No.	K25-2172	
Project Title	Novel Treatment of AML by Combination of Chemotherapy and CD112-Targeted Immunotherapy	
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(Form 2)

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IMSUT International Joint Usage/Research Center Project <International>

Joint Research Report (Annual/Project Completion)

Annual Report

Report

We investigated which surface molecules on leukemic cells could affect the exhaustion of $CD8^+$ T cells and NK cells. We found that CD112, also known as Nectin-2, expressed on leukemic cells, inhibits the cytotoxicity of NK cells. CD112 is one of the immune checkpoint molecules expressed in both hematologic malignancies and solid tumors.

Interestingly, CD112 interacts with three ligands: CD112R, TIGIT, and CD226 (also known as DNAM-1). It is known that the functions of $CD8^+$ T cells and NK cells are suppressed through interactions with CD112R or TIGIT, whereas these cells are activated via CD226/DNAM-1.

Therefore, we utilized the CRISPR-Cas9 system to generate CD112 knockout (KO) leukemic cells and found that NK cells exhibit higher cytotoxicity against CD112 KO cells compared to wild-type (WT) CD112-expressing cells. In addition, we generated CD112R or TIGIT deficient NK cells, which also exhibited higher cytotoxicity against leukemic cells compared with control NK cells.

Furthermore, when neutralizing antibodies against CD112 were added along with NK cells to leukemic cells, the cytotoxicity of NK cells was significantly enhanced compared to treatment with control antibodies

Additionally, we examined whether drug treatment affects CD112 expression on leukemic cells and the cytotoxicity of NK cells. We discovered that several anti-cancer drugs induce upregulation of CD112 expression. Consistently, NK cell cytotoxicity against drug-treated leukemic cells was reduced compared to untreated cells.

Among the anticancer drugs examined, decitabine induced greater upregulation of CD112 in several leukemic cell lines than other agents. Decitabine is a hypomethylating agent that inhibits DNA methylation. Therefore, we examined the DNA methylation status of the CD112 gene locus in leukemic cells and found that two loci were demethylated following decitabine treatment. We are currently investigating whether demethylation at these loci is associated with CD112 upregulation.

Taken together, these results suggest that leukemic cells that survive drug treatment upregulate CD112, thereby inhibiting NK cell cytotoxicity. However, combining anticancer therapy with neutralizing antibodies against CD112 may represent a promising strategy to target leukemic cells with high CD112 expression.