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Laser Immunotherapy: Novel Modality to Treat Cancer through Specific Antitumor Immune Response

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Abstract Treatment of metastatic cancer remains a great challenge and needs novel approaches. Combining a selective photothermal therapy with an active immunological stimulation, laser immunotherapy (LIT) was developed to induce systemic immune responses through local intervention. LIT consists of three major components: a near-infrared laser, a light-absorbing agent, and an immunological stimulant. Its effect relies on two major interactions: a selective photothermal interaction and an active immunological stimulation. The selective photothermal interaction can reduce the tumor burden and at the same time release the tumor antigens, which can induce specific antitumor immune response. The expression of heat shock protein and the application of immunoadjuvant further enhance the host immunity. It has been proved in pre-clinical studies that LIT could not only eradicate treated local tumors but also regress and eliminate untreated metastases at distant sites. Moreover, LIT is well tolerated and has shown to have many advantages for cancer treatment compared with other traditional modalities.

Key words medical optics; laser immunotherapy; cancer treatment; immunoadjuvant; cancer vaccine; glycated chitosan

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1 Introduction

Cancer is a major public health challenge in the world. Today, one in four deaths in the United States is due to cancer. During the past 10 years, the biggest progress in cancer treatment has been the development of monoclonal antibodies, which have emerged as effective therapeutic agents for an increasing number of human malignancies. Trastuzumab was approved for the treatment of breast cancer that overexpress Her2/neu by Food and Drug Administration (FDA) in 1998[1], and it has become a standard option for Her2-positive metastatic breast cancer patients. On April 30, 2010, FDA approved "Provenge", a cancer vaccine, for prostate cancer treatment[2]. It is just a start. Actually, various cancer vaccines are in clinical trials or under development[3]. It is believed that within 5 to 10 years cancer vaccines will become a major modality for cancer treatment.

Among all the cancer vaccines being developed, cell-based autologous cancer vaccines are the most promising one. But there are two major drawbacks for autologous cancer vaccines[4]: it is expensive to create a new, unique vaccine for each patient; cancer cells tend to mutate and cancer vaccine becomes less effective over time.

Laser immunotherapy (LIT) provides a convenient and efficient way to generate personalized tumor specific immunity based on the concept of *in situ* autologous whole-cells cancer vaccine. LIT, proposed in 1997, is a new approach for the cancer treatment[5]. Pre-clinical studies with using LIT showed that the combination of the selective photothermal and immunological interactions, both applied locally, could not only destroy the treated primary tumors but also eradicate untreated metastases at distant sites[6,7]. The clinical trials using LIT are under way and the preliminary clinical outcome is very promising.

2 Components of LIT

LIT contains three major components: a near-infrared laser (for non-invasive tumor irradiation), indocyanine green (ICG, a light-absorbing dye), and glycated chitosan (GC, a proprietary immunostimulant). It utilizes two major interactions: a se-

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lective local photothermal interaction, and an active systemic immunological stimulation[8,9].

Each component of the LIT plays a unique role on the induced antitumor immune response. ICG is a light-absorbing dye with an absorption peak at 800 ± 5 nm, which is injected into the center of the tumor before laser irradiation to enhance laser light absorption. A diode laser emitting 805-nm light is used. The laser energy is directed to the treatment site through an optical fiber. The output power density and the irradiation duration are modulated according to the local reaction. GC, as an immunoadjuvant, is injected into the center of the tumor and/or around the tumor to further stimulate immune response.

In LIT, the laser energy is high enough that the interaction between laser and light-absorbing dye is mainly photothermal effect. The interaction between laser and photosensitizer can induce photochemical reaction, which can induce antitumor immunity. The immune response can also be enhanced by some other immunostimulants. Korbelik *et al.*[10] reported the enhancement of the antitumor immune response to photodynamic therapy (PDT) by mycobacterium cell-wall extract (MCWE), which was a potent non-specific immunostimulant that elicited a local inflammatory response associated with antitumour activity. Uehara *et al.*[11] investigated the antitumor effect of PDT and/or local administration of a biological response modifier, the streptococcal preparation, on transplanted NR-S1 mouse squamous cell carcinoma. The results showed that PDT combination with local administration of OK-432 three hours before PDT induced the most favorable antitumor effect.

3 Selective Photothermal Effect of LIT

Thermal effect can be achieved by many different ablative techniques currently, including radiofrequency ablation (RFA)[12], laser induced thermotherapy[13,14], microwave ablation[15], and extracorporeal highintensity focused ultrasound (HIFU) ablation[16], which have been proved to be safe and effective in clinical applications.

