Refractory lung metastasis from breast cancer treated with multidisciplinary therapy including an immunological approach

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ABSTRACT

A suggestive case of metastatic disease from breast cancer is reported. The HER2-positive tumor was refractory to several agents, including anti-HER2 therapy, trastuzumab and lapatinib. After re-induction of trastuzumab in combination with activated natural killer cell injection therapy, tumor markers decreased, and finally a synergistic effect of taxane and capecitabine showed treatment response. This case suggests that multidisciplinary therapy including an immunological approach might be a breakthrough to refractory disease.

KEYWORDS

breast cancer, metastasis, natural killer cell, chemotherapy, trastuzumab

Introduction

Breast cancer can metastasize to lymph node, bone, lung, liver, and brain. Lung metastasis from breast cancer may not only decrease quality of life (QOL) but also threaten the patient's life. Therefore, it is important to control it.

Like endocrine therapy for estrogen-receptor (ER)-positive breast cancer, trastuzumab-based therapy is considered initially for tumors with overexpression of the HER-2/neu protein or amplification of the her-2/neu gene, which occur in 20-25% of metastatic breast cancers [1]. Treatment with trastuzumab, a humanized mAb directed against the extracellular domain of HER2, resulted in a response rate of 21% in patients pretreated with chemotherapy, as a single agent. Trastuzumab also increased the clinical benefit of chemotherapy in metastatic breast cancer with HER2 overexpression [2]. Furthermore, adjuvant use of trastuzumab reduced recurrence remarkably in primary breast cancer [3-4].

The activity of trastuzumab was found to depend on the engagement of Fc-receptor-expressing lymphocytes, indicating antibody-dependent cellular cytotoxicity (ADCC) as the major mechanism of antibody action [5-7]. ADCC is triggered by interaction between antibody-coated target cells and FcγRIII (CD16) on natural killer (NK) cells, which initiates a sequence of cellular events culminating in the

release of cytotoxic, granzyme-containing granules [8, 9]. Therefore, if we could increase the activity of NK cells, the efficacy of treatment with trastuzumab could be increased.

In this report, we present a case with refractory pulmonary metastasis, in which the tumor was successfully treated with trastuzumab, taxane, capecitabine, and activated NK cell injection therapy.

Case Report

A 55-year-old woman was treated in October 1999 for an invasive ductal carcinoma of the right breast by mastectomy (Auchincloss's procedure) at another hospital. Pathological examination showed pathological stage T2, positive lymph node for cancer (11/22), lymphatic invasion, and negative for ER and progesterone receptor (PgR). She was admitted to our hospital for adjuvant chemotherapy, which consisted of six courses of cyclophosphamide, methotrexate, and 5-fluorouracil (CMF).

In August 2001, a chest CT scan performed as a follow-up study showed an abnormal shadow in the lung, which was diagnosed as a metastatic carcinoma from the breast cancer using transbronchial lung biopsy (TBLB). Because the tumor was positive for

human epidermal growth factor receptor type 2 (HER2/neu) (3+ by an immunohistochemical test), intravenous treatment with docetaxel and trastuzumab was started. In January 2003, doxifluridine (5′DFUR) was added by oral administration, and Response Evaluation Criteria in Solid Tumors (RECIST) indicated a partial tumor response. In April 2004, we changed the treatment to capecitabine with trastuzumab because of the tumor marker elevation. However, four months later, a chest CT scan revealed that lung metastasis had progressed. Although four courses of epirubicin monotherapy were started in September 2004, we could not stop disease progression. In January 2005, the patient joined a clinical trial of lapatinib. Eleven months later, a follow-up chest CT scan showed disease progression, and the patient subsequently withdrew from the trial.

After multidisciplinary consultation and with the written consent of the patient, we initiated therapy by injecting activated natural killer (NK) cells [9, 10]. Blood was collected, and peripheral blood mononuclear cells (PBMCs) were isolated from the blood by Ficoll–Hypaque gradient centrifugation (Amersham Biosciences, Uppsala, Sweden) and washed twice with RPMI 1640, and the number of cells was counted. To generate activated NK cells, PBMCs were cultured in an anti-CD16-coated flask with AIM-V (Invitrogen, Tokyo, Japan) medium supplemented with 5% auto-plasma, 700

U/ml IL-2 (Chiron, Amsterdam, Netherlands), and 1 μl/ml OK432 (Chugai Pharmaceutical, Tokyo, Japan) for 24 h at 39°C. The cultured cells were then centrifuged at 1000 rpm for 10 min, and the supernatant was discarded. Next, the cells were again cultured in a flask (not coated with anti-CD16) in AIM-V medium supplemented with 5% auto-plasma and 700 U/ml IL-2 at 37°C for 2 to 3 weeks. During the culture periods, we added medium several times to expand and maintain the population of the activated NK cells; the purity of the NK cells was 92 to 94%. When the activated NK cells were injected intravenously by drip ($2x10^9$ cells/injection), blood was collected simultaneously. Then isolation and culture of NK cells were repeated at two-week intervals.

We also measured cytotoxic activity of NK cells by the following method. Freshly isolated PBMCs and activated NK cells were tested for cytotoxic activity at various effector-to-target (E/T) ratios in a Calcein-AM release assay using TERASCAN VP (Minerva Tech., Tokyo, Japan). We labeled the target cells, K562, with immunofluorescent-dye Calcein-AM solution (Do Jindo Lab., Kumamoto, Japan) and incubated for 30 min, washed the cells with PBS(-) and checked the fluorescence intensity of the cells. Target cells and effector cells were suspended with RPMI1640 and 10% fetal bovine serum at various E/T ratios and added into a 96-well plate, incubated

for 2 hours and again checked for fluorescence intensity.

