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In-Vivo Fluorescent and Bioluminescent Melanoma Graft Imaging in C57BL/6J Mice

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Introduction

Malignant melanomas—tumors that evolve from melanocytes (pigment-producing skin cells)—tend to metastasize to other organs, such as the lymph nodes, lungs, and brain, if not detected and removed at an early disease stage. Contrary to other skin cancers, such as basal cell carcinomas and squamous cell carcinomas, malignant melanomas are a deadly type of cancer. Although they are far less common than other skin cancers, they cause 75% of all skin-cancer related deaths (Cancer Research UK). Even though there are ongoing intensive research efforts in melanoma biology and possible treatments, investigations can benefit highly from the usage of in-vivo optical imaging techniques that offer the possibility to longitudinally monitor tumor growth inside an experimental animal, and thus allow the investigator to set up therapeutic studies and to evaluate treatment effects.

Furthermore, metastasis could be observed and validated in a representative environment using non-invasive methods without harming the animal(s). Using in-vivo imaging, fewer animals are needed to perform therapeutic studies compared to the classical approach in which a representative number of animals have to be sacrificed at different time points to compare post-mortem tumor growth. Also, this method allows the investigator to follow the same animal through repeated administrations of a therapeutic agent.

Material and Methods

B16 cells (ATTC: CRL-6475) were derived from a malignant melanoma developed in C57BL/6 mice, thus, creating a syngeneic model system in this strain. In culture, the line grows as a mixture of epithelial-like and spindle-shaped cells (ATCC). B16 cells grow well at several locations, i.e., subcutaneously and in the peritoneal cavity. They also form lung, liver, and kidney metastases when injected intravenously, rendering B16 a representative research model for malignant melanoma. The GFP-expressing fluorescent B16 line was established with an in-vitro transduction protocol using retroviral particles derived from the LZRS construct packaged in Phoenix cells. Tumor cells (5 x 105) were injected subcutaneously 30 minutes before image acquisition. Intraperitoneal and intravenous injections were similarly performed using 0.25-1 million cells of B16-F10-GFP or luciferase-expressing cells (B16-F10_Lux). In-vivo imaging and data analysis was performed with a Bruker In-Vivo MS FX PRO system and the Bruker Molecular Imaging software suite including MI 5.3.1 and Multispectral software. For spectral unmixing, (for details see Bruker BioSpin Application Notes AP0093 and AP0094) several fluorescence excitation projections were chosen (400–480 nm) in combination with a 535 nm wide-angle emission filter. Each projection was obtained with 1 x 1 binning, f-stop 2.8, and 20 seconds acquisition time. For anatomical co-registration, an X-ray image (10 seconds, 2 x 2 binning, f-stop 2.8) was performed.

Briefly, spectral unmixing provides a tool to separate signals derived from non-specific fluorescent origins (e.g., the autofluorescent properties of tissues, skin, fur, and body fluids) from target signals (e.g., GFP or DsRed).

For the acquisition of signals derived from luciferase-expressing tumors, 3 mg D-luciferin from 10 mg/mL stock, dissolved in sterile saline (BioSynth AG, Switzerland), was injected via the tail veins, and mice were imaged 10 minutes post injection. Acquisition of the luminescent signal was for 5 minutes (4 x 4 binning, f-stop 2.5).

Results & Discussion

With the application of in-vivo optical imaging, it was possible to obtain clear and distinct signals from both GFP- and luciferase-expressing B16-F10 grafts (see Fig. 1-3). While with a standard GFP-specific excitation/emission filter setting gave no clear identification of that a subcutaneous GFP signal was possible (Fig. 1), the Spectral unmixing function enabled the researchers to separate background autofluorescence from GFP-derived signals (Fig. 2). The signals obtained are congruent to ex-vivo investigations. Thus with spectral unmixing, as little as a half-million cells could be visualized in-vivo and non-invasively.

