RESEARCH ARTICLE

Effects of Thermotherapy on Th1/Th2 Cells in Esophageal Cancer Patients Treated with Radiotherapy

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Abstract

Background: To investigate the effects of double radiofrequency hyperthermia on Th1/Th2 cells in esophageal cancer patients treated with radiotherapy. Materials and Methods: 22 patients with esophageal cancer were divided into a radiotherapy group (10 cases) and a combined group (double radiofrequency hyperthermia combined with radiotherapy group, 12 cases). Both groups received conventional radiotherapy using a cobalt-60 therapy apparatus (TD60-66Gy/30-33F). Patients in the combined group also underwent double radiofrequency hyperthermia (2F/W, 8-10F). Before and after treatment, Th1, Th2, Tc1 and Tc2 cells in peripheral blood were determined with flow cytometry. Results: In the radiotherapy group, Th1 cell contents before and after radiotherapy were 17.5±5.26% and 9.69±4.86%, respectively, with a significant difference ($p<0.01$). The Th1/Th2 ratio was significantly decreased from 28.2±14.3 to 16.5±10.4 ($p<0.01$). In the combined group, Th1 cell content before radiotherapy was 15.9±8.18%, and it increased to 18.6±8.84 after radiotherapy ($p>0.05$), the Th1/Th2 ratio decreasing from 38.4±36.3 to 28.1±24.0 ($p>0.05$). Changes in Th2, Tc1 and Tc2 cell levels were not significant in the two groups before and after therapy ($p>0.05$). Conclusions: Double radiofrequency hyperthermia can promote the conversion from Th2 to Th1 cells, and regulate the balance of Th1/Th2 cells.

Keywords: Esophageal cancer - double radiofrequency hyperthermia - radiotherapy - Th1 - Th2

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Introduction

Esophageal cancer has a high incidence in China (Almhanna et al., 2013; Song et al., 2013), where most patients are diagnosed at middle or advanced stage (Zhao et al., 2007) and not suitable for surgery. Non-surgical treatment of esophageal cancer often relies on radiotherapy and multi-disciplinary comprehensive treatment. For a long time, radiotherapy employs conventional segmentation techniques, with a five-year survival rate of 10%. Local failure and distant metastasis are the main causes of radiotherapy failure.

T cells play a major role in tumor immunity, especially in patients with esophageal cancer. After radiotherapy, the body presents a long-term T cell dysfunction (Rotstein al., 1985). Persistent suppressed immune function can change host antitumor immune responses and cause local recurrence and distant metastases. Therefore, enhancing the immune function has certain clinical significance in improving the survival rate and life quality of patients. Thermotherapy has been discovered to ameliorate radiotherapy-induced T cell subset abnormalities, but its effects on Th1/Th2 cells and Tc1/Tc2 cells in patients underwent radiotherapy have not been reported yet. The objective of this study is to investigate the effects of double radiofrequency hyperthermia on Th1/Th2 cells and Tc1/Tc2 cells in esophageal cancer patients treated with radiotherapy.

Materials and Methods

Patients

22 patients with esophageal cancer treated in Nanjing Chest Hospital between December 2005 and April 2007 were divided into radiotherapy group and combined group (radiotherapy plus thermotherapy). There were 10 cases in radiotherapy group, including 8 males and 2 females, with age of 51-75 years (median 61.81 years). For pathological staging, there were 7 cases of phase III and 3 cases of phase IV. Combined group contained 12 cases, including 10 males and 2 females, with age of 50-73 years (median 61.92 years). There were 1 case of phase IIa, 7 cases of phase III and 4 cases of phase IV. All patients were diagnosed with pathological type of squamous cell carcinoma. This study was approved by the ethics committee of Nanjing Chest Hospital and written informed consent was obtained from each of the patients or their families.

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Treatment

Radiotherapy group received external radiation treatment in a total dose of TD64-66Gy with GWGP-80 cobalt-60 teletherapy machine. 2 ml of peripheral venous blood was collected before and after treatment, for detection of Th1/Th2 and Tc1/Tc2 cells. Combined group received external radiation treatment in a total dose of TD60-66Gy with GWGP-80 cobalt-60 teletherapy machine. 1 week later the double radiofrequency hyperthermia was performed, in which NLR002 heating machine was used twice per week for 3-4 weeks, and the anal temperature was 39.5-42.5°C.

