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Susceptibility of CD24$^+$ ovarian cancer cells to anti-cancer drugs and natural killer cells

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ABSTRACT

Natural killer cells are lymphocytes of the innate immune system that play a key role in the direct elimination of transformed or virus-infected cells. Recently, it has been reported that NK cells can attack cancer cells with stem cell-like properties. In this study, we isolated ovarian cancer cell lines CAOV3 and TOV21G with and without CD24, which has been reported as an ovarian cancer stem cell marker, and compared their drug resistance and susceptibility to NK cell lysis. The isolated CD24$^+$ CAOV3 and TOV21G cells were more resistant to cisplatin and doxorubicin anti-cancer drugs. Also, CD24$^+$ CAOV3 and TOV21G cells were more susceptible to NK cell lysis compared with CD24$^-$ cells. In order to identify reasons for the differing NK cell susceptibility, we examined NK cell-killing mechanisms against CD24$^+$ cancer cell lines by analyzing NKG2D ligands, MHC class I molecules, and natural cytotoxic receptor ligands expression on target cells. Consistently, CD24$^+$ CAOV3 and TOV21G cells showed up-regulated NKG2D ligands and down-regulated MHC class I molecule expression. These findings show that CD24$^+$ ovarian cancer cell lines are more resistant to antitumor drugs but are more susceptible to NK cell lysis; thus, NK cell immunotherapy might be useful in eliminating ovarian cancer stem cells and preventing tumor recurrence and metastasis.

1. Introduction

Natural killer (NK) cells are a part of the innate immune system that can eliminate virus-infected cells and transformed cancer cells without prior sensitization. Activated NK cells are effector cells that activate and regulate the adaptive immune system [1–3]. NK cells are activated when the appropriate receptors bind ligands on target cells and causes cell necrosis and apoptosis [4,5]. The major activating receptors are NKG2D and the NK cytotoxic receptors (NCRs) NKp30, NKp44, and NKp46 [6,7]. The inhibitory signaling of NK cells is also activated when the inhibitory receptors of NK cells bind MHC class I molecules on target cells. It is well known that the MHC class I molecule is down-regulated on cancer cells to avoid detection by the immune system; however, this phenomenon can leave cells more susceptible to NK cells, which recognize target cells lacking MHC class I molecules [8].

Recent studies have revealed that there is a small portion of cancer cells within tumors that has the properties of cancer stem cells. Cancer stem cells are defined by their ability to initiate tumors, self-renew, and give rise to differentiated progeny. Various tumor type cancer stem cells have been reported with cancer stem cell markers that are able to initiate tumors and that are more aggressive than cancer cells without cancer stem cell markers [9–12].

Identification of the first cancer stem cell originated from studies of leukemia stem cells [13,14]. Later, subsequent studies showed evidence of cancer stem cells by demonstrating that cancer stem cells from various cancer types with specific cell surface markers were more aggressive and better able to initiate tumors compared to cancer cells without cancer stem cell markers [15–17]. Al-Hajj et al. discovered CD44$^+$/CD24$^{-/low}$ as a breast cancer stem cell marker [15], and recently Gao et al. defined CD24$^+$ as an ovarian cancer stem cell marker [16]. In addition to the ability to self renew, seed tumors, and give rise to non-cancer stem cell progeny, cancer stem cells are more resistant to chemotherapy drugs and radiotherapy than are cancer cells [18].

According to Castriconi et al., glioblastoma (GBM) cells cultured in stem cell medium were more susceptible to IL-2-stimulated NK cells than were GBM cells cultured in non-stem cell medium [19]. Also, it has been previously reported that NK cells are cytotoxic to solid tumors such as breast and lung cancers [20,21]. However, no reports have been published on NK cell cytotoxicity or NK cell-killing mechanisms against ovarian cancer cells isolated with cancer stem cell markers.

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In this study, we isolated ovarian cancer cells with CD24 from CAOV3 and TOV21G cell lines. Isolated cancer cells were tested for their drug resistance and susceptibility to NK cell lysis. To reveal the different susceptibilities to NK cell cytotoxicity, we analyzed the expressions of NK cell activating receptors and inhibitory receptor ligands on target cell surfaces.

2. Materials and methods

2.1. NK cell isolation

Natural killer cells were isolated from the whole blood of healthy donors by negative selection using a RosetteSep NK enrichment antibody cocktail (StemCell Technologies, Vancouver, B.C., Canada). Fifty microliters of the antibody cocktail per 1 ml of whole blood was mixed and incubated for 20 min at room temperature. The blood sample was then diluted with the same volume of RPMI 1640 medium containing 2% FBS. The diluted blood with RPMI 1640 was placed onto a Ficoll-Paque (Amersham Pharmacia Biotech, Uppsala, Sweden) and centrifuged for 30 min at 1600 rpm at room temperature. The NK cell layer was collected and washed twice with RPMI 1640 containing 2% FBS. To verify the purity, NK cells were stained with FITC-conjugated CD3 and PE-conjugated CD56 (BD Pharmingen, San Diego, CA, USA) for 30 min without light and analyzed by BD LSRII. To activate NK cells, 20 ng/ml of recombinant IL-2 (ATgen, Seongnam Si, South Korea) was added for 18 h. The written and informed consents were obtained from all donors approved by the institutional review boards of Yonsei University Health System.

