

**Program Number: 323**

Downregulation of MicroRNA-451 Contributes to the Pathophysiology of Endometriosis in Both Women and Baboons

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**ABSTRACT**

Endometriosis is a gynecological disorder characterized by severe chronic pelvic pain and infertility. Although the causes of endometriosis associated infertility are not well defined it is evident that the presence of the disease results in aberrant gene expression in the eutopic endometrium (EUE) and the development of progesterone resistance. Recent studies also suggest that micro RNAs (miR) play a crucial role in gene expression as a consequence of endometriosis. Our miR microarray data generated using baboon model of endometriosis revealed that induction of endometriosis significantly alters the expression of miRs (miR 451, 181a, 200a, 19b, 424, 21, 29c and 141) in eutopic endometrium. miR-451 was highly down regulated ( $p < 0.000036$ ) at 3 months of disease and qRT-PCR results for miR-451 and its target mRNA's further validated the microarray data in both women and baboons. Objectives: 1) To localize and compare the uterine expression of miR-451 in EUE of control animals and baboons with endometriosis and to correlate these changes in miR-451 expression with the altered expression of the target genes 14.3.3 $\beta$  and Cox-2 at protein level. 2) To validate target specificity for miR-451 by performing in vitro 3'UTR luciferase assay using endometrial epithelial cells (EEC). Frozen sections (n=3) were processed for in situ hybridization (ISH) using double DIG labeled LNA probes. Formalin Fixed Paraffin Embedded (FFPE) sections were processed for immunohistochemistry (IHC) using specific antibodies against for 14.3.3 $\beta$ , Cox-2 and Ki67. EEC cells were cotransfected with wild type (wt) 3'UTR 14.3.3 $\beta$  vector and either with miR-451 over expression vector or empty vector. Mutated (mut) 3'UTR 14.3.3 $\beta$  vector was used to confirm the regulation of 14.3.3 $\beta$  by miR-451. ISH analysis revealed localized expression of miR-451 in the GE and luminal epithelium (LE) of the EUE of control animals during the mid-secretory phase. Induction of endometriosis resulted in significantly decreased signal intensity for miR-451 in GE and LE of EUE of the diseased animals. This was correlated with a marked increase in 14.3.3 $\beta$  and Cox-2 protein in the GE and surrounding stroma in EUE and ectopic lesions from animals with endometriosis. Both ISH and IHC data further validate our previous miR microarray and qRT-PCR data. Results of 3' UTR luciferase assay suggest a significant reduction in luciferase activity of the wt-14.3.3 $\beta$  vector and unaltered luciferase activity of mut-14.3.3 $\beta$  vector in the EEC cells co-transfected with miR-451 expression vector compared to empty vector. These in vitro observations further confirm the regulation of 14.3.3 $\beta$  expression by miR-451 and strongly support our in vivo findings from baboon model of endometriosis. Induction of endometriosis results in decreased expression of miR-451 in GE and LE of EUE of baboon with disease, which was also associated with increased expression of its predicted target proteins 14.3.3 $\beta$  and Cox-2. Observed inverse correlation between expression of miR-451 and 14.3.3 $\beta$  expression at both mRNA and protein level is well supported by the in vitro transfection studies. These two genes are known to enhance cell proliferation, decrease apoptosis and enhance inflammatory responses all of which are hallmarks of endometriosis. We propose that the marked and rapid down regulation of miR451 in both baboons and women contributes to these processes by altering target gene expression. (U54-HD40093).