論　文　内　容　要　旨

Biological implications of somatic DDX41 p.R525H mutation in acute myeloid leukemia

（急性骨髄性白血病における DDX41 p.R525H 体細胞変異の生物学的意義）

Experimental Hematology, 44(8): 745-754, 2016

主指導教員：一戸　辰夫　教授

（原爆放射線医科学研究所　血液・腫瘍内科学）

副指導教員：稲葉　俊哉　教授

（原爆放射線医科学研究所　がん分子病態研究分野）

副指導教員：本田　　浩章　教授

（原爆放射線医学研究所 疾患モデル解析研究分野）

角野 萌

（医歯薬保健学研究科　医歯薬学専攻）

Current comprehensive sequence approaches led to the identification of rare but reproducible somatic gene mutations in myeloid malignancies. Among them, there is a somatic mutation in the DDX41 gene encoding a DEAD-box type ATP-dependent RNA helicase. The somatic mutation in DDX41 is highly concentrated at c.G1574A (p.R525H) in the conserved motif VI, located at the C-terminus of the RecA-like helicase core domain where ATP interacts and is hydrolyzed. Therefore, it is likely that the p.R525H mutation in the DDX41 protein perturbs ATPase activity in a dominant-negative manner. In addition, more recently, germ line mutations in DDX41 were isolated in a subset of familial acute myeloid leukemia (AML) /myelodysplastic syndrome (MDS) pedigrees. However, the roles of these somatic and germline mutations in the pathogenesis of myeloid disease are not completely understood. In this study, we screened for the DDX41 mutation of CD34-positive tumor cells based on mRNA sequencing and identified the p.R525H mutation in three cases among 23 patients. Intriguingly, these patients commonly exhibited AML with peripheral blood cytopenias and low blast counts, suggesting that the mutation inhibits the growth and differentiation of hematopoietic cells.

In our study, although the enforced expression of WT DDX41 in cord blood derived cells had minimal effects, substitution of p.R525H induced cell cycle arrest, suggesting that this alteration interferes with cell growth in dominant-negative manner. In addition, as expected, the inhibitory effect of p.R525H DDX41 on cell cycle progression is due to the loss of ATPase activity. Unexpectedly, the cell cycle arrest is MDM2 and RB-dependent, but p53-independent. Our results further indicate DDX41 p.R525H mutation contributes to the pathogenesis of myeloid diseases through causing defects in rRNA synthesis and ribosomal biogenesis. We found that ribosome biogenesis was widely affected when cord blood-derived CD34-positive cells were enforcedly expressed with DDX41 p.R525H. Because it has been shown that some spliceosomal factors, such as hPrp43/DHX15 RNA helicase, also participate in pre-rRNA processing, DDX41 could play multiple roles. We think that this pathway can at least partly account for the development of AML with cytopenias harboring the DDX41 p.R525H mutation. Hematopoietic stem cells have a low level of protein synthesis compared with differentiating or growing progenitor cells. Assuming that AML stem cells with the DDX41 p.R525H mutation are constitutively in a low-protein synthesis status due to ribosomal stress, the cells could be able to be maintained under this stress, but they cannot proliferate or differentiate, which would explain in part the pathophysiology of a slowly growing AML.

In summary, we propose a mechanism of growth defect in hematopoietic cells triggered by p.R525H DDX41 occuring in the following order: (1) the p.R525H mutant inhibits pre-rRNA processing; (2) compromised ribosomal biogenesis as a result of impaired rRNA synthesis

causes a release of ribosomal protein that bind to MDM2; and (3) MDM2-mediated RB degradation is suppressed, eventually activating the RB pathway and resulting in the inhibition of E2F activity. Although this study uncovered a pathogenic role of p.R525H DDX41 in the slow growth rate of tumor cells, how the mutation induces AML development and inhibits cell differentiation is still not understood. Lethally irradiated mice transplanted with hematopoietic stem/progenitor cells overexpressing p.R525H DDX41 did not develop myeloid malignancy even in the p53 deficient background. Considering the late occurrence of AML in patients harboring the mutation, age-dependent epigenetic alterations or other somatic changes may be required for this mutation to transform hematopoietic cells fully.