2.2. $CD24^+$ cell isolation and culture

Ovarian cancer cells with CD24 were isolated. CAOV3 and TOV21G cells were incubated with FITC-conjugated CD24 monoclonal antibody (BD Pharmingen, San Diego, CA, USA). Then, BD FACS Aria II was used to isolate $CD24^-$ and $CD24^+$ ovarian cancer cells. The isolated $CD24^-$ and $CD24^+$ cancer cells were maintained in their original medium with 10% FBS for several passages and reanalyzed for purity using flow cytometry. The sorted $CD24^-$ and $CD24^+$ CAOV3 cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) with 10% fetal bovine serum, penicillin (100 U/ml), and streptomycin (100 μg/ml) (Gibco, Carlsbad, CA, USA) and incubated at 37 °C with 5% CO_2. The isolated $CD24^-$ and $CD24^+$ TOV21G were maintained in a 1:1 mixture of MCDB 105 (Sigma–Aldrich, St. Louis, MO, USA) and Medium 199 (Gibco, Carlsbad, CA, USA) with 10% FBS.

2.3. Chromium-51 release assay

The chromium-51 (Cr-51) release assay was performed. Cr-51 50 μCi (NEN, Boston, MA, USA) was added per 1×10^6 cancer cells and incubated for 2 h at 37 °C with 5% CO_2. Each cancer cell line (1×10^4) was plated into a 96-well microtiter plate. NK cells were added to each well at the indicated effector-to-target ratio. After target cells were co-cultured with NK cells for 4 h, cell-free supernatant was collected and measured the radioactivity (PerkinElmer, Boston, MA, USA). The percentage of NK cell cytotoxicity was calculated by the following formula: % cytotoxicity = (experimental Cr-51 release − spontaneous Cr-51 release)/(maximum Cr-51 release − spontaneous Cr-51 release) × 100. All the experiments were conducted in triplicate.

2.4. Flow cytometry

$CD24^-$ and $CD24^+$ CAOV3 and TOV21G were stained with the appropriate monoclonal antibodies. APC mouse anti-human CD44, PE mouse anti-human HLA ABC, and PE mouse anti-human MIC A/B monoclonal antibodies were purchased from BD Pharmingen (San Diego, CA, USA). PE anti-human IgG Fc monoclonal antibody was purchased from Biolegend (San Diego, CA, USA). NCR ligands and ULBP3 were analyzed by adding human recombinant proteins for 3 h in ice. Recombinant human NKp30/NCR3, NKp44/NCR2, NKp46/NCR1 Fc chimera proteins, PE anti-human ULBP1, ULBP2 monoclonal antibodies, and ULBP3 recombinant protein were purchased from R&D Systems (Minneapolis, MN, USA). PE anti-mouse IgG antibody was purchased from Sigma–Aldrich (St. Louis, MO, USA). Then, cells were washed and PE anti-human IgG Fc antibody was added for 30 min to detect the recombinant proteins bound on the target cell ligands, and then fluorescence was measured using BD LSR II.

2.5. MTT assay

The cell viability was measured using the MTT assay. $CD24^-$ and $CD24^+$ CAOV3 and TOV21G cells (7×10^3) were plated in 96-well microtiter plates for 18 h. Cisplatin and doxorubicin (Sigma–Aldrich, St. Louis, MO, USA) at different concentrations were added into each well. After 24 h of incubation at 37 °C with 5% CO_2, 25 μl/100 μl Thiazolyl Blue Tetrazolium Bromide (Sigma–Aldrich) was added and incubated for 4 h. All media were removed and DMSO (DuchefaBiochemie, Haarlem, Netherlands) was added. The samples were read at 570 nm. All the experiments were conducted in triplicate.

3. Results

3.1. Isolation of ovarian cancer cells with CD24

The cell surface expression of CD24 on ovarian cancer cells showed stem cell-like properties of capability for self-renewal and differentiation into heterogeneous cell types [16]. We isolated $CD24^-$ and $CD24^+$ cells from CAOV3 and TOV21G ovarian cancer cell lines. The isolated $CD24^-$ and $CD24^+$ cells were incubated for two passages, and then the percentages of $CD24^-$ and $CD24^+$ cells from each cell line were measured (Fig. 1). The percentages of $CD24^-$ and $CD24^+$ CAOV3 cells were 86.5% and 99%, respectively (Fig. 1A). The percentages of $CD24^-$ and $CD24^+$ TOV21G cells were 90.9% and 99.7%, respectively (Fig. 1B). The purity of all sorted $CD24^-$ and $CD24^+$ ovarian cancer cells was very high, which made it possible to conduct experiments to analyze the different characteristics of $CD24^-$ and $CD24^+$ cells. The maintained cells were constantly measured for CD24 cell surface expression and were maintained with high purity as the experiment proceeded, as $CD24^+$ cells tended to differentiate into $CD24^-$ cells.

