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ORIGINAL ARTICLE

Radiofrequency Ablation Does Not Induce the Significant Increase of $CD4^+CD25^+Foxp3^+$ Regulatory T Cells Compared with Surgical Resection in Hepal-6 Tumor Model

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Abstract Surgical resection (SR) and radiofrequency ablation (RFA) are all currently recognized as important and effective treatment in solid tumors. This study aimed to investigate change in level of $CD4^+CD25^+Foxp3^+$ regulatory T (Treg) cells in tumor-bearing mice after SR vs. RFA and the relationship of this level with tumor progression. Hepa1-6 tumor cells were inoculated subcutaneously into C57BL/6J mice. The population of Treg cells was measured by flow cytometry at selected post-SR or post-RFA times. Tumor growth was measured by rechallenge in the contralateral flank. The tumor volume was calculated and compared with that of a control group. The correlation between the population of Treg cells and tumor volume was analyzed. A significant increase in Treg cells was observed after SR compared with the preoperative level, while the level after RFA was relatively stable. A significant difference in tumor growth between the SR and RFA groups was observed in the initial postoperative phase but not in the later phase. A correlation was found between tumor volume and level of Treg cells. Our study revealed that RFA stabilizes the level of Treg during postoperative recovery, whereas SR activates the immunosuppressive reaction by upregulating the level of such cells, promoting tumor growth.

Keywords Radiofrequency ablation · Surgical resection · Tumor progression · $CD4^+CD25^+Foxp3^+$ regulatory T cells · Animal model

Introduction

Radiofrequency ablation (RFA) has been an important treatment for tumors, especially as curative therapy for hepatocellular carcinoma with a tumor diameter of $\leq$2 cm (Chen et al. 2006; Livraghi et al. 2008). Furthermore, RFA has been used successfully in the management of other malignancies, including renal cancer, lung cancer, and breast cancer (Crocetti and Lencioni 2010; Kinoshita et al. 2011; Tracy et al. 2010). These clinical studies raise the question of how RFA successfully destroys a tumor. Does it induce coagulation necrosis while also prompting a specific internal biological response?

Surgical resection (SR) is a traditional primary curative option available for patients with hepatocellular carcinoma. After resection, recurrence in the liver is often observed (Bruix and Sherman 2005), indicating microscopic residual tumor. Some studies have suggested that residual tumor growth is accelerated after resection (de Jong et al. 1996; Picardo et al. 1998; Stolar and Anderson 1980); however, the exact mechanism of this enhanced tumor growth remains unclear.

The interrelationship between tumors and the immune system is complex and confusing. Current information suggests that the immune system not only can protect the host against tumor development but also can select for tumors with lower immunogenicity and finally make them

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progression (Dunn et al. 2004). Tumor-specific immune killer cells can eliminate tumor cells, but the immune system also contains immune cells and cytokines that activate the progression of cancer. $CD4^+CD25^+Foxp3^+$ regulatory T (Treg) cells negatively regulate tumor immunity and contribute to tumor growth. Moreover, increased populations of Treg cells in tumor patients have been associated with negative prognosis and poor survival (Fu et al. 2007). In this study, we hypothesized that local RFA can reduce the population of $CD4^+CD25^+Foxp3^+$ Treg cells and induce the antitumor response, while SR impairs antitumor activity and upregulates Treg cells, ultimately contributing to tumor growth. Thus, the purpose of this study was twofold: (1) to compare the postoperative change in $CD4^+CD25^+Foxp3^+$ Treg cells after RFA vs. SR, and (2) to clarify the relationship between tumor growth and population of $CD4^+CD25^+Foxp3^+$ Treg cells.

Materials and Methods

Animal Tumor Model

C57BL/6J male mice, aged 6–8 weeks, were obtained from the medical experimental animal center of Sun Yat-Sen University. These animals were maintained and fed under specific pathogen-free conditions. All animals received care according to the criteria outlined in the guides approved by the China Association of Laboratory Animal Care and the Institutional Animal Care Committee.

The Hepa1-6 tumor model in C57BL/6J mice (Grimm et al. 2000) was chosen because these cells show reliable growth in the syngeneic host. Hepa1-6 cells are derived from the BW7756 mouse hepatoma that arose in a C57L mouse. Major histocompatibility complex class I and II expression is identical between C57L and C57BL/6J mice. The Hepa1-6 cell line was provided by Dr. Shunli Shen (Surgery Branch, First Affiliated Hospital of Sun Yat-Sen University). The cell line was maintained in RPMI-1640 cell culture media supplemented with 10 % heat-inactivated fetal bovine serum (Sijiqing, Hangzhou, China) and in a humidified atmosphere of 5 % CO_2 at 37 °C.