The relationship between hyperthermia and immunotherapy indicates an exciting prospect of thermal therapy. Evidence shows that raising the body temperature can favor the induction of an immune response against tumors[17]. Since ICG can selectively absorb the energy of 805-nm laser, photothermal effect of LIT is induced through the interaction between 805-nm laser and ICG, which can induce a high temperature increase in the target tissue. Based on the previous study[18], the highest temperature measured in the laser irradiated area could reach 67 ℃, which is in the range of cytotoxic temperature (> 43 ℃). The temperature increase can cause ablation of tumor cells directly, and tumor cells can undergo coagulative necrosis. In addition, tumor cells swell and break into pieces allowing antigen release, which can induce tumor-specific immune response in the host. The temperature of the area around the laser-irradiated region can reach 41 ℃ to 43 ℃ (heat shock temperature). The temperature of the area further away from the laser-irradiation center can be 39 ℃ to 41 ℃, which is called fever-range temperature. Different tissue temperatures can induce different immune responses in the host.

4 Antitumor Immune Response Induced by LIT

Evidence shows that temperature increase can favor the induction of immune responses against tumors[19,20]. It is believed that the induced immune responses are contributed to both humoral and cellular arms[21,22]. As mentioned above, cytotoxic temperature can create an antigen source for induction of an antitumor immune response. This is the most important step for LIT. It creates a large antigen load for the generation of antitumor immunity. These antigens include tumor associated antigens, thermal induced heat shock proteins (HSPs), and a large amount of self-antigens. Antigen presenting cells (APCs), particularly dendritic cells (DCs), can capture these antigens and migrate to lymph nodes, where they present these antigens to T cells, thus activating cytotoxic T-lymphocytes (CTLs). This process induces a specific cell-mediated antitumor immune response that is effective against tumor cell antigens.

HSPs work as "endogenous danger signals" in the immune surveillance system. Extracellular HSPs released from damaged cells can stimulate professional antigen-presenting cells, followed by cytokine release and expression of cell surface molecules[23,24]. In addition to stimulating innate immunity, extracellular HSPs can promote the cross-presentation of HSP-bound peptide antigens to major histocompatibility complex (MHC) class I mole-

cules in DCs, leading to efficient induction of antigen-specific CTL[25]. Granulocyte macrophage colony-stimulating factor (GM-CSF), tumor necrosis factor alpha (TNF-α), and interferon-gamma (IFN-γ) are also increased being treated by heat[26]. These cytokines further favor the induction of a tumor specific immune response. The roles of HSPs in stimulating both innate immunity and adaptive immunity can explain at least in part the molecular mechanism by which thermal stress bolsters the host immune system[27,28]. HSPs play a very important role in LIT-induced antitumor immune response.

Thermal interaction at heat shock temperature and fever-range temperature does not kill the tumor cells directly, but it can modify both immune cells and the tumor cells to make the endogenous tumor-specific immune response more easily to be induced and sustained. Hyperthermia exerts its immunomodulative effects through modulating the activities of immune cells [such as APCs, T cells, and nature killer (NK) cells] and increasing the immunogenicity of tumor cells.

However, as the immune systems of cancer patients are often compromised, tumor debris may not be sufficient in inducing a potent antitumor response[29]. Therefore, additional immunologic intervention is required to invoke the immune system to achieve an effective and protective immune response against residual tumor cells.

Glycated chitosan (GC) works as a novel nontoxic immunological stimulant in LIT to further enhance the antitumor immune response[30]. When mouse macrophages are incubated with GC, TNF-α secretion is increased. Such increase in secretion is clearly dose dependent[31]. Our recent study has shown that GC alone can activate dendritic cells and enhance antigen presentation (data are not published).

Tumor escape mechanisms are obstacles in tumor immunotherapy[32]. It is the reason of the limited success in current immunotherapeutic strategies[33]. LIT, as an autologous vaccine-like approach using whole tumor cells as the source of antigens, has one major advantage: the vaccine represents the whole spectrum of unique and shared antigens expressed by the individual. The photothermal effect induced by LIT can decrease local tumor burden in favor of effector T cells. Repeated LIT treatment cycles also enable the host to overcome mutations of tumor antigen, which is a possible mechanism of tumor escape[34].

5 Novel Features of LIT

LIT utilizes an autologous whole-cell cancer vaccine-like approach to stimulate antitumor immune response. The novelty of LIT lies in the synergistic use of laser photothermal therapy and active immunological stimulation.

