Adaptive Modulation of MHC Class I Expression and Immune Evasion to Cytotoxic Immunocytes in Cancer Cells

Yongxin Zhang, Ying Wang, William K. Decker, Zhenying Wang, Monica Zimmerman

***Abstract*— It has been well-demonstrated that cancer cells can escape from the immune surveillance of Cytotoxic T lymphocytes (CTL) and natural killer cells (NK cells) by modulating their MHC class I expression. In order to get insight into the mechanism in which cancer cells regulate their MHC class I expression in response to the attack of CTL and NK cells, different concentration of effector cells were used to examine the effects of low effector/target ratio on the MHC class I expression shifting, tanswells were used to separate effector cells and target cells in culture to check if the cell to cell contact is required for the MHC class I expression shifting, and intracellular flow cytometry was used to determine if MHC class I protein synthesis in cancer cells were also changed with their surface antigen in current studies. Our data indicate that (1) both elimination of target cells and direct regulation of MHC class I expression in target cells contributed to modulation of MHC class I expression in cancer cells; (2) effector cell mediated-regulation of MHC class I expression in cancer cells required cell to cell contact; (3) the shifting of surface MHC class I antigen on the cancer cells might be caused by the change of MHC class I protein synthesis in cancer cells; and (4) application of inadequate numbers of effector cells may induce immune evasion of cancer cells, a cautionary tale for future clinical studies.**

***Index Terms*— Cancer, Cell Therapy, T-cells, NK Cells, MHC**

I. INTRODUCTION

Cytotoxic immunocytes play critical role in preventing cancer development by the effective elimination of cancer cells [1], [2]. Cytotoxic T lymphocytes (CTL) and natural killer cells (NK cells) are two main subsets of these cytotoxic immunocytes which have the function of cancer immune surveillance [3]-[5]. As targets of CTL, the cancer cells need to express both MHC class I antigen and cancer specific antigen, while NK cells can recognize and kill MHC class I negative cells. Thus, CTL and NK cells respectively work on MHC class I positive and negative cancer cells, and build a very powerful barrier against cancer together [5]. However, some cancer cells have the potential capacity to escape from the attacks of these imuunocytes by modulating their MHC class I expression. We and others have demonstrated [5]-[10] MHC class I negative cells could become more dominant when these cells were under the immune selection pressure from CTL. Our recent studies also showed that the cancer cells could enhance their MHC class I expression to evade the attack of NK cells and in vitro and in vivo experiments demonstrated that the combined application of CLT and NK cells could significantly increase anticancer efficiency in cancer immunotherapy [5]. These data partially revealed the immunological mechanism of cancer development. Based on these findings, this study would further examine the conditions of the MHC class I shifting in cancer cells with CTL and NK cells, and clarify if the changes of MHC class I expression in cancer cells are only passive procedures provoked by immune section pressure or the cancer cells can also actively modulate their MHC class I expression to adapt to the environment with cytotoxic immunocytes.

Yongxin Zhang, corresponding author, Zyxell Inc., Carrollton, Texas 75007, USA. (972) 975-8600
Ying Wang, Zyxell Inc., Carrollton, Texas 75007, USA.
William K. Decker, Department of Pathology and Immunology, Baylor College of Medicine, Houston, TX 77030, USA.
Zhenying Wang, Zyxell Inc., Carrollton, Texas 75007, USA.
Monica Zimmerman, Carrollton, Texas 75007, USA.

II. MATERIAS AND METHODS

A. Human cancer cell culture

Lung cancer (PLs008, small cell lung cancer) primary tumor tissues obtained from the ZYX Biotech Company (Carrollton, TX, USA) were isolated and cultured as described previously [5], [11]. Cells were seeded (4 cultures/cell line) in 10 ml cell culture bags at 2×10^4/ml with magnetic beads for ZYX bioreactor culture.[5, 11, 12] At the end of the culture, cells were dissociated with enzyme-free cell dissociation buffer (Thermo-Fisher Scientific, Waltham, MA) and analyzed by flow cytometry for MHC class I antigen and CD45 expression.

