ELSEVIER

Blood 142 (2023) 1370–1371

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

602.MYELOID ONCOGENESIS: BASIC

Regulation of Metabolic Homeostasis By TRAF6 Contributes to the Leukemia Progression

Shinichiro Matsui, MDPhD[1,2], Chihiro Ri, MD[3], Lyndsey C. Bolanos, MBA, BSc[4], Kwangmin Choi, PhD[4], Asuka Shibamiya, MDPhD[3], Arata Ishii, MD PhD[3], Koji Takaishi, MD PhD[3], Nagisa Oshima-Hasegawa, MDPhD[3], Shokichi Tsukamoto, MDPhD[3], Yusuke Takeda, MD PhD[3], Naoya Mimura, MD PhD[5,3], Koutaro Yokote, MD PhD[2], Daniel T. Starczynowski, PhD[4,6,7], Emiko Sakaida, MDPhD[8,2], Tomoya Muto, MD PhD[9,3]

[1]Department of Hematology, Chiba University Hospital, Chiba-Shi, Japan
[2]Department of Endocrinology, Hematology and Gerontology, Chiba University Graduate School of Medicine, Chiba, Japan
[3]Department of Hematology, Chiba University Hospital, Chiba, Japan
[4]Division of Experimental Hematology and Cancer Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH
[5]Department of Transfusion Medicine and Cell Therapy, Chiba University Hospital, Chiba, Japan
[6]Department of Cancer Biology, University of Cincinnati, Cincinnati, OH
[7]Department of Pediatrics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH
[8]Department of Hematology, Chiba University Hospital, Chiba City, Japan
[9]Division of Cancer RNA Research, National Cancer Center Research Institute, Tokyo, Japan

TNF receptor associated factor 6 (TRAF6) is an E3 ubiquitin ligase that mediates innate immune signaling that converges on key mediators of Toll-like receptor and interleukin-1 signaling. Dysregulated TRAF6 expression and innate immune pathway activation is implicated in the pathogenesis of various myeloid malignancies. For instance, increased TRAF6-mediated signaling in hematopoietic stem and progenitor cells (HSPCs) results in myelodysplastic syndrome-like cellular features, such as the functional impairment of HSPCs. On the other hand, our previous study indicated that loss of TRAF6 induces the leukemic transformation of pre-leukemic cells (*Muto et al.* Cell Stem Cell). These paradoxical observations suggests that TRAF6 acts as both an oncogene and tumor suppressor gene in hematopoietic cells in a disease type- and context-dependent manner. However, the direct consequence of TRAF6 loss in AML cells, rather than in pre-leukemic cells, has not yet been explored. Therefore, the role of TRAF6 in the pathogenesis of AML remains obscure. In this study, we found elevated level of TRAF6 expression in AML cells, and determined the effect of TRAF6 loss on the cellular and molecular features of AML cells. We proposed a novel role of TRAF6 as a regulator of metabolic homeostasis, which is important for the progression of AML.

To determine the requirement of TRAF6 in AML, we first evaluated cell the effect of TRAF6 loss in AML cell lines on proliferative capacities. Multiple types of human AML cell lines showed significant growth impairment upon TRAF6-knockdown. Furthermore, transplantation of TRAF6-deficient HSPCs expressing the leukemic oncogene *MLL-AF9* to the recipient mice resulted in suppression of overt leukemia. These findings confirm that loss of TRAF6 suppresses leukemic cell function *in vitro* and *in vivo*.

To assess the molecular mechanism of inhibitory effect of TRAF6 loss in AML, we next performed gene expression analysis using human AML database and TRAF6-knockdown AML cell lines. Interestingly, we found that reduced levels of TRAF6 in AML is associated with changes in the expression of genes involved in mitochondria-related processes. Our extensive *in vitro* validation experiments including extracellular flux assay and metabolome analysis confirmed that TRAF6 loss in AML cells induces dynamic changes in metabolites and the impairment of mitochondrial function.

To identify the mechanism of metabolic alterations by TRAF6 loss in leukemia, we compared the upregulated genes in TRAF6-knockdown AML cells with the 130 previously identified essential genes for AML cell survival in vitro and in vivo (*Yamauchi et al.* Cancer Cell). We focused on O-GlcNAc transferase (*OGT*) as a gene associated in mitochondrial function and AML cell survival. Importantly, *TRAF6* gene expression in human AML patient samples was positively correlated with the expression of *OGT*. To confirm the importance of OGT in the regulation of mitochondrial function mediated by TRAF6 in leukemia, we utilized the inhibitor of O-GlcNAcase (OGA), a remover of O-GlcNAc from its substrates. The treatment with MK8719 (OGA inhibitor) mildly, but significantly, restored the number of TRAF6 knockdown AML cells, which was correlated with the changes in mitochondrial function, indicating that O-GlcNAc modification regulated by TRAF6 is important for AML progression. In

summary, We provide evidence for the oncogenic function of TRAF6 in leukemia, and shed light on the novel TRAF6/OGT/O-GlcNAc axis that regulates the metabolic reprogramming required for leukemogenesis.

Disclosures Bolanos: *Kurome:* Consultancy. **Mimura:** *Chigai:* Research Funding. **Starczynowski:** *Captor Therapeutics:* Consultancy; *Kurome Therapeutics:* Consultancy, Current equity holder in private company, Membership on an entity's Board of Directors or advisory committees, Patents & Royalties, Research Funding; *Treeline Biosciences:* Research Funding; *Sumitomo Pharma Oncology:* Research Funding; *Tolero Therapeutics:* Research Funding; *Kymera Therapeutics:* Consultancy. **Sakaida:** *Takeda:* Speakers Bureau; *Kyowa-Kirin:* Research Funding; *Chugai:* Research Funding; *Human Life Cord Japan:* Consultancy; *Ohara:* Consultancy; *Otsuka:* Consultancy; *Pfizer:* Consultancy, Speakers Bureau; *Novartis:* Consultancy, Speakers Bureau; *Bristol Myers Squibb:* Consultancy, Research Funding, Speakers Bureau; *Janssen:* Speakers Bureau.

https://doi.org/10.1182/blood-2023-178962