【Review Article】

The Present State of the Hyperthermia in Japan, and by What Kind of Way Should We Progress from Now on?

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日本のがん治療におけるハイパーサーミアの現状と今後の展望

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Abstract

Hyperthermia has a long history as a treatment modality for tumors, with the rapid development of heating devices for hyperthermia starting more than a century ago. Ideal heating devices would enable the targeted area to be heated in accordance with the depth and width of the tumor. However, hyperthermia alone does not show attractive results for cancer patients, but does appear to reinforce many therapeutic options, especially against for liver cancer. Hyperthermia is a potent sensitizer for radiotherapy, chemotherapy, and immune therapy, each of which has significantly advanced in this decade. A multimodal treatment approach that relies on hyperthermia combined with state-of-the-art radiation, chemotherapy, and immune therapy can result in great clinical benefits for pancreatic cancer patients. In a prior study, we demonstrated that hyperthermia enhances the cytotoxicity of gemcitabine, one of the key drugs for pancreatic cancer, by inhibiting gemcitabine -induced activation of nuclear factor kappa B (NF-kB). Considering these results, combining hyperthermia and chemotherapy could result in better treatment outcomes for pancreatic cancer patients.

Hyperthermia may reduce the metastatic potential of cancer cells and inhibit tumor metastasis. However, the underlying mechanisms through which hyperthermia inhibits cancer metastasis have yet to be fully elucidated. Epithelial-to-mesenchymal transition (EMT) plays a key role in tumor metastasis; therefore, we investigated and summarized the effects and underlying mechanisms of EMT in cancer cells. The results suggest that hyperthermia not only inhibits tumor growth, but also mediates the expression of EMT-related genes—including E-cadherin and vimentin. Elevated body temperature has been thought to play an important role in the regulation of immune responses, and accumulating evidence in thermal medicine indicates that hyperthermia could be a useful combination therapy to enhance the efficacy of cancer immunotherapy.

I believe that, in the years to come, a stronger focus on the research and development of these novel yet safe modals is what is required for a treatment breakthrough to emerge that ultimately results in longer survival and higher quality of life for many cancer patients.

要　旨

放射線療法，化学療法，免疫療法は，近年すばらしい進歩を遂げてきたが，これらの治療にハイパーサーミアを併用することは，さらにそれぞれの抗腫瘍効果を高めることが明らかとなってきている．たとえば，我々は，膵臓がんの key drug であるゲムシタビンの殺細胞効果をハイパーサーミアが増強することを見いたしている．また，ハイパーサーミアは，がん細胞の転移能を低下させるが，このメカニズムが長い間不明であった．上皮間葉移行（EMT）という現象が，がんの転移に重要であるが，この EMT をハイパーサーミアが抑制することが明らかとされた．さらに，近年最も注目を集めている免疫療法に関しては，体を温めることは，生体の免疫応答に非常に重要な役割を持っていることがわかっている．その上，ハイパーサーミアを免疫療法に併用することは，免疫療法をより効果的な治療法にする．本稿では，これらについて，私の研究データを基に概説する．

Key wards: hyperthermia, chemotherapy, NF-kB, Epithelial-Mesenchymal Transition, immunotherapy，温熱療法，化学療法，NF-kB，上皮間葉移行，免疫療法

I Introduction

Hyperthermia is old and is a novel cancer therapy. The treatment of burning off cancer with heat was performed around B.C. 2000, and it was the way near ablation currently performed now. This hyperthermia became science from the 1960s gradually. In 1970s, this treatment was investigated against for the cancer using microwaves, the clinical trial of hyperthermia had started completely by the West and a Japanese. First, the clinical trial of the concomitant with a radiotherapy preceded and it became an obvious that the curative effect which used a Radiotherapy and hyperthermia.

Whatever the contribution of Hypethermia about cancer treatment may be called, there is no adverse effect, a repeatedly treatment is made, and a sickness benefit is an inexpensive in it.

In this review, I would like to explain the basic and clinical approach about hyperthermia.

II Effective cases by using hyperthermia

At first, I mention clinical efficacy of hyperthermia in Japan.

