European Review for Medical and Pharmacological Sciences 2017; 21: 1027-1031

Oncogenic miR-155 down-regulated upon activation of antitumor cytotoxic T lymphocytes by the fusion of dendritic cells with breast carcinoma cells

J. ZHANG[1], Y. LI[2]

[1]The First Department of Breast Surgery, The Third Affiliated Hospital of Kunming Medical University, Tumor Hospital of Yunnan Province, Kunming, China
[2]The First Department of Medical Oncology, The Third Affiliated Hospital of Kunming Medical University, Tumor Hospital of Yunnan Province, Kunming, China

Abstract. – OBJECTIVE: MicroRNAs has been proved to play vital roles in many biological processes. In the present study, the expression profile of oncogenic miR-155 in the activated antitumor cytotoxic T lymphocytes generated by the fusion of dendritic cells with breast carcinoma cells has been demonstrated.

MATERIALS AND METHODS: Expression profile of oncogenic miR-155 was noted in antitumor cytotoxic T lymphocytes, which was generated by the fusion of dendritic cells (DC) with breast carcinoma cells (MCF-7). The fused cells were used to induce and generate antitumor CTL with the standard procedure. Flow cytometry, as well as qPCR analysis, were performed to identify the expression profile of oncogenic miR-155 in the generated antitumor CTL against breast cancer.

RESULTS: Compared with controls, the presence of MUC-1 and MHC-II in the DC/MCF-7 fused cells was confirmed by flow cytometry analysis. qPCR experimental results conclude that oncogenic miR-155 level was decreased in the antitumor CTL generated against breast cancer cells.

CONCLUSIONS: We observed that the oncogenic miR-155 level was down-regulated in the antitumor CTL against breast cancer, and it may be used as the effective treatment to eliminate the malignant cells in the breast cancer patients.

Key Words:
Dendritic cells, Breast cancer cells, MUC-1, MCF-7, MHC-II, Cytotoxic T lymphocytes.

Introduction

Dendritic Cells (DC) are one of the proficient Antigen Presenting Cells (APC) which initiate the primary immune responses[1,2]. To trigger T-cells, the DC express both MHC class I and MHC class II to produce secondary signals[3,4]. Reports clearly indicate that DC develops antitumor responses against tumor-associated peptides, which in turn induces the deterioration of disease both in the model system as well as in the clinical studies[5-10]. DC have the capability of inducing polyclonal immune response against multiple tumor antigens. DC have been pulsed by following methods: injected with tumor cell lysates, loaded with tumor peptides, acid-elution from tumor cells[11,12] and transfected through tumor cell RNA[13,14]. Hence, to induce polyclonal antitumor immune response, it is better to fuse tumor cells along with DC[15]. MicroRNA-373 has been reported to suppress the T-cell lymphocytes growth by repressing CCND1[16]. Rather, the key molecular mechanisms in context to micro RNAs were not clearly documented. More than 98% of non-coding DNAs (commonly called as 'junk' DNAs) are present in the form of short ncRNAs and long ncRNAs (lncRNAs), but the role and function of this specific RNAs, and their relevance to disease are still unclear. Recently, these short ncRNAs has been reported to perform following specific functions such as modulation of alternative splicing, chromatin remodeling and RNA metabolism, respectively. In contrast, other types of short RNA, namely microRNAs, play a vital role in signaling and regulatory functions. In short, microRNAs are basically, a non-coding RNA, which retards the gene expression at the posttranscriptional level by targeting the 3' UTR of mRNA sequences[17]. Xu et al[18] suggest that microRNAs have an important role in leukemogenesis. In contrast, single nucleotide polymorphisms in the promoter region of mir-133a-1 and in pre-mir-152 rs1707 have resulted in the risk of asthma[19]. Hence, it is essential

Corresponding Author: Yang Li, MD; e-mail: yangli5689@hotmail.com

to identify the signaling and regulatory mechanisms of microRNAs, which is an important target in cancer biology. In this paper, we aimed to fuse the human breast carcinoma cells with human DC and the fused cells activate autologous T cell proliferation followed by the induction of cytotoxic T lymphocytes (CTL) against breast tumor cells. The miR-155 expression levels were found out in the antitumor CTL against breast cancer.

