A Central Role of MG53 in Metabolic Syndrome and Type-2 Diabetes

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Accelerated Aging in China

### Percentage of People Over 60 (%)

<table>
<thead>
<tr>
<th>Year</th>
<th>0-15Y</th>
<th>16-59Y</th>
<th>&gt;60Y</th>
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<tbody>
<tr>
<td>2000</td>
<td>19.1%</td>
<td>67.3%</td>
<td>13.7%</td>
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<tr>
<td>2005</td>
<td>19.5%</td>
<td>62.5%</td>
<td>18%</td>
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<tr>
<td>2010</td>
<td>19.3%</td>
<td>57.9%</td>
<td>22.8%</td>
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<tr>
<td>2015</td>
<td>18%</td>
<td>57.9%</td>
<td>22.8%</td>
</tr>
<tr>
<td>2020</td>
<td>19.5%</td>
<td>57.9%</td>
<td>22.8%</td>
</tr>
<tr>
<td>2025</td>
<td>19.3%</td>
<td>57.9%</td>
<td>22.8%</td>
</tr>
</tbody>
</table>

Source: www.workercn.cn
Serious Unmet Medical Needs: Explosion of NCDs

**Diabetes** – 114 Mn in China

- Possible Asian DM phenotype with lower BMI onset, dissimilar glucose metabolism

**Hypertension** – 250 Mn in China:

- High incidence of complications
  - Stroke mortality in China/India is 160/125 vs. US 47, 100k population/year
Metabolic Syndrome: A Clinical Constellation

The IDF consensus worldwide definition of the metabolic syndrome (2005)
MS Increases the Risk of CVD and T2D

Scott M. Grundy, NATURE REVIEWS | DRUG DISCOVERY 5:295-309, 2006
Common Soil of MS: Insulin Resistance

- Obesity
- Dyslipidemia
- Hyperglycemia
- Hypertension
skeletal muscle accounts for 70-90% of insulin-stimulated glucose disposal

Nature Medicine, 10: 355–361, 2004
MG53: A Key Player in Insulin Resistance

Insulin

MG53

Hypertension
Coronary Heart Disease
Stroke
Diabetes
MG53 is Specifically Expressed in Cardiac and Skeletal Muscles

Northern blot of MG53 in *wt* mice.
1: subcutaneous adipose tissue;
2: visceral adipose tissue.
Upregulation of MG53 in Rodent Models with Metabolic Disorders

Song et al., *nature*, 2013
Transgenic Overexpression of MG53 Is Sufficient to Induce Obesity

**a**

<table>
<thead>
<tr>
<th></th>
<th>wt</th>
<th>mg53 TG</th>
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</thead>
<tbody>
<tr>
<td>Heart</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td></td>
<td></td>
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<tr>
<td>Brain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypothalamus</td>
<td></td>
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</tr>
<tr>
<td>Liver</td>
<td></td>
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<tr>
<td>Intestine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
<td></td>
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<tr>
<td>Visceral fat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testis</td>
<td></td>
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</tr>
</tbody>
</table>

**b**

**c.**

- **wt**
- **mg53 TG**

Body weight (g) vs. Age (weeks)
Transgenic Overexpression of MG53 Triggers Severe Insulin Resistance

**Graphs:**

**Graph a.**
- X-axis: Glucose (min)
- Y-axis: Glucose (mg/dl)
- Two lines: ○ wt, ● mg53 TG
- Significant differences marked with **

**Graph b.**
- X-axis: Insulin (min)
- Y-axis: Glucose (% of baseline)
- Two lines: ○ wt, ● mg53 TG
- Significant differences marked with **
Overexpression of MG53 Causes Hypertension and Metabolic Disorders
Transgenic Overexpression of MG53 Does Not Cause Skeletal Muscle Atrophy
Upregulation of MG53 Leads to Diabetic Cardiomyopathy

Diabetic cardiomyopathy is the major cause of death (50%) in patients with diabetes.

Common Soil: Insulin Resistance
Overexpression of MG53 Causes Cardiac Hypertrophy and Ventricular Dilation
Overexpression of MG53 Leads to Myocardial Lipid Accumulation
MG53 Ablation Attenuates HFD-induced Obesity

**A.**

Body weight (g) vs. Age (weeks)

- wt Chow
- mg53-/- Chow
- wt HFD
- mg53-/- HFD

**B.**

wt Chow  mg53-/- Chow  wt HFD  mg53-/- HFD

1 cm
Mg53 deficiency Prevents HFD-induced Glucose Intolerance

The graph shows the glucose levels over time in different conditions. The top graph displays glucose levels in milligrams per deciliter (mg/dl) and the bottom graph shows glucose levels as a percentage of baseline. The x-axis represents time in minutes (0-120), and the y-axis represents glucose levels.

