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Differential Effects of Transforming Growth Factor-β on NKG2D Ligands Expression and NK Cell-mediated Immune Responses in Primary and Metastatic Colon Cancer

Eun-Jung Yun[1,2], Yu-Rim Kim[1,2], Seong Jun Park[1,2], Sang-Yull Lee[1] and Jaeho Bae[1,2]*

[1]*Department of Biochemistry, Pusan National University School of Medicine, Yangsan 50612, Korea*
[2]*PNU GRAND Convergence Medical Science Education Research Center, Pusan National University School of Medicine, Yangsan 50612, Korea*

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Transforming growth factor-β (TGF-β) is a multifunctional cytokine that affects not only the survival and growth of cancer cells but also the activity of immune cells. Although it has been generally accepted that cancer cell-derived TGF-β could promote the survival and growth of early cancer cells and have immunosuppressive roles, it has been known that TGF-β has differential effects according to the type or stage of cancer cells. Therefore, it is hard to clearly define its role in cancer progression and immune responses. This study investigated the effects of TGF-β signaling on the expression of five NKG2D ligands and the NK cell-mediated anticancer immune response in the primary colon cancer cell line KM12C and its two metastatic cell lines, KM12SM and KM12L4A. At the surface protein level, exogenous TGF-β decreased the expression of MICA, MICB, ULBP1, and ULBP2, and galunisertib increased the expression of MICA, MIAB, ULBP1, ULBP2, and ULBP3 in KM12C. However, KM12SM and KM12L4A showed no significant changes in the expression of NKG2DLs after treatment with TGF-β or galunisertib. TGF-β signaling inhibition via galunisertib improved the NK cell-mediated anticancer immune response against KM12C but did not show a significant response to KM12SM and KM12L4A. Therefore, the suppression of TGF-β signaling could improve the NK cell-mediated anticancer immune response against KM12C. However, an increase in NKG2DLs expression and an enhanced NK cell-mediated cancer immune response is hard to expect due to the alteration of TGF-β signaling in KM12SM and KM12L4A.

Key words : Colon cancer, galunisertib, metastatic cells, NKG2D lignads, transforming growth factor-β

Introduction

Colon cancer is caused by obesity, heredity, eating habits, etc. and the incidence is increasing due to changes in westernized eating habits in South Korea [1]. It is mainly treated with surgical removal, radiotherapy, chemotherapy or their combination. Since immunotherapy has been showed limited effect to suppress the growth of colon cancer until now, it has been used as adjuvant therapy [5, 24].

Transforming growth factor-β (TGF-β) is produced in almost all cells of the human body and was known to stimulate interferon-gamma (IFN-γ) production, which can induce T and B lymphocyte proliferation as well as regulate the differentiation or activity of NK cells, dendritic cells, macrophages, and granular cells [10, 12, 16]. In addition, TGF-β contributed to the activity of cells such as regulatory T cells which had various immunosuppressive actions and T helper 17 cells which were related to inflammation and autoimmune [14, 29]. It is generally accepted that TGF-β derived from cancer cells can promote cancer growth and malignant progression through immune evasion during cancer progression. However, since there are reports that it had differential effects depending on the stage of cancer progression, it is hard to define clearly its role in cancer progression [22, 23]. These various functions of the TGF-β signaling occur through the SMAD-dependent pathway that is the major transporter of TGF-β signaling and the SMAD-independent pathways such as the

***Corresponding author**
Tel : +82-51-510-8085, Fax : +82-51-510-8086
E-mail : biosole@pusan.ac.kr

MAP kinase pathway, the Rho-like GTPase signaling pathway and the phosphatidylinositol-3-kinase/AKT pathway [18, 31].

Natural killer cells (NK cells) are lymphocytes belonging to the innate immune system that recognize and eliminate abnormal cells such as cancer cells or virus-infected cells, and have various inhibitory and activating receptors that regulate immune responses. Among them, NKG2D is a potent activation receptor expressed as surface proteins in NK cells, γδ T cells, and CD8+ αβ T cells, and combines with NKG2D ligands (NKG2DLs), which are expressed by response to external stimuli such as viral infection and genetic damage in target cells and several cytokines on target cells. These NKG2DLs include MHC class I polypeptide related chain proteins A and B (MICA and MICB), UL16 binding protein1 (ULBP1), ULBP2, ULBP3 and ULBP4 [9, 17, 20].

In previous studies, TGF-β has been reported to inhibit the expression of NKG2DLs in lung cancer cell lines and reduce cytotoxicity of NK cells, but its effect on metastatic cancer is not yet investigated [11].

In this study, we investigated the effect of TGF-β signal on the expression of NKG2DLs in primary KM12C colon cancer cell line and its metastatic KM12SM and KM12L4A cell lines, and ultimately, on the susceptibility of NK cells to these target cells.

Materials and Methods

Cell lines and reagents

Human colon cancer cell lines, KM12C, KM12SM and KM12L4A, were obtained from the Korean Cell Line Bank (Seoul, Korea). These cell lines were maintained in DMEM medium which was supplemented with 10% fetal bovine serum (FBS) (Gibco, Grand Island, NY, USA), 2 mM L-glutamine, 100 mg/ml streptomycin, and 100 U/ml penicillin. The NK-92 cell line was obtained from the American Type Culture Collection (Rockville, MD, USA) and cultured in an α-minimum essential modified medium supplemented with 12.5% (v/v) fetal bovine serum, 12.5% (v/v) horse serum, 2 mM L-glutamine, 0.1 mM 2-mercaptoethanol, 200 U/ml recombinant human interleukin-2, 100 μg/ml streptomycin and 100 U/ml penicillin. Cells were cultured in a humidified atmosphere at 37℃ and 5% CO_2. Recombinant human TGF-β was purchased from R&D system, Inc. (Minneapolise, MN, USA) and galunisertib were purchased from Selleckchem (Houston, TX, USA).

