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 be subsequently used in xenograft studies providing robust and long term expression without adversely affecting cellular behavior or function.

Aim of the Study

The aim of this study was to establish an improved, cost efficient, quick and simple method to genetically modify human cancer cells with a bioluminescent reporter gene that can be utilized for *in vivo* drug development.

Materials and Methods

Human BxPC-3 pancreatic cancer cells (ATCC) were stably transfected with a pSMARt-UBC-Luc and cultured for 4 weeks under selection. Colonies that 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 assay, parental and luciferase-labeled BxPC-3 cells were cultured in 96-well plates. A known chemotherapy drug, 0.1 μM gemcitabine (Lilly), was used as 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, 3×10^4 parental BxPC-3 and BxPC-3-luc cells were inoculated into the pancreas of athymic nude mice (Harlan, the Netherlands). Tumor-bearing mice were treated with vehicle or gemcitabine (60 mg/kg, q3dx4 i.p, one week pause, q3dx4 i.p). Tumor growth was followed 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 Goat IgG Human/Firefly Luciferase antibody, Novus Biologicals). Stained slides were scanned using Pannoramic slide scanner (3D Histech).

Transfection with a S/MAR vector

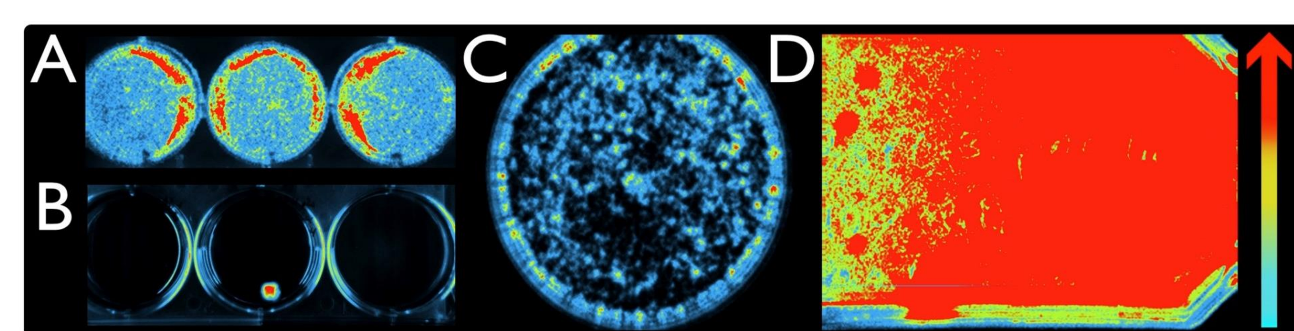
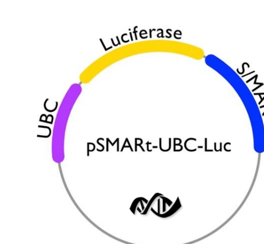


FIGURE 1. Generation of genetically modified cells. pSMARt-UBC-Luc was introduced into BxPC-3 cells using PeqFect reagent (PeqLab/VWR).

This figure represents the results of a typical experiment where cells can be imaged at each stage of the procedure. (A) Cells imaged 24 hours after transfection in a 6-well plate. (B) Within 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, Southern Blot analysis and PCR demonstrated that the DNA vector remained episomal and the expression cassette remained intact (data not shown).

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Histopathological assessment

