The 1st Technical & Educational Training on *"In vivo Imaging"* with the Luc Tg RAT for Asia-Pacific Berthold Technologies Sales Teams

(Keio University School of Medicine, Shinano-machi, Tokyo JAPAN on May 21-22, 2015)

International Expansion of *In vivo* Imaging Technology

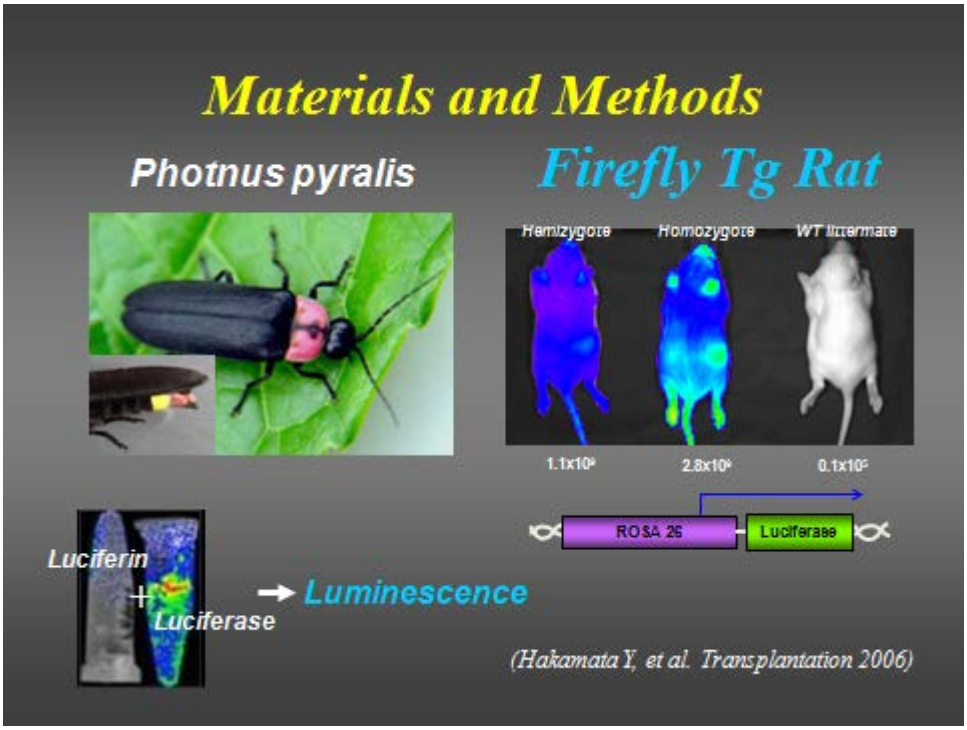
1. What the researchers expect?

Prof. Eiji Kobayashi (Keio University)

Introduction

The research method utilizing light as *in-vivo* imaging has benefits not only for scientifically observing the symptom occurring *in vivo* directly from our eyes but for giving us a chance to observe non-invasively experimental animals without sacrifice death from ethical point of view. I would like to propose that we should share these state-of-the-art tools internationally if we take contributions to further more patients into consideration.

In 2006 the author gave a birth to Luciferase (Luc) transgenic (Tg) rat, which the luminescent protein “Luciferase” emerges in the entire body of the rat through the optimization of transgenic technology. Administrating the substrate “Luciferin” to Luc Tg Rat, Luciferin is oxidized, which leads to the light emittance over the whole body. (Figure 1)



Currently not only European and American but Asian top-notch researchers have been developing their advanced medical researches through the usage of the Luc Tg Rat.

NightOWL developed by Berthold Technologies GmbH (Germany) is one of the *in vivo* imaging devices applying the chemical reaction of Luciferin-Luciferaze. There exist several *in vivo* imaging devices from various manufacturers in the world, which are all based on the above reaction. However, each one has its own characteristics. The objectives of this seminar is to further understand the usage of Luc Tg Rat the author has developed and how the researchers generate results out of it.

