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RESEARCH ARTICLE

Irradiation, Cisplatin, and 5-Azacytidine Upregulate Cytomegalovirus Promoter in Tumors and Muscles: Implementation of Non-invasive Fluorescence Imaging

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Abstract

Purpose: The cytomegalovirus (CMV) promoter is one of the most commonly used promoters for expression of transgenes in mammalian cells. The aim of our study was to evaluate the role of methylation and upregulation of the CMV promoter by irradiation and the chemotherapeutic agent cisplatin *in vivo* using non-invasive fluorescence *in vivo* imaging.

Procedures: Murine fibrosarcoma LPB and mammary carcinoma TS/A cells were stably transfected with plasmids encoding CMV and p21 promoter-driven green fluorescent protein (GFP) gene. Solid TS/A tumors were induced by subcutaneous injection of fluorescent tumor cells, while leg muscles were transiently transfected with plasmid encoding GFP under the control of the CMV promoter. Cells, tumors, and legs were treated either by DNA methylation inhibitor 5-azacytidine, irradiation, or cisplatin. GFP expression was determined using a fluorescence microplate reader *in vitro* and by non-invasive fluorescence imaging *in vivo*.

Results: Treatment of cells, tumors, and legs with 5-azacytidine (re)activated the CMV promoter. Furthermore, treatment with irradiation or cisplatin resulted in significant upregulation of GFP expression both *in vitro* and *in vivo*.

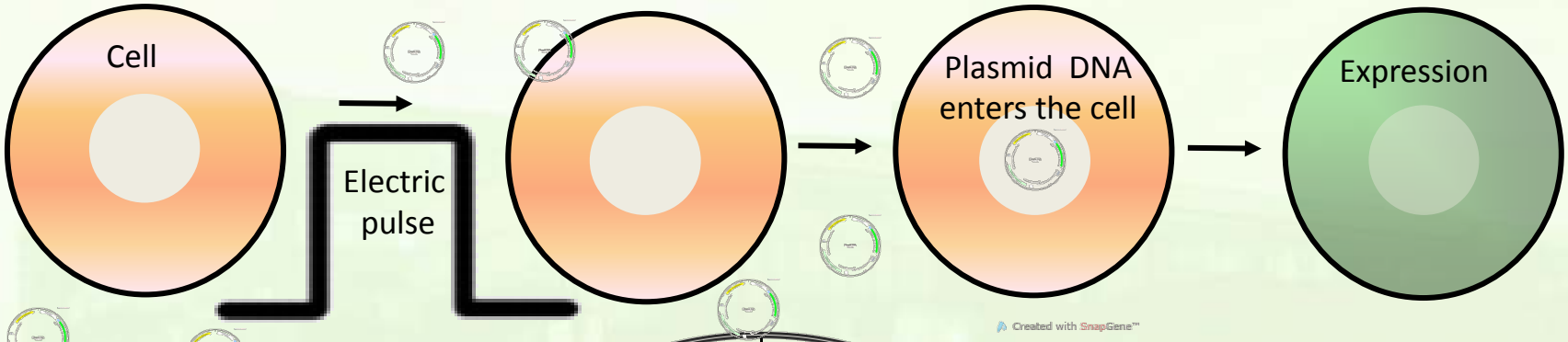
Conclusions: Observed alterations in the activity of the CMV promoter limit the usefulness of this widely used promoter as a constitutive promoter. On the other hand, inducibility of CMV promoters can be beneficially used in gene therapy when combined with standard cancer treatment, such as radiotherapy and chemotherapy.