Total RNA Extraction and Multiplex Reverse Transcription PCR (RT-PCR)

Total RNA extraction and reverse transcription PCR were performed according to previous study [19]. Total RNA was extracted from the cells using the RNeasy® Mini kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. cDNA was synthesized from 1 μg extracted total RNA using 100 pmol of random primers (Takara Bio Inc., Otsu, Japan) and 100 U M-MLV reverse transcriptase (Promega Corporation, Madison, WI, USA). The synthesized cDNA was subjected to PCR using the QIAGEN Multiplex PCR kit (Qiagen, Hilden, Germany). Seven pairs of primer were used to investigate the mRNA expression levels of NKG2D ligands : MICA, MICB, ULBP1, ULBP2 and ULBP3. β-actin (ACTB) and RPL19 were used as the loading control and the degradation marker, respectively (Bioneer Corporation, Daejeon, Korea). PCR analysis was separated and quantified by MultiNA (Shimadzu, Tokyo, Japan).

Flow Cytometry

Colon cancer cells were incubated with mouse anti-MICA, anti-MICB, anti-ULBP1-3 (R&D systems, Minneapolis, MN, USA), rabbit anti-TGF-β receptor1 (Rockland Immunochemicals, Inc., Limerick, PA, USA) and rabbit anti-TGF-β receptor2 monoclonal antibodies (Abcam, Cambridge, UK). 1 μg of the monoclonal antibodies (mAbs) and isotype control were sufficient to determine the surface expression of NKG2DLs and TGF-β receptor1 (TGF-βR1) and TGF-βR2. These were followed by the addition of goat anti-mouse PE-conjugated antibody and FITC goat anti-rabbit IgG (BD Phamingen Inc., San Diego, CA, USA). The analysis was performed using BD FACSCANTO II (Becton Dickinson and Company, Franklin Lakes, NJ, USA) and FlowJo software (BD Biosciences, Franklin Lakes, NJ, USA), the cell surface expression levels were quantified by the value of mean fluorescence intensity (MFI).

Western Blot Analysis

Western Blot Analysis was used to confirm the expression of TGF-βR1. Cells were lysed in a lysis buffer, and the cellular debris was removed by centrifugation at 15,000 rpm for 10 mins. The proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred onto a nitrocellulose membrane Hybond-ECL (GE Healthcare, Chalfont Saint Giles, UK), blocked with 5% skimmed milk, and incubated with anti-TGF-βR1 antibodies (Rockland Immunochemicals, INC. 1:1,000 dilution) and an anti-β-actin antibody

(Novus Biologicals, 1:10,000 dilution). Membranes were then incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG secondary antibody (Enzo Life Science Inc. Bucharest, Romania, 1:5,000 dilution) for 1 hr at room temperature and illuminated by enhanced chemiluminescence (Perkin-Elmer Life Science, Waltham, MA, USA). Expression levels were measured using an Amersham Imager 680 (GE Healthcare).

Flow Cytometry Analysis of the NK Cell-Mediated Cytotoxicity Assay

The colon cancer cell lines were labeled with 1 μM carboxyfluorescein succinimidyl ester (CFSE) (Invitrogen, Eugene, OR, USA) for 15 mins at 37℃ in a humidified incubator at 5% CO_2 and then washed three times. The NK-92 cells were co-cultured with CFSE-labeled colon cancer cell lines in round-bottomed 96-well plates for 4 hr and at appropriate NK-92 cells-to-cancer cells count ratios (5:1, 10:1). Propidium iodide (PI) (Sigma-Aldrich, St. Louis, MO, USA) was added to the co-cultured samples to distinguish dead cells. Dead cells were determined using FC500 flow cytometer (Beckman Coulter, CA, USA). All experiments were performed in triplicate.

Statistical Analysis

The mean folds of gene expressions and the standard errors were calculated to evaluate altered gene expression. A paired Student's *t*-test was performed to evaluate the significance. $p<0.05$ indicates a statistically significant value in all experiments.

Results

The mRNA expression of NKG2DLs after treatment with galunisertib in primary and metastatic colon cancer cell lines

The primary colon cancer cell line KM12C and its two metastatic colon cancer cell lines KM12SM and KM12L4A were treated with galunisertib, a TGF-β inhibitor, at 10 uM, and then the change in NKG2DLs mRNA expression over time was analyzed. The expressions of MICA, MICB, ULBP1, and ULBP3 were increased in KM12C, and in particular, the expression of MICA and ULBP1 was markedly increased (Fig. 1A). However, in metastatic cells KM12SM and KM12L4A, there was no significant change in the expression of NKG2DLs, except ULBP1 which decreased transiently in KM12SM (Fig. 1B, Fig. 1C). Since it was reported previously that the expression of NKG2DLs were increased in primary lung cancer, no responses in metastatic cell lines were interesting finding. It was predicted that TGF-β signaling which is involved in NKG2DLs expression has functional differences between primary and metastatic cancers, and that these differences may affect anticancer immune responses.