The device was used in regional hyperthermia, Thermotron RF8 which is made by Yamamoto VINITA Co. is shown in Fig.1 and this device is widely used for regional hyperthermia in Japan. Theses devices are working at about 100 hospitals in Japan.

This case is Hepatocellular carcinoma. Most part of tumor was caused necrotic change by hyperthermia combined with trans arterial chemoembolization (TACE) (Fig.2) [1]. Next case is multiple metastases from gastric cancer. Metastatic lesions could not be detected by CT scan after combination therapy using hyperthermia and chemotherapy (Fig.3) [2]. Third case is hepatic metastases from colonic cancer. The metastatic lesions almost disappeared by combination therapy using hyperthermia and chemotherapy. (Fig.4) [3]

We performed hyperthermia treatment with 114 patients for one week, in Kyoto Prefectural University of Medicine, and Takeda Clinic. However, clinical effect of hyperthermia alone is not so strong that in clinical setting hyperthermia is combined with radiation and/or chemotherapy.

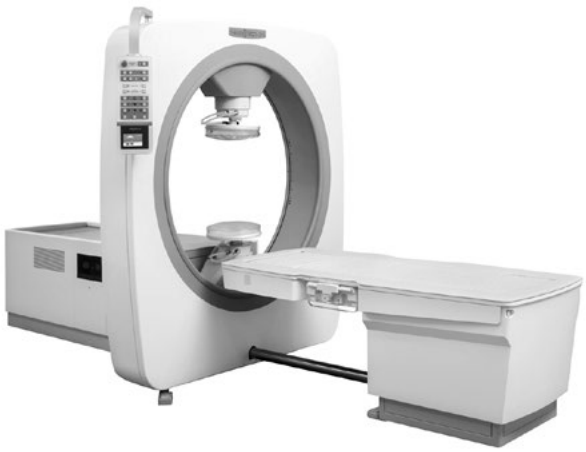

Fig.1 Radio-Frequency Dielectric Heater made by Yamamoto VINITA (Thermotron RF-8®)

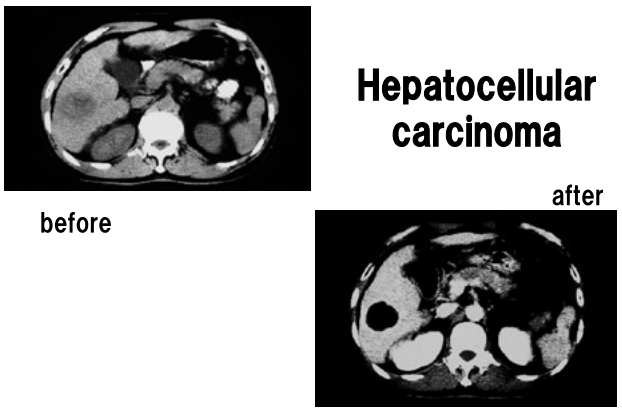

Fig.2 The case of Hepatocellular carcinoma

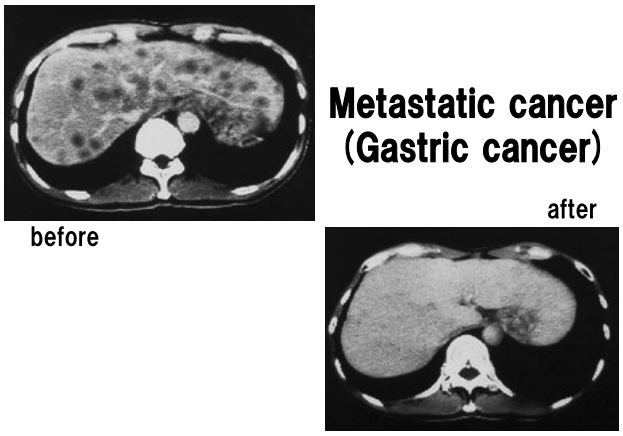

Fig.3 The case is multiple metastases from gastric cancer.

