Comparison of Firefly and Click Beetle Luciferase to Maximize Wavelength of Bioluminescence Imaging

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Abstract

This paper studied the relationship between the wavelength and the luciferase to determine whether Luc2 or CBR created a longer average wavelength. The wavelengths from previously conducted experiments on BLI were collected from a range of peer-reviewed academic papers and the mean wavelength of each luciferase was calculated along with a statistical test to test correlation. The results concluded that CBR produced an average wavelength of 642.67 nanometers (nm) which was higher that Luc2, which produced an average wavelength of 603.10 nm, meaning CBR, in terms of wavelength, would likely reduce photon absorption in the tissue more than Luc2 and therefore be more accurate at tracking cancer in comparison.

Introduction

Currently, breast cancer is the most common type of cancer in the world, affecting one in eight women in the United States alone. This type of cancer can often spread to other parts of the body, which can be difficult to track and, if gone unnoticed, is deadly. BLI is a tracking method that can be used in vivo (within the body) and in vitro (outside the body) using natural light emission properties of organisms. This process is able to visualize, track, and quantify tumor and other cell or bacterial growth. Currently, BLI is only used in small animals because surrounding tissue makes it harder for the photons to penetrate through and be detected. One of the factors to high photon absorption is a low photon yield. Hemoglobin and melanin in the body also tend to absorb blue to green light which has wavelengths averaging around 400 to 500 nm but absorption of wavelengths longer than 600 nm decrease significantly in comparison. Increasing the wavelength of the bioluminescence would make it easier for the photons to pass through the surrounding tissue. Longer wavelengths can penetrate through skin and tissue easier and farther because they are not as reflective and are also safer. Identifying a luciferase or luciferin that will produce a higher photon yield or increasing the wavelength of the bioluminescence would possibly make using BLI in larger animals or deeper tissue possible in the future.

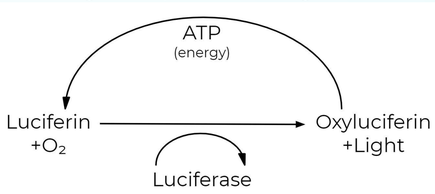

Fig 1: Chemical reaction of bioluminescence that leads to the production of light

Purpose

The purpose of this study was to identify the connection between luciferase and wavelength and to identify the optimal luciferase that would yield the longest wavelength by comparing a type of firefly luciferase (Luc2 or Fluc) and a type of red shifted click beetle luciferase (CBR) since longer wavelengths reduce photon absorption during in vivo use and are safer to the body than smaller, higher energy wavelengths.

Research Question

Is there a correlation between luciferase and wavelength and if so, which luciferase produces the average wavelength closest to infrared, Firefly luciferase (Luc2) or Click-beetle luciferase (CBR)?

Hypothesis

Alternative Hypothesis: There is a correlation between wavelength and luciferase and click beetle luciferase produces an averagely longer wavelength when compared to firefly luciferase, reducing harm to surrounding tissue and tissue absorption to improve accuracy on BLI use in vivo.
Null Hypothesis: Neither luciferase produces a longer average wavelength than the other or there is no significant correlation between which luciferase used in the experiment and the wavelength that is produced.

Methods

This study was completed using numerical data from various academic peer-reviewed papers that have been published within the last decade in order to ensure the data was relevant to current research. Utilizing systematic literature review and meta data analysis, all sources of data were obtained from the electronic databases of ScienceDirect, NCBI, EbscoHost, Google Scholar, ResearchGate, Nature, and PubMed.

The type of firefly luciferase used in this research was a type commonly used for BLI known as Luc2 and Fluc. The click beetle luciferase used was the red-light yielding click beetle luciferase, click beetle red or CBR. All wavelengths collected were quantitative values measured in nm containing three significant figures and were wavelength emitted at peak photon emission (λmax). In addition, all data was from in vivo tests tested in mice.

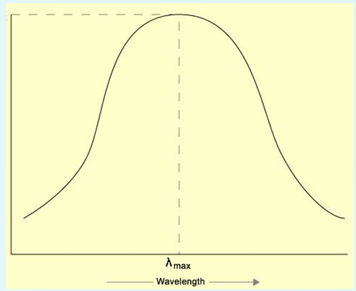

Fig. 2: λmax is pictured as the x-value of the peak of the normal curve

To find the average wavelength of each luciferase, a mean wavelength value was calculated for each luciferase. A two-tailed, two variable t-test was conducted using Excel on the wavelengths in order to test the correlation between the wavelength and luciferase.