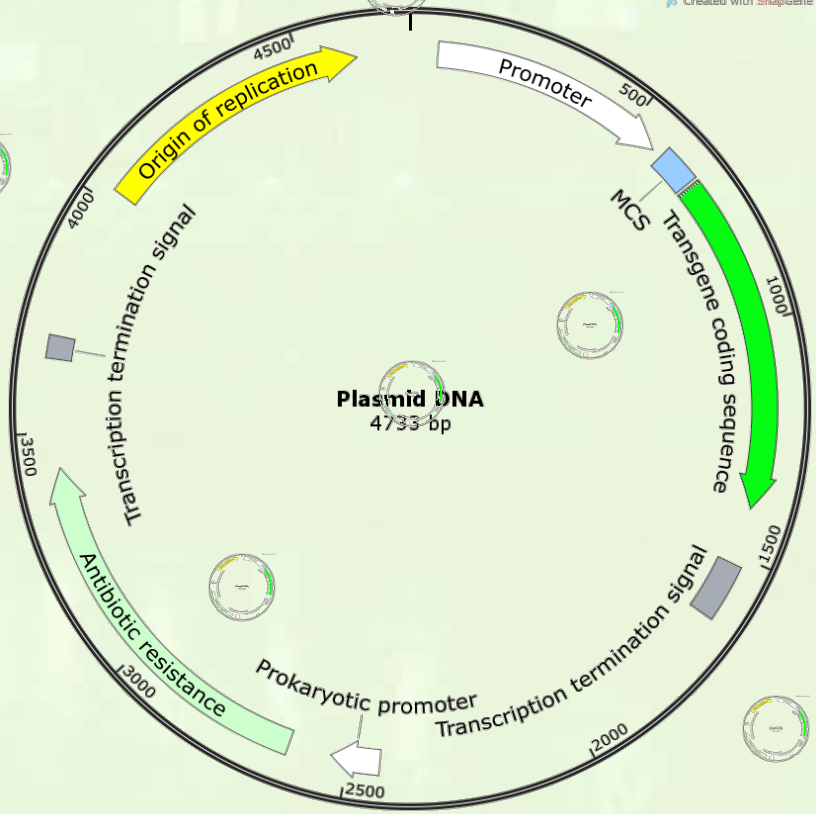
Key words: CMV promoter, Fluorescence imaging, Irradiation, Cisplatin, Demethylation, *In vivo*, Mice, Mammary carcinoma, Fibrosarcoma

Department of experimental oncology

- Translational research
  - Animal models
    - Gene therapy studies
      - Gene electrotransfer

Gene electrotransfer (GET)





