A microRNA-34a/FGF21 Regulatory Axis and Browning of White Fat

Jongsook Kim Kemper, Ph.D
Department of Molecular and Integrative Physiology,
University of Illinois at Urbana-Champaign, USA

2013 International Conference on Diabetes and Metabolism and 5th Asian Association for the Study of Diabetes, Seoul, Korea
The Global Epidemic of Obesity

- > 1/3 of adult population in USA are obese and 2/3 are overweight.
- Child obesity is rapidly growing.
Obesity: Disruption in Energy Balance

White Adipose Tissue (WAT) & Brown Adipose Tissue (BAT)

Adipose tissue (fat cell) is an endocrine organ that controls energy balance.

WAT (Bad Fat):
- Stores excess energy as Fat
- In particular, abdominal Fat

BAT (Good Fat)
- Packed with mitochondria
- Dissipates energy as heat (via UCP1)
- Newborns and small animals have BAT
- Also, adults have BAT (cold exposure)
- Activation of BAT would be an appealing option for weight-loss and fighting obesity
- BUT!!!
Third Type of Fat: “Beige Fat” Browning of WAT

Caption: Brown fat cells (stained brown with antibodies against the brown fat-specific protein UCP1) nestled amongst white fat cells.

Credit: Patrick Seale, University of Pennsylvania School of Medicine

P. Searle, U Penn
Beige Fat:

• Beige fats are detected by brown-fat specific UCP1 staining.
• Beige fats are distinct from the classical BAT (gene expression patterns, basal levels UCP1, myogenic myf5-negative cell lineage, ----).
• Beige fat depots are developed in WAT in response to various activators, including transcriptional regulators, hormones, and cold exposure (PRDM16, irisin, FGF21, cold exposure (activators of β-adrenergic receptor)-----).
Fibroblast Growth Factor-21 (FGF21)

- Upon cold exposure, FGF21 promotes fat browning in PGC-1α dependent manner.
- Both systemically and locally acts on WAT/BAT and liver to beneficially control metabolism and energy balance.
- Binds to the adipocyte FGF21 membrane receptor complex, FGFR1 and βKL.
- FGF21 resistance in obesity was proposed, but the mechanisms are not clear.

C. Cantor and J., Auwerx, review, Science, 2012
MicroRNAs (miRs)

- Small non-coding RNAs (20-25 nt)
- Binds to 3’ UTR of target mRNAs (negative gene regulators)
- Powerful cellular regulators
- Known that regulate >1/3 of genomes
- Aberrant expression in human disease
Elevated miR-34a causes metabolic problems in obesity!

Our recent published studies on miR-34a:
- J. Lee et al., JBC, 2010 (SIRT1 in liver)
- J. Lee and JK Kemper, Review, Aging, 2010
- T. Fu et al., PNAS, 2012 (bKL in liver)
- SE Choi et al., Aging Cell, 2013 (NAMPT in liver)
- SE Choi and JK Kemper, Review, Mol. and Cells, 2013
- T. Fu et al., MS in preparation (FGFR1, bKL in adipose)

Elevated miR-34a in obesity directly targets βKL in liver

Aberrantly elevated microRNA-34a in obesity attenuates hepatic responses to FGF19 by targeting a membrane coreceptor β-Klotho

Ting Fu*, Sung-E Choi*, Dong-Hyun Kim*, Sunmi Seok*, Kelly M. Suino-Powell*, H. Eric Xu*, and Jongsook Kim Kemper†

*Department of Molecular and Integrative Physiology, University of Illinois at Urbana-Champaign, Urbana, IL 61801; and †Laboratory of Structure Sciences, Van Andel Research Institute, Grand Rapids, MI 49503
miR-34a may directly target the both members of the FGF21 receptor

- bKL (a direct miR-34a target) is abundantly expressed in adipose tissue.
- The 3’UTR of FGFR1 mRNA also contains a potential miR-34a site.
- miR-34a is elevated in adipocyte as well as liver in obese patients.
Elevated adipocyte miR-34a in obesity contributes to FGF21 resistance by directly targeting the both members of the FGF21 receptor complex, FGFR1 and βKL.

