東北医科薬科大学

審査学位論文（博士）要旨

氏名（本籍）	リュウ　　ケンウェイ 劉　　健　偉　（中国）
学位の種類	博士（薬科学）
学位記番号	博薬学第 27 号
学位授与の日付	令和 6 年 3 月 8 日
学位授与の要件	学位規則第 4 条 1 項該当
学位論文題名	Deubiquitinase BRCC36 associates with FLT3-ITD to regulate its protein stability and intracellular signaling in acute myeloid leukemia （脱ユビキチン化酵素 BRCC36 は急性骨髄性白血病において FLT3-ITD との結合でタンパク質の安全性と細胞内シグナル伝達を制御する）
論文審査委員	主査　　教 授　山口　芳樹
	副査　　教 授　藤村　　務
	副査　　教 授　顧　　建国

Deubiquitinase BRCC36 associates with FLT3-ITD to regulate its protein stability and intracellular signaling in acute myeloid leukemia

（脱ユビキチン化酵素 BRCC36 は急性骨髄性白血病において FLT3-ITD との結合でタンパク質の安定性と細胞内シグナル伝達を制御する）

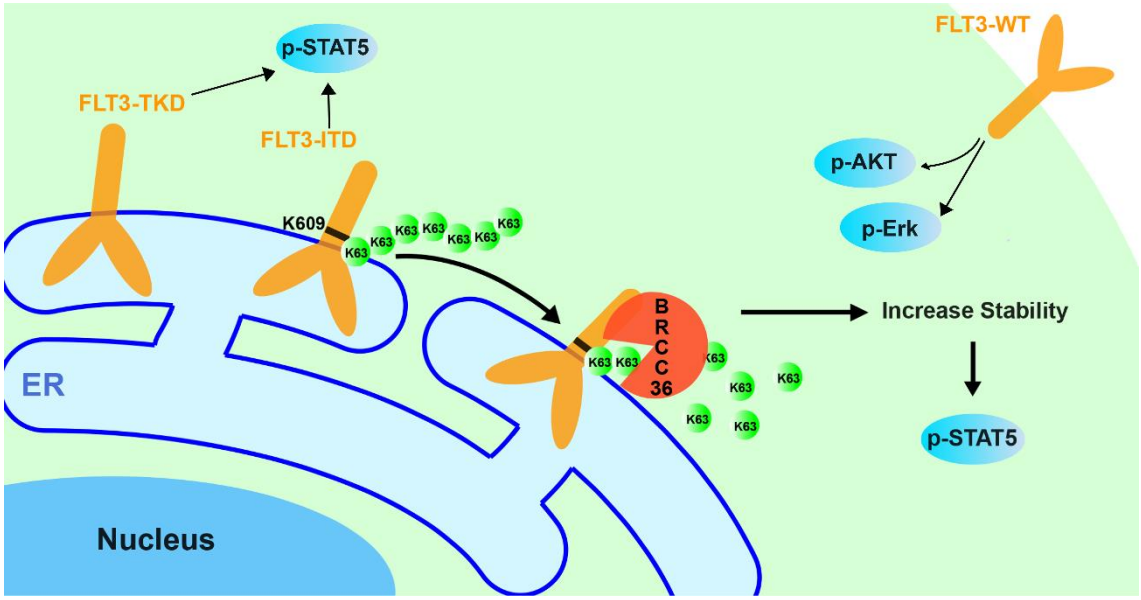
東北医科薬科大学　大学院薬学研究科

細胞制御学教室　劉健偉

Acute myeloid leukemia (AML) is adults' most common form of acute leukemia. It represents the deadliest type of this disease. Fms-like tyrosine kinase 3 (FLT3) is one of the most frequently mutated genes in AML. Internal tandem duplication (ITD) of the juxtamembrane domain of FLT3 is the primary kinase mutation in human AML, and the other predominant point mutation is the tyrosine kinase domain (TKD) mutation, as shown in the figure. It has been known that there are two forms of human FLT3; one is a mature form at around 150 kDa, which is thought to be fully N-glycosylated and is then expressed on the cell surface to activate MAPK signaling pathways efficiently. The other is an immature form at around 130 kDa, mainly localized in the ER. ITD and TKD can induce a robust activation of STAT5.

FLT3-ITD and FLT3-TKD exhibited distinct protein stability, cellular localization, and intracellular signaling. To understand underlying mechanisms, we performed proximity labeling with TurboID to identify proteins that regulate FLT3-ITD or TKD differently. The biotinylated proteins were enriched with streptavidin beads and identified by mass spectrometry. We found that BRCA1/BRCA2-containing complex subunit 36 (BRCC36), a specific K63-linked polyubiquitin deubiquitinase, was exclusively associated with ITD, not the wild type of FLT3 and TKD. Knockdown of BRCC36 resulted in decreased STAT5 phosphorylation and cell proliferation in ITD cells. Consistently, treatment with thiolutin, an inhibitor of BRCC36, specifically suppressed cell proliferation and induced cell apoptosis in ITD cells. Thiolutin

efficiently affected leukemia cell lines expressing FLT3-ITD cell viability and exhibited mutual synergies with quizartinib, a standard clinical medicine for AML. Furthermore, mutation of the lysine at 609 of ITD led to significant suppression of K63 polyubiquitination and decreased its stability, suggesting that K609 is a critical site for K63 ubiquitination specifically recognized by BRCC36 (figure). These data indicate that BRCC36 is a specific regulator for FLT3-ITD, which may shed light on developing a novel therapeutic approach for AML.



Schematic diagram of the differences in structure and localization among wild-type and two main variants of FLT3, and the proposed molecular mechanism for the specific interaction between FLT3-ITD and BRCC36 for regulating cell functions.

Reference

Jianwei Liu, Tomoya Isaji, Sachiko Komatsu, Yuhan Sun, Xing Xu, Tomohiko Fukuda, Tsutomu Fujimura, Shinichiro Takahashi and Jianguo Gu. BRCC36 associates with FLT3-ITD to regulate its protein stability and intracellular signaling in acute myeloid leukemia.

Cancer Sci. 2024. In press.