

Non-Invasive Direct Visualization of Luciferase-Luciferin Emitted Light Producing True Images from an Orthotopic Lung Cancer Xenograft Model in Nude Mice Using an Ultra-sensitive Low-light Camera and Optics

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Abstract. *Background/Aim:* In vivo imaging with luciferase-luciferin has been limited by the inability to visualize the low emitted light, with the signal quantified only by photon counting using a cumbersome highly-cooled CCD camera in a dark room. In the present study, we demonstrate direct visualization of the luciferase-luciferin signal from an orthotopic lung cancer in a nude-mouse xenograft model with a sensitive low-light camera and optics. *Materials and Methods:* Mouse Lewis-lung carcinoma cells expressing luciferase (LL/2-Luc2) were injected transcutaneously into the lung of a nude mouse. One week later after cell injection, luciferase imaging for emission at 560 nm was performed using the UVP Biospectrum Advanced system after i.v. injection of D-luciferin potassium salt. The intensity of the visualized light was measured and quantified with the instrument. *Results:* A week following the implantation of LL/2-Luc2 cells in nude mice, the luciferase-luciferin signal from LL/2-Luc2 tumors in the lung was sufficiently visible through the skin to produce true images. At fifteen minutes, the intensity peaked and then progressively dropped due to clearance of luciferin from the tumor. *Conclusion:* Using the UVP Biospectrum Advanced system we demonstrated non-invasive visualization of true images from luciferase-luciferin signals from an orthotopic lung-cancer mouse model. The luciferase-

luciferin emitted light was directly visible through the skin which is a major improvement over previous photon counting to detect the luciferase-luciferin signal.

In order to visualize tumor growth, metastasis, the tumor microenvironment (TME), and cancer-cell behavior in mouse models, luciferase-luciferin imaging has been widely used for *in vivo* cancer research (1-9). Emitted light from a chemical reaction between luciferase and its substrate D-luciferin has a very low background *in vivo* (4, 10). However, *in vivo* detection of the luciferase-luciferin signal was previously dependent on photon counting, instead of direct visualization of the emitted light, due to inadequate instrumentation to detect the very-low-light emission (11).

In the present study, we aimed to investigate non-invasive direct visualization of true images of luciferase-luciferin light emitted from lung-cancer cells expressing luciferase in a xenograft nude-mouse orthotopic model with the UVP Biospectrum Advanced imaging system (Analytik Jena US LLC, Upland, CA, USA).

Materials and Methods

Cell lines. The mouse Lewis-lung carcinoma cell line expressing luciferase (LL/2-Luc2) was obtained from the American Type Culture Collection (Manassas, VA, USA). The cells were cultured in Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 with the GlutaMAX™ supplement (GIBCO, Grand Island, NY, USA), 10% fetal bovine serum, and 100 IU/ml of penicillin/streptomycin.

Mice. Nude mice aged 8 weeks (AntiCancer, Inc., San Diego, CA, USA) were used in the present study. All mice were bred in a barrier facility with a high efficacy particulate air (HEPA)-filtered rack, in standard settings with 12-hour light/dark cycles. The present protocol was approved by the AntiCancer Inc. Institutional Animal Care and Use Committee, following the National Institutes of Health Guide for the Care and Use of Animals.

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Key Words: Luciferase, luciferin, orthotopic, lung cancer, Lewis-lung carcinoma, nude mouse, non-invasive imaging, direct visualization.



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Establishment of an orthotopic Lewis-lung carcinoma nude-mouse model. LL/2-Luc2 cells (1.0×10^6) in phosphate-buffered saline (PBS) (10 μ l) and Matrigel[®] Matrix 10 μ l (Corning Inc., Corning, NY, USA) were injected transcutaneously into the mouse lung. A 1 ml 27G syringe containing the cell mixture was inserted distally above the right axillary midline of the mouse, the needle was moved proximally through the skin, and then the needle was penetrated between the ribs into the lung. After applying negative pressure to the syringe and checking for air bubbles, the syringe was plunged and 20 μ l of the cell mixture was administered into the lung. The needle was withdrawn while negative pressure was applied.

Imaging. The BioSpectrum Advanced 900 imaging system was used in the present study (Analytik Jena US LLC). The system camera uses ultra-low-light F0.95 optics to capture light accurately and an ultra-cooled (-70°C) back-thinned 13 μm m-sized pixel CCD sensor to collect low-intensity light, suitable for luciferase-luciferin low-light detection as well as fluorescence.