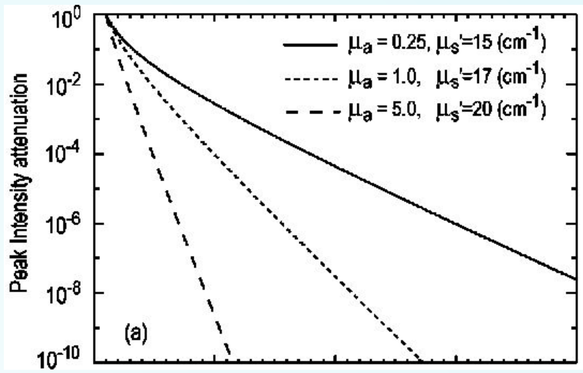

Fig. 3: (left) depicts 3 wavelengths tested at the same depth in vivo and their photon output. The solid black line represents a wavelength of 650 nm, the short dashed line is 590 nm, and the long dashed line is 550 nm.

Discussion

The average wavelength of the click beetle luciferase is longer than the average wavelength of Luc2 by a difference of 39.6 nm. In addition, the range for the p-value that this data was compared to was a value of 0.01. The p-value obtained of 0.000147 was below 0.01 giving an over 99 percent confidence that a strong correlation between wavelength and the type of luciferase used exists (Table 1). Therefore, the null hypothesis that there is no correlation between luciferase and wavelengths can be rejected. The alternative hypothesis that there is a correlation between the two can then be accepted. The data supports the hypothesis that CBR has an average longer wavelength than Luc2 and should therefore be used for in vivo imaging. The majority of the wavelengths used in this paper when using Luc2 were in the range of 590 nm to 615 nm. In comparison, the histogram of wavelengths produced using CBR had the highest number of wavelengths in the range of 615 nm to 640 nm.

Results

	Luc2	CBR
Mean Wavelength (nm)=	603	643
Standard Deviation (nm)=	27.7	50.1
p-value=	0.000147	

Table 1: (above) statistical values from the data in Table 2 (right). p-value calculated from a two-variable two-tailed t-test in Excel where Luc2 and CBR were the variables. Standard deviations were calculated for each variable as well as mean wavelengths. All values used three significant

Results

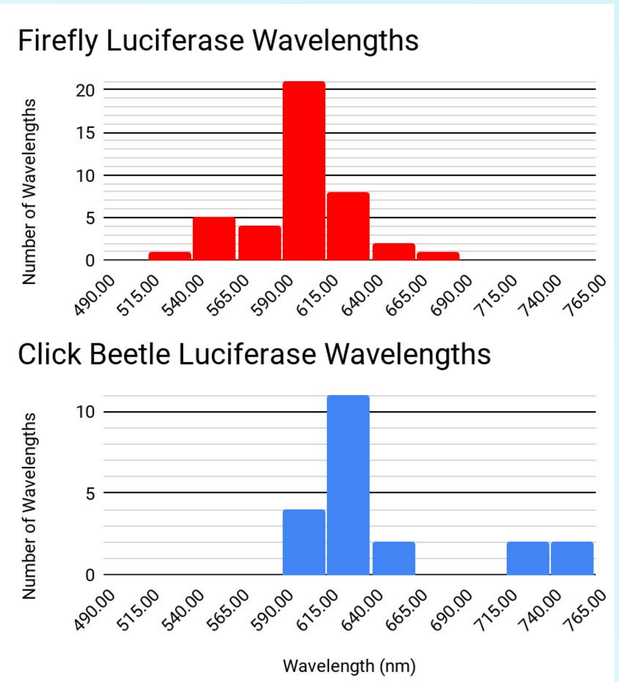

Fig. 4: (above) histograms of wavelengths found of Luc2 and CBR

Luc2	CBR
578	618
612	618
560	614
591	614
603	617
603	664
607	730
551	728
594	758
610	743
619	618
601	618
609	620
598	615
607	618
635	615
636	620
607	640
610	615
627	613
642	600
631	
625	
538	
600	
650	
559	
608	
678	
610	
580	
600	
612	
610	
617	
620	
612	
578	
560	
580	
600	
562	

Table 2: (right) wavelengths of Luc2 and CBR collected from various academic peer-reviewed papers measured in nanometers

Conclusion

Overall, this study concludes that click beetle luciferase in comparison to firefly luciferase yields longer wavelengths decreasing the photon absorption by surrounding tissues and reduce damage to the cells surrounding the area. Much of the current research is conducted using firefly luciferase. However, the results have determined that CBR should be used instead due to a longer average wavelength. Due to the decrease in energy of the photon, it would be able to travel deeper, perhaps make using BLI in larger subjects a possibility. If BLI were to be used in humans diseases such as breast cancer would be able to be tracked easier, safer, faster, and for a smaller cost than the methods currently used.