Clinical Experience of Integrative Cancer Immunotherapy with GcMAF

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Abstract. *Background: Immunotherapy has become an attractive new strategy in the treatment of cancer. The laboratory and clinical study of cancer immunotherapy is rapidly advancing. However, in the clinical setting, the results of cancer immunotherapy are mixed. We therefore contend that cancer immunotherapy should be customized to each patient individually based on their immune status and propose an integrative immunotherapy approach with second-generation group-specific component macrophage activating factor (GcMAF)-containing human serum. Patients and Methods: The standard protocol of our integrative cancer immunotherapy is as follows: i) 0.5 ml GcMAF-containing human serum is administered intramuscularly or subcutaneously once or twice per week for the duration of cancer therapy until all cancer cells are eradicated; ii) hyper T/natural killer (NK) cell therapy is given once per week for six weeks; iii) high-dose vitamin C is administered intravenously twice per week; iv) alpha lipoic acid (600 mg) is administered orally daily; v) vitamin D3 (5,000-10,000 IU) is administered orally daily. Results: By March 2013, Saisei Mirai have treated over 345 patients with GcMAF. Among them we here present the cases of three patients for whom our integrative immunotherapy was remarkably effective. Conclusion: The results of our integrative immunotherapy seem hopeful. We also plan to conduct a comparative clinical study.*

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Cancer is a complex disease characterized by uncontrollable growth and extension of cancer cells. More than 200 types of cancer are known. Because the pathological causes and clinical status of each cancer vary significantly, multimodal therapy that combines surgery, chemotherapy, radiation therapy and immunotherapy is thought to be more effective.

Cancer immunotherapy is the use of the immune system to eradicate cancer. In particular, it involves stimulating the patient's immune system to locate and eliminate the cells that are cancerous. The mechanisms of protection fall into two broad categories – innate immunity and adaptive immunity. There are several types of cells in the innate immune system: phagocytic neutrophils, macrophages, dendritic cells, mast cells and natural killer (NK) cells. The adaptive immune system is comprised of lymphocytes, T-cells and B-cells. These are able to recognize and remember specific pathogens, and their products, including antibodies.

Immunotherapy has become an attractive new strategy in the treatment of cancer (1). The laboratory and clinical study of cancer immunotherapy – such as dendritic cells, autologous lymphocyte-activated killer (LAK) cells, autologous NK cells, monoclonal antibodies, cancer peptide vaccines, cytokines, and biological response modifiers (BRM) – is rapidly advancing. However, in the clinical setting, the results of cancer immunotherapy are mixed.

We, therefore, contend that cancer immunotherapy should be multimodal and customized to each individual patient. We propose an integrative cancer immunotherapy based on second-generation group-specific component macrophage activating factor (GcMAF) as a promising candidate for a patient-friendly cancer immunotherapy.

Patients and Methods

At Saisei Mirai, we are currently treating patients with cancer with GcMAF-based cancer immunotherapy. We use GcMAF in combination with other immune system-related therapies, such as hyper T/NK cell therapy, intravenous high-dose vitamin C therapy, and alpha lipoic acid. All of these therapies aim to strengthen and activate the immune system and take a holistic approach to fighting cancer rather than a localized approach that is common with conventional therapies such as radiation and surgery. However, in cases where there is a large tumor load, localized therapies, preferably ones with minimal negative effects on the immune system, may be necessary to reduce tumor burden.

GcMAF. GcMAF is derived from the group-specific component (Gc) protein, a member of the albumin superfamily (2). GcMAF, also known as vitamin D binding protein-macrophage activating factor (DBP-maf), is a potent endogenous macrophage activator found naturally in the blood. Until recently, GcMAF was not used in cancer immunotherapy, in spite of it being a key player of the human innate immune system.

GcMAF activates macrophages *via* superoxide radical generation and phagocytic activation (3, 4), and has been demonstrated to have antiangiogenic and antitumor activity *in vivo* (5). GcMAF also directly inhibits the proliferation and migration of human prostate cancer cells, as well as human breast cancer cells, independently of its macrophage activation ability (6).

First-generation GcMAF. First-generation GcMAF is prepared by artificial enzymatic treatment of non-specific human Gc protein which is purified by vitamin D affinity chromatography. This may be made from pooled serum of many people's blood consisting of a mixture of Gc protein subtypes.

Clinical trials using first-generation GcMAF in patients with metastatic breast cancer (7), prostate cancer (8), and metastatic colorectal cancer (9) have been conducted. Cancer did not recur over a four- to seven-year period in all those administered weekly doses of 100 ng of GcMAF for seven to 19 weeks.

Second-generation GcMAF. Second-generation GcMAF is prepared by artificial enzymatic treatment of human serum without purification by vitamin D affinity chromatography. GcMAF is made from individual serum samples that are not pooled.

GcMAF therapy. We propose a multimodal integrative GcMAF-based immunotherapy which is characterized as the artificial exogeneous preparation of GcMAF using a sample of serum which is not purified using a vitamin D affinity column.