The expression of NKG2DLs surface protein after treatment with galunisertib in primary and metastatic colon cancer cell lines

The changes in surface protein expression of NKG2DLs were analyzed after 24 hr of treatment with TGF-β and galunisertib in colon cancer cell lines using flow cytometry. In KM12C, MICA, MICB, ULBP1 and ULBP2 tended to decrease after TGF-β treatment, and all five NKG2DLs surface proteins increased after galuniseritb treatment (Fig. 2A). This was nearly consistent with changes in the mRNA expression of NKG2DLs in KM12C treated with galunisertib. However, there was no significant change in the surface protein expression of NKG2DLs after treatment with TGF-β or galunisertib in KM12SM and KM12L4A (Fig. 2B, Fig. 2C). Since surface protein level is dependent on the level of transcription as well as protein stability, the differential effects of TGF-β on the expression of NKG2DLs was shown more clearly in the primary and metastatic cell lines.

Surface expression of TGF-βR1-2 and total protein level of TGF-βR1 in primary and metastatic colon cancer cell lines

We measured the surface proteins of two TGF-β receptors, TGF-βR1 and TGF-βR2, using flow cytometry to find whether differences in the expression of TGF-β receptors in primary and metastatic colon cancer cell lines could affect TGF-β signaling. There was no significant difference in TGF-βR1 and TGF-βR2 expression among three cell lines (Fig. 3A - Fig. 3C). Since TGF-βR1 were hardly detected the cell surface, the total amount of TGF-βR1 protein was checked again using Western blot. There was also no significant difference in the total amount of TGF-βR1 protein among three cell lines (Fig. 3D). It was found that different patterns of NKG2DLs expression did not result from the difference of TGF-βR1 and TGF-βR2 expression between primary and metastatic cell lines.

The NK cell-mediated immune response in primary and metastatic colon cancer cells after TGF-β and galunisertib treatment

