QUANTIFICATION AND ASSAY DEVELOPMENT OF PERIPHERAL CIRCULATING MICRO RNA USING A STANDARD CURVE APPROACH

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MicroRNAs (miRNAs) are small non-coding RNA molecules about 20-25 nucleotides in length. MicroRNAs are responsible for regulation of gene expression at a post-transcriptional level and is a rapidly growing area of investigation.

Study of miRNAs provides a challenge for researchers as circulating serum samples do not contain miRNAs at a level sufficient for detection by current technologies and thus must be purified and amplified for study. Circulating miRNA levels has been compared in relative concentration fold differences to known levels of control miRNAs, which has it's own set of limitations for potential clinical application.

During pilot study of cardiac regulating miRNA families, methods needed to be developed to quantify said miRNAs for differential study and further investigation. To accomplish this aim, standard curves with known concentrations of synthetic miRNA targets were generated by usage of TaqMan miRNA assays with miRNA-16 as an endogenous control. A standard curve approach allowed quantification of circulating miRNA levels in samples by plotting Ct values versus concentrations of synthetic miRNA in a standard curve. Fitting of the curve is used to approximate concentrations of endogenous miRNA from Ct values obtained with biological samples.

This method has been applied to study of circulating miRNA – 15b and -133b levels associated Left Ventricular Assist Device (LVAD) unloading in end stage heart failure patients. We found significant increase of those molecules ($p<0.005$) in serum specimens ($v=400\mu l$) of LVAD-non Responsive patients in a course of LVAD unloading comparison of patients non-responsive to LVAD unloading. Use of this method may allow further research into miRNAs as potential predictive and monitoring for LVAD responsiveness.