Inhibition of Cancer Regional Recurrence in Mice Using Pulsed Streamer Discharge

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Abstract: Cancer recurrence after surgery is a major problem for cancer treatment. Recently, cold atmospheric-pressure plasma attracts attention as a tool for cancer treatment. In this study, we demonstrate that plasma irradiation on surgical scars of cancer tumor in mouse leads to inhibition of cancer regional recurrence. Mouse melanoma B16F10 cancer cells were inoculated into mice to make cancer tumors. After resecting the tumors, a pulsed streamer discharge was irradiated to the surgical scar. The result indicates that tumor regional recurrence can be reduced by the 10 minutes plasma irradiation of the surgical scar of cancer tumor in mice.

Keywords: cold atmospheric-pressure plasma, mouse melanoma, recurrence

1. Introduction

Cancer recurrence after surgery is a major problem for cancer treatment. In spite of the efforts to remove all the cancer cells, some cancer cells remain, forming new tumors. Sometimes extra treatments such as chemotherapy and radiotherapy are carried out after surgery to reduce the risk of the cancer recurrence. However, these treatments may have some side effects.

Recently, cold atmospheric-pressure plasma attracts attentions as a new tool for cancer treatment [1]. Cold atmospheric-pressure plasma can be directly irradiated without thermal damage. Plasma medicine is expected to further enhance therapeutic effects when used in combination with conventional treatment methods.

In this study, we examined the effects of pulsed streamer discharge on suppressing cancer regional recurrence. In our previous study [2], tumor regional recurrence was suppressed by 5 days irradiation of pulsed streamer discharge to the tumor, followed by resection of the tumor. The 5 days tumor irradiation was expected to induce anti-tumor immunity in mice, suppressing tumor recurrence after the subsequent tumor resection. However, the effect of "one-time plasma irradiation after tumor resection," which is effective in actual clinical practice, has not yet been investigated. In this study, we examined the effect of one-time streamer irradiation of tumor scars in mice after surgery.

2. Methods

2.1. Plasma irradiation

Figure 1 shows the pulsed streamer discharge used for the treatment of mice after tumor resection. The discharge was generated by applying nanosecond high voltage pulses (25 kV, 20 ns, and 100 pps) between the rod electrode and the grounded plate electrode. The rod electrode was 3 mm in diameter with a hemispherical tip. The rod electrode was placed in a quartz tube of 4 mm inner diameter. Wet oxygen gas humidified using a water bubbler was flowed through the quartz tube at a flow rate of 0.5 L/min. The grounded metal tape around the quartz tube was used to stabilize the generation of streamer discharge by generating dielectric barrier discharge inside the quartz tube.

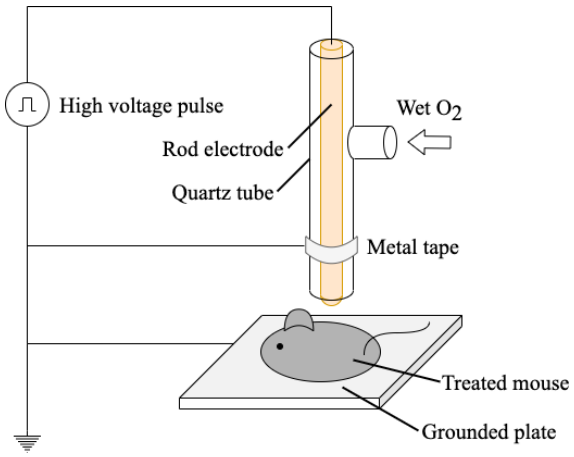

Fig. 1. Nanosecond pulsed streamer discharge.

2.2. In vivo experiment

Mouse melanoma B16F10 cancer cells purchased from RIKEN BioResource Center (Ibaraki, Japan) were cultured in RPMI media supplemented with 1% penicillin–streptomycin and 10% fetal bovine serum. C57BL/6J mice purchased from Oriental Yeast Co. (Tokyo, Japan) were injected subcutaneously at a concentration adjusted to contain 2×10^5 B16F10 cells per 100 µl. On the 12 days after the injection (Day 12), the skin of the mice was cut open and all visible cancerous tumors were removed. Although all visible tumors were removed, the periphery, where microscopic B16F10 cancer cells were expected to remain, was not resected to intentionally cause tumor recurrence.

The mice were divided into control and irradiated groups. In the irradiated group, the mouse was put on the grounded electrode and the excisional scar was irradiated with pulsed streamer discharge for 10 minutes under anesthesia immediately after excision of the cancerous tumors. The gap length between the rod electrode tip and the mouse surgical scur was adjusted to 8 mm. The control group was not irradiated for comparison. Then, the skin was sutured, and the growth of recurrent tumors from the excision scars was measured. The tumor regional recurrence in these two groups was compared.