- **wt Chow** (open circles)
- **wt HFD** (solid black circles)
- **mg53-/- Chow** (open squares)
- **mg53-/- HFD** (red squares with error bars)

Statistical significant differences are indicated by asterisks (*) and hashtags (#) on the graph.
MG53 Ablation Attenuates HFD-induced Hypertension

Blood pressure (mmHg)

Systolic

Diastolic

wt Chow
mg53-/ Chow
wt HFD
mg53-/ HFD

*
MG53 Ablation blocks HFD-induced Metabolic Disorders

A. Blood glucose (mg/dl)

- wt Chow
- mg53-/ Chow
- wt HFD
- mg53-/ HFD

* denotes statistical significance compared to control conditions.

B. Insulin (ng/ml)

- Fast
- Fed

* denotes statistical significance compared to control conditions.

C. Cholesterol (mg/dl)

- Fast
- Fed

* denotes statistical significance compared to control conditions.

Triglyceride (mg/dl)
MG53 Attenuates HFD-induced Fatty Liver

Chow

w t

mg53-/-

HFD

w t

mg53-/-

HE staining of Liver
MG53 Deficiency Prevents HFD-induced Pancreatic Dysfunction
MG53 Blocks Muscle Insulin Signaling

(a) Western blot analysis showing the effects of MG53 on muscle insulin signaling.

(b) Bar graphs illustrating the fold changes in phosphorylated and total protein levels for various signaling molecules under different conditions.

- Insulin: -/+ conditions.
- p-IRβ, pY-IRS1, p-Akt: Fold changes relative to wt.
- t-IRβ, t-IRS1, t-Akt: Fold changes relative to wt.

**P-values indicate statistical significance.**
MG53 Blocks Insulin-induced Glucose Uptake in C2C12 Myotubes and Cardiac Myocytes

**Graphs: A and B**

- **A**
  - 2-NBDG uptake (fold of Control)
  - insulin 0 min vs. insulin 30 min
  - Ad-GFP vs. Ad-MG53

- **B**
  - Glucose uptake (fold of Control)
  - insulin 0 min vs. insulin 30 min
  - Ad-GFP vs. Ad-MG53
**MG53 Ablation Restores Insulin Sensitivity in Mice on HFD**

<table>
<thead>
<tr>
<th></th>
<th>Chow</th>
<th>HFD</th>
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<tbody>
<tr>
<td><strong>wt</strong></td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><strong>mg53-/</strong></td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

**Insulin**
- wt Chow: -
- wt HFD: -
- mg53-/ Chow: +
- mg53-/ HFD: -

**p-IRS1** (fold of wt Chow)
- wt Chow: +
- wt HFD: +
- mg53-/ Chow: -
- mg53-/ HFD: -

**t-IRS1**
- wt Chow: +
- wt HFD: +
- mg53-/ Chow: -
- mg53-/ HFD: -

**p-Akt** (fold of wt Chow)
- wt Chow: +
- wt HFD: +
- mg53-/ Chow: -
- mg53-/ HFD: -

**t-Akt**
- wt Chow: +
- wt HFD: +
- mg53-/ Chow: -
- mg53-/ HFD: -

**p-GSK3β** (fold of wt Chow)
- wt Chow: +
- wt HFD: +
- mg53-/ Chow: -
- mg53-/ HFD: -

**t-GSK3**
- wt Chow: +
- wt HFD: +
- mg53-/ Chow: -
- mg53-/ HFD: -

**MG53**
- wt Chow: +
- wt HFD: +
- mg53-/ Chow: -
- mg53-/ HFD: -

**GAPDH**
- wt Chow: +
- wt HFD: +
- mg53-/ Chow: -
- mg53-/ HFD: -
Progression and Outcomes of MS

Grundy SM., *Journal of the American College of Cardiology*, 2006
Different T2D Profile in China vs USA

DiBonaventura, The burden of the Complicated T2DM Patient in China vs USA

- USA:
  - T2DM: 48%
  - T2DM + Hypertension: 14%
  - T2DM + Obesity: 17%
  - Other: 21%

