이학석사 학위논문

Anti-lymphoma activity of lenalidomide against non-germinal center B-cell subtype of diffuse large B-cell lymphoma

배중심 유형이 아닌 미만성 거대 B 세포 림프종 유형에 대한 레날리도마이드의 항암기작에 관한 연구

2012 년 8 월

서울대학교 대학원

의학과 협동과정 종양생물학과정

차 수 진

A thesis of the Degree of Master of Science

배중심 유형이 아닌 미만성 거대 B 세포 림프종 유형에 대한 레날리도마이드의 항암기작에 관한 연구

Anti-lymphoma activity of lenalidomide against non-germinal center B-cell subtype of diffuse large B-cell lymphoma

August 2012

The Department of Cancer biology

Seoul National University

College of Medicine

SooJin Cha

배중심 유형이 아닌 미만성 거대 B 세포 림프종 유형에 대한 레날리도마이드의 항암기작에 관한 연구

지도교수 허 대 석

이 논문을 이학석사 학위논문으로 제출함

2012 년 6 월

서울대학교 대학원

의학과 협동과정 종양생물학과 전공

차 수 진

차수진의 이학석사 학위논문을 인준함

2012 년 8 월

위 원 장 (인)

부위원장 (인)

위 원 (인)

Anti-lymphoma activity of lenalidomide against non-germinal center B-cell subtype of diffuse large B-cell lymphoma

by

SooJin Cha

(Directed by Dae Seog Heo, M.D.,Ph.D.)

A Thesis Submitted to the Interdisciplinary Graduate Program in Partial Fulfillment of the Requirements for the Degree of

Master of Science in Cancer Biology

at the Seoul National University

August 2012

Approved by Thesis Committee:

Professor _____Chairman

Professor _____Vice chairman

Professor _____

ABSTRACT

Purpose: Lenalidomide shows anti-tumor activity against B-cell malignancies. Although differential sensitivities to lenalidomide were observed between diffuse large B-cell lymphoma (DLBCL) subtypes, an *in vitro* efficacy of lenalidomide has not been verified in DLBCL cells. Here, we investigated the direct effect of lenalidomide on DLBCL cells as well as immunomodulatory properties using human NK cells.

Experimental Design: Direct effect of lenalidomide against DLBCL cell lines was evaluated by modified MTT or trypan blue exclusion assay. Immunoblotting was used to identify mechanism of growth inhibition. Human peripheral blood mononuclear cells (PBMCs) were prepared from healthy donors with leukoreduction system chambers. Primary NK cells were isolated from the PBMCs using anti-CD3 and anti-CD56 antibodies labeled with magnetic microbeads (MACS® separation). The cytotoxicity of lenalidomide-treated effector cells (PBMCs and NK cells) was analyzed by CD107a assay. NK cell-mediated growth inhibition was evaluated by Annexin V analysis. Flow cytometry was used to measure the expression patterns of lenalidomide-treated effector cells.

Results: We found that lenalidomide induced significant growth inhibition in non-GCB (non-germinal center B-cell) DLBCL subtype (0.738±0.070, mean±SD) compared with GCB DLBCL subtype (1.143±0.130, mean±SD) at 5μM of lenalidomide ($p<0.001$). Similar result obtained from trypan blue exclusion. After exposure to lenalidomide to non-GCB DLBCL cell lines for

three days, IRF4 expression decreased as well as NFκB signaling decreased in nucleus, whereas precursor form of NFκB increased in nucleus. When lenalidomide-treated NK cells were co-cultured with DLBCL cells, early apoptosis was induced more in lenalidomide-treated NK cells than lenalidomide-untreated NK cells. Although the percentage of $CD3^{-}CD56^{+}$ cell was similar between lenalidomide treated group and untreated group, CD56 expression on lenalidomide-treated NK cell was increased as a time-dependent manner (56.2±8.25 to 176.24±56.83, median ± SD at day0 to day7) and it means the number of $CD56^{bright}$ NK cells increased after exposure to lenalidomide. Although lenalidomide upregulated inhibitory KIR expressions (KIR2DL1, KIR2DL2/3, and KIR3DL1) on $CD56^{bright}$ NK cell subset, it has down-regulated inhibitory KIR expressions on $CD56^{dim}$ NK cell.

Conclusions: Lenalidomide showed antitumor activity against non-GCB DLBCL cells via decreased IRF4 expression through NFκB signaling pathway as well as modulation of NK cells to induce early apoptosis of DLBCL cells.

Keywords: Lenalidomide, DLBCL, immunomodulation

Student number: 2010-23730

CONTENTS

LIST OF FIGURES

LIST OF ABBREVIATION

DLBCL	diffuse large B-cell lymphoma
ABC	activated B-cell like
GCB	germinal center B-cell like
PMBL	primary mediastinal large B cell lymphoma
NF-κB	nuclear factor-kappa b
IRF4	interferon regulatory factor 4
PBMCs	peripheral blood mononuclear cells
KIR	killer-cell immunoglobulin-like receptor

INTRODUCTION

Lenalidomide is the second-generation immunomodulatory drugs (IMiDs) (1) and has been actively used in combination with dexamethasone for the treatment of relapsed (2, 3) or newly diagnosed (4) multiple myeloma (MM). Recently, lenalidomide maintenance prolonged survivals after high-dose chemotherapy with autologous stem cell transplantation in MM patients (5, 6) or in the setting of transplantation-ineligible MM (7) after the induction of melphalan, prednisolone, and lenalidomide. IMiDs including lenalidomide have direct anti-tumor activity as well as immunomodulatory properties. (1, 8) Particularly, immunomodulatory effects were induced by enhanced natural killer (NK) or NKT cell-associated cytotoxicity, (9) antigen-specific $CD8^+$ T-cell cytolysis,(8) and antibody-dependent cellular cytotoxicity. (10) Regarding tumoricidal effects, caspase 8-dependent apoptosis and down-regulation of nuclear factor kappa B (NF-κB) were observed in MM cells treated with IMiDs. (11) In addition, lenalidomide disrupted stromal supports in MM by decreasing interleukin-6 and vascular endothelial growth factor and inhibited the proliferation of MM cells directly via up-regulation of tumor suppressor genes.(8)