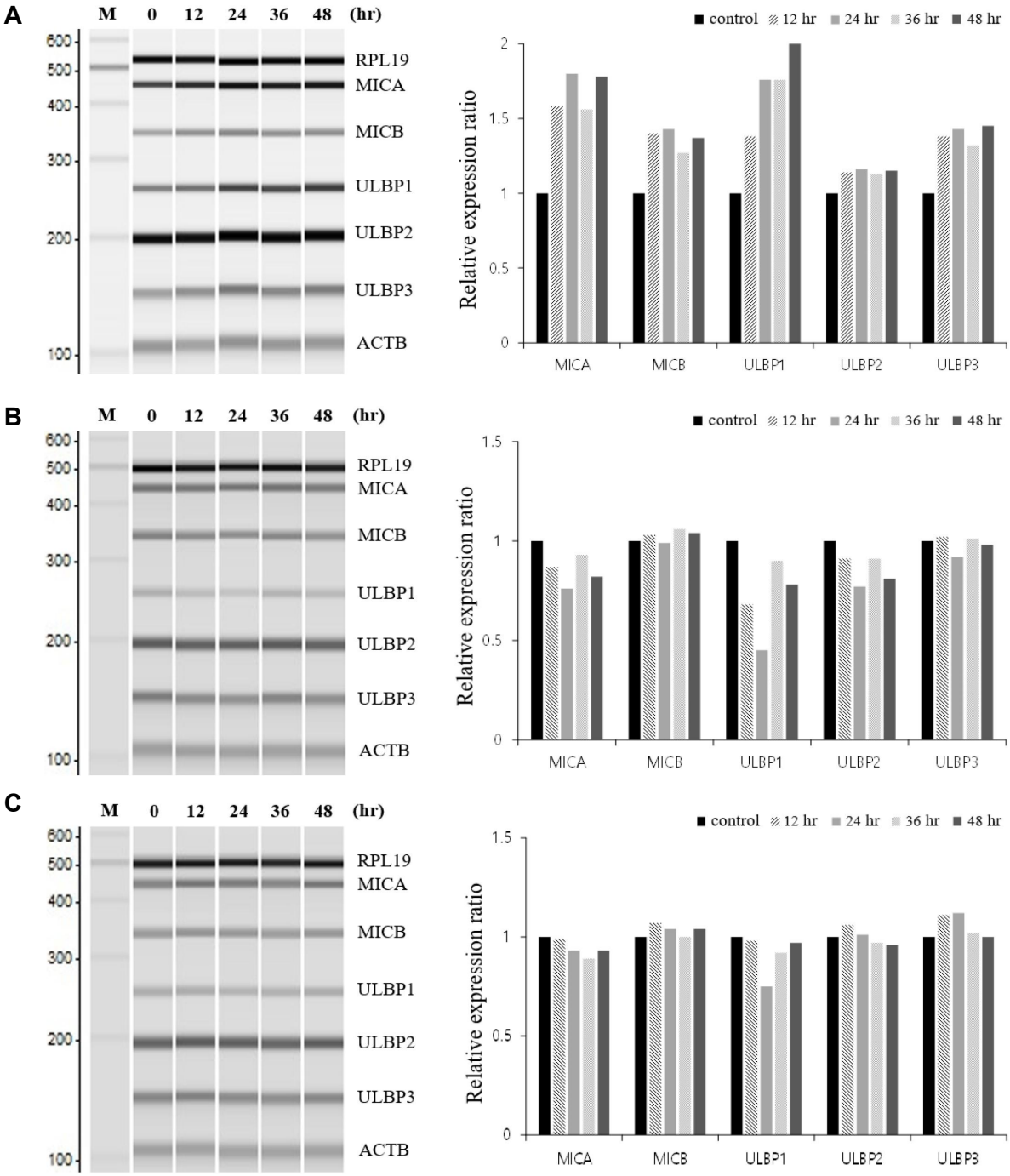

Fig. 1. The mRNA levels of NKG2DLs in primary and metastatic colon cancer cell lines after galunisertib treatment. The mRNA levels of NKG2DLs were analyzed using RT-PCR in colon cancer cell (A) KM12C, (B) KM12SM and (C) KM12L4A. Each colon cancer cells were treated with galunisertib 10 uM for 12, 24, 36 and 48 hr. The mRNA levels were normalized by ACTB and mRNA levels changes were presented as the mean fold in comparison to the controls.

In KM12C, galunisertib increased the surface expression of NKG2DLs and increased NK cell-mediated cytotoxicity by increasing NK cell sensitivity (Fig. 4A). Although the reason is unclear, there was no decrease in sensitivity to NK cells despite a decrease in NKG2DLs expression by TGF-β treatment. On the other hand, in KM12SM and KM12L4A, there were no significant changes in NK cell sensitivity after galunisertib and TGF-β treatment (Fig. 4B, Fig. 4C). These results showed that NK cell-mediated immune responses were different against to primary and metastatic colon cancer cell

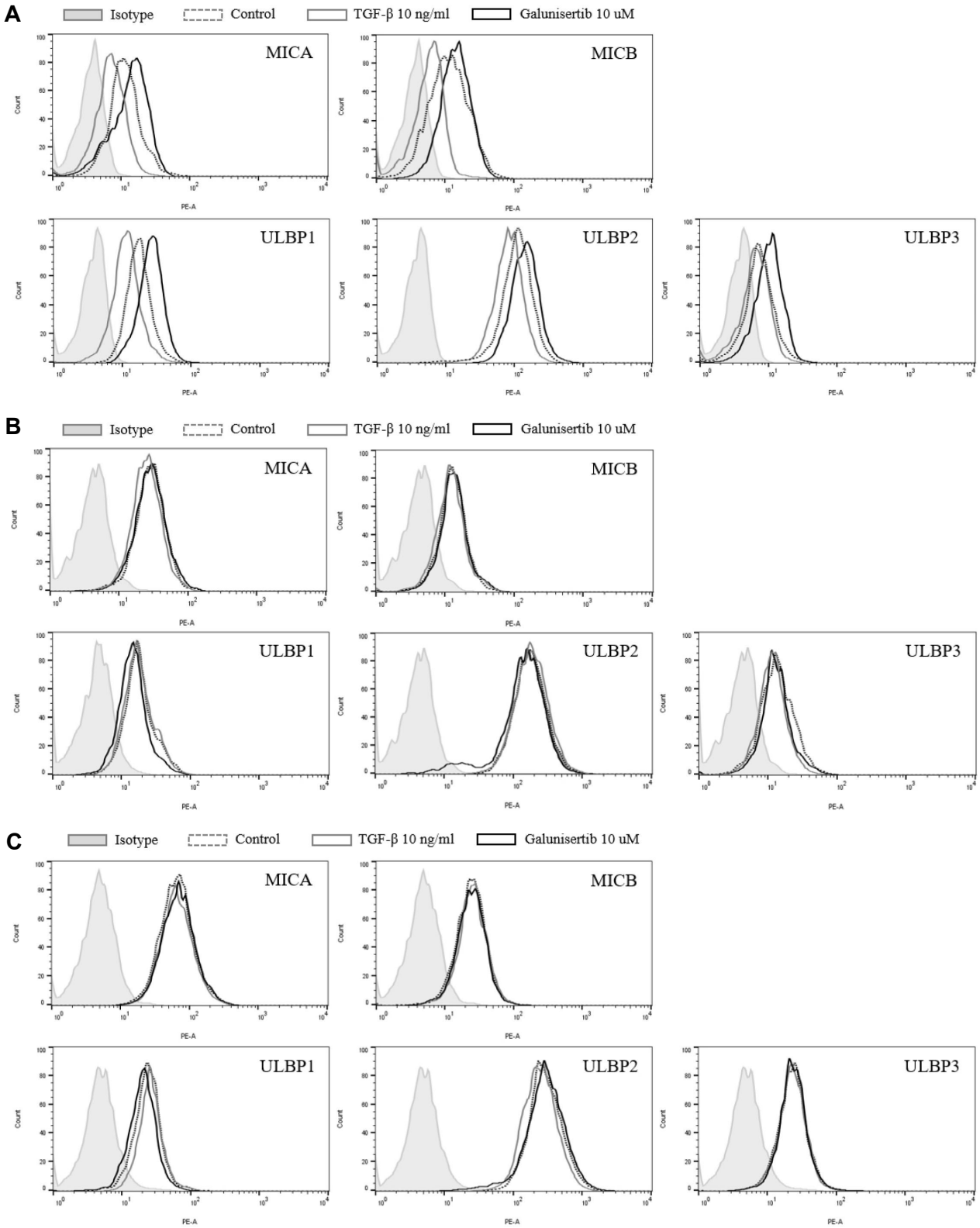

Fig. 2. The surface expression of NKG2DLs is altered by TGF-β and galunisertib in KM12C, but with different patterns in the two metastatic cancer cell lines. This histogram shows the surface expression of NKG2DLs in colon cancer cells (A) KM12C, (B) KM12SM, and (C) KM12L4A. They were treated with TGF-β 10 ng/ml and galunisertib 10 μM for 24 hr : Filled gray, dotted black, gray solid line and black solid line represent the isotype control, media-treated control, TGF-β and galunisertib treated samples, respectively.