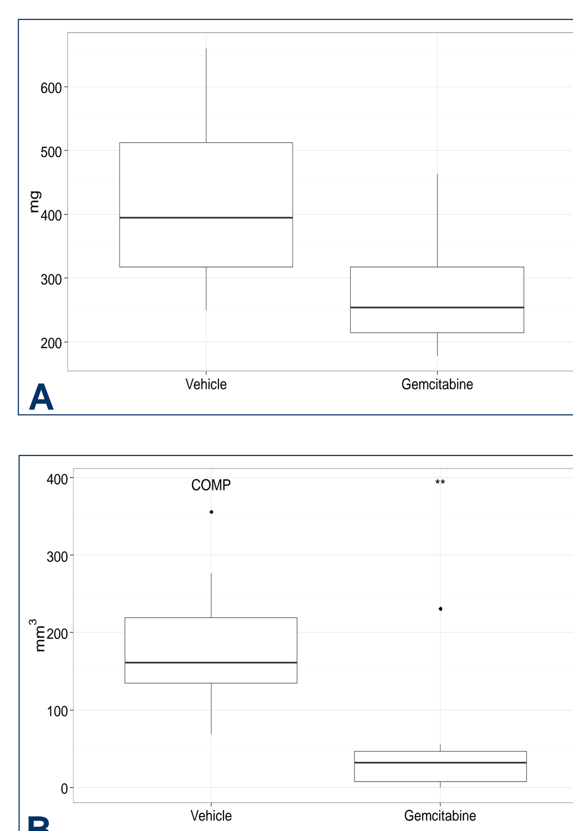
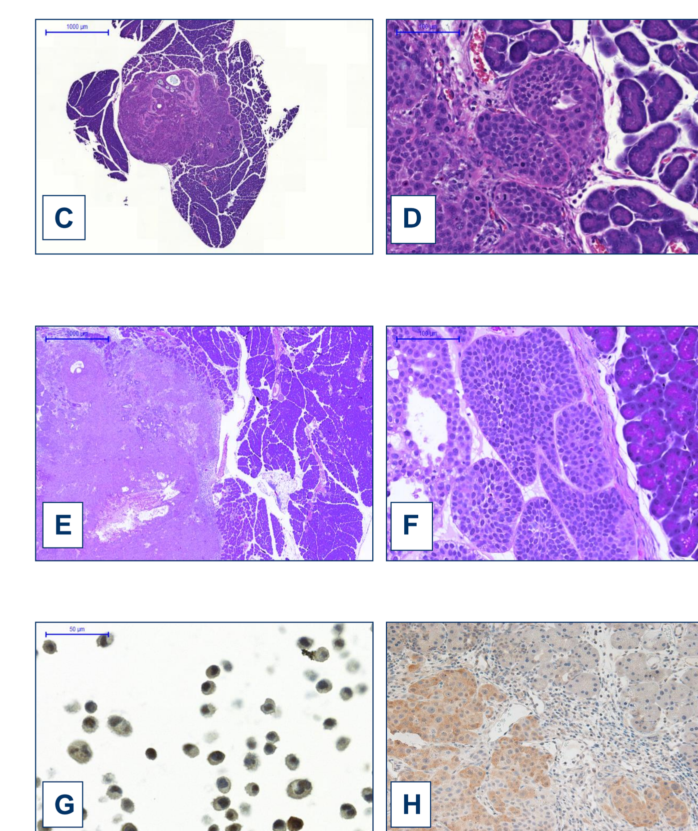


FIGURE 5. End-point analyses of tumor weight (A) and size (B) indicated gemcitabine efficacy. Data analysis were executed using Welch's t test or Mann-Whitney U test. Mann-Whitney U test was used only if the data was not normal even after a standard transformation (logarithmic, square root, or inverse).

Histopathological assessement of parental BxPC-3 xenograft (C-D) and BxPC-3-luc xenograft (E-F) (H&E-staining). IHC staining of BxPC-3-luc cell pellet (G) and BXPC-3-luc xenograft luciferase-staining (H).



