Title of the Thesis: Analysis of Role of “RNA Interference” and Mechanism by Which Rotavirus Counteract RNAi during Rotavirus Infection

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Abstract: RNA interference (RNAi) is the evolutionary conserved process which provides first level of defence against potentially harmful nucleic acids. This is triggered by double stranded RNA (dsRNA) leading to cleavage of mRNAs containing homologous sequences. Current study is focused on role of RNAi during rotavirus (RV) infection. RV is the single most common etiological pathogen of severe diarrhea in infants causing death of over half a million infants a year. In addition to RNAi study attempts were made to analyze efficacy of AGO2 for protection against RV.

Sixteen differentially regulated miRNAs were identified during RV infection of which hsa-miR-142-5p (upregulated) and hsa-miR-192; hsa-miR-194; hsa-miR-215 (downregulated) were validated by quantitative PCR. Exogenous expression of miR-192 mimic and miR-142-5p inhibitor significantly reduced viral propagation, indicating pro-viral and anti-viral role of miR-142-5p and miR-192 respectively. Functional studies of hsa-miR-142-5p identified its role in TGFβ signaling as TGFβRII and SMAD3 were degraded by this miRNA. Furthermore RV NSP5 was identified as regulator of hsa-miR-142-5p expression. TGFβ is induced during RV infection which may promote apoptosis by activation of non canonical pathways in HT29 cells. But in NSP5 or hsa-miR-142-5p overexpressing HT29 cells or during RV infection, TGFβ mediated non canonical pathways were blocked resulting in inhibition of early apoptosis and epithelial to mesenchymal transition. Overall the study has identified proviral potential of hsa-miR-142-5p for successful viral propagation and therapeutic potential to treat microsatellite stable colon cancer cell.

Six novel miRNAs, generated from RV genome were identified and validated by deep sequencing and miR-Q-RT-PCR respectively. Besides that Argonaute 2 and 1 protein were found to be proteasomally degraded by RV encoded NSP1. Degadation of these proteins finally resulted in blocking of RNAi during RV propagation. Further studies have identified that C-terminal domain of NSP1 was mainly responsible for Argonaute 2 degradation. However, N terminal domain of NSP1 has no role in Argonaute2 degradation. Over expression of Argonaute 2 resulted in blockage of rotavirus propagation.