Sample preparation and detection

Venous blood with lithium heparin as anticoagulant was added into RPMI-1640 medium supplemented with phorbol myristate acetate, ionomycin and monensin, followed by incubation in CO_2 for 4 h. Then the blood was transferred into two labeled tubes, which were added with CD3-PE-Cy5 and CD8-APC, respectively. After incubation, fixation medium was added and incubated. Normal saline was added, followed by centrifugation, and then the supernatant was removed. Permeabilization medium was added. Tube 1 was added with rat IgG-FITC and rat IgG-PE, and tube 2 was added with IFN-γ-FITC and IL-4-PE, followed by incubation. Normal saline was added, followed by centrifugation, and then the supernatant was removed. The prepared sample was mixed with normal saline, followed by detection with flow cytomety. CellQuest software was use to analyze the results.

Statistical analysis

Data was presented as mean±SD. Statistical analysis was performed with SPSS10.0 software. t-test and Chi-squared test were used for analysis of measurement and enumeration data, respectively. Comparison between groups used t-test after homogeneity test of variance.

Results

Flow cytometry results in radiotherapy group

Before and after radiotherapy, Th1 cell content in radiotherapy group was 17.54±5.26)% and (9.69±4.86)%, respectively, with significant difference between them (p<0.01). Th1/Th2 ratio was significantly decreased from 28.22±14.25 to 16.52±10.42 (p<0.01). After radiotherapy, Th2, Tc1 and Tc2 cell levels and Tc1/Tc2 ratio did not change markedly, compared with before radiotherapy (p>0.05) (Table 1, Figure 1).

Flow cytometry results in combined group

Before radiotherapy, Th1 cell content in combined group was (15.94±8.18)%, which was increased to

Table 1. Real-time qPCR Analysis ($2^{-\Delta\Delta Ct}$) of PTTG Gene in SCL-1 Cells

Group	PTTG mean Ct	β-actin mean Ct	ΔCt	ΔΔCt	$2^{-\Delta\Delta Ct}$
untreated	23.7	16.9	6.9	0.0	1.0
control siRNA	24.0	17.1	6.8	0.0	1.0
PTTG siRNA	31.1	17.2	13.9	7.0	0.0

Table 2. Real-Time qPCR Analysis ($2^{-\Delta\Delta Ct}$) of MMP-2 Gene in SCL-1 Cells

Group	MMP-2 mean Ct	β-actin mean Ct	ΔCt	ΔΔCt	$2^{-\Delta\Delta Ct}$
untreated	25.2	16.9	8.3	0.0	1.0
control siRNA	25.2	16.8	8.4	0.1	0.9
PTTG siRNA	27.4	17.0	10.4	2.1	0.2

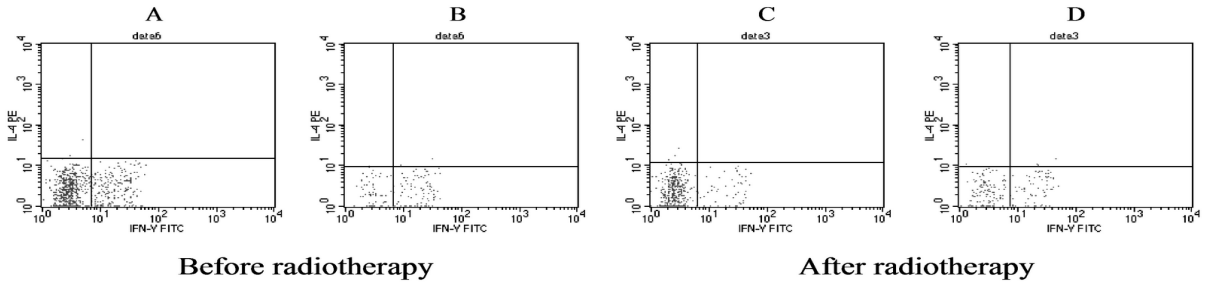

Figure 1. Flow Cytometry Results in Radiotherapy Group Before and After Treatment. A and B: high level of Th1 cells in 2 patients before radiotherapy; C and D: declined level of Th1 cells in the first patient after radiotherapy, without obvious change in the second patient

