Effects of Hyperthermia on the Host Immune System:  
From NK Cell-based Science to Clinical Application

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Abstract: Hyperthermia has been known to enhance the host immune responses against cancer through several mechanisms. One of the mechanisms is sensitization of cancer cells to natural killer (NK) cells through enhancement of NK cell-activating ligands on cancer cells. However, NK cell number and cytotoxicity in patients with cancer is often low. Therefore, NK cell-based adoptive immune cell therapy can be applied for cancer patients after hyperthermia. In this paper, we discuss these findings from our data. Moreover, we present the clinical usefulness of combining hyperthermia, immune cell therapy, and low-dose chemotherapy as palliative treatment for advanced stages of cancer, which are refractory to conventional therapy in the context of NK cell-mediated immune responses.

Key Words: hyperthermia, immunotherapy, NK cell, MICA/B, palliative cancer therapy

Introduction

This manuscript is based on a presentation at the symposium of the 28th Annual Meeting of Japanese Society for Thermal Medicine, “Immunological Science of Hyperthermia - basic and clinical medicine” in September 2011.

Hyperthermia has been reported to activate the host immune system against cancer¹⁰. Although the effects this activation are not fully understood, several mechanisms of tumor regression have been
postulated as follows (Fig. 1): increase of direct cytotoxicity of effector cells such as natural killer (NK) cells against cancer; enhancement of maturation signals into immature dendritic cells (DC) via the production of heat shock protein (HSP) -tumor antigen complex in tumors; facilitation of the presentation of tumor antigens to CD8+ T cells to produce cytotoxic T lymphocytes (CTL); decrease of regulatory T cell number in tumor tissues and blood; increase of expression level of adhesion molecules in endothelial venules; enhancement of the trafficking of lymphocytes, such as T cells and cytokine-producing NK cells, to secondary lymphoid tissues; and sensitization of cancer cells to immune effector cells, such as NK cells, CTL via major histocompatibility complex class I-related chain A/B (MICA/B) molecule, and major histocompatibility complex (MHC) class I antigen.

The number of NK cells increase in tumor sites after hyperthermia treatment in mouse model. Moreover, NK cells eliminate tumor cells. Hyperthermia treatment of human blood is known to increase the number of NK cells and cause their higher cytotoxic activity.

In this paper, we discuss a mechanism to increase sensitivity of cancer cells to NK cell cytotoxicity by hyperthermia. We also discuss how NK cell-based immune therapy can be used to kill cancer cells that have been sensitized by hyperthermia. Furthermore, we present the clinical effects of hyperthermia when combined with immune cell therapy (especially NK cell-based immunotherapy) as palliative treatment for advanced stages of cancer.

![Fig. 1. Regional hyperthermia induces the activation of immune system against cancer. NK cells: natural killer cells, iDC: immature dendritic cells, mDC: mature dendritic cells, CTL: cytotoxic T lymphocytes, Treg: regulatory T cells, HEV: high endothelial venules, HSP: heat shock protein, Ag: antigen, MHC: major histocompatibility complex, MICA/B: major histocompatibility complex class I-related chain A/B, IFN: interferon, ICAM-1: intercellular adhesion molecule 1, CCL: chemokine (C-C motif) ligand, CD: cluster of differentiation.](image-url)
**Up-regulated expression of NK cell-activating ligands on cancer cells by hyperthermia**

NK cells have many activating and inhibitory receptors, which recognize specific ligands as expressed on target cells\(^{17}\). Most activating ligands on target cells are recognized by NK cell-activating receptors and can be enhanced by thermal stress to the cells. On the other hand, MHC class I antigen expressed on autologous cells are engaged by NK cell-inhibitory receptors. A balance of these activating and inhibitory receptor signals through these ligands regulates the effector function of NK cells.

We found that hyperthermia induced an up-regulated expression of NK cell-activating ligands (such as MICA/B) in several cancer cell lines (Fig. 2A). Hyperthermia did not induce increased expression of MHC class I antigen in these cell lines (Fig. 2B). Furthermore, hyperthermic cancer cell lines increased NK cell cytotoxicity in proportion to MICA/B expression level (Figs. 2C and 2D). Other researchers have observed that cellular stresses, such as heat shock induced by hyperthermia, enhanced the expression of NK cell-activating ligand\(^{12}\), but not the expression of MHC class I antigen that has been exposed to

![Graph A](image1.png) ![Graph B](image2.png) ![Graph C](image3.png) ![Graph D](image4.png)

