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Title(English)	
著者(和文)	宮原瞳
Author(English)	Hitomi Miyabara
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報告番号 For administrative use only	乙 第　　号	氏　名 Name	宮原　瞳

（要　旨）
(Summary)

This thesis describes the development of highly sensitive *in vivo* optical imaging system for analyzing tumor stromal cells with hypoxia-inducible factor (HIF) activity.

Chapter 1 summarized the background of this study, which explains how the hypoxic tumor microenvironment activates the transcription factor HIF in cancer cells and stromal cells within tumors, and how these HIF-active cells are involved in tumorigenesis, proliferation, metastasis, and invasion. Although some efforts have been made to develop transgenic (Tg) mice with HIF-dependent bioluminescence reporter genes to analyze the behavior of HIF-active cells in a spatiotemporal manner, previous systems have suffered from low detection sensitivity of specific signals. Therefore, this study purposed to generate Tg mice with highly sensitive reporter gene and to use these mice to identify the types of tumor-infiltrating cells with HIF activity.

Chapter 2 focused on development and evaluation of Tg mice with highly sensitive reporter gene to detect HIF-active cells. The Tg mice, named HVA-Tg, have a reporter gene consisting of a promoter sequence containing the enhancer sequence hypoxia response element (HRE), the green fluorescent protein Venus, and the modified firefly luciferase AkaLuc (HVA) that express Venus-AkaLuc fusion proteins in a HIF activity-dependent manner. In HVA-Tg, HIF-active cells can be visualized using AkaBLI, an ultra-sensitive *in vivo* bioluminescence imaging technology that produces a highly biopermeable light upon reaction of AkaLuc with the D-luciferin analog substrate Akalumine-HCl (Aka-HCl). When HIF activity was induced in HVA-Tg, sufficient bioluminescence signals were obtained from HIF-activated cells upon administration of substrate, but substrate-derived autoluminescence was also observed in the liver, independent of Venus-AkaLuc expression.

Chapter 3 discussed the evaluation of autoluminescence signals in *in vivo* imaging using AkaBLI. Because the autoluminescence was observed after Aka-HCl administration to non-Tg, I evaluated the level of substrate-derived autoluminescence. The signal from Aka-HCl reacting with hydrogen peroxide, which is abundant in tumors, was significantly lower than the signal generated by the Aka-HCl-luciferase reaction. Furthermore, signals from tumor-bearing non-Tg mice were measured after substrate administration and was less than 3000 p/s/cm^2/sr. Therefore, signals higher than this value are considered to be due to HVA reporter activity.

Chapter 4 described *in vivo* imaging of tumor-infiltrating cells with HIF activity. HVA- Tg were

successfully used for noninvasive monitoring of bioluminescence signals from HIF-active tumor stromal cells in both syngeneic tumor models： an orthotopic breast cancer using cell line E0771 and a subcutaneous tumor model using lung cancer cell line LLC. Furthermore, immunohistochemical analysis revealed that the localization of HIF-active cells in the tumors largely overlapped with that of macrophage marker F4/80-positive cells.

Chapter 5 showed the analysis of the cell type of tumor-infiltrating HIF-active stromal cells. Using flow cytometry, I investigated the Venus-AkaLuc-positive cell population in E0771 tumors and found that the majority of these cells were F4/80-positive macrophages.

Chapter 6 summarized the results and discussed future prospects for using HVA-Tg to understand the tumor microenvironment and its application to drug discovery.

In summary, the development of a new method for analyzing the behavior of tumor- infiltrating HIF-active stromal cells by *in vivo* imaging may open new avenues for research into the understanding of the role of these cells in tumor malignancy and for the development of drugs targeting these cells.