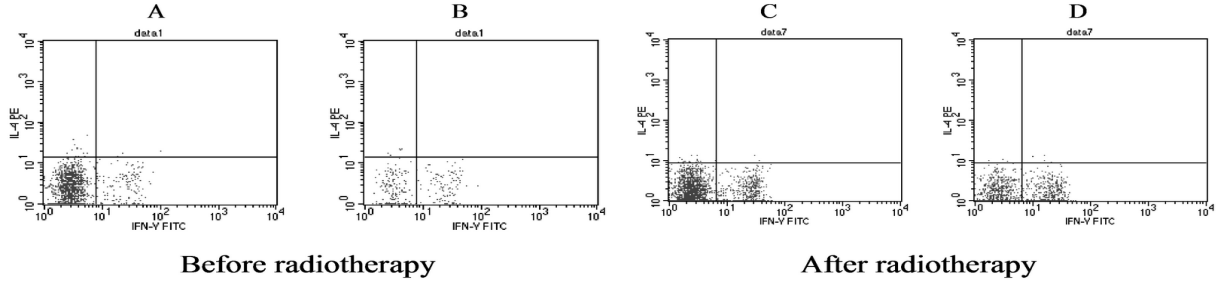

Figure 2. Flow Cytometry Results in Combined Group Before and After Treatment. A and B: high level of Th1 cells in 2 patients before radiotherapy; C and D: increased level of Th1 cells in 2 patients after radiotherapy

Table 3. Real-Time qPCR Analysis ($2^{-\Delta\Delta Ct}$) of MMP-9 Gene in SCL-1 Cells

Group	MMP-9 mean Ct	β-actin mean Ct	ΔCt	ΔΔCt	$2^{-\Delta\Delta Ct}$
untreated	24.7	16.9	7.8	0.0	1.0
control siRNA	25.1	17.2	7.9	0.1	1.0
PTTG siRNA	28.3	18.2	10.1	2.2	0.2

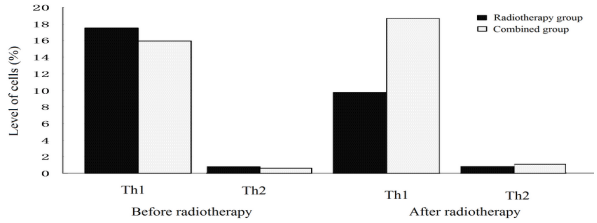

Figure 3. Comparison of Th1 and Th2 Cell Level between Two Groups Before and After Radiotherapy

(18.64±8.84) after radiotherapy. Th1/Th2 ratio decreased from 38.39±36.34 to 28.10±24.03, but the differences between before and after radiotherapy were not significant ($p>0.05$). After radiotherapy, Th2, Tc1 and Tc2 cell levels and Tc1/Tc2 ratio did not change distinctly, compared with before radiotherapy ($p>0.05$) (Table 2, Figure 2).

Comparison between combined group and radiotherapy group

After radiotherapy, Th1 cells significantly decreased in radiotherapy group ($p<0.01$), but slightly increased in combined group ($p>0.05$), with significant difference between two groups ($p<0.05$). This indicated that, thermotherapy could increase the Th1 cell level in esophageal cancer patients under radiotherapy. There was no significant difference of Th2 cell level between two groups before or after radiotherapy ($p>0.05$) (Figure 3).

Discussion

It is found that thermotherapy can enhance the efficacy of radiotherapy through its complementary effect on radiotherapy (Cui et al., 2012), direct effect on tumor cells (Wang et al., 2012; Zhou et al., 2012) and regulation of immune function. Thermotherapy targets cell membrane and cytoskeleton, while radiotherapy targets DNA, so the combined treatment can exert synergistic effect from different mechanisms (Saga et al., 2002; Bottaro and Liotta, 2003; Griffin et al., 2010; Dings et al., 2011). Heating can ameliorate the hypoxia status of tumor cells. The radiation and heating act with cells in different phases. S phase cells are sensitive to heat but not to radiation. Thermotherapy can cause the redistribution of tumor cell cycle (Zolzer and Streffer, 2001; Yuguchi et al., 2002). Hyperthermia also inhibits the repair of sublethal damage and potentially lethal damage induced by radiation. Thermotherapy turns tumors into huge heat storage due to their vascular disorders and slow blood flow, with temperature increase of 5-10°C. When the temperature reaches 42.5°C, the tumor cells and tissue are directly destroyed. Thermotherapy can initiate T cell immunity and enhance antitumor immune function in patients. It leads to elevated synthesis of HSP, which induces immune cell activation and promotes Th cells differentiation into Th1 cells (Wang et al., 2001). After thermotherapy, tumor cells degrade and the necrotic decomposition products are absorbed by human body as an antigen to stimulate immune system to produce antitumor immunity.