**Fig. 2.** Expression rate of MICA/B molecule (A) and MHC class I antigen (B) in cancer cell lines was shown after treatment at 37°C (gray column) and 42°C (black column) for 16 hours in human cancer cell lines as follows: SKBR3 derived from breast carcinoma, Daudi and Raji derived from Burkitt lymphoma, C1AK newly derived from colon carcinoma by us, and UB2MT newly derived from uterus body carcinoma by us. Cytotoxicity of NK cells to UB2MT cells (C) was in proportion to expression rate of MICA/B molecule in UB2MT cells (D) after thermal treatment. Expanded NK cells were prepared by 14 day cultivation using BINKIT (Biotherapy Institute of Japan, Tokyo, Japan)\(^{10}\). The cytotoxicity of the expanded NK cells was measured against UB2MT cells at an effector-to-target ratio 6:1 in a calcen-AM release assay using a Terascan VP (Minerva Tech., Tokyo, Japan) as previously described\(^{10}\). A representative experiment was shown. Similar results were obtained in three independent experiments.
elevated temperatures on cancer cell lines. Hyperthermia also results in transcriptional up-regulation of MICA on tumor target cells in a manner that correlates with increased sensitivity to cytolysis. These data suggest that hyperthermia can induce higher sensitivity of cancer cells to an attack of NK cells. If NK cell activity can be increased, then tumor regression might be more effective.

**Activation of NK cells by hyperthermia and NK cell therapy**

According to epidemiologic data, cytotoxic activity of NK cells in blood is different among healthy individuals; low activity is associated with increased cancer risk. Cytotoxic activity of NK cells in cancer patients is significantly lower compared to healthy individuals. If NK cell number and cytotoxicity is low, then tumor regression cannot be induced even when hyperthermia induces NK cell-activating ligands on cancer cells. Therefore, NK cell-based adoptive immune cell therapy can be considered for relatively immunosuppressed patients with cancer after hyperthermia.

To use NK cells for immune cell therapy, BINKIT (Biotherapy Institute of Japan, Tokyo, Japan) can be used to prepare the *ex vivo* expanded, highly activated NK cells by the method described earlier. The expanded NK cells had much higher activity than NK cells that were freshly isolated from peripheral blood mononuclear cells (Fig. 3). The expanded NK cells killed cancer cell lines expressing the MHC class I antigen, which is an inhibitory ligand of NK cell cytotoxicity and the CD133 molecule (a marker of cancer stem cells) (Fig. 3). Of interest, elevated temperatures enhanced the expression of the MICA/B molecule on CD133+ cells and increased NK cell-mediated cytotoxic activity on a CD133+ cell (Fig. 2c). These data suggest that a combination treatment using hyperthermia with NK cell-based immune cell therapy can possibly kill cancer stem cells, which are known to be resistant to radiotherapy and chemotherapy.

**Fig. 3.** Expanded NK cells have significantly higher cytotoxicity than freshly purified NK cells from peripheral blood mononuclear cells against cancer cell lines. Expanded NK cells were prepared as described in Fig. 2 (*, p<0.01). Freshly purified NK cells were isolated from peripheral blood mononuclear cells by negative selection using Dynabeads Untouched Human NK cells (VERITAS, Tokyo, Japan). The average percentages of CD3-CD56+ NK cells in expanded NK cells and purified NK cells were 90.5% and 97.2%, respectively. The cytotoxicity of effector cells against UB2MT cells was measured at an effector-to-target ratio 3:1 as described in Fig. 2. Values are expressed as the mean ± SD of four samples of a representative experiment. Similar results were obtained in three independent experiments. Student’s t-test was used to compare results between expanded NK cells and freshly purified NK cells.
In addition to NK cells, γδT cells and αβT cells express NK cell-activating receptors such as the receptor of natural killer group 2, member D (NKG2D) \(^{24}\). NKG2D receptor mediates cytotoxicity of these effector cells to cancer cells expressing MICA/B\(^{23}\). To compare the cytotoxicity with NK cells, γδT cells, and αβT cells, we expanded these cells and measured their cytotoxic efficacy against cancer cells. NK cell-dominantly expanded cells have the higher cytotoxicity regardless of expression of MHC class I antigen on the target cells, UB2MT (Fig. 4). The higher cytotoxicity of expanded NK cells compared with expanded γδT cells and expanded αβT cells was observed against UB2MT as well as other cancer cell lines, such as K562, Daudi, Raji, and C1AK (submitted). These data suggested that ex vivo expanded, activated NK cells are suitable for injecting into blood to kill the immune-sensitized cancer cells by hyperthermia.

**Clinical usage of hyperthermia combined with immune cell therapy**

The above data suggests that the combination of hyperthermia with NK cell-based combined immune cell therapy may be more effective against cancer because of NKG2D-MICA/B interaction. Although hyperthermia itself is insufficient to cause significant tumor regression clinically, regional hyperthermia combined with low-dose chemotherapy has been reported to be safe and effective\(^{25,26}\). Such more frequent and low-dose drug administrations compared with conventional chemotherapy has been proposed as metronomic chemotherapy which is expected to restore anticancer immune response through these inhibitory effects on regulatory T cells\(^{27}\). Moreover, DNA-damaging agents, including cisplatin and gemcitabine, induce the expression of NKG2D ligands such as MICA/B\(^{28,29}\).

