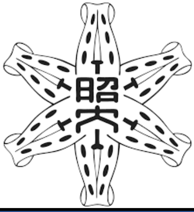


Effect of Short-Chain Fatty Acids on Head and Neck Cancer

Masakazu Murayama, MD[1]; Astuo Kuramasu, Prof.[1]; Sei Kobayashi, Prof.[2]; Toshikazu Shimane, Prof.[3]
Hitome Kobayashi, Prof.[3]; Yuji Kiuchi, Prof.[4]; Kiyoshi Yoshimura, Prof.[1]

[1]Department of Clinical Immuno Oncology, Clinical Research Institute for Clinical Pharmacology and Therapeutics, Showa University.
[2]Department of Otorhinolaryngology, Showa university Fujigaoka Hospital.
[3]Department of Otorhinolaryngology - Head and Neck Surgery, Showa University School of Medicine.
[4]Department of Pharmacology, Showa University School of Medicine.

ABSTRACT

◎Introduction
Recently, approaches to the tumor microenvironment, especially to intestinal bacteria, have been considered important to improve the response rate of cancer therapy. In this study, we focused on short-chain fatty acids-A (SCFA-A) as a metabolite of intestinal bacteria, and clarify the effects of SCFA-A on immune activity and tumor growth.

◎Methods
Human T3M-1 oral cancer cells and human T cells from healthy volunteers were mono- or co-cultured. They were treated with SCFA-A for 72 h. The changes in proliferation of human cancer cells and T cells, and phenotype of T cells were analyzed using flow cytometry. And the gene expression of T cells was analyzed by qPCR. SCFA-A was administered to bone marrow macrophages or M1 macrophages derived from mouse, and gene expression and phenotype of them were analyzed by qPCR and flow cytometry.

◎Results
SCFA-A inhibited tumor cell growth, but not T cells. The frequency of Ki67, ICOS, and PD-1 expression in T cells and gene expression of *IFNG*, *ICOS*, and *PDCD1* in T cells were increased. The frequency of Foxp3 expressing in T cells and gene expression of *FOXP3* in T cells were decreased. The gene expression of CD80 and the frequency of CD86 expression in macrophages was increased. The gene expression of CD206 and the frequency of CD163 expression in macrophages was decreased. SCFA-A upregulated gene expression of IFNG and NOS2 in M1 macrophages. This increase in gene expression was suppressed under the combination of MPN.

◎Conclusions
SCFA-A has the potential to exhibit anti-tumor effects by activating T cells and M1 macrophages, as well as demonstrating a direct growth inhibitory effect on T3M-1 cells.
These finding may support the development of novel cancer therapies that improve the response rate.

CONTACT

[name] Masakazu Murayama
[organization] Showa University Hospital
[address]1-5-8, Hatanodai, Shinagawa-ku, Tokyo, 142-8666, JAPAN
[email]nomurmur@med.showa-u.ac.jp
[phone]+81 80-4630-1886

INTRODUCTION

Recently, **immune checkpoint inhibitors** (**ICIs**) have become the core of new treatment option for head and neck cancer. However, **Low response rates** have become a serious problem, and **approaches to the tumor microenvironment** are important.[1]

Gut microbiota has been shown to **enhance the anti-tumor effects** of cancer immunotherapy.[2]

Short-chain fatty acids (**SCFAs**), metabolites of intestinal bacteria, are thought to **influence anti-tumor immunity**.[3,4]

The effects of each **SCFAs** on cancer and immune cells are still unclear.

In this study, we focused on **short-chain fatty acid A** (**SCFA-A**) among seven types of short-chain fatty acids and clarified the effects of SCFA-A on immune activity and tumor growth.

METHODS AND MATERIALS

Experiment① The effect of SCFA on the proliferation of cancer cells and T cells (Fig. 1)
SCFA-A was added to human oral cancer T3M-1, human T cells, or co-cultures of them for 72 hours. The numbers of T cells and cancer cells were measured by flow cytometry.

Experiment ② The effect of SCFA-A on the phenotype of T cells in co-culture with cancer cells (Fig. 2)
SCFA-A was added to co-cultures of T3M-1 and human T cells for 72 hours. The phenotype of the T cells was analyzed by flow cytometry.

Experiment ③ The effect of SCFA-A on gene expression of T cells (Fig. 3)
SCFA-A was added to human T cells for 72 hours. The gene expression of the T cells was measured by qPCR.

Experiment ④ The effect of SCFA-A on gene expression or phenotype of macrophages (Fig. 4)
a, b. Bone marrow cells were stimulated with M-CSF to induce macrophages, and SCFA-A was added. The gene expression of *CD80* and *CD206* was measured by qPCR. The frequency of cells expressing CD86 and CD163 was measured by flow cytometry.

Experiment ⑤ (Fig. 5)
Bone marrow cells were stimulated with M-CSF to induce macrophages, and SCFA-A was added together with either BHB, GLP, or MPN. The same analysis as in Fig. 4 was performed using qPCR.

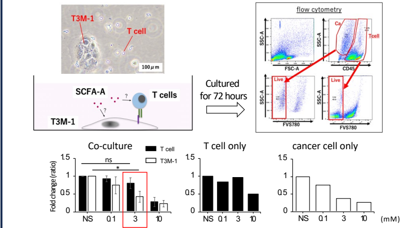
Experiment ⑥ Functional effects of SCFA-A on M1 macrophages (Fig. 6)
Bone marrow cells were stimulated with M-CSF to differentiate into macrophages, and then IFN-γ and LPS were added to induce M1. (a) SCFA-A, or (b) SCFA-A+MPN were added, and gene expression of IFNG and NOS2 were measured by qPCR.