Diffuse large B-cell lymphoma (DLBCL) is a heterogeneous subtype of non-Hodgkin's lymphoma (NHL) (12) and is divided into germinal-center B-cell like (GCB) and activated B-cell like (ABC) subtypes using gene expression profiling. (13, 14) Although rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone (R-CHOP) is the standard treatment of DLBCL, 10 - 25% of patients and nearly 40% of high-risk patients are incurable in the rituximab era. (15) In addition, patients with ABC-DLBCL had worse outcomes compared with those with GCB-DLBCL. (13) Dysregulated NF-κB

activation was down-regulated by bortezomib, which was translated into clinical activity against non-GCB DLBCL in combination with R-CHOP (16) or dose-adjusted doxorubicin-based chemotherapy. (17) Since phase II studies of lenalidomide in relapsed or refractory aggressive NHL demonstrated overall response rate (ORR) of 35% with ORRs of 19-28% in DLBCL,(18, 19) patients with non-GCB DLBCL benefited from lenalidomide compared with those with GCB DLBCL (ORR 52.9 vs 8.7%; and median progression-free survival [PFS] 6.2 vs 1.7 months).(20) Although moderate growth inhibition (40%) and apoptosis (10-26%) were observed in DLBCL cells treated with lenalidomide, these were originated from GCB-DLBCL. (21) In addition, differential sensitivities to lenalidomide have not been well elucidated between GCB- and ABC-DLBCL cells. Here, we investigated the tumoricidal effect of lenalidomide on GCB- and non-GCB DLBCL cells as well as immunomodulatory properties using human NK cells.

MATERIALS AND METHODS

NK cell preparation

Human peripheral blood mononuclear cell (PBMC) was prepared from healthy donors with leukoreduction system chambers. PBMC were isolated by using Ficoll-Hypaque (Amersham Pharmacia Biotech, Piscataway, NJ, USA) density gradient centrifugation. Primary NK cell was isolated from the PBMC by two-step immunomagnetic separation system using anti-CD3 and anti-CD56 antibodies labeled with magnetic microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany). This procedure was approved by the Institutional Review Board of Seoul National University Hospital (H-1105-081-362).

Cell lines and cell culture

The diffuse large B cell lymphoma (DLBCL) cell lines (SU-DHL4, SU-DHL10, RC-K8, U2932, U2940) were purchased from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ; Germany) and U266, SU-DHL6, K562 were purchased from American Type Culture Collection (ATCC; Rockvillle, MD, USA). These cell lines were cultured in RPMI 1640 medium supplemented with 10% heat-inactivated (55'C, 30min) fetal bovine serum (Gibco-BRL), 2mM L-glutamine and 1% gentamicin solution (Gibco-BRL).

Reagents and Antibodies

Lenalidomide (Celgene Corporation, Summit, NJ, USA) was dissolved in dimethyl sulfoxide (DMSO) and diluted in media for working solution. Cells were stained with fluorescein isothiocyanate (FITC)-, phycoerythrin (PE)- or

allophycocyanin (APC)-conjugated monoclonal antibodies and these were CD56 (clone B159), CD16 (clone 3G8), KIR2DL1 (clone HP-3E4), KIR2DL2,3 (clone CH-L), KIR3DL1 (clone DX9) and CD107a (clone H4A3). All samples were analyzed by FACSCalibur flow cytometer (Becton-Dickinson, Franklin Lakes, NJ, USA) and FlowJo software ver.7.6.5 (Tree Star, Ashland, OR, USA).

Anti-NFκB p65 (clone C-20; Santa Cruz Biotechnology, Inc, CA, USA), anti-NFκB p50 (clone C-19; Santa Cruz), anti-IRF4 (Clone EP5699; EPITOMICS, Inc, CA, USA), and GAPDH (clone 14C10; Cell signaling, Danvers, MA, USA) were used for western blotting.

Cell proliferation assay

Cell proliferation assay was assessed by measuring the conversion of the tetrazolium salt (WST-8) to formazan according to the manufacturer's instructions (CCK-8; Dojindo, Kumamoto, Japan). Briefly, target cells in RPMI-1640 were plated into 96-well round bottom plates and treated with lenalidomide at different concentrations 0.01μM to 10μM. After indicated time points, 20 μl of WST-8 solution were added to each well and the plates were incubated for an additional 1h - 4h at 37 ˚C. The absorbance of each plate at 450 nm represented a direct correlation with the cell number in this analysis, and was measured by Eon™ Microplate Spectrophotometer (BioTek Instruments, Inc, Winooski, VT). Each experiment was done in over quadruplicate. The result was expressed with relative cell activity (%) calculated by [test value / control]×100, where test value was the absorbance of the test sample and control was the absorbance of the control sample.

Western blotting

Cells were cultured with lenalidomide at 10μM for 72 hours and proteins extracted from upon $5x10^6$ cells. Cytosol and nuclear proteins were extracted by a Pierce NE-PER Nuclear and Cytoplasmic Extraction kit (Thermo Fisher Scientific, Loughborough, UK). For total lysate of cells, cell lysis cocktail was used. Protein concentrations were determined by using a Bradford's method and Eon™ Microplate Spectrophotometer (BioTek Instruments). Proteins were loaded on NuPAGE 4-12% Bis-Tris gels (Invitrogen) and transferred to PVDF membranes. After blocking membranes by skim milk, primary and secondary antibodies were labeled. Detection of results was analyzed by LAS-3000 imaging system (Fuji Photo Film Co., Stamford, CT, USA) according to the manufacturer's instructions.