Tumor inoculation was performed subcutaneously with 4×10^6 Hepa1-6 cells in 100 mL of serum-free Dulbecco's Modified Eagle's Medium into the right flank of mice. Mice were monitored daily, and tumor volumes were measured once every 2 days with a digital caliper using the sphere formula: $(\pi \times L \times W \times [L + W]/6)$, where L and W are tumor length and width, respectively. When tumors reached an approximate volume of 500 mm^3 (largest diameter: 10.01 mm; smallest diameter: 7.36 mm), the mice were randomly assigned to an RFA group ($n = 27$), an SR group ($n = 26$), or a control group ($n = 5$).

RFA and SR

Animals were anesthetized using 4.3 % chloral hydrate and shaved properly at the tumor area and on the contralateral flank. After placement and proper attachment of the mouse pars dorsalis onto a plate electrode, an incision was made at the tumor area. An RFA 21-G needle with an active tip of 10 mm was inserted into the tumor under direct visualization. After placement of the needle, an RF lesion generator system (RFS-1500; MEDSPHERE, Shanghai, China) provided power of 5 W for 80 s under temperature control. This procedure protocol is determined by the outcome of preliminary experiment, which can lead to pathologically complete ablation. After 2–3 treatments, the skin was sutured and feeding was continued under the specific pathogen-free conditions.

The SR and control groups underwent the same anesthetization and incision processes to maintain the same conditions. All tumors in the SR group were completely excised, all tumors in the RFA group were completely ablated, and all tumors in the control group were allowed to continue growing. This was confirmed pathologically after sectioning.

Study Design Protocol

To compare the RFA and SR groups in terms of the postoperative population of $CD4^+CD25^+Foxp3^+$ Treg cells, the mice were killed at 1 day ($n = 5$), 4 days ($n = 5$), 7 days ($n = 5$), or 14 days ($n = 5$) after RFA or SR. The designated time points were determined by the outcome of a preliminary experiment. The rest of the mice in the RFA ($n = 7$) and SR ($n = 6$) groups and those in the control group ($n = 5$) were rechallenged subcutaneously with 1×10^7 cells at 1 day after treatment. They were monitored daily, and tumor volumes were measured once every 2 days. Nineteen days after tumor implantation, the animals were killed. The spleens of all killed mice were sampled, and then lymphocytes were separated with hydroxypropylmethyl cellulose (EZ-Sep™ Mouse 1×, Dakewe, Shenzhen, China).

Flow Cytometric Analysis

Cell suspensions of spleen lymphocytes were prepared according to standard protocols and then stained using the following antibodies: APC anti-mouse CD4, PE anti-mouse CD25, Alexa Fluor 488 anti-mouse/rat/human Foxp3, and appropriate isotype controls. All these antibodies were purchased from Biolegend Pharmingen (San Diego, CA, USA). Intranuclear staining of Foxp3 was performed according to the product application.

Statistical Analysis

Data for population of $CD4^+CD25^+Foxp3^+$ Treg cells were analyzed using the Student's *t* test, assuming unequal variance. Tumor volume measurement data from the tumor rechallenge study were analyzed using non-parametric methods because the data were not normally distributed. Group distributions were compared using the Kruskal–Wallis test; the control group was compared with each of the other two groups. Correlation between the population of $CD4^+CD25^+Foxp3^+$ Treg cells and tumor volume was analyzed using the Spearman test. Data were considered statistically significant if $p < 0.05$.