If so, downregulation of elevated miR-34a in obesity should restore FGF21 signaling, which results in beneficial effects on metabolism and energy balance.
Inverse correlation between FGFR1/bKL and miR-34a levels in WAT and BAT in obesity
miR-34a directly targets the 3’UTR of FGFR1 mRNA

Overexpression of miR-34a

Downregulation of miR-34a
In vivo role of elevated adipocyte miR-34a in obesity:
Downregulation of miR-34a using lenti-anti-miR-34a (L-34ai) injection

Inject Lentivirus

<table>
<thead>
<tr>
<th>Days</th>
<th>5d</th>
<th>8d</th>
<th>12d</th>
<th>14d</th>
<th>20d</th>
</tr>
</thead>
<tbody>
<tr>
<td>ND or HFD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sacrifice

q-RTPCR
IB
Staining

miR-34a

Rel miRNA level

WAT
BAT

ND (Lenti-E)
HFD (Lenti-E)
HFD (Lenti-34ai)

Body Weight

Body Weight (g)

Start

(g)

0 10 20 30 40 50 60

ND
HFD, LAE
HFD, LA 34ai

Food Intake

Food Intake / per day

g / per day

0 1 2 3

5d 8d 12d

NS
NS
NS
Downregulation of miR-34a (lenti-anti-miR-34a) reduced adiposity in diet-induced obese mice

Types of Adipose Tissue
BAT
WAT:
Visceral Epididymal WAT (eWAT):
  perirenal WAT (prWAT)
  gonadal WAT (gWAT)
Subcutaneous inguinal WAT (iWAT)

Adipose Tissue

Adipose Weight

(g)

0

0.5

1

1.5

BAT  prWAT  gWAT  iWAT

ND(L-E)  HFD (L-E)  HFD(L-34ai)
Anti-miR-34a in obesity promotes fat browning: UCP1 staining

**Adipose Size**

<table>
<thead>
<tr>
<th>ND (L-E)</th>
<th>HFD (L-E)</th>
<th>HFD (L-34ai)</th>
</tr>
</thead>
</table>

**Mitochondrial Number**

<table>
<thead>
<tr>
<th>ND (L-E)</th>
<th>HFD (L-E)</th>
<th>HFD (L-34ai)</th>
</tr>
</thead>
</table>

**mDNA/nDNA**

<table>
<thead>
<tr>
<th>ND (Lenti-E)</th>
<th>HFD (Lenti-E)</th>
<th>HFD (Lenti-34ai)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>0.5</td>
<td>1.0</td>
</tr>
</tbody>
</table>
Anti-miR-34a in obesity promotes browning in WAT (beige fat)

Mitochondrial UCP1 (IHC)

<table>
<thead>
<tr>
<th></th>
<th>ND(L-E)</th>
<th>HFD(L-E)</th>
<th>HFD(L-34ai)</th>
</tr>
</thead>
<tbody>
<tr>
<td>prWAT He UCPI</td>
<td><img src="50um" alt="Image" /></td>
<td><img src="50um" alt="Image" /></td>
<td><img src="50um" alt="Image" /></td>
</tr>
<tr>
<td>Visceral eWAT</td>
<td><img src="50um" alt="Image" /></td>
<td><img src="50um" alt="Image" /></td>
<td><img src="50um" alt="Image" /></td>
</tr>
<tr>
<td>gWAT He UCPI</td>
<td><img src="50um" alt="Image" /></td>
<td><img src="50um" alt="Image" /></td>
<td><img src="50um" alt="Image" /></td>
</tr>
<tr>
<td>Subcutaneous iWAT</td>
<td><img src="50um" alt="Image" /></td>
<td><img src="50um" alt="Image" /></td>
<td><img src="50um" alt="Image" /></td>
</tr>
</tbody>
</table>

Brown gene expression

- prWAT
  - Ucp1: ***
  - Pgc1α: *
  - Prdm16: **

- gWAT
  - Ucp1: **
  - Pgc1α: *
  - Prdm16: *

- iWAT
  - Ucp1: ***
  - Pgc1α: **
  - Prdm16: *
Citrate Synthase (CS) Activity is restored by anti-miR-34a

CS catalyzes the first step in the TCA cycle in mitochondria and its activity is a marker for mitochondrial oxidative function.
Anti-miR-34a in obesity induces true beige fats in all types of WAT: detection of CD137 (Beige fat-specific marker)
Anti-miR-34a in obesity induces beige fats in all types of WAT: detection of CD137 (Beige fat-specific marker)
Mechanisms?