3. Results and discussions

Figure 2 shows the changes in tumor volume in the control and irradiation groups after recurrence. The vertical axis represents the tumor volume measured as an ellipsoid, and the horizontal axis represents the number of days elapsed since the cancer cells were injected.

In the control group, all five mice had cancer recurrence, whereas in the irradiated group, only one had cancer recurrence. One of the irradiated group died before recurrence on day 7, and finally three of the five mice survived without recurrence in the irradiated group.

Figure 3 shows the probability of recurrence-free survival. Mice surviving without recurrence were treated with survivors. One mouse in the irradiated group died before recurrence as mentioned above and is treated as dead in Figure 3.

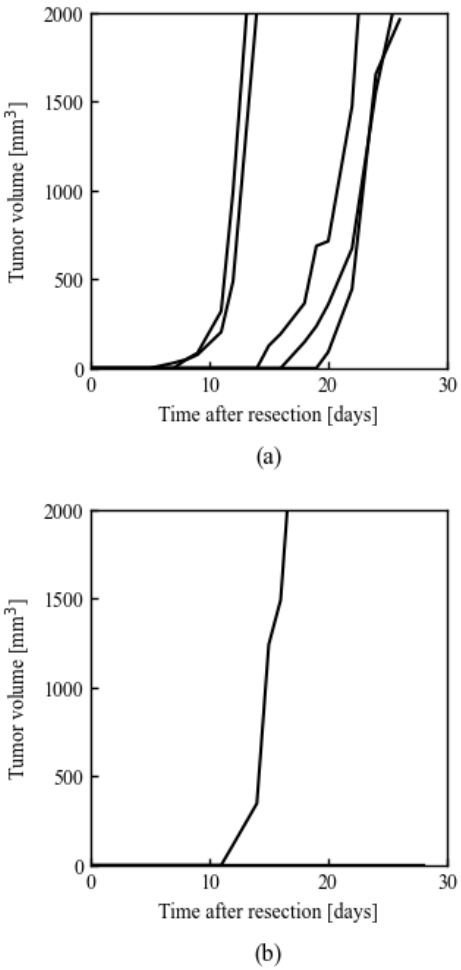

Fig. 2. Growth of recurrent tumors. (a) Control ($N = 5$) (b) Plasma ($N = 5$).

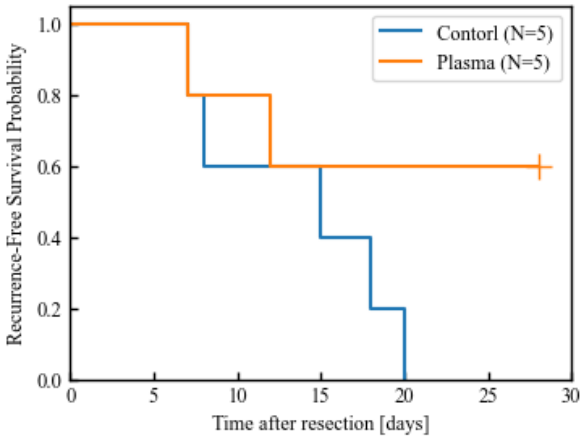

Fig. 3. Recurrence-free survival probability.

There are two possible reasons why the plasma irradiation inhibited the recurrence. One is that the plasma irradiation to the scar immediately after surgical resection may have killed invisible cancer cells that could not be removed by surgery. Another is that the immune system of the mice was activated, as we demonstrated in the previous study [2].

As this experiment alone was not enough to give a statistically significant difference, two more similar experiments were performed. If all the results were combined, a significant difference of $p < 0.05$ was obtained with a log-rank test between the control and plasma irradiated groups for the recurrence-free survival probability. In total, the plasma irradiation approximately doubled the survival rate in all three experiments. In the other two experiments, however, the tumors were resected eight days after injection.

4. Conclusion

In this study, we examined the effect of plasma irradiation after resection of B16F10 cancerous tumors in mice. The results showed that the plasma irradiation had a significant effect on inhibition of cancer regional recurrence. This is the first time to demonstrate that a single 10-minute irradiation of the scar after excision using pulsed streamer discharge has a suppressive effect of regional recurrence. This result may lead to the possibility of suppressing the recurrence of cancer in combination with surgery and plasma treatment in clinical practice.

In future studies, it is necessary to explore better irradiation conditions that are more effective in suppressing recurrence and to elucidate the mechanism by which plasma irradiation suppresses recurrence.

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