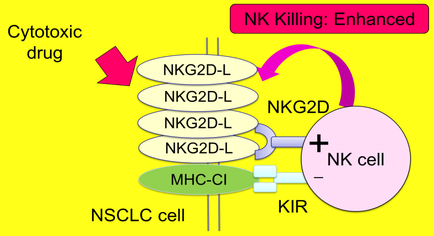
Cytotoxic anticancer drug enhances NK cell-mediated cytotoxicity via the DNA stress induced NKG2D ligands in non-small-cell lung cancer cells

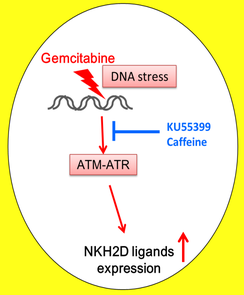
Department of General Thoracic Surgery, Kawasaki Medical School, Kurashiki, Japan
Riki Okita, Koichiro Yasuda, Ai Maeda, Takuro Yukawa, Shinsuke Saisho, Katsuhiko Shimizu, Masao Nakata.

Conclusions

- Cytotoxic drug Gemcitabine-induced upregulation of the expression of NKG2D ligands enhances the NK cell-mediated cytotoxicity.



- Gemcitabine upregulates the expression of NKG2D ligands in NSCLC cells via ATM-ATR pathway.



Background

MICA, MICB, and ULBPs are the ligands of NKG2D expressing in tumor cell. The main role of the NK cell is immunosurveillance dependent on NKG2D-NKG2D ligand interaction

Lanier LL, Nat Med 2001

DNA stress induced ATM-ATR signaling regulates the expression of NKG2D ligand.

Gasser S, et al. Nature 2005

Aim

To assess the effects of anticancer drug on the NK cell-mediated cytotoxicity against NSCLC cells.

Materials and Methods

- NSCLC cell lines:

A549, PC-9, RERF-LC-AI, RERF-LC-KJ, LC2/ad

Regents:

- Cytotoxic drugs;

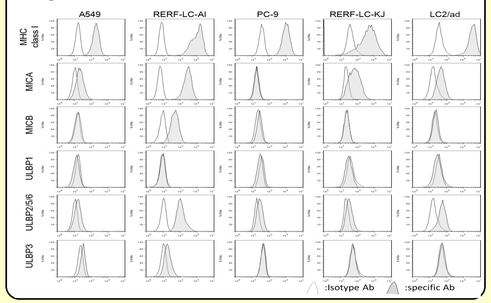
Gemcitabine (GEM), Docetaxel (DTX), Vinorelbine (VNR), Pemetrexed (PEM),

- ATM-ATR inhibitors:

Caffeine (Caf), KU55399 (KU)

- Expression of NKG2D ligands: Flow cytometry
- Cell signalling: Flow cytometry
- Cytotoxicity: LDH release and CD107a assays

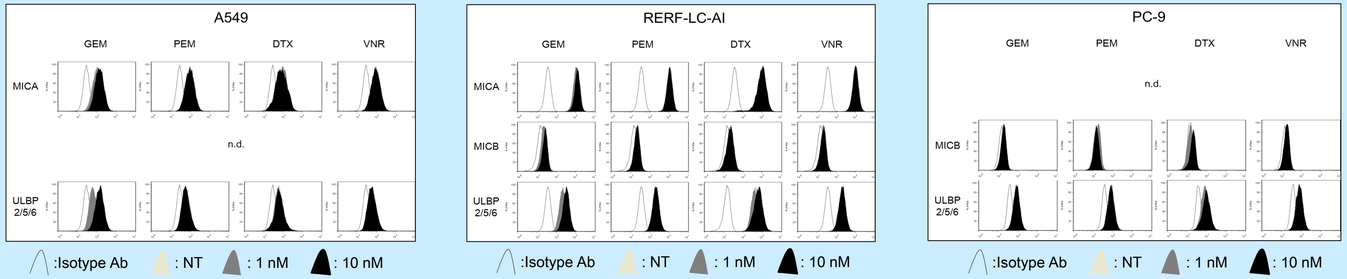
The expressions of MHC class I and NKG2D ligands in NSCLC cell lines



Potential Conflicts of Interest:
Dr. Masao Nakata has received research funding from Astra Zeneca for this study.
All other authors declare no conflicts of interest.

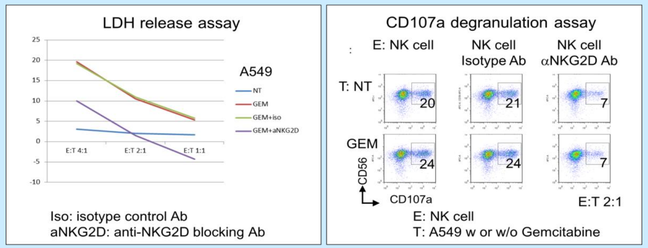
Results

NSCLC cells were treated with or without 1 to 10nM of Gemcitabine (GEM), Pemetrexed (PEM), Docetaxel (DTX) or Vinorelbine (VNR) for 24 hours, then the expressions of NKG2D ligand were assessed by flow cytometry.



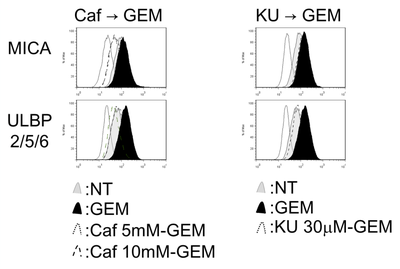
*All drugs showed no effect on the expression of NKG2D ligands in other two cell lines; RERF-LC-KJ and LC2/ad.

Gemcitabine enhanced NK cell-mediated cytotoxicity via NKG2D-NKG2D ligand interaction in A549 cells



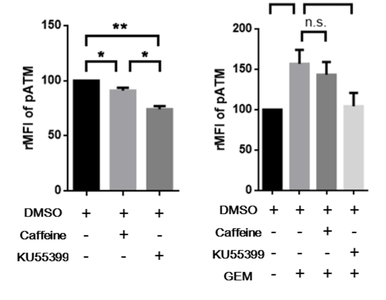
A549 cells with or without Gemcitabine (10 nM) for 24 hours were subjected to LDH release assay and NK cell degranulation assay using IL-2 activated NK cells as effector cells. The IL-2 activated NK cells were pretreated with blocking Ab of NKG2D or isotype control Ab before the cytotoxicity assay. E:T ratio; effector/target ratio.

Gemcitabine-induced upregulation of the expression of NKG2D ligand is regulated via ATM-ATR pathway in A549 cells.



A549 cells were pretreated with caffeine (Caf) or KU55399 (KU) followed by Gemcitabine (GEM) then the expressions of NKG2D ligand were assessed by flow cytometry.

Both ATM-ATR inhibitors blocked Gemcitabine-induced NKG2D ligands in A549 cells.



A549 cells were pretreated with Caf or KU followed by GEM then the phosphorylated ATM was assessed by flow cytometry. GEM-induced pATM was blocked by KU55399.