Materials and Methods

Culture of Human MCF-7, DCs, Monocytes and T Cells for Cell Fusion

Human breast carcinoma cell lines MCF-7 (ATCC) were grown in Dulbecco's Modified Eagle Medium (DMEM) Sigma-Aldrich (St. Louis, MO, USA) as described in the paper[20]. The peripheral blood mononuclear cells (PBMC) were mixed in RPMI medium with human serum (10%) Invitrogen (San Diego, CA, USA) and incubated for 1.5 h. The non-adherent cells were removed, and the remaining T cells were isolated by nylon-wool separation method. The attached cells were cultured for 6 days in RPMI medium (Sigma-Aldrich, St. Louis, MO, USA) along with 1000 U/ml of granulocyte-macrophage colony-stimulating factor (GM-CSF; Sigma-Aldrich, St. Louis, MO, USA) and 500 U/ml of IL-4 (Sigma-Aldrich, St. Louis, MO, USA). The non-attached and loosely adherent cells were harvested by repeated washes to generate the dendritic cells (DC). Tightly attached monocytes were harvested by slight treatment of Trypsin (Sigma-Aldrich, St. Louis, MO, USA). DCs were mixed with human breast carcinoma cells (MCF-7) at the ratio of 10:1 and were incubated in serum-free RPMI medium containing 50% PEG (Sigma-Aldrich, St. Louis, MO, USA) for 5 min. The cells were re-suspended in RPMI medium and incubated at 37°C for 7-14 d. The protocols for the culture of human MCF-7, DCs, Monocytes and T cells, as well as Cell Fusion analysis were referred from the paper of Gong et al[20].

Flow Cytometry Analysis

The protocols for the flow cytometry analysis were referred from Gong et al[20] with slight modifications. In brief, after digestion with collagenase at 37°C for 15 minutes, cells were squashed and their suspension were passed through 40 μM nylon mesh (Sigma-Aldrich, St. Louis, MO, USA). The processed cells were analyzed in flow cytometry with sorting of MUC-1 and MHC-II cells. Cells were washed with PBS and incubated with primary antibodies against MUC1 and MHC class II (Sigma-Aldrich, St. Louis, MO, USA) for 1 h at 4°C. The cells were washed with PBS and incubated with fluorescein conjugated goat anti-mouse IgG (Sigma-Aldrich, St. Louis, MO, USA) for 30 min at 4°C followed by incubation with phycoerythrin-conjugated anti-MHC class II (Sigma-Aldrich, St. Louis, MO, USA) or anti-MUC-1 (Sigma-Aldrich, St. Louis, MO, USA) for 1 h at 4°C. Samples were then washed, fixed and detected in a FACS Calibur (Becton Dickinson Immunocytometry Systems, San Jose, CA, USA). Appropriate irrelevant antibodies (isotypes) (Sigma-Aldrich, St. Louis, MO, USA) were used as controls for the flow cytometry experiments.

RNA Extraction

Total RNA was extracted from the cells using TRIzol reagent method (Invitrogen, San Diego, CA, USA) as per the manufacturer's instruction. Cells were homogenized in TRIzol reagent and the RNA concentrations and RNA quality were determined by NanoDrop (NanoDrop 2000, Wilmington, CA, USA). The RNA integrity was assessed by agarose gel electrophoresis (Bio-Rad, Hercules, CA, USA). The isolated total RNA was stored at -80°C for further experiments.

Quantification of miR-155 Levels Using qRT-PCR

The microRNA, miR-155 level was analyzed using TaqMan MicroRNA assays. Total RNA was isolated from the antitumor CTL cells followed by recovering small RNA fractions (<200 nucleotides) using the mirVANA PARIS miRNA Isolation Kit (Ambion, Life Technologies, Foster City, CA, USA). The integrity of the RNA was checked using NanoDrop at the absorbance of 260 nm. qRT-PCR was performed with the threshold cycle (Ct) as the fractional cycle number at which fluorescence exceeds the fixed threshold of 0.2. Quantitative miR-155 expression was analyzed using dCt (the Ct value normalized to internal 'housekeeping' miRNAs such as miR-24 and miR-103) and ddCt (difference between the dCt of positive population and that of the negative population) values for each of the miRNAs. The percentage of expression was calculated using the formula 2-ddCt. Total RNA with the concentration of 10 ng was used to measure the miR-155.

Statistical Analysis

All the results were statistically significant. Statistical analysis such as mean, average and standard deviation were performed using Microsoft office 2007. $p < 0.05$ was considered statistically significant.

