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Gaussia Luciferase-a Novel Bioluminescent Reporter for Tracking Stem Cells Survival, Proliferation and Differentiation in Vivo

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Abstract

Transduction of bone-marrow derived human mesenchymal stem cells with lentivirus vectors expressing a novel and naturally secreted bioluminescent reporter was undertaken as an approach to track stem cells survival, proliferation as well as differentiation using bioluminescent imaging techniques. A self inactivating lentivirus vector expressing both Gaussia luciferase (a novel secreted luciferase that is over 2000 times brighter than firefly or Renilla luciferase) and green fluorescent protein (Lenti-Gluc-GFP) was used for these studies. Transduction of human mesenchymal stem cells with this lentivirus vector at an MOI of 30 resulted in approximately 100% transduction as assessed by GFP fluorescence. Luciferase activity in the conditioned media was found to be directly proportional to the number of stem cells suggesting that Gluc could be a useful marker for assessing stem cells growth and survival in vivo. Stem cells-expressing Gluc mixed with matrigel and implanted subcutaneously in nude mice could be easily visualized and tracked over time using standard in vivo bioluminescence imaging. Since Gaussia luciferase is naturally secreted, the extent of cell survival and proliferation in vivo could be assessed by measuring its levels in few microliters of blood. Similarly, circulating stem cells (injected intravenously) could also be monitored over time by measuring the activity of Gluc in the blood. We have also used a Gaussia luciferase based reporter plasmid systems (pSiscreen system from Targeting Systems) for the effectiveness of siRNAs in silencing target genes in stem cells. Once an siRNA "hit" is found, it can be co-transfected to stem cells expressing Gluc and the effect of gene silencing on stem cell survival or fate in vivo can be monitored by in vivo bioluminescence imaging together with quantitative assessment of Gluc activity in the blood.

Objectives

1) Transduction of mesenchymal stem cells with a lentivirus vectorexpressing a novel secreted bioluminescent luciferase reporter gene (Gaussia luciferase, Gluc)

2) Evaluation of Gaussia luciferase as an indicator of cell survival and proliferation

3) Evaluation of Gaussia luciferase as a reporter to study tracking and survival of stem cells in vivo

Advantages of Gluc reporter

• Gluc is secreted, therefore activation can be monitored by assaying few microliter of conditioned medium with no need for cell lysis.

• Gluc is 2000-fold more sensitive than commonly used reporters such as luciferases from Renilla or firefly and the secreted alkaline phosphatase (SEAP).

• Gluc can detect as few as 10 mammalian cells expressing it .

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Lentivirus vector expressing both GFP and CFP

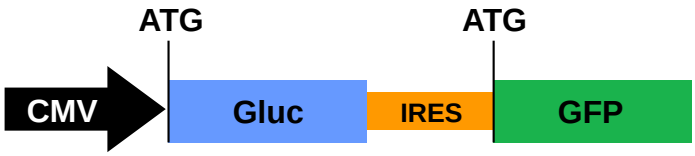

***Figure 1 :** Gluc and GFP separated by an internal ribosomal entry site (IRES) we cloned in a self inactivating lentivirus vector. Lentiviral vectors expressing Gaussia luciferase, red and green firefly luciferase and GFP are commercially available from Pluristem Innovations (www.pluristeminnovations.com)

Gluc level in blood is linear with respect to implanted cell number

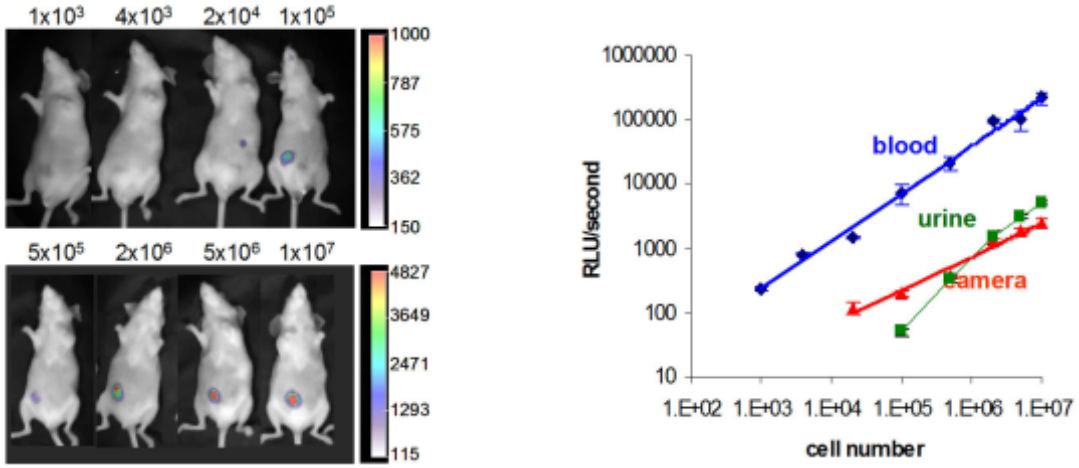

***Figure 2 :** (Left) Different numbers of Gli36 human glioma cells expressing-Gluc (Gli36-Gluc) were implanted subcutaneously in mice and 3 days later, mice were injected i.v. with coelenterazine (4 mg/kg body weight) and imaged with CCD camera. (right) Total relative light units (RLU) per second was calculated for tumors in (red line). Gluc activity was measured in 5 µl blood (blue line) or urine (green) after addition of 100 µL 100 µM coelenterazine and acquiring photon counts using a luminometer.

