Aptamer Proteomic and MicroRNA Profiling for Biomarker Discovery in Cardiac Allograft Vasculopathy

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INTRODUCTION

- Cardiac allograft vasculopathy (CAV) is common after heart transplantation and limits successful long-term outcomes.
- Biomarkers may aid noninvasive diagnosis, prognostication, and surveillance of CAV.
- The Slow Off-rate Modified Aptamer (SOMAscan) proteomic and NanoString nCounter microRNA (miRNA) assays provide rapid high precision evaluation of many analytes, capturing potentially important biological functions in CAV pathogenesis.
- We aimed to identify potential biomarkers for CAV utilizing these novel biomarker discovery technologies.

METHODS

- A nested case-control study design was used (12 patients in each group).
- CAV (Cases) was defined on angiography according to ISHLT CAV_3 and controls were defined as ISHLT CAV_0.
- Serum samples were obtained at a mean of 8 ± 43 days from angiography.
- Biomarker assays were performed on serum samples according to standard operating procedures for SOMAscan and Nanostring platforms.
- Comparisons were performed between CAV and Control groups using unpaired t-tests and a p-value <0.05 considered significant. Top candidate markers were further analyzed utilizing pathway software analysis.

RESULTS

- Most patients were male (71%) and median age was 57 years (Table 1).
- Compared to controls, patients with CAV were significantly later post-transplant and had greater allosensitization with higher class II cPRA (Table 1).
- Cases and controls were otherwise well matched in all other baseline demographic (Table 1).
- The SOMAscan identified 29 candidate proteins which were differentially expressed in CAV vs control groups (Figure 1 and 2).
- The Nanostring assay revealed 27 miRNAs significantly up- or down-regulated in patients with CAV.

Table 1: Patient Characteristics

	ALL N = 24	CAV N = 12	CONTROL N = 12	P-VALUE
Age, years	57 (46-68)	57 (49-68)	56 (41-66)	0.55
Male	17 (71)	8 (67)	9 (75)	1.00
Years post-transplant	9.5 (1.1-17.9)	18 (16-22)	1.1 (1-4)	<0.01
Donor-recipient sex mismatch	4 (18)	3 (27)	1 (9)	0.59
Diabetes	7 (29)	1 (8)	6 (50)	0.07
Myocardial infarct post-transplant	4 (17)	4 (33)	0 (0)	0.09
Calculated panel reactive antibody Class I, % Class II, %	 0 (0-0) 0 (0-61)	 0 (0-0) 58 (0-78)	 0 (0-5) 0 (0-0)	 0.75 <0.01
Treated Rejection Cellular mediated ≥3A/2R Antibody mediated	 4 (17) 1 (4)	 3 (25) 1 (8)	 1 (8) 0 (0)	 0.38 0.37
Left ventricular ejection fraction, %	56 ± 8	53.4 ± 10	59 ± 4	0.10
Low density lipoprotein mmol/L	1.6 (1.1-2)	1.2 (1.1-1.9)	1.7 (1.3-2.5)	0.22
Glomerular filtration rate, ml/min	57 (46-84)	53 (25-74)	65 (52-84)	0.21
Medications Cyclosporine/Tacrolimus Mycophenolic acid Prednisone Sirolimus/Everolimus Aspirin Statin	 24 (100) 21 (88) 19 (79) 0 (0) 22 (92) 22 (92)	 12 (100) 9 (75) 9 (75) 0 (0) 11 (92) 10 (83)	 12 (100) 12 (100) 10 (83) 0 (0) 11 (92) 12 (100)	 - 0.22 1.00 - 0.76 0.48