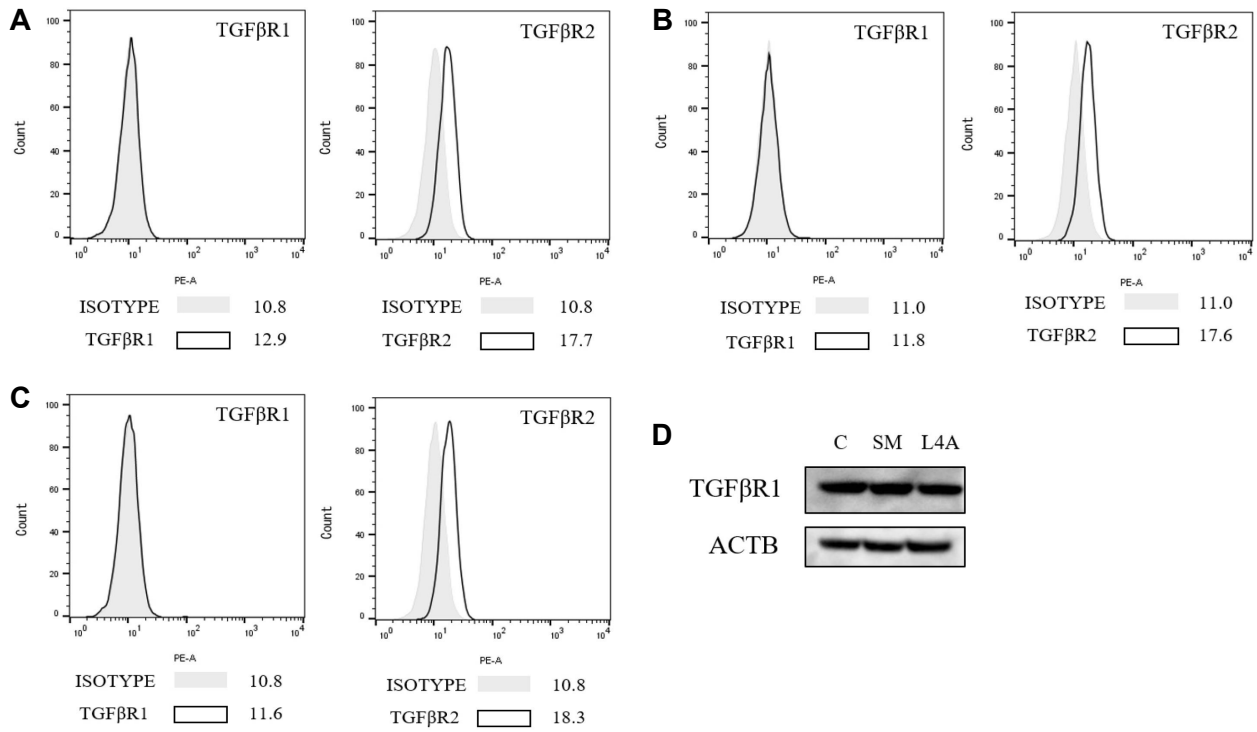

Fig. 3. TGF-βR1/2 protein expression in colon cancer cell lines. The TGF-β receptor surface protein expression of colon cancer cells was analyzed by flow cytometry using specific antibodies for TGF-βR1 and TGF-βR2 in (A) KM12C, (B)KM12SM and (C)KM12L4A. Filled gray represent the isotype control, Solid line represent TGF-βR1 or TGF-βR2. (D)The total amount of TGF-βR1 protein in each colon cancer cells were measured by Western Blot using anti-TGF-βR1 monoclonal antibodies and ACTB was used as the loading control.

