The Role of Orphan Nuclear Receptor SHP in Fibrotic Kidney Disease

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Keimyung University School of Medicine
Daegu, Korea
1. Introduction of Diabetic nephropathy
2. Role of SHP in metabolic disease
3. Role of SHP in Fibrotic kidney disease
1. Introduction of Diabetic nephropathy
2. Role of SHP in metabolic disease
3. Role of SHP in Fibrotic kidney disease
End Staged Renal Disease

Adjusted prevalent rates & annual percent change

Rate per million population

Symbols: one-year % change

US Renal Ddata System 2009
Comparison of ESRD incidence & prevalence worldwide

Incidence

Prevalence

all rates are unadjusted. Data from Israel, Japan, & Taiwan are dialysis only.

US Renal Data System 2008
Diabetes was the primary cause of ESRD for 54 percent of new patients in 2007 in US.

US Renal Data System 2009
About 45% of patients of ESRD have diabetes in Korea and Japan.
Diabetic nephropathy

- The single most common cause of ESRD in Korea, Japan, Europe and United States

- A major cause of illness and death in diabetes

- The hallmark of diabetic nephropathy
  Persistent albuminuria (>300 mg/24 hr): overt nephropathy
  Microalbuminuria (30-300 mg /24hrs): incipient nephropathy
The accumulation of extracellular matrix proteins is the key feature of chronic fibrotic kidney disease including diabetic nephropathy.

- Kimmelstiel and Wilson
  : pathologic sign of nodular sclerosis

[Images: H & E Stain, PAS stain]
Pathology of Diabetic nephropathy

Glomerulopathy
- Mesangial expansion
- Glomerular hypertension
- Diffuse thickening of the GBM
- Broadening of foot processes
- Podocyte loss
- Reduced slit pore proteins
- Glomerulomegaly
- Kimmelstiel-Wilson lesion
- Adhesions to Bowman’s capsule
- Neovascularization
- Nodular and diffuse glomerulosclerosis

Tubulopathy
- Tubular hyperplasia and hypertrophy
- Progressive and cumulative atrophy
- Thickening of the TBM
- Epithelial mesenchymal transition
- Accumulation of lysosomal bodies
- Armani-Ebstein lesion
- Reduced tubular brush border
- Increased tubular salt reabsorption
- Increased Na⁺/H⁺ antiporter activity
- Impaired tubular acidification
- Abnormal tubuloglomerular feedback
- Decreased endocytosis of protein
- Abnormal lysosomal processing
- Impaired uptake of organic ions
Pathogenesis of Diabetic nephropathy

High glucose

- AGEs
- Glucosamine
- Angiotensin II
  - Oxidative stress
- PKC
- Endothelin
- Thromboxane

TGF-β1

- Mechanical stretch
- CTGF

Cell hypertrophy
- ECM accumulation

Glomerulosclerosis, arteriolar thickening and tubulointerstitial fibrosis

Renal function failure

Transcriptional activation of transforming growth factor-beta1 in mesangial cell culture by high glucose concentration.


Expression of TGF-β is elevated in multiple forms of experimental and human kidney disease, ranging from diabetic nephropathy and glomerulonephritis to tubulointerstitial nephritis


Increased renal production of transforming growth factor-beta1 in patients with type II diabetes.

Sharma K et al., Diabetes 46: 854-859, 1997

TGF-β1 siRNA suprresses the tubulointerstitial fibrosis in the kidney of ureteral obstruction.

Hwang MY et al., Experimental and molecular pathology 81 48-54, 2006
PAI-1 is the main physiologic inhibitor of the tissue and urokinase plasminogen activator and is considered to be the most important inhibitor of fibrinolysis.
PAI-1 and Diabetes

- Proinsulin-like molecules
- Glucose
- Insulin
- Increased VLDL triglyceride
- Low serum HDL cholesterol
- Insulin resistance
- Hypertension
- Glucose intolerance
- Obesity
- Increased transcription

Endothelial cell
Nucleus

Exon 1
PAI-1 gene

PAI-1

NEJM, 2000
Multifunctionality of PAI-1 in fibrogenesis: Evidence from obstructive nephropathy in PAI-1–overexpressing mice
Matsuo S et al. Kidney Int 2005

