Pathway Analysis for Discovery of Rejection Mechanism and Biomarkers in Intestinal Transplantation

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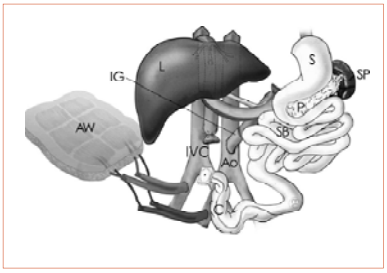
Intestinal transplantation is valuable for treatment of patients with massive abdominal catastrophes. To date about 2000 intestinal transplants have been performed all over the world (of them 300 in the Miami Transplant Institute). Patient survival is 80%, 60%, 48% at 1, 3, and 5 years post-transplant [1]. Rejection is the most common reason of late grafts failure and death. If diagnosed early and timely and aggressively treated, severe rejection and graft failure can be prevented. Currently, there are no known early stage biomarkers of intestinal rejection.

In this pilot study, we used the minimally invasive procedure (gene expression analysis of peripheral whole blood) followed by the pathway enrichment analysis with MetaCore 6.0 (GeneGo, Inc.) and Pathway Studio 7.0 (Ariadne Genomics, Inc) for determination of the putative candidate biomarkers of the early stage of intestinal rejection and for understanding of the mechanism of rejection.

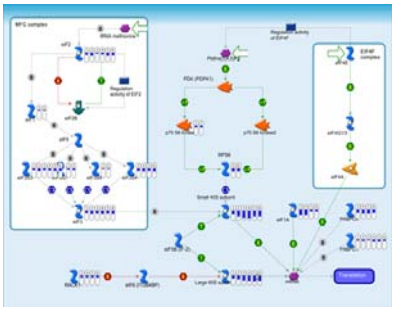
We analyzed peripheral blood from 3 intestinal transplant patients that received an intestinal graft due to mesenteric vein thrombosis (n=2) or Crohn's disease (n=1). Peripheral blood (whole blood) was drawn at different time points post-transplantation. The patient samples were compared to a pool of healthy volunteers.

Results were compared with pathology of contemporaneous allograft biopsies. A total of 11 samples was collected: **Patient A**: sample 1 (day of transplant), sample 2 (minimal rejection), sample 3 (severe rejection); **Patient B**: sample 4 (no rejection), sample 5 (minimal rejection), sample 6 (mild rejection), sample 7 (mild rejection), sample 8 (mild rejection); **Patient C**: sample 9 (mild rejection), sample 10 (mild rejection), sample 11 (severe rejection). Maintenance immunosuppression for all patients was tacrolimus and steroids. Importantly, none of the patients was on rapamycin.

RNA samples extracted from peripheral blood samples were analyzed with Illumina Human-6 Expression BeadChip microarrays. The resulting microarray data was analyzed using Illumina Beadstudio software and then by MetaCore and Pathway Studio.

