中文題目：急性骨髓性白血病 NPM 基因突變患者以階層式群集分析顯示不同免疫表現型和預後的預測

英文題目：Hierarchical cluster analysis reveal distinct immunophenotype and predict prognosis in AML patients with NPM gene mutation

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前言： Acute myeloid leukemia(AML) is a group of heterogeneous disease characterized with increasing immature progenitors in the bone marrow and peripheral blood. Prior reports suggested a relationship between surface antigens with prognosis of AML, however, there were some debates in subsequent studies. The conflicting result of immunophenotype and FAB classification in AML possibly contributed to that the mutant genes were not known before. Recently, the leukemogenesis had made progress and several mutant genes were found such as *NPM, CEBPA,* and *FLT3.*

In this study, we analyzed 63 AML patients with *NPM* mutation, clustering analysis of the immunophenotype expression profile with gene mutation, and correlated with cytogentic changes, prognosis and clinical characteristics.

材料及方法：

Patients

There were 63 patients diagnosed as acute myeloid leukemia and subgroup according to FAB criteria at the National Taiwan University Hospital from 1987 to 2002. The medical records were reviewed. The study was approved by the Institutional Review Board of the National Taiwan University Hospital.

Immunophenotyping

A panel of monoclonal antibodies to myeloid associated antigens including CD13, CD33, CD11b, CD15, CD14, and CD41a, as well as lymphoid associated antigens including CD2, CD5, CD7, CD19, CD10, and CD20, and lineage nonspecific antigens HLA-DR, CD34,

and CD56 was used to characterize the phenotypes of the leukemic cells. Expression of surface antigens on the leukemic cells was shown by flow cytometry or by an indirect immunoalkaline phosphatase method as described before. The cut-off values for positive result of the markers were more than 30% by flow cytometry or more than 20% by indirect immunoalkaline phosphatase method.

Analysis of genetic mutation of *NPM, FLT3, JAK2, PTPN11*, *NRAS* and *KRAS*

Mononuclear cells were isolated from BM aspirates by Ficoll-Hypaque gradient centrifugation. The genomic DNA was extracted by high quality PCR kit (Qiagen) with a standard protocol. Mutations of target gene were detected by genomic DNA polymerase chain reaction (PCR) and direct sequencing using a previously described method. Abnormal sequencing results were confirmed by at least two repeated analyses.

Statistics

Comparisons were made with the ANOVA and the Chi-square test. Hierarchical cluster analysis was performed with agglomeration schedule, and cluster distance measured with Binary Square Euclidean distance. Dendrogram were plotted with average linkage method (between groups). Survival curves were plotted by use of the Kaplan–Meier method; differences between curves were analyzed by the log-rank test. All statistical analyses were performed by use of SPSS 13.0 for Windows (SPSS, Chicago, IL). Values of $P < 0.05$ were considered significant.

結果:

Clinical characteristics of *NPM* gene mutation

The gene mutations were screened in a total of 324 patients with AML. There are 49 children (age less than 18 year old) and 275 adult. The patients with *NPM* were older than the others($p<0.05$). The frequency of *NPM* gene mutations were 63 cases (19.4%). The patients with *NPM* mutation were HLADR(-)CD33(+) CD34(-) ($p<0.05$).

Mutational analysis in the patients with AML

FLT3/ ITD could interact with each subtype of Class II gene mutations, but were

particularly associated with *NPM* mutations (p<0.001). *FLT3 / TKD* was closely related to *NPM* mutations (p=0.03). *PTPN11* mutations were more frequently detected in patients with *NPM* mutations than others(p=0.035). Combined the gene mutations, patients with *NPM(+)FLT3(+)* had shorter relapse free interval than patients with *NPM(+)FLT3(-)* (14 vs 91 months, p<0.05).

Hierarchical cluster analysis of *FLT3/ITD, NPM*, *CEBPA,* and *AML1* gene mutation

The cluster analysis of study cases was performed based on the expression profile of eight surface markers (HLADR, CD34, CD13, CD33, CD7, CD14, CD15, CD56). The clustering result displayed by the Treeview dendrogram. The rows represent individual cases and the columns are the results of expression of individual surface markers. The blue color indicates positive expression and yellow color indicates negative.

Forty-seven *NPM* mutation patients had complete immunophenotype data were enrolled to the hierarchical cluster analysis. The clustering analysis divided the patients with *NPM* mutation into three groups based on the expression profiles of immunophenotype(Figure 1a), which we have designated as cluster groups I(red), II(green), and III(blue). Three distinct immunophenotype could be found, most patients in group I showed CD34(-)CD15(+), group II showed HLA DR(-)CD34(-), and group III showed HLADR(+)CD34(+) and variable CD7 expression. Four patients with *PTPN11* mutation were within group I *NPM* mutation(p=0.014), the patients with *FLT3/ITD* were less common in group I than group II and III(25% vs 75% vs 69%, p=0.021), but the frequency of *FLT3/TKD*, and *NRAS* mutation among form group I to group III was not significantly different. These three groups revealed significantly different relapse free survival (median: not reach vs 15 months vs 3 months, p<0.001, Figure 1b) and overall survival (median: not reach vs 94 months vs 8 months, p<0.001, Figure 1c).

討論及結論:

In conclusion, the NPM mutation was one of the most common mutations in AML. NPM mutation had distinct immunophenotype of HLADR(-)CD33(+) CD34(-) (p<0.05).

Hierarchical cluster analysis of the immunophenotype profile of NPM gene mutation could revealed three distinct groups. The three groups could predict the prognosis in AML patients with NPM mutation. Exploring the interactions of gene mutations and correctly identifying the prognostic groups by immunophenotypic profiles may help us more understand the pathogenesis of leukemia and benefit further therapeutic strategy.