B. Cell Enumeration and Flow Cytometry

Total cell counts were determined by hemacytometry following Trypan blue staining and flow cytometry with propidium iodide staining using standard procedure. CTL enumeration was conducted using flow cytometry, and cell markers were stained by means of established standard protocols [18]-[22]. Specifically, lung cancer cells were stained with FITC-anti-human HLA-A,B,C antibody (Biolegend, San Diego, CA, Mouse IgG_{2a}, κ) and PE-anti-human CD45 (Biolegend, Mouse IgG_1, κ). CTLs were stained with PerCP-anti-human CD8 (Biolegend, Mouse IgG_1, κ) [5], [11], [12-16]. Activated CTLs were stained with PerCP-anti-human CD8 (Biolegend, Mouse IgG_1, κ) and PE-anti-human CD137 (Biolegend, Mouse IgG_1, κ) [5], [11], [17-19]. NK cells were defined as $CD3^-CD56^+$ based on staining with PerCP-anti-human CD3 (Biolegend, Mouse IgG_{2a}, κ), FITC-anti-human CD56 (Biolegend, Mouse IgG_1, κ) and PE-anti-human CD69 (Biolegend, Mouse IgG_1, κ) to determine activation status[20-23]. Suitable isotype controls from the same manufacturer were applied. Cells were stained (including intracellular staining for the analysis of MHC class I antigen expression in cancer cells) and analyzed as described previously [5], [11], [12-16]. All cells from pre-

and post- culture used for the staining were derived from the same lot (cells from the same tissue and processed in the same container at the same time). Flow-Check™ Fluorospheres (Beckman Coulter, Inc. Cat#6605359) were used to calibrate the flow cytometer prior to each use. In addition to the percentage of positive cells, mean fluorescence intensity (MFI) was used to evaluate the MHC class I expression.

C. *Immune effector expansions*

Commercially available pairs of autologous human cancer cells (small cell lung cancer [PLs008]) [5], [11] and peripheral blood mononuclear cells (PBMC, [Mnc013], ZYX Biotech) from a normal healthy donor (as allogenic NK cell targets) were used in this study. Cancer cells were approximately 80% MHC class I-positive and 100% CD45-negative. All mononuclear cell fractions contained at least 10^8 $CD3^+CD45^+$ cells. Cytokines and other reagents for CTL and NK cell culture and detection have been described previously. [5, 11, 12-16] PLs008 cancer cells were used as stimulators for CTL expansions and as targets for CTL and NK cell lytic assays. Autologous PBMC were bioreactor-sorted [5], [11] to separate $CD3^+$ and $CD3^-$ fractions which were then stimulated to generate CTL and NK cell effectors respectively. Established protocols [5], [11], [12-16] with slight modifications were used in this study. Briefly, (1) $CD3^+$ and $CD3^-$ cells from the mononuclear fraction were separated in the bioreactor. The $CD3^+$ cells were maintained in the original reaction chamber (1st RC) for CTL expansion whereas the $CD3^-$ cells were sorted into an auxiliary reaction chamber (2nd RC) for NK cell expansion. (2) For CTL expansions, cancer cells were grown and enriched in cell culture bags for 6 days as described above. Irradiated (20 Gy) cancer cells were cultured with the corresponding autologous $CD3^+$ cells in the ZYX Bioreactor [5], [11] in 8 ng/mL IL-2 and 10 ng/mL IL-7 for 12 days, as determined previously [5], [11]. (3) For NK cell expansions, autologous or allogenic $CD3^-$ cells were cultured in 8 ng/mL IL-2, 50 ng/ml IL-12 and 10 ng/ml IL-18 for 12 days as determined previously [5], [11], [12-16]. All cytokines were purchased from PeproTech (Rocky Hill, NJ). (3) Following cell expansion, $CD8^+$ cells were isolated from the 1st RC (for CTL enrichment) and $CD56^+$ cells were isolated from the 2nd RC (**for NK** cell enrichment) by the automated bioreactor-embedded cell sorter using anti-CD8 and anti-CD56 magnetic separation beads (Miltenyi Biotec, San Diego, CA) as described previously.[5, 11] After isolation, purities of $CD8^+$ cells and $CD56^+$ cells were > 95% and > 92%, respectively, and $CD8^+CD137^+$ activated CTL[17]-[19] and $CD56^+CD69^+$ activated NK cell was > 88% and > 81%. Controls consisted of SEB-stimulated cells, un-stimulated cells, and/or cells stimulated with autologous tumor cells.