CD107a assay

Both PBMC and DLBCL cell lines were exposed to lenalidomide at a dose of 10 μM for 72 hours to evaluate the effect of lenalidomide on cytotoxic lymphocytes, DLBCL cell lines, or both. After 72 hours-exposure to lenalidomide or placebo, PBMC and DLBCL cell lines were co-cultured at an effector/target (E:T) ratio of 10:1 with FITC-conjugated anti-CD107a antibody for 4 hours at 37 ˚C. After 1hr incubation, Golgistop™ containing monensin (BD Bioscience) was added and incubated last 3 h of the culture. After incubation, cells were stained with APC-conjugated anti-CD56 (BD) for determination of CD107a expression on NK cells. Expression of CD107a was represented by percent of fluorescence of FITC-CD107a and it was analyzed by flow cytomerty.

Flow cytometry analysis

Flow cytometry analysis was performed using FACSCalibur (Becton Dickinson, USA) to determine the expression of cell surface molecules on lenalidomide or placebo-treated PBMCs.

Apoptosis assay

Isolated NK cells were treated with lenalidomide at 10 μM and incubated for 5 days at 37˚C. After NK cells were co-cultured with target cells (E:T=3:1) for 30min or 1.5hours, cells were stained with FITC-Annexin V (BD). Stained cells were analyzed by flow cytometry.

Statistics

All experiments were performed at least 3 timesand the signigicance of differences between experimental groups was calculated using the Student *t* – test.

RESULTS

Lenalidomide inhibits proliferation in non-GCB subtypes of DLBCL cell lines

To evaluate direct killing effect of lenalidomide to DLBCL cell lines, these were treated with lenalidomide at a concentration of 0.01μM to 10μM for 5 to 7 days. Lenalidomide significantly decreased of the proliferation rates at 5 μM of control cells (U266, 0.607±0.153%) as well as non-GCB DLBCLs (RC-K8, 0.761±0.020%; U2932, 0.757±0.106%; and U2940, 0.695±0.059%) as compared with those of GCB-DLBCL cells (SU-DHL4, 1.111±0.023%; SU-DHL6, 1.062±0.115%; and SU-DHL10, 1.254±0.155%) ($P < .001$) (Figure 1A). Although cell number and viability were decreased in non-GCB DLBCL (U2932 and U2940) and U266 cells, these were not changed in SU-DHL4 cells (Figure 1B).

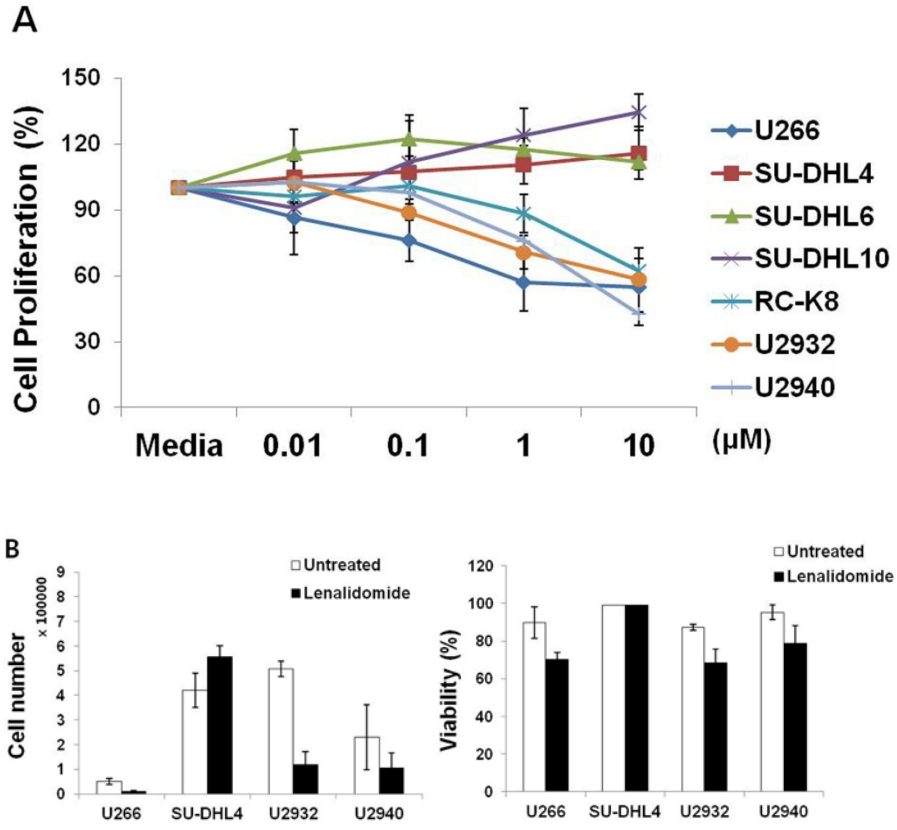

Figure 1. Cell proliferation assay of lenalidomide-treated DLBCL cells. GCB DLBCL cells (SU-DHL4, SU-DHL6, SU-DHL10), non-GCB DLBCL cells (RC-K8, U2932, U2940), and U266 (control, multiple myeloma) were exposed to lenalidomide at various concentration. (A) Cell viability assay using WST-8 after exposure to lenalidomide, (B) Trypan blue exclusion assay at day 7 (mean±SD).

