Inosine shows antitumor effects in head and neck cancer

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ABSTRACT

Introduction
In recent years, the intestinal microbiota has come to be important in order to improve the response rate of cancer immunotherapy. In this study, we focused on inosine as a metabolite of intestinal bacteria to clarify its effects on immunity and tumor growth.

Methods
T cells taken from healthy volunteers were stimulated with inosine and then gene expression of T cells was analyzed by qPCR after 6 hours. Phenotype of T cells was also analyzed after 72 hours by flow cytometry. In addition, human T cells (5 × 105 cells) and human T3M-1 Cl-10 oral carcinoma cells (5 × 104 cells) were co-cultured with stimulation with inosine. The changes in proliferation of human T cell and human carcinoma cell were analyzed after 72 hours.

Results
Inosine increased Granzyme B gene expression in human T cells and decreased PD-1 and TIM-3 gene expression. It also suppressed PD-1 and TIM-3 expression in CD4-positive and CD8-positive T cells. They also suppressed differentiation into regulatory T cells. In cell proliferation assays, inosine promoted T cell proliferation by about 50% and inhibited oral carcinoma cell proliferation by about 50%.

Conclusion
Inosine promoted activity and suppressed exhaustion of human T cells, and inhibited differentiation into regulatory T cells in this study. Inosine also showed inhibition of human T3M-1 Cl-10 oral carcinoma cells proliferation. These findings may support the development of new cancer therapies that improve the response rate.

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INTRODUCTION

Immune checkpoint inhibitors (ICIs) are potentially able to be strong strategy to treat the Head and Neck Cancer, however they lack enough their efficacy, so the search for factors that can improve their efficacy is needed.

Recently, it has been recognized that the composition of the intestinal microflora influences the therapeutic effect and that modification of the intestinal microflora by fecal transplantation enhances the therapeutic effect of ICIs.

The effect of intestinal bacterial metabolites such as short-chain fatty acids (SCFA) on immunity has been suggested as a mechanism for this effect.

In this study, we focused on inosine as a metabolite of intestinal bacteria to clarify its effects on immunity and tumor growth.

METHODS

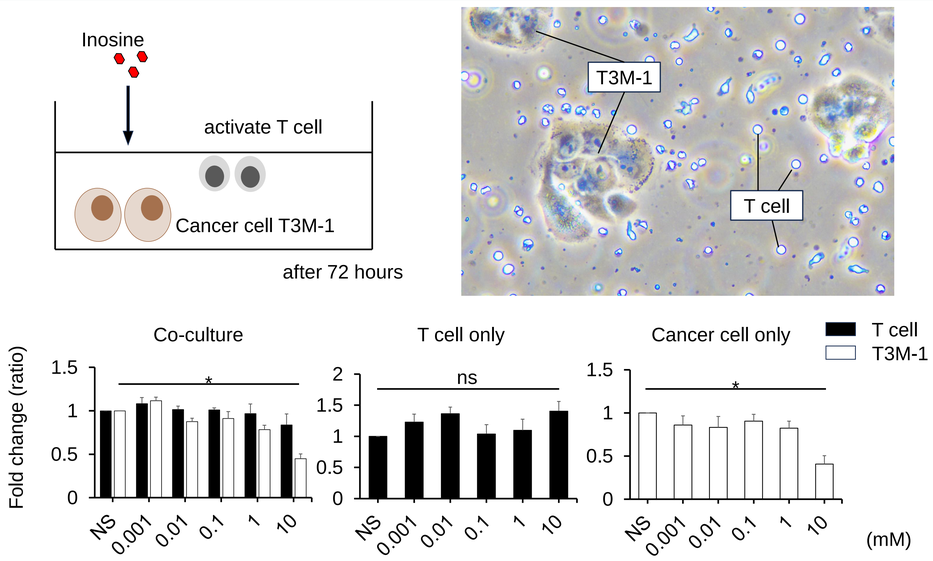
①The effect of inosine on the proliferation of T cells and cancer cells (Fig. 1)
Human T cells and human T3M-1 oral carcinoma cells were stimulated with inosine and changes in proliferation were analyzed after 72 hours.

②The effect of inosine on gene expression of T cells (Fig. 2)
Human T cells were stimulated with inosine and gene expression were measured by PCR after 6 hours.

③The effect of inosine on the phenotype of T cells (Fig. 3)
Human T cells were stimulated with inosine and phenotype were analyzed by flow cytometry after 72 hours.

