Novel luciferase labeling technique to improve imaging of orthotopic pancreatic cancer model

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Introduction

Bioluminescence-labeling allows sensitive non-invasive sequential imaging of tumor development and early metastasis. However, current methods for the genetic modification of cells typically use integrating genotoxic viruses that can potentially disrupt the molecular behavior of cancer cell lines due to their random nature of integration. Here, we utilized a DNA vector that comprises a S/MAR (Scaffold/Matrix Attachment Region) element to stably modify cells that can subsequently be used in xenograft studies providing robust and long term expression without adversely affecting cellular behavior or function.

Materials and Methods

Human BxPC-3 pancreatic cancer cells (ATCC) were stably transduced with a pSMARt-UCB-Luc and cultured for 4 weeks under selection. Colonies first formed after this period were isolated and expanded in normal medium and evaluated for luciferase expression and molecular integrity of the DNA vector.

For in vitro proliferation assays, parental and luciferase-labeled BxPC-3 cells were cultured in 96-well plates. A known chemotherapeutic drug (e.g. gemcitabine (3µM)) was used as an reference compound. The cells were cultured for 5 days and the effects of the gemcitabine was identified by measuring the cells at days 1, 3 and 5 using a WST-1 proliferation kit (Roche Diagnostics) and a CellTiter-Glo luminescent cell viability assay (Promega). For in vivo studies, 3 x 10⁵ parental BxPC-3 and BxPC-3-Luc cells were inoculated into the pancreas of athymic nude mice (Harlan, Elektra SLCD). Tumor-bearing mice were treated with vehicle or gemcitabine (80 mg/kg, p.o. 1 time, weeks 6-13). Tumor growth was monitored by bioluminescence imaging (BLI) once a week (IVIS Lumina 2. Caliper Life Sciences). After sacrifice, orthotopic tumors were characterized using histology (H&E staining) and immunohistochemistry (Polyclonal IgG Human/Fetal Luciferase antibody, Novus Biologicals). Stained slides were scanned using Panoramic slide scanner (3D Histech).

FIGURE 1. Generation of genetically modified cells. pSMARt-UCB-Luc was introduced into BxPC-3 cells using PEG-fect reagent (Poly/science). This figure represents the results of a typical experiment where cells can be imaged at each stage of the procedure. (A) Cells imaged 48 hours after transfection in a 8-well plate. (B) Within 4 weeks, colonies of cells expressing luciferase can be isolated and seeded into 10 cm petri dishes (C) and expanded ad infinitum (D). Illustrates a confluent flask of BxPC-3 cells robustly expressing the transgene luciferase. The arrow represents the increasing intensity of bioluminescence (from blue to red) of transgenic Luciferase expression.

Three months following the initial transfection procedure, total DNA was isolated from the BxPC-3-Luc cell line by plasmid rescue (Promega). Southern blot analysis and PCR demonstrated that the DNA vector remained episomal and the expression cassette remained intact (data not shown).

FIGURE 2. Proportionality determined by measuring number of viable cells (A) and number of metabolically active cells (B). Control compound gemcitabine (c) inhibits proliferation of both parental and luciferase-labeled cells combined with base line control (BL).

FIGURE 3. Cell number correlates with luminescence output. Serial dilutions of BxPC-3-Luc cells were made. Luminescence was recorded 10 minutes after reagent addition. Values represent the mean of four replicates for each cell number. There is a linear relationship between the luminescence signal and the number of cells from 0 to 100,000 cells per well.

In vitro analyses

Current Xenografts: Construction of gene transfer vectors is lengthy and costly. S/MAR DNA Xenografts: Construction of S/MAR vector is versatile and simple.

Generation of stable cell lines using viral vectors is lengthy and costly. S/MAR DNA xenografts are half the cost and can be used for short or long term studies.

Integration of viral vectors into DNA results in chromosomal instability and therefore does not affect genetic information of the cells. S/MAR DNA xenografts can be frozen for extended periods and before use. All forms of S/MAR xenografts can be quantified using BLI

Interaction of oncogenic viral vector with host immune system will affect radiance readings. S/MAR DNA xenografts can therefore be used in multiple studies on the same animal.

Subcutaneous xenografts are required for calibration measurements. Formation of tumors is easily monitored using BLI imaging and can be monitored.

Conclusions

In conclusion, S/MAR DNA vectors are able to generate genetically modified cells without the limitation of random genomic integration, whilst providing extra-large plasmids to mitotic stability and robust and sustained transgene expression. When utilized in orthotopic xenograft studies, these luciferase-expressing cells form a reliable and essential non-invasive imaging platform that improves substantially efficacy testing of anticancer drug candidates.

Histopathological assessment

FIGURE 5. End-point analyses of tumor weight (A) and size (B) indicated gemcitabine efficacy. Data analysis were executed using Welch’s t test or Mann-Whitney U test. Mann-Whitney U test was used only if the data was not normal even after a standard transformation (logarithmic, square root, or inverse).

Histopathological assessment of parental BxPC-3 xenograft (C), BxPC-3-Luc xenograft (D) (H&E-staining), IHC staining of BxPC-3-Luc cell pellet (E) and BxPC-3-Luc xenograft luciferase-staining (F).

Acknowledgements

We thank Ms. Minna Auramo, Mrs Natalia Halinen-Kittila, Mr. Yuonen Koraki, Mr. Riikka Kyttman, Ms. Antti Luokkala, Mr. Jari Sepanen and Mrs. Johanna Ægna for their technical assistance. This work financially supported by the Eurostars Programme (E83-08-05).

References


Figure 4. (A) BxPC-3-Luc tumor growth was monitored once a week and at sacrifice with IVIS Lumina 2. Images were taken 10 minutes after subcutaieous injection (luciferin 3 mg/mouse ip). Results are shown as tumor area, radius and tumor flux. (B) Reference compound (gemcitabine) inhibited tumor growth but the difference compared with vehicle was not statistically significant. The tumor growth curves were analyzed using a mixed model with fixed effects for treatment and day as well as a random effect for the interaction. The hypotheses were tested using model contrasts and p-values were adjusted for multiple comparisons. Zero values in the data were replaced by a very small value (1/2 of the detection limit) in order to apply the logarithmic transform to the flux and average radiation measurements.

Orthotopic pancreatic in vivo tumor model

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