3.2. Cisplatin and doxorubicin resistance of $CD24^-$ and $CD24^+$ ovarian cancer cells

The drug resistance of sorted CD24 cancer cell lines from CAOV3 and TOV21G cells was measured using MTT assay. $CD24^-$ and $CD24^+$ CAOV3 and TOV21G cells were treated with cisplatin, and the cell viability was measured. At almost all concentrations, $CD24^+$ cells were considerably more resistant to cisplatin compared with $CD24^-$ cells. As the concentration increased, the differences between $CD24^-$ and $CD24^+$ cells were also significantly increased (Fig. 2A). Therefore, CAOV3 and TOV21G cells with CD24 were more resistant to cisplatin at all concentrations compared to the cell lines without CD24.

Next, $CD24^-$ and $CD24^+$ CAOV3 and TOV21G cells were incubated with doxorubicin to analyze their chemotherapy drug resistance. Consistently, all tested $CD24^+$ ovarian cancer cell lines were

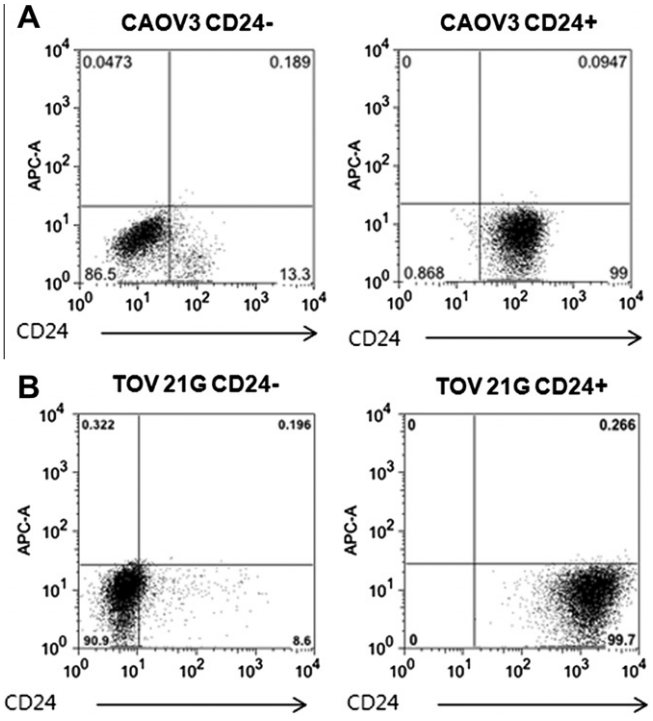

Fig. 1. Isolation of ovarian cancer cell lines with CD24. CAOV3 and TOV21G cell lines were sorted with $CD24^-$ and $CD24^+$. After two passages, all cell lines were stained with FITC-conjugated CD24 antibody. (A) CAOV3 cells without CD24 are shown on left and CAOV3 cells sorted with CD24 are shown on right. (B) TOV21G cells without CD24 cells are shown on left and with CD24 cells are shown on right.

highly resistant to doxorubicin compared with $CD24^-$ cells (Fig. 2B). $CD24^+$ CAOV3 cells were at least three times more resistant to doxorubicin compared to $CD24^-$ cells. Additionally, $CD24^-$ CAOV3 cells were shown to be susceptible to doxorubicin because, even at very low concentrations of the drug, the cell viability was less than 20% compared to the more than 70% of $CD24^+$ cells that survived at the same doxorubicin concentration. These results showed that $CD24^+$ CAOV3 and TOV21G cells were highly resistant to doxorubicin.

3.3. The susceptibility of $CD24^-$ and $CD24^+$ ovarian cancer cells to NK cells

To observe the susceptibility of $CD24^-$ and $CD24^+$ CAOV3 and TOV21G cells, each cell line was co-cultured with freshly isolated NK cells. $CD24^+$ CAOV3 and TOV21G cells were more susceptible to NK cells compared with $CD24^-$ cells (Fig. 2C). More than 15% of $CD24^+$ CAOV3 cells were killed, whereas freshly isolated NK cells lysed less than 5% of $CD24^-$ CAOV3 cells. Also, $CD24^+$ TOV21G cells were lysed by NK cells at a level that was three times higher than $CD24^-$ cells at the 1:10 target-to-effector ratio. Therefore, $CD24^+$ CAOV3 and TOV21G cells were more susceptible to the freshly isolated NK cells.

