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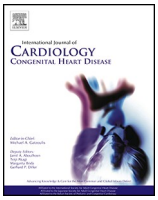

Novel immunologic mechanisms for Fontan-associated liver disease

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ABSTRACT

Introduction: Single ventricle congenital heart disease resulting in Fontan palliation has led to improved survival, however, Fontan-associated liver disease (FALD) is ubiquitous in this population by adulthood. While lymphopenia has been associated with the degree of FALD, potential immunologic mechanisms remain unstudied, and were the focus of this study.

Methods: Single-nuclei RNA-seq (snRNA-seq) data from liver samples of adolescent Fontan and control patients were analyzed with specific focus on lymphocytes and natural killer (NK) and T-cell fractions.

Results: Liver samples from Fontan patients demonstrated upregulation of endothelial cells (ECs: 4.2 ± 1.0 vs. 13.6 ± 3.4 %, p = 0.037) and total lymphocytes (0.7 ± 0.1 vs. 3.6 ± 0.7 %, p = 0.007), more specifically in NK and T-cells (NK: 0.29 ± 0.16 vs. 1.40 ± 0.64 %, p = 0.028 and T-cell: 0.28 ± 0.04 vs. 1.80 ± 1.01 %, p = 0.034). Enhanced genes important in T-cell activation and differentiation were demonstrated, as well as those involved in cell-to-cell adhesion and lymphocyte migration. Supporting lymphocyte trafficking, ECs demonstrated amplification of critical chemotactic and lymphocyte recruitment genes. Increased time from Fontan palliation was associated with more dramatic lymphocytic transcriptomic changes.

Conclusions: Hepatic changes in adolescent Fontan patients suggest that T-cells are contributing to the early development and possible progression of FALD.

1. Introduction

Children born with a single ventricle represent the most complex form of congenital heart disease, yet with Fontan palliation most survive to adulthood. Fontan circulation results in both late cardiac and extra-cardiac disease, in particular, Fontan-associated liver disease (FALD), which is ubiquitous in this population by late childhood. Traditional markers of liver function (i.e. the Model for End-stage Liver Disease/MELD score) have failed to identify severe FALD, and the etiology of this unique form of liver disease is poorly understood. Lymphopenia has been reported commonly in Fontan physiology [1,2], and we recently demonstrated that it is linked to liver dysfunction [3]. However, potential immunologic underpinnings to FALD remain unstudied, and were the focus of this study.

2. Methods

2.1. Data processing

Single-nuclei RNA-seq (snRNA-seq) de-identified data from liver samples of adolescent Fontan (n = 4, 13–18 y/o) and control patients (n = 2, 13–14 y/o) were downloaded from a publicly available repository (GSE223843) [4] and reanalyzed, obviating the need for institutional review board review. This data was re-processed via the Seurat v5 package (Yuhan Hao, 2023). Briefly, nuclei were filtered for mitochondrial reads <10 %, counts >400, and features >250. Doublets were removed with DoubletFinder (v2.0.4) and samples were integrated using the canonical correlation analysis from the Seurat package. Cells were clustered and identified using the *FindClusters* and *FindMarkers* function in Seurat. Pseudo-bulk RNA-seq analysis was performed using DESeq2 (v1.40.2), and gene set enrichment was performed using the fgsea package (v1.26.0).

Abbreviations: EC, Endothelial cells; FALD, Fontan-associated liver disease.

