News Release

Title

Ferroptosis induced by plasma-activated Ringer's lactate solution prevents oral cancer progression

Key Points

- We discovered that the killing effect of plasma-activated Ringer's lactate (PAL) is specific to OSCC cells and that PAL induces ferroptosis.
- PAL prevents cancer progression and improves the survival rate by decreasing LOX expression in cancer cells and changing collagen matrix formation.

Summary

This study aimed to investigate the effect of plasma-activated Ringer's lactate solution (PAL) on cancer cells, considering iron metabolism and the tumor microenvironment.

PAL killed oral squamous cell carcinoma (OSCC) cells at lower concentrations than epithelial cells and fibroblasts in vitro. The killing effect was prevented by deferoxamine and ferrostatin-1. Apoptosis, necroptosis and autophagy were excluded. After PAL treatment, iron increased in the cells, and lipid peroxidation occurred. PAL dramatically decreased the lysyl oxidase levels. Furthermore, PAL inhibited both migration and invasion. PAL reduced the incidence rate of SCC and improved the survival rate without causing severe side effects in vivo. PAL treatment resulted in lower expression of GPx4 and higher expression of 8-OHdG and 4-HNE. The type of cell death was ferroptosis. Furthermore, the study results indicate that PAL decreased lysyl oxidase (LOX) expression in cancer cells and the amount of collagen. To our knowledge, this is the first study to evaluate the effect of PAL on oral cancer with a focus on iron and collagenous matrix formation.

PAL could provide a sustainable and effective therapy for oral cancer patients. These data support the further clinical development of PAL treatment for oral cancer.

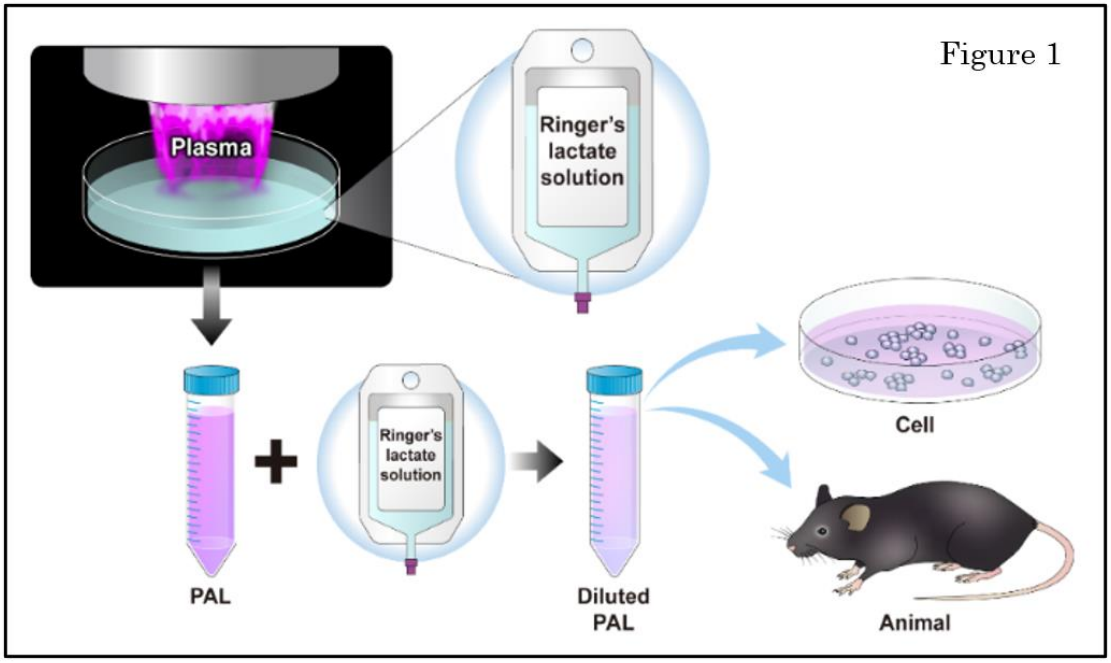
Research Background

The number of patients with oral cancer has been increasing worldwide, and the 5-year survival rate of patients with tongue cancer is 67.1%. This rate has not improved since 2010, even though the prognosis is relatively good if patients receive prompt and early treatment. Surgery is the first choice of treatment. However, some patients cannot undergo surgery because of their general condition or medical history, and chemotherapy and radiotherapy are thus performed instead. These treatments are particularly effective at preserving tissue but can cause severe side effects in some patients, necessitating treatment discontinuation. Furthermore, preventing recurrence after primary treatment is important to maintain the quality of life of patients and increase their survival rates. Although the morbidity associated with oral

cancer, which affects approximately 2% of patients, is not as high as that of other cancers, the reduction in quality of life after treatment is severe. Therefore, developing sustainable, effective and harmless novel treatments is important as a key research effort.

