論文の内容の要旨

論文題目　Prognostic value of somatic mutations at remission in acute myeloid leukemia（急性骨髄性白血病の寛解期に検出される遺伝子変異の臨床的意義）

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[Background]

Intensive induction chemotherapy renders complete remission (CR) in 70% of patients with acute myeloid leukemia (AML), however, half of these patients eventually experience relapse. Therefore, predicting the risk of relapse is an imminent challenge in AML.

Risk stratification of AML patients has been attempted mostly based on the pretreatment biomarkers such as cytogenetic abnormalities and somatic mutations as represented by ELN classification, yet, the single-timepoint snapshot of molecular aberrations at diagnosis does not take account of the heterogeneous behavior of individual AML subclones in response to therapies. Post-treatment biomarkers may be more powerful to predict the risk of relapse, since they do take account of how leukemic cells respond to chemotherapy in AML patients. Indeed, residual cytogenetic abnormalities at CR are considered to be an indicator for risk of relapse, and detection of minimal residual disease (MRD) by quantitative polymerase chain reaction or multicolor flow cytometry have been shown to be useful for prognostication. Recently, there has been a growing interest in using somatic mutations as a molecular MRD marker in AML. However, except for the use of *NPM1* mutation, the utilization of other somatic mutations in MRD assessment has been confounded, partly because some mutations (*DNMT3A*, *TET2*, and *ASXL1*) are often preleukemic-origin and persistence of these mutations does not necessarily represent “residual leukemia”, but rather, it might reflect the regression towards clonal hematopoiesis of indeterminate potential (CHIP). In contrast, a study by Klco and colleagues showed that persistence of somatic mutations at remission was predictive of worse survival in AML. Getta and colleagues also showed that persistent somatic mutations prior to allogeneic stem cell transplantation (allo-SCT) were associated with poor outcome.

These data suggest that, although clinical utility of an individual mutation as an MRD marker remains elusive, persistence of somatic mutations as a whole or as a group of selected genes may serve as a molecular MRD marker in AML.

[Method]

Patients

We analyzed previously untreated AML patients who received idarubicin plus cytarabine (IA)-based frontline intensive induction chemotherapy and attained CR around day 30. Sixty patients (46%) underwent allo-SCT at first CR.

DNA sequencing

Somatic mutations in the paired bone marrows obtained at diagnosis and at day 30 CR were detected by targeted capture DNA sequencing for 295 genes (median 257x coverage in pre-treatment samples and 575x coverage in CR samples). We defined three levels of mutation clearance (MC) based on the variant allele frequency (VAF) of residual mutations at CR: (1) MC2.5, if at least one mutation persisted with a VAF of < 2.5%, (2) MC1.0, if at least one mutation persisted with a VAF of < 1%, and (3) complete mutation clearance (CMC) if there were no persistent mutations.

MRD assessment by multicolor flow cytometry

MRD was also assessed by multicolor flow cytometry on the same CR marrow as part of the routine clinical workup. Briefly, cells were stained with standardized 7- to 8- colored fluorescence combinations and were analyzed on FACSCanto II with FACSDiva software (BD Biosciences) and FCS Express (De Novo Software). The data was interpreted by in-house board certified hematopathologists.

Statistical analysis

Overall survival (OS) was calculated from the date of CR to the date of death from any causes. Patients who were alive were censored on the date of last follow-up. Event-free survival (EFS) was calculated from the date of CR to the date of relapse, or death for those who did not have relapse. Patients who were alive without relapse were censored on the date of last follow-up. Cumulative incidence of relapse (CIR) was calculated from the date of CR to the date of relapse, considering death without relapse as a competing event. Categorical variables were compared using chi-square or Fisher's exact test. Continuous variables were analyzed by a *t* test or a Mann-Whitney U test. A Kaplan-Meier plot was used to visualize survival distributions. Differences in survival between groups were analyzed using a log-rank test. Gray's method was used for CIR analysis. Adjustment for multiple testing was performed by either Bonferroni or Benjamini-Hochberg method. For multivariate analysis, we used a Cox proportional hazards model for OS and EFS, and a Fine-Gray proportional hazards model for CIR. We considered $p<0.05$ as statistically significant.