Results

Cell Fusion Analysis

The study has shown that the oncogenic miR-155 level was down-regulated in the antitumor CTL against breast cancer. In order to generate the antitumor cytotoxic T lymphocytes (CTL), blood samples were carefully collected from the metastatic breast cancer patients. From the blood samples monocytes, dendritic cells (DC) and T cells were isolated and cultured. After isolation, monocytes from the blood sample were used to generate DC, whereas human breast cancer cells (MCF-7) were used to induce antitumor CTL. Cell fusion experiments were performed by fusing DC with breast carcinoma cells (MCF-7) using the standard procedure as described before.

Flow Cytometry Analysis

To further explore the expression of MUC-1 (breast cancer marker) in MCF-7/DC fused cells, flow cytometry experiment was performed. MCF-7, DC and MCF-7/DC fused cells were harvested and placed in RPMI medium. The data of flow cytometry was shown in Figure 1. The sorted cells illustrate the expression of MUC-1 in breast carcinoma cells and obviously not in the DC. Also, the data (Figure 1) shows that after the fusion of MCF-7 with DC, the fused cells express MUC-1. In contrast, the expression of MHC class – II (marker for DC) was noted in the DC as well as MCF-7/DC fused cells, but not in the breast carcinoma cells (MCF-7). The result clearly indicates the co-expression of MUC-1 and MHC-II in the MCF-7/DC fused cells. The MCF-7/DC fused cells effectively stimulate the T-cells, which induce the generation of antitumor CTL against breast carcinoma cells.

qRT-PCR Analysis of miR-155

As we have generated the antitumor CTL against breast carcinoma cells, to identify the expression pattern of miR-155 in the antitumor CTL cells, qRT-PCR was performed. The data of qRT-PCR was shown in Figure 2. The data shows the down-regulation of oncogenic miR-155 in the antitumor CTL cells. The experiments were repeated thrice; same results were obtained as mentioned in Figure 2. The relative expression of miR-155 was reduced in the antitumor CTL against breast carcinoma. Evidently, the breast carcinoma cells (MCF-7) show the expression of miR-155, whereas the antitumor CTL against breast cancer shows the down-regulation of miR-155.

Discussion

Breast cancer is one of the second most common cancers, which cause death in women population[21]. Molecular analysis in the context of micro RNAs and its relation with cancer is not well studied. It was already reported that long non-coding RNAs (lncRNAs) such as MALAT-1, H19 and HOTAIR have play an important role in carcinogenesis or cancer growth[22]. Also, micro RNAs have a vast role in the development of cancer. It was reported that $CD44^{+}$ positive human prostate cancer cells expressed miR-34a at levels of ~25-70%[22], which illustra-

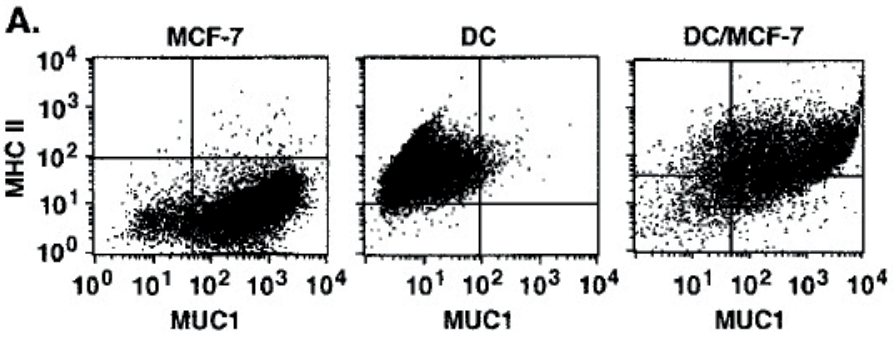

Figure 1. Flow cytometry analysis. Flow cytometry analysis of MCF-7, DC and DC/MCF-7 fused cells with the markers MUC-1 and MHC-II were shown in Figure.

