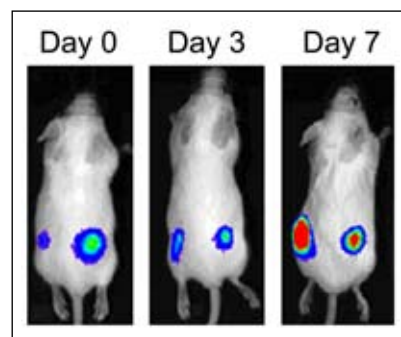




Applications in Chemical Toxicity

Introduction

Caliper is developing bioluminescent transgenic mice and cell lines to follow the time course and severity of response to potentially toxic chemical agents. These LPTA animal models and Bioware cell lines link promoters from chemically-inducible genes to firefly luciferase. The reporters are activated during acute or chronic chemical treatment, and the bioluminescent signal can be detected using Caliper's highly-sensitive IVIS Imaging System and Living Image software. Response to potential toxins can be followed noninvasively *in vivo* in Caliper's transgenic mice expressing luciferase, or in mice implanted with cell lines transformed with a luciferase reporter. Furthermore, the response *in vivo* can be correlated with *in vitro* response in these cell lines.



Chemical toxicity can be assessed in four areas with these transgenic LPTA animal models and Bioware cell lines:

- Oxidative Stress
- Immunotoxicity
- Genotoxicity
- Endocrine Disruption

Hypoxia and Oxidative Stress LPTA Animal Models

A suite of LPTA animal models are being developed to assess chemicals for their potential to induce oxidative stress when the chemicals are administered systemically or locally. These LPTA animal models use promoters from genes induced by oxidative stress to drive luciferase. The models include:

γ GCS (gamma Glutamyl Cysteine Synthetase):

CD1-Tg (γ Gcs-luc) The γ Gcs-luc model uses 15.3 kb of the mouse γ GCS gene promoter fused to the luciferase gene. This gene codes for the rate-limiting enzyme for the synthesis of glutathione, an important antioxidant. γ GCS is induced by oxidative stress and is regulated in parallel with HO1. We have shown that this reporter is induced in response to both chloroform and cadmium chloride.

VEGFR2 (Vascular Endothelial Growth Factor Receptor-2):

FVB/N-Tg (*Vegfr2-luc*) VEGFR2 is the cognate receptor for the peptide VEGF and mediates VEGF stimulation of angiogenesis in vascular endothelial cells. Angiogenesis is an important component of the response to inflammation in several disease states including rheumatoid arthritis. The *Vegfr2-luc* transgenic mouse uses 4.5kb from the promoter of the mouse VEGFR2 gene to drive luciferase expression. The *Vegfr2-luc* reporter is induced during cutaneous wound healing, and in the ear following treatment with oxazolone.

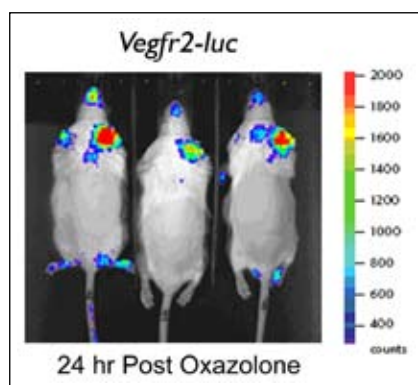


Figure 1. After initially sensitizing the mouse to oxazolone by topical application to the abdomen, the right ears of *Vegfr2-luc* mice were treated with oxazolone, while the left ear was untreated, and the mice were imaged 24 hours later. Oxazolone clearly induced the luciferase in the treated compared with control ear.

SOD1(Superoxide Dismutase-1): CD1-Tg (*Sod1-luc*) Xen

SOD1 is a critical enzyme responsible for the elimination of superoxide radicals and is considered to be a key anti-oxidant. The gene is induced by oxidative stress and serves a protective function. This LPTA model under development uses 15.5 kb of the mouse SOD1 gene promoter to drive luciferase activity.

Oxidative Stress Bioware Cell Lines

In addition to the LPTA animal models, a selection of Bioware cell lines have been developed to assess chemicals for their potential to induce oxidative stress. These cell lines can be implanted in animals or used *in vitro*. In the area of oxidative stress the cell lines include:

Cell Line VEGF (Vascular Endothelial Growth Factor): *hVEGF-luc/PC-3M*

The human VEGF promoter was fused to luciferase and the PC-3M human tumor cell line was stably transfected with this construct. When the *hVEGF-luc/PC-3M* cell line was implanted subcutaneously into SCID mice, the reporter was induced, likely reflecting the inadequate oxygen supply to the transplanted cells during the early stages of tumor growth.