[Result]

Pattern of mutation clearance was different among mutated genes and molecular pathways

In the pre-treatment samples, a total of 428 high-confidence somatic mutations (250 single-nucleotide variants [SNVs] and 178 small insertions and deletions [indels]) were detected in 73 genes in 122 patients (93%) (median 3 mutations per patient). The most frequently mutated genes were *NPM1* in 37 patients (28%), followed by *DNMT3A* in 32 (24%), *FLT3* in 29 (22%), and *CEBPA* in 20 patients (15%). The median VAF of the pretreatment mutations was 0.30.

In the paired CR samples, 125 mutations (101 SNVs and 24 indels) were detected in 35 genes in 64 patients, including 119 mutations that were also detected in the pretreatment samples, and 6 CR-specific mutations that were undetectable at pre-treatment. The median VAF of the mutations in CR samples was 0.06. MC2.5 was attained in 75 patients (57%), MC1.0 in 64 (49%), and CMC in 59 (45%). Rate of MC varied by the mutated genes. Mutations in *NPM1*, *CEBPA,* and *FLT3* showed a high rate of MC, whereas *ASXL1*, *DNMT3A, TET2, TP53,* and *SRSF2* mutations showed poor MC.

By molecular pathway, mutations in hematopoietic transcription factors or receptor tyrosine kinase genes had higher MC rates, whereas mutations associated with CHIP, DNA methylation and RNA splicing had lower MC rates.

Analysis of longitudinal samples obtained during and after consolidation chemotherapy showed that consolidation chemotherapy was beneficial in clearing some of the residual mutations but it may not be effective in clearing preleukemic mutations.

Clearance of somatic mutation at CR is associated with better survival and lower risk of relapse

With a median follow-up duration of 35.2 months, 51 patients (39%) relapsed and 49 (37%) patients died. Those who achieved MC1.0 and CMC had significantly better OS (2-year OS 75% vs. 61% in MC1.0 vs. non-MC1.0, p=0.0465; 2-year OS 77% vs. 60% in CMC vs. non-CMC, p=0.0303), and lower CIR (2-year CIR 26% vs.46% in MC1.0 vs. non-MC 1.0, p=0.0349; 2 year-CIR 24% vs. 46% in CMC vs. non-CMC, p=0.03), while there was no significant difference in any of the above outcomes by MC2.5.

We also analyzed the role of allo-SCT in patients with and without persistent mutations. Among the patients who did not attain CMC, allo-SCT at first CR improved OS with borderline significance (2-year OS 69.6% with allo-SCT vs. 51.1% without allo-SCT, p=0.0495). In contrast, allo-SCT does not seem to have conferred survival benefit in patients who attained CMC (p=0.343).

Correlation between mutation clearance and flow cytometry based MRD

Flow cytometry based MRD (flow-MRD) data at CR was available in 125 patients (95%), and 94 (75%) patients attained negative flow-MRD at CR. Patients with negative flow-MRD had

significantly better EFS (P<0.001), OS (P=0.0176) and CIR (P=0.00637). Among patients with negative flow-MRD at CR, long-term outcome appeared to be different based on the CMC status, although the survival difference was not statistically significant (4-year OS 72.1% with CMC vs. 37.4% without CMC, p=0.138). When preleukemic mutations such as *DNMT3A*, *TET2*, and *ASXL1* were removed from the analysis, both MC1.0 and CMC predicted better OS in flow-MRD negative patients, suggesting that MRD assessment based on somatic mutations maybe complementary to flow-MRD.

Multivariate analysis

Multivariate analysis adjusting for age, cytogenetic risk, allo-SCT, and flow-MRD revealed that patients with CMC had significantly better EFS (HR 0.43, p=0.0083), OS (HR 0.47, p=0.04), and CIR (HR 0.27, p<0.001) compared with patients who did not attain CMC. These prognostic associations became more pronounced when preleukemic mutations such as *DNMT3A*, *TET2*, and *ASXL1* were removed from the analysis.

[Conclusion]

Clearance of somatic mutation at CR, particularly in non-preleukemic gene, was associated with significantly better survival and lower risk of relapse. Somatic mutations in non-preleukemic genes may serve as a molecular MRD marker in AML.