We examined the clinical effects of hyperthermia when combined with NK cell-based immune cell
therapy as palliative treatment on advanced stages of cancer at the Tokyo Clinic and the Southern Tohoku General Hospital between April 2007 and March 2009 retrospectively. Fifty-two patients with advanced cancer refractory to conventional therapy were treated with a combination of hyperthermia and NK cell- and CTL-based immune cell therapy with low-dose chemotherapy. Regional hyperthermia using an 8-MHz capacitive heating device (Thermotron RF8 by Yamamoto Vinita, Osaka, Japan) with low-dose chemotherapy (Three cases did not receive chemotherapy) was performed every one or two weeks. Ten mg of cisplatin (max : 20 mg) dissolved in 500 ml of saline, 20 mg of docetaxel (max : 40 mg) dissolved in 250 ml of saline, 20 mg of irinotecan (max : 40 mg) dissolved in 250 ml of saline, or 600 mg of gemcitabine (max : 800 mg) dissolved in 100 ml of saline were given intravenously 30 to 60 min with hyperthermia for each patient every two weeks.

The treatment regimen of S-1 consisted of four week cycles in which 40 mg of oral S-1 per square meter of body-surface area per day was given for two weeks and no chemotherapy was given for the following two weeks. The appropriate concentration of these anti-cancer drugs was decided individually for each patient in consideration of the general conditions, renal function, and liver function. The total doses of each drugs which were actually given to patients for three months are shown in Table I. *Ex vivo* expanded, autologous NK cells and CTL (prepared in the manner described previously) were infused intravenously every two weeks during non-chemotherapy weeks. All patients who fulfilled the following requirements were selected for analysis: (1) case refractory to

<table>
<thead>
<tr>
<th>Tumor origin</th>
<th>After treatment for 3 months</th>
<th>After treatment for 6 months</th>
<th>Total dose of anti-cancer drugs for 3 months</th>
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<tbody>
<tr>
<td></td>
<td>CR</td>
<td>PR</td>
<td>SD</td>
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<tr>
<td>esophagus, head &amp; neck</td>
<td>0</td>
<td>1</td>
<td>3</td>
</tr>
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<td>lung</td>
<td>0</td>
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</tr>
<tr>
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<tr>
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<td>3</td>
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<td>1</td>
<td>0</td>
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<tr>
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<td>2</td>
<td>1</td>
</tr>
<tr>
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<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total case</td>
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<td>16</td>
</tr>
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</table>

The response of measurable target lesions was objectively evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST) guidelines.

CR : complete response, PR : partial response, SD : stable disease, PD : progressive disease
CDDP : cisplatin, TXT : docetaxel, CPT-11 : irinotecan, GEM : gemcitabine
conventional therapy; (2) at least four times of immune cell therapy received; (3) Eastern Cooperative Oncology Group (ECOG) performance status of 2 or less at the time of the first infusion of immune cells; and (4) informed consent obtained before beginning treatment.

After three months of treatment, objective responses were identified in 18 of 52 patients (35%), including one complete response (CR) and 17 partial responses (PR) as shown in Table I. Sixteen patients had stable disease (SD), whereas 18 had progressive disease (PD). Disease control rate was 66% including CR, PR, and SD. After treatment for six months, the objective responses and disease control rate were 25% and 52%, respectively.

The treatment was well tolerated for all cases. No adverse reaction over grade 1 (defined according to the Common Toxicity Criteria of the National Cancer Institute) was observed. It was not clear if there is any tendency to better control with higher doses because of the limited cases of each tumor origin.

These preliminary results suggest that hyperthermia combined with NK cell- and CTL-based immune cell therapy with low-dose chemotherapy was effective against advanced cancer that is refractory to conventional therapy, and without severe adverse effects.

More studies using the combined therapy for various kinds of cancers need to be done to determine which cancer is suitable for adaptation and how this therapy is beneficial to sustain the lives of patients and maintain their good quality of life.

Conclusion

To optimize the utility of hyperthermia in treating cancer, a large number of clinical data about hyperthermia when combined with NK cell-based immune cell therapy and chemotherapy must be collected to evaluate hyperthermia's usefulness in cancer immunotherapy.

One could not expect the effect of standard chemotherapy alone on tumor regression because of cases refractory to conventional therapy, but when combined with hyperthermia and immune cell therapy through NK cell-mediated cancer killing, it may induce effective immune responses and cause tumor regression. The appropriate combination of hyperthermia with immune cell therapy and chemotherapy has to be determined to maximum NK cell-mediated immune responses.

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References