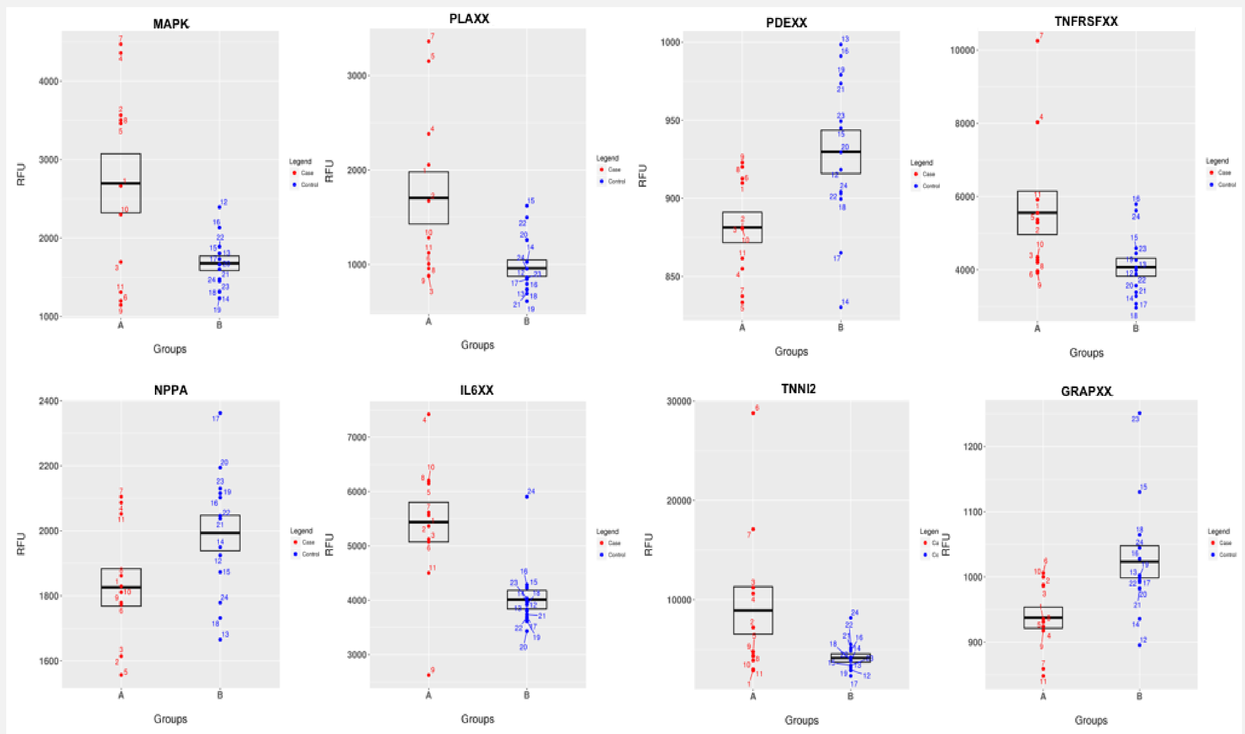

Figure 1. Potential candidate biomarkers for the diagnosis of CAV

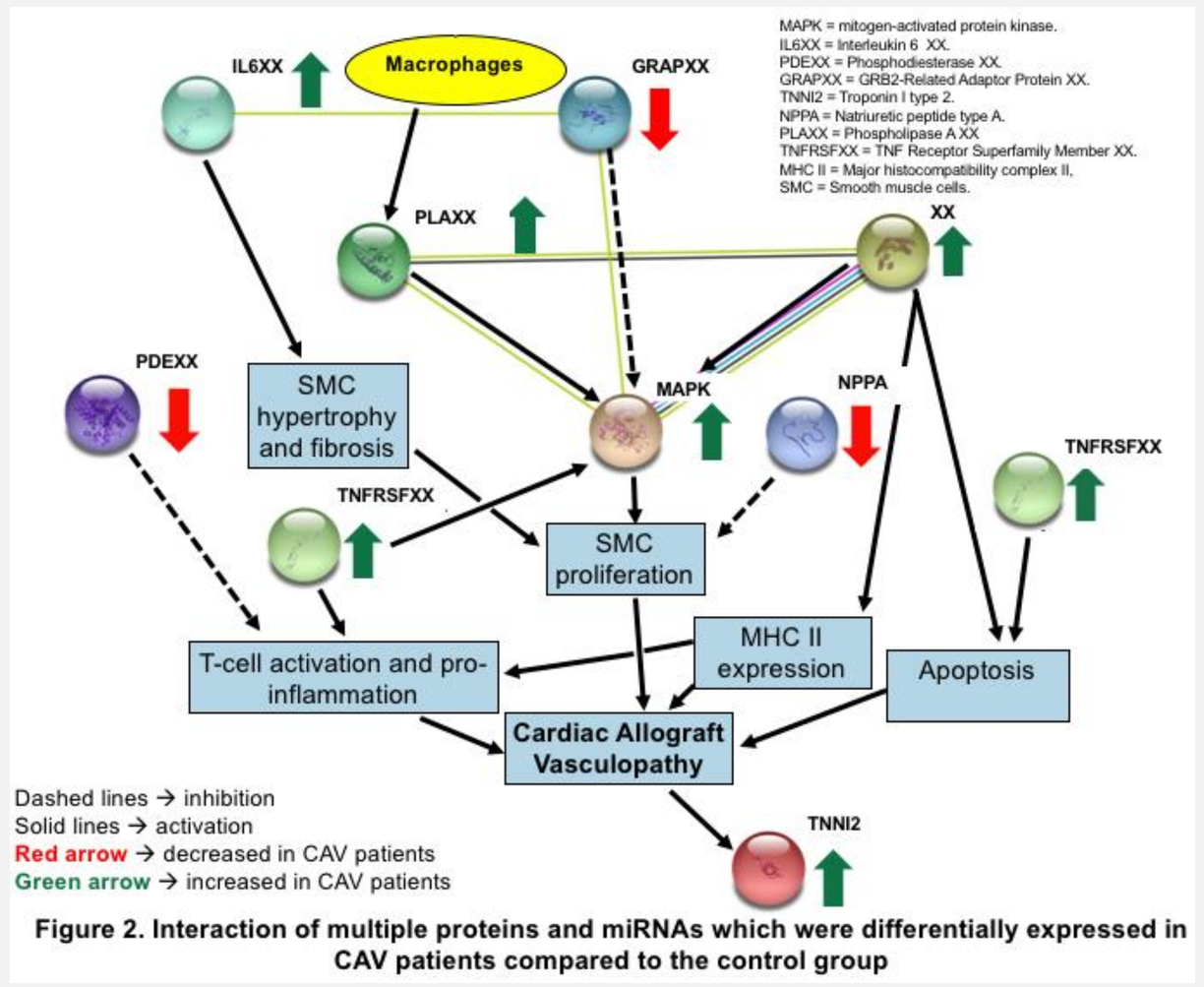

Figure 2. Interaction of multiple proteins and miRNAs which were differentially expressed in CAV patients compared to the control group

CONCLUSION

- This proof-of-principle study demonstrates the potential application of proteomics and miRNA technology to identify a panel of biologically relevant biomarker candidates for CAV.
- Our results require prospective validation in a larger cohort of patients.

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