Next to GFP acquisitions, imaging a luciferase-expressing model was also tested and the bio-luminescence reaction was captured using different cell densities (i.e., cell numbers) as well as injection sites. As little as 250,000 cells could be imaged in deep tissue such as the intraperitoneal area (see Fig. 3A). When different injection routes were compared, it became clear that intravenous injection of tumor cells leads to slower establishment of lesions (Fig. 3B); whereas, intraperitoneal spread is rapid. At this point in time, subcutaneous injection leads to the development of palpable and easily imaged lesions (data not shown).

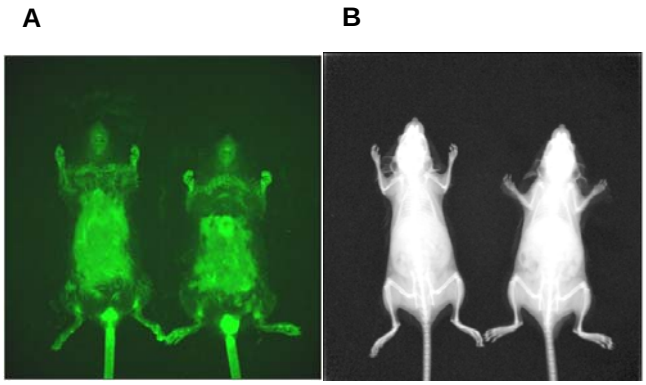

Figure 1. Imaging without spectral unmixing. (A) An image acquired with the ex 470 ± 10 nm/em, 535 ± 17.5 nm filter set illustrates the strong background derived by the mouse autofluorescence. A clear GFP-derived signal could not been identified. (B) The respective X-ray image.

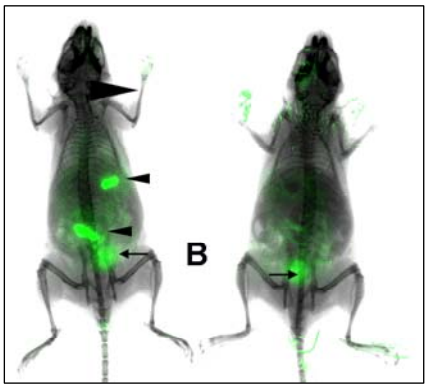

Figure 2. After spectral unmixing and co-registration with an X-ray image. Fur and background fluorescence is unmixed and subtracted, producing a much clearer representation of the target signal. Bladder signal remains, as it overlaps with the wavelengths for GFP detection. The arrowheads indicate specific signals by GFP-positive subcutaneous lesions in the left mouse. Non-specific signals from the bladder (arrows) and urine (seen on anus and paws) is evident in the right mouse.

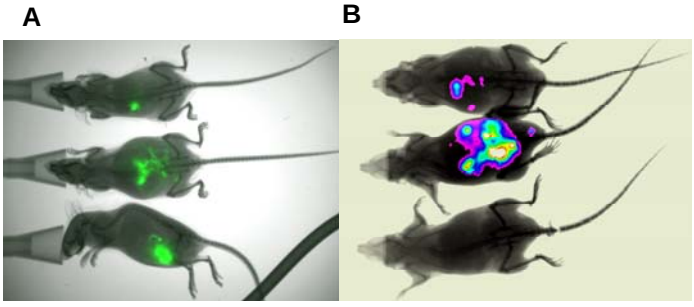

Figure 3. Detection of B16-Lux colonies injected intravenously and into the intraperitoneal cavity. (A) Varying cell doses ranging from 0.25 million cells (top mouse), 0.5 million (center mouse), and 1 million cells (bottom mouse) were injected into the peritoneal cavity and imaged. (B) 5 x 105 cells were injected intravenously (top) and intraperetonially (center). As a control, only saline was injected IP and IV into the mouse at the bottom. Mice were imaged 18 days past injection with the In-Vivo MS FX PRO imager.

Conclusion

Non-palpable, subcutaneous GFP-positive cell deposits, consisting of as few as half a million cells can be detected using the In-Vivo MS FX PRO Imaging System. This system facilitates conduction of temporal therapeutic response experiments starting immediately upon tumor cell injection. Similarly, the imager can also image deep tissue lesions after intraperitoneal or intravenous injection using luciferase-expressing melanoma cells. X-ray imaging also allows for precise anatomical localization of tumor grafts.

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