IL-2-activated NK cells were tested for cytotoxicity against CAOV3 and TOV21G cells with and without CD24. IL-2-activated NK cells also showed higher specific lysis against $CD24^+$ CAOV3 and TOV21G cells compared with $CD24^-$ cells (Fig. 2D). $CD24^+$ CAOV3 cells showed a two-fold higher specific lysis at the 1:10 ratio, and the difference was more obvious than with the freshly isolated NK cells. Importantly, the susceptibility of CAOV3 cells to freshly isolated NK cells was very low, even with $CD24^+$ cells. However, when NK cells were activated with IL-2, the susceptibility increased, showing significant differences between $CD24^-$ and $CD24^+$ cells. $CD24^+$ TOV21G cells also showed higher susceptibility at the effector-to-target ratio of 1:1. There were no differences between $CD24^-$ and $CD24^+$ cells at the 1:10 ratio because IL-2 activated NK cells were able to kill up to 80% of the target cells under these conditions. Therefore, $CD24^+$ CAOV3 and TOV21G cells were more susceptible to IL-2-stimulated NK cells.

3.4. MHC class I expression

The expression level of MHC class I molecules on $CD24^-$ and $CD24^+$ CAOV3 and TOV21G cells was measured. $CD24^+$ CAOV3 and TOV21G cells showed down-regulated MHC class I molecules on their cell surface compared with $CD24^-$ cells. The $CD24^-$ CAOV3 mean PE value was 2093 and with $CD24^+$ cells was 1148 (Fig. 3A).

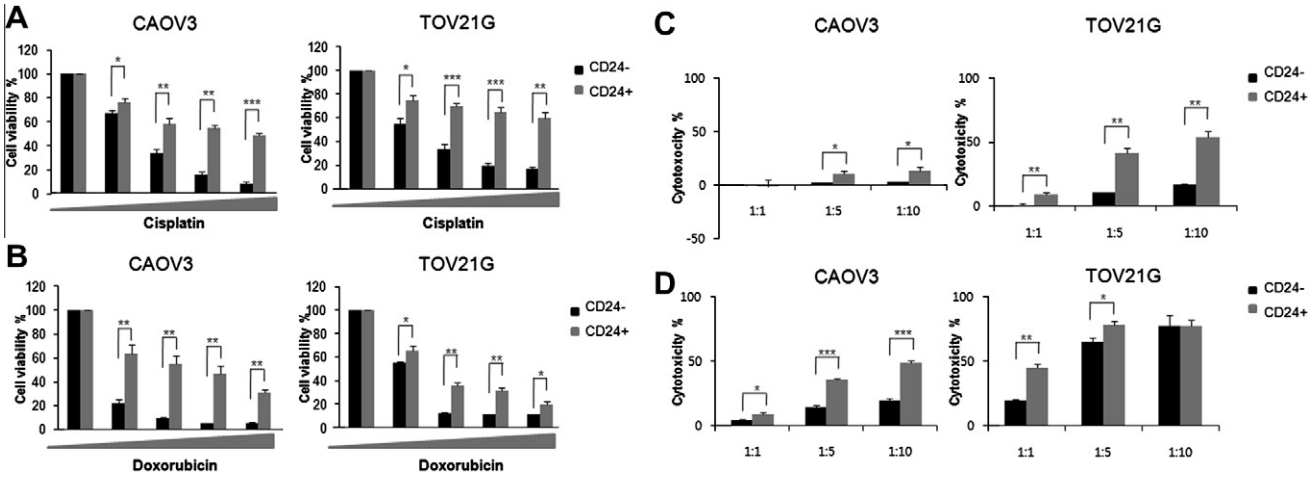

Fig. 2. Drug resistance and susceptibility to lysis of NK cells of ovarian cancer cell lines with $CD24^-$ and $CD24^+$. $CD24^-$ and $CD24^+$ CAOV3 and TOV21G cells were co-cultured with cisplatin and tested for the cell viability. (A) $CD24^-$ and $CD24^+$ CAOV3 and TOV21G cells were co-cultured with cisplatin. (B) $CD24^-$ and $CD24^+$ CAOV3 and TOV21G cells were co-cultured with doxorubicin. $CD24^+$ CAOV3 and TOV21G cells were highly resistant to anti-cancer drugs. $CD24^-$ and $CD24^+$ CAOV3 and TOV21G cells were tested for their susceptibility to the lysis of NK cells. (C) Freshly isolated NK cells were applied for 4 h, and then the cytotoxicity was measured as indicated target to NK cell ratio. Both cell lines showed that $CD24^+$ cells were more susceptible to the lysis of NK cells. (D) IL-2 activated NK cells were applied to the target cells. Again, $CD24^+$ ovarian cancer cells were more susceptible to the lysis of NK cells. The black bars refer to $CD24^-$ cells, and gray bars refer to $CD24^+$ cells. All experiments were conducted in triplicate. $^*P < 0.5$, $^{**}P < 0.1$, $^{***}P < 0.001$.