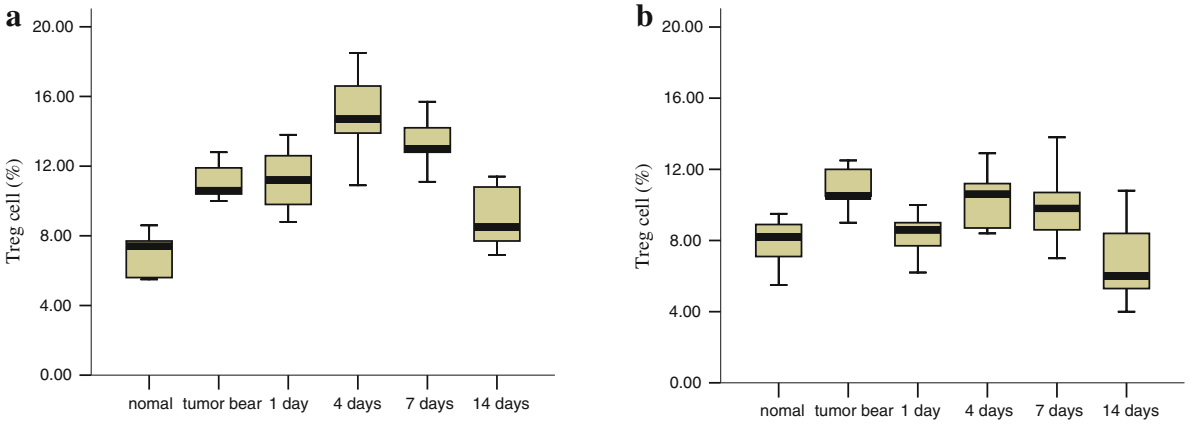
Results

Significant Postoperative Increase in $CD4^+CD25^+Foxp3^+$ Treg Cells after SR Compared with RFA

The level of $CD4^+CD25^+Foxp3^+$ Treg cells increased in the SR group during the initial postoperative period, especially at 4 and 7 days (14.92 ± 2.87 and 13.16 ± 2.06 %, respectively), compared with the preoperative level (11.14 ± 1.17 %); the differences were statistically significant ($p = 0.005$ and 0.031, respectively). In the longer post-treatment period, however, the population gradually returned to the level of normal mice (the level at 14 days was 9.06 ± 1.96 %; $p = 0.098$; Fig. 1a).

The RFA group, however, maintained a relatively stable level of $CD4^+CD25^+Foxp3^+$ Treg cells (the levels at 1, 4, and 7 days were 8.30 ± 1.44, 10.36 ± 1.86, and 9.98 ± 2.55 %, respectively; $p = 0.05$, 0.672, and 0.472, respectively). This change was different from the trend of the SR group during the whole postoperative recovery period. Moreover, at 14 days, the level was also equal to the level of normal mice (6.90 ± 2.70 vs. 7.84 ± 1.58 %, $p = 0.462$; Fig. 1b).

In comparison of the SR and RFA groups, no significant differences were observed in population of Treg cells in normal mice, tumor-bearing mice, at 7 days, and at 14 days ($p > 0.05$), but at 1 day and 4 days the population was significantly increased in the SR group ($p = 0.029$ and 0.017, respectively; Figs. 1, 2).



	Normal	Tumor-bearing	1 day	4 days	7 days	14 days
RFA group	7.84 ± 1.58	10.90 ± 1.39	8.30 ± 1.44	10.36 ± 1.86	9.98 ± 2.55	6.90 ± 2.70
SR group	6.96 ± 1.36	11.74 ± 2.38	11.24 ± 2.03	14.92 ± 2.87	13.16 ± 2.06	9.06 ± 1.96
p	0.374	0.541	0.029	0.017	0.062	0.186

RFA: radiofrequency ablation; SR: surgical resection.

Note: Data are given as mean ± SD percentage of Treg cells.

Fig. 1 Difference in postoperative trend of $CD4^+CD25^+Foxp3^+$ Treg cells between RFA and SR groups. **a** Trend in population of $CD4^+CD25^+Foxp3^+$ Treg cells after surgical resection: increase at 1, 4, and 7 days with a peak at 4 days and return to baseline at 14 days. **b** Trend in population of $CD4^+CD25^+Foxp3^+$ Treg cells after radiofrequency ablation: stabilization and potential decrease at 14 days