- China:
  - T2DM: 56%
  - T2DM + Hypertension: 9%
  - T2DM + Obesity: 8%
  - Other: 27%
Tracking Insulin Sensitivity in Multiple Organs

- **Liver**
  - Gluconeogenesis
  - Glycogenolysis
  - Insulin
  - Glucose

- **Pancreas**
  - Insulin-dependent glucose uptake
  - Adiponectin
  - Resistin
  - TNF-α

- **Intestine**
  - Glucose

- **Muscle**
  - Glucose

- **Fat**
  - Glucose
  - Lipolysis
  - Adiponectin
Muscle Insulin Resistance Is the Earliest Defect in mg53 TG mice

a

<table>
<thead>
<tr>
<th></th>
<th>Skeletal muscle</th>
<th>Liver</th>
<th>Visceral fat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>wt</td>
<td>mg53 TG</td>
<td>wt</td>
</tr>
<tr>
<td>Insulin</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>p-Akt</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>t-Akt</td>
<td></td>
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<td></td>
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<tr>
<td>MG53</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>GAPDH</td>
<td></td>
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</tbody>
</table>

b

![Graph showing p-Akt/t-Akt (fold of WT) for Skeletal muscle, Liver, and Visceral fat with statistical significance marked with asterisks.](image)

- **: Significant difference

**Insulin**

- - : No Insulin
- + : Insulin Present
Aberrant Muscle Insulin Signaling Precedes Systemic Insulin Resistance in Mice on HFD for 1 week
Systemic Insulin Resistance in *mg53 TG* mice at 38 weeks of age

**Graph:**
- **Title:** p-Akt/t-Akt (fold of wt)
- **Y-axis:** p-Akt/t-Akt (fold of wt)
- **X-axis:** Insulin (-, +)
- **Legend:**
  - *wt* (white bars)
  - *mg53 TG* (red bars)

**Immunoblot:**
- **Tissues:** Skeletal muscle, Liver, Visceral fat
- **Proteins:** Insulin, p-Akt, t-Akt, MG53, GAPDH
- **Conditions:** wt, mg53 TG
- **Comparisons:**
  - *Skeletal muscle*:
    - Insulin
    - p-Akt
    - t-Akt
    - MG53
    - GAPDH
  - *Liver*:
    - Insulin
    - p-Akt
    - t-Akt
    - MG53
  - *Visceral fat*:
    - Insulin
    - p-Akt
    - t-Akt

**Statistical Significance:**
- **Skeletal muscle:**
  - Insulin: + vs. -
- **Liver:**
  - Insulin: + vs. -
- **Visceral fat:**
  - Insulin: + vs. -

**Notes:**
- The graph shows a significant increase in p-Akt/t-Akt ratio for *mg53 TG* compared to wt in various tissues.
- The immunoblot images confirm the expression levels of the proteins under different conditions.
Systemic Insulin Resistance-induced by HFD (35 Weeks)

**Skeletal muscle**

<table>
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<tr>
<th>Chow</th>
<th>HFD</th>
</tr>
</thead>
<tbody>
<tr>
<td>wt</td>
<td>mg53-wt</td>
</tr>
</tbody>
</table>

- Insulin: - + - + - + - +
- p-Akt:  
- t-Akt:  

**Liver**

<table>
<thead>
<tr>
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<th>HFD</th>
</tr>
</thead>
<tbody>
<tr>
<td>wt</td>
<td>mg53-wt</td>
</tr>
</tbody>
</table>

- Insulin: - + - + - + - +
- p-Akt:  
- t-Akt:  

**Visceral fat**

<table>
<thead>
<tr>
<th>Chow</th>
<th>HFD</th>
</tr>
</thead>
<tbody>
<tr>
<td>wt</td>
<td>mg53-wt</td>
</tr>
</tbody>
</table>

- Insulin: - + - + - + - +
- p-Akt:  
- t-Akt:  

**Graphs**

- Insulin levels across different conditions.
- p-Akt/t-Akt ratios in different tissues and conditions.

*Significance levels:*

- * p < 0.05
- ** p < 0.01
- *** p < 0.001
Posttranslational Downregulation of Both IR and IRS1 in MS Models

![Bar graph a](image)

![Bar graph b](image)

![Bar graph c](image)

![Bar graph d](image)
Is MG53 an E3 Ligase?