Plasmid = small, circular, double stranded, extra-chromosomal DNA

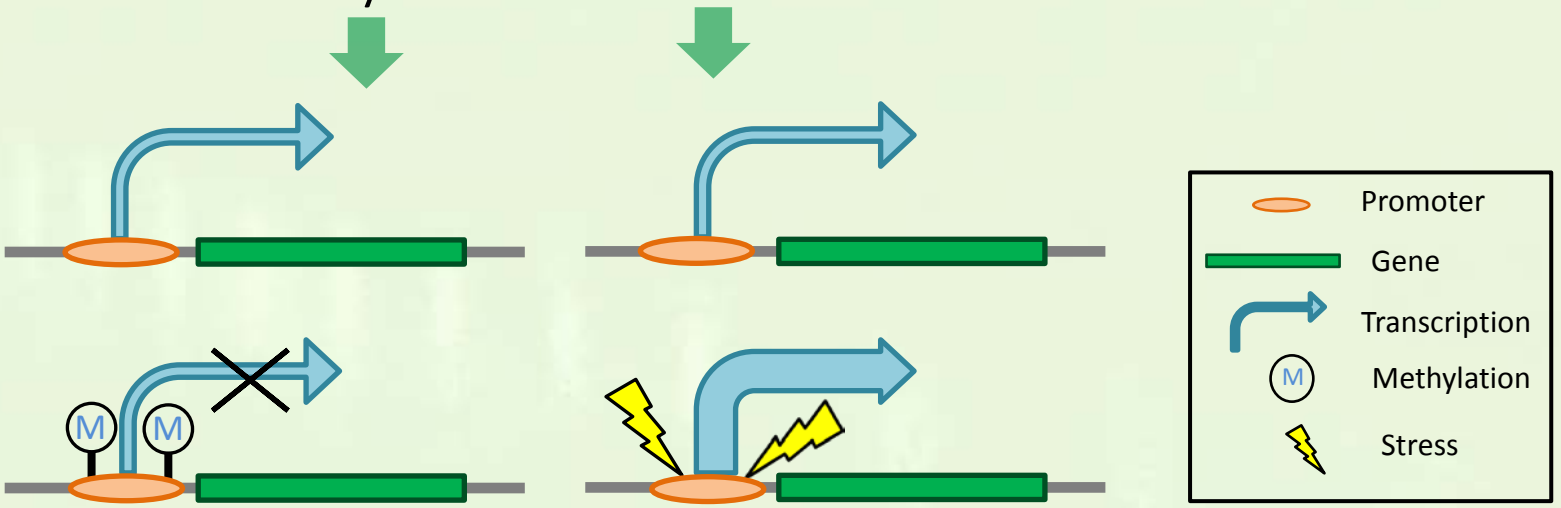
Cytomegalovirus (CMV) promoter

Optimal promoter should provide sustained and well-defined expression levels of the transgene.

CMV promoter is the most commonly used promoter in gene expression vectors used for research or clinical purposes.

- IN THEORY: strong, constitutive, unregulated, pan-specific promoter.
  - IN PRACTICE: its transcriptional activity
    - is strongly dependent on the host-cell transcriptional environment,
    - can be changed under specific conditions like:

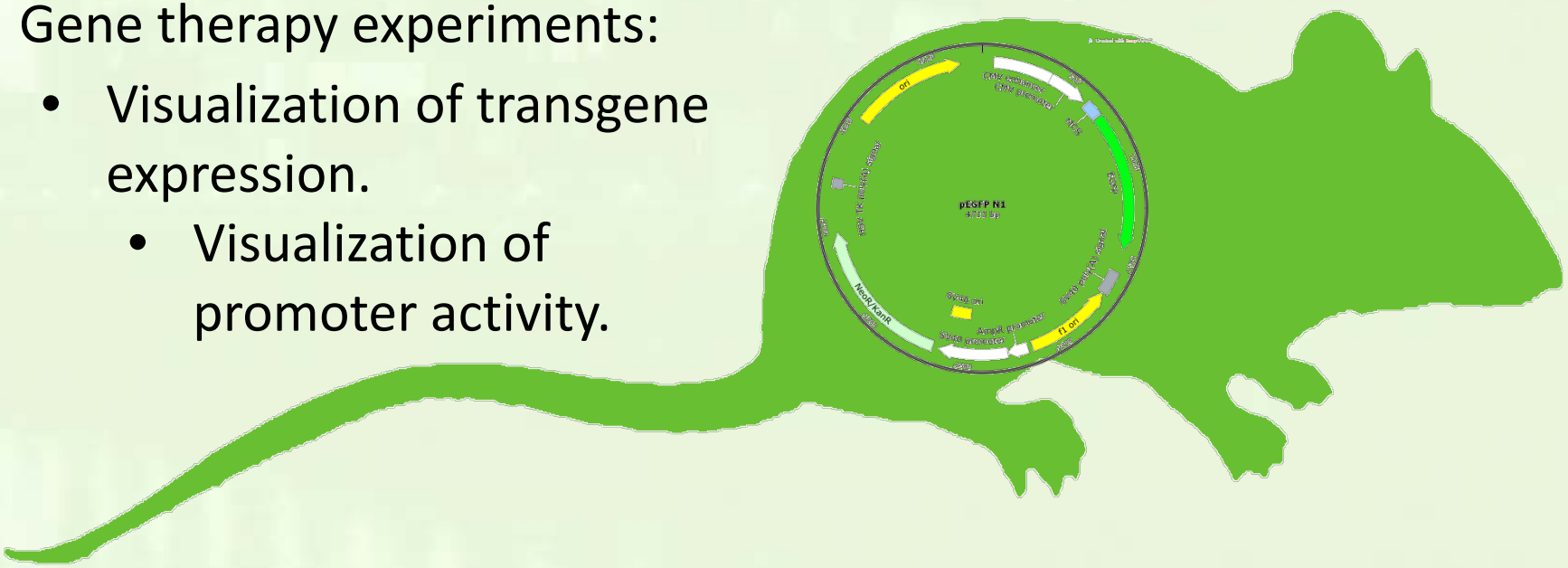
methylation and stress.