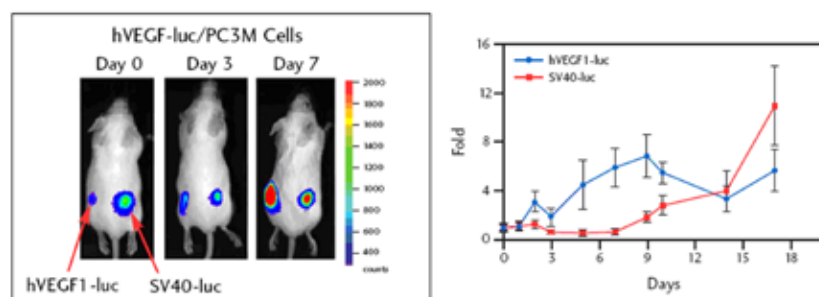


Figure 2. Male SCID mice were implanted s.c. with 3×10^6 *hVEGF1P-luc/PC-3M* cells on the left flank and *SV-40-luc/PC-3M* cells on the right flank. Growth was monitored for 17 days. *hVEGF-luc* expression increased rapidly during the first week after cells were implanted, as compared to the constitutive *SV-40-luc*. The graph illustrates Fold expression relative to Day 0.

Immunotoxicity and Inflammation LPTA Animal Models

A set of LPTA animal models designed to respond to agents or manipulations that activate or suppress the immune system have been developed. These models are useful for screening of topically administered irritants as well as systemically administered agents that might modulate the immune system.

NF κ B-Response Elements: *NF κ B-RE-luc*

The *NF κ B* signaling pathway is a ubiquitous signaling pathway involved in inflammation and apoptosis. The *NF κ B-luc* transgenic mouse uses a set of response elements for *NF κ B* to regulate a minimal promoter driving luciferase activity. This reporter system is induced by a variety of stimuli that produce inflammation or apoptosis. A transgenic animal model was created in an academic laboratory using *NF κ B* response elements to drive luciferase expression (Karlsen and Blomhoff et al., *J. Immunology*, 2002). This *in vivo* luciferase reporter was shown to respond to a variety of stimuli including injection of bacterial lipopolysaccharide, ultraviolet light, and monoclonal antibody induced arthritis.

iNOS (Inducible Nitric Oxide Synthase): FVB/N-Tg (*iNos-luc*) Xen

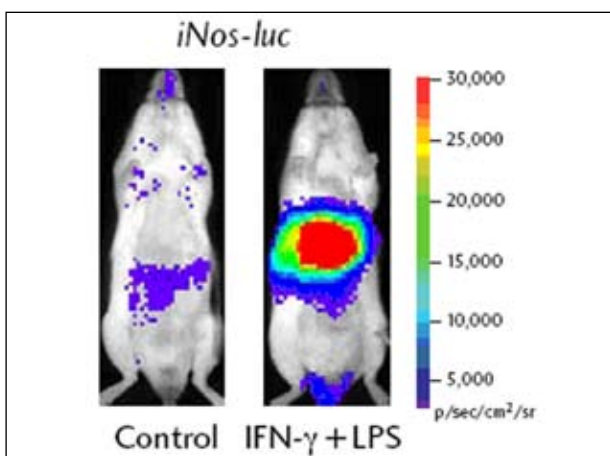
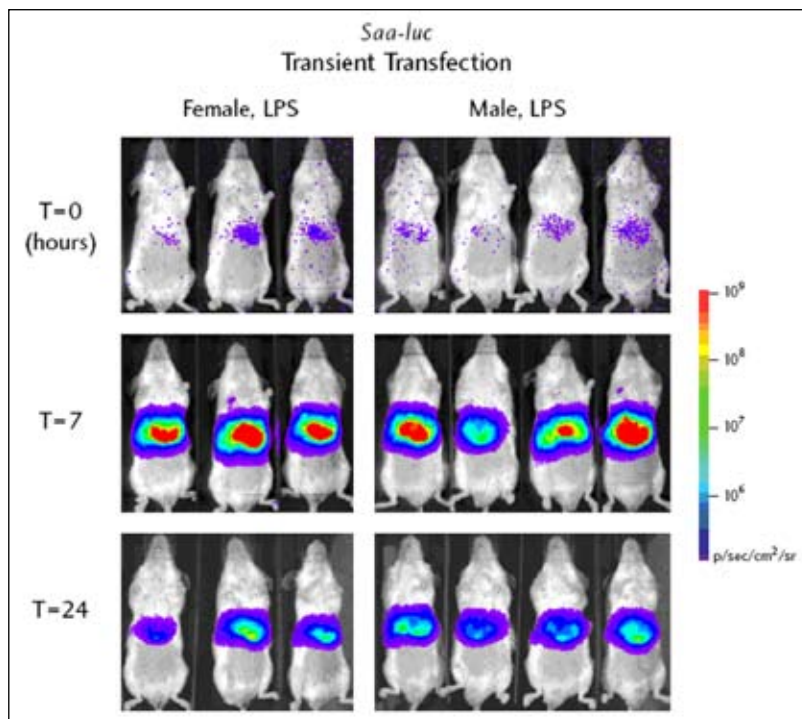