Lenalidomide downregulated NF-κB related signaling pathway in non-GCB DLBCL cells

NF-κB-related molecules including interferon regulatory factor-4 (IRF4) were evaluated in DLBCL cells at baseline and at 72 hour-exposure to lenalidomide. When non-GCB DLBCL cells that over-expressed NF-κB signal pathway were treated with lenalidomide, nuclear REL A (p65) and p50 subunit decreased, but cytosolic ones increased. In addition, precursor form of NF-κB1 (p105) increased both in nucleus and cytosol of non-GCB DLBCL cells (RC-K8, U2932, and U2940) (Figure 2). Interestingly, IRF4 was down-regulated in non-GCB DLBCL cells treated with lenalidomide.

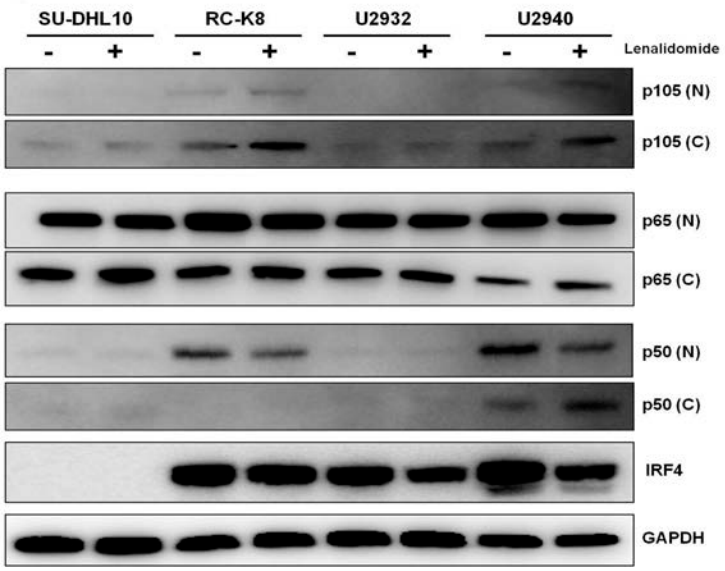

Figure 2. Alteration of IRF4 and NF-κB molecules after treatment with lenalidomide at 10 μM for 72 hours. Nucleic and cytosolic NF-κB expressions were compared according to the lenalidomide treatment. IRF4 expression was represented as whole protein. SU-DHL10 is GCB DLBCL, RC-K8, U2932 and U2940 are non-GCB DLBCL cell lines.

Lenalidomide induced degranulation of cytotoxic lymphocytes against DLBCL cell lines

Because immunomodulatory properties were mediated by T and NK cells, cell surface translocation of CD107a was performed using PBMC treated with lenalidomide. The percentage of CD107a was increased in lenalidomide-treated PBMC-K562 group (mean±SD, 10.08±4.93%) compared to untreated PBMC-K562 group (12.73±5.8). The GCB DLBCL cell line, SU-DHL10 showed similar percentage of CD107a between untreated (3.12±0.65%) and lenalidomide-treated group (3.49±0.89%). After exposure to lenalidomide, CD107a expression increased in Non-GCB DLBCL cell lines including RC-K8 (1.87±1.37 to 2.65±1.24%), U2932 (4.55±2.74 to 6.34±3.58%), and U2940 (3.84±1.26 to 4.82±1.57%) (Figure 3A and 3B).

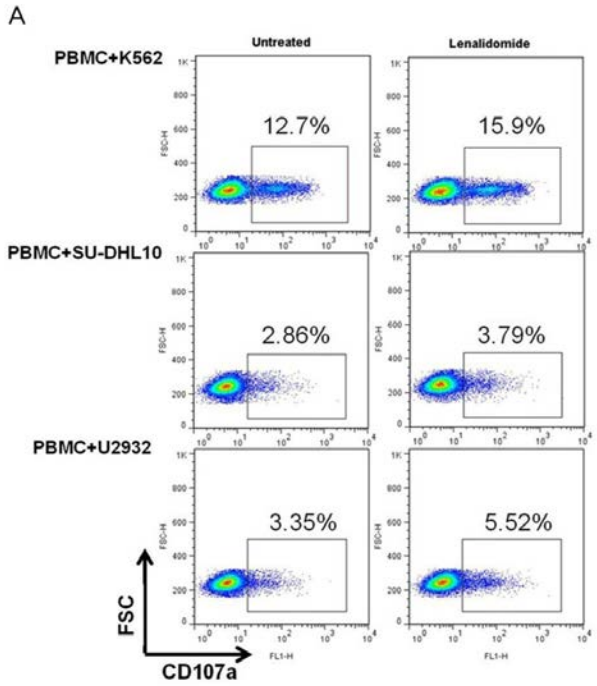

Figure 3. CD107a expression on lenalidomide-treated cytotoxic lymphocytes against DLBCL cells. CD107a expression on lenalidomide-treated PBMC cultured with lenalidomide-treated target cells was assessed by flow cytometry. (A) Dot plot of CD107a expression of PBMC was shown. PBMCs were gated and analyzed. Representative plots were showed.

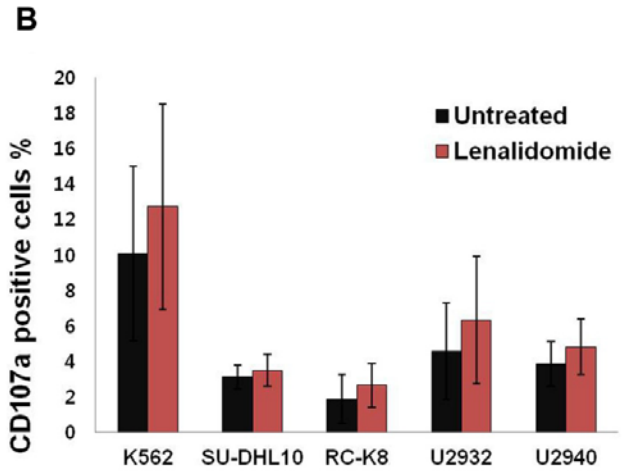

Figure 3. CD107a expression on lenalidomide-treated cytotoxic lymphocytes against DLBCL cells. CD107a expression on lenalidomide-treated PBMC cultured with lenalidomide-treated target cells was assessed by flow cytometry. (B) The percentage of CD107a positive cells was evaluated average of percentage of each cell lines. Bar graph represents mean ±SD.

