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ORAL ABSTRACTS

201.GRANULOCYTES, MONOCYTES, AND MACROPHAGES

Deep Proteomic Analysis Reveals Shared Terminal Mechanisms for Familial Hemophagocytic Lymphohistiocytosis and Lymphoma-Associated Hyperinflammation

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Background: Hemophagocytic lymphohistiocytosis (HLH) is a hyperinflammatory syndrome increasingly observed in patients with hematologic malignancies (HM-HLH). Familial HLH (FHL) is seen in young children and is typically caused by mutations affecting the perforin pathway of lymphocyte cytotoxic function, leading to excessive CD8+ T cell activation and interferon-gamma production. Though HM-HLH has clinical similarities to FHL, little is known regarding the pathophysiology of HM-HLH. We previously developed the 'Optimized HLH Inflammatory (OHI) index,' combining soluble CD25 and ferritin elevations, to improve recognition of hyperinflammation and prognostication in HM patients (Zoref-Lorenz et al., Blood; 2022). Based on these findings, we hypothesize that OHI+ HM's share similar immune pathophysiology with FHL and aim to validate this hypothesis using an unbiased proteomic approach.
Methods: We conducted an unbiased proteomic analysis of samples from Schneider Children's, Meir, Sheba, and Beilinson Medical Centers. Our cohort included FHL patients, healthy pediatric controls, and patients with HMs, classified as HM-OHI+(sCD25>3,900 U/mL and ferritin>1,000 ng/mL) or HM-OHI-. SomaLogic proteomics platform, an aptamer-based method, assessed 7,596 serum proteins representing 6,412 genes. Unsupervised clustering techniques (primary component analysis and unbiased hierarchical clustering) were used to group patient results. Gene set enrichment analysis (GSEA) of the hallmark set was used to identify enriched biologic pathways in HM-OHI+ and FHL. Differential expression analysis (Student t-test) identified differentially expressed proteins (DEPs, $q<0.1$, $p<0.05$) between FHL vs. healthy controls and HM-OHI+ vs. HM-OHI-. Venn analysis revealed overlapping DEPs between FHL and OHI+ HM-HLH patients. GSEA with hallmark pathways evaluated the overlapping genes in each pairwise comparison within the entire sets and the intersecting genes. Spearman's correlation analysis examined the relationship between fold changes of proteins and normalized enrichment scores of hallmark GSEA comparisons within intersecting proteins and genes of FHL and HM-OHI+. Logistic regression created an equation for the linear curve of these correlations.
Results: We analyzed 42 patients, including 14 HM-OHI+ and 14 HM-OHI- patients, matched by malignancy type, 7 FHL patients, and age-matched controls. Unsupervised hierarchical clustering (Figure 1A) showed that HM-OHI+ patients clustered with FHL patients, while healthy controls and HM-OHI- patients clustered together. GSEA analysis identified common enriched biological pathways between HM-OHI+ and FHL: interferon-γ response, IL6 JAK STAT3 signaling, IL2 STAT5 signaling, and oxidative phosphorylation (Normalized enrichment scores of 1.82:1.92; 1.76:2.23; 1.76:1.76 and 1.68:1.78 respectively for HM-OHI+ and FHL). In pairwise statistical comparisons, 841 differential proteins were shared between FHL and HM-OHI+. HM-OHI+ and FHL exhibited highly correlated fold changes ($R=0.97$, $p<0.0001$, $Y= 1.362 X-0.2597$, Figure 1B), with highly significant elevated proteins in HM-OHI+ vs. HM-OHI- that are key in the mechanism of FHL: T cell activation (GRZB, FC-1.6 $q=0.01$; GRZH: FC-1.5, $q= 0.002$), IFN-γ response (CXCL10, FC-2.1,$q=0.001$), and macrophage activation (CD14: FC- 1.6,

q=0.001; CSF1:FC- 2.1, 1<0.0001; IL1RL: FC- 1.8,q=0.0009). The intersecting genes in the GSEA analysis revealed a high correlation of the biological pathways (R=0.94, p<0.0001, Y=1.282X+0), with the IFN-γ response, the known driver of FHL being the most significantly over-enriched pathway in both pairwise comparisons (q<0.25). These findings indicate shared pathophysiology mechanisms between HM-OHI+ and FHL. We validated key proteins using ELISA as an orthogonal method. **Conclusions:** This study establishes a common downstream mechanism of hyperinflammation in hematologic malignancies and FHL, closely linked to the IFN-γ response, and validates the OHI index as a diagnostic tool for HM-HLH. Understanding the shared pathophysiology between HM-OHI+ and FHL sheds light on the poor prognosis of HM-associated hyperinflammation and emphasizes the need to study the upstream mechanisms of HM-HLH to define targets for improved therapeutic approaches.

