The use of *in vivo* bioluminescence for designing synthetic gene delivery systems

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Introduction

Bioluminescence imaging (BLI) is a powerful versatile tool for monitoring transgene expression in living animals. BLI can be combined with biofluorescence imaging (BFI) to provide further spatial and temporal information. Here, we show some applications of BLI and BFI that have been used to evaluate synthetic gene delivery systems (SGDS) for *in vivo* gene transfer to the lungs or the skeletal muscles.

Materials and Methods

Animal experimentations are carried out following protocols approved by the Institutional Animal Care and Research Advisory Committee. Original cationic lipids (UMR CNRS 6521, Brest) and Firefly Luciferase-encoding plasmid DNA (pDNA) are assembled to form supramolecular aggregates called lipoplexes. These are administrated in mice (Janvier Labs) following a specific delivery route. BLI is done thereafter *via* non-invasive imaging of luciferase expression (with emission at 560 nm) thanks to a CCD camera (NightOWL NC320, Berthold). Following an intraperitoneal injection of D-Luciferin, animals are anesthetized and laid inside an acquisition chamber. Luminescence images are captured with a binning and for an exposure time depending on the intensity of the signals. Luminescence is quantified within the regions of interest in the unit of photons/sec. For BFI, the same device is used following an adequate acquisition procedure with appropriate filters.

Results and Discussion

BLI allows to evaluate – in a semi-quantitative way – *in vivo* luciferase expression. This allows to distinguish between effective and non-effective SGDS[1], notably depending on the administration route (Fig.1). BLI can be challenged by measuring luciferase expression in organ/tissue homogenates, thus highlighting some technical limitations (e.g. tissue absorption and sensitivity threshold). BFI allows to track fluorescent lipoplexes once administered in animals and to correlate biodistribution with transgene expression[1,2]. BLI also emphasizes the critical role of the pDNA to deliver[3]; when its sequence is optimized, transgene expression can be monitored after single or multiple administration(s) throughout the lifespan of animals.

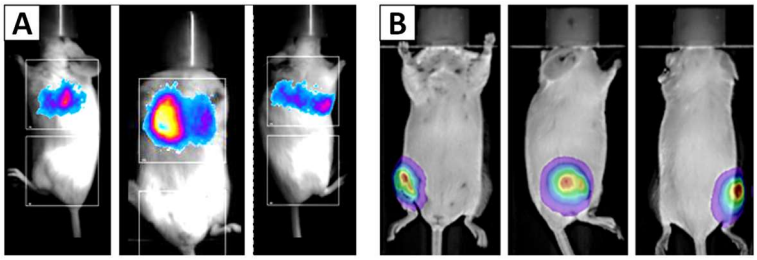

Figure 1: Examples of *in vivo* bioluminescence in living mice following efficient gene delivery to the lungs (A) or to the muscles in the hind limb (B). Each mouse was imaged according to three positions. Luminescence intensity is color-coded.

Conclusion

Besides some limitations/drawbacks, BLI has many practical advantages for *in vivo* screening of gene delivery systems. It allows the identification of efficient SGDS following general (systemic) or local (aerosol, hydrodynamic limb vein) administrations in living animals. Future developments in this field could consist in multiplexing luminescent and fluorescent reporters for gaining deeper insights into the potential/fate of SGDS.

Bibliography

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