Case Report

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Long-Term Cryopreservation of an Autologous Immunocomplex of Dendritic Cells and Cancer Antigen (HiDCV-OS1) that Stimulates Antitumor Immunity Against Chemotherapy-Resistant Ovarian Cancer

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ABSTRACT

In recent years, therapies that use cellular products made from autologous tissues for medical treatment have been developed. Although novel treatments for ovarian cancer, such as a PARP inhibitor, have been developed and the prognosis has improved, for the relapse of ovarian cancer, efficient treatments are not developed for the relapse yet. We started a clinical trial of autologous cell processed products (HiDCV-OS1), which were made from ovarian cancer cells and dendritic cells by using hemagglutinating virus of Japan envelope (HVJ-E). The quality of HiDCV-OS1 was evaluated using cell viability and fusion rate, which were measured using surface antigen markers and flow cytometry. The cryopreservation time was four years, considering the recurrence period. HiDCV-OS1 could be stored for 4 years while maintaining cell viability and fusion rate. The fusion rate of HiDCV-OS1 before and after 4 years of cryopreservation was 30.4%–35.3%, and no changes such as deterioration were observed. Cell viability upon thawing during storage was stable, ranging from 67.0% to 86.0%. When HiDCV-OS1, which had been cryopreserved for 4 years, was administered to patients who had relapsed, some antitumor effects were obtained. HiDCV-OS1, consisting of autologous tumor tissue and dendritic cells with HVJ-E, was cryopreserved for four years, after which the thawed cells maintained their fusion rate and showed a certain anti-tumor effect, suggesting that they may have antigen-presenting potential.

Keywords: Cryopreservation; Personalized Medicine; Vaccine; Treatment; Ovarian Cancer; Hemagglutinating Virus of Japan Envelope

Introduction

In recent years, therapies that use cellular products made from autologous tissues have been developed [1]. In Japan, regenerative medicine is practiced in accordance with two regulations [2]. Ovarian cancer (OC) has a longer time to recurrence than other cancers, which has been extended in recent years with the arrival of novel drugs such as PARP inhibitors [3]. However, when OC relapses, it undergoes repeated remissions and progressions even after treatment is restarted, and eventually resistance develops; therefore, new and improved treatments are needed. Therefore, we established a novel therapeutic strategy using an autologous immunocomplex of dendritic cells (DCs) and cancer antigen (HiDCV-OS1). At the time of the initial surgery, the patient's tumor cells and DCs were fused with HVJ-E for high efficiency and without antigen destruction [4,5], and the immunocomplex was cryopreserved. HiDCV-OS1 was thawed and used when OC relapsed. HiDCV-OS1 was used in patients suffering from chemotherapy-resistant OC (jRCTc051190054) in Japan. This is the first report on the long-term cryopreservation of vaccine dendritic cells for cancer treatment.

Materials and Methods

Production of HiDCV-OS1 using Patient-Derived OC and Mononuclear Cells

Pre-treatment of Tumor Tissue: The OC was cut into small pieces (14.6 g ± 2.5 g) and dissociated completely using a gentleMACS octo Dissociator with Heaters (Miltenyi Biotec, K.K., Tokyo, Japan). A cloudy cell layer was obtained using the Ficoll–Paque centrifugation method. OC was isolated using Dynabeads CD45 (Thermo Fischer Scientific K. K., Tokyo, Japan). 1.2 x 10^6 cells of OC with STEM-CELLBANKER GMP grade (Nippon Zenyaku Kogyo Co.,Ltd. Fukushima, Japan) was added to 1.8 mL cell cryopreservation tubes and frozen at 80°C. The cells were transferred to -150.0°C ± 15.0°C for storage within 3 days. The cells were thawed and irradiated with 50-Gy radiation (TX-2500, Nanogray, inc. Osaka, Japan).

Mononuclear Cell Isolation and DC Maturation: Apheresis (COBE-Spectra, Terumo BCT, Tokyo, Japan) was performed to obtain mononuclear cell components 1.3 ± 0.14 x 10^9 cells) and incubated for approximately 7 days. Immature DCs were then collected.

Promotion of DC Maturation: AIM-V medium (Thermo Fischer Scientific K. K., Tokyo, Japan) and 25 mNAU/mL of HVJ-E were added to DC and incubated for 24 h for DC maturation.

Cryopreservation of DC: DC was suspended in STEM-CELLBANKER GMP grade at a rate of 2 million cells per 1.2 mL, placed in 1.8 mL cell cryopreservation tubes, and frozen at 80 °C. The cells were transferred to -150.0°C ± 15.0°C for storage within 3 days.

Preparation of HiDCV-OS1: Mixed at a ratio of 1 x 10^6 mature dendritic cells to 5 x 10^5 OC cells with 250 mNAU of HVJ-E and then shaken with a shaker (75 rpm) at 4°C for 10 min. The cells were then shaken with a shaker (75 rpm) for 20 min at 37°C.