D. *Analysis of cancer cell MHC class I expression*

PBMC from both a patient and healthy donor were processed for the activation and expansion of cancer-specific CTL, autologous NK cells, and allogenic NK cells. Cancer cell seeding density was 0.2 x 10^6/ml; CTL, NK cell, and autologous PBMC seeding density was 0.5 - 1.0 x 10^6/ml. IL-2 was added at a concentration of 8ng/ml during the cytolytic expansion. Cells were cultured for 10 days and cells were examined for their MHC class I expression by flow cytometry at the end of co-culture.

E. *Transwell assay*

For examining the effects of the contacts between these immunocytes and cancer cells on the MC class I antigen expression on cancer cells, 0.2x10^6/ml lung cancer cells (in the lower chamber) and 1x10^6/ml CTLs or NK cells (in the upper chamber, transwell) were co-cultured in 10 ml per well of 6-well Falcon multi-well plates with a surface area of 9.62 cm^2 in Falcon polyurethane cell culture inserts (upper chamber pre-coated with Collagen I/fibronectin/BSA, 0.4 mm pore diameter, BD Labware, Franklin Lakes, NJ) [25]. MHC class I expression on the $CD45^{neg}$ cancer cells were also examined with flow cytometry on day 5 and day 10.

F. *Data analysis*

Analysis of variance was used to compare the numbers of CTL and NK cell and the percent cell lysis between different groups. SAS and SAS Statview (SAS Institute, Cary, NC, USA) were used to perform analyses. A p-value < 0.05 was considered to be significant. All experiments are the result of six independent repetitions unless stated otherwise.

III. RESULTS

A. *Impact of low concentration of CTL and NK cell on cancer cell MHC class I expression in co-culture.*

A higher effector: target ratio (E:T ratio) is required for CTLs to function well in inhibiting or killing their target cells [5], [11]. When the lung cancer cells expressing MHC class I (HLA-A, B, C) at a pre-culture MFI of 162 (average of 6 detections with 83% of cells MHC class I-positive) were co-cultured, the higher E:T ratio resulted in MHC class I shift of the surviving cells. We investigated whether such a shift [5] also occurs at lower E:T ratios. Figures 1A and 1B show that, while target cell growth was not significantly inhibited, MHC class I expression was still greatly decreased in the co-culture with CTLs and increased with NK cells when the E:T ratio was reduced by 50% to 2.5:1. These data suggest that inadequate administration of cytotoxic immunocytes in cancer cell therapy or partial inhibition of immune responses to cancer cells in the body provide enough selection pressure to promote immune evasion of cancer cells.

B. *Mechanism by which tumor MHC class I expression is regulated.*

The shifts [5] observed in cancer cell MHC class I expression could be passive due to simple selection

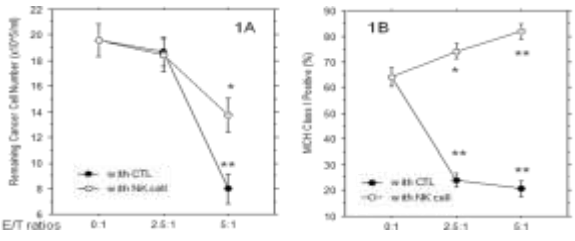

Figure 1. Effects of low E:T ratios on MHC class I expression. Lung cancer cells co-cultured with activated cytotoxic T lymphocytes (CTLs) and/or natural killer (NK) cells. Figures 1A and 1B show the effects of different E:T ratios on remaining cancer cell numbers (1A) and the percentage of MHC class I positive cells (1B), respectively. These two figures exhibit that the target cells were not significant inhibited (1A, p>0.2) but MHC class I expression was still greatly decreased (1B) in the co-culture with CTLs (p<0.01) and increased with NK cells (p<0.05) when the E/T ratio was at 2.5:1, and both remaining cell number and MHC class I expression were significantly affected (p<0.01) when the ET ratios reached 5:1. Error bars = ±SEM. **P* < 0.05, ***P* < 0.01.