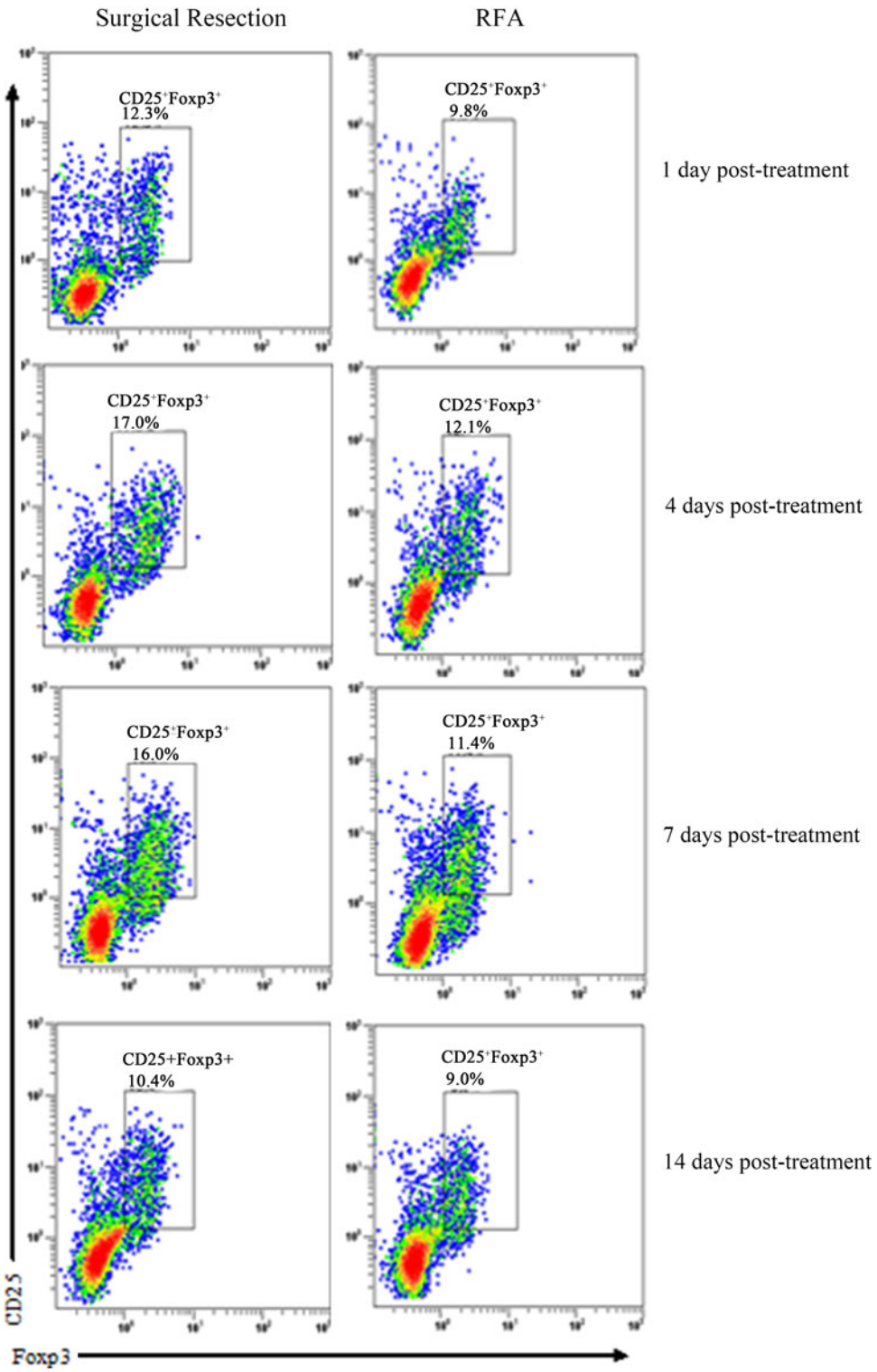


Fig. 2 Representative density plots for the level of $CD4^+CD25^+Foxp3^+$ Treg cells in the two groups (gated on $CD4^+$ T cell)

Different Growth Trend of Contralateral Tumor Rechallenge Following Complete Eradication of Primary Tumors with RFA and SR

After complete eradication of the tumor lesion with SR and RFA, tumor rechallenge was performed in the contralateral flank. A significant difference in tumor growth was observed between the SR and RFA groups in the initial postoperative phase ($p < 0.05$ at 3, 5, 7, 9, and 11 days) but not at the later phase ($p > 0.05$ at 13, 15, 17, and 19 days; Fig. 3).

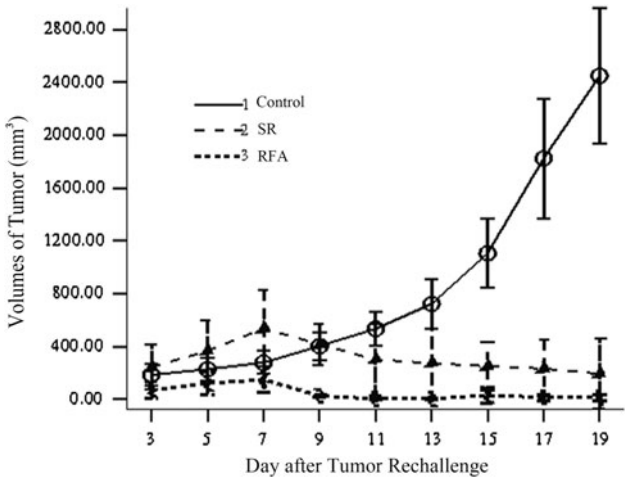


Fig. 3 Tumor growth in the three groups in contralateral rechallenge. A significant difference was observed between the radiofrequency ablation (*RFA*) and surgical resection (*SR*) groups at 3, 5, 7, 9, and 11 days using the Kruskal–Wallis test ($p < 0.05$). The control group showed a gradual increase over time, whereas the RFA and SR groups showed a general decrease

