Klf5 Regulates Cardiac PPARα and Med13 and affects Fatty Acid Metabolism And Obesity

Konstantinos Drosatos, PhD
Assistant Professor
Laboratory of Metabolic Biology
Center for Translational Medicine
Department of Pharmacology

School of Medicine
Temple University
Name: Konstantinos Drosatos
Title: Klf5 Regulates Cardiac PPARα and Med13 and affects Fatty Acid Metabolism And Obesity

No conflict of interest to be disclosed
Sepsis/endotoxic shock

* Systemic Inflammatory Response Syndrome

- **Systemic inflammatory** response that follows **bacterial infection**.
- **Hypotension** and **organ failure**.

*SIRS = 2 of the following: Temperature ≥38°C or ≤36°C, HR ≥90 beats/min, Respirations ≥20/min, WBC count ≥12,000/mm³ or ≤4,000/mm³ or >10% immature neutrophils*
Sepsis-associated cardiac dysfunction etiology

Inflammatory Hypothesis
- ↑ TNFα
- ↑ IL-1
- ↑ IL-6

Energetic Hypothesis
- ↓ Fatty acid oxidation

Cardiac dysfunction

LBP
LPS
CD-14
TLR4
NF-κB
PPARα
Sepsis-associated cardiac dysfunction etiology

Inflammatory Hypothesis
- ↑TNFα
- ↑IL-1
- ↑IL-6

Energetic Hypothesis
- ↓Fatty acid oxidation

Cardiac dysfunction

- LBP
- LPS
- CD-14
- TLR4
- NF-κB
- PPARα
### Peroxisome Proliferator Activating Receptors (PPARs)

**Nuclear receptors family members**

<table>
<thead>
<tr>
<th>Isoforms</th>
<th>Major function</th>
<th>Natural ligands</th>
<th>Synthetic ligands</th>
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<tr>
<td>PPARα</td>
<td>Fatty acid oxidation</td>
<td>Fatty acids</td>
<td>Fibrates, WY14643</td>
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<tr>
<td>PPARγ</td>
<td>Lipogenesis &amp; Fatty acid oxidation</td>
<td>Fatty acids, Prostanoid</td>
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<tr>
<td>PPARδ</td>
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<td>Prostacyclins, FAs</td>
<td>GW0742</td>
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</tbody>
</table>
**Stimulation of cardiac fatty acid oxidation and energy production treats septic cardiac dysfunction**

**An Integrated Clinico-Metabolomic Model Improves Prediction of Death in Sepsis**
Raymond J. Langleyl,1,2 Ehrnaim L. Tsallik,1,2,3,4,5 Jennifer C. van Velkinburgh,1,5 Seth W. Glickman,1,6 Brandon J. Rice,1 Chunping Wang,2 Bo Chen,3 Lawrence Carin,7 Arturo Suarez,8 Robert P. Mohney,9 Debra H. Freeman,2 Mu Wang,10 Jinsam You,10 Jacob Wulff,9 J. Will Thompson,9 M. Arthur Moseley,1 Stephanie Reisinger,11 Brian T. Edmonds,12 Brian Grinnell,12 David R. Nelson,13 Darrell L. Dinwiddie,14-16 Neil A. Miller,17-19 Carol J. Saunders,19 Sarah S. Soden,20 Angela J. Rogers,15,16 Lee Gazoulian,15 Laura E. Frederlenburg,15 Anthony F. Massaro,15 Rebecca M. Baron,15 Augustine M. K. Chol,15 G. Ralph Corey,2 Geoffrey S. Ginsburg,9 Charles B. Cairns,8 Ronny M. Otero,8 Vance G. Fowler Jr.,3 Emanuel P. Rivers,8 Christopher W. Woods,3,4,5 Stephen F. Kingsmore,1,19

**Inhibition of c-Jun-N-terminal Kinase Increases Cardiac Peroxisome Proliferator-activated Receptor α Expression and Fatty Acid Oxidation and Prevents Lipopolysaccharide-induced Heart Dysfunction**2,3
Konstantinos Drosatos2, Zoi Drosatos-Tampakaki2, Rafael Khan2, Shunichi Homma3, P. Christian Schulze3, Vassilis I. Zannis4, and Ira J. Goldberg2