Non-invasive fluorescence imaging

Conduction of non-invasive and longitudinal studies of dynamic biological processes.

- Gene therapy experiments:
  - Visualization of transgene expression.
    - Visualization of promoter activity.



fluorescence intensity ∝ reporter gene expression ∝ promoter activity

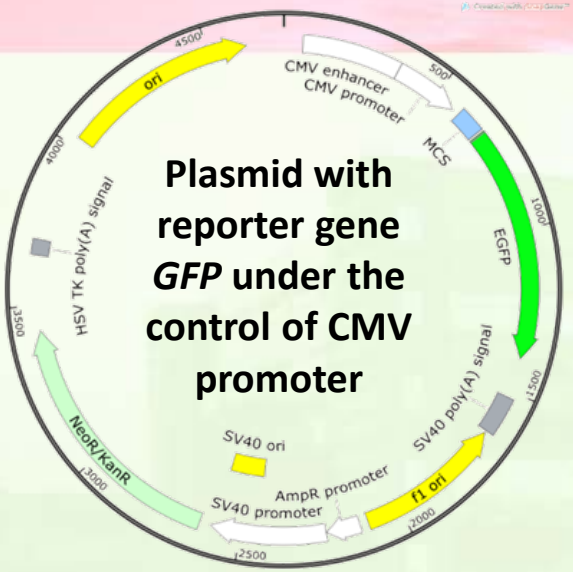
Aim

To evaluate the role of methylation and upregulation of the CMV promoter by stress *in vivo* using non-invasive fluorescence imaging.

Long-term follow-up of reporter gene fluorescence in the animals and consequently, the activity of promoters that control reporter gene expression.

fluorescence intensity ∝ reporter gene expression ∝ promoter activity

Methods

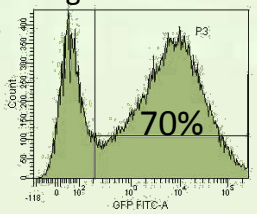


Plasmid with reporter gene *GFP* under the control of CMV promoter

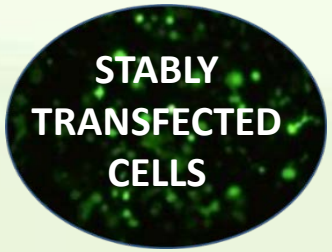
***In vitro* GET**

2 months of culturing under increasing concentrations of selection agent geneticin

Flow citometry fluorescence histogram



GFP expression in **TS/A** cells (mouse mammary adenocarcinoma)

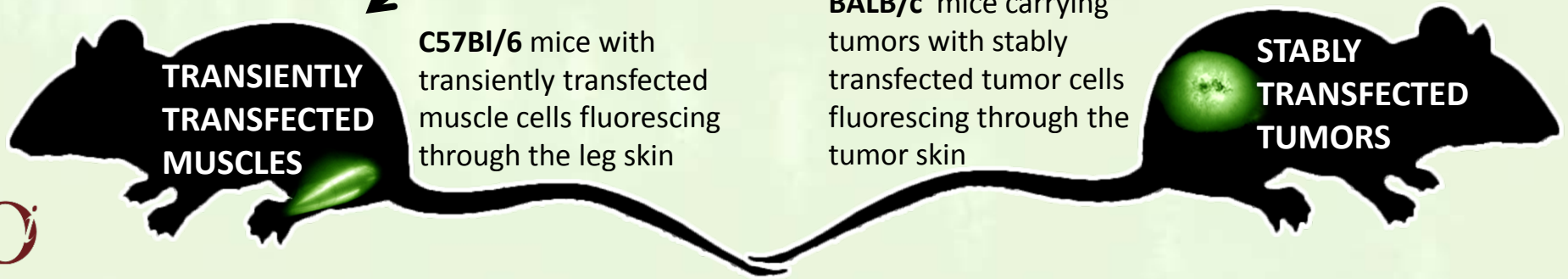


Propagation of stably transfected cells *in vitro*

Subcutaneous injection of stably transfected cells

***In vivo* GET to muscle**

Preparation of reporter gene experimental models



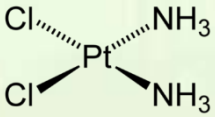
C57Bl/6 mice with transiently transfected muscle cells fluorescing through the leg skin