Lenalidomide-treated NK cell induced early apoptosis in DLBCL cell lines

After isolated NK cell were treated lenalidomide at a dose of 10μM with IL-2 50U/ml for 5 days, lenalidomide-treated NK cells induced early apoptosis in DLBCL cell lines (Annexin-V, mean±SD% before and after lenalidomide): SU-DHL4 (60±8.89 *vs.* 74±0.58%), U2932 (61±7.07 *vs.* 74±2.52%), and U2940 (68±14.5 vs. 84±5.69%) (Figure 4).

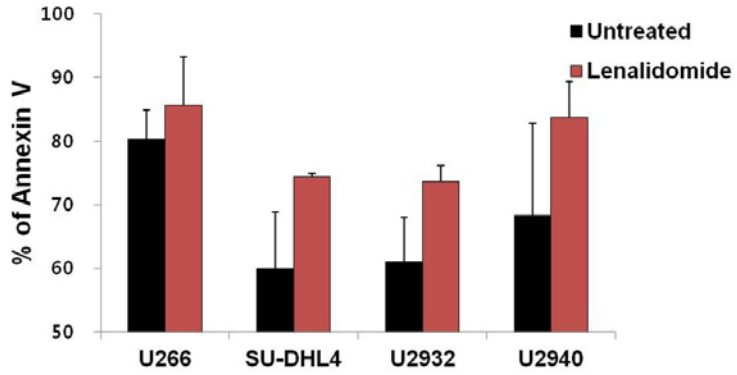

Figure 4. Apoptosis assay by Annexin V staining. Isolated NK cell is used as effector cell, DLBCL cell line is used as target cell. After NK cell was cultured with lenalidomide at 10μM and IL-2 50U/ml for 5 days, NK cell have been co-cultured with target cells. The bar graph is represented by mean±SD.

Lenalidomide modulates KIR expression in CD56^{bright} NK and CD56^{dim} NK cells

Next, we identified modulation of KIR expressions in lenalidomide-treated PBMC or NK cells without IL-2 support. When PBMC was treated lenalidomide for 7 days, mean fluorescent intensity (MFI) of CD56 increased from day0 to day7 (56.2±8.25 to 176.24±56.83, MFI±SD) although the number of CD3-CD56+ NK cells were not changed (Figure 5A and Figure 5B).

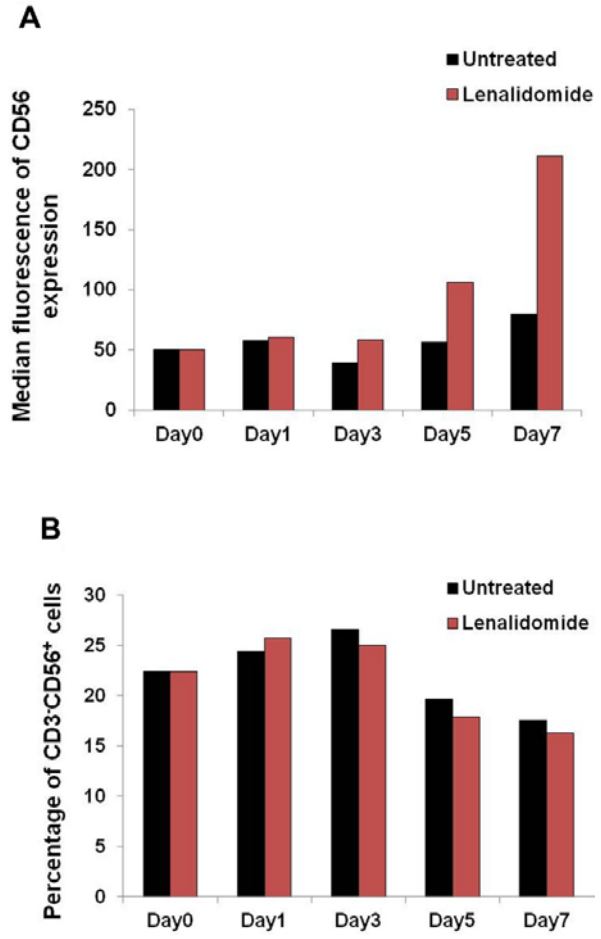

Figure 5. Alteration of CD56 expression of lenalidomide-treated NK cells

(A) The bar graph showed CD56 expression on healthy donor PBMCs treated with 10 μM of lenalidomide from day 0 to day 7. (B) $CD3^{-}CD56^{+}$ cells was not changed when lenalidomide treated until day 7. Representative data is showed by median fluorescent intensity. Three independent experiments were done using different PBMCs derived from 3 donors.

In addition, lenalidomide down-regulated KIR expression in CD3-CD56+NK cell (MFI of KIR2DL1, 37.7 to 15.5; KIR2DL2/3, 303 to 163; KIR3DL1, 370 to 257) (Figure 6A) as well as in $CD56^{dim}$ NK cells (KIR2DL1, 28.1 to 17.3; KIR2DL2/3, 157 to 49.1; KIR3DL1, 346 to 201) (Figure 6B). However, KIR2DL2/3 and KIR3DL1 expressions decreased in $CD56^{bright}$ NK cell (KIR2DL2/3, 18.8 to 51.5; and KIR3DL1, 22.3 to 136) (Figure 6C).