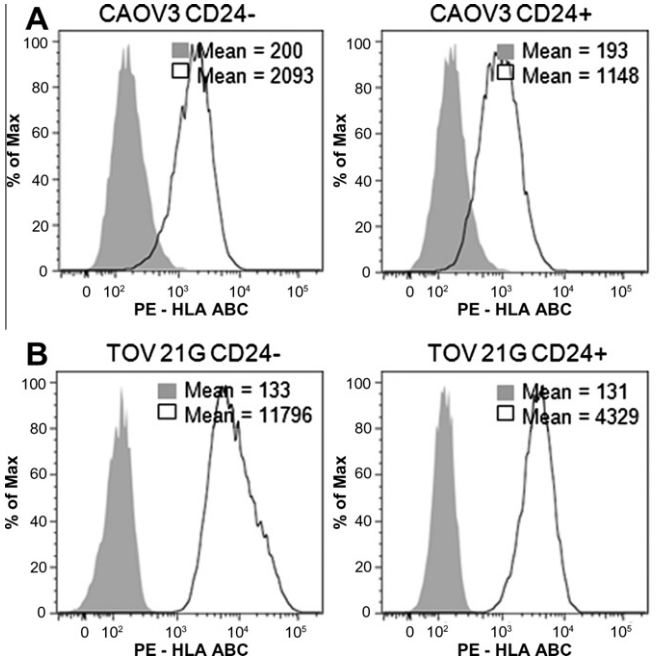

Fig. 3. Analysis of MHC class I molecule expression. CD24$^-$ and CD24$^+$ CAOV3 and TOV21G cells were stained with PE-conjugated HLA-ABC monoclonal antibody. The mean PE values are indicated on each graph. (A) MHC class I molecule expression on CD24$^-$ CAOV3 (left) and CD24$^+$ CAOV3 (right). (B) MHC class I molecule expression on TOV21G cells sorted with CD24$^-$ (left) and CD24$^+$ (right). The gray shadow refers to the control group, and the black line refers to the sample stained with HLA-ABC antibody.

CD24$^+$ TOV21G cells expressed levels that were three times less than that of CD24$^-$ cells (Fig. 3B). Again, CD24$^+$ CAOV3 and TOV21G cells showed similar results, which expressed a lower level of MHC class I molecules.

3.5. NKG2D and NCR ligand expression

MICA/B, ULBP1, ULBP2, and ULBP3 expression was measured on target cell surfaces. CD24$^+$ CAOV3 cells expressed four times the level of ULBP1 and five times the level of ULBP3 compared with CD24$^-$ cells (Fig. 4B and D). CD24$^+$ TOV21G cells expressed five times the level of MIC A/B. ULBP3 expression on CD24$^+$ cells was also higher than that of CD24$^-$ cells. The mean FITC values were 2164 and 3415, respectively (Fig. 4A and D). Overall, CD24$^+$ CAOV3 and TOV21G cells expressed higher levels of all NKG2D ligands compared with CD24$^-$ cells.

The cell surface expression of NKp30, NKp44, and NKp46 ligands were measured on target cells. CD24$^+$ CAOV3 cells expressed higher levels of NCR ligands. Especially, NKp44 ligand expression on CD24$^+$ cells was significantly higher compared with CD24$^-$ cells (Fig. 4F). CD24$^-$ and CD24$^+$ TOV21G cells expressed similar levels of NCR ligands (Fig. 4E–G). Therefore, NCR ligand expression was not significant to compare the different characteristics of CD24$^-$ and CD24$^+$ ovarian cancer cell lines.

4. Discussion

In this study, we isolated ovarian cancer cells with and without CD24 and tested for drug resistance and susceptibility to the lysis by freshly isolated and IL-2-stimulated NK cells. The expression