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Gluc level in the blood to monitor cell proliferation and death

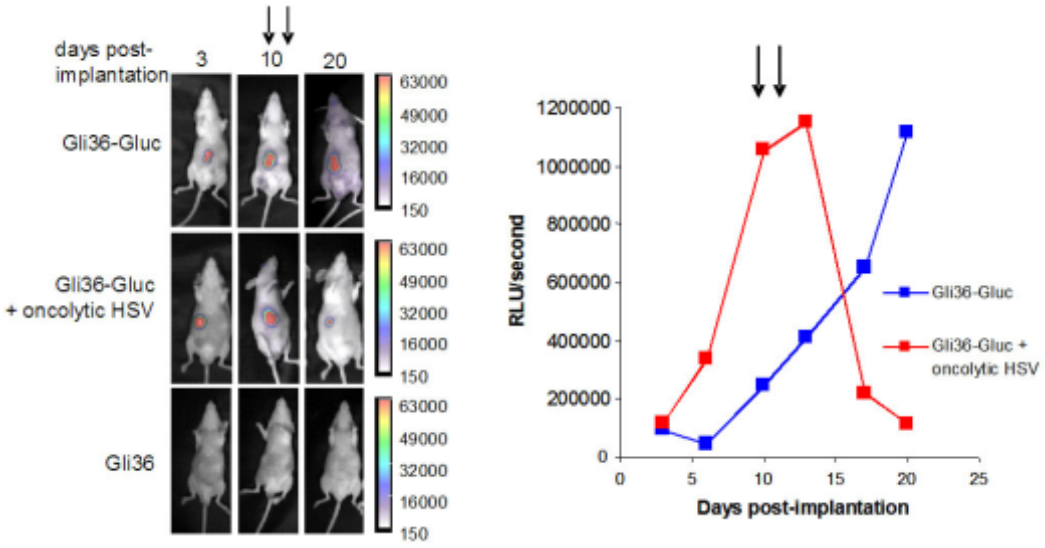

***Figure 3 :** Mice were implanted with one million Gli36-Gluc cells subcutaneously and tumor growth was monitored by both in vivo bioluminescence imaging (left) and the Gluc blood assay (right). At day 10 and 13 post-implantation, one set of mice was injected intra-tumorally (arrows) with an oncolytic HSV vector and another set with PBS (blue line). Gluc blood level from tumors treated with virus decreased showing that Gluc blood assay can be used to monitor cell death.

Efficient transduction and in vivo imaging of mesenchymal stem cells with a lentivirus expressing Gaussia luciferase and GFP

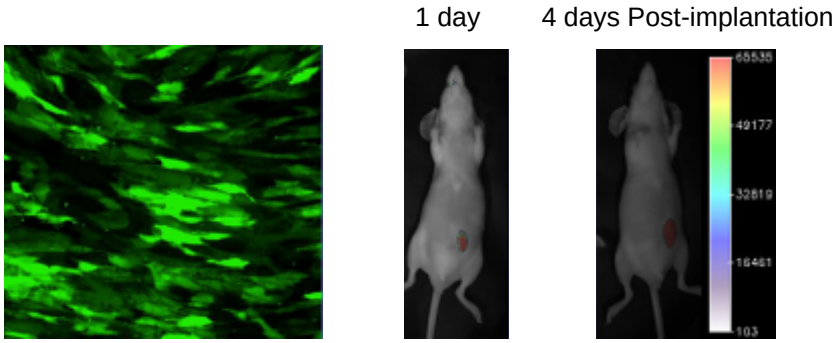

***Figure 4 :** Primary human bone marrow-derived mesenchymal stem cells were transduced with a lentivirus vector carrying the expression cassette of Gaussia luciferase and GFP, separated by an IRES element, under control of the CMV promoter (VMV-Gluc-IRES-CFP) at an MOI of 30. The results indicate that the transduction efficiency was nearly 100% (left). One million of these cells were mixed with matrigel and implanted subcutaneously in nude mice. At different time points, mice were injected with coelenterazine and imaged using a CCD camera (right). The signal increased over time showing that these cells proliferated in vivo.