Orthotopic pancreatic *in vivo* tumor model

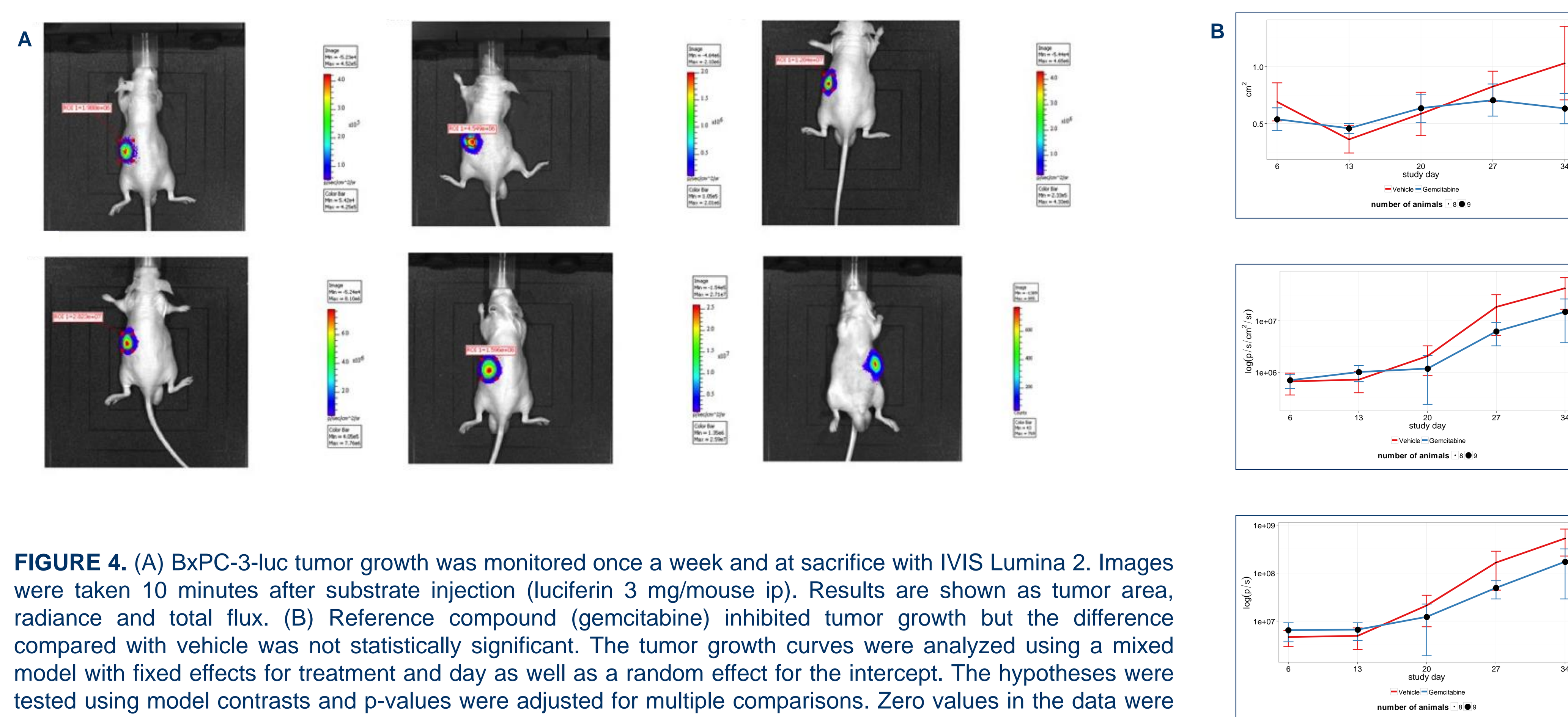


FIGURE 4. (A) BxPC-3-luc tumor growth was monitored once a week and at sacrifice with IVIS Lumina 2. Images were taken 10 minutes after substrate injection (luciferin 3 mg/mouse ip). Results are shown as tumor area, radiance and total flux. (B) Reference compound (gemcitabine) inhibited tumor growth 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 intercept. 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.

In vitro analyses

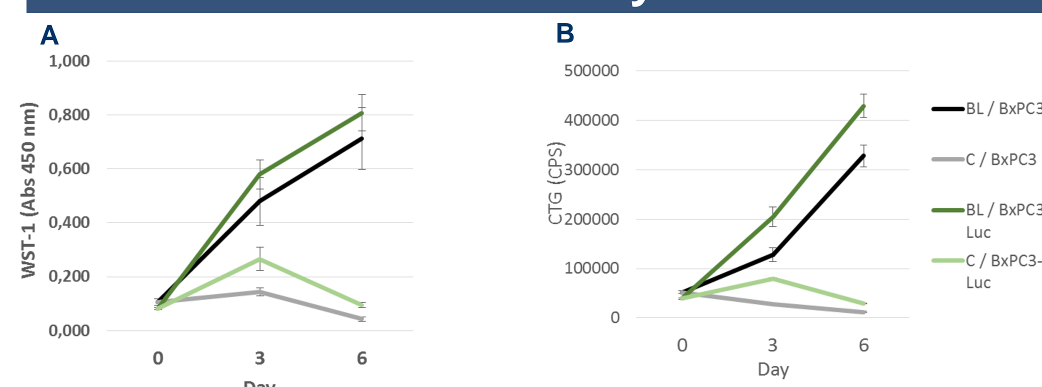


FIGURE 2. Proliferation was determined by measuring number of viable cells (A) and number of metabolically active cells (B). Control compound gemcitabine (c) inhibits proliferation of both parental and luciferase-labeled cells combined with base line control (BL).

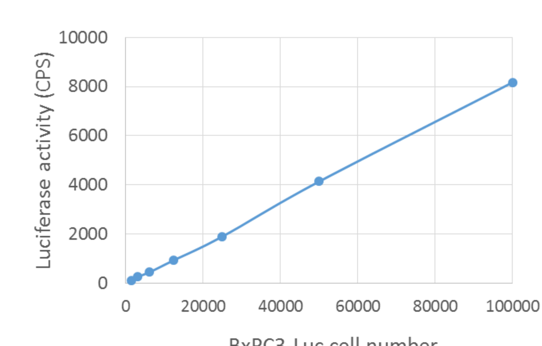


FIGURE 3. Cell number correlates with luminescence output. Serial dilutions of BXPC-3-luc cells were made. Luminescence was recorded 10 minutes after reagent addition. Values represent the mean of four replicates for each cell number. There is a linear relationship between the luminescence signal and the number of cells from 0 to 100.000 cells per well.

Summary

Current Xenografts	S/MAR DNA Xenografts
Construction of gene transfer vectors is lengthy and costly	Construction of S/MAR vectors is versatile and simple
Generation of stable cell lines using viral vectors is lengthy and costly	Generation of stable cell lines using S/MAR DNA is cost-efficient and takes only a month
Integration of viral vectors/pDNA alters genetic background of tumor cells	S/MAR DNA remains episomal and therefore does not affect genetic information of the cells
Viral vectors have a small insert capacity	S/MAR DNA has an unlimited insert capacity
Interaction of oncolytic viral vector with host's immune system	S/MAR DNA is non-immunogenic
Subcutaneous xenografts are required for caliper measurement	All forms of S/MAR xenografts are quantifiable using BLI
Large groups of animals required to as it is not known whether all animals will develop a tumor	Formation of tumors are easily visualized with BLI in the same animal
Metastasis is infrequent in subcutaneous xenografts and if do occur, are undetectable	Metastases are easily visualized 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-chromosomal 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.