pressure or could be an active regulatory process employed by tumor cells as a defense mechanism. To distinguish between these two hypotheses, MHC class I-positive cells and MHC class I-negative cells were separated by cell sorting and co-cultured with CTLs and NK cells (Figure 2-4), respectively. This pre-separation of highly positive from strictly negative populations eliminated the possibility of simple selection pressure as the mechanism of MHC class I shift in subsequent experiments. Despite pre-separation, the MHC class I-positive cells still exhibited a significant reduction in MHC class I expression when co-cultured with CTLs (Figures 2A and 2B, Figures 3A and B, and Figures 3a3 and 3c3) in comparison to the no effector control. Similarly, the MHC class I-negative cells significantly increased MHC class I expression (Figure 2C and 2D, Figure 3C and 3D, and Figure 4b3 and 4b4) when cultured with NK cell effectors. These results suggested some level of bona fide active regulation of tumor cell surface MHC class I.

Contact and/or adhesion between effectors and target cells is critical for cell killing [25]-[31]. However, it remains unclear whether cell contact is also required for effector cell modulation of MHC class I antigen expression in cancer cells. To address this issues, MHC class I positive and negative cancer cells were co-cultured with CTLs and NK cells in transwells [24]. As shown in figure 2, in the co-culture with CTL, the MHC class I MFI level (figure 2A and 2B) declined significantly in the culture with CTLs mixed with cancer cells but not in the cultures without effectors or in the cultures with effectors separated from target cells by transwells. The percentage (Figures 3A and 3B, Figure 4a1-a4 and 4c1-c4) of MHC class I positive cancer cells decreased from > 98% to 65%, while MHC class I in no effector and transwell co-cultures decreased only non-significantly. Similarly, in the co-culture with NK cells (Figures 2C, 2D, 3C, 3D, 4b1-b4 and 4d1-Cd4), MHC class I expression was up-regulated in the cultures in which NK cells and cancer cells were mixed but not in cultured in which effectors and targets were separated by transwells. These data suggest the contact between cancer cells and effector cells is critical for regulation of MHC class I expression on cancer cells.

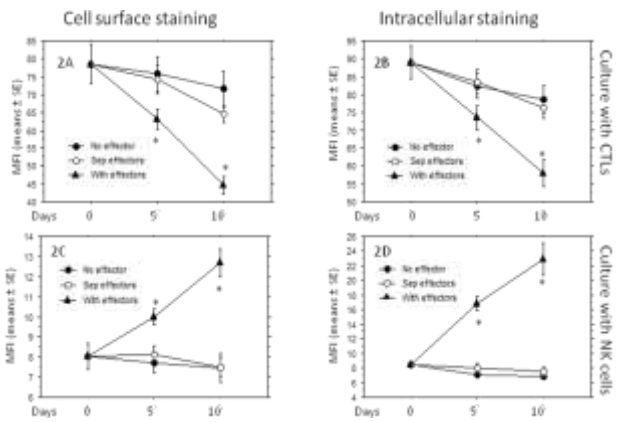

Figure 2. Effect of contact between CTL effectors and targets on the mean fluorescence intensity (MFI) of MHC class I antigen in cancer cells. Small cell lung cancer cells were sorted with ZYX bioreactor using positive selection program for MHC class I positive cell isolation and the eluted cells were further sorted using negative selection program for the isolation of highly purified MHC class I negative cells. Cancer cells were seeded in 6 well plates with/without 1 x 10^6 effectors (activated CTLs or NK cells). Transwells were used to separate the target cancer cells and effectors. Target cells were seeded in the lower chambers, and effectors were seeded in the upper chambers. MHC class I-positive cells were used to test the effect of CTL on MHC class I expression, and MHC class I-negative cells were used to evaluate the effect of NK cells on the MHC class I expression. Each group comprised six independent cultures. MHC class I MFI of effectors and cancer cells cultured alone (No effectors), together (With effectors), or together separated by a transwell (Sep effectors). All cells were stained for MHC lass I and CD45, and CD45 negative cells were analyzed to assess the MHC class I expression of cancer cells. In 2A and 2B, cancer cells were cultured with CTLs, and in 2C and 2D, cancer cells were cultured with NK cells; in 2A and 2C, cancer cell surface staining for MHC class I expression was conducted for flow cytometry analysis, in 2B and 2D, cancer cells were intracellularly stained for MHC class I expression analysis. 2A and 2B respectively exhibit the MHC class I-positive cancer cells in contact with CTLs significantly decrease their MHC class I expression on the surface of the cells and inside the cell in comparison with other groups. 2C and 2D show the MHC class I negative cancer cells in contact with NK cells (With effectors) significantly increases the MHC class I expression in both surface stain and intracellular stain when compared to other groups. The cancer cells separated from effectors by transwell (Sep effectors) were not significantly affected by effector cells for their MHC class I expression in comparison with the cultures without effectors (No effectors, $p>0.05$). Data are represented as means ±SE; *$p< 0.05$.