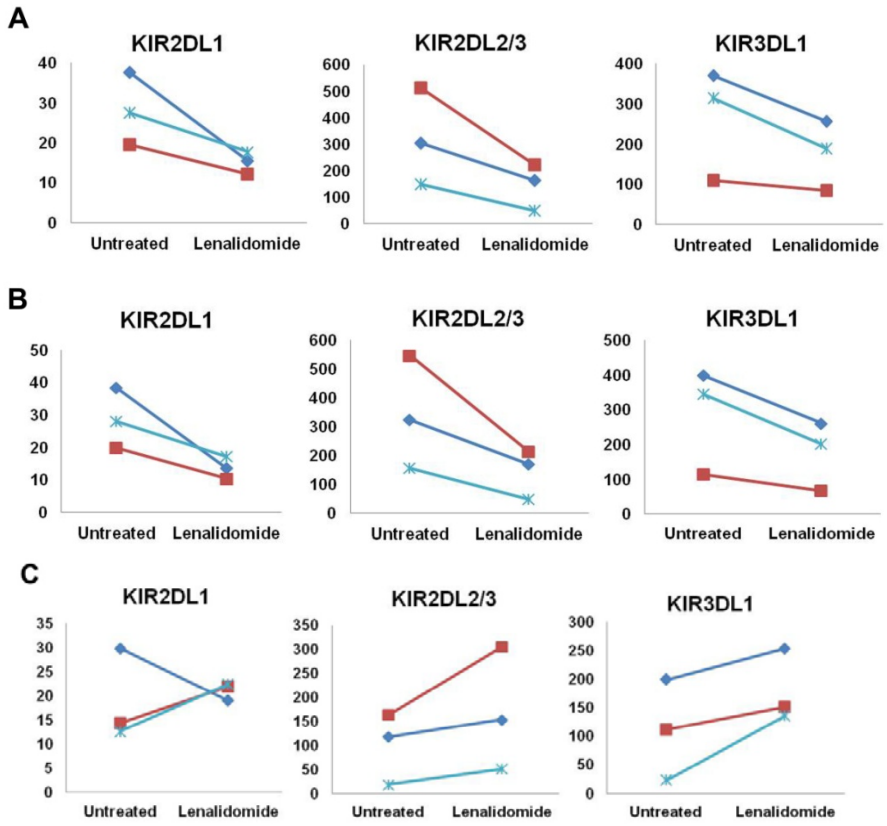

Figure 5. KIR expression of lenalidomide-treated $CD56^{+}$ NK cells.

Three independent experiments were done using different PBMCs derived from 3 donors. Inhibitory KIR expression on $CD3^{-}CD56^{+}$ NK cells (A), $CD56^{dim}$ NK cells (B), and $CD56^{bright}$ NK cells (C) were represented at day 5. Values are represented by mean fluorescent intensity.

DISCUSSION

Our study demonstrated that lenalidomide shows tumoricidal activity against non-GCB DLBCL cells as well as immunomodulatory properties associated with NK cells. Lenalidomide significantly inhibited non-GCB DLBCL cells compared with GCB DLBCL cells via down-regulation of NF-kB pathway and IRF4. In addition, lenalidomide enhanced NK cell cytotoxicity and modulated KIR expressions in NK cells.

Because ABC DLBCL is dependent on constitutive activation of NF-κB pathway, NF-κB inhibitor has been implicated in an attractive target for ABC DLBCL. (22) Therefore, indirect inhibition of NF-κB by bortezomib enhanced the efficacy of chemotherapy in patients with non-GCB DLBCL. (17) In line with NF-κB inhibition by IMiDs in MM, (11) lenalidomide showed higher response rate in patients with non-GCB DLBCL than in those with GCB DLBCL in one retrospective study. (20) Although early study identified that lenalidomide could inhibited proliferation of DLBCL B-cell lymphoma cell lines (21), there was no *in vitro* data regarding the efficacy of lenalidomide in DLBCL subtypes. Here, non-GCB DLBCL cells were more sensitive to lenalidomide than GCB DLBCL cells. Theses sensitivities were mediated by down-regulation of canonical NF-κB pathway where high expression was observed in non-GCB DLBCL in this study like IMiDs-associated inhibition of NF-κB in MM. (11) Considering molecular and therapeutic similarities between MM and ABC DLBCL, IRF4 was also down-regulated in non-GCB DLBCL cells like MM. (23) In this way, non-GCB DLBCL was more beneficial from lenalidomide treatment compared with GCB DLBCL.

Immunomodulatory effects of lenalidomide were mediated by

modulating cytotoxic lymphocytes such as $CD8^+$ T and NK cells. (8) Similarly, degranulation capacity expressed as CD107a assay minimally increased in PBMC treated with lenalidomide in our study. In addition, lenalidomide instigated NK cells to induce apoptosis in non-GCB DLBCL cells in comparison with GCB DLBCL cells. Recent studies demonstrated that lenalidomide increased CD56 expression on $CD3^-CD56^+$ NK cell and suggested an increased $CD56^{bright}$ NK cell population. (24, 25) $CD3^-CD56^+$NK cells were subdivided into cytotoxic $CD56^{bright}$ NK and cytokine-producing $CD56^{dim}$ NK cells (26) that had different NK cell receptors. Our result indicated that inhibitory KIR expressions were decreased in $CD3^-CD56^+$ NK cells as well as $CD56^{dim}$ NK cells, whereas those were increased in $CD56^{bright}$ NK cells. Because $CD56^{bright}$ NK cells function as regulatory NK cells by producing various cytokines and it has low level of KIR expression than $CD56^{dim}$ NK cells, up-regulation of inhibitory KIR expression on lenalidomide-treated $CD56^{bright}$ NK cells might regulate activated tumor B-cells in DLBCL. In contrast, lenalidomide might enhance the cytotoxicity of $CD56^{dim}$ NK cells by decreasing inhibitory KIR expressions. However, these are not conclusive because selection of $CD56^{bright}$ and $CD56^{dim}$ NK cells is difficult for functional studies. Combination of lenalidomide and anti-inhibitory KIR antibody may augment NK cell-associated immunomodulation in ABC DLBCL, like a previous preclinical model in MM. (27)

