibility is ineffective assay primers, either by design or due to degradation from numerous freeze-thaw cycles. Further work is needed to dissect this issue. Once the expression levels of the lncRNAs can accurately be measured, we aim to test our CR2 lncRNA knockdown protocol on subjects with and without the SNP using 3 different siRNAs designed with Integrated DNA Technologies' custom siRNA generator.