Presenter Disclosure Information Elements

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FINANCIAL DISCLOSURE:
Grants/Research Support: Regulus Therapeutics
Consultant: Regulus Therapeutics
The Transcriptional Repressor MAFG Regulates Cholesterol Catabolism

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Division of Cardiology
David Geffen School of Medicine
UCLA

Irvine H. Page Young Investigator Award
5.8.15
Cholesterol Catabolism to Bile Acids Is Controlled by the Nuclear Receptor FXR

FXR Ligands:
- Bile Acids
- GW4064
- GSK2324

FXR

Liver

Cholesterol

Bile acids

Gall Bladder

Target genes

de Aguiar Vallim et al.
Cell Metab. 2013 (Review)
FXR Mediates Many of Physiologic Effects of Bile Acids

- Bile Acid Homeostasis
- Circulating Lipids
- Glucose Homeostasis
- Hepatic Lipid Metabolism
- Lipid Absorption
- Intestinal Barrier Function
- Hepato-protection

References:
- de Aguiar Vallim et al. Cell Metab. 2015
- Tarling, Ahn and de Aguiar Vallim ATVB 2015
- de Aguiar Vallim et al. Circ Res. 2013
- Bennett*, Vallim* et al. Cell Metab. 2013
FXR Activation Represses Hepatic Bile Acid Synthetic Genes

Bile Acid Synthesis Pathway

Liver

Cholesterol

CYP7A1

CYP27A1

CYP7B1

HSD3B7

AKR1D1

AKR1C14

CYP27A1

SLC27A5

AMACR

ACOX2

HSD17B4

SCP2

Cholic Acid

Chenodeoxycholic Acid

MCA

n=7-9mice/group
Activated FXR Functions to Increase Transcription. How Does FXR Mediate Repression?
FXR Activation Induces Several Putative Transcriptional Repressors

Repressed Genes
(500 genes >2 fold)

Cyp8b1

Cyp7a1

诱导基因
(250 genes >2-fold)

MafG

Crip2

Shp

Zfp385
Induction of Putative Transcriptional Repressors By FXR Agonists Is Dependent upon FXR

Liver

Shp  MafG  Crip2  Zfp385

GW4064
GSK2324
FXR

WT  KO  WT  KO  WT  KO  WT  KO

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n=7-9mice/group
de Aguiar Vallim et al. Cell Metab. 2015
Overexpression of *MafG*, but not *Crip2* or *Zfp385* Represses Hepatic *Cyp8b1* Expression

**Liver**

![Bar chart](image.png)

<table>
<thead>
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<th>Ad-Control</th>
<th>Ad-Crip2</th>
<th>Ad-MafG</th>
<th>Ad-Zfp385</th>
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n= 7-8 mice/group
MafG Over-expression Reduces Cholic Acid and Increases Muricholic Acid Levels

\[\text{α-Muricholic Acid (αMCA)}\]
\[\text{β-Muricholic Acid (βMCA)}\]
\[\text{Cholic Acid (CA)}\]

n = 7-8 mice/group

de Aguiar Vallim et al.
Cell Metab. 2015
MafG<sup>+/−</sup> Mice Have Increased Cyp<sub>8b1</sub> Expression and Increased Cholic Acid Levels

Liver

![Liver diagram]

**MafG**

MafG

Lamin A/C

mRNA (Fold Change)

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<thead>
<tr>
<th></th>
<th>WT</th>
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<tr>
<td>MafG</td>
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n=9-11 mice/group (littermates)
ChIP-Seq Analysis Identified MAFG Response Elements in Cyp8b1

MAFG Structure

BLRP Tag  Basic Region  Leucine Zipper (DBD)

Homology Region

n= 6/7 mice/group
MAFG Is a Transcriptional Repressor of Bile Acid Metabolism Genes

Cyp7b1  Cyp27a1  Acox2  Akr1c14  Akr1d1  Amacr  Hsd3b7  Hsd17b4  Scp2  Slc27a5

Repressed
Unchanged

mRNA (Fold Change)

