Monitoring of Renilla Luciferase Activities in-vitro and in-vivo

Ivo Que[1]

Introduction

Firefly and *Renilla* luciferases, because of their distinct evolutionary origins, have dissimilar enzyme structures and substrate requirements. These differences make it possible to selectively discriminate between their respective bioluminescent reactions. Firefly luciferase is a 61kDa monomeric protein that does not require post-translational processing for enzymatic activity. Thus, it functions as a genetic reporter immediately upon translation. Photon emission at 560 nanometer (nm) is achieved through oxidation of beetle luciferin in a reaction that requires ATP, Mg^{2+} and O_2 (Figure 1). Under conventional reaction conditions, the oxidation occurs through a luciferyl-AMP intermediate that turns over very slowly. As a result, this assay chemistry generates a "flash" of light that rapidly decays after the substrate and enzyme are mixed. *Renilla* luciferase, a 36kDa monomeric protein, may function as a genetic reporter immediately following translation. The luminescent reaction catalyzed by *Renilla* luciferase utilizes O_2 and coelenterateluciferin (coelenterazine) (Figure 1). In the assay, the kinetics of the *Renilla* luciferase reaction provides a stabilized luminescent signal that decays slowly over the course of the measurement.

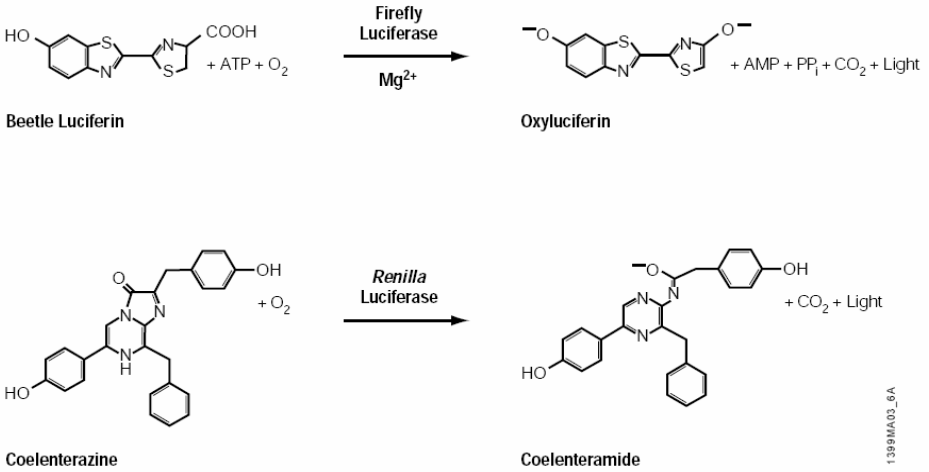

Figure 1: Bioluminescent reactions catalyzed by firefly and *Renilla* luciferase

[1]Leiden University Medical Centre, Department Endocrinology, The Netherlands; IQue@lumc.nl

Renilla luciferase has a spectral peak at 480 nm and is sodium dependent and ATP independent.

Experiment

Bhaumik and *Gambhir* have recently demonstrated that *Renilla* luciferase (Rluc) is a promising bioluminescence reporter gene that can be used for noninvasive optical imaging of reporter gene expression in living mice, with the aid of a cooled charged couple device (CCD) camera.

In our study, we explore the expression of a novel EnduRen™ and ViviRen™ *Renilla* luciferase reporter gene (Promega) in vitro and in vivo.

B02F11-N11 cancer cells were transfected with pCMV-luc and/or pCMVRluc plasmid.
72 hours after transfection and trypsinization the cells were washed in PBS. 10^4 cells were used for the in vitro experiment (Figure 2).