BALB/c mice carrying tumors with stably transfected tumor cells fluorescing through the tumor skin

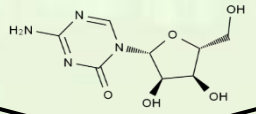
Methods

Radiotherapy
Irradiation

Chemotherapy
Cisplatin



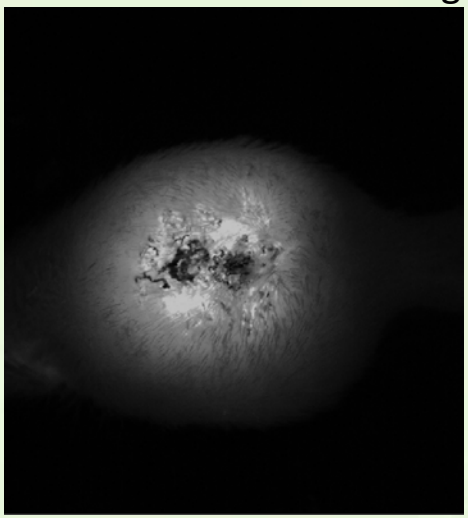
Demethylation
5-aza-2dC

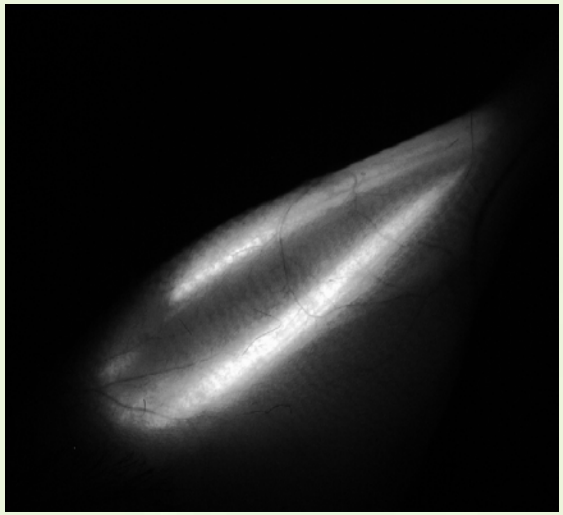


noninvasive fluorescence imaging

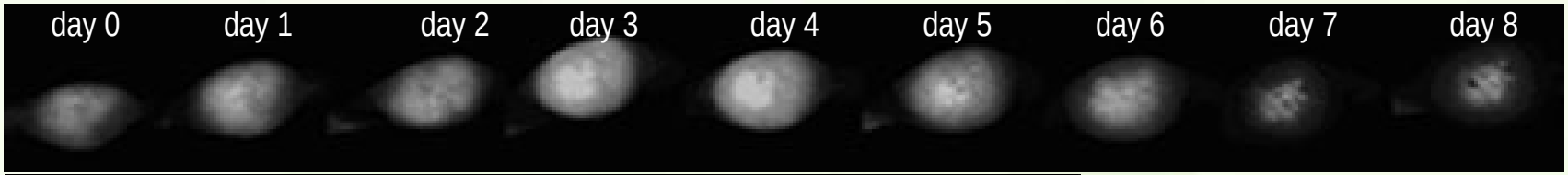
Fluorescence stereomicroscope

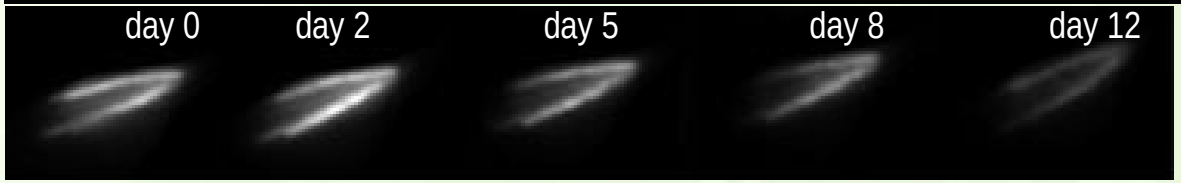
Fluorescent image of the tumor and muscle