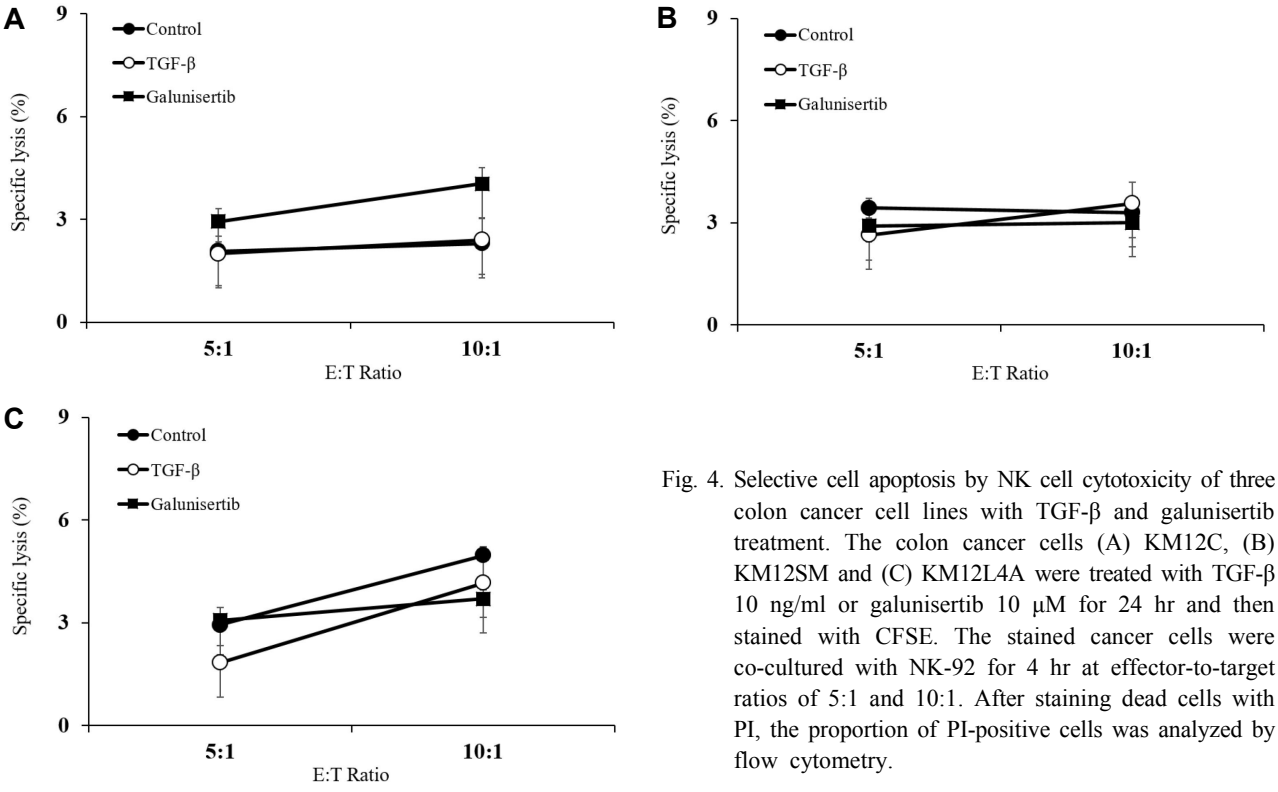

Fig. 4. Selective cell apoptosis by NK cell cytotoxicity of three colon cancer cell lines with TGF-β and galunisertib treatment. The colon cancer cells (A) KM12C, (B) KM12SM and (C) KM12L4A were treated with TGF-β 10 ng/ml or galunisertib 10 μM for 24 hr and then stained with CFSE. The stained cancer cells were co-cultured with NK-92 for 4 hr at effector-to-target ratios of 5:1 and 10:1. After staining dead cells with PI, the proportion of PI-positive cells was analyzed by flow cytometry.

lines after modulation TGF-β signaling.

Discussion

TGF-β is an obscure cytokine that promotes apoptosis in primary cancer, helps cells proliferation and migration in metastatic cancer, and regulate the immune response differentially depending on the degree of tumor progression [22, 23]. The mechanism of action of TGF-β according to the tumor stage is not clear and further study is needed. In normal cells or early cancer cells, TGF-β induced cyclin-dependent kinases inhibitors such as $p16^{INK4A}$, $p15^{INK4B}$ and $p27^{KIP1}$ and arrest the cell cycle in the G1 phase. It induced transcription factors such as c-myc, NF-κB, and ID1, and promoted cell growth and apoptosis also [7, 8, 21, 28]. Whereas, in late cancer cells, inhibition of TGF-β signaling results suppressed cell cycle and tumor growth through the overexpression of cyclin D and c-myc. The anticancer immune responses were altered by TGF-β signaling. In early cancer, TGF-β suppressed T cell differentiation and inhibited myeloid cell proliferation and differentiation through reduction of IFN-γ secretion. In late cancer, TGF-β suppressed the differentiation and activity of immune cells such as cytotoxic T lymphocytes, regulatory T cells, NK cells and B cells. The TGF-β also induced IL-17 secretion which might be involved in cancer growth, metastasis and angiogenesis [2, 30].

Previously it was reported that TGF-β decreased the expression of NKG2DLs and NKp30 in lung cancer cells and NK cells, respectively [4, 6]. It was presumed that TGF-β helped the evade immune responses by suppression of NK cells mediated immune responses in cancer cells. Based on these previous reports, it was necessary to investigate whether TGF-β inhibition could regulate NK cell activity in metastatic cancer cells as if in primary cancer cells.

In this study, it was showed the changes in mRNA transcription and surface protein expression levels of five NKG2DLs and NK cell-mediated anticancer effects upon the presence of TGF-β inhibitors only on primary, not on metastatic cell lines. Similar to previous studies, exogenous TGF-β inhibited the surface expression of NKG2DLs (MICA, MICB, ULBP1 and ULBP2), and galunisertib increased surface expression of mRNA and NKG2DLs in primary colon cancer cell line. However, there was no significant change in the expression of NKG2DLs when TGF-β or galunisertib was treated in two types of metastatic colon cancer cell lines despite no difference of the expression of TGF-βR1 and TGF-β R2 among primary and metastatic colon cancer cell lines.