NK cell activity in the patient was extremely low before injection of activated NK cells (Fig. 1); however, the activity increased after therapy began. Simultaneously, we resumed intravenous treatment with trastuzumab. The concentrations of carcinoembryonic antigen (CEA) and carbohydrate antigen 15-3 (CA15-3) had decreased for six months, but began to increase following trastuzumab treatment. We added capecitabine to trastuzumab; however, these tumor markers continued to increase. At the same time, skin and bone metastases emerged. Therefore, weekly intravenous injection of paclitaxel was added, and we also started hyperthermia for the skin metastases. After that, NK cell activity increased again, and the tumor markers decreased. A chest CT scan in November 2006 showed that the lung metastases had reduced (Fig. 1). NK cell activity remained at a high level and the progression-free survival was about 10 months.

Discussion

In the current report, lung metastasis which had been refractory to anti-HER2 therapy responded to the combination therapy of trastuzumab, capecitabine, paclitaxel, and

activated NK cell injection therapy.

There are some reports that indicated a relation between the activity of NK cells and disease progression. When breast cancer cells were inoculated into NOD/SCID mice that possessed NK cell activity, only a small tumor grew at the inoculation site and no organ metastasis was shown. However, when NOD/SCID/γc^{null} (NOG) mice lacking T-cell, B-cell, and NK cell activity were inoculated with breast cancer cells, there was efficient formation of a relatively large tumor and spontaneous organ metastasis [10]. An epidemiological study demonstrated that the natural cytotoxic activity of PBMCs was significantly associated with reduced cancer risk [11]. We recently reported that NK cell activity in metastatic breast cancer patients was significantly reduced compared with that of healthy controls [12]. Furthermore, several exploratory studies have shown that NK cell therapy contributes to reduce the tumor burden in human cancers [13].

Antibody-dependent cellular cytotoxicity (ADCC) by NK cells is mediated by the binding of FcγRIII (CD16) to the Fc portion of the antibody, which initiates a sequence of cellular events culminating in the release of cytotoxic, granzyme-containing granules [8]. Trastuzumab is known to mediate ADCC against a HER2/neu-positive breast cancer target [5-7]. In the present case, after induction of activated NK cell injection therapy and trastuzumab, the NK cell activity of the patient recovered and subsequently the

level of CEA decreased. This result indicated that activity of NK cells might influence the efficacy of trastuzumab.

Capecitabine is enzymatically converted to 5-fluorouracil in the tumor by thymidine phosphorylase (TP) [14]. We previously reported that docetaxel-containing regimens modulate TP in primary breast cancer tissues in a neoadjuvant setting [15]. Therefore, it was suggested that the combination of taxane and capecitabine was synergistically effective. Some experiments using human cancer xenografts have indicated this synergic effect and some clinical trials have also suggested that combination therapy of taxane and capecitabine provided favorable effects compared with capecitabine alone [16-17]. In the present case, the tumor responded to combination therapy of trastuzumab, docetaxel, and doxifluridine as a first-line therapy after recurrence. Furthermore, the combination therapy of paclitaxel and capecitabine was also effective in reducing re-growth of the tumor after induction of activated NK cell injection and trastuzumab.

Some reports have also shown that taxanes increased NK cell cytotoxicity. Paclitaxel induced mRNA and protein production of perforin and the activation of nuclear factor-κB (NF-κB) in NK cells in vitro [18]. In a taxane treatment group, NK cell cytotoxicity was 39% higher than that in a non-taxane treatment group [19]. Furthermore, several studies have indicated that hyperthermia therapy may increase NK

cell activity [20]. Although paclitaxel and hyperthermia might modulate the activity of NK cells, the relation between the modulation and the treatment response is still unclear.

In addition to downregulation of NK cell activity, various other mechanisms such as PTEN and phosphoinositol kinase abnormalities have been suggested to explain trastuzumab resistance [21, 22]. Therefore knowing the individual mechanism of resistance to anti-HER2 therapy such as trastuzumab or lapatinib is warranted to maximize the effect of anti-HER2 therapy.

Interestingly, although this patient was resistant to anti-HER2 therapy and showed an extremely low level of NK cell activity before NK cell injection therapy, NK cell activity increased and response was obtained after the patient received the combined treatment with highly purified and well activated NK cells and trastuzumab. Furthermore, adding TP-inducible chemotherapy and TP-targeting chemotherapy to the treatment yielded an objective response. Our case report indicates that multidisciplinary therapy, including an immunological approach, might be a breakthrough to refractory metastasis.

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Conflicts of interest

All authors declared they have no financial support that may pose a conflict of interest.

Informed consent

We obtained consent for publication in print and electronically from the patient's husband.

Figure legend:

Figure 1

This figure shows changes of tumor markers and NK cell activity in the patient during activated NK cell injection therapy. It also shows images from a chest CT scan above the graph. NK cell activity in the patient was extremely low before injection of activated NK cells. However, her NK cell activity increased after activated NK cell injection therapy began. Because of disease progression, we also initiated trastuzumab, capecitabine, and paclitaxel with activated NK cell injection therapy. Then tumor markers became smaller, and the lung metastasis was reduced.