論文の内容の要旨

論文題目　BAALC potentiates oncogenic ERK pathway through interactions with MEKK1 and KLF4.

（急性骨髄性白血病細胞において BAALC は MEKK1 および KLF4 との相互作用を介して腫瘍形成性 ERK 経路を活性化している）

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Acute myeloid leukemia (AML) is a clonal disorder characterized by differentiation arrest and accumulation of immature myeloid progenitors in the bone marrow, resulting in hematopoietic failure. Although modern chemotherapy together with hematopoietic stem cell transplantation has improved survival of patients with AML and offered a cure in a subset of patients, more than half of young adults and about 90% of elderly patients still finally succumb to their diseases. Thus, novel therapeutic approaches based on molecular characteristics of individual patients are urgently needed. To date, several poor prognostic factors for AML have been reported, which include gene mutations in TET2, ASXL1 or DNMT3A and overexpression of ERG, EVI1, MN1 or BAALC. Among these genes, pathogenesis of brain and acute leukemia, cytoplasmic (BAALC) is mostly unknown. Previous reports suggest that in normal hematopoiesis, the expression of BAALC is limited to the immature fraction, i.e. $CD34^{+}$ fraction rich in hematopoietic stem and progenitor cells. In a large cohort of

patients with AML, high BAALC expression was associated with expressions of stem cell markers. From these facts, it is suggested that BAALC is related to immaturity of hematopoietic cells. In this report, we unveiled the molecular function of BAALC in propagation and maintenance of AML cells and show promising therapeutic targets for BAALC-high AML.

When BAALC was overexpressed in HEL cells, a BAALC-low expressing AML cell line, they showed increased proliferative capacity with accelerated cell cycle progression. In contrast, knockdown of BAALC by shRNA in Kasumi-1 cells, a BAALC-high expressing AML cell line, decreased its proliferative capacity and the cell cycle progression was decelerated. To gain insight into molecular functions of BAALC, we next explored interacting partners of BAALC with a yeast two-hybrid system on a proteome-wide scale. Using BAALC as bait and a cDNA library from adult human tissues as prey, we identified mitogen-activated protein (MAP) kinase kinase kinase 1 (MEKK1) and Krüppel-like factor 4 (KLF4) as potential binding partners of BAALC. These interactions were further affirmed by in vitro pull-down assay. Considering that MEKK1 and KLF4 are both involved in extracellular signal-regulated kinase (ERK) pathway, we postulated that BAALC induces leukemia cell proliferation by potentiating ERK signaling. Through phosphorylated protein screening of cytoplasmic signaling-related molecules, we indeed found that forced expression of BAALC specifically activated ERK pathway. Co-immunoprecipitation assay revealed that BAALC binds to MEKK1 and works as an adaptor protein, which inhibits the interaction between ERK and its specific phosphatase MAP kinase phosphatase 3 (MKP3/DUSP6). Previous data suggest that prolonged ERK pathway activation induces

monocytic differentiation as well as proliferation of AML cells. It is of note that excess ERK pathway potentiation induces accumulation of KLF4 in the nucleus and this step is critical for monocytic differentiation of AML cells. We discovered in immunofluorescence assay that BAALC keeps KLF4 in the cytoplasm and inhibits the KLF4-dependent differentiation of leukemia cells. Finally, we developed a BAALC-high AML model mice using NOD/SCID/gamma (NSG) mouse to investigate the combinational effect of MEK inhibition and KLF4 induction, and found that MEK inhibition synergizes with KLF4 induction to suppress the growth of AML cells in vivo. Our data provide a molecular basis for the role of BAALC in regulating proliferation and differentiation of AML cells and present novel therapeutic targets for BAALC-high AML.