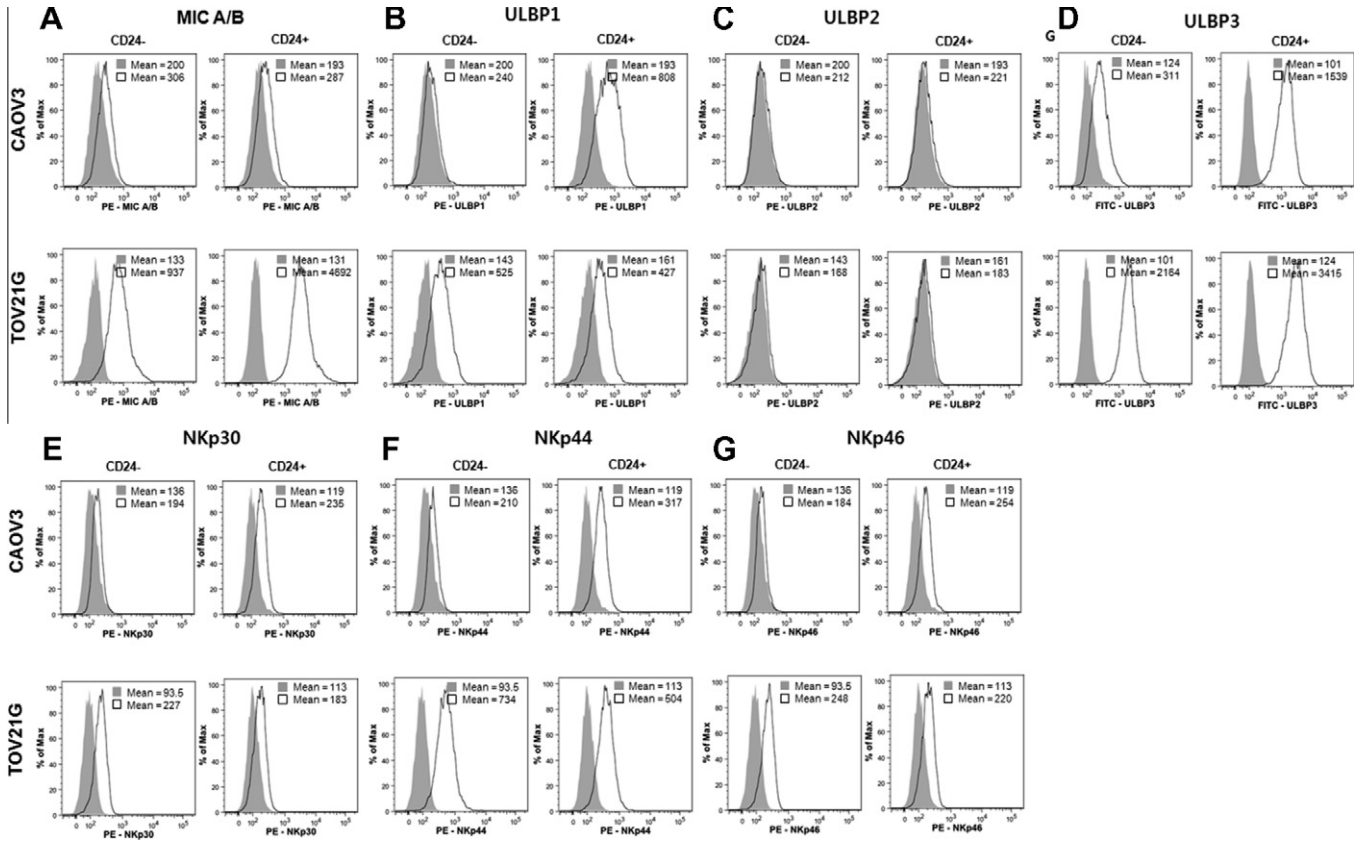

Fig. 4. Analysis of NKG2D and NCR ligands expression. MIC A/B, ULBP1, ULBP2, and ULBP3 expression on CD24$^-$ and CD24$^+$ CAOV3 (first row) and TOV21G (second row) were measured. (A–D) MIC A/B, ULBP1, ULBP2, and ULBP3 expression on CD24$^-$ CAOV3 and TOV21G (left) and CD24$^+$ CAOV3 and TOV21G (right). NKp30, NKp44, and NKp46 ligand expression on CD24$^-$ and CD24$^+$ CAOV3 and TOV21G were measured. (E–G) NCR expression on CD24$^-$ CAOV3 and TOV21G (left) and CD24$^+$ CAOV3 and TOV21G (right). Fc-conjugated chimera human recombinant protein of NKp30, NKp44, and NKp46 were used, and then PE-anti-human IgG Fc antibody was added. The mean PE values are indicated on each graph. The gray shadow refers the control group, and the black line refers the sample stained with specific antibodies.

levels of cell surface molecules on $CD24^-$ and $CD24^+$ ovarian cancer cells were tested to identify reasons for the differential susceptibility values to NK cell cytotoxicity. Here, we showed that the chemotherapy drug-resistant $CD24^+$ CAOV3 and TOV21G were effectively killed by both freshly isolated and IL-2-stimulated NK cells. Also, $CD24^+$ CAOV3 and TOV21G cells showed up-regulated NKG2D ligands and down-regulated MHC class I molecules.

It is a well-established theory that cancer stem cells are highly resistant to chemotherapy drugs. In this study, among many ovarian cancer stem cell markers, we specifically chose CD24 as a target cell surface molecule. First we used two widely used chemotherapy drugs, cisplatin and doxorubicin, to test drug resistance in $CD24^-$ and $CD24^+$ ovarian cancer cell lines. Drug resistance may differ depending on the cell type and drugs used; however, overall, ovarian cancer cell lines with CD24 were highly resistant to the anti-cancer drugs compared with cancer cells without CD24. Previous studies revealed that cancer stem cells express increased levels of ABC drug transporters, which efflux chemotherapy drugs from cancer cells to resist against anti-cancer drugs [18].