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Toll-Like Receptor-Mediated Inflammatory Signaling Reprograms Cardiac Energy Metabolism by Repressing Peroxisome Proliferator-Activated Receptor γ Coactivator-1 Signaling
Joel Schilling, Ling Lai, Nandakumar Sambandam, Courtney E. Dey, Teresa C. Leone and Daniel P. Kelly

PPARγ Activation Prevents Sepsis-Related Cardiac Dysfunction and Mortality in Mice
Konstantinos Drosatos, Raffay S. Khan, Chad M. Trent, Hongfeng Jiang, Ni-Hujing Sun, William S. Blumer, Shunichii Homma, P. Christian Schulze and Ira J. Goldberg
Inhibition of JNK prevents inhibition of cardiac PPARα and FAO and protects cardiac function in sepsis

LPS-induced activation of JNK suppressed PPARα gene expression and Fatty Acid Oxidation

Inhibition of JNK stimulated PPARα gene expression, prevented suppression of FAO and improved cardiac function, despite elevated inflammation

Drosatos K. et. al., Journal of Biological Chemistry, Oct 2011
What is the role of c-Jun in *ppara* gene regulation?
Does c-Jun bind on the PPARα promoter and inhibit PPARα gene expression?

-800bp
AGTG CCTGAGCTGGACACCAGTCAGCCCCCTCAACCCCTGAAAGCTATGCAGAGGGGCGACA

-800bp
TCACGGGACTCGACTGTGCACTGGGGGAGTAGGACCTTCGCACTACGTCTCCCCGGTGT

   AP1 (0.82)     KLFs (0.92)
                    
   Sense

   Anti-sense

-740bp
GCCGGGGGGCAGCGCAGAGCGAGGGTGAGGTCAAGCCGCCGCCTCCCCCTGCAGGACCGTC

   KLFs (0.96)     AP1 (0.92)
                    
   Sense

   Anti-sense
KLFs – Krüppel-like factors

- Zinc-finger proteins.
- Repressing or activating transcriptional function.
- KLF2, KLF3, KLF7 and KLF15 inhibit PPARγ expression and adipogenesis
- KLF4, KLF5 and KLF6 induce PPARγ and adipogenesis
- KLF5 promotes cardiac hypertrophy
- KLF5+/− mice have increased FA oxidation in muscle and are resistant in diet-induced obesity although they consume more food.
KLF5 and KLF6 show the most prominent increase in LPS-treated C57BL/6 mice.
PPARα is reduced and KLF5 is increased in LPS-treated mouse cardiomyocyte-derived cell line (HL-1)

Gene expression (fold-change)

CTRL LPS CTRL LPS CTRL LPS

PPARα KLF5 KLF6

*p<0.05 vs CTRL

William C. Claycomb
LSU Health Sciences Center
Hypothesis: c-Jun and KLF5 are negative regulators of PPARα
c.a. c-Jun decreases while KLF5 increases PPARα gene expression levels in HL-1 cells

**Gene expression (fold-change)**

*AdGFP* vs. *AdKLF5* **p<0.01** vs. *AdGFP* ***p<0.001** vs. *AdGFP*  

*n≥5*
Potential KLF and c-Jun binding sites on PPARα promoter

-792/-772 bp (PPARα promoter)

-719/-698 bp (PPARα promoter)
c-Jun and KLF5 compete for binding on PPARα promoter
LPS-induced binding of c-Jun on PPARα promoter prohibits binding of KLF5

-792/−772 bp (PPARα promoter)

* p<0.05 vs. CTRL

CTRL (cJun Ab)  LPS (cJun Ab)  CTRL (KLF5 Ab)  LPS (KLF5 Ab)
Generation of cardiomyocyte-specific KLF5-/- mouse model

αMHC-Cre-KLF5+/+

X

αMHC-Cre-KLF5+/-

KLF5loxP/loxP

Provided by Prof. Jeffrey Whitsett
Cincinnati Children’s Hospital Medical Center

αMHC-KLF5-/-
αMHC-KLF5-/- have normal cardiac function
Cardiomyocyte-specific ablation of KLF5 reduces cardiac PPARα gene expression

Gene expression (fold-change)

- Heart
- Skeletal muscle
- Intestine
- Kidney

PPARα

- CTRL
- αMHC-KLF5/-

*p<0.05 vs. CTRL
**p<0.01 vs. CTRL
n=5
Cardiomyocyte-specific ablation of KLF5 reduces cardiac PPARα and FAO-related gene expression.
Reduced cardiac PPARα is associated with cardiac lipotoxicity

Is diabetes associated with changes in cardiac KLF5 and PPARα?