Figure 3. Male *iNos-luc* mice injected with lipopolysaccharide and interferon gamma and imaged 6 hours later show a strong induction of the luciferase signal in liver Kupffer cells.

Inducible nitric oxide synthase (iNOS) is expressed in a variety of cell types but is primarily induced in circulating macrophage/monocytes and in resident macrophage cells in liver and intestine. The *iNos-luc* transgenic mouse uses a 1.3kb promoter fragment of the mouse iNOS gene to drive luciferase expression. This model has been shown to respond to LPS induced sepsis by activating *iNos-luc* in Kupffer cells in the liver. Furthermore, zymosan injected subcutaneously into an airpouch, or intraarticularly into the knee joint, induces the luciferase signal, presumably by recruiting and activating monocytes. Local skin irritation produced by oxazolone in the delayed type hypersensitivity test, or with formalin applied to the footpad, also induces the luciferase signal.

SAA (Serum Amyloid A-1): BALB/C-Tg (*Saa-luc*)

Serum amyloid A1 (SAA) is a liver specific acute phase response gene that is highly induced in both acute and chronic inflammatory diseases. In addition, it is an important component of the protein deposited in some amyloidosis disease states. The BALB/C-Tg (*Saa-luc*) mouse under construction uses 7.9kb of the murine SAA promoter to drive luciferase expression (Figure 4). Transient liver transfection studies with the same construct being used to build the transgenic mouse produces a reporter that is highly induced by lipopolysaccharide.



IL2 (Interleukin-2): CD1-Tg (*Il2-luc*)

10.7 kb of mouse interleukin-2 promoter was fused to the firefly luciferase gene and the construct was used to create a transgenic mouse in the CD1 mouse strain. This model is currently in development.

Figure 4. FVB/N mice were transiently transfected by the hydrodynamic transfection protocol with a plasmid containing the *Saa-luc* construct. Eight days after transfection, the mice were challenged with LPS, inducing the luciferase signal in liver.

Genotoxicity LPTA Animal Models and Bioware Lines

In vivo genotoxicity studies often require long-term exposure to agents in order to identify potential toxicity. Caliper is developing a set of LPTA animal models and Bioware cell lines, designed to rapidly assess the potential for a chemical to cause DNA damage. These models use the promoters for genes induced by DNA damage, fused to luciferase, to report on the induction of DNA repair mechanisms. The genes selected are highly induced by DNA damage and apoptosis in mammalian cells.

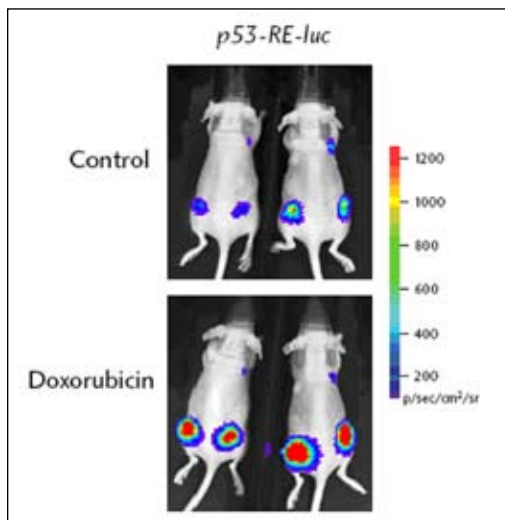


Figure 5. A549 cells containing the p53-RE-luc construct were implanted subcutaneously in Nude mice. Mice were then injected with either saline or doxorubicin and imaged 6 hours after injection. Doxorubicin clearly induced the luciferase reporter.