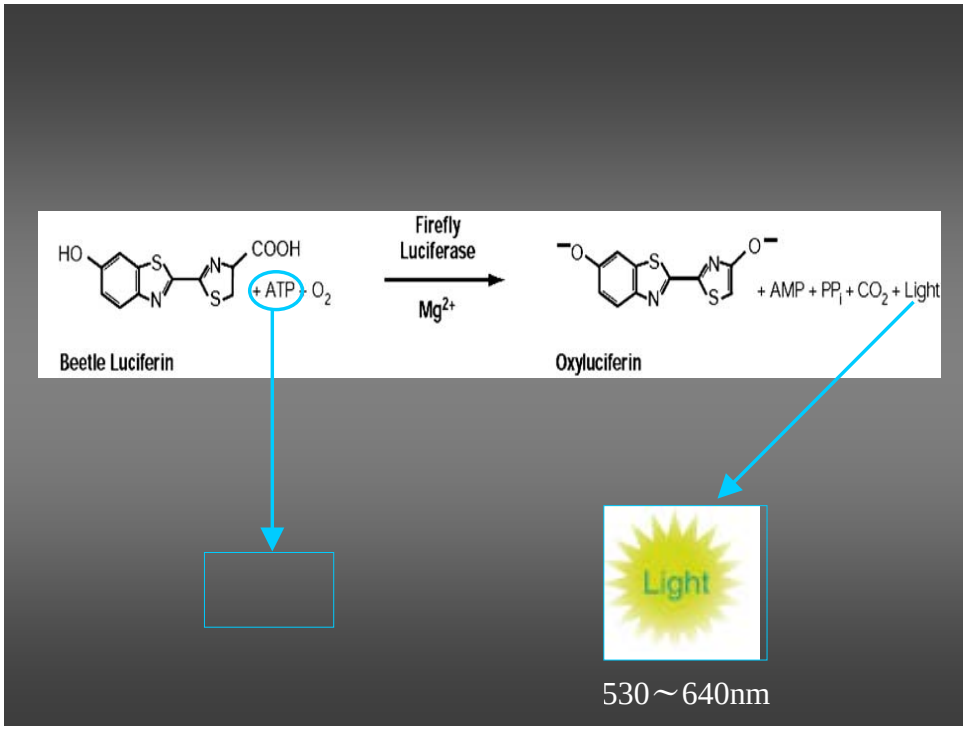
In vivo administration of Luciferin

This section explains how to administrate Luciferim to rats, which will be detailed from the researchers’ point of view.

Figure 2 shows the chemical formula of luminescence. It reacts against Luciferin and

emits light depending of ATP and density of oxygen.

(Figure 2)



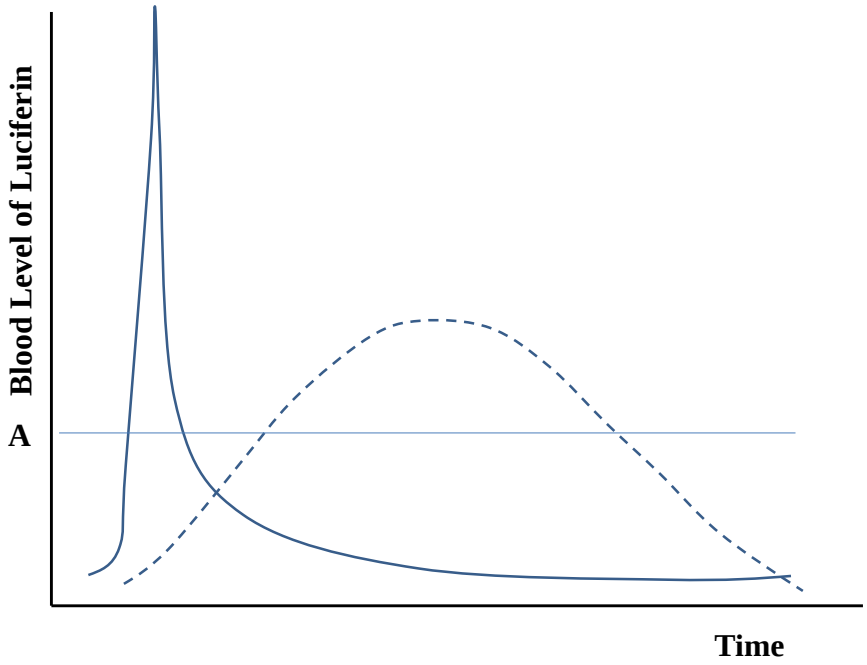
The rat for *in vivo* is alive under anesthesia, to which we need to add sufficient amount of Luciferin (to the extent that the luminescent strength reaches the maximum).

In the beginning of the year 2000's when the in vivo imaging device such as IVIS has been developed, mice (around 20g physical weight) smaller in size compared with rats have been used mainly. Furthermore, Luchiferin has been intraperitoneally administrated because of its easy operation. Nevertheless, in the field of regenerative medicine the shift from mice to rats with 10 times bigger in physical size has been occurring for regenerating organs and evaluating the amount of blood flow.

Here is the question; does the amount of Luciferin increase 10 times compared to that of mice? The answer is "Yes". Luciferin is relatively expensive as a reagent, which gives headache to the researchers. The number of researchers would be increased if the manufacturers distributing imaging devices overcame the reagent price barriers.

The author has solved the problem through having modified administrating method of Luciferin. The below figure shows the scientific proof (Figure 3).