Hyper T/NK cell therapy. In a previous study, tumor-infiltrating lymphocytes (TIL) were reported to be effective in experimental and clinical research of advanced cancer (10, 11). TILs recognize specific antigens expressed by autologous tumor cells. Sekine and colleagues developed a feasible method to obtain large numbers of activated or effective TILs (12). Administration of these expanded TILs demonstrates clinical activity in some patients with several types of cancers, making it a useful adoptive immunotherapy.

We developed a cultivation method with autologous plasma from patients with specific antibodies for membrane antigens of NK cells based on Sekine et al's techniques and our own clinical results. This cultivation method is used not only to obtain activated T-lymphocytes, but also 'hyper T-cells' and NK cells. Hyper T-cells, a name we coined, are unique immature multipotent T-cells with various capabilities. Hyper T-cells have a broader specificity for antigens expressed by autologous tumor cells, are able to proliferate, and maintain their activity for long periods *in vivo* (13). Therefore, expansion of hyper T-cells has the potential for being a suitable and important factor in adoptive immunotherapy against cancer. NK cells are a unique subset of lymphocytes, distinct from T-lymphocytes. They contribute to essential immune systems, such as host antimicrobial and antitumor immunity, without requirement for prior immune sensitization of the host (14). NK cells are promising effector cells for immunotherapy against cancer. For immunotherapy, three cell types–T-lymphocytes, hyper T-cells, and NK cells–are cultured simultaneously. We developed a simple method of simultaneously culturing T-lymphocytes, hyper T-cells, and NK cells, which together seem to be important for effective immune therapy. Using these cells in combination provides the advantage of one cell type to compensate for the disadvantage of using the other alone – their synergistic actions contribute to the eradication of tumor cells.

High-dose vitamin C therapy. The recommended dietary allowance (RDA) of vitamin C for women is 75 mg/day and for men is 90 mg/day. While the regular RDA dose of vitamin C is employed as a nutritional supplement, high-dose vitamin C (50-100 g) is advocated as an anticancer agent. Concentrations of 1,000-5,000 μmol/l are selectively cytotoxic to tumor cells *in vitro* (15, 16). Plasma concentration of vitamin C after intravenous infusion of 50-100 g of vitamin C reaches about 3,000-4,000 μg/ml or 17,000-22,700 μmol/l. Tumoricidal ascorbate concentrations can be achievable in the human body without significant side-effects.

Alpha lipoic acid. Alpha-lipoic acid is approved in Germany as a drug for the treatment of polyneuropathies, such as diabetic and alcoholic polyneuropathies, and liver disease. Alpha-lipoic acid has antioxidant effects. It is reduced intracellularly to dihydrolipoic acid, which in cell culture regenerates by reduction of antioxidant radicals, such as vitamin C and vitamin E (17). Alpha-lipoic acid enhances the antitumor efficacy of ascorbate to the point where significant tumor cell killing can occur at concentrations achievable by intravenous infusion (18).

Integrative cancer immunotherapy with GcMAF. The standard protocol of our integrative cancer immunotherapy is as follows: i) GcMAF (0.5 ml) is administered intramuscularly once or twice per week; ii) hyper T/NK cell therapy is given once per week for six weeks; iii) high-dose vitamin C is administered intravenously twice per week; iv) alpha lipoic acid (600 mg) is administered orally daily; and v) vitamin D3 (5,000-10,000 IU) is administered orally daily.

Results

At the end of April 2011, Saisei Mirai started treating patients with cancer using second-generation GcMAF produced from human serum. By March 2013, Saisei Mirai have treated 345 patients using GcMAF. Among them, we present the cases of three patients for whom our integrative immunotherapy was remarkably effective.

Patient 1. A 71-year-old man was diagnosed with thymic carcinoma with lung metastasis. The patient received 24 weeks of the integrative immunotherapy. No progression of the cancer was found 12 months after completion of the therapy.

Patient 2. A 74-year-old man was diagnosed with prostate cancer with multiple bone metastases. He received 12 weeks of the integrative immunotherapy combined with hyperthermia therapy. Bone scintigram results nine months after initiation of the therapy were normal and metastatic tumors had disappeared.

Patient 3. A 72-year-old woman was diagnosed with metastatic liver cancer after sigmoidectomy and bilateral oophorectomy. She received 24 weeks of the integrative immunotherapy combined with 55 Gy of radiation. There was no evidence of local recurrence or metastatic disease on Positron Emission Tomography (PET) and Computed Tomography (CT) scans 12 months after initiation of the therapy.

Discussion

We have used this multimodality integrative immunotherapy-based approach in nearly 400 patients with cancer. The results of this integrative immunotherapy look hopeful. We also plan to conduct a comparative clinical study to clarify its efficacy compared to that of several other integrative immunotherapies using different Gc protein subtypes, different concentrations of GcMAF, and different macrophage statuses to find the relationship between each therapy and the curative effect of GcMAF-containing human serum. We aim to determine the optimal combination of the integrative immunotherapy from the results of these clinical and analytical studies.