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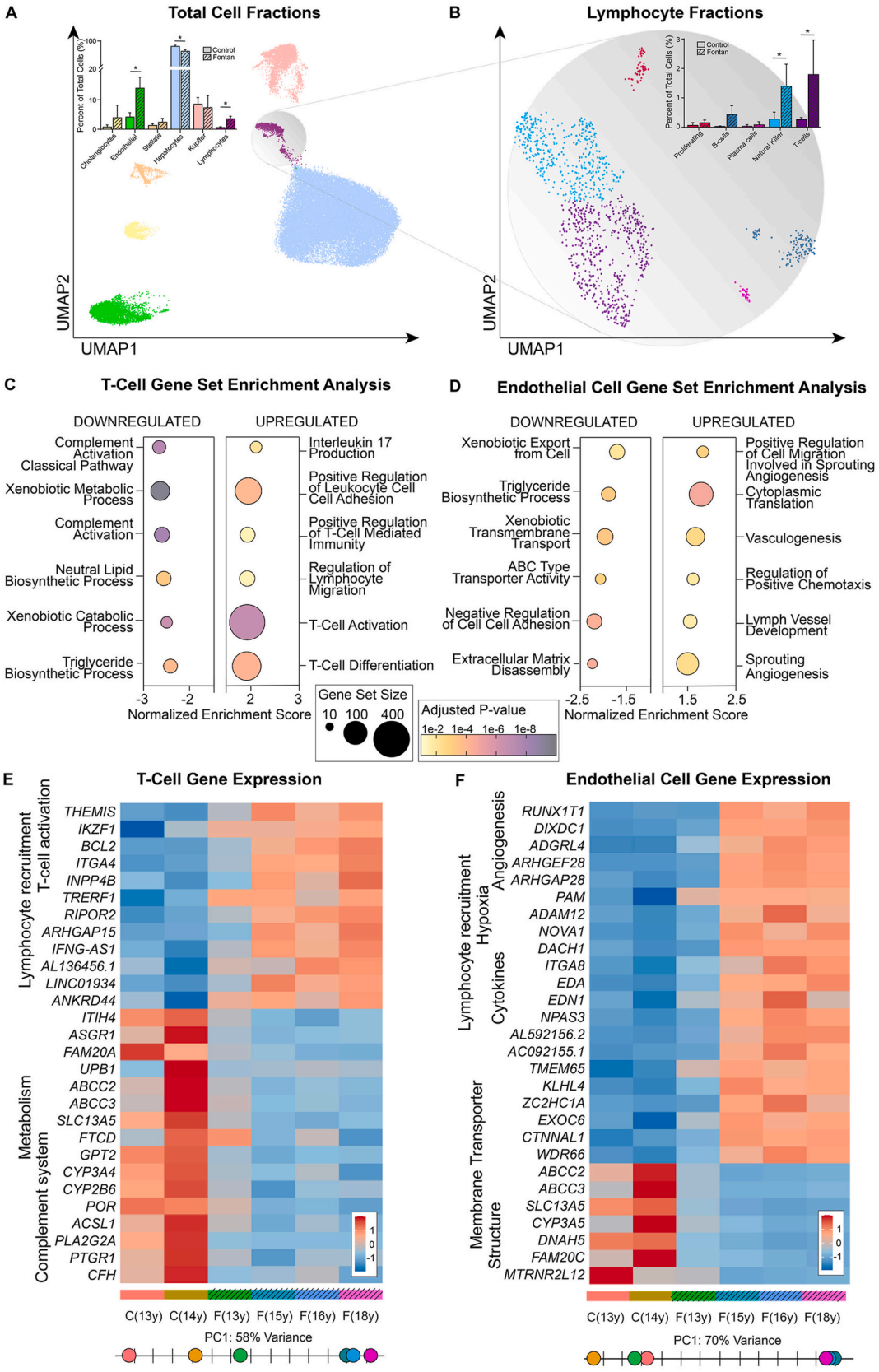

Fig. 1. Immunologic Changes in Fontan-Associated Liver Disease (**A**) Uniform Manifold Approximation and Projection (UMAP) plots demonstrate cell types identified in the single-nuclei RNA-ATAC seq data analysis of adolescent Fontan (n = 4) and Control (n = 2) liver samples. Quantitative data is shown in the inset. (**B**) UMAP plot for lymphocyte populations identified within the lymphocyte cluster are shown. Quantitative data is included in the inset. Gene Set Enrichment Analysis demonstrating significantly up- and down regulated pathways in hepatic T-cells **(C)** and endothelial cells **(D)** in FALD and control patients. Heatmap for genes significantly up- or downregulated in hepatic T-cells **(E)** and endothelial cells **(F)** of FALD patients. PCA plots at the bottom of **(E)** and **(F)** demonstrate progression in the activation of gene programs in T-cells and endothelial cells, respectively, with time from Fontan palliation.

2.2. Statistical analysis

Analysis and statistics were performed in R (v.4.3.1). Data are reported as mean ± standard deviation. A Student's t-test was used to detect differences between control and Fontan patients, and a p-value of <0.05 was considered statistically significant.

3. Results

While hepatocytes were attenuated in Fontan samples (84 ± 2 vs. 69 ± 4 %, $p = 0.010$), endothelial cells (ECs) were upregulated (4.2 ± 1.0 vs. 13.6 ± 3.4 %, $p = 0.037$), as were total lymphocytes (0.7 ± 0.1 vs. 3.6 ± 0.7 %, $p = 0.007$, Fig. 1A). More specifically, with respect to lymphocytes, upregulation was demonstrated in natural killer (0.29 ± 0.16 vs. 1.40 ± 0.64 %, $p = 0.028$) and T-cell (0.28 ± 0.04 vs. 1.80 ± 1.01 %, $p = 0.034$) populations without changes in B-cells (Fig. 1B). In support of Fontan liver T-cell upregulation, we found that the most enhanced genes functioned in T-cell activation and differentiation (*THEMIS*, *IKZF;* Fig. 1C and D), along with those involved in leukocyte cell to cell adhesion and lymphocyte migration (ITGA4, RIPOR2; Fig. 1C and D) [5]. We also observed an increase in resident T-cell markers (*ITGA4*, *INPP4B*, *TRERF1*, Fig. 1D) [6]. In further support of lymphocyte trafficking, critical chemotactic and lymphocyte recruitment genes (*DACH1*, *NOVA1*) were amplified in ECs, including those that encode for selectins (endothlin-1, which increases expression of E-selectin) and integrins (*ITGA8*, *ITGB5*, *SELP*), which are essential for active trafficking of T-cells into tissue; concomitantly, we demonstrated attenuation of negative cell to cell adhesion regulators (Fig. 1E and F) [7]. We also observed increases in angiogenic pathways, lymphangiogenic pathways, and pro-inflammatory cytokines (*EDN1*, *EDA*, *BMP4*) in ECs. To further characterize the importance of upregulated genes in ECs and T-cells, principal component analysis (PCA) was performed and revealed an age-dependent upregulation in both the EC and T-cell activation transcriptome. The transcription profile of ECs and T-cells became less like the control samples with increasing time from Fontan palliation, suggesting time-dependent progressive EC and T-cell activation in FALD.