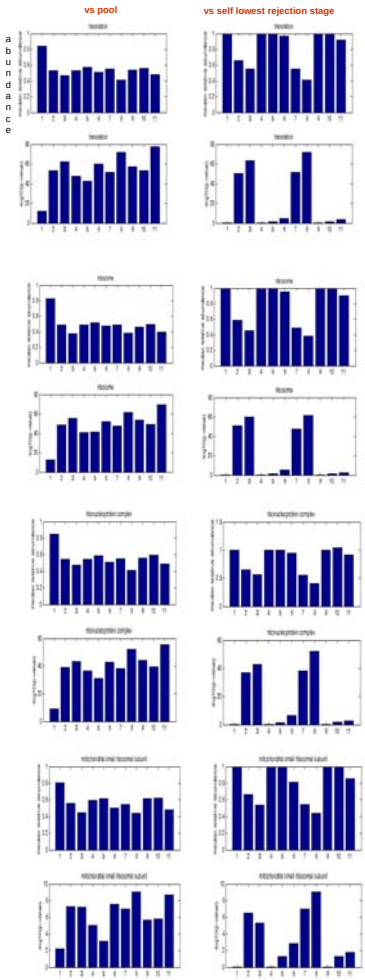


Regulation of Translation Initiation Pathway

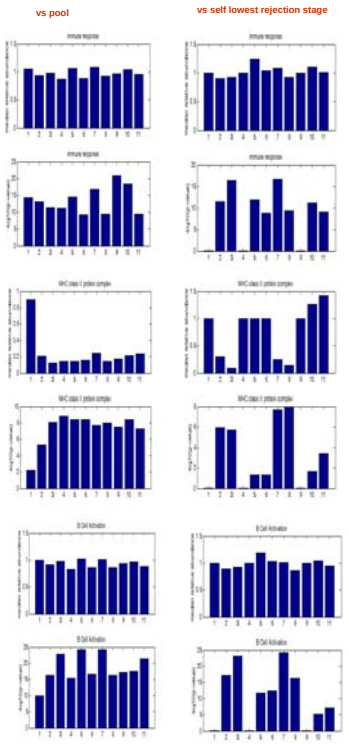


Blue thermometers indicate down-regulated genes in 6 conditions

Gene Set Enrichment Analysis [2] with Pathway Studio

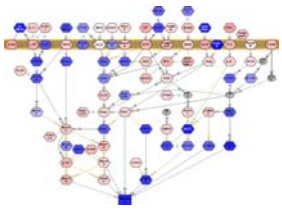


Gene Set Enrichment Analysis (Cont)

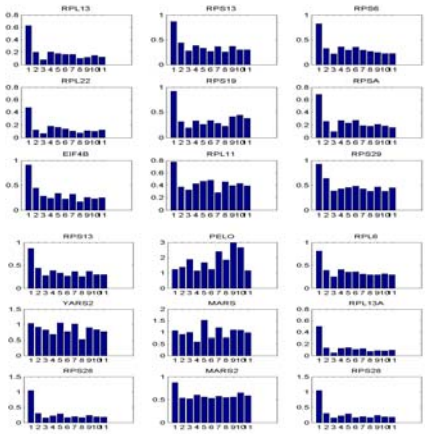


1-A transpl. day, 2-A min, 3-A severe, 4-B no rejection, 5-B min, 6-B mild1, 7-B mild2, 8-B mild3, 9-C mild1, 10-C mild2, 11- C severe

Translation Control Pathway (Patient C severe rejection)

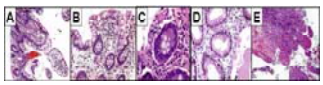


Expression of Genes Involved in Translation (vs pool)



Our hypothesis, derived from this project, is that down-regulation of translation in peripheral blood mononuclear cells occurs early during the course of rejection, precedes any clinically detectable signs of rejection and correlates with the severity of the rejection episode as determined by concurrent graft biopsies. We hypothesize that rejection episode is acting through increased inflammation feeding back to the neutrophils to shut down cell signaling and further inflammatory processes in these cells. It will be verified in the ongoing study with increased number of patients and with isolation of peripheral blood cell sub-populations.

Cellular rejection in small bowel allografts

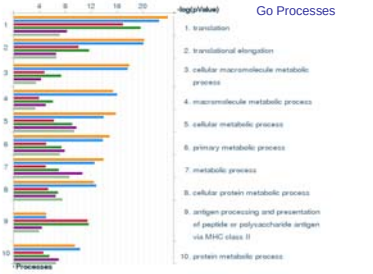


A. Small bowel allograft, no evidence of acute rejection; B. Indeterminate for acute rejection-Minimal rejection; C. Acute cellular rejection –Mild; D. Acute cellular rejection – Moderate; E. acute cellular rejection - Severe.

Enrichment Analysis with MetaCore

GeneGo Processes

Go Processes



Conditions versus normal pool

1-C mild, 2-C severe, 3-B mild, 4-B min, 5-A min, 6-A severe, 7-A transplant day

Importantly, no enrichment for "A transplant day"

References

1. Tzakis A et al.100 Multivisceral Transplants at a Single Center. *Ann Surg* 2005;242: 480–493.
2. Subramanian A et al. Gene Set Enrichment Analysis: A Knowledge-based Approach for Interpreting Genome-wide Expression Profiles. *PNAS* 2005; 102: 15545-15550.

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