REFERENCES

1. N Engl J Med. 2016 Nov 10;375(19):1856-67.
2. Science. 2018 Jan 5;359(6371):97-103.
3. JAMA Netw Open. 2020 Apr 1;3(4):e202895.
4. Nat Commun. 2020 May 1;11(1):2168.
5. Gastroenterology. 2013 Aug;145(2):396-406.e1-10.

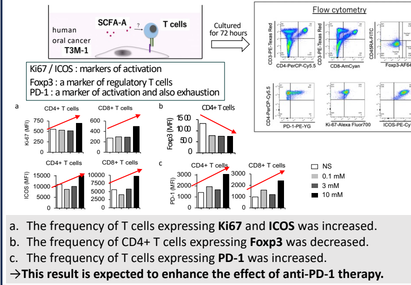
RESULTS

Fig. 1 SCFA-A inhibited tumor cell growth, but not T cells.



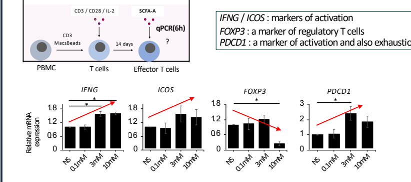
SCFA-A inhibited tumor cell growth, but not T cells.
We thought that not only the direct anti-tumor effect of **SCFA-A**, but also the immune activity of T cells by SCFA-A might be affected.

Fig. 2 SCFA-A induced activation of effector T cells and may suppressed regulatory T cells.



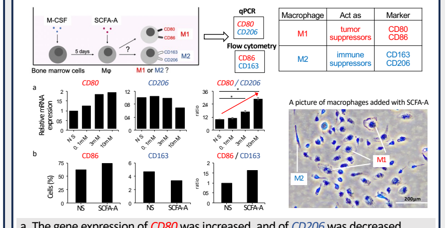
a. The frequency of T cells expressing **Ki67** and **ICOS** was increased.
b. The frequency of CD4+ T cells expressing **Foxp3** was decreased.
c. The frequency of T cells expressing **PD-1** was increased.
→This result is expected to enhance the effect of anti-PD-1 therapy.

Fig. 3 The qPCR of mono-culture T cells largely replicated those with the FCM analysis in co-culture setting.



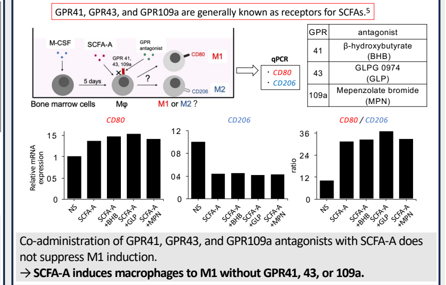
As with flow cytometry (Fig. 2),
SCFA-A upregulated gene expression of ***IFNG, ICOS,*** and ***PDCD1***.
The gene expression of ***FOXP3*** was decreased.

Fig. 4 SCFA-A induces macrophages to M1, which acts on antitumor effects.



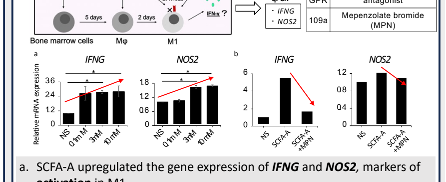
a. The gene expression of *CD80* was increased, and of *CD206* was decreased.
b. The frequency of CD86-expressing cells was increased, and of CD163-expressing cells was decreased.
→ SCFA-A induces macrophages to M1.

Fig. 5 SCFA-A induces macrophages to M1 without GPR41, 43, or 109a.



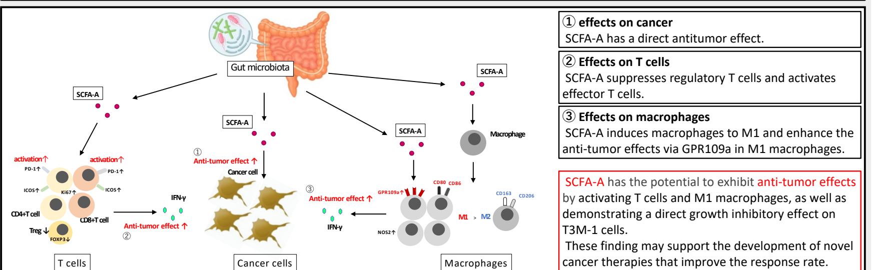
Co-administration of GPR41, GPR43, and GPR109a antagonists with SCFA-A does not suppress M1 induction.
→ SCFA-A induces macrophages to M1 without GPR41, 43, or 109a.

Fig. 6 SCFA-A enhances antitumor effects via GPR109a in M1 macrophages.



a. SCFA-A upregulated the gene expression of ***IFNG*** and ***NOS2***, markers of **activation** in M1.
b. SCFA-A-induced increase in gene expression of ***IFNG*** and ***NOS2*** was suppressed under the combination of MPN, GPR109a antagonist.
→SCFA-A enhances anti-tumor effects via GPR109a in M1 macrophages

CONCLUSIONS



① **effects on cancer**
SCFA-A has a direct antitumor effect.

② **Effects on T cells**
SCFA-A suppresses regulatory T cells and activates effector T cells.

③ **Effects on macrophages**
SCFA-A induces macrophages to M1 and enhance the anti-tumor effects via GPR109a in M1 macrophages.

SCFA-A has the potential to exhibit anti-tumor effects by activating T cells and M1 macrophages, as well as demonstrating a direct growth inhibitory effect on T3M-1 cells.
These finding may support the development of novel cancer therapies that improve the response rate.