Cryopreservation of HiDCV-OS1: One million cells/mL of HiDCV-OS1 STEM-CELLBANKER GMP grade was added to 1.8 mL cell cryopreservation tubes and frozen at 80°C. The cells were transferred to -150.0°C ± 15.0°C for storage.

Assessment of the HiDCV-OS1 Quality

Viability: Cells stored for variable periods (3, 12, 24, 96 months) were tested for viability using the trypan blue exclusion test.

Fusion rate of HiDCV-OS1: The percentage of HiDCV-OS1 cells was calculated by detecting CD11c- and CD326- or CD90-positive cells using flow cytometric analysis (Table 1).

Table 1: List and addition volume of the antibodies.

	Negative ctrl.	Positive ctrl.	Sample
Antibodies	PE/Cy7 Mouse IgG1, κ Isotype Ctrl 4 μL	PE and Cy7 anti-human CD11c 2 μL	PE and Cy7 anti-human CD11c 2 μL
	PE Mouse IgG2b, κ Isotype Ctrl 4 μL	PE Mouse IgG2b, κ Isotype Ctrl 0.62 μL	PE anti-human CD326 (EpCAM) 5 μL
	APC Mouse IgG1, κ Isotype Ctrl 5 μL	APC Mouse IgG1, κ Isotype Ctrl 5 μL	APC anti-human CD90 (Thy1) 5 μL

Note: APC, allophycocyanin; CD, cluster of differentiation; Cy, carboxylic acid; EpCAM, epithelial-specific cell adhesion molecule; IgG, immunoglobulin G; PE, phycoerythrin; Thy1, thymus cell antigen 1.

Results

The viability and fusion rate of cryopreservation start date and results up to 4 years after storage are presented in Table 2, and the HiDCV-OS1 subpopulation is shown in Table 3.

Table 2: Viability and fusion rate of HiDCV-OS1 after long-term storage.

	Viability (%)	Fusion rate (%)
Day 0	-	30.4
3 months	67.0	30.1
12 months	73.0	36.5
24 months	86.0	34.2
96 months	71.4	35.7

Table 3: Subpopulation (%) of HiDCV-OS1.

	Thy-1+, EpCAM-	Thy-1+, EpCAM+	Thy-1-, EpCAM-	Thy-1-, EpCAM+
Day 0	30.4	0	69.6	0
3 months	30.1	0	69.9	0
12 months	36.5	0	63.4	0
24 months	34.2	0	65.7	0
96 months	35.3	0.4	64.3	0

Note: EpCAM, epithelial-specific cell adhesion molecule; Thy1, thym.

Discussion

We showed the viability and cell fusion rates of HiDCV-OS1 after four years of cryopreservation. The specification criteria were the quality of HiDCV-OS1 after cryopreservation based on previous reports on antitumor efficacy [6]. In fact, all HiDCV-OS1 stocks met these criteria. To produce HiDCV-OS1, we used HVJ-E, which has high fusion efficiency between DCs and cancer cells, high antigen-presenting ability, and high antitumor effect [6]. While OC has a prolonged time between initial treatment remission and relapse [3], there is a lack of treatment options when the disease recurrence. Therefore, the use of HiDCV-OS1 after recurrence is expected to have an antitumor effect as a novel therapeutic tool. It is thought to be difficult to obtain sufficient tumor volume to produce the necessary amount of HiDCV-OS1 at that time, and the general condition of relapsed patients is often poor, and sufficient monocytes cannot be collected to differentiate into dendritic cells. There is a concern that the properties of tumor cells may change during recurrence and HiDCV-OS1 production. However, tumor cells that have acquired resistance to chemotherapy probably derived from cancer stem cells and express Thy-1. Because HiDCV-OS1 contains cancer antigens derived from Thy-1-positive cells, it can be expected to generate antitumor immunity even after recurrence [7,8]. Therefore, it is reasonable to prepare and store cell preparations for future administration in case of recurrence when the patient is well. HiDCV-OS1, which was stored for approximately 4 years, was used in patients with recurrent OC. There were no serious adverse events, and some efficacy was observed [9,10]. The relapsed patient was alive 387 days after the administration of HiDCV-OS1.

Conclusion

HiDCV-OS1, consisting of autologous tumor tissue and dendritic cells with HVJ-E, was cryopreserved for four years, after which the thawed cells maintained their fusion rate and showed a certain anti-tumor effect, suggesting that they may have antigen-presenting potential.

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Authorship Contribution

Conceptualization, Y. K. and K. S.; data curation, M. S., T. N. and K. S.; investigation, T. N., CY. C., J. F., H. H., K. O., K. S. and T. K.; writing-original draft preparation, M. S.; writing-review and editing, K. S.; project administration, T. N., K. S. and T. K.; supervision, Y. K.

Conflict of Interests

Conflicts of interest related to this study have been reviewed and appropriately managed by the Institutional Conflicts of Interest Committee.

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