Table 1 Correlation between tumor volume and level of $CD4^+CD25^+$ $Foxp3^+$ Treg cells in rechallenged mice

Mouse	Tumor volume (mm^3)	Treg cell (%)	*r*	*p*
SR group (mean ± SD)	191.89 ± 103.82	8.00	0.812	0.050
Mouse 1	690.80	9.90		
Mouse 2	62.80	6.40		
Mouse 3	12.56	6.80		
Mouse 4	175.84	8.50		
Mouse 5	175.84	8.80		
Mouse 6	33.49	7.60		
RFA group (mean ± SD)	11.61 ± 8.69	6.69	0.180	0.699
Mouse 1	62.80	7.10		
Mouse 2	4.19	6.40		
Mouse 3	12.56	6.80		
Mouse 4	1.41	7.90		
Mouse 5	0.38	5.40		
Mouse 6	0.00	7.60		
Mouse 7	0.00	5.60		
Both groups (mean ± SD)	94.82 ± 87.93	7.29	0.621	0.023

SR surgical resection, *RFA* radiofrequency ablation

Relationship Between Tumor Volume and Level of $CD4^+CD25^+Foxp3^+$ Treg Cells with Contralateral Tumor Challenge

At 19 days, we killed all the rechallenged mice and investigated the population of $CD4^+CD25^+Foxp3^+$ Treg cells in their spleens. We discovered a correlation between the tumor volume in the contralateral flank and the level of $CD4^+CD25^+Foxp3^+$ Treg cells ($r = 0.621$, $p = 0.023$): the larger the tumor volume, the higher the level of $CD4^+CD25^+Foxp3^+$ Treg cells. Moreover, we found that this correlation was statistically significant in the SR group ($r = 0.812$, $p = 0.050$) but not in the RFA group ($r = 0.180$, $p = 0.699$; Table 1; Fig. 4).

Discussion

RFA is a minimally invasive therapy used for local tumor destruction that generates large amounts of tumor debris (Garcea et al. 2003), while SR is one of the most important therapies for most solid tumors. Using a newly developed tumor animal model, we investigated the difference between RFA and SR in terms of the impact on $CD4^+CD25^+Foxp3^+$ Treg cells. The results show that RFA stabilizes the level of $CD4^+CD25^+Foxp3^+$ Treg cells, whereas SR can increase the population of $CD4^+CD25^+Foxp3^+$ Treg cells, resulting in potential tumor growth and progression in the initial postoperative recovery phase.

Preliminary research revealed that SR resulted in an increase in growth factor expression compared with RFA, especially hepatocyte growth factor and basic fibroblast growth factor (Meredith et al. 2006). However, the rapid tumor progression after SR and the tumor diminution after RFA seen in some clinical cases cannot be fully explained (Sanchez-Ortiz et al. 2003). Many studies have suggested that RFA can act as a strong "adjuvant" for antitumor responses (Ali et al. 2005; Den Brok et al. 2004; Wissniowski et al. 2003) and induce antigen-presenting cell infiltration and amplification of weak tumor-induced immunity (Dromi et al. 2009). RFA can induce a tumor-specific proliferative T-cell response and even transplantable protective immunity, but this occasionally stimulated immune response may not be sufficient to prevent tumor relapse (Zerbini et al. 2006); thus it also cannot fully explain the positive impact of RFA on antitumor responses. The present study is the first to show the comparison of different tumor immunosuppressive reaction between RFA and SR.