Methods

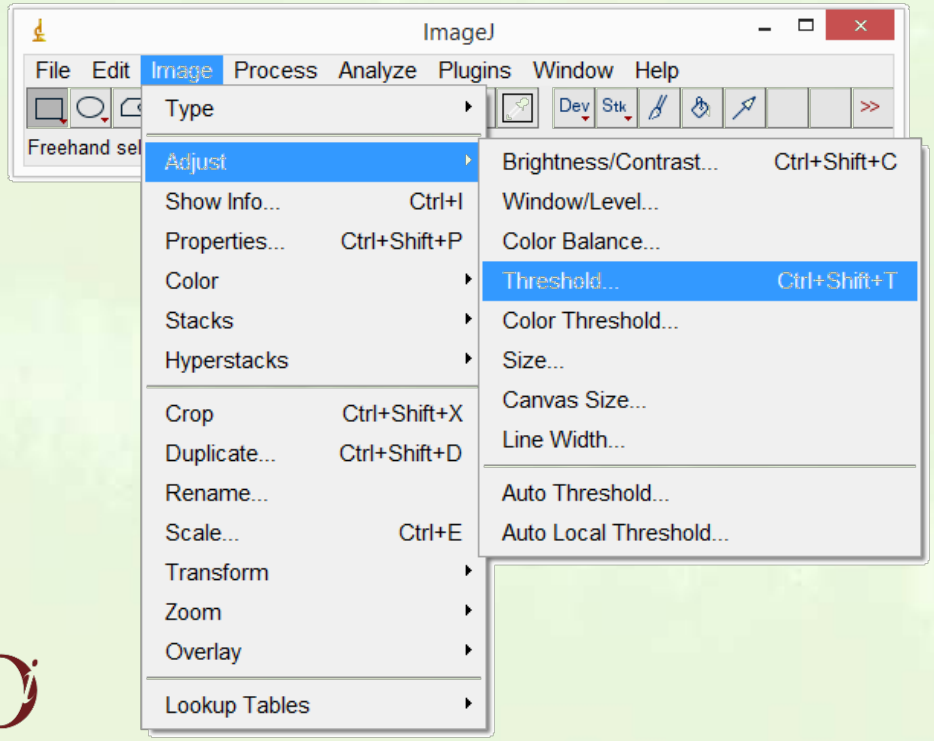


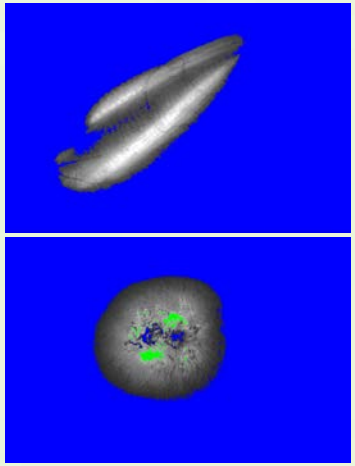


Fluorescence quantification using Image J software

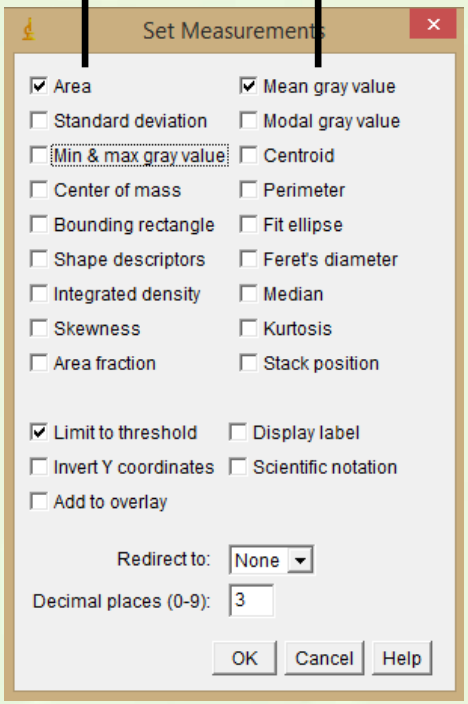
= mean fluorescence intensity