Cyp7b1  Cyp27a1  Acox2  Akr1c14  Akr1d1  Amacr  Hsd3b7  Hsd17b4  Scp2  Slc27a5

Ad-Ctr  Ad-MG  Ad-Ctr  Ad-MG  Ad-Ctr  Ad-MG  Ad-Ctr  Ad-MG  Ad-Ctr  Ad-MG  Ad-Ctr  Ad-MG  Ad-Ctr  Ad-MG  Ad-Ctr  Ad-MG  Ad-Ctr  Ad-MG  Ad-Ctr  Ad-MG

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Liver

Cholesterol

CYP7A1

CYP8B1

AKR1D1

AKR1C14

CYP27A1

SLC27A5

AMACR

ACOX2

HSD17B4

SCP2

Cholic Acid

Chenodeoxycholic Acid

MCA
Summary – Identification of MAFG as a Novel Regulator of Bile Acid Synthesis and Metabolism

- FXR functions as the master regulator of bile acid synthesis by repressing bile acid synthetic genes.

- We used FXR agonists, whole body and tissue-specific FXR knockout mice and ChIP-Seq to identify 3 novel FXR target genes, including *MafG*, that encode transcriptional repressors.

- *MafG* overexpression represses several bile acid synthesis genes, including *Cyp8b1*, resulting in reduced cholic acid levels and altered biliary bile acid composition.

- MAFG ChIP-Seq identified MAFG response elements in multiple bile acid synthetic genes.

- *MafG* silencing with antisense oligonucleotides or *MafG*+/− mice have increased *Cyp8b1* expression and altered biliary bile acid composition.
Acknowledgments

Vallim / Tarling Lab

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Collaborators

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Peter Tontonoz

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Tohoku University
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Hozumi Motohashi

Isis Pharmaceuticals
Richard Lee
Mark Graham

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American Heart Association®
Post-doctoral fellowship
Scientist Development Grant

UCLA/UCSD DRC
Sponsored Research Agreement
Redundant Pathways Controlling Bile Acid Synthesis

- SHP WT
- SHP KO

Cyp7a1 mRNA (Fold Change)
- GSK2324
- n=7-9 mice/group

Cyp8b1 mRNA (Fold Change)
- iFXR

MafG<sup>-/-</sup> Mice Have a Compensatory Mechanism

n=9-11 mice/group (littermates)
MAFG Overexpression Represses Multiple Genes of Bile Acid Metabolism

Repressed Genes (1.5-fold)

Cyp8b1
Cyp27a1
Cyp7b1

Induced Genes (1.5-fold)

Ad-Control

Ad-MAFG

KEGG PATHWAY TERM (Repressed Genes)

- Complement and coagulation cascades
- Primary bile acid biosynthesis
- Steroid hormone biosynthesis
- Androgen and estrogen metabolism
- Nitrogen metabolism
- Arachidonic acid metabolism
- Adipocytokine signaling pathway

de Aguiar Vallim et al. Cell Metab. 2015
MAFG Has Multiple Binding Partners

Hepatocyte

Nucleus

MAFG - MAFG

MARE

Repression

MAFG - MAFF

MARE

Repression
MafF Is Transcriptionally Regulated by FXR

![Graph showing the regulation of MafF mRNA by FXR and GSK2324](image-url)
MAFG ChIP-Seq Analysis Identified MAREs in BA Genes

de Aguiar Vallim et al. Cell Metab. 2015
MafG Over-expression Represses Multiple Genes of Bile Acid Metabolism

de Aguiar Vallim et al. Cell Metab. 2015
Nuclear Receptor Superfamily

Steroid Receptors
- GR: glucocorticoid
- MR: mineralcorticoid
- PR: progesterone
- AR: androgen
- ER: estrogen