GADD45 α and β (Growth Arrest and DNA-Damage Inducible 45alpha and beta):

CD1-Tg(*Gadd45 α -luc*) Xen, CD1-Tg(*Gadd45 β -luc*) Xen

GADD153 (Growth Arrest and DNA-Damage Inducible 153): CD1-Tg(*Gadd153-luc*) Xen

These LPTA animal models for genotoxicity are under development.

In addition to the LPTA animal models, a Bioware cell line has been developed to assess chemicals for their potential to induce DNA damage. This cell line can be implanted in animals or used *in vitro*.

Cell Line p53 Response Elements: *Ip53-RE-luc/A549*

P53 response elements were used to drive a minimal promoter fused to the firefly luciferase gene. A549 human tumor cell lines were stably transfected with this construct. When these cells were implanted subcutaneously into nude mice, the reporter was induced with the anti-cancer agent doxorubicin.

Endocrine Disruption LPTA Animal Models

Caliper is developing a set of LPTA animal models for the rapid *in vivo* assessment of potential disruption of androgen or estrogen signaling. These LPTA animal models use mouse or human promoters that are highly regulated by androgens or estrogens to control luciferase expression.

KAP (Kidney Androgen Regulated Protein): FVB/N-Tg (*Kap-luc*)

The KAP gene is a kidney specific gene of unknown function that is transcriptionally regulated by androgens. Approximately 1.5 kb of the mouse KAP promoter was fused to the luciferase gene. This transgenic reporter is up-regulated by androgens and down-regulated by antiandrogens such as cyproterone.

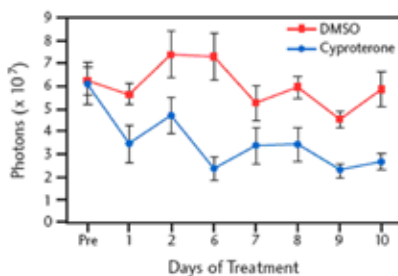
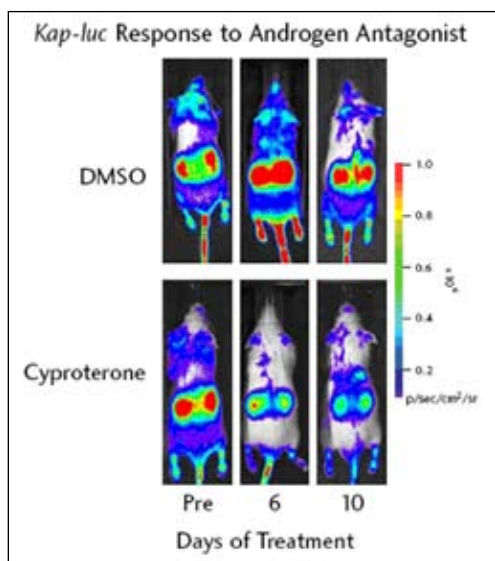


Figure 6. *Kap-luc* mice were injected either daily with the anti-androgen, cyproterone, or with the vehicle DMSO. Injection of cyproterone significantly reduced the luciferase signal in the kidney of treated mice.

PS2 (Trefoil Peptide-1): FVB/N-Tg (*pS2-luc*)

Trefoil peptide-1 is a gene involved in mucin secretion from the gastrointestinal tract. It is also highly induced in human mammary tumors, and is highly regulated by estrogen. A 2.0 kb human promoter fragment from this gene has been fused with the luciferase gene and an LPTA animal model is in development.

Chemical Toxicity LPTA Animal Model Features

These models can report the activity *in vivo* of specific signal transduction pathways in response to chemical agents. The responses and analyses are rapid. Typical experiments are run in a single day and produce valuable information that can be used in determining peak response times and duration of response to a chemical. This information provides alternative and/or complementary information to measurements of circulating markers for responses to toxicants as well as histopathological analyses.

Contact Information:

If you have any questions regarding these cell lines please contact Caliper at 508.497.6592 or e-mail: reagents@caliperLS.com

Disclaimer

For LPTA animal model lines CYP3a11, CYP3A4 rat, Epx, Vegfr2 and Vegf: These product lines and their use are claimed by pending U.S. and foreign patent applications owned by Caliper Life Sciences, Inc.

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