= transfection area

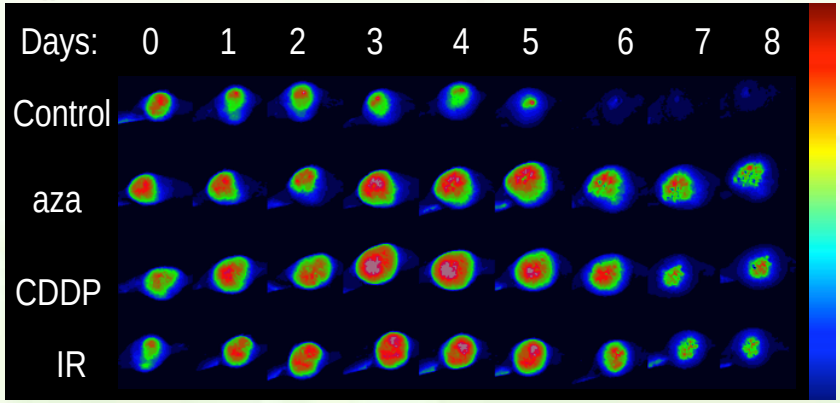


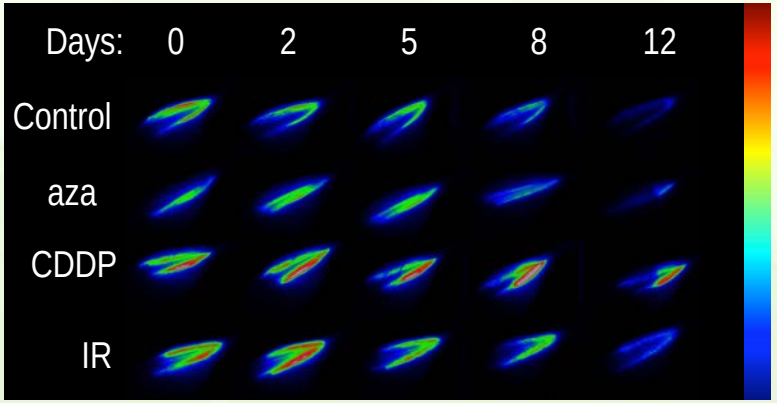


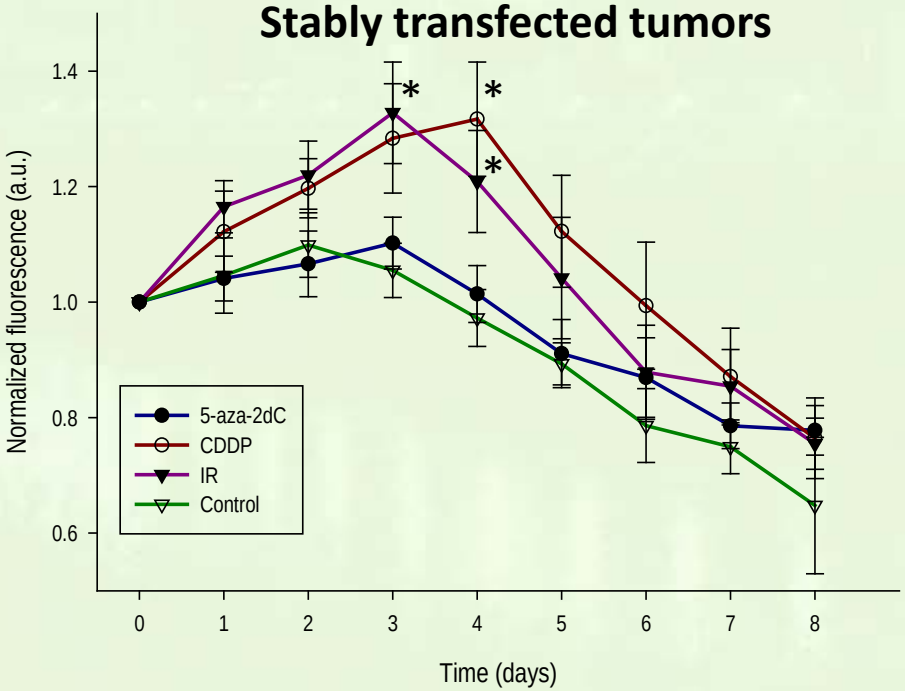
Blue = background fluorescence
Green = necrotic areas of the tumor