When intracellular MHC class I antigen was analyzed with flow cytometry [5], [12], it was found that the expression of intracellular MHC class I shifted in parallel with that of cell surface MHC class I and was significantly down-regulated by CTLs (Figures 2B, 3B, 4c1-c4, $p < 0.01$) yet significantly up-regulated by NK cells (Figures 2D, 3D, 4d1-d4, $p < 0.05$), suggesting that cancer cells might evade or retard attack from CTLs and NK cells by regulating MHC class I synthesis.

IV. DISCUSSION

The phenotypic heterogeneity among cancer cells, caused by genetic or epigenetic alterations, affects immune response [32]-[35], in which the percentage of various types of MHC class I loss could be variable [35]. The diversity of MHC class I expression in cancer is closely associated to CTL and NK cell-mediated

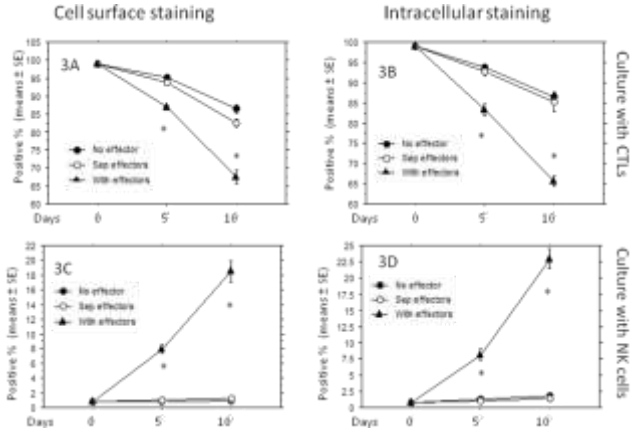

Figure 3. Effect of contact between CTL effectors and targets on the percentage of MHC class I positive cancer cells. Cell culture condition is the same as that for Figure 2. In 3A and 3B, cancer cells were cultured with CTLs and in 3C and 3D cancer cells were cultured with NK cells; in 3A and 3C, cancer cell surface staining for MHC class I expression was conducted for flow cytometry analysis, in 3B and 3D, cancer cells were intracellularly stained for MHC class I expression analysis. 3A and 3B exhibit the cancer cells in contact with CTLs significantly reduce MHC class I-positive cells in both the surface stain and intracellular stain when compared with other groups. 3C and 3D show the cancer cells in contact with NK cells (With effectors) significantly increases the MHC class I-positive cells in MHC class I-negative cells in both surface stain and intracellular stain when compared to other groups (*$p<0.01$). The cancer cells separated from effectors by transwell (Sep effectors) were not significantly affected by effector cells for their MHC class I expression in comparison with the cultures without effectors (No effectors, $p>0.05$). Data are represented as means ±SE; *$p< 0.05$.