4. Discussion

In this limited sample we demonstrate a potentially novel immunologic mechanism for FALD characterized by hepatic lymphocytic infiltration and activation. Our data also suggests that a time-based coefficient exists and may explain differential FALD severity.

FALD is a unique and only relatively recently recognized late extracardiac manifestation that results from Fontan physiology [8]. In other forms of liver disease, more specifically in steatotic liver disease (SLD), lipid accumulation and lipotoxicity are thought to trigger liver injury, which leads to inflammation and subsequent fibrosis [9,10]. Recent data has suggested that the high central venous pressures observed in Fontan physiology may incite central hepatocyte changes that lead to fibrosis and the early initiation of FALD [4]. Our data would support that although the provoking source of FALD may differ from SLD, downstream inflammatory and immune-based mechanisms may be responsible for the propagation and progression of FALD. In support of this, we demonstrated increases in the frequency of T-cells in Fontan liver, and evidence of upregulation of T-cell activation and differentiation. We also showed concomitant changes in the EC transcriptome to facilitate T-cell recruitment from the periphery to the liver. Taken together, this data suggests that ECs are involved in recruiting T-cells from the systemic circulation to the liver, where they are differentiating and activating, potentially mediating FALD.

In further support for a "T-cell immune mediated mechanism" for FALD, many patients with Fontan physiology also demonstrate peripheral lymphopenia. This may suggest that attenuation in circulating lymphocytes could be due to peripheral (liver) recruitment. While the findings are correlative, the hepatic changes in adolescent Fontan patients suggest that T-cells are contributing to the early development and possible progression of FALD. The small number and age-restrictive nature of the samples analyzed present one limitation of this study. However, future simultaneous investigation of systemic and hepatic lymphocytes in older patients with more significant FALD will facilitate better understanding of T-cell mediated mechanisms associated with this unique form of liver disease. In conclusion, this study supports the need for further investigation of immune-based mechanisms in FALD.

CRediT authorship contribution statement

Austin Angelotti: Writing – original draft, Formal analysis, Data curation. **Maninder Dhesi:** Formal analysis, Data curation. **Shyam S. Bansal:** Writing – review & editing, Conceptualization. **Elisa A. Bradley:** Writing – review & editing, Writing – original draft, Conceptualization.

Data availability statement

Data is available to download using the GEO database (GSE223843).

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Declaration of competing interest

None.

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NA.

References

[1] Mattes M, Connor J, Kelly SS, Schwartz MC. Lymphopenia in patients with single-ventricle heart disease after the fontan operation. Congenit Heart Dis 2016;11:270–5.
[2] Morsheimer MM, Rychik J, Forbes L, et al. Risk factors and clinical significance of lymphopenia in survivors of the fontan procedure for single-ventricle congenital cardiac disease. J Allergy Clin Immunol Pract 2016;4:491–6.
[3] Scandinaro AL, McCann MD, Chaudhry A, Kunselman A, Bradley EA, Davidson Jr WR. Lymphopenia in the adult population with fontan physiology: a potential new marker for disease assessment. CJC Open 2024;6:773–80.
[4] Hu P, Rychik J, Zhao J, et al. Single-cell multiomics guided mechanistic understanding of Fontan-associated liver disease. Sci Transl Med 2024;16:eadk6213.
[5] Prasad M, Brzostek J, Gautam N, Balyan R, Rybakin V, Gascoigne NRJ. Themis regulates metabolic signaling and effector functions in CD4(+) T cells by controlling NFAT nuclear translocation. Cell Mol Immunol 2021;18:2249–61.
[6] Chen ZY, Mortha A. INPP4B ensures that ILC1s and NK cells set up a productive home office. J Exp Med 2024;221.
[7] Shintani A, Fukai S, Nobusawa R, et al. Dach1 transcription factor regulates the expression of peripheral node addressin and lymphocyte trafficking in lymph nodes. Curr Res Immunol 2022;3:175–85.
[8] Daniels CJ, Bradley EA, Landzberg MJ, et al. Fontan-associated liver disease: proceedings from the American college of cardiology stakeholders meeting, october 1 to 2, 2015, Washington DC. J Am Coll Cardiol 2017;70:3173–94.
[9] Rinella ME, Lazarus JV, Ratziu V, et al. A multisociety Delphi consensus statement on new fatty liver disease nomenclature. Ann Hepatol 2024;29:101133.
[10] Lee E, Korf H, Vidal-Puig A. An adipocentric perspective on the development and progression of non-alcoholic fatty liver disease. J Hepatol 2023;78:1048–62.