Micro-RNA and mRNA profiles associated with ectopic germinal center formation in thymus samples of patients with autoimmune myasthenia gravis

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BACKGROUND: Myasthenia gravis (MG) is an autoimmune neuromuscular disorder caused by antibodies directed against proteins present at the post-synaptic surface of neuromuscular junction (NMJ). A characteristic pathology of patients with early onset MG is thymic hyperplasia with ectopic germinal centers (GC). However, mechanisms that trigger and maintain thymic hyperplasia are poorly characterized. Micro-RNAs (miRNA) are small, non-coding RNAs that are increasingly appreciated to be involved in the pathology of several autoimmune diseases. In order to determine the central mechanisms involved in the pathology, thymus samples from MG patients were assessed by histology and grouped based on appearance of GC compared to samples without them. MiRNA and mRNA profiles were evaluated.

METHODS: Thymic specimens collected during the course of the NIH-supported study of thymectomy (MGTX, U01 NS4268) were used for histological analysis to grade the degree of thymic hyperplasia, grade 0, no observed GC and grade 1 to 4 with varying numbers of GCs. MiRNA and mRNA were evaluated using GeneChip® miRNA 4.0 Array and GeneChip® Human Transcriptome Array 2.0, respectively. Partek Genomic Suite 6.6 and Transcriptome Analysis Console 2.0 programs were used to identify candidates that were differentially expressed in grade 0 versus grades 1 to 4. ANOVA p-value <0.05 and FDR<0.05 was determined as significant. Further validation by qRT-PCR was done. Ingenuity Pathway Analysis was used to identify miRNA targets, pathways involved and reciprocal expression pairing of miRNA-mRNA. IHC was performed to check localization of proteins.

CONCLUSIONS:
❖ Grade 0 and grade 1-4 patients have distinct mRNA and miRNA profile demonstrating separation of groups.
❖ Deregulation of 100 annotated mRNA and 34 mature miRNA.
❖ Reciprocal expression pairing of miRNA and mRNA observed.
❖ Maintenance of autoimmunity is supported by regulatory pathways known to be involved in neoplasia.

Fig.1 Germinal center activity in MG thymus

Fig.2 Gradation of thymus samples

Fig.3 Differential mRNA expression in MG thymus

Fig.4 Cluster analysis of small RNA expression profile stratifying samples across the grades

Fig.5 Reciprocal Pairing of miRNA and mRNA

Fig.6 mRNA and miRNA regulating cellular functions in germinal center formation