P1301 BIOLOGICAL SIGNIFICANCE OF UCK2 IN HTLV-1-INFECTED CELLS IN ATL LEUKEMOGENESIS AND ACQUIRED RESISTANCE TO AZACITIDINE

Topic: 20. Lymphoma Biology & Translational Research

Tatsuro Watanabe1, Yuta Yamamoto2, Kazuharu Kamachi1, 2, Yuki Kurahashi1, 2, 3, Nao Yoshida-Sakai1, 2, Hiroshi Ureshino1, 2, Yuki Fukuda-Kurahashi1, 3, Hideaki Nakamura4, Eisaburo Sueoka5, Shinya Kimura1, 2

1Department of Drug Discovery and Biomedical Sciences, Saga University, Saga, Japan; 2Division of Hematology, Respiratory Medicine and Oncology, Department of Internal Medicine, Faculty of Medicine, Saga University, Saga, Japan; 3OHARA Pharmaceutical Co., Ltd., Shiga, Japan; 4Department of Transfusion Medicine, Saga University Hospital, Saga, Japan; 5Department of Clinical Laboratory Medicine, Saga University, Saga, Japan

Background: Adult T-cell leukemia-lymphoma (ATL) is an aggressive hematological malignancy of CD4+ T-cells transformed by human T-cell lymphotropic virus-1 (HTLV-1). We have reported that regional DNA hypermethylation in HTLV-1-infected T-cells reflects the disease status of ATL and the anti-ATL effects of DNA demethylating agents, azacitidine (AZA) and decitabine (Blood 2020, 136: 871-884). We recently generated AZA-resistant (AZA-R) cells from ATL cell lines via long-term drug exposure and found down-regulation of uridine cytidine kinase2 (UCK2) correlated with lower susceptibility to AZA (Int J Cancer 2022, 150: 1184-1197). UCK1 and UCK2 are involved in mono-phosphorylation of uridine and cytidine in pyrimidine nucleotide biosynthesis. Both proteins also catalyze mono-phosphorylation of AZA. Overexpression of UCK2 has been observed in several types of solid tumor tissues.

Aims: Here, we aimed to understand the role of UCK2 in HTLV-1-infected cells during multi-step carcinogenesis of ATL and the biological significance of inactivation of UCK2 in AZA-R cells.

Methods: All studies using human samples were performed in accordance with the guidelines set out in the Declaration of Helsinki and were approved by the institutional review board at Saga University (2018-03-02). Peripheral blood mononuclear cells (PBMCs) were isolated from peripheral blood samples from healthy volunteers, HTLV-1 carriers and patients with ATL. ATL cell lines were cultured in RPMI-1640 medium containing 10% FBS.

Results: We found that UCK2 gene expression was higher in HTLV-1-infected T-cells than in normal T-cells using the gene expression datasets GSE55851 and GSE33615. UCK2 protein expression was increased in HTLV-1-infected T-cells (CADM1+ cells) isolated from patients with ATL compared with uninfected T-cells (Fig. A). We also found UCK2 gene expression was upregulated via activation of T-cell receptor (TCR) signaling pathway in normal CD4+ T-cells (Fig. B). Somatic alterations and epigenetic modifications activating TCR signaling have been identified in ATL cells. Therefore, we think UCK2 protein is overexpressed through dysregulated TCR signaling in HTLV-1-infected cells during ATL leukemogenesis. We have successfully established AZA-R cell lines, whose UCK2 expression was lost or decreased. Although they proliferated normally in vitro, the knockdown of UCK2 dramatically suppressed cell growth of ATL cell lines (Fig. C). Since UCK2 is involved in pyrimidine nucleotide biosynthesis, we then performed metabolome analysis in AZA-R cells. Although uridine and cytidine were accumulated in AZA-R cells, possibly due to loss of UCK2, the amount of UTP and CTP was almost same with parental cells. On the other hands, dihydroorotic acid (DHO) was decreased and orotate was increased in AZA-R cells (Fig. D). DHODH catalyzes the oxidation of DHO to orotic acid and is a rate-limiting enzyme in de novo pyrimidine nucleotide synthesis. AZA-R cells were more susceptible to BAY2402234, a DHODH inhibitor (Fig. E), indicating the activation of pyrimidine de novo synthesis in AZA-R cells.

Image:
Summary/Conclusion: Taken together, we think UCK2 supports the vigorous cell proliferation not only in normal TCR-stimulated T-cells but also in HTLV-1-infected cells. Inactivation of UCK2 makes cells resistant to AZA but suppresses the cell growth possibly due to the pyrimidine nucleotide starvation. AZA-R cells with reduced UCK2 expression proliferate normally in vitro by the activation of pyrimidine de novo synthesis.