MG53:
- **RING**
- **B-box**
- **Putative coiled-coil**
- **PRY**
- **SPRY**

**Features**:
- **Ring:** E3 ligase?
- **B-box:** no clear function
- **Coiled-coil:** oligomerization
- **SPRY:** protein interaction
MG53 E3 Ligase Targets IR and IRS1 for Ubiquitin-dependent Degradation

(a) Lysate
IP: IRβ
IB: ubiquitin
95 kDa

(b) Lysate
IP: IRS1
IB: ubiquitin
170 kDa

(c) Lysate
IP: IRβ
IB: ubiquitin
95 kDa

(d) Lysate
IP: IRS1
IB: ubiquitin
170 kDa
Sequence Analysis of MG53 N-terminus

RING B-box Putative coiled-coil PRY SPRY

C14A

Homo sapiens: MSAAPGLVQELSCLQLQLDAPVTAECSHKSFRCALGRVAGEPAADGTVLGCCQAPTRQPQALSTNLQ 70
Mus musculus: MSAAPGLVQELSCLQLQLDAPVTAECSHKSFRCALIRVAGEPAADGTVLGCCQAPTRQPQALSTNLQ 70
Xenopus laevis: MSTPQLQGMQKELQQLQLLELFRPVTENLGCHTQPQGTLSCTGPKNQDNSVSTETCSSFTLQINK 72

ΔRING (lacking 1-56 aa)

Ring finger ‘cross-brace’ motif
Ring Finger Domain Is Required for MG53-mediated IR and IRS1 Ubiquitination

**a**

<table>
<thead>
<tr>
<th>Lysate</th>
<th>IB: Flag</th>
<th>IB: Myc</th>
<th>Flag-FL-MG53</th>
<th>Flag-ΔRING</th>
<th>Flag-C14A</th>
<th>IR-Myc</th>
<th>HA-Ub</th>
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<tbody>
<tr>
<td>+ - - -</td>
<td>- + - -</td>
<td>- - + -</td>
<td>+ + + +</td>
<td>+ + + +</td>
<td>+ + + +</td>
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**b**

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<tr>
<th>Lysate</th>
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<th>Flag-FL-MG53</th>
<th>Flag-ΔRING</th>
<th>Flag-C14A</th>
<th>IRS1-Myc</th>
<th>HA-Ub</th>
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**170 Kd**

**IRβ (Ub)_n**

**95 Kd**
Central Role of E3 Ubiquitin Ligase MG53 in Insulin Resistance and Metabolic Syndrome

- Dietary factors
- Hypertension
- Genetic defects
- Stress...

Insulin Resistance, MS, Obesity, T2D and CVD
Summary

1. MG53 expression is universally elevated in various rodent, nonhuman primate models and *humans* with insulin resistance and metabolic disorders.

2. Transgenic overexpression of MG53 in mice is *sufficient* to cause severe insulin resistance and metabolic syndrome.

3. Upregulation of MG53 is indispensable for diet-induced insulin resistance and metabolic disorders.

4. Thus, upregulation of MG53, a novel E3 ligase, is both necessary and sufficient to target insulin resistance and resultant metabolic syndrome.
Ongoing Translational Research

Man (disease detection, prevention, intervention and possible reversal; human tissue bank)

Rodent Disease Models (KO, KI, TG models, in vivo physiology and pathology, cell signaling circuitries)

Nonhuman Primate disease models

Molecules (medical genetics; high-throughput gene sequencing and proteomics; bioinformatics)
NHP Model of Spontaneous Metabolic Syndrome
Insulin Resistance in MS Monkeys
Upregulation of MG53 in NHPs and Humans with MS

NHP

<table>
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<tr>
<th></th>
<th>Control</th>
<th>MS</th>
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<tr>
<td>MG53</td>
<td><img src="image1" alt="Image" /></td>
<td><img src="image2" alt="Image" /></td>
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<tr>
<td>GAPDH</td>
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Human

<table>
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<th>Control</th>
<th>MS</th>
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<tr>
<td>MG53</td>
<td><img src="image5" alt="Image" /></td>
<td><img src="image6" alt="Image" /></td>
</tr>
<tr>
<td>GAPDH</td>
<td><img src="image7" alt="Image" /></td>
<td><img src="image8" alt="Image" /></td>
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</table>

MG53 protein (fold of control)

- NHP: *p < 0.05
- Human: **p < 0.01
Human Population Genetic Study
Target Validation: MG53 as A Promising Target for Metabolic and CV Diseases

Discovery   Development
Discovery to Delivery

IP Portfolios
University

Healthcare Delivery
Thank You!