There are several reports that established cancer cells themselves were insensitive to TGF-β [3, 25]. Most human colon cancer cells had defects to transduce TGF-β signaling, therefore resistant to TGF-β apoptosis or TGF-β-mediated growth inhibition. These defects often result by deletion or mutation of receptors such as TGF-βR1 and TGF-βR2, or downstream molecules such as SMAD2 or SMAD4 [13, 15, 26, 27].

In order to elucidate the difference in TGF-β signaling that affects NKG2DLs expression differently depending on the degree of cancer cells progression, it was thought that further study might be needed to find defects of downstream molecules of TGF-β signaling in metastatic cell lines.

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The Conflict of Interest Statement

The authors declare that they have no conflicts of interest with the contents of this article.

References

1. Alexander, D. D., Miller, A. J., Cushing, C. A. and Lowe, K. A. 2010. Processed meat and colorectal cancer: a quantitative review of prospective epidemiologic studies. *Eur. J. Cancer Prev.* **19**, 328-341.
2. Becker, C., Fantini, M. C. and Neurath, M. F. 2006. TGF-beta as a T cell regulator in colitis and colon cancer. *Cytokine Growth Factor Rev.* **17**, 97-106.
3. Calon, A., Tauriello, D. V. and Batlle, E. 2014. TGF-beta in CAF-mediated tumor growth and metastasis. *Semin. Cancer Biol.* **25**, 15-22.
4. Castriconi, R., Cantoni, C., Della Chiesa, M., Vitale, M., Marcenaro, E., Conte, R., Biassoni, R., Bottino, C., Moretta, L. and Moretta, A. 2003. Transforming growth factor beta 1 inhibits expression of NKp30 and NKG2D receptors: consequences for the NK-mediated killing of dendritic cells. *Proc. Natl. Acad. Sci. USA.* **100**, 4120-4125.
5. Chau, I. and Cunningham, D. 2006. Adjuvant therapy in colon cancer--what, when and how? *Ann. Oncol.* **17**, 1347-1359.
6. Crane, C. A., Han, S. J., Barry, J. J., Ahn, B. J., Lanier, L. L. and Parsa, A. T. 2010. TGF-beta downregulates the activating receptor NKG2D on NK cells and CD8+ T cells in glioma patients. *Neuro Oncol.* **12**, 7-13.
7. Freudlsperger, C., Bian, Y., Contag Wise, S., Burnett, J., Coupar, J., Yang, X., Chen, Z. and Van Waes, C. 2013. TGF-beta and NF-kappaB signal pathway cross-talk is

mediated through TAK1 and SMAD7 in a subset of head and neck cancers. *Oncogene* **32**, 1549-1559.
8. Hannon, G. J. and Beach, D. 1994. p15INK4B is a potential effector of TGF-beta-induced cell cycle arrest. *Nature* **371**, 257-261.
9. Jamieson, A. M., Diefenbach, A., McMahon, C. W., Xiong, N., Carlyle, J. R. and Raulet, D. H. 2002. The role of the NKG2D immunoreceptor in immune cell activation and natural killing. *Immunity* **17**, 19-29.
10. Laouar, Y., Sutterwala, F. S., Gorelik, L. and Flavell, R. A. 2005. Transforming growth factor-beta controls T helper type 1 cell development through regulation of natural killer cell interferon-gamma. *Nat. Immunol.* **6**, 600-607.
11. Lee, Y. S., Choi, H., Cho, H. R., Son, W. C., Park, Y. S., Kang, C. D. and Bae, J. 2021. Downregulation of NKG2DLs by TGF-beta in human lung cancer cells. *BMC Immunol.* **22**, 44.
12. Letterio, J. J. and Roberts, A. B. 1998. Regulation of immune responses by TGF-beta. *Annu. Rev. Immunol.* **16**, 137-161.
13. Li, F., Cao, Y., Townsend, C. M. Jr. and Ko, T. C. 2005. TGF-beta signaling in colon cancer cells. *World J. Surg.* **29**, 306-311.
14. Li, M. O. and Flavell, R. A. 2008. TGF-beta: a master of all T cell trades. *Cell* **134**, 392-404.
15. Markowitz, S., Wang, J., Myeroff, L., Parsons, R., Sun, L., Lutterbaugh, J., Fan, R. S., Zborowska, E., Kinzler, K. W. and Vogelstein, B. 1995. Inactivation of the type II TGF-beta receptor in colon cancer cells with microsatellite instability. *Science* **268**, 1336-1338.
16. Meadows, S. K., Eriksson, M., Barber, A. and Sentman, C. L. 2006. Human NK cell IFN-gamma production is regulated by endogenous TGF-beta. *Int. Immunopharmacol.* **6**, 1020-1028.
17. Mistry, A. R. and O'Callaghan, C. A. 2007. Regulation of ligands for the activating receptor NKG2D. *Immunology* **121**, 439-447.
18. Mu, Y., Gudey, S. K. and Landstrom, M. 2012. Non-Smad signaling pathways. *Cell Tissue Res.* **347**, 11-20.
19. Park, S. W., Bae, J. H., Kim, S. D., Son, Y. O., Kim, J. Y., Park, H. J., Lee, C. H., Park, D. Y., Kim, J. Y., Lee, M. K., Chung, B. S., Kim, S. H. and Kang, C. D. 2007. Comparison of level of NKG2D ligands between normal and tumor tissue using multiplex RT-PCR. *Cancer Invest.* **25**, 299-307.
20. Paul, S. and Lal, G. 2017. The Molecular Mechanism of Natural Killer Cells Function and Its Importance in Cancer Immunotherapy. *Front. Immunol.* **8**, 1124.
21. Polyak, K., Kato, J. Y., Solomon, M. J., Sherr, C. J., Massague, J., Roberts, J. M. and Koff, A. 1994. p27Kip1, a cyclin-Cdk inhibitor, links transforming growth factor-beta and contact inhibition to cell cycle arrest. *Genes Dev.* **8**, 9-22.
22. Principe, D. R., Doll, J. A., Bauer, J., Jung, B., Munshi, H. G., Bartholin, L., Pasche, B., Lee, C. and Grippo, P. J. 2014. TGF-beta: duality of function between tumor prevention and carcinogenesis. *J. Natl. Cancer Inst.* **106**, djt369.
23. Roberts, A. B. and Wakefield, L. M. 2003. The two faces of transforming growth factor beta in carcinogenesis. *Proc. Natl. Acad. Sci. USA.* **100**, 8621-8623.
24. Saltz, L. B. 2010. Adjuvant therapy for colon cancer. *Surg. Oncol. Clin. N. Am.* **19**, 819-827.
25. Syed, V. 2016. TGF-beta Signaling in Cancer. *J. Cell. Biochem.* **117**, 1279-1287.
26. Thiagalingam, S., Lengauer, C., Leach, F. S., Schutte, M., Hahn, S. A., Overhauser, J., Willson, J. K., Markowitz, S., Hamilton, S. R., Kern, S. E., Kinzler, K. W. and Vogelstein, B. 1996. Evaluation of candidate tumour suppressor genes on chromosome 18 in colorectal cancers. *Nat. Genet.* **13**, 343-346.
27. Wang, J., Han, W., Zborowska, E., Liang, J., Wang, X., Willson, J. K., Sun, L. and Brattain, M. G. 1996. Reduced expression of transforming growth factor beta type I receptor contributes to the malignancy of human colon carcinoma cells. *J. Biol. Chem.* **271**, 17366-17371.
28. Warner, B. J., Blain, S. W., Seoane, J. and Massague, J. 1999. Myc downregulation by transforming growth factor beta required for activation of the p15 (Ink4b) G(1) arrest pathway. *Mol. Cell. Biol.* **19**, 5913-5922.
29. Yang, L., Anderson, D. E., Baecher-Allan, C., Hastings, W. D., Bettelli, E., Oukka, M., Kuchroo, V. K. and Hafler, D. A. 2008. IL-21 and TGF-beta are required for differentiation of human T(H)17 cells. *Nature* **454**, 350-352.
30. Yang, L., Pang, Y. and Moses, H. L. 2010. TGF-beta and immune cells: an important regulatory axis in the tumor microenvironment and progression. *Trends Immunol.* **31**, 220-227.
31. Zhang, Y. E. 2009. Non-Smad pathways in TGF-beta signaling. *Cell Res.* **19**, 128-139.