CTRL (saline)  Diabetes (STZ)

C57BL/6
Streptozotocin-induced diabetes induces mild cardiac dysfunction in C57BL/6 mice.
Diabetes reduces cardiac KLF5 and PPARα gene expression

![Graph showing gene expression changes](image)

- **KLF5** and **PPARα** gene expression
- **fl/fl** vs. **fl/fl + STZ**
- *p<0.05 vs. fl/fl, n=4

![Western blot images](image)

- KLF5 ~ 50 kDA
- β-actin

**School of Medicine**

**Center for Translational Medicine**
Glucose elevation drives KLF5 and PPARα downregulation

HL-1 cells

CTRL

Glucose (1 mg/ml, 24h)

KLF5

PPARα

Glut1

Glut4

PDK4

AOX

Cpt1b

LCAD

VLCAD

n = 5-6

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Hyperglycemic ob/ob mice have reduced cardiac KLF5 and PPARα.
Glucose is reabsorbed prior to excretion through kidneys

Nature Reviews Drug Discovery (July 2010)
SGLT2 inhibition — a novel strategy for diabetes treatment
Edward C. Chao & Robert R. Henry
Correction of hyperglycemia with SGLT2 inhibition restored KLF5 and PPARα levels in C57BL/6 mice

Pharmacological inhibitor
Dapagliflozin

SGLT2

Anti-sense-SGLT2 oligo

Pharmacologic inhibition of SGLT2 – glucose lowering treatment

**p<0.01 vs. CTRL
***p<0.001 vs. CTRL
n=7
αMHC-KLF5⁻/⁻ mice gain more weight in HF diet

Body weight (g)

Floxed

αMHC-KLF5⁻/⁻

WT

*p<0.05, n=5-7
αMHC-KLF5−/− mice gain more weight in HF diet
High fat-fed αMHC-KLF5−/− mice have larger adipocytes in WAT and higher lipid accumulation in BAT
HFD-fed αMHC-KLF5-/- mice have increased expression of lipid metabolism markers in WAT
A change in cardiomyocytes promotes DIO!

αMHC-KLF5−/−

HF diet

More profound body weight increase
αMHC-MED13−/− mice gain more weight in HF diet

αMHC-KLF5−/− mice have reduced cardiac MED13 expression without changes in miR-208
Adenovirus-mediated overexpression of KLF5 increases MED13 gene expression in HL-1 cells
KLF5 binds the promoter of mMED13 gene
Proposed model

**Increased systemic metabolic rate**

*MED13 promoter* → *KLF binding site* → *KLF5* → *MED13* → Increased systemic metabolic rate

**Reduced systemic metabolic rate** → **Obesity**

*MED13 promoter* → *KLF binding site* → *KLF5* → *MED13* → Reduced systemic metabolic rate

**Obesity**
Summary

1. **KLF5** and **cJun compete** for binding on **PPARα** gene promoter in sepsis.

2. **KLF5** is a **positive regulator** of **PPARα** gene expression that is driven by hyperglycemia.

3. **KLF5** is a **positive regulator** of **MED13** gene expression.

4. **Cardiomyocyte-specific KLF5 deletion** leads to **downregulation** of **MED13** and accelerates **diet-induced obesity**.
Long-term plan

**Activation**

**KLF5**

**↑ Cardiac PPARα**
- Improved cardiac fatty acid oxidation
- Alleviation of cardiac lipotoxicity

**↑ Cardiac MED13**
- Improved systemic metabolism
- Inhibition of obesity
Acknowledgements

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K99/R00 award, 2012-2017