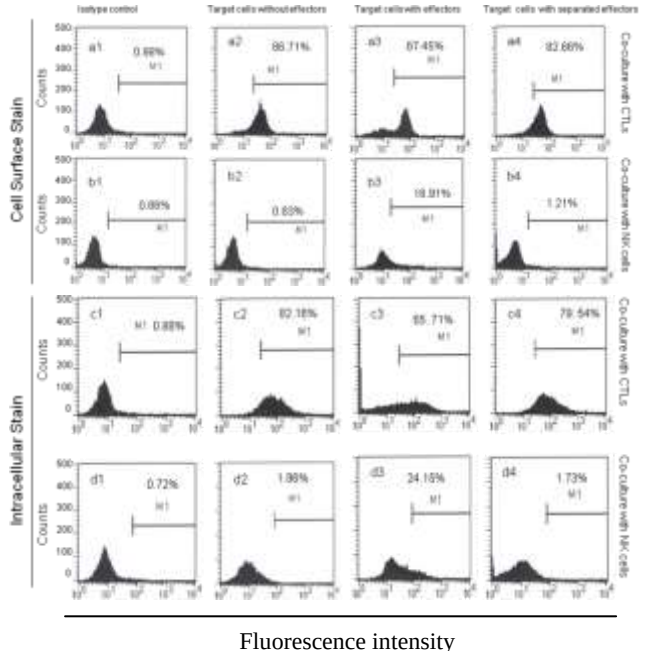

Figure 4. Representative flow cytometry histograms of MHC class I expression in cancer cells. Cell culture condition is the same as that for Figure 2. On day 10 following culture: in a1-a4, MHC class I- positive cancer cells were examined in the co-culture with CTLs and cells surface MHC class I antigen was analyzed; in b1-b4, MHC class I- negative cancer cells with NK cells and cell surface MHC class I antigen was analyzed; in c1-c4, MHC class I- positive cancer cells with CTLs and intracellular MHC class I antigen was analyzed; and in d1-d4, MHC class I- negative cancer cells with NK cells and intracellular MHC class I antigen was analyzed. a1-d1 are isotype controls; a2-d2 are controls containing only target cancer cells; a3-d3 are co-cultures of cancer cells and effector cells (a3 and c3 for CTL, b3 and d3 for NK cell); and a4-d4 are co-cultures in which cancer cells and effector cells were separated by transwells.

anticancer immunity [34], [35]. The molecular alternations underling MHC class I loss could be reversible or irreversible [32], [36]-[39] and are mainly resulted from immune selection pressure of antitumor $CD8^+$ T cells [34], [35]. Consistent with these findings, the data in our current studies exhibited that PLs008 lung cancer cells could increase or decrease their MHC class I expression when they co-cultured with different cytotoxic imunocytes. In addition to the CTL-related MHC class I reduction in cancer cells, we also found NK cell-associated MHC class I enhancement, and both immune selection and direct regulation of CTLs and NK cells to the MHC class I expression of cancer cells would contribute to the immune evasion of the cancer cells.

Our results indicated significant modulation in expression of MHC class I in many cancer cells and cancer cell lines in response to co-culture with immune effectors as well as the existence of a potential mechanism for evading the host immune response as suggested by previous studies [5], [32]-[40]. The data further reveal that the expression of MHC class I antigens was modulated under certain conditions, particularly under the stress of CTL or NK cell attack, leading to cancer cell immune evasion and proliferation of resistant cells.

We also found significant evidence that the mechanism by which tumor cells modulate surface MHC expression in response to cytolytic attack is an active process. This apparent ability of cancer cells to adapt in response to selection pressure from immunocyte effectors could be an important mechanism by which cancer cells evade immune surveillance and a critical reason for failures of previous anticancer cell therapies. This is consistent with our previous studies in which when we co-cultured cancer cells with cancer-specific CTLs and NK cells together, the number of remaining cancer cells was significantly reduced in comparison to co-cultures with CTLs or co-cultures with NK cells alone [5]

Our data showed the MHC class I expression did not only shift on the surface of the cancer cells in response to the attack of cytotoxic lymphocytes, but also had corresponding intracellular changes, suggesting that the MHC class I protein synthesis was up- or down-regulated, and a further studies on this regulation signaling pathway could be helpful for the development of new strategies in the prevention of cancer immune evasion.

One of the reasons for the failure of cancer immune therapy is that the cancer patients in clinical trials are often at very late stage of the disease, and such patients have a huge amount of cancer cells in their bodies. In these cases, the immunocytes used for the adoptive transfer are often inadequate to effectively kill cancer cells. Our current study indicates the inadequate immunocytes co-cultured with cancer cells could potentially induce immune evasion of cancer cells and might result in a higher possibility for the failure of succeeding cell therapy in clinic. Therefore, for a more ideal therapeutic effect, CTL and NK cells should be administrated after most cancer cells are removed by surgery, chemotherapy or/and radiotherapy.