It has long been recognized that the level of $CD4^+CD25^+Foxp3^+$ Treg cells increases in the peripheral blood of patients with various cancers (Sasada et al. 2003; Somasundaram et al. 2002; Wolf et al. 2003). Increased $CD4^+CD25^+Foxp3^+$ Treg cells may impair the function of cytotoxic T lymphocytes, promote tumor progression, and lead to a poor prognosis (Gao et al. 2007). We found in a preliminary study that baseline Hepa1-6 tumor-bearing mice had a significantly increased population of

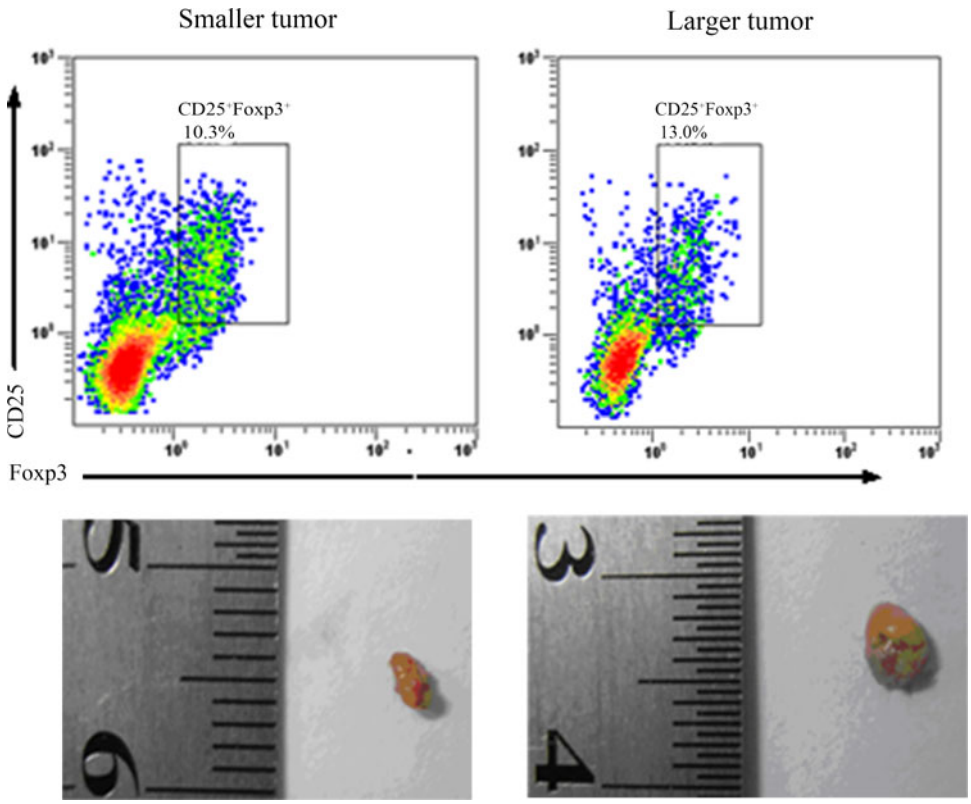


Fig. 4 Representative data for relationship between tumor volume and level of $CD4^+CD25^+Foxp3^+$ Treg cells. The T cell populations for the smaller and larger tumors were 10.3 and 13.0 %, respectively (gated on $CD4^+$ T cell)

$CD4^+CD25^+Foxp3^+$ Treg cells compared with non-tumor-bearing controls (data not shown). After SR, the level of $CD4^+CD25^+Foxp3^+$ Treg cells significantly increased and peaked at 4 days before returning to the level of normal mice because of tumor eradication. A possible explanation for this increase is that the host experiences SR as a serious attack, which strongly activates the immune system. To prevent inflammatory disorder and maintain homeostasis, $CD4^+CD25^+Foxp3^+$ Treg cells are developed in the thymus or induced from naïve T cells in the periphery (Bluestone and Abbas 2003) and suppress the proliferation and/or cytokine production of the effector T cells. In addition, Treg cells prevent $CD8^+$ cells from differentiating into cytolytic effector cells in vivo without affecting their proliferation or interferon γ production (Mempel et al. 2006); ultimately, the increase of Treg cells disables the antitumor effect and induces tumor progression. Unlike SR, RFA is a minimally invasive treatment that does not stimulate the regulatory portion of the immune system as much as SR and, therefore, keeps $CD4^+CD25^+Foxp3^+$ Treg cells at a relatively stable level throughout the post-operative recovery period, except for a weak increase at 4 and 7 days.

We further confirmed that the elevated level of $CD4^+CD25^+Foxp3^+$ Treg cells may contribute to tumor growth. The rechallenged mice in the SR group had significantly larger tumor volumes compared with RFA-treated animals and controls at 3, 5, and 7 days. Moreover, the rechallenged mice in the RFA group had the smallest tumor volumes. This phenomenon indicated that tumor growth in the SR group was faster than in the other two groups. However, the difference between the SR and RFA groups was not statistically significant over longer time periods. The trend coincides with the post-treatment change in $CD4^+CD25^+Foxp3^+$ Treg cells, implying that higher levels of $CD4^+CD25^+Foxp3^+$ Treg cells may result in enhanced tumor growth.