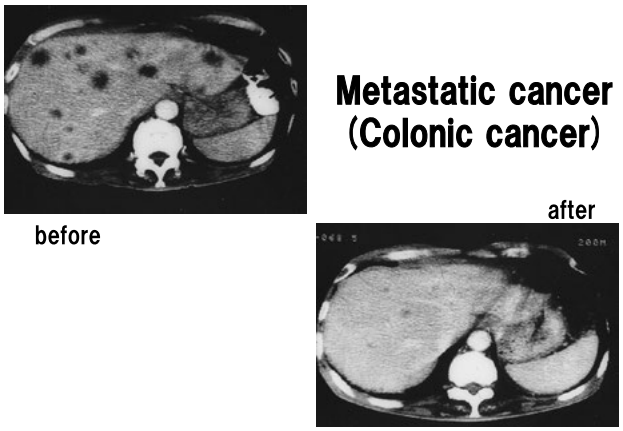

Fig.4 The case is hepatic metastases from colonic cancer.

III Hyperthermia combined with chemotherapy

The effects of hyperthermia for chemotherapy is shown. Anti-cancer agents such as Gemcitabine (GEM) or CPT-11 are known to activate the transcription factor NF-kB (Fig.5) [4]. It was reported that activation of NF-kB in cancer cells attenuates apoptosis induced by the chemotherapy [4]. I found that heat treatment inhibits NF-κB activation induced by anti-cancer agents and enhances chemotherapy-induced apoptosis of cancer cells [4].

Inhibition of NF-kB induced by hyperthermia is one of the mechanisms by which hyperthermia enhances effects of chemotherapy.

IV Clinical trial

Gemcitabine (GEM) was widely used as standard regimen of unresectable pancreatic cancer. However, the overall objective response rate remains low and additional improvement is clearly needed. We recently found that hyperthermia inhibited GEM-induced activation of NF-κB [5], resulting in the enhancement of the GEM cytotoxicity. The clinical efficacy of the combination of GEM and hyperthermia was estimated in untreated patients with unresectable pancreatic cancer [5]. The protocol of combination therapy is shown in Fig.6.

In this study, median survival times (MST) were 198 days for GEM group and 327 days for the combination therapy of GEM and Hyperthermia (GEM+HT) group (Fig.7) [5]. Patients treated with GEM and HT had significantly better survival than the GEM alone.

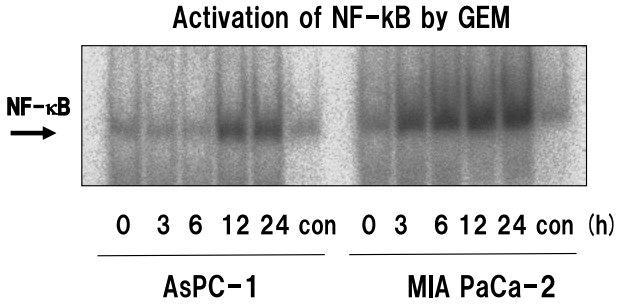

Fig.5 Activation of NF-kB by GEM at pancreatic cancer cell lines (AsPC-1 and MIA PaCa-2) Activation of NF-kB was investigated by Electrophoretic Mobility Shift Assay (EMSA) 0, 3, 6, 12, 24 h after GEM treatment, con. indicates control lane of EMSA

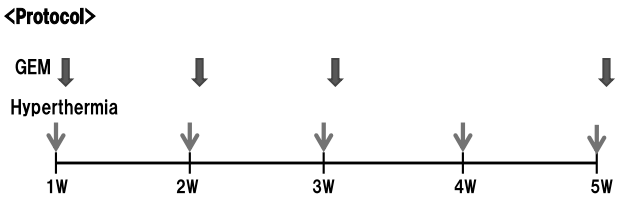

Fig.6 The protocol of combination therapy of GEM and hyperthermia in untreated patients with unresectable pancreatic cancer

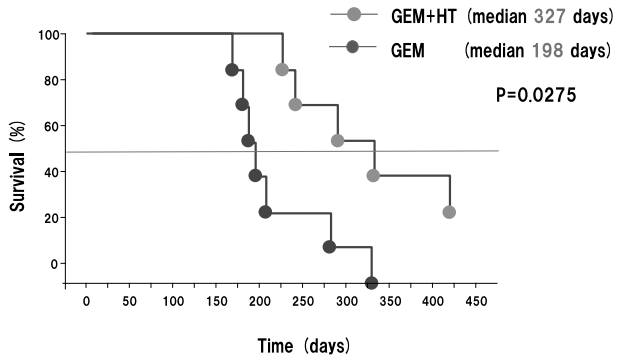

Fig.7 Kaplan-Meier graph for overall survival in patients with unresectable pancreatic cancer