In summary, our study demonstrated that (1) both elimination of target cells and direct regulation of MHC class I expression in target cells contributed to effector modulation of MHC class I expression in cancer cells; (2) effector cell mediated-regulation of MHC class I expression in cancer cells required cell to cell contact; (3) the shifting of surface MHC class I antigen on the cancer cells might be caused by the change of intracellular MHC class I expression; and (4) application of inadequate numbers of effector cells may induce immune evasion of cancer cells, a cautionary tale for future clinical studies. Additional in vitro and in vivo studies will be necessary to validate these findings and to translate them into clinical practice.

REFERENCES

[1] Ryungsa Kim, Manabu Emi, and Kazuaki Tanabe. Cancer immunoediting from immune surveillance to immune escape. Immunology. 2007 May; 121(1): 1–14. doi: 10.1111/j.1365-2567.2007.02587.x

[2] Dass S. Vinay, et al. Immune evasion in cancer: Mechanistic basis and therapeutic strategies. Seminars in Cancer Biology. 2015; 35: S185–S198

[3] Raval RR, et al. Tumor immunology and cancer immunotherapy: summary of the 2013 SITC primer. J Immunother Cancer. 2014; May 14; 2:14.

[4] Kwong ML, et al. Adoptive T-cell transfer therapy and oncogene-targeted therapy for melanoma: the search for synergy. Clin Cancer Res. 2013; 19(19):5292-9.

[5] Yongxin Zhang, Ying Wang, William Decker, et al. Inhibition of cancer cell immune evasion by combined application of cytotoxic T-lymphocytes and natural killer cells. Journal of Translational Research. 2017 (to be published)

