Mouse model of human Barth syndrome

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Background

- Barth (BTHS) syndrome is X-linked genetic disorder.
- Common symptoms include:
  - Cardiomyopathy
  - Skeletal myopathy
  - Neutropenia
  - 3-methylglutaconic aciduria
- BTHS caused by mutations in tafazzin (taz) gene.
- Taz gene is located on Xq28.
- Taz encodes mitochondrial acyltransferase.
- Mutations in taz gene cause cardiolipin deficiency.
- Cardiolipin is mitochondrial phospholipid and constitutes about 20% of inner mitochondrial membrane.
MODELS OF TAFAZZIN DEFICIENCY

• Yeast;
• Drosophila;
• Zebrafish;
• Various cell cultures.

Urgent need for mouse model of Barth syndrome

• Several “floxed” alleles of taz have been generated in mouse ES cells;
• Problems lay with germline transmission of “floxed” taz allele.
Tafazzin tet-on shRNA transgenic mice were generated by TaconicArtemis in 2009.

Work was initiated and funded by Barth Syndrome Foundation (www.barthsyndrome.org).

Mice available from Jackson Laboratory:
Gt(ROSA)26Sor<tm37(H1/tetO-RNAi:Taz)Arte
RECOMBINASE MEDIATED CASSETTE EXCHANGE

ROSA 26 (RMCE)

\[
\text{Exon 1} \quad \text{SA} \quad \text{ZsGreen-pA} \quad \text{Hyg}^R \quad \text{FLPe} \quad \text{FRT} \quad \text{Exon 2}
\]

\[
\text{Exon 1} \quad \text{SA} \quad \text{Neo}^R \quad \text{H1-tet0-shRNA:taz} \quad \text{tet-R} \quad \text{FRT} \quad \text{Exon 2}
\]

\[
\text{Resulted locus}
\]

site-specific recombination by FLPe

exchange vector
Dox

H1-tet0-shRNA:taz

shRNA

Taz RNA

Taz
Important Questions:

1. How efficient is shRNA mediated silencing in different tissues?
2. How tight is regulation by Dox?
3. What are side-effects of chronic Dox administration?
4. Is it reversible?
Induction of Taz knockdown

Doxycycline was administered with rodent chow, formulated by Purina Mills (625 mg / kg)

-5 days 0 4 days

Dox

WT

X

Taz shRNA-Tg

Dox
TAFAZZIN KNOCKDOWN

Un-silencing

WT + Dox
TG − Dox
TG + Dox
TG +/− Dox 1 M
TG +/− Dox 2½ M
Cardiolipin analysis

Control Heart

Taz-knockdown heart

Control Sk. Muscle

Taz-deficient Sk. Muscle

Acehan et al. JBC 2010 (in press)
CARDIOLIPIN IN HEART AND MUSCLE

**Heart**

- **NTG**
- **Taz-KD**

**Skeletal muscle**

- **NTG**
- **Taz-KD**

Acehan et al. JBC 2010 (in press)
CARDIOLIPIN IN HEART AND MUSCLE

Heart

Skeletal muscle

4x(18:2)
MLCLs IN HEART AND MUSCLE

Heart

Skeletal muscle

MLCL, nmol / mg w.w.

NTG

Taz-KD
CARDIAC MRI

Control   Taz Knockdown

Acehan et al. JBC 2010 (in press)
ECHOCARDIOGRAPHY (8M)

ACEHAN ET AL. JBC 2011
LOSS OF TAZ ALTERS CARDIAC PARAMETERS
Wall thickness and LV wall mass

IVS;d

LVPW

LV mass

EF

NTG

Taz knockdown
CONTROL HEART

TAFAZZIN KNOCKDOWN HEART

Acehan et al. JBC 2011
Questions Answered:

1. How efficient is shRNA mediated silencing?
   An shRNA mediated silencing of taz is very efficient in heart, muscle, liver and brain.

2. How tight is regulation by Dox?
   Dox very efficiently controls shRNA expression in heart, skeletal muscle and liver. In brain control is less tight and we observed ~35% reduction of taz mRNA level.

3. What are side-effects of chronic Dox administration?
   We didn’t find any adverse effects of prolonged dox administration in control animals.

4. Is it reversible?
   We found that withdrawal from dox restores taz level to 75-90% of normal in 2.5 months.
Impact of Cardiolipin on IMM

Claypool et al. 2008
Dox shRNA-induction causes significant TAZ knockdown

Soustek et al., HGT, 2011
TAZKD Results in Impaired Contractility in the Soleus

Soustek et al., HGT, 2011
TAZKD Results in Reduced Cardiac Function

Soustek et al., HGT, 2011
Transduction of pTR-Myc-TAZ-FL in HEK293 Cells

Western Blot: C-Myc Antibody (1:200)
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