Based on the promising result of the pilot study, a phase 2 clinical study was conducted. Stage IVa means T4 without distant metastasis, and stage IVb means patient has a distant metastasis. When the proportional-hazards analyze was conducted about age, sexuality, Performance Status (PS), and stage, the significant difference was acquired by only stage [6]. In the group of stage IVa patients, MST was 17.7 months. On the other hand, MST of stage IVb patients were 5.2 months [6]. This result about stage IVa is better than the treatment group of chemo-radiation therapy (CRT) with much report for MST 8 to 10 months [7, 8]. The merit of GEM combined with hyperthermia are at first, an enhanced effect of GEM, at the second, since not only a primary focus but most livers can be warmed, a liver metastasis nest is also made to a target, at the third, a heating -- for a wide reason, we can control a growth of micro-metastases, such as a lymph node and hepatic micro metastases.

V My question about a reason with GEM+ hyperthermia effective in Stage IVa

My hypothesis is that hyperthermia prevents the liver metastasis from the primary focus of a pancreatic carcinoma, and a peritoneum sowing. In the invasive transition process of a cancer cell, epitheliocytes carry out a shape variation at a mesenchyme system, and epitheliocytes lose the property and carry out a behavior like a mesenchyme system. This phenomenon is called epitheliums mesenchyme transition (Epithelial-Mesenchymal Transition, EMT) [9, 10]. In general, it is well known inflammation, oxidative stress, and hypoxia were EMT inducing factors [11–14]. My hypothesis is that hyperthermia can suppress EMT. Cancer cell expressed E-cadherin. When TGF-b or GEM stimulation was received, cancer cell change to mesenchyme cell, which disappeared E-cadherin, and expressed vimentin [15, 16]. If hyperthermia inhibits EMT, after stimulation of TGF-b or GEM, cancer cell is still epithelial cell, namely it has an E-cadherin but dose not have vimentin. In Fig.8 [17], red color shows E-cadherin. When TGF-b, which induces EMT, is added, E-cadherin disappeared. When the heat treatment was performed, as compared with the TGF-b group, the reduction of E-cadherin was inhibited [17]. How about vimentin, which is the marker of mesenthymal cell. In Fig.9 [17], red color shows vimentin. TGF-b and GEM stimulation induce the vimentin. However, heat treatment inhibits vimentin expression.

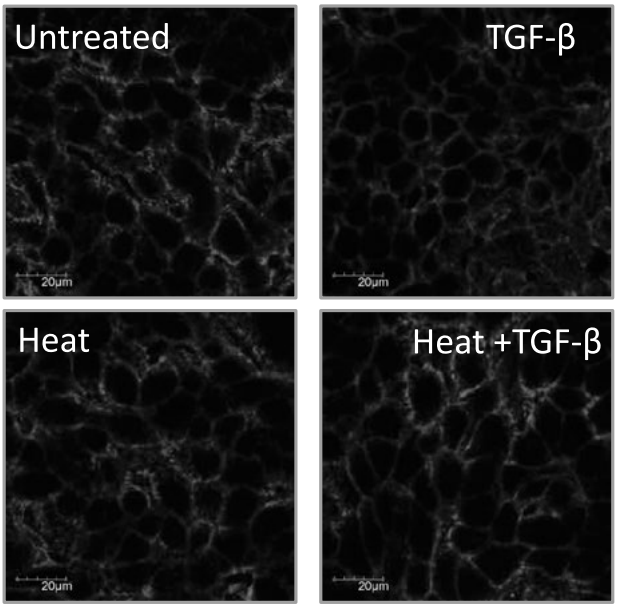

Fig.8 Effect of heat on TGFb- induced E-cadherin disappearance (MIAPaCa-2)

