Differential mRNA expression in ectopic germinal centers of myasthenia gravis thymus

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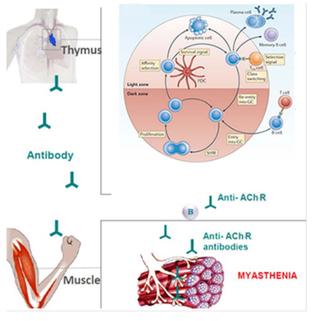
BACKGROUND: Myasthenia gravis (MG) is an autoimmune neuromuscular disorder resulting in weakness of voluntary muscles. It is caused by antibodies directed against proteins present at the post-synaptic surface of neuromuscular junction. A characteristic pathology of patients with early onset MG is thymic hyperplasia with ectopic germinal centers (GC). However, mechanisms that trigger and maintain thymic hyperplasia are poorly characterized.

OBJECTIVE: We assessed the differential mRNA expression profiles in MG thymus samples with and without GC. We evaluated pathways involved in GC maintenance. We studied expression and regulation of GC specific transcript *RGS13.*

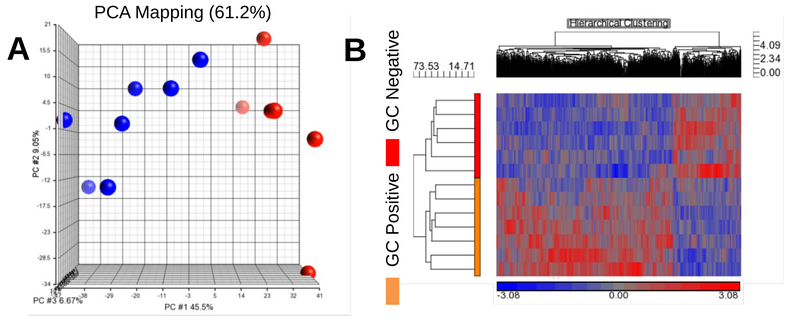
Fig.1 Germinal center activity in MG thymus



Adapted from B. A. Heesters et. al., 2014, Nature Reviews Immunology

METHODS: Thymic specimens collected during the course of the NIH-supported study of thymectomy (MGTX, U01 NS4268) were used for histological analysis and grouped based on presence (GC positive) or absence of GC (GC negative). Transcription profiling was done using GeneChip® Human Transcriptome Array 2.0. Partek Genomic Suite 6.6 and Transcriptome Analysis Console 2.0 programs were used to identify candidates that were differentially expressed. ANOVA p-value <0.05 and FDR<0.05 was determined as significant. Further validation by qRT-PCR was done. IHC was performed to study localization of selected proteins.
Gene ontology (GO) enrichment analysis and Ingenuity Pathway Analysis (IPA) core analysis was used to identify pathways, molecular and cellular functions of the transcripts with respect to GC formation.

Fig.2 Distinct mRNA expression pattern in MG thymus samples with ectopic GC



Differential mRNA expression profiles of thymus samples with and without germinal centers. (A) Principal Component Analysis plot (Blue GC positive, Red GC negative) and (B) Hierarchical clustergram (ANOVA p<0.05, no fold change). GC positive n=7; GC negative n=6.

Fig.3 Differentially expressed genes belong to functional pathways important in GC formation

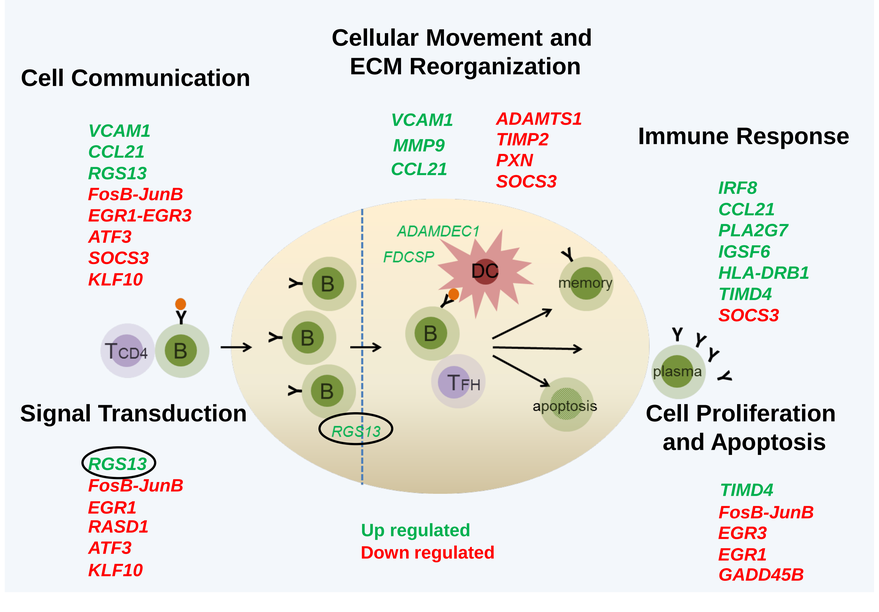


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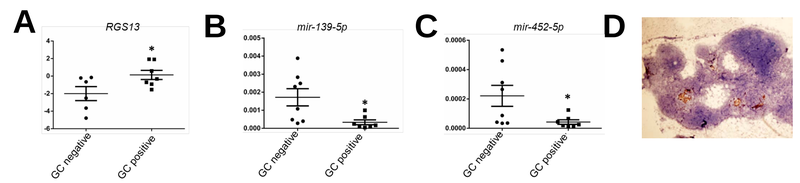
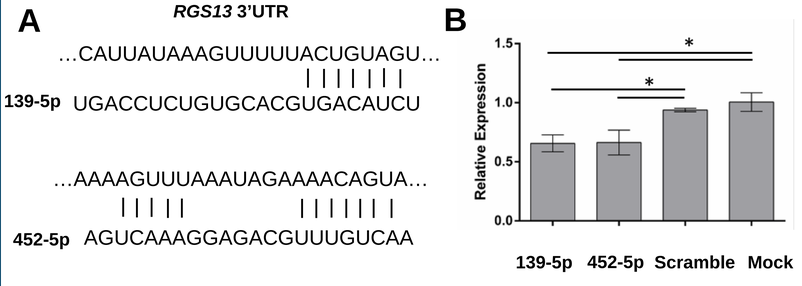
Fig.4 *RGS13* is overexpressed in ectopic GC



(A) Increase in *RGS13* expression as determined by qRT-PCR is associated with decrease in (B) miR 139-5p and (C) miR 452-5p in GC positive samples. (D) Immunohistochemistry of thymus samples using RGS13 antibody confirms its expression in GC.

Fig.5 *RGS13* expression is regulated by miRNAs



(A) 3'UTR of *RGS13* gene has predicted binding sites for miR-139-5p and miR-452-5p. (B) qRT-PCR analysis *RGS13* expression in Raji cells post transfection with miRNA mimics. p<0.05 is considered as significant.

CONCLUSIONS:

- Thymus samples with GC demonstrate unique gene expression pattern
- The differentially expressed transcripts belong to pathways involved in immune response, cell signaling, cellular movement, cell proliferation and apoptosis. These functions are important for GC formation
- GC B-cell specific RGS13 expression is higher in GC positive group and is reciprocally regulated by miRNA 139-5p and 452-5p