In conclusion, we demonstrated that lenalidomide showed anti-proliferative effect in non-GCB DLBCL cell lines through down-regulation of canonical NF-κB signals as well as IRF4. Furthermore, lenalidomide enhanced degranulation of cytotoxic granules against DLBCL cells and augmented NK cell-mediated apoptosis of non-GCB DLBCL cells. In addition, lenalidomide up-regulated CD56 expression as well as inhibitory KIR expressions in $CD56^{bright}$ NK cells.

REFERENCES

1. Bartlett JB, Dredge K, Dalgleish AG. The evolution of thalidomide and its IMiD derivatives as anticancer agents. Nat Rev Cancer. 2004 Apr;4(4):314-22.

2. Dimopoulos M, Spencer A, Attal M, Prince HM, Harousseau JL, Dmoszynska A, et al. Lenalidomide plus dexamethasone for relapsed or refractory multiple myeloma. N Engl J Med. 2007 Nov 22;357(21):2123-32.

3. Weber DM, Chen C, Niesvizky R, Wang M, Belch A, Stadtmauer EA, et al. Lenalidomide plus dexamethasone for relapsed multiple myeloma in North America. N Engl J Med. 2007 Nov 22;357(21):2133-42.

4. Zonder JA, Crowley J, Hussein MA, Bolejack V, Moore DF, Sr., Whittenberger BF, et al. Lenalidomide and high-dose dexamethasone compared with dexamethasone as initial therapy for multiple myeloma: a randomized Southwest Oncology Group trial (S0232). Blood. 2010 Dec 23;116(26):5838-41.

5. McCarthy PL, Owzar K, Hofmeister CC, Hurd DD, Hassoun H, Richardson PG, et al. Lenalidomide after stem-cell transplantation for multiple myeloma. N Engl J Med. 2012 May 10;366(19):1770-81.

6. Attal M, Lauwers-Cances V, Marit G, Caillot D, Moreau P, Facon T, et al. Lenalidomide maintenance after stem-cell transplantation for multiple myeloma. N Engl J Med. 2012 May 10;366(19):1782-91.

7. Palumbo A, Hajek R, Delforge M, Kropff M, Petrucci MT, Catalano J, et al. Continuous lenalidomide treatment for newly diagnosed multiple myeloma. N Engl J Med. 2012 May 10;366(19):1759-69.

8. Davies F, Baz R. Lenalidomide mode of action: linking bench and clinical findings. Blood Rev. 2010 Nov;24 Suppl 1:S13-9.

9. Hsu AK, Quach H, Tai T, Prince HM, Harrison SJ, Trapani JA, et al. The immunostimulatory effect of lenalidomide on NK-cell function is profoundly inhibited by concurrent dexamethasone therapy. Blood. 2011 Feb 3;117(5):1605-13.

10. Wu L, Adams M, Carter T, Chen R, Muller G, Stirling D, et al. lenalidomide enhances natural killer cell and monocyte-mediated antibody-dependent cellular cytotoxicity of rituximab-treated CD20+ tumor cells. Clin Cancer Res. 2008 Jul 15;14(14):4650-7.

11. Mitsiades N, Mitsiades CS, Poulaki V, Chauhan D, Richardson PG, Hideshima T, et al. Apoptotic signaling induced by immunomodulatory thalidomide analogs in human multiple myeloma cells: therapeutic implications. Blood. 2002 Jun 15;99(12):4525-30.

12. Stein H, Warnke RA, Chan WC, Jaffe ES. Diffuse large B-cell lymphoma, not otherwise specified. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, et al., editors. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. Lyon, France: IARC; 2008. p. 233-7.

13. Alizadeh AA, Eisen MB, Davis RE, Ma C, Lossos IS, Rosenwald A, et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature. 2000 Feb 3;403(6769):503-11.

14. Rosenwald A, Wright G, Chan WC, Connors JM, Campo E, Fisher RI, et al. The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma. N Engl J Med. 2002 Jun 20;346(25):1937-47.

15. Ziepert M, Hasenclever D, Kuhnt E, Glass B, Schmitz N, Pfreundschuh M, et al. Standard International prognostic index remains a valid predictor of outcome for patients with aggressive CD20+ B-cell lymphoma in the rituximab era. J Clin Oncol. 2010 May 10;28(14):2373-80.

16. Ruan J, Martin P, Furman RR, Lee SM, Cheung K, Vose JM, et al. Bortezomib plus CHOP-rituximab for previously untreated diffuse large B-cell lymphoma and mantle cell lymphoma. J Clin Oncol. 2011 Feb 20;29(6):690-7.

17. Dunleavy K, Pittaluga S, Czuczman MS, Dave SS, Wright G, Grant N, et al. Differential efficacy of bortezomib plus chemotherapy within molecular subtypes of diffuse large B-cell lymphoma. Blood. 2009 Jun 11;113(24):6069-76.

18. Wiernik PH, Lossos IS, Tuscano JM, Justice G, Vose JM, Cole CE, et al. Lenalidomide monotherapy in relapsed or refractory aggressive non-Hodgkin's lymphoma. J Clin Oncol. 2008 Oct 20;26(30):4952-7.