In a previous study, Castriconi et al. showed that GBM cells cultured in stem cell conditions were more susceptible to lysis by IL-2-activated NK cells [19]. Since different cancer stem cell markers were identified on several cancer cells, it seemed necessary to investigate the ovarian cancer stem cell characteristics to show that drug resistant ovarian cancer stem cells were effectively killed by NK cells compared with cancer cells without cancer stem cell markers in order to be able to apply NK cell immunotherapy to target ovarian cancer stem cells.

In this study, we isolated $CD24^-$ and $CD24^+$ ovarian cancer cells to determine the different characteristics of each cell lines with and without CD24 and show that $CD24^+$ ovarian cancer cells are more resistant to chemotherapy drugs but more susceptible to freshly isolated and IL-2-activated NK cells. This study showed that $CD24^+$ CAOV3 and TOV21G cells were highly susceptible to lysis by both freshly isolated and IL-2-activated NK cells. To reveal the reasons for the higher susceptibility of $CD24^+$ ovarian cancer cells to NK cells, the target cell surface molecules were analyzed.

The data showed that $CD24^+$ ovarian cancer cells decreased expression of MHC class I molecules and increased expression of NKG2D ligands. It is well known that MHC class I molecules are down-regulated on cancer cells in order to avoid the effects of the adaptive immune system; however, NK cells recognize target cells lacking MHC class I molecules [8]. Therefore, since $CD24^+$ CAOV3 and TOV21G cells were more susceptible to NK cells, the level of down-regulated MHC class I molecules compared to that on $CD24^-$ cells might be one of the reasons for high susceptibility to NK cell lysis.

NKG2D is an important receptor that initiates NK cell-killing mechanisms. Our data demonstrated that $CD24^+$ CAOV3 and TOV21G cells expressed a significantly higher level of NKG2D ligands compared with $CD24^-$ cells. In particular, MIC A/B, ULBP1, and ULBP3 were highly expressed on $CD2sss4^+$ CAOV3 and TOV21G cells. There is a published report demonstrated that NKG2D prevents tumor initiation and controls tumor formation [22]. Also, NKG2D contributes to graft rejection in transplanted stem cells with up-regulated expression of NKG2D ligands [23]. Therefore, NKG2D plays a key role in preventing tumor initiation and also in eliminating cancer stem cells due to their stem cell-like properties.

NCRs are critical NK cell receptors to kill target cells, however, only NKp44 ligand was significantly expressed on $CD24^+$ CAOV3 cells, while no other NCR ligands were up-regulated on $CD24^+$ cells compared with $CD24^-$ cells.

In this study, we used CAOV3 and TOV21G cell lines. $CD24^+$ CAOV3 and TOV21G cell lines showed cancer stem cell properties that were drug-resistant and susceptible to NK cell lysis. Also, $CD24^+$ CAOV3 and TOV21G cells showed increased NKG2D ligands and decreased MHC class I molecules on their cell surfaces. Gao et al. revealed that $CD24^+$ cells have cancer stem cell properties [18], and other studies identified that CD44, CD117, and CD133 are ovarian cancer stem cell markers [24,25].

Based on the results of CAOV3 and TOV21G cells, we concluded that $CD24^+$ cells had cancer stem cell properties that are more resistant to chemotherapy drugs and susceptible to the lysis of NK cells. Also, NKG2D/ligand interaction is a major death mechanism in eliminating ovarian cancer cells with $CD24^+$ cells. In the present study, IL-2-activated NK cells killed the majority of both $CD24^-$ and $CD24^+$ cancer cells at the 1:10 target-to-effector ratio. If IL-2-activated NK cells were applied, then it might be possible to eliminate drug-resistant ovarian cancer stem cells that remain after chemotherapy or radiotherapy and thus prevent tumor recurrence. Furthermore, conducting the experiments using different ovarian cancer cell lines and primary ovarian cancer cells isolated with $CD24^-$ and $CD24^+$ and with other ovarian cancer stem cell markers CD44, CD117, and CD133 are necessary for further study to conclude that ovarian cancer stem cells are resistant to antitumor drugs but more susceptible to the lysis of NK cells in order to apply NK cells to treat ovarian cancer stem cells.

