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POSTER ABSTRACTS

506.BONE MARROW MICROENVIRONMENT

Oncogenic RAS Signaling Promotes an Immunosuppressive Niche in Acute Lymphoblastic Leukemia

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Emerging evidence suggests that genomic aberrations in cancer cells may impact the composition of the tumor microenvironment leading to a tumor-specific supporting niche. Previous genomic profiling studies have identified somatic alterations in RAS pathway genes that drive treatment resistance in B-cell acute lymphoblastic leukemia (B-ALL): yet it is unknown whether such genetic events may specifically hijack the leukemic niche.

Here, we analyzed bulk RNA-sequencing data of bone marrow mononuclear cells from 185 pediatric B-ALL patients entering our center and performed mutation calling of RAS pathway genes, namely *KRAS, NRAS, BRAF* and *PTPN11*. Transcriptomic analysis revealed attenuated immune responses and decreased T cell activation in samples harboring RAS mutations (n= 62) compared with wild type cases (n= 123). To further explore this finding at single-cell resolution, we sorted bone marrow mononuclear cells from 8 primary B-ALL pediatric patient samples (4 RAS mutant and 4 wild type), enriching on the hematopoietic and non-hematopoietic compartments, followed by single-cell RNA-sequencing using the 10x Genomics platform. Twelve cell populations were identified by the expression of *bona fide* markers, mapping different immune and stromal cells in the ALL landscape. Specifically, the RAS-mutant microenvironment was defined by dysfunctional CD8+ T-cells, characterized by high expression of *TIM-3*, *TIGIT* and *LAG-3*, as well as regulatory T cells, displaying expression of *CTLA-4*, *ICOS* and *FOXP3*, suggesting a RAS-mediated immunosuppressive mechanism.

In light of these changes, we examined flow cytometry data of bone marrow mononuclear cells collected at diagnosis from our cohort of 185 patient samples. Whereas the overall proportion of lymphoid subsets (T, B and NK cells) was not significantly changed among RAS mutant and wild type groups, we detected a decrease in myeloid subsets (neutrophils, eosinophils, and monocytes) in RAS mutant ALL, which was more prominent in monocytes. Cross comparison of the transcriptional state of monocytes in our single-cell data unravel that monocytes in the RAS mutant niche displayed a non-classical phenotype, characterized with high expression of the immune checkpoint *TIM-3*, which is a major driver of T cell exhaustion.

Taken together, the bone marrow microenvironment of ALL patients harbouring oncogenic RAS lymphoblasts is defined by dysfunctional immune cell populations. Further studies on the cellular dynamics in the niche will provide the basis for the development of therapeutic opportunities to target the immune micro-environment in RAS mutant ALL.

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