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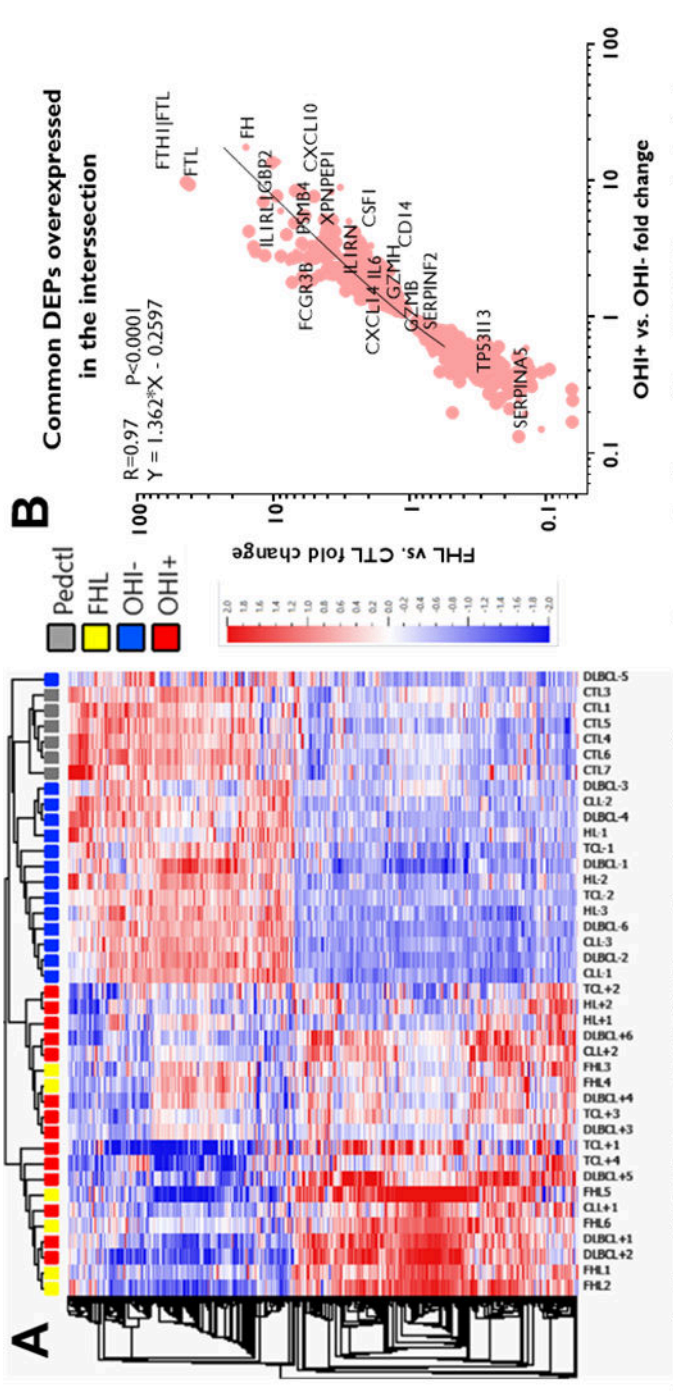

Figure 1. Familial HLH and HM-OHI+ patients share the same immunologic profile. (A) Unbiased clustering analysis of the proteins with q<0.05 ,1456 proteins, comparing HM-OHI+ and HM-OHI-) are shown on a heat map. (B) Common differentially expressed proteins (DEPs) shared between HM-OHI+ vs. HM-OHI- and FHL vs. pediatric controls (q<0.1, p<0.05) are shown in a scatter plot of the fold changes of these pairwise comparisons. Spearman's correlation test and linear logistic regression were used to calculate correlation and the regression line, respectively. Pedctl- healthy pediatric controls; FHL- familial hemophagocytic lymphohistiocytosis; OHI- Optimized HLH/hyper Inflammatory index.

Figure 1