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References

[1] A. Cerwenka, L.L. Lanier, Natural killer cells, viruses and cancer, Nat. Rev. Immunol. 1 (2001) 41–49.
[2] W.M. Yokoyama, S. Kim, A.R. French, The dynamic life of natural killer cells, Annu. Rev. Immunol. 22 (2004) 405–429.
[3] R.H. Wiltrout, B.J. Mathieson, J.E. Talmadge, C.W. Reynolds, S.R. Zhang, R.B. Herberman, J.R. Ortaldo, Augmentation of organ-associated natural killer activity by biological response modifiers. Isolation and characterization of large granular lymphocytes from the liver, J. Exp. Med. 160 (1984) 1431–1449.
[4] I. Voskoboinik, M.A. Dunstone, K. Baran, J.C. Whisstock, J.A. Trapani, Perforin: structure, function, and role in human immunopathology, Immunol. Rev. 235 (2010) 35–54.
[5] S.P. Cullen, M. Brunet, S.J. Martin, Granzymes in cancer and immunity, Cell Death Differ. 17 (2010) 616–623.
[6] W.M. Yokoyama, Recognition structures on natural killer cells, Curr. Opin. Immunol. 5 (1993) 67–73.
[7] L.L. Lanier, NK cell receptors, Annu. Rev. Immunol. 16 (1998) 359–393.
[8] J.C. Sun, L.L. Lanier, Cutting edge: viral infection breaks NK cell tolerance to "missing self", J. Immunol. 181 (2008) 7453–7457.
[9] J. Monteiro, R. Fodde, Cancer stemness and metastasis: therapeutic consequences and perspectives, Eur. J. Cancer 46 (2010) 1198–1203.
[10] T. Reya, S.J. Morrison, M.F. Clarke, I.L. Weissman, Stem cells, cancer, and cancer stem cells, Nature 414 (2001) 105–111.
[11] J. Stingl, C. Caldas, Molecular heterogeneity of breast carcinomas and the cancer stem cell hypothesis, Nat. Rev. Cancer 7 (2007) 791–799.
[12] S. Bomken, K. Fiser, O. Heidenreich, J. Vormoor, Understanding the cancer stem cell, Br. J. Cancer 103 (2010) 439–445.
[13] D. Bonnet, J.E. Dick, Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell, Nat. Med. 3 (1997) 730–737.
[14] B.J. Huntly, D.G. Gilliland, Leukaemia stem cells and the evolution of cancer-stem-cell research, Nat. Rev. Cancer 5 (2005) 311–321.
[15] M. Al-Hajj, M.S. Wicha, A. Benito-Hernandez, S.J. Morrison, M.F. Clarke, Prospective identification of tumorigenic breast cancer cells, Proc. Natl. Acad. Sci. USA 100 (2003) 3983–3988.
[16] M.Q. Gao, Y.P. Choi, S. Kang, J.H. Youn, N.H. Cho, $CD24^+$ cells from hierarchically organized ovarian cancer are enriched in cancer stem cells, Oncogene 29 (2010) 2672–2680.
[17] M. Dean, T. Fojo, S. Bates, Tumour stem cells and drug resistance, Nat. Rev. Cancer 5 (2005) 275–284.
[18] M. Dean, T. Fojo, S. Bates, Tumour stem cells and drug resistance, Nat. Rev. Cancer 5 (2005) 275–284.
[19] R. Castriconi, A. Daga, A. Dondero, G. Zona, P.L. Poliani, A. Melotti, F. Griffero, D. Marubbi, R. Spaziante, F. Bellora, L. Moretta, A. Moretta, G. Corte, C. Bottino, NK cells recognize and kill human glioblastoma cells with stem cell-like properties, J. Immunol. 182 (2009) 3530–3539.

[20] C.S. Alvarado, H.W. Findley, W.C. Chan, R.S. Hnath, A. Abdel-Mageed, R.C. Pais, M.H. Kutner, A.H. Ragab, Natural killer cells in children with malignant solid tumors. Effect of recombinant interferon-alpha and interleukin-2 on natural killer cell function against tumor cell lines, Cancer 63 (1989) 83–89.
[21] K. Itoh, C.D. Platsoucas, C.M. Balch, Monocyte- and natural killer cell-mediated spontaneous cytotoxicity against human noncultured solid tumor cells, Cell Immunol. 108 (1987) 495–500.
[22] M.J. Smyth, J. Swann, E. Cretney, N. Zerafa, W.M. Yokoyama, Y. Hayakawa, NKG2D function protects the host from tumor initiation, J. Exp. Med. 202 (2005) 583–588.
[23] K. Ogasawara, J. Benjamin, R. Takaki, J.H. Phillips, L.L. Lanier, Function of NKG2D in natural killer cell-mediated rejection of mouse bone marrow grafts, Nat. Immunol. 6 (2005) 938–945.
[24] S. Zhang, C. Balch, M.W. Chan, H.C. Lai, D. Matei, J.M. Schilder, P.S. Yan, T.H. Huang, K.P. Nephew, Identification and characterization of ovarian cancer-initiating cells from primary human tumors, Cancer Res. 68 (2008) 4311–4320.
[25] M.D. Curley, V.A. Therrien, C.L. Cummings, P.A. Sergent, C.R. Koulouris, A.M. Friel, D.J. Roberts, M.V. Seiden, D.T. Scadden, B.R. Rueda, R. Foster, CD133 expression defines a tumor initiating cell population in primary human ovarian cancer, Stem Cells 27 (2009) 2875–2883.