One week later after LL/2-Luc2-cell injection into the mice, D-luciferin potassium salt (Gold Biotechnology, St. Louis, MO, USA) (150 mg/kg) was injected intravenously into the tail vein. Images emitting at 560 nm were obtained every 5 min after the injection of D-luciferin. Image exposure time required 30 s. The intensity of the visualized light was measured and quantified with the UVP Biospectrum Advanced system (12-14).

Results

One week after the implantation of LL/2-Luc2 cells in nude mice, the luciferase-luciferin signal was captured through the skin to produce true images (Figure 1). The intensity of the emitted light was 130.99, 130.62, 136.58, 95.39, 76.66, and 58.24 obtained at 5 min intervals, respectively. The intensity of emitting light peaked at 15 minutes and gradually decreased and was 58.24 at 30 min (Figure 2 and Figure 3). After the mouse was euthanized by cervical dislocation, the mouse was dissected, and the tumor location was confirmed in the lung. There was no obvious tumor formation in the subcutaneous or thoracic regions, and the tumor was located on the surface of the middle lobe of the lung (Figure 4).

Discussion

The present study demonstrated a direct visualization and quantification of true images from luciferase-luciferin-emitted light captured non-invasively from an orthotopic lung cancer in a nude-mouse model using the UVP Biospectrum Advanced imaging system. The luciferase-luciferin emitted light decreased after peaking at 15 minutes because of the clearance of D-luciferin from the tumor.

The present study demonstrated an important improvement over previous studies with luciferase-luciferin imaging *in vivo* which relied on photon counting because the instrumentation could not collect sufficient very-low light to produce a visible image (11).

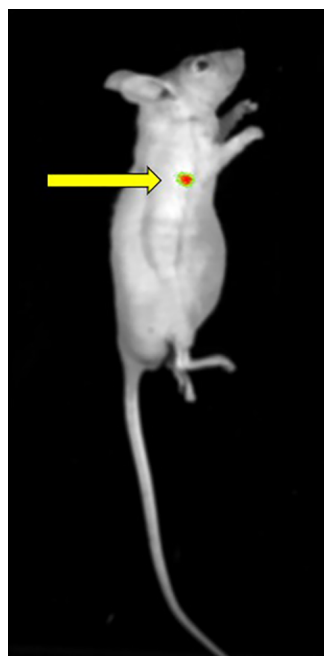


Figure 1. Direct true image of luciferase-luciferin-emitted light from a Lewis-lung carcinoma (LL/2-Luc2) implanted orthotopically in a nude mouse captured at 15 min after injection of luciferin. Exposure time was 30 s. (Arrow; luciferase-luciferin-emitted light true image).

The instrument used in the present study comprised optics and a camera that could detect very low amounts of light. This is essential for luciferase-luciferin imaging *in vivo* because the signal is so weak and in the present study the signal must be imaged non-invasively from the lung. A low-light-gathering instrument such as the one used in the present study is essential for luciferase-luciferin imaging *in vivo*. In order to have reproducible results, imaging must be timed before luciferin is cleared from the tumor and the image intensity decreases.

Conclusion

The current study demonstrated non-invasive direct visualization of emitted low light from luciferase-luciferin to produce true images from an orthotopic lung cancer mouse model using the UVP Biospectrum Advanced system. The direct images needed only 30 s of exposure time. The present results are a major improvement in luciferase-luciferin *in vivo* imaging.

Conflicts of Interest

AW, NC, and SG are employees of Analytik Jena. KM, SM, MS, BMK, and RMH are non-salaried associates of AntiCancer Inc. AntiCancer Inc. uses mouse models of cancer for contract research.

