Peran Mutasi Gen FLT3 Pada Resistensi Sel Leukemia Mieloblastik Akut Terhadap Terapi Induksi D3A7 Serta Hubungannya Dengan Penanda Sel Punca Leukemia = The Role of FLT3 Gene Mutation in The Resistance of Acute Myeloid Leukemia Cells to D3A7 Induction Therapy and Its Association with Leukemia Stem Cell Markers

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Abstrak

Penelitian ini bertujuan untuk menganalisis peran mutasi gen FLT3 dalam resistensi terapi induksi D3A7 pada pasien LMA, melalui pengamatan perubahan struktur protein reseptor FLT3, aktivitas jalur persinyalan downstream FLT3 yaitu PI3K/AKT, menggunakan persentasi jumlah sel yang berapoptosis dan proliferasi sebagai indikator dalam mekanisme multidrug resistance terhadap terapi induksi D3A7. Selain itu dilakukan juga analasis peran sub populasi sel punca leukemia CD34+CD38-CD123+ dan marka ALDH dalam resistensi terapi Induksi D3A7, untuk mengetahui hubungannya denagn resistensi terapi induksi D3A7. Metode Deteksi mutasi gen FLT3 dilakukan dengan PCR-sekuensing dari sampel darah sumsum tulang pasien LMA de novo yang telah selesai diberikan terapi induksi D3A7. Sikuens gen yang termutasi kemudian dianalisis menggunakan studi in silico untuk menilai dampak dari mutasi gen terhadap perubahan struktur dan aktivitas pengikatan protein terhadap regimen sitarabin. Analisis aktivitas fosforilasi protein PI3K dan AKT dilakukan dengan metode sandwich ELISA. Penghitungan persentasi jumlah sel yang mengalami apoptosis dan proliferasi, serta deteksi sel punca leukemia dengan penanda CD34+, CD38-, CD123+, dan ALDH menggunakan Flowcitometry. Hasil Ditemukan mutasi baru Ins_572G573 (Insersi-G) pada domain juxtamembran dari protein reseptor FLT3 dengan frekuensi sebesar 30% dari total 20 pasien LMA yang direkrut, sementara frekuensi mutasi FLT3-ITD yang diperoleh sebesar 20%. Kelompok pasien dengan mutasi gen FLT3 mengalami peningkatan fosforilasi protein PI3K dan AKT yang bermakna secara statistik, mengalami peningkatan rerata persentasi jumlah proliferasi sel dan penurunan rerata jumlah apoptosis sel dibandingkan kelompok tanpa mutasi. Kelompok pasien dengan outcome terapi resistensi juga mengalami peningkatan fosforilasi protein PI3K dan AKT, penurunan rerata jumlah sel yang mengalami apoptosis dan peningkatan rerata jumlah sel yang berproliferasi. Penanda sel punca leukemia CD34+, CD38-, CD123+, dan ALDH memiliki hubungan tidak bermakna dengan resistensi terapi induksi D3A7. Kesimpulan Penelitian ini menunjukkan bahwa mutasi gen FLT3 tidak berhubungan lansung pada resistensi terapi D3A7. Namun perubahan struktur protein akibat mutasi berperan penting dalam mekanisme resistensi melalui aktivasi jalur pro-proliferasi dan anti papoptosis dari persinyalan PI3K/AKT. Salain itu, penanda sel punca leukemia tidak berhubungan dengan resistensi terapi induksi D3A7.

.....Introduction This study aims to analyze the role of FLT3 gene mutations in the resistance of AML therapy induction with D3A7 in patients, through observing changes in FLT3 receptor protein structure, the activity of downstream FLT3 signaling pathways such as PI3K/AKT, and using the percentage of apoptotic and proliferative cells as indicators in the mechanism of multidrug resistance against D3A7 induction therapy. Additionally, the study also analyzes the role of leukemia stem cell subpopulations CD34+CD38-CD123+ and ALDH markers in resistance to D3A7 induction therapy, to understand their relationship with resistance to D3A7 induction therapy. Method Detection of FLT3 gene mutations was performed by PCR-

sequencing from bone marrow blood samples of de novo AML patients who had completed D3A7 induction therapy. Sequences of the mutated genes were then analyzed using in silico studies to assess the impact of gene mutations on structural changes and protein binding activity with cytarabine regimens. Analysis of PI3K and AKT protein phosphorylation activity was conducted using sandwich ELISA. Calculation of the percentage of cells undergoing apoptosis and proliferation, as well as detection of leukemia stem cells marked by CD34+, CD38-, CD123+, and ALDH, was performed using Flow cytometry. Results A novel mutation, Ins_572G573 (Insertion-G), was found in the juxtamembrane domain of the FLT3 receptor protein with a frequency of 30% among a total of 20 recruited AML patients. Meanwhile, FLT3-ITD mutation frequency was obtained at 20%. Patients with FLT3 gene mutations showed statistically significant increases in PI3K and AKT protein phosphorylation, as well as higher average percentages of proliferating cells and lower average percentages of apoptotic cells compared to the non-mutation group. Patients in the therapy-resistant outcome group also exhibited increased PI3K and AKT protein phosphorylation, decreased average percentages of apoptotic cells, and increased average percentages of proliferating cells. However, leukemia stem cell markers CD34+, CD38-, CD123+, and ALDH did not show statistically significant associations with resistance to D3A7 induction therapy. Conclusion This study indicates that FLT3 gene mutations do not directly correlate with resistance to D3A7 therapy. However, structural changes in proteins due to mutations play a crucial role in resistance mechanisms through the activation of pro-proliferation and anti-apoptosis pathways via PI3K/AKT signaling. Additionally, leukemia stem cell markers are not associated with resistance to D3A7 induction therapy.