1103. Single-cell RNA sequencing Reveals the Spatial Heterogeneity in BTKi-Resistant Richter Transformation Patients

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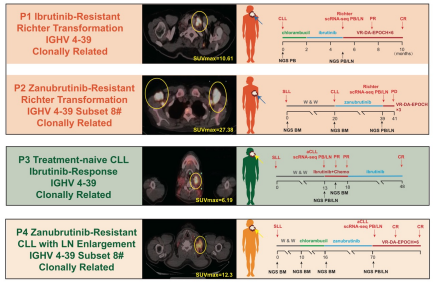
Introduction

- Richter transformation (RT) remains a clinical challenge with poor prognosis to standard treatment. The underlying mechanisms has not been fully disclosed yet, especially the spatial heterogeneity of tumor cells and immune microenvironment when progressed after novel targeted agents like Bruton's Tyrosine Kinase inhibitors (BTKi).

Methods

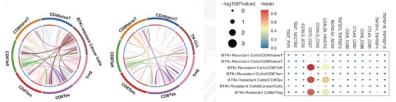
- Single-cell RNA sequencing (scRNA-seq) of paired lymph node (LN) and peripheral blood (PB) was performed among 4 CLL patients and bioinformatics analysis was conducted. Patient 1 and Patient 2 were treated with BTKi (P1 ibrutinib for 3 months and P2 zanubrutinib for 19 months) and then progressed to RT. Patient 3 were initially diagnosed as aCLL and Patient 4 were treated with zanubrutinib for 54 months and then progressed to accelerated CLL (aCLL).



Results

1. BTKi-resistant RT showed MYC targeted pathways activation and metabolic reprogramming, high proliferative cells enriched in LN showed downregulation of MHC class I. GSVA analysis showed significant activation of MYC targeted pathways and oxidative phosphorylation (OXPHOS) in RT. Trajectories of tumor cells were constructed and cells from PB were mainly distributed in the front of trajectories with the enrichment of G1 phase cells, while cells from LNs were mainly distributed in the terminal with S and G2M cells, indicating that the BTKi-resistant aggressive LNs were evolved from PB indolent CLL cells. The expression of MHC genes were displayed in these trajectories and showed gradual downregulation of MHC class I genes including HLA-A、HLA-B、HLA-C and HLA-E in those high proliferative cells, indicating that those BTKi-resistant proliferative cells escape from immune surveillance by the downregulation of MHC class I.

2. BTKi-resistant RT showed strengthened crosstalk between tumor and T cells, the activation of CD70-CD27 and LGALS9 (galectin-9)-HAVCR2 (TIM3) contributed to the enrichment of exhausted T cells in LN. Distribution of T cell subsets between PB and LN showed great spatial heterogeneity, especially in RT after BTKi progression. PB of all four patients showed relatively similar T cells distribution, with CD4+ naïve T cells and CD8+ effector memory T cells being two dominant clusters. However, LN of two RT patients displayed significant enrichment of CD8+ exhausted T cells while CD4+ naïve T cells were dominant cluster in LN of treatment-naïve CLL. Construction of cell-cell interaction displayed that RT showed significantly higher number of interactions with T cell subsets than treatment-naïve CLL, especially the interactions with CD8+ effective and exhausted T cells. Further analysis of receptor-ligand interactions between T cell subsets and tumor cells were conducted and activation of CD70-CD27 and LGALS9 (galectin-9)-HAVCR2 (TIM3) were found between BTKi-resistant tumor cells and CD8+ effective and exhausted T cells, indicating the underlying mechanism contributing to immune escape of RT.



Results

Spatial distribution of CD8+ exhausted T cells showed great heterogeneity, with terminally enhausted T cells specifically enriched in LN. Monocle 2 analysis showed the evolutionary trajectories of T cells subsets. CD8+ naïve T cells were distributed in the initial point of the evolutionary trajectories while CD8+ exhausted T cells showed heterogeneous evolution, with two different evolutionary trajectories in the terminal points. The heterogeneity of CD8+ exhausted T cells were further explored and we found four clusters of exhausted T cells manifesting heterogeneous characteristics. Herein, exhausted CD8+ T cells in PB were mostly Cluster 1, showing highest cytotoxicity and lowest exhausted feature. Cluster 2 was distributed scantly in PB while mostly in LN, showing highest cycling and proliferation score. Cluster 3 was LN specific exhausted T cells, showing highest exhausted score and lowest cytotoxicity score, indicating its terminally exhausted characters.

Conclusion

This study disclosed the spatial heterogeneity of tumor and immune microenvironment between PB and LN among BTKi-resistant RT patients. High proliferative tumor cells enriched in LN showed downregulation of MHC class I and strengthened crosstalk with T cells, contributing to T cell exhaustion and immune escape through the activation of CD70-CD27 and LGALS9-HAVCR2 crosstalk. In summary, we proposed the possible immune escape mechanism of proliferative LN progression under BTKi-resistant treatment and provide novel treatment targets to overcome the drug resistance