論文審査の要旨

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Changes in intracellular activation-related gene expression and induction of Akt contribute to acquired resistance towards nelarabine in CCRF-CEM cell line

（CCRF-CEM 細胞株における細胞内活性化関連遺伝子発現の変化と Akt の誘導がネララビンに対する獲得抵抗性に寄与している）

Nelarabine is a purine nucleoside analog used to treat relapsed/refractory T-cell ALL/lymphoma (T-ALL/T-LBL), with satisfactory effects. Drug resistance is a major problem in treatment with nelarabine, and its resolution requires elucidation of the underlying mechanisms. In the current study, nelarabine-resistant sub-clones of the human T-cell ALL cell line CCRF-CEM were established to investigate the factors related to the acquisition of nelarabine resistance *in vitro* and identify strategies to overcome resistance to nelarabine, focusing on intracellular activation-related genes, changes in apoptosis, and signaling pathways.

Two nelarabine-resistant subclones, Clone 1 and Clone 2, were established by serial incubation with an increasing dose of nelarabine followed by limiting dilutions. Expression of genes and protein related to nelarabine intracellular activation, apoptosis induction, and changes in signaling pathways were investigated. Therapies with PI3K/Akt inhibitor, MEK/ERK inhibitors, and other classes of chemotherapy were done to see its effect on resistant subclones. This study showed that:

1. Resistant subclones showed downregulation of ENT1, DCK, DGUOK (in Clone 2), and upregulation of SAMHD1.
2. Resistant clones did not show caspase 3 activation and PARP cleavage after nelarabine treatment.
3. Bcl2 family genes in sensitive and resistant cells responded differently after nelarabine treatment. CCRF-CEM showed upregulation of BCL2, BAD, and BAX. Clone 1 showed no changes in the Bcl2 family expression, while Clone 2 showed upregulation of Bcl-xL upon 10 μM nelarabine and upregulation of BAD and BAX in high dose nelarabine treatment.
4. Upregulation of p-Akt in untreated cells and upon nelarabine treatment were observed in resistant clones, notably in Clone 2.
5. The combined therapy of nelarabine with PI3K/Akt pathway inhibitors increased drug toxicity, reduced p-Akt levels, and induced cell death in resistant clones.
6. p-ERK was upregulated after treatment with nelarabine, although not statistically significant; without significant effect on cell viability after treating the cells with the MEK/ERK inhibitors.
7. Cross-resistance in resistant clones was observed with ara-C and not with vincristine, daunorubicin, or etoposide treatment.

In summary, the present study showed that in nelarabine resistant cell model, resistance involved changes in the expression of genes related to nelarabine activation, inhibition of apoptosis, and changes in the response of Bcl2 family genes and Akt induction. The use of other chemotherapeutic agents or combination therapy with PI3K/Akt pathway inhibitors may be used to overcome acquired drug resistance during treatment.

よって本研究は学位論文として十分な価値を有するものと判定した。