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Peripheral Blood Transcriptome in Patients with Sarcoidosis-Associated Uveitis

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Abstract

Introduction—Sarcoidosis has traditionally been thought of as a compartmentalized disease – the inflammatory milieu within pulmonary granulomas harbors products of activated genes leading to the manifestation of the autoimmune process. However, recent research has shown that such a compartmentalized view of sarcoidosis may not be entirely accurate and that distinguishing biomarkers may be identified from the peripheral blood.[1]

Methods—Twenty participants were recruited from a convenience sample from the Francis I. Proctor Foundation at the University of California, San Francisco (UCSF). This study was approved by the UCSF Institutional Review Board and adhered to the tenets of the Declaration of Helsinki. Participants had peripheral whole blood drawn into PAXgene blood RNA tubes (QIAGEN, Germantown, MD) and prepared and stored at −80C according to manufacturer's recommendations. Samples were deidentified and laboratory personnel handing samples and interpreting data were masked. Differential gene expression was performed to identify host transcriptome signatures.[2] Briefly, analysis of sequenced data was made using a rapid computational pipeline developed in-house to classify host genes. Quality filtered RNA transcripts were aligned to the ENSEMBL CRCh38 human genome using STAR2. Genes were filtered to include only protein-coding genes that were expressed in at least 25% of the patients. Gene count data were analyzed with DESeq2.[3] Differentially expressed genes with false discovery rate (FDR) <0.01 were considered as notable.

Results—Ten participants with uveitis compatible with sarcoidosis (8 with pulmonary involvement, 1 with CNS involvement, and 1 with conjunctival granulomas), 9 participants with Vogt-Koyanagi-Harada (VKH)-associated uveitis, and 9 healthy controls were enrolled into the study. Ten genes exhibited at least two-fold difference in expression in sarcoidosis participants compared to controls (Table 1 and Figure 1). When examining all differential genes identified (FDR<0.01) in patients with sarcoidosis compared to controls, there was no overlap of these

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differentially expressed genes in VKH compared to controls or in VKH compared to sarcoidosis patients (Figure 1C).

Conclusions—We compared the peripheral blood signatures of patients with sarcoid-associated uveitis to those with another granulomatous, but clinically distinct disease, VKH and control patients. Sarcoidosis exhibited a unique transcriptome compared to controls. Additionally, there was not overlap in differentially expressed genes when comparing sarcoidosis to VKH.

A limitation of the present study is that the peripheral blood of patients with sarcoidosis and VKH were obtained at various disease states. Of the 10 sarcoidosis patients, 4 had uveitis that was in a medication-free remission, 2 had controlled inflammation on topical or intraocular steroids, and 4 had controlled inflammation on systemic immunosuppression. Those using systemic immunosuppression or in medication-free remission may exhibit transcriptome signatures different from those with active or uncontrolled inflammation. In pulmonary tuberculosis, the peripheral blood transcriptome reverts to that of controls after treatment.[4] However, autoinflammatory transcriptomes may persist unlike infectious processes. Four of our patients described were in a medication-free remission with respect to their uveitis, though the transcriptome of these patients resembled that of the other sarcoidosis patients. It is possible that there was sub-clinically active disease elsewhere in their body responsible for the persistence of their transcriptional profile.

Our sarcoidosis patients had extraocular involvement and disease burden outside of the eye likely plays a role in the peripheral blood transcriptome that we identified. Future studies where patients with ocular disease alone, but in whom a suspicion for sarcoidosis exists (based on International Workshop of Ocular Sarcoidosis features) may employ examination of inflamed aqueous or vitreous.[5,6]

Genes that are differentially expressed may be clinically useful as non-surgical disease biomarkers for sarcoidosis. Moreover, such differential gene expression may also allow for the stratification of patients based on disease severity, disease location, and may inform future therapeutic targets.

