【Grant-in-Aid for Scientific Research (S)】
Biological Sciences (Biological Sciences)

Title of Project: Development of New *in vivo* Imaging Technologies by Using "Biological Optical Window"

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Research Project Number : 26221004 Researcher Number : 50271896
Research Area : Experimental Animal
Keyword : Research bio-resources

【Purpose and Background of the Research】

In this project, we aim to improve non-invasive *in vivo* fluorescent imaging technology by developing mice that enable monitoring of angiogenesis, tissues fibrosis and intensity of pain. We have achieved this using a novel fluorescent protein, iRFP and its derivatives, which have excitation and emission wavelengths in the "biological optical window".

【Research Methods】

1. Development of the fundamental technology required to improve the efficiency of *in vivo* imaging.
1-1. Development of a mouse expressing proteins that fluoresce in the near-infrared : We have developed a fluorescent observation method using iRFP and its derivatives.
1-2. Development of iRFP that enables repeated and stage-specific observation: We have developed *in vivo* imaging technology that enables repeated and stage-specific observation of fluorescence by developing Deg-iRFP.
1-3. Development of a custom-made melanin inhibition method: We developed technology to create albino mice by introducing a point mutation into the Tyrosinase gene using the CRISPR/Cas9 system.
1-4. Development of a custom-made body hair inhibition method: Body hair becomes an inhibitory factor when a mouse is studied by fluorescence. We have established a technique that enables introduction of the hair less ($HR^{hr}$) mutation using the CRISPR/Cas9 system.

2. Development of mice that enable the monitoring of various clinical conditions.
2-1. Development of a mouse that enables tracking of specific cells with iRFP: We expressed iRFP only in specific cell groups and in a variety of previously developed Cre-driver mice to produce a mouse that enables tracking of specific cells expressing iRFP.
2-2. Development of a mouse that enables monitoring of angiogenesis: We have developed a mouse which enables angiogenesis to be followed *in vivo* and in a stage-specific manner by inserting iRFP or Deg-iRFP into the murine Flk1 and Flt1 genes.
2-3. Development of a mouse that enables monitoring of fibrosis: We have developed a mouse that enables stage-specific *in vivo* monitoring of type 1 collagen transcription. Collagen production increases during fibrosis after tissue damage.
2-4. Development of a mouse that enables monitoring of neural activity (intensity of pain): Using the previously described Deg-iRFP, we have developed a mouse that enables us to stage-specifically monitor neural activity history.

【Expected Research Achievements and Scientific Significance】

We attempt to expand the range of applications for *in vivo* fluorescent imaging technology, is a technique widely used in life sciences research, by developing a less invasive technique that utilizes the biological optical window. Furthermore, we envisage that our techniques will lead to pain reduction in experimental animals and reduce the number of animals required to conduct statistically valid analyses.

【Publications Relevant to the Project】

- Mizuno S, Dinh TT, Kato K, Mizuno-Iijima S, Tanimoto Y, Daitoku Y, Hoshino Y, Ikawa M, **Takahashi S.** Sugiyama F, Yagami KI. Simple generation of albino C57BL/6J mice with G291T mutation in the tyrosinase gene by the CRISPR/Cas9 system. ***Mamm Genome.*** 2014.
- Tran TNM, Tanaka J, Hamada M, Sugiyama Y, Sakaguchi S, Nakamura M, **Takahashi S**, Miwa Y. *In vivo* image analysis using iRFP transgenic mouse. ***Exp Animal.*** *in press.*

【Term of Project】 FY2014-2018

【Budget Allocation】 88,500 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.md.tsukuba.ac.jp/basic-med/anatomy/embryology/index.html