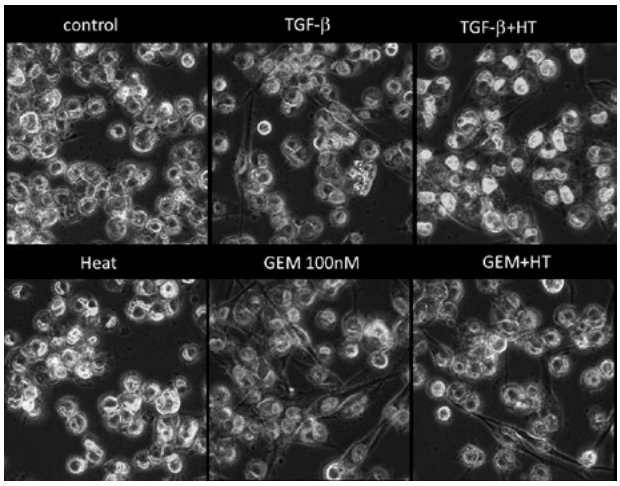

Fig.9 Effect of heat on TGFb- / GEM- induced vimentin expression (MIAPaCa-2)

From the result of basic experiments, it has been clarified that EMT is inhibited by hyperthermia [17].

In conclusion, hyperthermia combined with chemotherapy works on the reduction of target tumor and inhibition of metastasis via the suppression of EMT.

VI Effects of hyperthermia on immune response

The mechanisms by which cancer cells could escape from host immune system are explained below. In general, cancer cells express a low level of MHC class I and cancer antigens [18, 19]. But we and other groups [20–23] reported that hyperthermia can increase both MHC class I, cancer antigen expression, and other protein. Cancer cells also produce immune suppressive cytokines that exert systemic effects on immune cell function [24, 25]. Effect of hyperthermia on immune suppressive cytokines and regulatory T cell (Treg) is unknown. Therefore, we investigated the effect of hyperthermia on immune suppressive factors such as IL-10, TGF-β, and VEGF in the tumor tissue and Treg infiltration. Colon 26 cells were injected subcutaneously. When tumor size reached a mean of 5mm in diameter, mice were treated with hyperthermia, 43 degrees for 1 hour. Tumors were dissected 24 hours after hyperthermia, and RT-PCR, ELISA, and immunohistochemistry were assessed for the immune suppressive factors. The mRNA level and protein level of TGF-β1, IL-10 and VEGF in tumor were all significantly reduced by hyperthermia [26]. To visualize Treg in the tumor site, we performed immunohistochemical staining of Foxp3 expression. The number of Foxp3 positive lymphocytes (Treg) decreased by hyperthermia. Western blotting analysis revealed that expression of Foxp3 protein decreased when mice were treated with hyperthermia [26]. Thus, hyperthermia blocks the accumulation or induction of regulatory T cells into the tumor site.

As shown above, hyperthermia enhances anti-cancer immune activity by several mechanisms. Therefore, the mechanism of cancer cell killing of hyperthermia might have not only the cell death induced by heat but also an indirect action through the immune activation.

Moreover, hyperthermia could enhance immunotherapy in cancer treatment because hyperthermia has the potential to overcome the cancer-associated immunosuppression. Namely, hyperthermia suppresses Treg induction [26]. Hyperthermia inhibits the immune suppressive cytokines production in tumor tissues, and hyperthermia increases the expression of cancer antigen [26].

VII New immune cell therapy using naïve T cells.

In order to check a synergistic effect of hyperthermia and immune therapy, we examined the effect of hyperthermia combined with adoptive immunotherapy using naïve T cell in cancer-bearing mice. A protocol for in vivo experiment are indicated in Fig 10. Mice were injected subcutaneously