Radiotherapy is important as main measure in non-surgical treatment of esophageal cancer. Local failure and distant metastasis are the primary causes of conventional radiotherapy failure. Non-surgical treatment attempts to reduce tumor recurrence. Tumor recurrence and metastasis are not only associated with tumor biological behavior, but also with the immune status of human body. Radiotherapy weakens immune function in patients even 4 months after treatment. The immune function is still lower than before after 8 months from radiotherapy (Rotstein et al., 1985). The long-term weakened T cell immune function after radiotherapy can change the host antitumor immune responses, and may be a comprehensive factor for local recurrence and distant metastases. Research has shown that, prognosis of patients with esophageal cancer underwent radiotherapy is closely related to cellular immune function after radiotherapy (Morita et al., 2001). Previous detection of peripheral blood T-lymphocyte subsets in patients with esophageal cancer before and after radiotherapy revealed that, CD8+ cells and CD4+/CD8+ cell ratio decreased obviously in radiotherapy group, while thermotherapy improved radiotherapy-induced T cell subset abnormalities. So the immune function of patients was enhanced, with improved antitumor ability of host.

Further research has shown that, activated CD4+ T cells secret TNF and IFN-γ, which increase MHC class I molecule expression in tumor cell and sensitivity of tumor cell to CTL killing (Kobayashi et al., 2013). IFN-γ directly inhibits tumor cell proliferation and tumor angiogenesis, as well as works on tumor cell immune escape mechanisms to suppress tumor cell proliferation. Braumüller et al. (2013) have demonstrated that, TNF and IFN-γ can induce tumor cell senescence. In addition, Tsukui et al. (1996) and Magdalena et al. (2004) have discovered down-regulated cytokines secreted by Th1 cells in many tumors and Th1 to Th2 shift. It seems that Th1 cells are essential in tumor immunity (Mosmann et al., 1986; Abbas et al., 1996; Westerterp et al., 2008). Once Th1/Th2 cell ratio unbalances and shifts to Th2 cells, the consequent immunosuppressive state will seriously interfere the anti-tumor immunity. Therefore, reduction of Th1 cells is considered as one manifestation of low immunity in patients with tumor, while radiotherapy for esophageal cancer results in decreased Th1 cell ratio in patients (Wang and Chen, 2009).

The effects of radiotherapy plus thermotherapy on Th1/Th2 cells in patients with tumor are seldom reported before. Moreover, the effects of radiotherapy on Th1/Th2 cells in patients with esophageal cancer have not been reported. Therefore, we combined thermotherapy with radiotherapy to treat patients with esophageal cancer and monitored their Th1/Th2 cells before and after treatment, to explore its effects on Th1/Th2 cells. Ahlers et al. (2005) detected T cell subsets and related cytokines, and discovered that Th1 cells increased 24h

after thermotherapy. Our research also showed that, after treatment, the peripheral blood Th1 cells significantly reduced in radiotherapy group, but not in combined group. Th1 cells in radiotherapy group statistically differed from those of combined group after treatment, indicating that thermotherapy could abate the reduction of Th1 cells in cancer patients induced by radiotherapy. This, on the other hand, confirmed that thermotherapy could enhance the cellular immune function in esophageal cancer patients with radiotherapy. The change of Tc1 and Tc2 cells was not significant in radiotherapy or combined group before and after treatment. Whether the radiotherapy and thermotherapy have no effect on Tc1 and Tc2 cells still needs further investigation.

Antigen presenting cells activate T cells, stimulate immune effect and cause a chain reaction of immunologic effector cells, through a series of activities (9). Activated T cells markedly increased after radiotherapy in both groups, especially in combined group. However, whether this suggests a more active state of T cell subsets in patients still requires further study. In conclusion, double radiofrequency hyperthermia can improve the effects of radiotherapy on T cell subsets in esophageal cancer patients and promote the shift from Th2 to Th1.

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