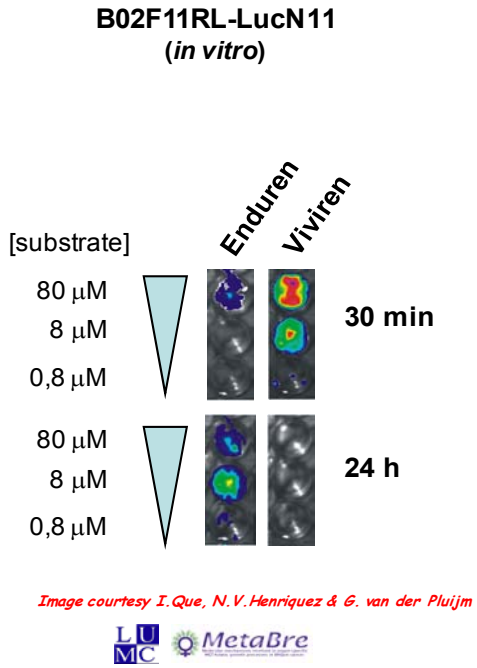

Figure 2: Light production of 10E4 B02F11RL-LucN11 cancer cells exposed to EnduRen™ and ViviRen™

Balb C nude mouse was anesthetized with isoflurane and followed by injection of two concentrations of B02F11(RL)-lucN11 cells at different place of mammary fat pads.
1 minute after injection of 10 µl *Renilla* substrate (subcutaneous, intraperitoneal and tail vein) the mouse was placed in the LB 981 NightOWL front illuminated molecular imaging system under anesthesia and measured. The acquisition time was 1 minute with 7x7 binning (Figure 3).

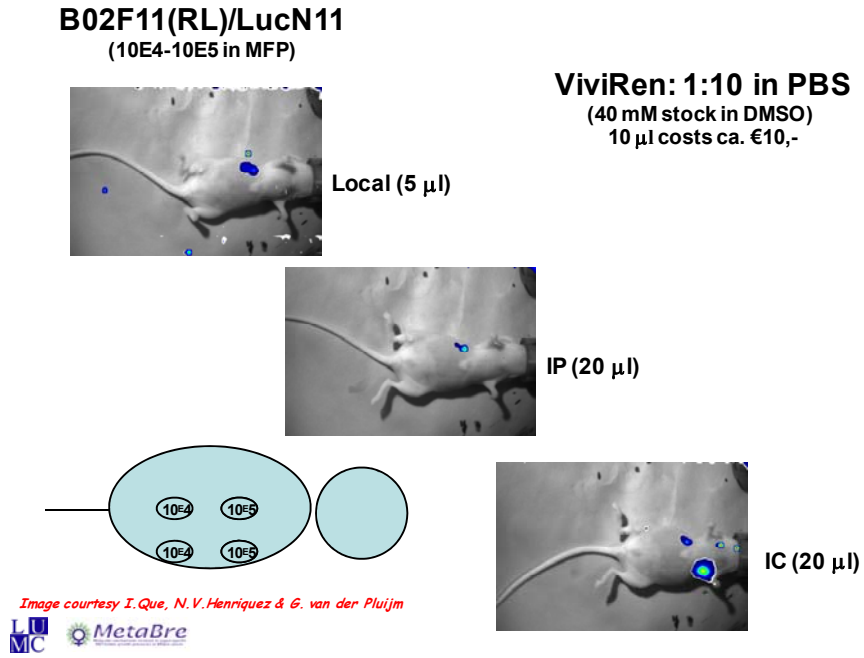

Figure 3: Expression of different concentration B02F11 RL-luc N11cancer cells in mammary fat pads, subcutaneous, intraperitoneal and tail vein injection of ViviRen™ substrate

Balb C nude mouse, expressing B02F11RL-lucN11 bone tumor, was anesthetized with Isoflurane and injected with 20 µl ViviRen™ substrate via tail vein and the bioluminescence was measured with the LB 981 NightOWL at different time points (pixel bining 7x7).