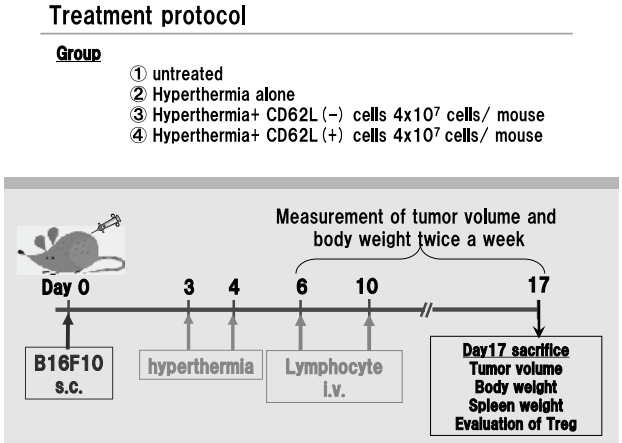

Fig.10 Exmerimental protocol

with B16 melanoma cells and treated with adoptive immunotherapy combined with hyperthermia. Mice were treated with hyperthermia on day 3 and day 4. On day 6 and day 10, mice were treated with intravenous adoptive transfer of naïve (CD62L+) or effector (CD62L-) CD8 T cells. At Day 17, mice were sacrificed and analyzed. Figure 11 [27] shows the change in the tumor volume of each group. The tumor volume significantly decreased in naïve T cell (CD62L+) transfer therapy combined with hyperthermia, compared to effector (CD62L-) T cell transfer therapy combined with hyperthermia or hyperthermia alone. According to this preclinical study, we performed phase I study of a naive T cell transfer therapy about 5 years ago [28], we have tried the combination therapy with naive T cell transfer therapy and hyperthermia in clinical.

One case of advanced gastric cancer, who rejected standard chemotherapy, received hyperthermia and naïve T cell transfer therapy. The patient was 62 years old man. He appealed abdominal discomfort, and went to the neighboring clinic. In that clinic, he received Gastric fiber (GF) and CT at 23th June in 2013. By GF, he had the advanced gastric cancer, which occupied from middle body to antrum, mainly anteriol to lessor curvature including gastric angle. CT found liver metastasis and ascites. From a cytological examination of the ascites, he was diagnosed peritonitis carcinomatosa. The final diagnosis was advanced gastric cancer with liver metastasis, and peritonitis carcinomatosa.

About the treatment, at first, he had agreed to receive "S-1+CDDP" for 1 cycle which was one of the standard chemotherapy in Japan [29]. He took S-1 for 1 week. However, adverse effects were very severe, he rejected to continue chemotherapy. So, we selected the hyperthermia +naïve T cell transfer therapy. He received naive T cell transfer therapy and hyperthermia from August 3rd, 2013 to the end of March, 2014, for 8 month. After this treatment, I rechecked advanced gastric cancer by GF at 23th April 2013, surprisingly I could not find the gastric cancer, I found normal gastric mucosa without any malignant lesion. CT also showed the disappearance of liver metastasis and ascites. Furthermore, there were no recurrences of gastric cancer, liver metastasis, or peritonitis carcinomatosa in June 2015. And his performance status was very good.

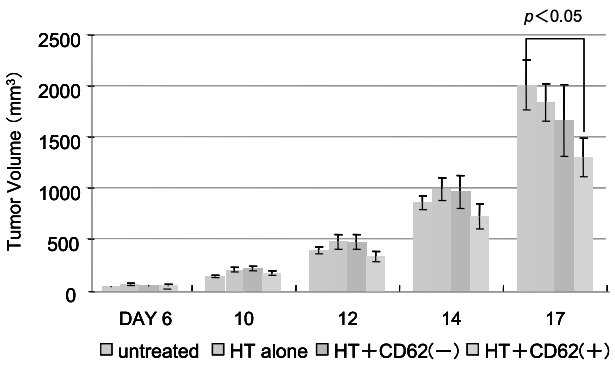

Fig.11 Anti-tumor effect of hyperthermia and adoptive transfer of Naïve T lymphocyte CD62L+ indicates naïve T cell, CD62L- indicats effector T cell

VIII Conclusion

Since the combination with hyperthermia and chemotherapy is useful, we should use them together as much as possible. Hyperthermia itself can inhibit EMT, resulting in the prevention of metastasis. Hyperthermia plus immunotherapy are also expectable because I sometimes experienced almost recover cases like the presented case of the advanced gastric cancer. Now I expect the combination therapy of hyperthermia plus immunotherapy extremely.

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