RXR Heterodimers
- T<sub>3</sub>R: thyroid hormone
- RAR: all-trans RA
- VDR: 1,25-(OH)<sub>2</sub>-VD
- PPARα: fatty acids /
- PPARγ: 15d-Δ<sub>12,14</sub>-PGJ
- EcR: ecdysone
- FXR: bile acids
- CAR: androstane
- LXR: oxysterol
- PXR/SXR: xenobiotics

Dimeric Orphan Receptors
- RXR
- COUP
- HNF-4
- TR2
- TLX
- GCNF

9-cis RA

Monomeric / Tethered Orphan Receptors
- NGFI-B
- SF-1
- Rev-erb
- ROR
- ERR

Olefsky JBC 2001
Activated FXR Mediates Negative Feedback of Bile Acid Synthesis
Recent Discovery that FXR Activation Represses Almost all Bile Acid Synthesis Genes

de Aguiar Vallim et al. Cell Metab. 2015
A New Mechanism for the Repression of Bile Acid Synthesis by FXR

Does MAFG bind to the Cyp8b1 promoter and repress transcription?
Natural FXR Ligands

- Chenodeoxycholic acid (CDCA)
- Cholic acid (CA)

However bile acids are promiscuous and also activate:
- Other nuclear receptors (PXR, VDR and CAR)
- GPCR (TGR5)
The Farnesoid X Receptor (FXR): The Master Regulator of Bile Acid Metabolism

Low Bile Acid Levels
- Impaired Lipid Absorption
- Gallstones

High Bile Acid Levels
- Dyslipidemia
- Cholestasis
- Cancers
- Diarrhea

Impaired Lipid Absorption

Gallstones
FXR Activation Represses Two Critical Bile Acid Synthetic Genes

Reviewed in Vallim et al. Cell Metab. 2013

Cyp7a1 Cyp8b1

GW4064 FXR Agonists GSK2324

n=7-9mice/group

FXR Agonists
GW4064 GSK2324
FXR Activation with GW4064 or GSK2324 Induces MAFG Protein Levels

n=7-9 mice/group
MafG Is a Direct FXR Target Gene
Regulation of MafG by FXR Is Rapid and Requires Hepatic FXR

MafG mRNA (Fold Change)

GSK2324  Veh  30'  1h  2h  4h

n= 6 mice/group
In vivo Studies: Silencing MafG in Mice Causes De-repression of Cyp8b1

Collaboration with Richard Lee and Mark Graham (ISIS Pharmaceuticals)
The Cyp8b1 Promoter Is Repressed by MafG Overexpression

de Aguiar Vallim et al. Cell Metab. 2015
Silencing *MafG* with Antisense Oligonucleotides (ASOs) also Increases *MafF*

Collaboration with Drs. Richard Lee and Mark Graham (ISIS Pharmaceuticals)
MafG Is Expressed in Several Metabolic Tissues

- Adrenal
- Aorta
- BAT
- Brain
- Heart
- Duodenum
- Jejunum
- Ileum
- Colon
- Kidney
- Liver
- Lung
- Muscle
- Skin
- Spleen
- Thymus
- WAT

MafG mRNA (Fold Change)
BACH1 is a MAFG Binding Partner.
FXR-mediated Transcriptional Repression

- FXR
- RXR
- FXRE
- SHP
- MAFG
- FGF15
- FGFR4
- β-Klotho
- Cyp7a1
- Cyp8b1

Hepatocyte

Nucleus
DRC Funded Publications

MAFG Is a Transcriptional Repressor of Bile Acid Synthesis and Metabolism

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http://dx.doi.org/10.1016/j.cmet.2015.01.007

Original Article

The Nuclear Receptor FXR Uncouples the Actions of miR-33 From SREBP-2

Elizabeth J. Tarling Hannah Ahn, Thomas Q. de Aguiar Vallim

Published in ATVB 2015
GSK2324 Treatment of Mice Activates FXR Target Genes in all Four FXR Expressing Tissues

mRNA (Fold Change)