[6] Copier J, Bodman-Smith M, Dalgleish A. Current status and future applications of cellular therapies for cancer. Immunotherapy. 2011; 3(4):507-16.
[7] Grupp SA, et al. Adoptive cellular therapy. Curr Top Microbiol Immunol. 2011; 344:149-72.
[8] Austin Doyle, et al. Markedly decreased expression of class I histocompatibiity antigens protein, and rNA in human small-cell lung cancer. J. Exp. Med.1985; 161:1135.
[9] Mariusz Kaczmarek, et al. Analysis of expression of MHC class I molecules and TAP genes in malignant human cell lines. Folia Histochemica et Cytobiologica. 2007; 45:205.
[10] A. D. Wells, et al. Restoration of MHC class I surface expression and endogenous antigen presentation by a molecular chaperone. Scand J Immunol. 1997; 45(6):605-12.
[11] Yongxin Zhang, et al. Cancer Specific CTL Expansion with ZYX Bioreactor. Journal of Clinical & Cellular Immunology 2016; 7:398.
[12] Yongxin Zhang, et al. Restoration of Retarded Influenza Virus-specific Immunoglobulin Class Switch in Aged Mice. J Clin Cell Immunol 2016; 7:403.
[13] Mbawuike IN, Zhang Y, Couch RB. Control of mucosal virus infection by influenza nucleoprotein-specific CD8+ cytotoxic T lymphocytes. Respir Res. 2007; 27:8.
[14] Zhang Y, Qiu J, Zhou Y, Farhangfar F, Hester J, Lin AY, Decker WK. Plasmid-based vaccination with candidate anthrax vaccine antigens induces durable type 1 and type 2 T-helper immune responses. Vaccine. 2008; 26(5):614-22.
[15] Yongxin Zhang, et al. Apoptosis and Reduced Influenza A Virus Specific CD8+ T Cells in Aging Mice. Cell Death and Differentiation. 2002; 9(6):651.
[16] Biao Zheng, Yongxin Zhang, Innocent Mbawuike, et al. Rectification of age-associated deficiency in cytotoxic T cell response to influenza A virus by immunization with immune complexes. J Immunol. 2007; 179 (9):6153-9.
[17] Matthias Wolfl, et al. Activation-induced expression of CD137 permits detection, isolation, and expansion of the full repertoire of $CD8^+$T cells responding to antigen without requiring knowledge of epitope specificities. Blood. 2007; 110: 201
[18] Simeone E, Ascierto PA. Immunomodulating antibodies in the treatment of metastatic melanoma: the experience with anti-CTLA-4, anti-CD137 and anti-PD1. J Immunotoxicol. 2012; 9(3):241-7.
[19] Li SY, Liu Y. Immunotherapy of melanoma with the immune costimulatory monoclonal antibodies targeting CD137. Clin Pharmacol. 2013; 5(Suppl 1):47-53.
[20] Gilles Benichou, et al. Natural killer cells in rejection and tolerance of solid organ allografts. Curr Opin Organ Transplant. 2011; 16(1): 47–53.
[21] Juan P. Cata, et al. Potential Use of Natural Killer Cell Transfer Therapy in the Perioperative Period to Improve Oncologic Outcomes. Scientifica. 2015; 2015:1
[22] Naoyuki Sakamoto, et al. Phase I clinical trial of autologous NK cell therapy using novel expansion method in patients with advanced digestive cancer. Sakamoto et al. J Transl Med. 2015;13:277.
[23] EIICHI ISHIKAWA, et al. Autologous Natural Killer Cell Therapy for Human Recurrent Malignant Glioma. ANTICANCER RESEARCH. 2004; 24:1861-1872
[24] Zhenxiang Wang, Ying Wang, Farhang Farhangfar, Monica Zimmer, Yongxin Zhang. Enhanced Keratinocyte Proliferation and Migration in Co-culture with Fibroblasts. PLoS ONE. 2012; 7(7).
[25] Bellone M, Calcinotto A. Ways to enhance lymphocyte trafficking into tumors and fitness of tumor infiltrating lymphocytes. Front Oncol. 2013; 3:231.
[26] Brenner MK. Will T-cell therapy for cancer ever be a standard of care? Cancer Gene Ther. 2012; 19(12):818-21.
[27] Rezvani K, et al. Cancer vaccines and T cell therapy. Biol Blood Marrow Transplant. 2013; 19(1 Suppl):S97-S101.
[28] Shi H, Liu L, Wang Z. Improving the efficacy and safety of engineered T cell therapy for cancer. Cancer Lett. 2013; 28; 328(2):191-7.
[29] Franks HA, et al. New anticancer immunotherapies. Anticancer Res. 2012; ;32:2439-53.
[30] Bernatchez C, Radvanyi LG, Hwu P. Advances in the treatment of metastatic melanoma: adoptive T-cell therapy. Semin Oncol. 2012; 39(2):215-26.
[31] Pedrazzoli P, et al. Is adoptive T-cell therapy for solid tumors coming of age? Bone Marrow Transplant. 2012; 47(8):1013-9.
[32] Kaczmarek M, Frydrychowicz M, Rubis B, et al. Analysis of expression of MHC class I molecules and TAP genes in malignant human cell lines. *Folia Histochem Cytobiol* 2007; 45:205-14.
[33] Wells AD, Rai SK, Salvato MS, Band H, Malkovsky M. Restoration of MHC class I surface expression and endogenous antigen presentation by a molecular chaperone. *Scand J Immunol* 1997; 45:605-12.
[34] Federico Garrido, Natalia Aptsiauri, Elien M Doorduijn, Angel M Garcia Lora, and Thorbald van Hall.The urgent need to recover MHC class I in cancers for effective immunotherapy. *Curr Opin Immunol* 2016; 39: 44–51.
[35] Garrido F., Romero I., Aptsiauri N., Garcia-Lora A.M. Generation of MHC class I diversity in primary tumors and selection of the malignant phenotype. *Int J Cancer* 2016; 138: 271–280.
[36] Fisher R, Pusztai L, Swanton C. Cancer heterogeneity: implications for targeted therapeutics. *Br J Cancer* 2013; 108:479–85.
[37] De Sousa EMF, Vermeulen L, Fessler E, et al. Cancer heterogeneity—a multifaceted view. *EMBO Rep* 2013; 14:686–95.
[38] Garrido F, Cabrera T, Aptsiauri N. "Hard" and"soft" lesions underlying the HLA class I alterations in cancer cells: implications for immunotherapy. *Int J Cancer* 2010; 127:249–56.
[39] Garrido F, Algarra I, Garcia-Lora AM. The escape of cancer from T lymphocytes: immuno-selection of MHC class I loss variants harboring structural-irreversible "hard" lesions. *Cancer Immunol Immunother* 2010; 59:1601–6.
[40] Doyle A, Martin WJ, Funa K, et al. Markedly decreased expression of class I histocompatibiity antigens protein, and RNA in human small-cell lung cancer. *J Exp Med* 1985; 161:1135-51