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Blood 142 (2023) 2695–2696

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

503.CLONAL HEMATOPOIESIS, AGING AND INFLAMMATION

The Roles of Immune Cells Derived from Clonal Hematopoiesis in Colorectal Cancer Metastasis

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[Backgrounds]
Clonal hematopoiesis (CH) has been observed in over 20% of patients diagnosed with solid cancers, and it has been associated with a poor prognosis in most cases, with the exception of colorectal cancer (CRC) patients who tend to have a favorable prognosis. Among the various gene mutations detected in CH, *TET2* mutations are particularly prevalent. Previous research has demonstrated that the impact of *TET2*-mutated CH immune cells depends on the specific types of cancer tissues involved. Specifically, studies have shown that Tet2-KO myeloid cells can inhibit the progression of melanoma while promoting the growth of hepatoma and lung cancers. However, there is a limited understanding of the roles of *TET2*-mutated CH immune cells on CRC progression.
[**Methods**]
We conducted an experiment focusing on CRC and CH using CRC organoid cells transplanted into the spleens of different types of *Tet2* conditional knockout mice: *VAV1Cre* (with *Tet2* gene deletion in all hematopoietic cells), *LysMCre* (myeloid cells), *CD19Cre* (B lymphocytes), and *CD4Cre* (T lymphocytes). After a period of 30 days, we collected livers for analysis. The extent of liver metastasis tumor burden (LMTB) was determined by counting the number of tumor foci on 10 hematoxylin-stained slides, with an interval of 80 μm between each slide. We utilized immunohistochemistry (IHC) and flow cytometry (FC) to analyze immune cells. The number of positive cells was automatically counted in 10 fields at 20x magnification. Furthermore, we performed whole transcriptome analysis (WTA) on sorted CD4+, CD8+, CD11b+, and CD19+ cells, respectively.
[Results]
LMTB was significantly lower in *VAV1Cre* and *CD4Cre* mice compared to the control mice (*VAV1Cre* vs. control: 55.65±35.62 vs. 89.93±34.57 foci/1000 mm², $p<0.05$; *CD4Cre* vs. control: 50.19±30.39 vs. 85.98±7.92 foci/1000 mm², $p<0.05$). However, the LMTB of *CD19Cre* and *LysMCre* mice was comparable to that of control mice. The differential gene expression of WTA of CD8+ cells revealed that 243 genes were up-regulated, while 189 genes were down-regulated in both of *VAV1Cre* and *CD4Cre* mice compared to the control mice. Among the down-regulated genes, a gene group consisting of *Pdcd1, Tigit, Lag3* and *Havcr2*, which encode inhibitory molecules associated with exhausted T cells exhibited enrichment (Enrichment score =3.77) based on the analysis using DAVID. Ingenuity Pathway Analysis further indicated that these molecules were downregulated by the upstream regulator TOX. Furthermore, T cell exhaustion signaling pathways were downregulated in both *VAV1Cre* and *CD4Cre mice* using gene set enrichment analysis. The immunofluorescence results demonstrated that the number of CD8-T cells expressing PDCD1 and HAVCR2 was decreased in *VAV1Cre* mice and *CD4Cre* compared to the control mice (*VAV1Cre* and *CD4Cre* vs control; 5.91±2.50 and 12.86±5.3 vs. 35.15±8.21 cells/100 CD8-T cells, $p<0.01$ for PDCD1; 4.51±4.06 and 13.65±7.84 vs. 24.51±9.94 cells/100 CD8-T cells, $p<0.01$ for HAVCR2). Moreover, the number of CD8-T cells expressing TOX was also significantly decreased in *VAV1Cre* mice and *CD4Cre* compared to the control mice (*VAV1Cre* and *CD4Cre* vs control; 5.19±7.48 and 11.31±14.43 vs. 35.15±16.50 cells/100 CD8-T cells; $p<0.01$).
[**Conclusions**] The depletion of the *Tet2* gene in hematopoietic cells resulted in a reduction of exhausted CD8+ T cells and suppressed the liver metastasis of CRC organoid cells. These findings provide insights into the favorable prognosis observed

in CRC patients with CH. Considering the high prevalence of CH in cancer patients, our results suggest that studying the roles of CH-immune cells could shed new light on understanding the cancer microenvironment from a novel perspective.

Disclosures Chiba: *Thyas:* Research Funding; *Astellas:* Research Funding; *Kyowa Kirin:* Research Funding; *Chugai Pharmaceutical:* Honoraria; *Bayer Pharma:* Honoraria; *Eisai Co., Ltd.:* Honoraria. **Sakata-Yanagimoto:** *Eisai Co., Ltd.:* Research Funding; *Mundipharma K.K.:* Research Funding; *Otsuka Pharmaceutical.:* Research Funding; *Kyowa Kirin Co.,Ltd.:* Honoraria.

https://doi.org/10.1182/blood-2023-185813