초록 : 원발성 및 전이성 대장암에서 TGF-beta가 NKG2D 리간드 발현과 NK 세포 매개 면역반응에 미치는 영향

윤은정[1,2] · 김유림[1,2] · 박성준[1,2] · 이상률[1] · 배재호[1,2]*

([1]부산대학교 의과대학 생화학과, [2]부산대학교 의과대학 PNU GRAND 융합의학교육연구센터)

Transforming growth factor-β (TGF-β)는 암세포의 생존과 성장뿐만 아니라 면역세포의 활성에도 영향을 미치는 다기능 사이토카인이다. 일반적으로 암세포에서 유래된 TGF-β는 초기 암세포의 생존과 성장을 촉진하고 면역억제 효과가 있다고 받아들여지고 있지만 TGF-β는 세포의 종류나 단계에 따라 다른 효과를 가진 것으로 알려져 있다. 따라서 암 성장에 미치는 TGF-β의 작용기전은 아직 명확하게 정의하기 어렵다. 이 연구에서는 원발성 대장암 세포주인 KM12C와 이들의 두 전이성 세포주인 KM12SM과 KM12L4A에서 TGF-β 신호전달이 5개의 NKG2D 리간드 발현과 NK 세포 매개 항암 면역 반응에 미치는 영향을 조사했다. 외인성 TGF-β에 의해 KM12C의 MICA, MICB, ULBP1 및 ULBP2의 표면 단백질 발현 수준이 감소하였고 TGF-β 억제제인 galunisertib에 의해 MICA, MIAB, ULBP1, ULBP2 및 ULBP3의 발현이 증가하였다. 그러나 KM12SM과 KM12L4A에서는 TGF-β 또는 galunisertib에 의한 유의성 있는 NKG2DLs의 변화를 보지못하였다. Galunisertib를 통한 TGF-β 신호전달 억제는 KM12C에 대한 NK 세포 매개 항암 면역 반응을 개선했지만 KM12SM과 KM12L4A에 대한 유의성 있는 반응을 나타내지 않았다. 따라서 TGF-β 신호 전달을 억제하면 KM12C에 대한 NK 세포 매개 항암 면역 반응은 개선할 수 있지만 KM12SM 및 KM12L4A에서는 TGF-β 신호 전달 억제를 통한 NKG2DLs 발현의 증가 및 향상된 NK 세포 매개 암 면역 반응을 기대하기는 어려울 것으로 생각된다.