In the present study, we found a trend of a positive correlation between tumor volume and level of $CD4^+CD25^+Foxp3^+$ Treg cells. The greater the population of $CD4^+CD25^+Foxp3^+$ Treg cells, the larger the tumor volume. An increased level of $CD4^+CD25^+Foxp3^+$ Treg cells indicates a poor prognosis, although we did not follow up survival state. Several studies had similar results (Gao et al. 2007; Kim et al. 2009; Nakamura et al. 2009; Sinicrope et al. 2009). However, in-depth analysis revealed that the correlation in the RFA group was not strong ($r = 0.180$). There may be more complex mechanisms affecting tumor growth in the RFA group.

Immunotherapy has demonstrated some clinical activities in tumors treatment. As new therapy, RFA combined immunotherapy maybe a potential effective treatment.

Some initial clinical researches have showed the positive signal (Ma et al. 2010; Weng et al. 2008). However, they have not discovered the incentivising tendency on overall survival time. So the complex biological mechanisms of RFA need to be further clarified. Moreover, depletion of $CD4^+CD25^+$ or $CD4^+CD25^+Foxp3^+$ Treg cells affords enhanced immune activation, resulting in improved anti-tumor immunity in a mouse model (Heier et al. 2008). If we proceed to the combination therapy of RFA and depletion of Treg cell, is this therapy successful in clinical practice? Can it enhance the effect of RFA treatment? We need more researches in the future work.

Although the present study showed a correlation between tumor growth and level of $CD4^+CD25^+Foxp3^+$ Treg cells and different trends after SR and RFA, it has some limitations. First, this was an animal model study, with the tumor cells inoculated by artificial means rather than appearing and developing naturally. Thus, the applicability of the results to the natural state is unknown. Second, we measured the population of $CD4^+CD25^+Foxp3^+$ Treg cells only in the spleen. The immune status inside tumor and draining lymph nodes is pretty important for tumor growth and metastasis. The present study showed the systemic, but not local, host immune response after RFA or SR. The immune status of intratumor and draining lymph nodes after treatment also need to be clarified in the sequential study. Third, the present study was only focused on the influence of the early change of Treg cells on tumor growth. Because many factors, including nutrient, environment and infection and so on, can impact the long-term survival of tumor-bearing mice, the survival status was not followed. We will deeply study the relationship between immune response and survival in the future.

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References

Ali MY, Grimm CF, Ritter M et al (2005) Activation of dendritic cells by local ablation of hepatocellular carcinoma. J Hepatol 43:817–822

Bluestone JA, Abbas AK (2003) Natural versus adaptive regulatory T cells. Nat Rev Immunol 3:253–257

Bruix J, Sherman M (2005) Management of hepatocellular carcinoma. Hepatology 42:1208–1236

Chen MS, Li JQ, Zheng Y et al (2006) A prospective randomized trial comparing percutaneous local ablative therapy and partial hepatectomy for small hepatocellular carcinoma. Ann Surg 243:321–328

Crocetti L, Lencioni R (2010) Radiofrequency ablation of pulmonary tumors. Eur J Radiol 75:23–27

De Jong KP, Sloof MJ, de Vries EG et al (1996) Effect of partial liver resection on tumour growth. J Hepatol 25:109–121

Den Brok MH, Sutmuller RP, van der Voort R et al (2004) In situ tumor ablation creates an antigen source for the generation of antitumor immunity. Cancer Res 64:4024–4029

Dromi SA, Walsh MP, Herby S et al (2009) Radiofrequency ablation induces antigen-presenting cell infiltration and amplification of weak tumor-induced immunity. Radiology 251:58–66

Dunn GP, Old LJ, Schreiber RD (2004) The three Es of cancer immunoediiting. Annu Rev Immunol 22:329–360

Fu J, Xu D, Liu Z et al (2007) Increased regulatory T cells correlate with CD8 T-cell impairment and poor survival in hepatocellular carcinoma patients. Gastroenterology 132:2328–2339

Gao Q, Qiu SJ, Fan J et al (2007) Intratumoral balance of regulatory and cytotoxic T cells is associated with prognosis of hepatocellular carcinoma after resection. J Clin Oncol 25:2586–2593