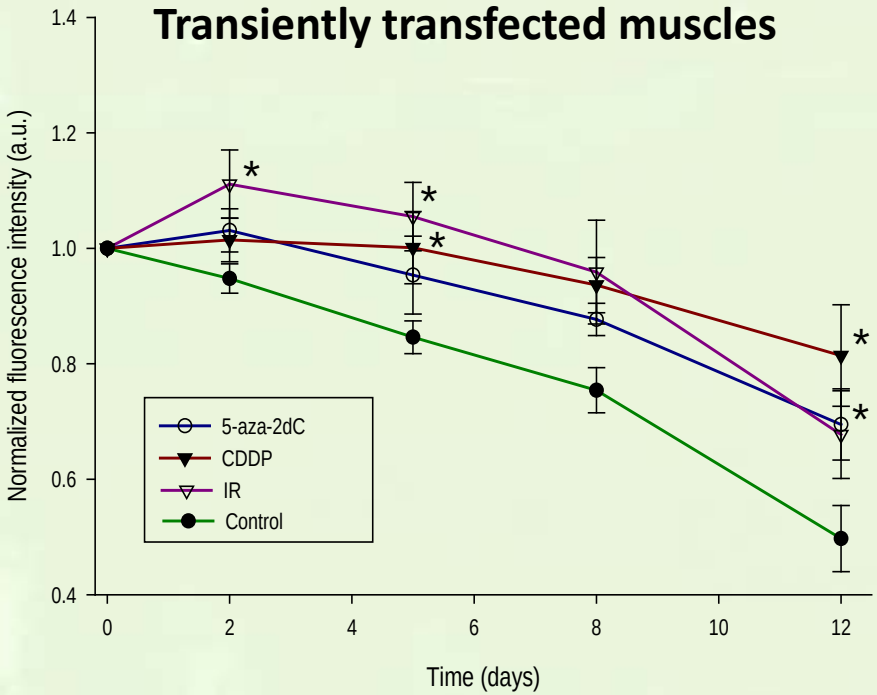


Results









Exposure of tumors and leg muscles to irradiation , cisplatin or 5-aza-2dC resulted in upregulation of the GFP expression.

U. Kamensek et al, Mol Imaging Biol, 2011

Conclusions

CMV promoter can be altered by different treatments.

- Observed alterations in the activity of the CMV promoter limit the usefulness of this widely used promoter as a constitutive promoter.
  - Proper choice of promoter linked to the gene of interest is critical for the success of gene therapy.

Non-invasive fluorescence imaging is an appropriate and convenient method to monitor the activity of the promoter *in vivo*.

- Compliance with the **3Rs** principle:
  - **R**eduction of the number of animals needed to conduct the experiment:
    - no need to sacrifice the subject at each time point to obtain the measurements,
    - every subject can serve as its own control – standardisation.
  - **R**eplacement: invasive method with non-invasive
  - **R**efinement: non-invasive method

Thanks!

Javna agencija
za raziskovalno dejavnost
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