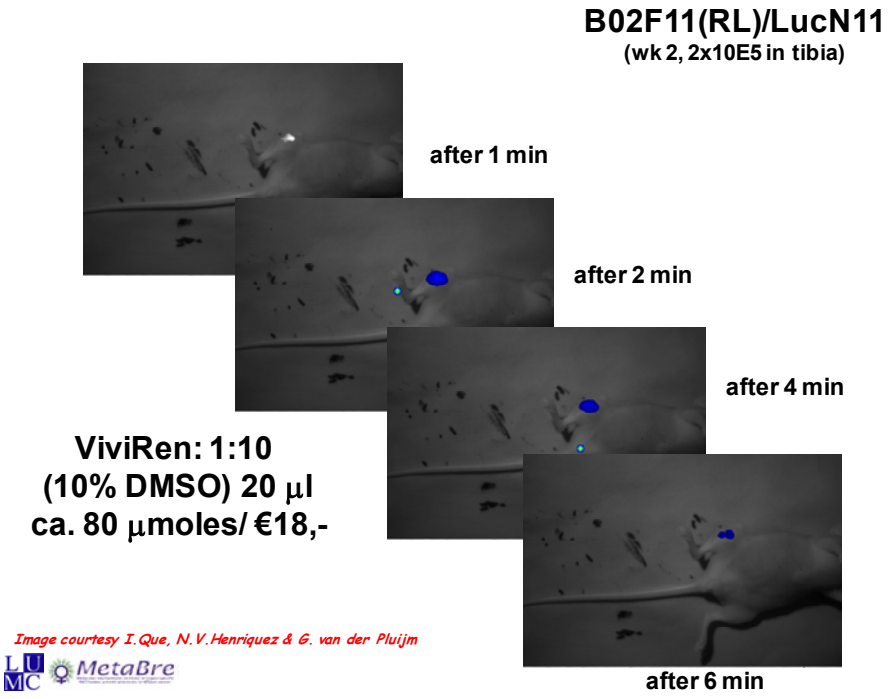

Figure. 4: Kinetics of light production from mice with bone tumor after tail vein injection of ViviRen™ substrate

Note

It's important to know which type *Renilla* luciferase you are using, there are many *Renilla* luciferase types from different companies available. The measurement must done directly after injection of the substrate and for the first time I will suggest to do time kinetics.
It's also important to maintain healthy cells. Some cell lines become more sensitive to transfection agents after a large number of passages. Perform also a control transfection by varying cell confluence and using different transfection reagents. Cell toxicity will be increased by low cell density and by too much transfection reagent.

Renilla luciferase is **not** soluble in water. It's soluble in ethanol, methanol and DMSO, but the concentration must be as low as possible. It is advisable not to inject a substrate dissolve in 70% ethanol in a mouse!

Summary

Bioluminescence imaging (BLI) of luciferase reporters, firefly and *Renilla* luciferase, provides sensitive and quantitative detection of cellular response with minimal post-transfection processing.
Renilla luciferase catalyzes the oxidation of coelenterazine by oxygen to produce light. The expression of the encoding gene enzyme can be detected at very low level.

Material

- Balb C nude mouse (CBy/cby.CQ-fox1<nu>/J)
- LB 981 NightOWL Molecular Imaging System (BERTHOLD Technologies)
- EnduRen™/ViviRen™ (Promega)
- pCMV-luc plasmid
- pCMVRluc plasmid

Literature

- S.Bhaumik and S.S.Gambhir : *Optical imaging of Renilla Luciferase reporter gene expression in living mice; PNAS; vol.1; page 377-382; jan 2002.*
- Promega descriptions of EnduRen/ViviRen
- M. Otto-Duessel, V. Khankaldyyan, I. Gonzales-Gomez, M.C. Jensen, W.E. Laug and
- M. Rosol: *In vivo testing of Renilla Luciferase substrate analogs in an orthotopic murine model of human glioblastoma; Mol. Imaging 2006; Apr-Jun;5(2); 57-64.*