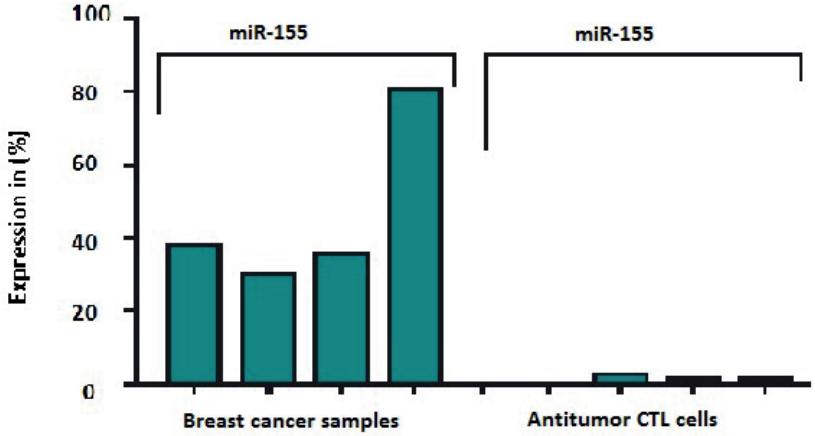

Figure 2. qRT-PCR analysis. RNA was isolated from the antitumor CTL cells as well as breast cancer cells and subjected to qRT-PCR. CTL shows decreased miR-155 expression when compared with that of breast cancer cells.

tes the crucial role of micro RNAs related to cancer. With this point of view, present experiment targeted the oncogenic miR-155 and studied the expression level of miR-155 after the fusion of DC/MCF-7 cells. The flow cytometry analysis of MUC-1 and MHC-II experiments show interesting results. The data shown in Figure 1, illustrates that MUC-1, which is the molecular marker for the breast cancer cells, was expressed in the MCF-7 cells, that indicates the cells are breast cancer cells. At the same time it is interesting that MUC-1 is also expressed in the DC/MCF-7 fused cells. Similarly, MHC-II is the DC marker, which expressed in the DC cells and also in the DC/MCF-7 fused cells, indicates that the fused cells are immune cells. Co-expression of MUC-1 and the MHC-II in the DC/MCF-7 fused cells implies that the immune cells process the breast cancer cells and present the MUC-1 antigen, which is one of the key findings in the present experiment. The MCF-7/DC fused cells induce the T-cells, followed by the generation of antitumor CTL against breast carcinoma cells. These antitumor CTL down-regulates the expression of oncogenic miR-155. The qPCR results were shown in Figure 2, which indicates and confirms the down-regulation of miR-155 expression in the antitumor CTL. The qPCR results clearly indicate that breast cancer cells has the expression of miR-155, rather the expression of miR-155 was down-regulated in the antitumor CTL cells. It has been reported that CD44 positive human prostate cancer cells expressed miR-34a at levels of ~25-70%[22]. A similar strategy has been demonstrated in the immune competent mice having prostate cancer[23], but in the present study the miR-155 level is decreased in the antitumor CTL. Though the breast cancer cells (MCF-7) show the higher expression of oncogenic miR-155, the level is decreased in the antitumor CTL.

Conclusions

The oncogenic miR-155 level was down-regulated in the antitumor CTL. Hence, it may be used as an effective treatment to eliminate the breast cancer malignant cells in the breast cancer patients.

Conflict of interest

The authors declare no conflicts of interest.

References

1) Steinman RM. The dendritic cell system and its role in immunogenicity. Annu Rev Immunol 1991; 9: 271-296.
2) Young JW, Steinman RM. The hematopoietic development of dendritic cells: a distinct pathway for myeloid differentiation. Stem Cells 1996; 14: 376-387.
3) Young J, Koulova L, Soergel S, Clark E, Steinman R, Dupont B. The B7/BB1 antigen provides one of several costimulatory signals for the activation of CD4+ T lymphocytes by human blood dendritic cells in vitro. J Clin Invest 1992; 90: 229.
4) Inaba K, Witmer-Pack M, Inaba M, Hathcock K, Sakuta H, Azuma M, Yagita H, Okumura K, Linsley P, Ikehara

S. The tissue distribution of the B7-2 costimulator in mice: abundant expression on dendritic cells in situ and during maturation in vitro. J Exp Med 1994; 180: 1849-1860.

5) Celluzzi CM, Mayordomo JI, Storkus WJ, Lotze MT, Falo L. Peptide-pulsed dendritic cells induce antigen-specific CTL-mediated protective tumor immunity. J Exp Med 1996; 183: 283-287.

6) Mayordomo J, Zorina T, Storkus W, Zitvogel L, Celluzzi C, Falo L, Melief C, Ildstad S, Kast WM, Deleo A. Bone marrow-derived dendritic cells pulsed with synthetic tumour peptides elicit protective and therapeutic antitumour immunity. Nat Med 1995; 1: 1297-1302.

7) Paglia P, Chiodoni C, Rodolfo M, Colombo MP. Murine dendritic cells loaded in vitro with soluble protein prime cytotoxic T lymphocytes against tumor antigen in vivo. J Exp Med 1996; 183: 317-322.

