TACONIC

LPTA ANIMAL MODELS

LPTA Animal Model *RIP-luc Xen*

Information

Mouse Strain:	FVB/N-Tg(RIP-luc)-Xen
Common Name:	*RIP-luc*
Genotype:	Hemizygous
Background Strain:	FVB/N
Coat Color:	Albino

Key Applications

- Insulin Production
  - Fasting, high fat feeding, Streptozotocin treatment, Insulin resistance
- Pancreatic islet transplantation

Description of Model:

A light producing animal model (LPTA®) animal model FVB/N-Tg (RIP-luc)-Xen, commonly called RIP-luc, carries a 780 bp rat insulin promoter (RIP), 800 bp human β–globin intron 2, and modified firefly luciferase cDNA (Promega pGL-3). Luciferase expression of the reporter was highest in pancreas, with low levels observed in male and female gonads. The reporter is inducible during high-fat feeding and onset of diet-induced obesity. The model provides for the study of transcriptional regulation of the insulin promoter, indications of possible changes in pancreatic-cell mass and function, and can serve as donors for islet transplantation studies. white blood cells. The reporter is constitutively expressed and is not significantly inducible.

Origin

The background strain is FVB/N from Taconic. The transgenic line was created by microinjection method using FVB donor embryos. Founding lines were characterized at Caliper Life Sciences

Applications

The RIP-luc LPTA® animal model is useful in studying changes in insulin production resulting from fasting, high-fat feeding, streptozotocin treatment, or therapy using compounds that sensitize the animals to insulin, and may be used as donor animals for studying pancreatic islet transplantation.

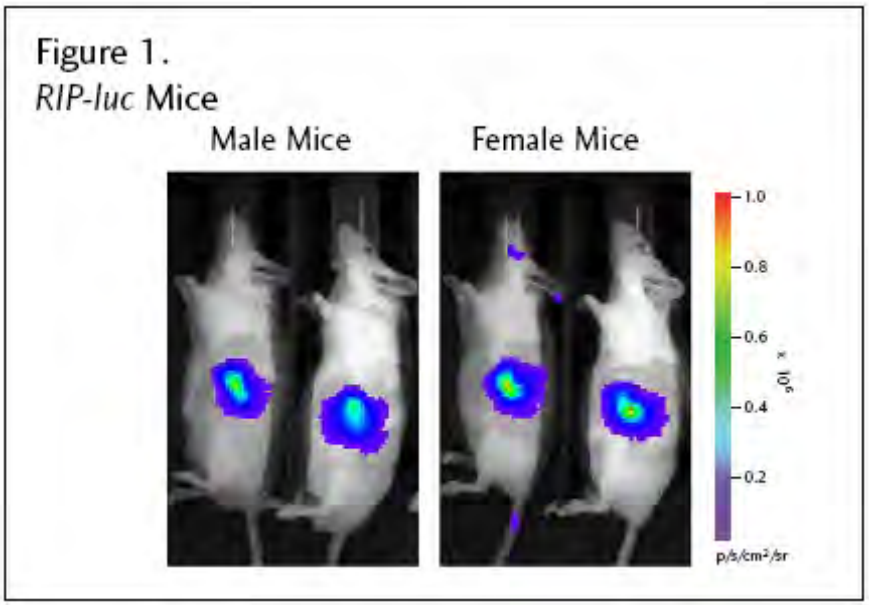

Figure 1: Optimal detection of luciferase expression in pancreas during imaging is achieved by positioning mice right flank down and shaved left flank facing the lens. To quantify luciferase expression, mice are fasted for approximately five hours and then anesthetized prior to injection of luciferin. Optimal imaging occurs between 10 and 20 minutes following intraperitoneal injection of luciferin.

Example Phenotype

The coat color of the LPTA® animal model is albino. RIP-luc mice behave as normal FVB/N mice, and are observed to have litters of 8–12 pups. At >6 weeks of age, adult mice weights were appropriate: 20-30g.

Imprinting and Chromosomal Localization

A number of mice tested positive for luciferase by PCR were found to have little or no luciferase expression following luciferin injection. Examination of the parentage of the imaged animals showed that transgenic offspring from male transgenic parents are more likely to have high luciferase expression than if the transgene was donated by a female transgenic parent. The difference is likely due to genomic imprinting, where chromosomal regions can be turned off, often by methylation, during gamate formation. The RIP-luc transgene was found to integrate on Chromosome 6, as identified by in situ hybridization

Baseline Expression study

The RIP-luc transgene was induced to express luciferase in a group of 10 male and 12 female age-matched adult mice for 20 days. Groups were fed either a chow or high-fat (30% fat by weight; Research Diets #D12492) diet. Mice were fasted five hours prior to