This type of integrative immunotherapy can be of benefit to patients and is a promising treatment. We expect that the described immunotherapy using second-generation GcMAF will play a central role in future treatments against human cancer, both alone and in combination with other therapies, such as sonodynamic and photodynamic therapy.

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References

1 Mellman I, Coukos G and Dranoff G: Cancer immunotherapy comes of age. Nature *480*: 480-489, 2011.
2 Yamamoto N and Homma S: Vitamin D_3-binding protein (group-specific component) is a precursor for the macrophage-activating signal factor from lysophosphatidylcholine-treated lymphocytes. Proc Natl Acad Sci USA *88*: 8539-8543, 1991.
3 Yamamoto N and Kumashiro R: Conversion of vitamin D_3-binding protein (group-specific component) to a macrophage-activating factor by the stepwise action of beta-galactosidase of B-cells and sialidase of T-cells. J Immunol *151*: 2794-2802, 1993.
4 Yamamoto N, Lindsay DD, Naraparaju VR, Ireland RA and Popoff SN: A defect in the inflammation-primed macrophage-activation cascade in osteopetrotic rats. J Immunol *15*: 5100-5107, 1994.
5 Kisker O, Onizuka S, Becker CM, Fannon M, Flynn E, D'Amato R, Zetter B, Folkman J, Ray R, Swamy N and Pirie-Shepherd S: Vitamin D-binding protein-macrophage-activating factor (DBP-maf) inhibits angiogenesis and tumor growth in mice. Neoplasia *5*: 32-40, 2003.
6 Pacini S, Punzi T, Morucci G, Gulisano M and Ruggiero M: Effects of vitamin D-binding protein-derived macrophage-activating factor on human breast cancer cells. Anticancer Res *32*: 45-52, 2012.
7 Yamamoto N, Suyama H, Yamamoto N and Ushijima N: Immunotherapy of metastatic breast cancer patients with vitamin D-binding protein-derived macrophage-activating factor (GcMAF). Int J Cancer *122*: 461-467, 2008.
8 Yamamoto N, Suyama H and Yamamoto N: Immunotherapy for prostate cancer with Gc protein-derived macrophage-activating factor, GcMAF. Transl Oncol *1*: 65-72, 2008.
9 Yamamoto N, Suyama H, Nakazato H, Yamamoto N and Koga Y: Immunotherapy of metastatic colorectal cancer with vitamin D-binding protein-derived macrophage-activating factor, GcMAF. Cancer Immunol Immunother *57*: 1007-1016, 2008.
10 Rosenberg SA, Spiess P and Lafreniere R: A new approach to the adoptive immunotherapy of cancer with tumor-infiltrating lymphocytes. Science *233*: 1318-1321, 1986.
11 Rosenberg SA, Lotze MT, Muul LM, Chang AE, Avis FP, Leitman S, Linehan WM, Robertson CN, Lee RE, Rubin JT, Seipp CA, Simpson CG and White DE: A progress report on the treatment of 157 patients with advanced cancer using lymphokine-activated killer cells and interleukin-2 or high-dose interleukin-2 alone. N Engl J Med *316*: 889-897, 1987.
12 Sekine T, Shiraiwa H and Yamazaki T: A feasible method for expansion of peripheral blood lymphocytes by culture with immobilized anti-CD3 monoclonal antibody and interleukin-2 for use in adoptive immunotherapy of cancer patients. Biomed Pharamocother *47*: 73-78, 1993.
13 Utsuyama M, Hirokawa K, Kurashima C, Fukayama M, Inamatsu T, Suzuki K, Hashimoto W and Sato K: Differential age-change in the numbers of $CD4^+CD45RA^+$ and $CD4^+CD29^+$ T-cell subsets in human peripheral blood. Mech Ageing Dev *63*: 57-68, 1992.
14 Ljunggren HG and Malmberg KJ: Prospects for the use of NK cells in immunotherapy of human cancer. Immunology *7*: 329-339, 2007.
15 Padayatty SJ, Riordan HD, Hewitt SM, Katz A, Hoffer LJ and Levine M: Intravenously administered vitamin C as cancer therapy: Three cases. CMAJ *174*: 937-942, 2006.
16 Chen Q, Espey MG, Krishna MC, Mitchell JB, Corpe CP, Buettner GR, Shacter E and Levine M: Pharmacologic ascorbic acid concentrations selectively kill cancer cells: Action as a pro-drug to deliver hydrogen peroxide to tissues. Proc Natl Acad Sci USA *102*: 13604-13609, 2005.
17 Packer L, Witt EH and Tritschler HJ: α-Lipoic acid as a biological antioxidant. Free Rad Biol Med 19: 227-250, 1995.
18 Casciari JJ, Riordan NH, Schmidt TL, Meng XL, Jackson JA and Riordan HD: Cytotoxicity of ascorbate, lipoic acid, and other antioxidants in hollow fibre *in vitro* tumours. Br J Cancer *84*: 1544-1550, 2001.

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