(Figure 3)



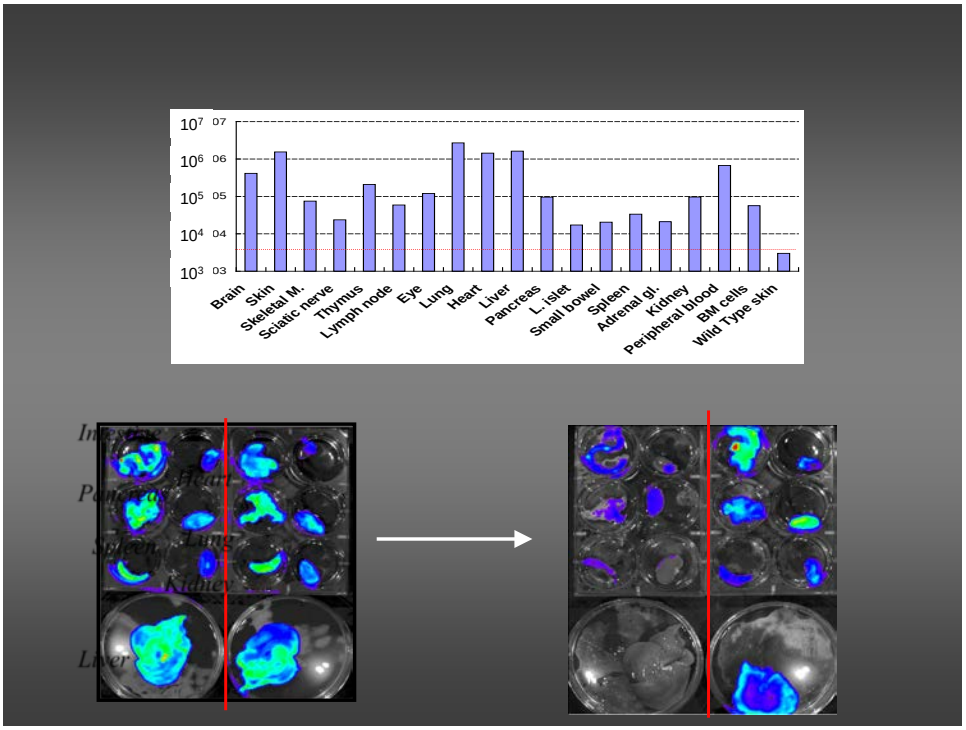
The density of Liciferin is shown that the curved straight line is administrated in vein and the curved dotted line is intraperitoneally done. AUC: Area under the curve shows the amount of Luciferin. When the density reaches at the level “A”, the luminescent strength stays flat. It shows that it is recommended to administrate diluted Luciferin into vein so that we get stable luminescence from rats with relatively big in size.

It should be seriously considered that this kind of information would help researchers and contributes to the further implementation of the device accuracy as well.

Ex vivo Luciferin Administration

Luc Tg Rat the author has developed has been well known throughout the top-notch research institutes all over the world as a tool for academic co-researches. Herein after, even in Europe it will be concentrated on researches. One of the most effective applications is to indirectly measure ATP amount changes kept in preserved organs by measuring luminescent level continuously the resected organ immerged in medium (Figure 4).

(Figure 4)



In that case, it is indispensable to realize that we optimize the characteristics favorable for NightOWL. Luciferin-Luciferase reaction excels in quantitative analysis but the outline of light-emitting cells, tissues and organs becomes vague. And when it is placed in the multiple-hole dish, the device catches the light on the border of the holes and ends up with instrumental errors. As the optical axis of NightOWL high sensitive camera is designed to avoid catching scattered light, it is important to seed cells in nearby dishes to prevent from catching the leaked light. Also the plastic materials located around the dishes cause halation but we can avoid it by making the edges black to decrease the halation ratio (The dishes with edges painted in black are on sale but it is good enough to paint it in black by felt pen).

The author strongly believes that this kind of user-friendly knowledge also improve the performance of device and be a helpful partner for the researchers as well.

2. Handling of laboratory animals

Prof. Yoji Hakamata(Nippon Veterinary and Life Science University)

1) Over view of animal experimentation　（Power Point presentation）

2) Procedure of laboratory animal (Website streaming)
http://www.procedureswithcare.org.uk/administration-of-substances/
Handling
Administration

3) Wet hand practice (**Natsume Rat** model)
Handling of laboratory animals and inhalation anesthesia device system
Intragastric administration
Intravenous administration to tail vein
Blood collection from tail vein

3. Resection of Organs and Tissues from Luc Tg RAT and Cell Preparation

Prof. Shin Enosawa (National Center for Child Health and Development)

1) Picking of Organ/Tissues (Movie)

(In resecting order)

a. Blood

b. Abdominal Cavity

Liver

Pancreas

Spleen

Kidney, Adrenal Gland

Genital Organs (Testicle, Epididymis, or Ovary, Uterus)

Digestive Organs (Stomach, Duodenum, Jejunum, Ileum, Cecum, Large Intestine)

c. Thoracic Cavity/Cervical Region

Thymus Gland

Heart

Lung

Thyroid Gland

Salivary Gland

d. Head

Brain (Cerebrum, Cerebellum, Medulla Oblongata)

e. Others

Lymph Node (Armpit, Groin)

Skeletal Muscle

Subcutaneous Fat

2) Lymphocytic Segregation from Whole Blood (Movie)

3) Segregation of Hepatocyte (Movie)

4) Observation of Luminescent Organs, Tissues and Cells (Workshop)