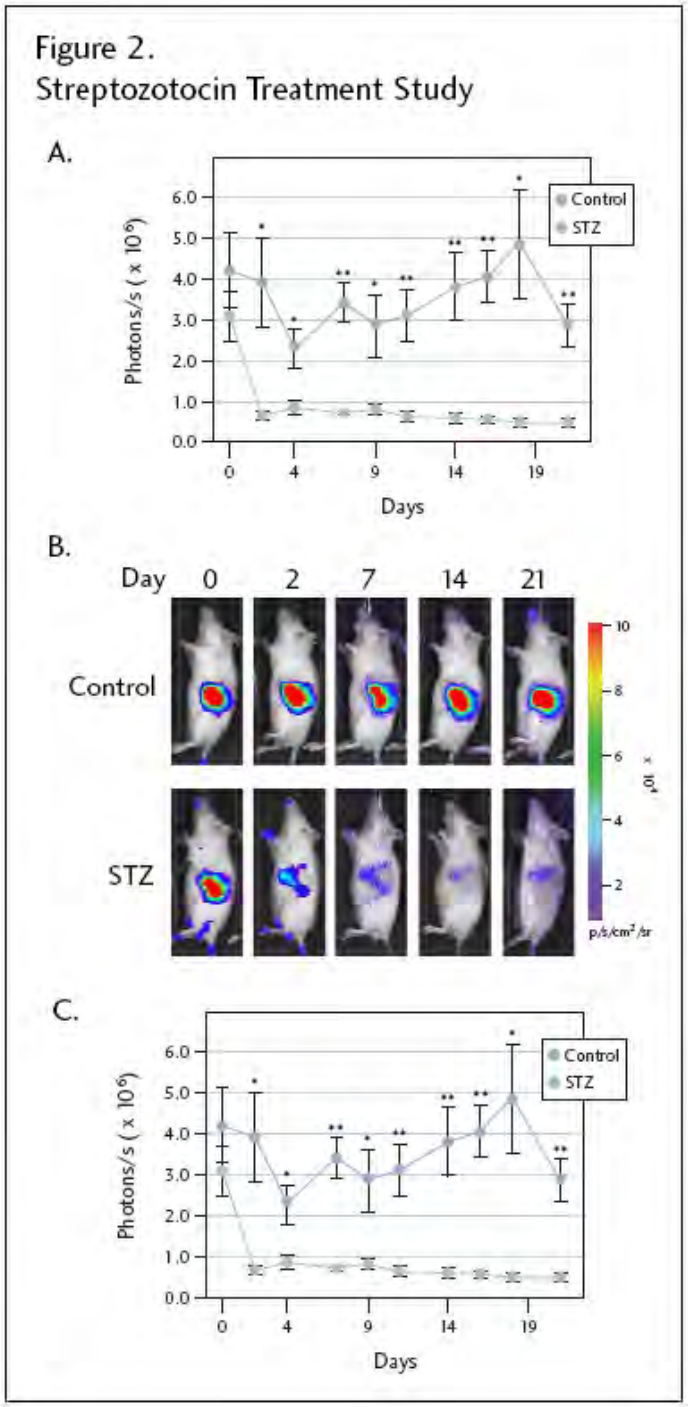

Figure 2. 3-Week streptozotocin treatment study results. The luciferase signal decreased in STZ-treated mice within 2 days of treatment, as pancreatic b-cells were killed by STZ and the animals became diabetic (A). STZ-treated mice showed a significant reduction in luciferase expression (A, B) in homogenized pancreatic tissue in control (8.90±2.30 RLU/mg protein) and STZ treated (0.74±0.19RLU/mg protein) mice. STZ-treated mice became hyperglycemic within 2 days of treatment (C), had reduced circulating C-peptide levels, and lost more wei ht han control animals.

measurement and were 12 weeks of age at study onset.

High fat-fed mice gained significant weight compared to chow-fed animals. During chow feeding, baseline luciferase expression was found to have little variability over time. During high fat feeding, luciferase signal was found to increase as the mice gained weight. This is likely due to progressive peripheral insulin resistance associated with the diet-induced obesity. Fasting circulating glucose and insulin levels did not change during the course of the study for any of the diet groups

Streptozotocin Treatment Study

Male mice were followed for three weeks following intraperitoneal injection of STZ (175 mg/kg). Control (n=10) and STZ-treated (n=9) mice were used in this study. Immunohistochemical staining for insulin and glucagon identified a decrease in islet insulin from STZ-treated pancreatic islets.

Pancreatic Islet Transplantation Study

Intraheptatic autologous islet transplantation using donor islets from the RIP-luc strain was performed in streptozotocin-induced diabetic FVB/N mice. Amelioration of the hyperglycemic state ensued, and was associated with intense luciferase expression from islets implanted in the liver. Prior to islet transplantation, STZ-treated mice had blood glucose levels greater than 350mg/dl. One week following islet transplantation blood glucose levels had stabilized at 120-150 mg/dl.

Genotype

The transgenic lines are hemizygous. The presence of the *RIP*-luc transgene was determined by PCR. The 1 kb PCR product from the sequence internal to the luciferase gene was amplified using forward primer:

LF2 (5'- TGGATTCTAAAACGGATTACCAGGG -3')
and reverse primer:
LR2 (5'- CCAAAACAACAACGGCGGC -3'),

both at 0.4 µM in the reaction mix. PCR conditions: 97ºC 5:00 min; 94.5ºC 0:40 min; 58ºC 1:30 min; 72ºC 1:30 for 35 Cycles; 72ºC 10:00; hold at 4ºC until analysis.

References

1. Soares MB, Schon E, Henderson A, Karathanasis SK, Cate R, Zeitlin S, Chirgwin JM, Efstratiadis A. RNA-mediated Duplication: The Rat Preproinsulin I Gene is a Functional Retroposon. Mol Cell Biol. 1985; 5:2090-2103.
2. Efrat S, Surana M, Fleischer N. Glucose Induces Insulin Gene Transcription in a Murine Pancreatic Beta-cell line. J Biol Chem. 1991 Jun 15; 266:11141-11143.
3. Smith SJ, Zhang H, Clermont AO, Powers AC, Kaufman DB, Purchio AF, West DB. In Vivo Monitoring of Pancreatic-Cell Mass and Function in a Transgenic Mouse Model. Mol Imaging. 2006 Apr-Jun;5(2):65-75