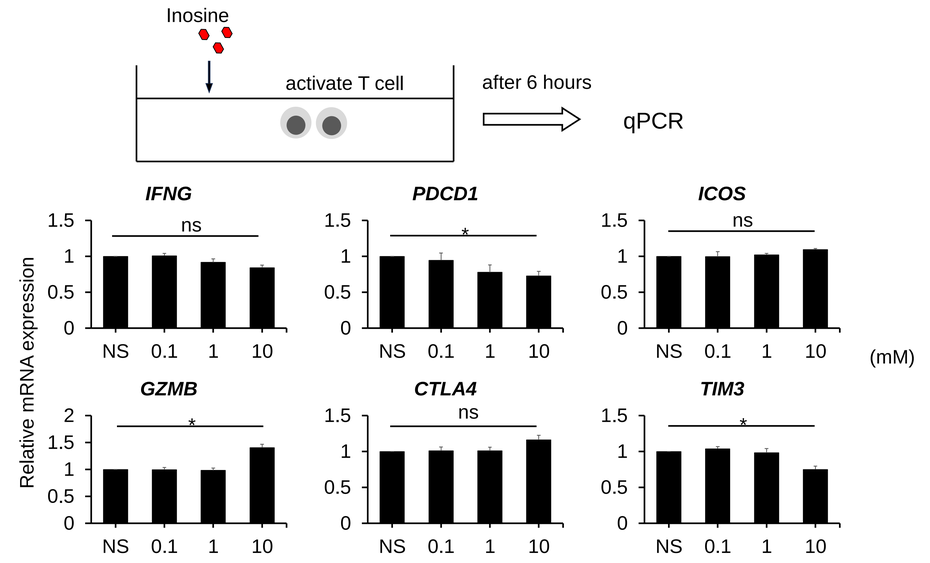
RESULTS

Fig. 1 The effect of inosine on the proliferation of T cells and cancer cells.



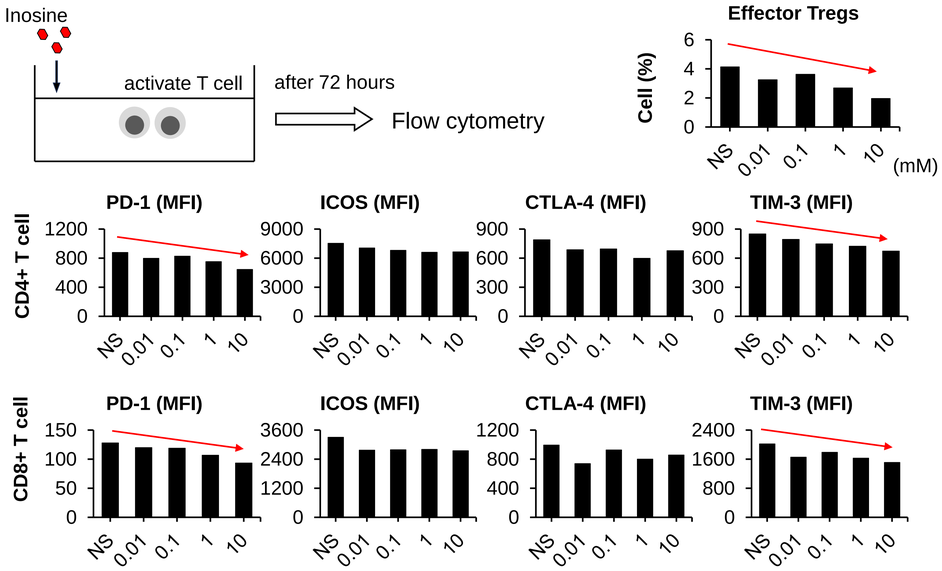
Inosine promoted T cell proliferation by about 50% and inhibited cancer cell proliferation by about 50%.
In co-culture, only cancer cells were suppressed.

Fig. 2 The effect of inosine on gene expression of T cells.



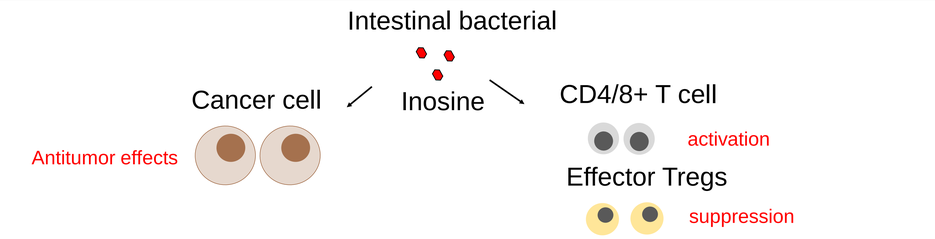
Inosine increased gene expression of granzyme B, a marker of cytotoxicity.
Inosine decreased gene expression of PD-1 and TIM-3, markers of T cell exhaustion.

Fig. 3 The effect of inosine on the phenotype of T cells.



Inosine suppressed PD-1 and TIM-3 expression in CD4-positive and CD8-positive T cells.
Inosine suppressed differentiation into regulatory T cells.

CONCLUSION



Inosine promoted activity and suppressed exhaustion of human T cells, and inhibited differentiation into regulatory T cells.
Inosine also showed inhibition of human T3M-1 Cl-10 oral carcinoma cells proliferation.
These findings may support the development of new cancer therapies that improve the response rate.