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Mining the serum proteome for non-invasive monitoring of kidney allograft rejection

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Introduction: We hypothesized that protein biomarkers released from rejecting allograft tissues can be detected early in the systemic circulation.

Approach: We outline our modifications in the on-going search for biomarker panel that could accurately predict complications during kidney allograft rejection. In order to increase validity of identified allograft rejection-specific biomarkers, we used high-throughput protein array platforms and applied Systems Biology approach.

Methods: Serum samples were collected prospectively from 4 groups of patients (n=25 in each group); Group 1: recipients of kidney transplant requiring kidney biopsy for renal dysfunction, Group 2: transplant recipients with stable function, Group 3: chronic kidney disease patients awaiting transplant, and Group 4: healthy individuals. Serum was labelled with the fluorescent dye Cy3 and assayed on phosphoprotein microarray platform from Full moon Biosystems. For subsequent validation by quantitative Reverse Capture Protein Microarray platform, we used individual serum samples that were spotted in serial dilutions on a glass slide and probed with the specific antibodies for predicted biomarker proteins and correlated with the severity of the disease.

Results: Using bioinformatics algorithms, we were able to identify multiple candidate graft rejection-specific biomarkers. Lower levels of Ubiquitin, p38MAPK, histone H3.1 and Tak1 and higher levels of ATM, p38MAPK, HDAC8, SAPK/JNK, GSK3a-b, NFkappa B and RelB pointing to an altered p53 Signaling pathway were associated with group 3 and group 1 patient serum. Among the tested phosphorylated proteins, phospho-species of SAPK/JNK and RelB were elevated in group 2 vs. group 3 and group 1.

Conclusion: Data suggested that these novel analytes in the serum, together or independently, may constitute a robust and quantitative serum proteomic signature for rejection of renal allografts. We conclude that detection of allograft rejection by affinity proteomics offers a promising non-invasive tool for the surveillance and early detection of kidney allograft rejection.

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