So far,
Anti-miR-34a in diet-induced obese mice:
• reduced adiposity
• increased mitochondrial number and activity
• increased UCP1 expression
• increased browning of WAT (true beige fats)

Question:
How downregulation of miR-34a in obesity restores FGF21 signaling and promotes fat browning of WAT?
Downregulation of miR-34a restored FGF21 signaling in WAT in vivo

Days: 5d, 8d, 12d, 14d, 16d

- Inject Lentivirus
- Begin
- Collect WAT IB Assay
- Sacrifice

FGF21 signaling (Erk)

<table>
<thead>
<tr>
<th></th>
<th>ND</th>
<th>HFD</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-E</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FGFR1/βKL</td>
<td>1</td>
<td>1.11</td>
</tr>
<tr>
<td>βKL</td>
<td>0.63</td>
<td>0.55</td>
</tr>
<tr>
<td>FGFR1</td>
<td>0.38</td>
<td>0.53</td>
</tr>
<tr>
<td></td>
<td>0.55</td>
<td>0.53</td>
</tr>
</tbody>
</table>

FGF21 signaling (Erk)

<table>
<thead>
<tr>
<th></th>
<th>ND</th>
<th>HFD</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-E</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P-Erk</td>
<td>1</td>
<td>4.22</td>
</tr>
<tr>
<td>T-Erk</td>
<td>0.16</td>
<td>0.66</td>
</tr>
<tr>
<td></td>
<td>0.43</td>
<td>0.43</td>
</tr>
<tr>
<td></td>
<td>2.51</td>
<td>4.09</td>
</tr>
</tbody>
</table>

FGF21

βKL
Downregulation of miR-34a decreased Ac of PGC-1α in WAT in vivo

**Diagram:**
- Ac-PGC-1α → FGF21
- miR-34a

**Table: SIRT1 Protein**
<table>
<thead>
<tr>
<th></th>
<th>ND</th>
<th>L-E</th>
<th>HFD</th>
</tr>
</thead>
<tbody>
<tr>
<td>FGF21</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SIRT1</td>
<td>1</td>
<td>0.86</td>
<td>0.10</td>
</tr>
<tr>
<td>Actin</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Note:**
- Kd values are indicated for each condition.
Both FGF21 signaling and SIRT1 are important for anti-miR-34a-mediated PGC-1α deAc and fat browning in adipocyte.
Both FGF21 signaling and SIRT1 are important for anti-miR-34a-mediated PGC-1α deAc and fat browning in adipocyte.
Summary

This study identifies miR-34a a novel target for adipocyte FGF21 signaling, providing exciting potential options for treating obesity-related diseases.

Ting Fu et al., MS in preparation
Acknowledgements

J. Kim Kemper lab

Ting Fu
Sunmi Seok, Ph.D.
Sung-E Choi, Ph.D.
Sam Huang
Subodh Kumar, Ph.D.
Dong-Hyun Kim, Ph.D.
Sangwon Byun, Ph.D.
Sanghoon Kwon, Ph.D.
Danny Ryerson
Erica Yu
David Tkac

Supported by grants from NIH (DK062777, DK095842), AHA Grant in Aid, and ADA Basic Science Award
Extra energy is stored as FAT (TG) in adipose tissue
Downregulation of miR-34 (Lenti-34ai) reduced TG in both liver and WAT in diet-induced obese mice

