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Microarray-based Gene Expression Profiling of Abdominal Aortic Aneurysm

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WHAT THIS PAPER ADDS

This microarray-based gene expression profiling study demonstrates differential expression of previously undescribed transcripts in abdominal aortic aneurysms (AAA), with functional roles in proteolysis, inflammation, and apoptotic processes. These were differentially expressed in AAA compared with screened controls, were modulated by surgery, and expressed in matched aortic biopsies and vascular smooth muscle. The transcriptional changes discovered may carry prognostic value and indicate underlying pathological processes of AAA.

Objective/Background: Microarray-based gene expression profiling studies may detect transcriptional signatures carrying prognostic value in abdominal aortic aneurysms (AAA). A gene expression profiling study was conducted to compare individuals with AAA with screened controls.

Methods: The peripheral blood transcriptome was compared between 12 individuals with AAA and 12 age- and sex-matched controls using microarray. Validation by Taqman real-time quantitative (qPCR) was performed in an independent group as described. Peripheral blood RNA was hybridized to Illumina microarrays, each representing 37,846 genes, allowing comparison of gene expression between cases and controls. Eleven differentially expressed genes were re-quantified by qPCR in the independent group with AAA ($n = 95$), controls ($n = 92$), pre- and postendovascular AAA repair (EVAR, $n = 31$); or open AAA repair ($n = 13$), AAA wall biopsies ($n = 11$), and in matched smooth muscle cultures ($n = 7$).

Results: Microarray detected 47 significantly differentially expressed genes in AAA after correction for multiple testing ($p < .05$). These genes conferred roles in regulation of apoptosis, proteolysis, the electron transport chain, leukocyte migration, and the humoral immune response. Gene quantification in the independent group demonstrated three genes to be downregulated in AAA compared with controls: *MSN*, *PSMB10*, and *STIM1*; however, their expression remained unchanged post-AAA repair. *PSMB10* was the only gene conferring a consistent direction of effect in both the discovery and validation analyses (downregulated). *EIF3G*, *SIVA*, *PUF60*, *CYC1*, *FIBP*, and *CARD8* were downregulated post-EVAR. Expression of all 11 genes of interest was detected in aortic biopsies and matched smooth muscle cultures.

Conclusion: This study demonstrates differential expression of transcripts in peripheral blood of individuals with AAA, with functional roles in proteolysis, inflammation, and apoptotic processes. These were modulated by aneurysm exclusion from the circulation and expressed in matched aortic biopsies and smooth muscle cultures. These observations further support the key roles for these pathways in the pathogenesis of AAA.

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INTRODUCTION

Recent literature has advocated genome-wide approaches, including expression profiling, as fundamental to deciphering abdominal aortic aneurysm (AAA) pathogenesis and subsequent development of clinical tools.[1,2] The modalities available for performing gene expression profiling, also known as whole transcriptome analysis, include real-time quantitative polymerase chain reaction (qPCR) arrays and DNA microarrays. Evidence from previous array studies of inflammatory and neoplastic diseases have revealed transcriptional signatures in peripheral blood that have conferred prognostic value.[3—5] Over 80% of the peripheral blood transcriptome may be shared with that of any given tissue; thereby reflecting system-wide translational processes.[6] This suggests these techniques may be used to identify biomarkers of AAA in peripheral blood, with the potential to become diagnostic and prognostic tools. It is possible that circulating mononuclear cells are directly involved in low-grade chronic inflammation related to AAA. Among previous transcriptomic studies of AAA using aortic

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tissue and peripheral blood, the differential expression of genes involved in immune function, inflammation, angiogenesis, apoptosis, proteolysis, blood coagulation, lipid metabolism, and oxygen transport have been demonstrated.[7–14] Small numbers of samples have limited these studies and none have demonstrated replication of their findings in larger cohorts. The aim of this study was therefore to first perform microarray-based gene expression profiling comparing individuals with AAA with screened controls, and perform a subsequent PCR-based validation study in a separate cohort of individuals. It was hypothesised that the peripheral blood gene expression profile of individuals with AAA would be significantly different from those with a normal-calibre aorta. Second, it was hypothesised that differentially expressed genes identified in blood would be expressed in matched aortic aneurysmal wall, as well as be modulated by aneurysm exclusion from the circulation by surgery.