19. Witzig TE, Vose JM, Zinzani PL, Reeder CB, Buckstein R, Polikoff JA, et al. An international phase II trial of single-agent lenalidomide for relapsed or refractory aggressive B-cell non-Hodgkin's lymphoma. Ann Oncol. 2011 Jul;22(7):1622-7.

20. Hernandez-Ilizaliturri FJ, Deeb G, Zinzani PL, Pileri SA, Malik F, Macon WR, et al. Higher response to lenalidomide in relapsed/refractory diffuse large B-cell lymphoma in nongerminal center B-cell-like than in germinal center B-cell-like phenotype. Cancer. 2011 Nov 15;117(22):5058-66.

21. Hernandez-Ilizaliturri FJ, Reddy N, Holkova B, Ottman E, Czuczman MS. Immunomodulatory drug CC-5013 or CC-4047 and rituximab enhance antitumor activity in a severe combined immunodeficient mouse lymphoma model. Clin Cancer Res. 2005 Aug 15;11(16):5984-92.

22. Yamasaki A, Eimer S, Okochi M, Smialowska A, Kaether C, Baumeister R, et al. The GxGD motif of presenilin contributes to catalytic function and substrate identification of gamma-secretase. J Neurosci. 2006 Apr 5;26(14):3821-8.

23. Fraering PC, Ye W, LaVoie MJ, Ostaszewski BL, Selkoe DJ, Wolfe MS. gamma-Secretase substrate selectivity can be modulated directly via interaction with a nucleotide-binding site. J Biol Chem. 2005 Dec 23;280(51):41987-96.

24. Netzer WJ, Dou F, Cai D, Veach D, Jean S, Li Y, et al. Gleevec inhibits beta-amyloid production but not Notch cleavage. Proc Natl Acad Sci U S A. 2003 Oct 14;100(21):12444-9.

25. Moon RT, Kohn AD, De Ferrari GV, Kaykas A. WNT and beta-catenin signalling: diseases and therapies. Nat Rev Genet. 2004 Sep;5(9):691-701.

26. Liu C, Li Y, Semenov M, Han C, Baeg GH, Tan Y, et al. Control of beta-catenin phosphorylation/degradation by a dual-kinase mechanism. Cell. 2002 Mar 22;108(6):837-47.

27. Liu C, Kato Y, Zhang Z, Do VM, Yankner BA, He X. beta-Trcp couples beta-catenin phosphorylation-degradation and regulates Xenopus axis formation. Proc Natl Acad Sci U S A. 1999 May 25;96(11):6273-8.

28. Aberle H, Bauer A, Stappert J, Kispert A, Kemler R. beta-catenin is a target for the ubiquitin-proteasome pathway. EMBO J. 1997 Jul 1;16(13):3797-804.

국문 초록

서론: Lenalidomide 는 B 세포 림프종에서 직접 암세포를 공격하거나 암세포 주변의 미세환경을 조절하여 암세포를 공격하는 기작을 가지는 약물이다. 현재 미만성 거대 B 세포 림프종의 유형에 따라 lenalidomide 의 반응성이 다르다는 것이 알려져 있으나 그 기전에 대한 연구는 충분히 되어 있지 않다.

방법: Colorimetric 실험방법을 통하여 lenalidomide 가 미만성 거대 B 세포 림프종에 미치는 직접적인 영향을 연구하였고, 유세포분석기를 이용하여 lenalidomide 가 건강한 사람의 말초혈액단핵구에 미치는 영향과 lenalidomide 를 처리한 말초혈액단핵구가 미만성 거대 B 세포 림프종에 미치는 영향을 연구하였다.

결과: Lenalidomide 는 GCB 세포 유형에서는 세포증식에 영향을 주지 않았지만 non-GCB 세포 유형에서 유의하게 세포증식을 감소시켰다($p<0.001$). 또한, non-GCB 세포 유형에서 lenalidomide 는 NF-κB (p50, p65)의 단백질의 양을 변화시켰는데, 핵에서는 감소하고 세포질에서는 증가하는 양상을 보였다. 더불어 IRF4 의 단백질도 함께 감소하는 양상을 확인할 수 있었다.

Lenalidomide가 함유된 배지에서 자연살세포를 배양한 후 미만성 거대 B세포와 함께 배양하였더니 조기에 세포자멸사가 유도되었으며 이는 GCB유형보다 non-GCB 유형에서 보다 더 잘 유도되는 경

향을 보였다. 한편, lenalidomide를 말초혈액단핵구에 처리하여 7 일 동안 배양하면서 관찰한 결과 자연살세포의 절대적인 수치가 증가하지는 않았으나 CD56 발현 정도가 증가하는 것을 관찰할 수 있었다. 이와 같은 결과는 lenalidomide가 $CD56^{bright}$ 자연살세포의 비율을 증가시키는 것이라고 볼 수 있다. 또한, inhibitory KIR가 변화하는 양상도 관찰할 수 있었는데, $CD56^{dim}$ NK세포에서는 감소하였고 $CD56^{bright}$ 자연살세포에서는 증가하는 양상을 확인하였다.

결론: lenalidomide 는 non-GCB 미만성 거대 B 세포에서 세포증식을 억제시켰다. 항암효과의 기전으로는, IRF4 와 NF-κB 신호전달 체계의 변화가 암세포에 직접 영향을 미친 것이 주요 기전으로 생각되며, 환자의 자연살세포에 영향을 끼쳐 림프종세포의 세포자멸사를 유도한 부분도 기여한 것으로 관찰되었다.

주요어 : Lenalidomide, 미만성 거대 B 세포 림프종, non-GCB
학 번 : 2010-23730