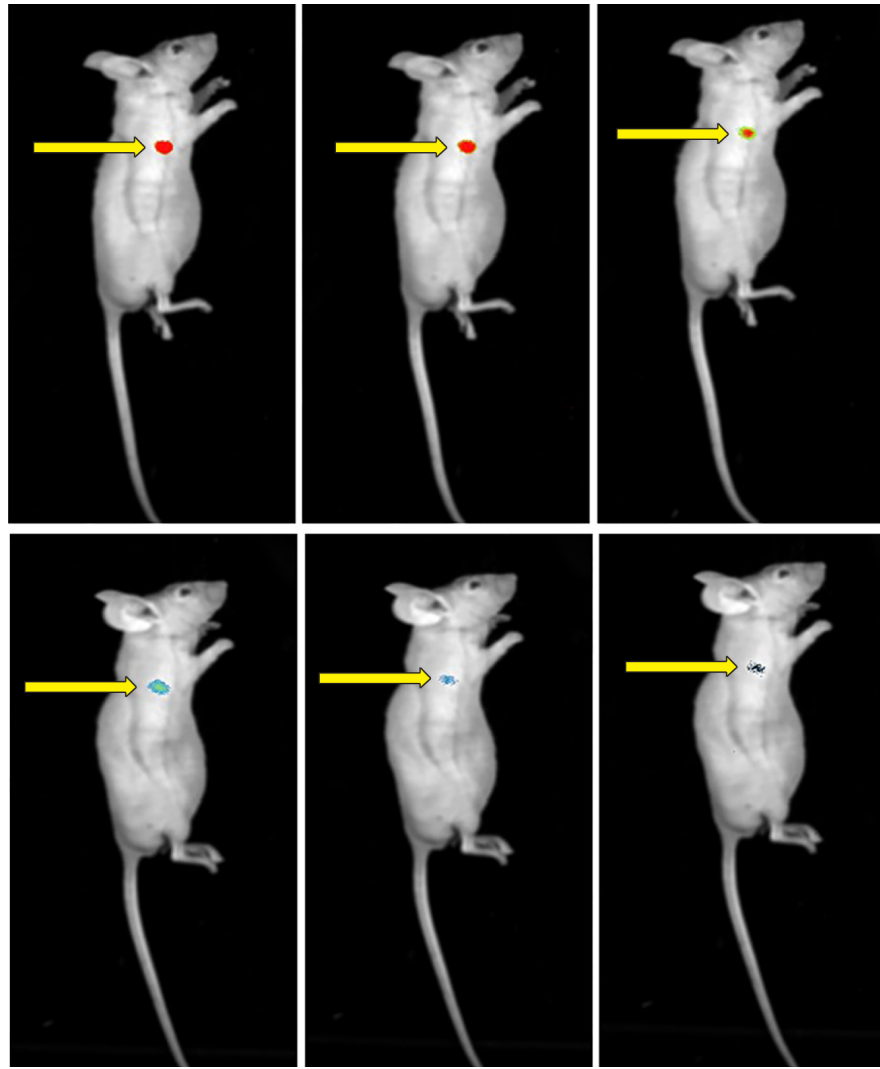


Figure 2. Time-course of direct true luciferase-luciferin images captured from a Lewis-lung carcinoma (LL/2-Luc2) orthotopic nude-mouse model. Images were captured at 5 min intervals over 30 min going from left to right and top to bottom. (Arrows; luciferase-luciferin-emitted light true image). Exposure time was 30 s.

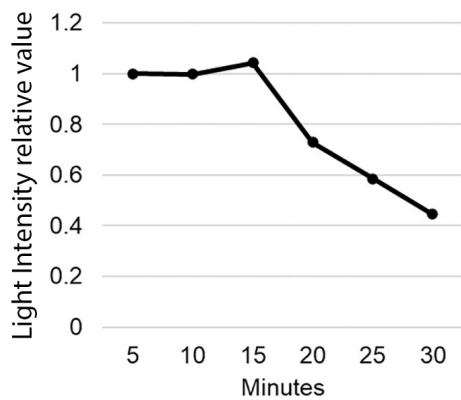


Figure 3. Time-course of the intensity of the emitted light from luciferase-luciferin in the Lewis-lung carcinoma (LL/2-Luc2) in the orthotopic mouse models shown in Figure 2.

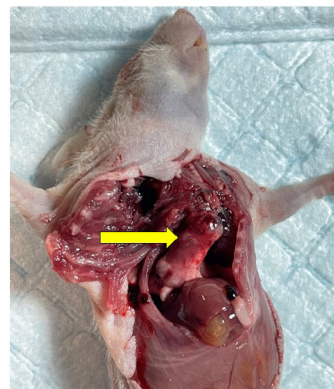


Figure 4. Location of the orthotopic Lewis-lung tumor (LL/2-Luc2) on the surface of the middle lobe of the right lung of a nude mouse. (Arrow; tumor in the lung).

Authors' Contributions

KM, SG, AW, and NC performed experiments. KM and RMH wrote this article. SM, MS, and BMK critically reviewed this article.

