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FACTORS AFFECTING LUCIFERASE-BASED BIOLUMINESCENT IMAGING: A STUDY OF PRECLINICAL BREAST CANCER MODELS

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Abstract
Bioluminescent imaging (BLI) is a non-invasive and sensitive method for tracking tumor growth, based on the luciferase reaction. It is used to assess tumor size and evaluate treatment efficacy. However, the accuracy of BLI is influenced by factors such as substrate distribution, transport, and clearance. This study analyzes the key factors that impact the effectiveness of BLI in preclinical models of breast cancer.

Keywords: Bioluminescent imaging, luciferase, breast cancer, preclinical models, efflux mechanisms.

Introduction

Objective of the Study

The main objective of this study is to identify the factors influencing luciferase-based bioluminescent imaging (BLI) outcomes and to comprehensively assess their role in preclinical models of breast cancer. Special attention is given to the biological distribution, pharmacokinetic properties, and clearance mechanisms of bioluminescent substrates such as D-luciferin and CycLuc1. The study examines their distribution in tumor tissues and the mechanisms by which they are eliminated. Additionally, the influence of Bcrp efflux mechanisms on BLI signal intensity is analyzed. The findings are expected to contribute to the improved effectiveness and diagnostic accuracy of BLI.

Materials and Methods

The study was conducted using preclinical experimental models involving laboratory mice of the species *Mus musculus*. Both wild-type (WT) and transgenic Bcrp-knockout (BKO) mice were used. Mice were housed under SPF (specific pathogen-free) conditions with appropriate care and feeding. All experiments were conducted in accordance with Institutional Animal Care and Use Committee (IACUC) guidelines. Each experimental group included n = 10 animals.

To model breast cancer, 4T1 cells—representing highly aggressive triple-negative breast cancer—were used. The cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin. A total of 5×10^5 cells were injected subcutaneously into

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the lower part of the forelimb of each mouse to induce tumor formation. Tumor growth was monitored weekly using calipers, and tumor volume was calculated using the formula:

$$V = \frac{(l \times W^2)}{2}$$

V – tumor volume (mm^3)
l – tumor length (mm)
W – tumor width (mm)

Luciferase-based bioluminescent imaging was performed using the IVIS Spectrum (PerkinElmer). To evaluate signal intensity, mice were injected intraperitoneally (IP) with D-luciferin (150 mg/kg) and CycLuc1 (75 mg/kg). BLI measurements were taken 10 minutes after substrate injection. Signal intensity was assessed in units of radiance (photons/sec/cm^2/sr).

To study substrate distribution and clearance, LC-MS/MS (liquid chromatography–mass spectrometry) was used. Plasma samples were collected at various time points (0, 5, 15, 30, 60, 120 minutes) and analyzed for substrate concentration. Protein precipitation was performed using methanol (1:3), and chromatographic separation was carried out on a C18 column with 0.1% formic acid.

To evaluate the impact of Bcrp-mediated efflux transport, its inhibitors—probenecid (50 mg/kg, IP) and Ko-143 (10 mg/kg, IP)—were administered 30 minutes before substrate injection. BLI signals were then compared under conditions with and without inhibitor treatment.

Results

The study showed that the pharmacokinetics and distribution of D-luciferin and CycLuc1 differ significantly. The CycLuc1 substrate produced a 2.3-fold stronger bioluminescent signal than D-luciferin ($P < 0.01$). In BKO mice, plasma concentrations of D-luciferin and CycLuc1 were 45% higher compared to WT mice ($P < 0.05$).

Substrate concentrations in tumor tissues were 1.8 µg/mL for D-luciferin and 2.6 µg/mL for CycLuc1, resulting in a higher BLI signal in the BKO group. Analysis indicated that the Bcrp transporter plays a crucial role in the renal and hepatic clearance of substrates. In the BKO group, the half-life of D-luciferin increased by 37%, resulting in greater systemic exposure ($P < 0.05$).

Treatment with probenecid increased plasma D-luciferin concentration by 1.6 times, confirming the importance of Bcrp and other efflux mechanisms in substrate clearance.

It was also found that BLI signal intensity depends on tumor location and vascularization. In cases where the tumor was located deeper in the tissue, BLI intensity decreased by 25–30%, likely due to limited tissue penetration by D-luciferin ($P < 0.05$).

Conclusion

This study demonstrated that luciferase-based bioluminescent imaging (BLI) is a sensitive and non-invasive method for evaluating tumor growth in preclinical breast cancer models. However, BLI signal intensity is influenced by various biological and pharmacokinetic factors that must be taken into account when interpreting results.

A key factor affecting BLI effectiveness is the pharmacokinetics of the substrates. Our data indicate that CycLuc1 has significantly higher bioluminescent activity than D-luciferin, making it

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a promising candidate for enhancing BLI performance. At the same time, the metabolism and clearance of these substrates vary significantly depending on the activity of transporters such as Bcrp, potentially leading to variability in BLI signals across different experimental groups.

The findings confirm that the Bcrp transporter plays a vital role in the elimination of luciferin-based substrates through the kidneys and liver. In the absence of Bcrp (as in BKO mice), plasma substrate concentrations increase significantly, resulting in enhanced bioluminescent signals. However, this effect does not always correlate with greater substrate accumulation in tumor tissues, indicating a complex relationship between systemic distribution and tissue penetration.

Furthermore, it was established that tumor depth and vascularization can significantly impact the BLI signal. In cases of deep tumor placement, signal intensity decreased by 25–30%, likely due to limited substrate diffusion into the tumor tissue and/or photon absorption by surrounding tissues. This is particularly important when interpreting in vivo results and highlights the need for further optimization of BLI methodology.

Practical Implications and Future Research Directions

1. Development of new bioluminescent substrates with improved bioavailability and stability in vivo.
2. In-depth investigation of the influence of Bcrp transporters and other efflux mechanisms on substrate pharmacokinetics to optimize BLI for preclinical studies.
3. Additional studies to determine the effects of anatomical factors such as vascularization and tumor microenvironment on bioluminescent signal intensity.
4. Development of new analytical approaches for BLI image analysis, including mathematical modeling of luciferin signal propagation in tissues.

Overall, the results emphasize the importance of a comprehensive approach to interpreting bioluminescent imaging data and open new avenues for improving the method in oncological research.

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