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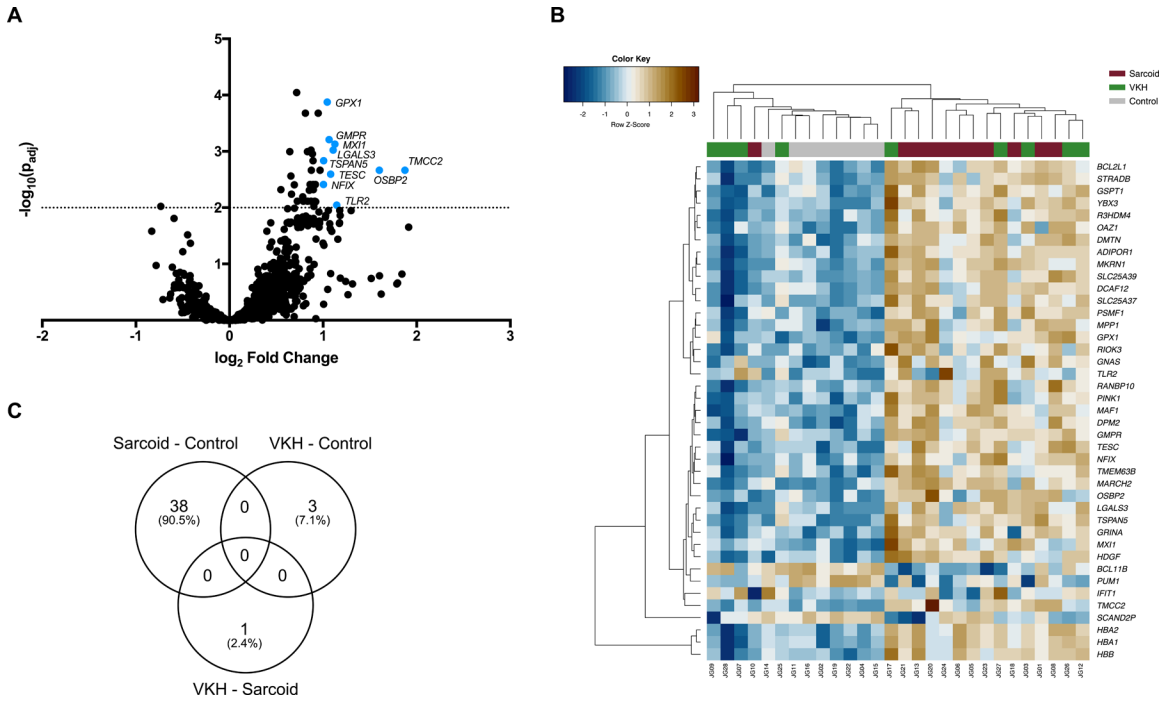

Figure 1. Peripheral blood transcriptomes between patient groups.

A) Volcano plot of the relative abundance distributions of transcripts for 19 patients. The x-axis shows the $\log_2$ fold of relative abundance ratio between patients with sarcoidosis and patients without uveitis. The y-axis shows the negative $\log_{10}$ of adjusted P-values.

B) Heatmap of differentially expressed transcripts between control, VKH, and sarcoidosis patients. Normalized expression levels, arranged by unsupervised hierarchical clustering, reflecting over-expression (brown) or under-expression (blue) of genes (rows) for each peripheral blood sample (columns). 38 transcripts identified for control compared to sarcoidosis and 3 transcripts identified for control compared to VKH using FDR <0.01.

C) Venn diagram showing the lack of overlapping differential genes between patient groups.

Table 1.

Genes that exhibited ≥ 4-fold expression in sarcoidosis compared to controls (False Discovery Rare (FDR) < 0.01).

Gene*	Full name*	Function*	Possible connection(s) to sarcoidosis
GPX1	Glutathione peroxidase 1	Catalyzes the reduction of organic hydroperoxides and hydrogen peroxide by glutathione, and thereby protect cells against oxidative damage	Peroxiredoxins, including glutathione peroxidase 1, are expressed in the lung. Expression of peroxiredoxins is elevated in sarcoidosis.[7]
GMPR	Guanosine monophosphate reductase	Catalyzes the irreversible and NADPH-dependent reductive deamination of GMP to IMP. The protein also functions in the re-utilization of free intracellular bases and purine nucleosides.	Role in cellular metabolism.[8,9]
LGALS3	galectin 3	Plays a role in numerous cellular functions including apoptosis, innate immunity, cell adhesion and T-cell regulation. The protein exhibits antimicrobial activity against bacteria and fungi.	Biomarker associated with fibrosis.[10]
MXI1	MAX interactor 1	Transcriptional repressor thought to negatively regulate MYC function and is therefore a potential tumor suppressor. This protein inhibits the transcriptional activity of MYC by competing for MAX, another basic helix-loop-helix protein that binds to MYC and is required for its function.	Associated with cell growth and maintaining differentiated state as well as may be associated with downregulation of interleukin 8, which is involved in neutrophil chemotaxis.[11,12]
NFIX	Nuclear factor I X	A transcription factor that binds the palindromic sequence 5'-TTGGCNNNNNGCCAA-3 in viral and cellular promoters. The encoded protein can also stimulate adenovirus replication in vitro.	Involved in macrophage function and B lymphopoiesis.[13,14]
OSBP2	Oxysterol binding protein 2	Binds oxysterols such as 7-ketocholesterol and may inhibit their cytotoxicity.	Associated with inflammation and may be a biomarker for some tumor metastases.[15]
TESC	Tescalcin	Plays important roles related to chromatin remodeling, transcriptional regulation, and epigenetic modification.[2]	Associated with cell growth and differentiation.[16]
TLR2	Toll-like receptor 2	Fundamental role in pathogen recognition and activation of innate immunity. Leads to an up-regulation of signaling pathways to modulate the host's inflammatory response. This gene is also thought to promote apoptosis in response to bacterial lipoproteins. This gene has been implicated in the pathogenesis of several autoimmune diseases.	Associated with mediating granulomatous inflammation.[17–19]
TMCC2	Transmembrane and coiled-coil domain family 2	Involved in protein binding.	Associated with mediating inflammation.[20]
TSPAN5	Tetraspanin 5	Mediates signal transduction events that play a role in the regulation of cell development, activation, growth and motility.	Associated with T lymphocyte transmigration.[21]

*National Center for Biotechnology Information https://www-ncbi-nlm-nih-gov.ucsf.idm.oclc.org/guide/genes-expression/ accessed April 1, 2020.