中文題目：在506位原發性急性骨髓性白血病病患中*DNMT3A*基因突變在致癌機轉上的角色與臨床表徵

英文題目： *DNMT3A* Mutations in 506 *de novo* Acute Myeloid Leukemia Patients: Distinct Clinical-Biologic Features and Prognostic Implication

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Background:

Acute myeloid leukemia (AML) is a relentless hematologic malignancy characterized by precursor cells overproduction and impaired differentiation with great variability in the pathogenesis and clinical course. *DNMT3A* encodes the enzyme DNA methyltransferase (DNMT) 3A which play a crucial role in epigenetic regulation. Recently, mutations in *DNMT3A* were identified in patients with hematologic diseases, including AML. In this study, we aimed to investigate the role of *DNMT3A* mutation in the leukemogenesis of *de novo* AML patients.

Materials and Methods:

Mutation analysis of *DNMT3*A exons 2-23 was performed by polymerase chain reaction and direct sequencing in 506 *de novo* AML patients. Their interaction with clinical parameters, chromosomal abnormalities and mutations of Class I, such as *C-KIT, FLT3/*ITD*, FLT3/*TKD*, NRAS, KRAS, JAK2* and *PTPN11,* Class II, such as *CEBPA, MLL/*PTD and *AML1/RUNX1* and other mutations, such as *WT1, NPM1, ASXL1, IDH1, IDH2* and *TET2* were analysed for total patients before chemotherapy.

Results:

DNMT3A mutations were identified in 14% of total patients and 22.9% of patients with normal karyotype (CN-AML). Excluding the eight single nucleotide polymorphisms (P9P,

S267S, G291G, A398A, P385P, L422L, V435V, and V563V) that were detected in 316 patients but did not alter the amino acid residues and the seven missense mutations (C586W, P896L, G543C, Y735C, A644T, G699D and G707D) that were found in six patients but with uncertain biologic significance because they were not reported previously and could not be verified due to lack of matched marrow samples at complete remission (CR), 30 different kinds of *DNMT3A* mutations were identified in 70 patients. Twelve were missense mutations, eight were nonsense mutations, nine were frame-shift mutations and one, in-frame mutation. The most common mutation was R882H (26 patients), followed by R882C (15 patients), R882S (3 patients), R736H (3 patients) and R320X (2 patients).

DNMT3A mutations were closely associated with older age, higher white blood cell (WBC) and platelet counts at diagnosis. Patients with FAB M5 subtype of AML had the highest incidence (50%, P<0.0001) of *DNMT3A* mutation followed by those with M4 subtype (22.6%, P=0.0026). *DNMT3A* mutations were positively associated with the expression of CD13 and CD14, but inversely associated with the expression of CD34 on the leukemic cells.

With regarding to cytogenetics, *DNMT3A* mutations occurred more frequently in patients with intermediate-risk cytogenetics (19.5%) than in those with favorable karyotype or unfavourable cytogenetics (2.4%, P=0.0069). There was also a significant difference in the incidence of the *DNMT3A* mutation among patients with normal karyotype (22.9%), simple abnormalities with one or two changes (6.2%) and complex cytogenetics with three or more abnormalities (3.9%, P<0.0001). None of the patients with t(8;21), t(15;17) inv(16) or 11q23 translocation showed *DNMT3A* mutation.

To investigate the interaction of gene mutations in the pathogenesis of adult AML, a complete mutational screening of 16 other genes was performed. Among the 70 patients with *DNMT3A* mutations, 68 (97.1%) showed additional molecular abnormalities at diagnosis. Fifteen had one additional change, 37 had two, 13 had three and three had four. The most common associated molecular event was *NPM1* mutation (38 cases), followed by *FLT3*-ITD (30 cases), *IDH2* mutation (16 cases) and *FLT3*-TKD (9 cases). Patients with *DNMT3A* mutations had significantly higher incidences of *NPM1* mutation, *FLT3*-ITD, *IDH2* and *PTPN11* mutations than those with *DNMT3A*-wild type (54.3% *vs.* 15.3%, P<0.0001; 42.9% *vs.* 19.3%, P<0.0001; 22.9% *vs.* 9.1%, P=0.0016; and 10% *vs.* 3.5%; P=0.007, respectively). On the contrary, *CEBPA* was rarely seen in patients with *DNMT3A* mutations (4.3% *vs.* 14.7%, P=0.0134)**.** Among the 68 patients with concurrent other genetic alterations, fifty-one (75%) had at least one concomitant Class I mutations; 16

(23.5%), Class II mutations; and 38 (54.3%), *NPM1* mutations which behave more like Class II mutations. Totally, 40 patients (58.8%) had concurrent both Class I and Class II or *NPM1* mutations at diagnosis.

With a median follow-up of 55 months (ranges, 1.0 to 160), patients with *DNMT3A* mutation had significantly poorer overall survival (OS) and relapse-free survival (RFS) than those without *DNMT3A* mutation (median, 14.5 months *vs*. 38 months, P =0.013, and medium, 7.5 months *vs.* 15 months, P=0.012, respectively). In the subgroup of 130 younger patients (less than 60 years) with CN-AML, the differences between patients with and without *DNMT3A* mutation in OS (median, 15.5 months *vs.* not reached, P= 0.018) and RFS (median, 6 months *vs.* 21 months, P=0.004) were still significant. Multivariate analysis demonstrated that *DNMT3A* mutation was an independent poor prognostic factor for OS and RFS among total patients (HR 2.218, 95% CI 1.333-3.692, P=0.002 and HR 2.898, 95% CI 1.673-5.022, P<0.001, respectively) and CN-AML group (HR 2.303, 95% CI 1.088-4.876, P=0.029 and HR 3.496, 95% CI 1.773-6.896, P<0.001, respectively). Further, a scoring system incorporating *DNMT3A* mutation and eight other prognostic factors, including age, WBC count, cytogenetics, and gene mutations (*NPM1/FLT3*-ITD, *CEBPA*, *AML1/RUNX1*, *WT1*, and *IDH2* mutations*)*, into survival analysis was proved to be very useful to stratify AML patients into different prognostic groups (P<0.001).

DNMT3A mutations were serially studied in 316 samples from 138 patients, including 35 patients with distinct *DNMT3A* mutations and 103 patients without mutation at diagnosis. Among the 34 patients with *DNMT3A* mutations who had ever obtained a CR and had available samples for study, 29 lost the original mutation at remission status, but five retained it; all these five patients relapsed finally within a median of 3.5 months and died of disease progression, suggesting presence of leukemic cells. In the 13 patients who had available samples for serial study at relapse, all patients regained the original mutations, including mutant clone was found by TA cloning in one patient. Among the 103 patients who had no *DNMT3A* mutation at diagnosis, none acquired *DNMT3A* mutation at relapse, while karyotypic evolution was noted at relapse in 39% of them

Conclusion:

DNMT3A mutations could be detected in a substantial proportion of patients with *de novo* AML and were closely associated with distinct clinical and biological features and was a poor prognostic factor in AML patients. Furthermore, the mutation may be a potential biomarker for monitoring of minimal residual disease.