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Gluc blood assay to monitor circulating stem cells

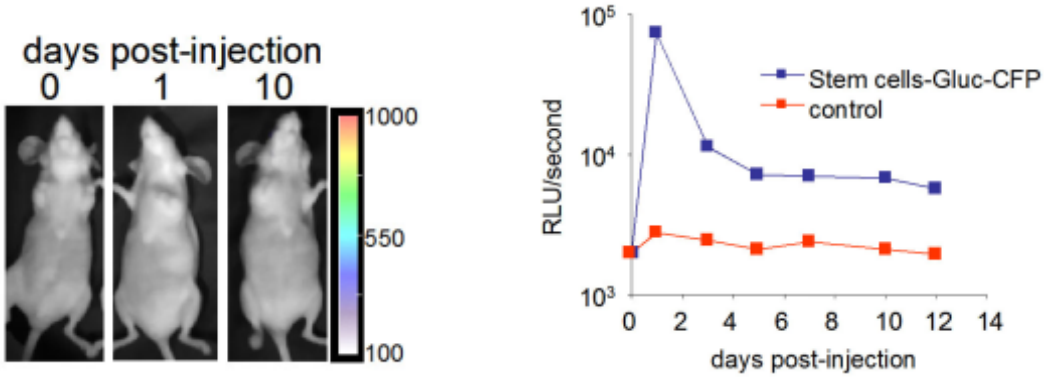
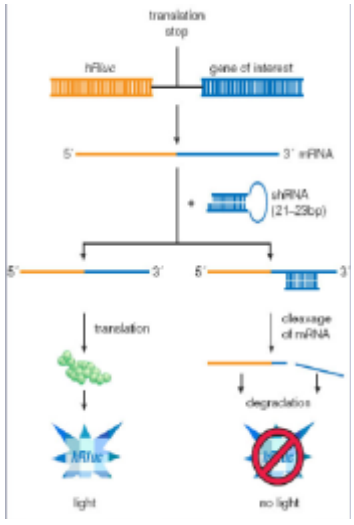
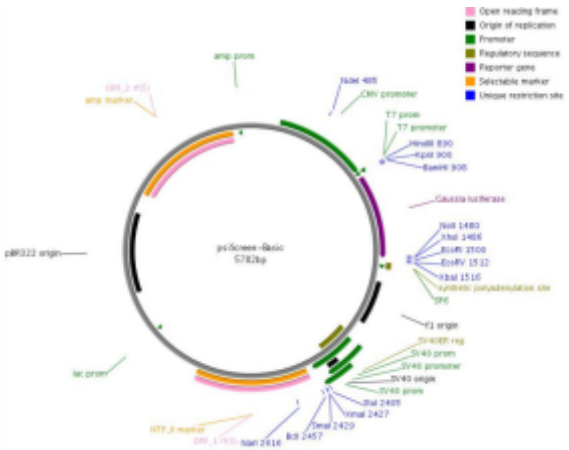


***Figure 5 :** One millions stem cells expressing Gluc and GFP or PBS control were injected i.v. in nude mice. Prior to injection and at several time-points the Gluc activity was monitored using the CCD camera and in 20 µL blood samples using the luminometer. At no time point the CCD camera was able to detect the stem cells, however, the Gluc level in blood indicated that a significant number of cells survived the injection and did not proliferate.

Gaussia luciferase expression vectors can be used to screen siRNAs for their effectiveness in silencing target genes in stem cells



Gaussia Luciferase expression



No Luciferase activity

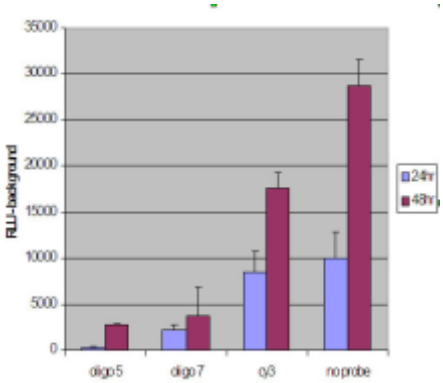


***Figure 6 :** The psiScreen vectors are designed with Gluc as the primary reporter gene and the target gene subcloned into an MCS site downstream of the luciferase gene after the stop codon. Measurement of decreased Gluc activity serves as an indicator of RNA interference (left and middle). Screening of different siRNAs (small interfering siRNAs) against p53 using the psiScreen system in supernatants of human mesenchymal stem cells expressing Gluc (right). Gaussia luciferase based psiScreen systems as well as several products using gaussia luciferase and other luciferases for studying gene expression are commercially available from Targeting Systems, CA (www.targetingsystems.com

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Ongoing Work

• Gaussia luciferase expression in stem cells will be used as a tool to study stem cell differentiation and quantitaive expansion of stem cells in a bioreactor

• Evaluating the ability of several recombinant proteins to differentiate mesenchymal cells engineered with a lentiviral vector expressign GFP and Gaussia luciferase.

Conclusions

• Gaussia luciferase is a powerful tool for studying stem cell growth and survival

• Gaussia luciferase can be used to track the fate of small numbers of implanted stem cells in vivo using bioluminescent imaging techniques.

• Gaussia luciferase could be potentially useful for studying gene silencing in stem cells

• Gaussia luciferase could potentially also be useful to study stem cell differentiation in vivo

Wurdinger T, Badr C, Weissleder R, Breakefield X and Tannous B. Gaussia luciferase for ex vivo monitoring of in vivo processes. Nature Methods (in press)