Garcea G, Lloyd TD, Aylott C et al (2003) The emergent role of focal liver ablation techniques in the treatment of primary and secondary liver tumours. Eur J Cancer 39:2150–2164

Grimm CF, Ortmann D, Mohr L et al (2000) Treatment of hepatocellular carcinoma by breaking immunological ignorance towards a-fetoprotein using genetic immunization in mice. Gastroenterology 119:1104–1112

Heier I, Hofgaard PO, Brandtzaeg P et al (2008) Depletion of $CD4^+CD25^+$ regulatory T cells inhibits local tumor growth in a mouse model of B cell lymphoma. Clin Exp Immunol 152:381–387

Kim WY, Jeon YK, Kim TM et al (2009) Increased quantity of tumor-infiltrating FOXP3-positive regulatory T cells is an independent predictor for improved clinical outcome in extranodal NK/T-cell lymphoma. Ann Oncol 20:1688–1696

Kinoshita T, Iwamoto E, Tsuda H et al (2011) Radiofrequency ablation as local therapy for early breast carcinomas. Breast Cancer 18:10–17

Livraghi T, Meloni F, Di Stasi M et al (2008) Sustained complete response and complications rates after radiofrequency ablation of very early hepatocellular carcinoma in cirrhosis: Is resection still the treatment of choice? Hepatology 47:82–89

Ma H, Zhang Y, Wang Q et al (2010) Therapeutic safety and effects of adjuvant autologous RetroNectin activated killer cell immunotherapy for patients with primary hepatocellular carcinoma after radiofrequency ablation. Cancer Biol Ther 9:903–907

Mempel TR, Pittet MJ, Khazaie K et al (2006) Regulatory T cells reversibly suppress cytotoxic T cell function independent of effector differentiation. Immunity 25:129–141

Meredith K, Haemmerich D, Qi C et al (2006) Hepatic resection but not radiofrequency ablation results in tumor growth and increased growth factor expression. Ann Surg 245:771–776

Nakamura R, Sakakibara M, Nagashima T et al (2009) Accumulation of regulatory T cells in sentinel lymph nodes is a prognostic predictor in patients with node-negative breast cancer. Eur J Cancer 45:2123–2131

Picardo AM, Karpoff HM, Ng BM et al (1998) Partial hepatectomy accelerates local tumor growth: potential roles of local cytokine activation. Surgery 124:57–64

Sanchez-Ortiz RF, Tannir N, Ahrar K et al (2003) Spontaneous regression of pulmonary metastases from renal cell carcinoma after radio frequency ablation of primary tumor: an in situ tumor vaccine? J Urol 170:178–179

Sasada T, Kimura M, Yoshida Y et al (2003) $CD4^+$ $CD25^+$ regulatory T cells in patients with gastrointestinal malignancies. Possible involvement of regulatory T cells in disease progression. Cancer 98:1089–1099

Sinicrope FA, Rego RL, Ansell SM et al (2009) Intraepithelial effector ($CD3^+$)/regulatory ($FoxP3^+$) T-cell ratio predicts a clinical outcome of human colon carcinoma. Gastroenterology 137:1270–1279

Somasundaram R, Jacob L, Swoboda R et al (2002) Inhibition of cytolytic T lymphocyte proliferation by autologous $CD4^+CD25^+$ regulatory T cells in a colorectal carcinoma patients is mediated by transforming growth factor-beta. Cancer Res 62:5267–5272

Stolar CJ, Anderson KD (1980) Growth of mouse hepatoma following liver resection. J Pediatr Surg 15:513–516

Tracy CR, Raman JD, Donnally C et al (2010) Durable oncologic outcomes after radiofrequency ablation: experience from treating 243 small renal masses over 7.5 years. Cancer 116:3135–3142

Weng DS, Zhou J, Zhou QM et al (2008) Minimally invasive treatment combined with cytokine-induced killer cells therapy lower the short-term recurrence rates of hepatocellular carcinomas. J Immunother 31:63–71

Wissniowski TT, Hansler J, Neureiter D et al (2003) Activation of tumor-specific T lymphocytes by radiofrequency ablation of the VX2 hepatoma in rabbits. Cancer Res 63:6496–6500

Wolf AM, Wolf D, Steurer M et al (2003) Increase of regulatory T cells in the peripheral blood of cancer patients. Clin Cancer Res 9:606–612

Zerbini A, Pilli M, Penna A et al (2006) Radiofrequency thermal ablation of hepatocellular carcinoma liver nodules can activate and enhance tumor-specific T-cell responses. Cancer Res 66:1139–1146