8) Hsu FJ, Benike C, Fagnoni F, Liles TM, Czerwinski D, Taidi B, Engleman EG, Levy R. Vaccination of patients with B-cell lymphoma using autologous antigen-pulsed dendritic cells. Nat Med 1996; 2: 52-58.

9) Nestle FO, Alijagic S, Gilliet M, Sun Y, Grabbe S, Dummer R, Burg G, Schadendorf D. Vaccination of melanoma patients with peptide-or tumor lysate-pulsed dendritic cells. Nat Med 1998; 4: 328-332.

10) Nieda M, Nicol A, Kikuchi A, Kashiwase K, Taylor K, Suzuki K, Tadokoro K, Juji T. Dendritic cells stimulate the expansion of bcr-abl specific CD8+ T cells with cytotoxic activity against leukemic cells from patients with chronic myeloid leukemia. Blood 1998; 91: 977-983.

11) Ribas A, Bui L, Butterfield L, Vollmer C, Jilani S, Dissette V, Glaspy J, McBride W, Economou J. Antitumor protection using murine dendritic cells pulsed with acid-eluted peptides from in vivo grown tumors of different immunogenicities. Anticancer Res 1998; 19: 1165-1170.

12) Liau LM, Black KL, Prins RM, Sykes SN, DiPatre P-L, Cloughesy TF, Becker DP, Bronstein JM. Treatment of intracranial gliomas with bone marrow-derived dendritic cells pulsed with tumor antigens. J Neurosurg 1999; 90: 1115-1124.

13) Nair SK, Boczkowski D, Morse M, Cumming RI, Lyerly HK, Gilboa E. Induction of primary carcinoembryonic antigen (CEA)-specific cytotoxic T lymphocytes in vitro using human dendritic cells transfected with RNA. Nat Biotechnol 1998; 16: 364-369.

14) Boczkowski D, Nair SK, Snyder D, Gilboa E. Dendritic cells pulsed with RNA are potent antigen-presenting cells in vitro and in vivo. J Exp Med 1996; 184: 465-472.

15) Gong J, Chen D, Kashiwaba M, Kufe D. Induction of antitumor activity by immunization with fusions of dendritic and carcinoma cells. Nat Med 1997; 3: 558-561.

16) Tian Y, Jia C, Li Y, Wang Y, Jiang L, Liu A, Wu J, Zhu Y, Hu Y, Haznedaroglu I. Eur Rev Med Pharmacol Sci 2016; 20: 4435-4444.

17) Bao J, Lin L. MiR-155 and miR-148a reduce cardiac injury by inhibiting NF-κB pathway during acute viral myocarditis. Eur Rev Med Pharmacol Sci 2014; 18: 2349-2356.

18) Xu L, Guo Y, Cen J, Yan W, He H, Niu Y, Lin Y, Chen C, Hu S. Overexpressed miR-155 is associated with initial presentation and poor outcome in chinese pediatric acute myeloid leukemia. Eur Rev Med Pharmacol Sci 2015; 19: 4841-4850.

19) Zhou P, Li Y, Ma Z, Li Z, Chen F, Jiang Y. Single nucleotide polymorphisms in the promoter region of mir-133a-1 and in pre-mir-152 rs1707 may contribute to the risk of asthma in a Chinese Han population. Eur Rev Med Pharmacol Sci 2016; 20: 2642-2649.

20) Gong J, Avigan D, Chen D, Wu Z, Koido S, Kashiwaba M, Kufe D. Activation of antitumor cytotoxic T lymphocytes by fusions of human dendritic cells and breast carcinoma cells. Proc Natl Acad Sci 2000; 97: 2715-2718.

21) Independent UK Panel on Breast Cancer Screening. The benefits and harms of breast cancer screening: an independent review. Lancet 2012; 380: 1778-1786.

22) Liu C, Kelnar K, Liu B, Chen X, Calhoun-Davis T, Li H, Patrawala L, Yan H, Jeter C, Honorio S. The microRNA miR-34a inhibits prostate cancer stem cells and metastasis by directly repressing CD44. Nat Med 2011; 17: 211-215.

23) Shi Z, Li X, Hao L, Zhao Y, Wang Y, Dong B, Chen W, Zhang Z, Wang Y, Fu Q. Cbl-b gene silencing in splenic T lymphocytes as a therapeutic strategy to target the prostate cancer RM-1 cell tumors in immune competent mice. Eur Rev Med Pharmacol Sci 2014; 18: 3819-3830