論 文 の 内 容 の 要 旨

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論 文 題 目

Aberrant methylation of protocadherin 17 and its prognostic value in pediatric acute lymphoblastic leukemia.
（小児急性リンパ性白血病におけるプロトカドヘリン 17 遺伝子の異常メチル化と予後因子としての有用性の検討）

（論文の内容の要旨）

Background: The cure rate of childhood acute lymphoblastic leukemia (ALL) has improved over the past four decades. However, the prognosis of approximately 20% of patients with ALL remains poor because of disease recurrence. In recent years, many studies have attempted to identify new clinical biomarkers that can predict high-risk patients and serve as targets for novel therapeutic interventions using gene-expression microarrays, DNA-methylation arrays, and next-generation sequencing. DNA methylation and histone modifications are two major epigenetic mechanisms regulating gene expression. Hypermethylation of CpG islands in the promoter region of tumor suppressor genes that results in transcriptional silencing plays an important role in lymphoid-lineage leukemogenesis and may be an important contributor toward relapse. In addition, DNA methylation profiling is useful for subtype classification of newly diagnosed ALL patients and prediction of outcome and relapse risk. This study was aimed at examination of the methylation status of cadherin superfamily genes and its prognostic value for ALL relapse.

Patient and methods: Bone marrow (BM) cells were aspirated from 40 children with B-cell precursor (BCP) ALL diagnosed between 1995 and 2008 at Shinshu University. Six ALL cell lines were used. Peripheral blood (PB) mononuclear cells (MNCs) from healthy adult volunteers and BM MNCs from ALL patients at complete remission (CR) were used as controls. We used Infinium Methylation 450K Array to assess the genome-wide DNA methylation status. Methylation status of each individual gene was then determined by combine bisulfite restriction analysis (COBRA) and genome bisulfite sequencing. mRNA expression was evaluated by reverse transcriptase PCR (RT-PCR) and quantitative real-time PCR.

Results: Cadherin superfamily genes including *cadherin (CDH)1*, *protocadherin (PCDH)8*, and *PCDH17* were selected for analyzing methylation status. In 40 B-cell precursor (BCP) ALL samples at onset, the methylation frequencies of *CDH1*, *PCDH8*, and *PCDH17* were 62.5%, 55%, and 30%, respectively. *CDH1* and *PCDH8* methylation was observed in all leukemic cell lines whereas *PCDH17* methylation was detected in 4 of them. *CDH1* and *PCDH8* methylation was also detected in 80% and 20% of control BM samples, respectively. On the contrary, *PCDH17* was unmethylated in all control BM samples. The correlation between methylation status and event-free survival (EFS) or overall survival (OS) was evaluated. *PCDH17* methylation-positive group was profoundly inferior to that of *PCDH17* methylation-negative group: 33% (95% CI, 10–59) vs. 75% (95% CI, 55–87); $P = 0.005$. A significant difference in the OS was also found between the two groups: 50% (95% CI, 21–73) vs. 82% (95% CI, 62–92); $P = 0.016$. Conversely, there were no substantial correlations between neither the methylation status of *CDH1* and EFS or OS, nor the methylation status of *PCDH8* and EFS or OS. By multivariate analyses, only *PCDH17* methylation was associated with increased risk for relapse and mortality in patients with BCP ALL [HR, 5.23; $P = 0.016$ for relapse and HR, 8.22; $P = 0.016$ for mortality]. *CDH1* and *PCDH8* methylation did not influence ALL outcomes.

Conclusion: *PCDH17* methylation at onset was closely related to poor prognosis, and thus it could be used as a new biomarker to predict relapse in BCP ALL.