FACULTY ABSTRACTS

Advances in the microenvironment and biology of Waldenström macroglobulinemia

Mu Hao[1,2,*], Hao Sun[1,3], Wenjie Xiong[1], Shuhua Yi[1], Steven Treon[3] and LuguiQiu[1,2,*]

1. State Key Laboratory of Experimental Hematology, National Clinical Research Center for Blood Diseases, Haihe Laboratory of Cell Ecosystem, Institute of Hematology & Blood Diseases Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Tianjin 300020, China; 2. Gobroad healthcare Group; 3. The Bing Center for Waldenström's Macroglobulinemia, Dana-Farber cancer institute.

Introduction: Waldenström macroglobulinemia (WM) is a rare and incurable indolent B-cell malignancy. The molecular pathogenesis and the role of the immunosuppressive microenvironment in WM development are still incompletely understood.

Main points: Single-cell RNA-sequencing (scRNA-seq) was utilized to characterize the multicellular landscape in the bone marrow (BM) of patients with Waldenström macroglobulinemia (WM), aiming to explore its underlying molecular features. A total of 22 WM samples were analyzed, including 15 newly diagnosed WM (NDWM), 5 relapsed WM (RRWM), 2 post-treatment WM, and 1 sample exhibiting both newly diagnosed and post-treatment characteristics. Moreover, 7 healthy donor controls were included. Additionally, the study encompassed a total of 2 matched pairs of bone marrow and peripheral blood (BM&PB), comprising 1 WM samples and 1 healthy donor control. Overall, the analysis encompassed a population of 199,758 single cells. Our data unveiled substantial heterogeneity among malignant cells in WM, as well as the dynamic co-evolution between WM and immune cells, which play crucial roles in disease development and progression.

In all stages of WM disease, there is a reduction in myeloid cells and an increase in lymphoid cells. T/NK cells show a significant elevation in the NDWM stage, which further increases after treatment but decreases during relapse. HSPC, Myeloid, and erythroid progenitor cells are notably diminished in the NDWM stage and further decreased during relapse. Following treatment, HSPC and erythroid progenitor cells recover, while Myeloid remains depleted. During relapse, HSPC and erythroid progenitor cells decrease again, whereas myeloid cell count increases. B/PC cells as tumor cells in WM are elevated in the ND stage but decrease after treatment before rising again during relapse. Subsequently, B and plasma cells were isolated for cluster analysis once again resulting in 19 clusters from a total of 38,315 cells. Notably, cluster 14 emerged as a novel cluster observed exclusively in RRWM (relapsed WM) samples. Differential gene expression analysis revealed 2,913 DEGs between cluster 14 and other tumor cells. GSEA hallmark analysis further indicated the activation of the MYC signaling pathway specifically within cluster 14, suggesting its association with WM relapse. Additionally, longitudinal samples from a WM patient before and after treatment with BTKi were utilized to investigate mechanisms underlying drug resistance against BTKi therapy by comparing differences within tumor cell populations pre- versus post-treatment. Our findings identified specific BTKi-resistant clusters enriched within samples obtained after BTKi treatment initiation.

Significantly high expression levels of the PRKCE gene were observed within BTKi resistance clusters (C5-C7 and C9-C10). Given Teron's group's survival data analysis results suggest a plausible association between elevated PRKCE levels and BTKi resistance in patients with WM. Furthermore, through single-cell resolution analysis techniques employed here have led to identification two distinct subpopulations among malignant cells characterized by their expression patterns for T-cell marker genes - specifically those expressing both CD19 and CD138 alongside CD3. Our pseudotime-ordered analyses have indicated that these malignancies marked by co-expression are indicative precursors at an early stage within the context of differentiating from typical WM-B cell lineages. Subsequent validation via colony formation assays has further supported this notion regarding the potential roles played by these specific malignancies as precursors within the context of Waldenström

macroglobulinemia (WM). Building upon our observations related to abnormal expressions seen amongst T-cell markers found on tumor-associated cellular populations linked to Waldenström macroglobulinemia (WM), we posit a working hypothesis implicating prolonged activation stemming from tumor antigen-driven processes leading towards establishment immunosuppressive microenvironments which contribute significantly towards disease pathogenesis within this setting. As such our investigation has sought out elucidating putative molecular underpinnings responsible for immune cell dysfunctions witnessed amidst cases involving Waldenström macroglobulinemia while also identifying instances where precursor exhausted states manifest amongst certain subsets belonging to cytotoxic T-cells alongside noting functional deletions occurring across natural killer (NK) cellular populations too. In addition to these insights highlighted above concerning dysfunctional immune responses encountered during cases tied back towards Waldenström macroglobulinemia(WM), it is worth emphasizing how targeting efforts directed against inhibitory receptor molecule known as 'CD47' could hold promise when seeking ways aimed at restoring normalcy amidst compromised immune functions.

In conclusion, this study has demonstrated that the comparison of gene expression profiles between distinct stages of B cells in patients with WM can provide valuable insights into the underlying biology of this disease and potential therapeutic targets for overcoming resistance to BTK inhibitors and immune cell dysfunction.

A biography

Mu Hao, M.D./Ph.D.
Professor of State Kay Lab of Experimental Hematology,
Institution of Hematology and Blood Disease Hospital, Chinese Academy of Medical Science.

Professor Hao obtained her PhD from Sichuan University of China in 2007 and subsequently completed postdoctoral training in Professor Zhan’ s lab at the University of Iowa, USA. Her research primarily focuses on elucidating the pathogenesis of multiple myeloma and Waldenström macroglobulinemia (WM). WM is a rare and incurable indolent B-cell malignancy, with its molecular pathogenesis and the role of the immunosuppressive microenvironment remaining incompletely understood. Through her study, it was demonstrated that comparative microarray profiles offer comprehensive insights into WM biology. These findings have implications for advancing novel therapies targeting aberrant T-cell markers in WM. Furthermore, her research contributes to a deeper understanding of the biological heterogeneity of tumor cells and the immunosuppressive microenvironment in WM, potentially informing the development of innovative immunotherapies targeting pre-exhausted CD8-T cells in WM. Drug resistance remains a key challenge leading to refractory and relapsed cases in both WM and MM. Professor Hao's current research interests include functional investigation of oncogenes and non-coding RNAs involved in disease development as well as drug resistance, along with exploring tumor microenvironment interactions with tumor cells during the pathogenesis and progression of WM.