GSK2324

FXR

WT

KO

WT

KO

Liver

Intestine

Kidney

Adrenal Gland

Shp

Bsep

Fgf-15

Ostβ

Ostβ

n=8-10 mice/group
Bile Acid Metabolism Is Profoundly Altered in Isolated Primary Hepatocytes

![Graph showing mRNA (Fold Change) over time post isolation.](image-url)
Bile Acids and the Nuclear Receptor FXR

**Nuclear Hormone Receptors**

- **FXR Controls:**
  - Bile Acid Metabolism:
    - Synthesis, Absorption, Conjugation
  - Lipid and Lipoprotein metabolism
  - FXR was recently shown to mediate effects of bariatric surgery (Ryan et al Nature 2014).
  - FXR ligands in Phase II clinical studies for steatosis.
Loss of Cyp8b1 in Mice Mimics Bile Acid Profile Seen after MafG Overexpression
MafG over-expression in HepG2 cells also leads to reduced Cyp8b1 expression.

Most cell lines do not express Cyp7a1 and Cyp8b1.

- Hep3B (Human)
- Hepa1-6 (Mouse)
GSK2324 Treatment of Mice Activates FXR Target Genes in all Four FXR Expressing Tissues

Liver

Intestine

Kidney

Adrenal Gland

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Loss of Function Studies Show MafG Silencing Increases *Cyp8b1* Expression

![Graph showing mRNA expression changes for MafG and Cyp8b1](image-url)

Primary Mouse Hepatocytes
In Human Cells, MafG Silencing also Increases *CYP8B1* Expression
Bile Acids and the Nuclear Receptor FXR

Nuclear Hormone Receptors

FXR Controls:

- Bile Acid Metabolism:
  - Synthesis, Absorption, Conjugation

- Lipid and Lipoprotein metabolism.

- FXR ligands in clinical studies for steatosis.

Natural FXR Ligands

- CDCA
- Cholic acid (CA)

Synthetic FXR Ligands

- GW4064 (Bass et al 2011)
- GSK2324
The Nuclear Receptor FXR Uncouples the Actions of miR-33 From SREBP-2

Elizabeth J. Tarling, Hannu Ahl, Thomas Q. de Aguiar Vallim
MafG Over-expression Reduces Cholic Acid Levels

Bile Acid Levels (%)

Ad-Control Ad-MafG

α-MCA
β-MCA
CA
ACA
CDCA
DCA

n= 7-8 mice/group
Complexity of Bile Acid Synthesis

Liver:
- CYP7A1
- HSD3B7
- AKR1D1
- AKR1C14
- CYP27A1
- SLC27A5
- AMACR
- ACOX2
- HSD17B4
- SCP2

Cholic Acid

Peripheral Tissues:
- CYP27A1
- CYP7B1

Chenodeoxycholic Acid

MCA

Alternate BA Synthesis Pathway

Classic BA Synthesis Pathway
MafG may Regulate Hepatic Bile Acid Synthesis Independently of Shp
In Human Cells, MafG Is also Regulated by FXR and MafG Overexpression Represses CYP8B1

HepG2 Cell Line
FXR Agonists Are Potent Lipid-Lowering Agents

GW4064 @ 60mpk
GSK2324 @ 60mpk

n=7-9 mice/group
FXR Represses Almost all Enzymes of

Bile Acids: Cholic Acid, Chenodeoxycholic Acid, MCA

Repressed: CYP7A1, CYP27A1, CYP7B1
Unchanged: AKR1D1, AKR1C14, SLC27A5, AMACR, ACOX2, HSD17B4, SCP2
Induced: CYP8B1

de Aguiar Vallim et al. Cell Metab. 2015
In vivo Studies: Silencing MafG in Mice Causes De-repression of Cyp8b1

Collaboration with Drs. Richard Lee and Mark Graham (ISIS Pharmaceuticals)
ChIP Analysis Identified a MARE at the Cyp8b1 Promoter