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References

- Burgos JS, Rosol M, Moats RA, Khankaldyyan V, Kohn DB, Nelson MD, Laug WE: Time course of bioluminescent signal in orthotopic and heterotopic brain tumors in nude mice. *Biotechniques* 34(6): 1184-1188, 2003. DOI: 10.2144/03346st01
- Nogawa M, Yuasa T, Kimura S, Kuroda J, Sato K, Segawa H, Yokota A, Maekawa T: Monitoring luciferase-labeled cancer cell growth and metastasis in different *in vivo* models. *Cancer Lett* 217(2): 243-253, 2005. DOI: 10.1016/j.canlet.2004.07.010
- Jenkins DE, Oei Y, Hornig YS, Yu SF, Dusich J, Purchio T, Contag PR: Bioluminescent imaging (BLI) to improve and refine traditional murine models of tumor growth and metastasis. *Clin Exp Metastasis* 20(8): 733-744, 2003. DOI: 10.1023/B:CLIN.000006815.49932.98
- Choy G, Choyke P, Libutti SK: Current advances in molecular imaging: Noninvasive *in vivo* bioluminescent and fluorescent optical imaging in cancer research. *Mol Imaging* 2(4): 153535002003031, 2003. DOI: 10.1162/15353500200303142
- Rehmentulla A, Stegman LD, Cardozo SJ, Gupta S, Hall DE, Contag CH, Ross BD: Rapid and quantitative assessment of cancer treatment response using *in vivo* bioluminescence imaging. *Neoplasia* 2(6): 491-495, 2000. DOI: 10.1038/sj.neo.7900121
- Morita N, Haga S, Ohmiya Y, Ozaki M: Long-term *ex vivo* and *in vivo* monitoring of tumor progression by using dual luciferases. *Anal Biochem* 497: 24-26, 2016. DOI: 10.1016/j.ab.2015.12.007
- Zhu H, Kauffman ME, Trush MA, Jia Z, Li YR: A simple bioluminescence imaging method for studying cancer cell growth and metastasis after subcutaneous injection of Lewis lung carcinoma cells in syngeneic C57BL/6 mice. *React Oxyg Species (Apex)* 5(14): 118-125, 2018. DOI: 10.20455/ros.2018.813
- Bhang HE, Gabrielson KL, Laterra J, Fisher PB, Pomper MG: Tumor-specific imaging through progression elevated gene-3 promoter-driven gene expression. *Nat Med* 17(1): 123-129, 2011. DOI: 10.1038/nm.2269
- Cekic C, Day YJ, Sag D, Linden J: Myeloid expression of adenosine A2A receptor suppresses T and NK cell responses in the solid tumor microenvironment. *Cancer Res* 74(24): 7250-7259, 2014. DOI: 10.1158/0008-5472.CAN-13-3583
- Lee H, Kim YP: Fluorescent and bioluminescent nanoprobe for *in vitro* and *in vivo* detection of matrix metalloproteinase activity. *BMB Rep* 48(6): 313-318, 2015. DOI: 10.5483/bmbrep.2015.48.6.054
- Rice BW, Cable MD, Nelson MB: *In vivo* imaging of light-emitting probes. *J Biomed Opt* 6(4): 432, 2001. DOI: 10.1117/1.1413210
- Mizuta K, Gallagher S, Wang A, Chang N, Morinaga S, Sato M, Kang BM, Hoffman RM: Head-to-head comparison of green fluorescent protein (GFP) imaging with luciferase-luciferin imaging *in vivo* using single-nanometer laser-excitation tuning and an ultra-low-light-detection camera and optics demonstrates the superiority of GFP. *Anticancer Res* 44(7): 2823-2826, 2024. DOI: 10.21873/anticancer.17094
- Kubota Y, Wang A, Chang N, Tarantino S, Gallagher S, Aoki Y, Masaki N, Obara K, Morinaga S, Tsunoda T, Hoffman RM: Precise non-invasive imaging mouse model of pancreatic cancer: very narrow band-width laser fluorescence excitation of green fluorescent protein provides ultra-bright tumor images with no skin autofluorescence. *Cancer Diagn Progn* 4(1): 30-33, 2024. DOI: 10.21873/cdp.10281
- Kubota Y, Aoki Y, Wang A, Chang N, Tarantino S, Gallagher S, Tsunoda T, Hoffman RM: Non-invasive fluorescence imaging of breast cancer metastasis to the brain in an orthotopic nude-mouse model with very-narrow-band-width laser excitation of red fluorescent protein resulting in an ultra-bright signal without skin autofluorescence. *In Vivo* 38(1): 69-72, 2024. DOI: 10.21873/invivo.13411

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