<table>
<thead>
<tr>
<th></th>
<th>ND(L-E)</th>
<th>HFD (L-E)</th>
<th>HFD(L-34ai)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Oil Red O</strong></td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
</tr>
<tr>
<td><strong>Liver</strong></td>
<td><img src="image4.png" alt="Image" /></td>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
</tr>
<tr>
<td><strong>WAT</strong></td>
<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
<td><img src="image9.png" alt="Image" /></td>
</tr>
</tbody>
</table>
Summary

Obesity

- Directly targets FGFR1 and βKL
- Impaired FGF21 signaling
- Directly targets SIRT1
- Elevated PGC-1 Ac
- Dysfunctional Tx activity of PGC-1α

Obesity + Anti-miR-34a

- Restored FGFR1/βKL/FGF21 signaling
- Increased SIRT1
- Decreased PGC-1 Ac
- Transcription Program of Beige Fat

Ting Fu et al., MS in preparation
Summary

1. miR-34a directly binds to the 3’UTR of both FGFR1 and bKL mRNAs.
2. Elevated adipocyte miR-34a attenuates FGF21 signaling in obesity.
3. Downregulation of miR-34a reduces adiposity in obese mice.
4. Downregulation of miR-34a increases mitochondrial number and function and promotes fat browning (beige fat) in all types of WAT.

5. Mechanisms: Downregulation of miR-34a by lenti-anti-miR-34a
   Restored FGFR1, bKL, and SIRT1 levels in WAT
   Restored FGF21 signaling
   Deacetylation of PGC1-α and increased Tx activity
   Upregulation of brown genes (UCP1, PGC-1α, PRDM16, ---)
   Promotes development of Beige Fat in WAT

6. identifies miR-34a a novel target for adipocyte FGF21 signaling, providing exciting potential targets for treating obesity-related diseases.

Ting Fu et al., MS in preparation
Downregulation of miR-34 (Lenti-34ai) reduced TG in both liver and WAT in diet-induced obese mice.
Mechanisms?
How downregulation of miR-34a in obesity promotes fat browning?

- Increased FGFR1/βKL levels
- Restored FGF21 signaling
- Increased SIRT1 levels
- Decreased Ac of PGC-1α (a key Tx activator of Mito biogenesis and fat browning)
- Promotes transcriptional program of fat browning
miR-34a attenuates FGF21 signaling in adipocytes (3T3)

Overexpression of miR-34a

Downregulation of miR-34a
Inverse correlation between FGFR1/bKL and miR-34a levels in obesity
However, there are limited amounts of BAT in adult humans, BAT is substantially reduced in obese humans, and more importantly, the physiological significance of BAT in adults has just begun to be appreciated, so it is not clear whether targeting the classical BAT would be a therapeutically effective and ideal approach.
Brown Adipose Tissue Activity
(PET-CT with $^{18}$F-FDG)

- Lean, Thermoneutral
- Lean, Cold Exposure
- Overweight, Cold Exposure
miR-34a hampers Glucose uptake in adipocytes

Done by Dr. SungE Choir
NDT Scramble

AntiAmiR34a

ND (Saline)  HFD (Oligo)  Anti-miR34a

H&E

Oil Red

GTT

Glucose (mg/dl)

Time (min)

**  *

ND (Saline)  HFD (Anti-SC)  HFD (Anti-miR-34a)

ITT

Time (min)

**  **  *

*
Figure 4-25  The electron transport system and ATP synthesis  (1) Chemical energy released during metabolism is captured by high-energy electrons. (2) When these high-energy electrons move through the electron transport system, some of their energy is used to transport H⁺ from the matrix into the intermembrane space. (3) By the end of the electron transport system, the electrons are back to their normal energy state. They combine with H⁺ and an oxygen atom to form water. (4) Potential energy from the H⁺ ions concentrated in the intermembrane space is converted to kinetic energy when H⁺ pass through the protein known as ATP synthase. The kinetic energy is captured in the high-energy bond of ATP.
Increased energy expenditure through uncoupled respiration

During mitochondria oxidative phosphorylation pathway, H⁺ leak (which is mediated by UCPs), uncouples the processes of electron transport/proton-gradient generation and ATP synthesis. The energy that is derived from this uncoupling process is released as Heat.