MATERIALS AND METHODS

The experimental workflow of this study comprised two stages. Stage 1 involved an initial expression microarray discovery study with immediate qPCR validation of findings in the same samples (technical replication of top-ranked genes). Stage 2 quantified genes of interest in an independent, larger group of individuals. Individuals were recruited from the University Hospitals of Leicester NHS Trust. Screened controls were defined as having a normal-calibre aorta ($\leq$2.4 cm), as the risk of developing AAA is negligible under these conditions.[15] Cases were defined as those with radiological evidence of small AAA (3–5.4 cm) and large AAA ($\geq$5.5 cm). Systemically well white men $\geq$60 years of age were included. Exclusion criteria included the clinical or radiological presence of peripheral arterial disease, neoplasia, and chronic autoimmune/inflammatory conditions, which might confound gene expression profiling results. Preoperative patients were consented for repeat blood samples $\geq$6 months postsurgery. This minimal interval of 6 months was chosen to avoid early postoperative physiological changes confounding gene expression profiles. Further exclusion criteria applied at this stage included those with evidence of endoleak, as this would technically suggest incomplete AAA exclusion from the circulation. Ethical approval for the study was obtained from the Leicestershire, Northamptonshire, and Rutland Research Ethics Committee (ref: 6891). All participants provided written informed consent.

Sample collection and processing

Peripheral blood samples were obtained in a closed collection system PAXgene tubes (Preanalytix, Hombrechtikon, Switzerland) designed to stabilise the mRNA profile for storage at −20 °C. Peripheral blood and matched biopsies from the anterior aneurysm sac (at its maximal diameter) were obtained from patients undergoing elective open repair of nonruptured AAAs. Biopsies were lavaged with ribonuclease-free water to remove blood and debris from contaminating subsequent analyses. Biopsies were divided in two; one sample being immediately stored in RNALater solution (Ambion, Waltham, MA, USA) to stabilise the mRNA profile for storage at −80 °C, and one placed in minimum essential medium on ice for immediate smooth muscle culture. The medial smooth muscle layer was dissected under microscopy, segmented into 1-mm^3 explants and incubated in Medium 199 + 20% fetal calf serum and incubated for at 37 °C, in 5% humidified CO_2. Cells were passaged by routine trypsinisation, centrifugation, and resuspension in medium. Cells from passages 4–8 were analysed for RNA expression.

RNA extraction

Total RNA extraction from blood/cultured cells and tissue was performed with a spin column-based PAXgene Blood RNA Kit/RNeasy Micro Kit (Qiagen, Hilden, Germany) and phenol-based kit (TRIzol; Invitrogen, Carlsbad, CA, USA). RNA samples were purified by exposure to DNAse I enzyme to digest genomic DNA. High RNA purity was confirmed via microfluidic-based electrophoresis (Agilent 2100 Bioanalyzer platform; Agilent Technologies, Santa Clara, CA, USA).

Microarray platform

Human HT-12 BeadChips (Illumina, San Diego, CA, USA) were processed with a Direct Hybridization Assay and scanned on a BeadArray Reader. Each BeadChip contained multiple presynthesized full-length 50-mer oligonucleotide probes representing 37,846 genes on microsphere-based microarrays. RNA was amplified and biotinylated for hybridization with the BeadChips. Differential gene expression and ontology results were generated with GenomeStudio V2009.1 (Illumina) and GeneSpring GX v11.0 (Agilent Technologies). Cubic spline normalisation was applied to raw array to normalise data between BeadChips. Genes were considered to be positively expressed on the array by exhibiting a detection score of $\geq$.99, as a criterion previously described.[7] Gene functions were analysed by manually tallying their associated biological processes, as listed by the Gene Ontology (GO) database.

PCR technique

Evidence from repetitive cDNA hybridizations has demonstrated that single microarray output is subject to substantial variability.[16] Therefore, the expression of top-ranked protein encoding genes in replicate samples was validated. A probe-specific two-step TaqMan qPCR assay was utilised to validate microarray results and to perform extended replication of genes of interest (Applied Biosystems, Foster City, CA, USA). Genes for extended replication were chosen based on ranking of differential expression and biological annotation relevant to vascular disease. Gene expression was determined by the comparative Ct method and compared between cases and controls with the Mann–Whitney *U* test, utilising Prism software v5.03 (GraphPad, La Jolla, CA, USA). Spearman's rank