Nonthermal plasma (NTP), or low-temperature plasma, is a contradictory condition with high energy at near-body temperature that was invented via engineering in the 1990s. NTP is currently being used in preclinical experiments in various fields. NTP can be applied in two ways: directly on lesions or indirectly using Ringer's lactate solution irradiated with NTP, which is called plasma-activated Ringer's lactate solution (PAL). Recently, some researchers have shown that PAL is effective against ovarian and pancreatic cancers in vivo.

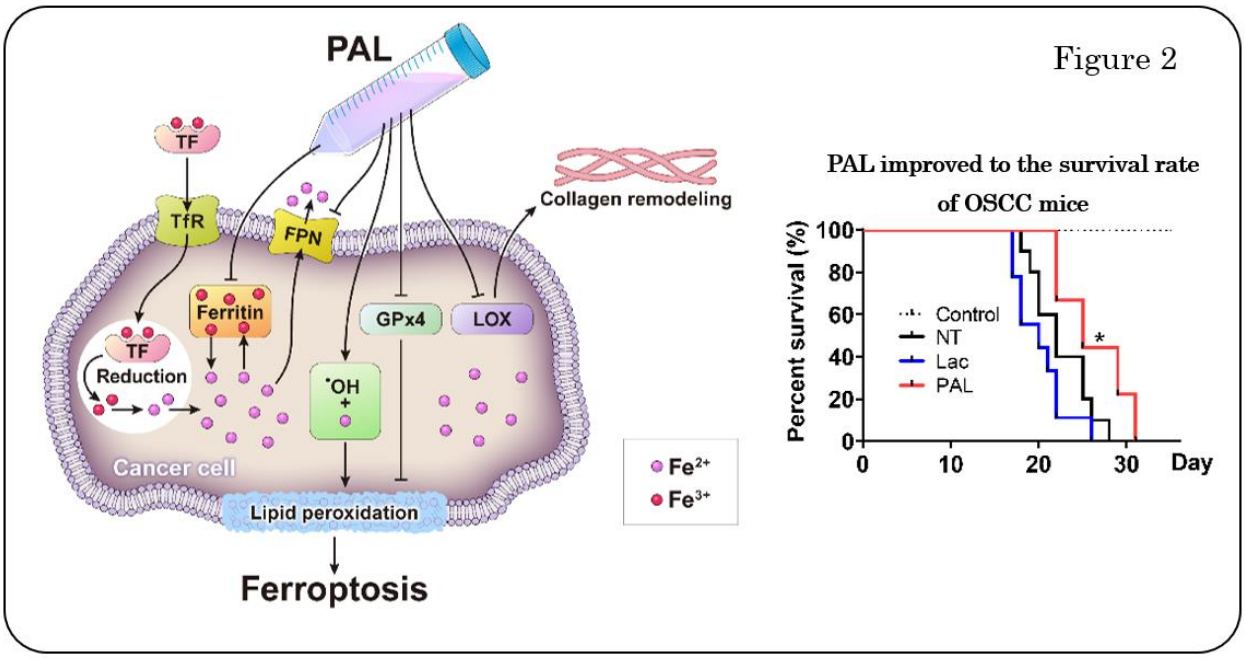
It has been considered that cancer cells contain Fe^{2+} more than nontumorous cells and that is a key factor of the killing effect of PAL. In addition, some researchers have reported that collagen created around cancer cell is closely related with cancer metastasis.



Scheme of PAL production and application method (Figure 1):PAL is made from applying plasma to Ringer's lactate solution

Research Results

We used three OSCC cell lines, one keratinocyte cell line and two fibroblast lines, and cell viability assays, immunoblotting, flow cytometry and transmission electron microscopy were performed to evaluate the effect and type of cell death. The effect of PAL treatment on lysyl oxidase (LOX) expression was investigated in vitro and in vivo. Tamoxifen-inducible Mob1a/b double-knockout mice were used for the in vivo experiment. We found that PAL specifically kills OSCC cells and that the type of cell death is ferroptosis. Furthermore, the results indicate that PAL prevents cancer progression and improves the survival rate by decreasing LOX expression in cancer cells and changing collagen matrix formation.



Schematic of the plasma-activated Ringer's lactate solution (PAL) treatment of oral cancer cells.

PAL upregulates catalytic Fe2+ in cancer cells, influencing iron metabolism and lipid peroxidation and leading to ferroptosis. Furthermore, PAL downregulates LOX in cancer cells, leading to the suppression of cancer progression. Fe, ferrum; TF, transferrin; TfR, transferrin receptor; FPN, ferroportin; •OH, hydroxyl radical; GPx4, glutathione peroxidase 4; LOX, lysyl oxidase; PAL, plasma-activated Ringer's lactate solution.

Research Summary and Future Perspective

We found that PAL specifically kills OSCC cells and induces ferroptosis. Furthermore, the results indicate that PAL prevents cancer progression and improves the survival rate by decreasing LOX expression in cancer cells and changing collagen matrix formation (Figure 2). PAL could provide a sustainable and effective therapy for patients with oral cancer. These data support the further clinical development of PAL treatment for oral cancer.

Publication

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Japanese ver.

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