5.1 Unique Approach in Inducing Personalized Anti-tumor Immune Responses

In comparison with conventional immunotherapies and cancer vaccination approach, it is the entire components from the whole tumor cells exposed by LIT at the treatment sites that provide the targeted immunological stimulation in the host, without pre-selection and processing of tumor-specific antigens. In fact, this approach allows the host immune system to select the desirable specific tumor components, resulting in an *in situ* autologous whole-cells cancer vaccination. Because the antigen exposed by LIT is from the patients' own tumor cell, it is more effective to generate personalized specific antitumor immune response.

5.2 Non-Invasive Selective Photothermal Interaction

For treating melanoma and breast cancer, the laser irradiation is processed from the surface of the skin. The laser energy is delivered to the treatment site with an optical fiber. The fiber tip maintains a certain distance from the surface of the treatment site. Selective photothermal tumor destruction of LIT is achieved by using intratumoral administered light absorbing dye that strongly absorb the near-infrared light.

5.3 Low Toxicity

LIT is essentially a local, non-invasive treatment which only induces local discomforts. According to the results from our preliminary clinical trials, the most common adverse effects were blisters at the treatment sites. No grade 4 toxicity was observed. This will especially benefit patients who cannot receive surgery, or not responding to or cannot receive chemotherapy or radiation therapy.

5.4 Option for Repeated Treatments

Because of the low toxicity, the treatment of LIT can be repeated many times as long as viable tumor tissue can be found and targeted. In clinical

application, some of the patients received 6 cycles of LIT at most. Repeated treatments help overcome the immunosuppression, even when the cancer cells mutate during the course of the treatment. This is very important for cancer immunotherapy.

5.5 Easy Operation

Unlike other methods to make cancer vaccine, the process of LIT is simple. The local injection of light-absorbing agent and GC is a very straightforward procedure. The technique of laser irradiation is easy for doctors to master. The equipment is small enough to be stored in a briefcase and transported, which means it can be used in medical centers as well as in small clinics.

5.6 Low Cost

Unlike FDA approved monoclonal antibodies, the components of LIT, such as the light-absorbing agent and GC are relatively inexpensive. The infrared laser used in LIT costs significantly less than commonly used medical equipments in other therapies such as radiation, proton therapy, and gamma knife. In addition, LIT can be performed on an outpatient basis, significantly reducing the cost to patients and to hospitals.

6 Clinical Application of LIT

Currently, LIT has been applied to treat late-stage, metastatic melanoma patients; our preliminary data show that the efficacy of LIT is far better than that of currently available modalities[35,36]. Recently we started clinical trials for late-stage breast cancer patients, who have failed in other available modalities; our results showed that LIT was capable of reducing the size of treated primary breast tumors and untreated metastases in the lungs and livers[37]. It has been proved in a phase I clinical study that LIT is well tolerated. The most common adverse effects were rash and pruritus at the treatment sites. No grade 4 toxicity was observed. In clinical application, LIT can be repeated as needed because of the low toxicity of LIT.

LIT is also promising for early-stage cancer treatment followed by surgical removal. The combination of LIT with surgery will not decrease the efficacy of each modality. Moreover, it may have the following advantages: it makes the surgery operation easier. By coagulating the tissue, the haemorrhage during surgery operation will decreased; LIT-induced antitumor immune response will deplete the potential tumor lesions, which may not be detected and therefore not removed by surgery. This will help to reduce the risk of recurrence and metastasis after surgery. In early-stage cancer, the immune system of the patients is still intact, the tumor burden is relatively low, and the possibility of immune escape is small. These features of early-stage cancer patients allow LIT prime the immune response more efficiently.

To further improve the efficacy of LIT, we can combine LIT with many other immunotherapy modalities, such as DCs[38,39], low-dose cyclophosphamide[40], and high-dose GM-CSF[41,42]. The efficiency of LIT will eventually lead to its clinical applications for almost all solid tumors. Potential clinical indications of LIT include: cervical cancer and colon cancer treatment combining endoscope technique; primary tumors or metastasis in liver, lung and brain using interstitial irradiation technique.

7 Summary

As a newly invented technology, LIT still has many unknown fields to be investigated. However, the future of LIT is promising. Its treatment effect has been validated by cellular investigations, animal studies, and preliminary clinical trials. Specific antitumor immune response can be induced by LIT. With further understanding of its fundamental mechanism and continuous clinical trials, it is believed that LIT will become a standard method for cancer treatment and benefit more and more cancer patients, particularly those who have metastatic tumors and have failed in conventional therapies.

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