PAI-1 directly promotes tissue fibrosis by promoting the migration of monocytes/macrophages, transdifferentiated tubular epithelia, and myofibroblasts
Huang Y et al. JCI 2003

PAI-1 deficiency retards diabetic nephropathy
Nicholas SB. et al. Kidney Int. 2005
1. Introduction of Diabetic nephropathy

2. Role of SHP in metabolic disease

3. Role of SHP in Fibrotic kidney disease
Atypical orphan nuclear receptor that lacks conventional DNA binding domain

Highly expressed in liver, heart, pancreas and Kidney.
Function of SHP

- SF-1
- HNF4
- TTF
- FXR
- LXR
- ER
- ERRγ
- SHP
- PPARγ
- CAR/PXR
- LRH/LXR
- RARRXR
- HNF-4
- TR
- ER
- LXR
- AR
- GR
- Obesity, Diabetes
- Drug metabolism (CYP2B)
- Cholesterol metabolism (CYP7A gene)
- Insulin secretion
- diverse cellular effects
- Cell differentiation
- (Cholesterol)
- (Sex steroid)
- (Glucose Metabolism)
Mechanism of repression of SHP

1. DNA binding inhibition
2. Coactivator competition
3. Recruitment of corepressor
Glucotoxicity in the INS-1 Rat Insulinoma Cell Line Is Mediated by the Orphan Nuclear Receptor Small Heterodimer Partner

Park KG et al. Diabetes 2007
Glucotoxicity in the INS-1 Rat Insulinoma Cell Line Is Mediated by the Orphan Nuclear Receptor Small Heterodimer Partner

Park KG et al. Diabetes 2007
Role of SHP in ER stress induced β-cell dysfunction

A

B

C

Ad-ATF6  Ad-GFP

Dose (mol) 0 10 50 100 50 100

ATF6

Insulin

PDX-1

MafA

BETA2

β-actin

Ad-ATF6  Ad-GFP

Dose (mol) 0 10 50 100 50 100

Insulin secretion (ng/3x10^5 cells/h)

Adenovirus - ATF6 GFP

3 mmol/l 16.7 mmol/l

* *

Ad-GFP  Ad-ATF6

Time (h) 0 6 12 24 12 24

ATF6

SHP

Insulin

18S

Ad-GFP  Ad-ATF6

Dose (mol) 0 10 50 100 50 100

ATF6

SHP

Insulin

PDX-1

18S

siRNA - SHP

Con

Seo HY et al., Endocrinology 2008
The Nuclear Receptor **SHP** Mediates Inhibition of Hepatic Stellate Cells By FXR and **Protects Against Liver Fibrosis**

_Fiorucci S. et al. Gastroenterology 2004_

Loss of Orphan Receptor **Small Heterodimer Partner** Sensitizes Mice to Liver **Injury from Obstructive Cholestasis**

_Park YJ et al. hepatology, 2008_

**Metformin Inhibits Hepatic Gluconeogenesis Through AMP-Activated Protein Kinase–Dependent Regulation of the Orphan Nuclear Receptor** **SHP**

_Kim YD, Diabetes, 2008_
Fenofibrate Differentially Regulates PAI-1 Gene Expression via AMPK–Dependent Induction of SHP

A

B

C

D

Chanda. et al. Hepatology 2009
Orphan nuclear receptor SHP inhibits angiotensin II- and TGF-β Stimulated PAI-1 expression in VSMCs

Lee KM et al. EMM 2009
• liver fibrosis & hepatic injury
• tumor growth
• gluconeogenesis
• lipid and cholesterol metabolism

• Glucotoxicity
• ER stress induced β-cell dysfunction

• TGF-β induced PAI-1 expression

SHP
1. Introduction of Diabetic nephropathy
2. Role of SHP in metabolic disease
3. Role of SHP in Chronic fibrotic kidney
Therefore, we examined whether SHP prevents renal fibrosis in the unilateral ureteral obstruction (UUO) model and elucidated its mechanism in cultured renal cells.
Unilateral urinary obstruction (UOO) surgery.
The expression levels of SHP in UUO kidney

A

<table>
<thead>
<tr>
<th>Gene</th>
<th>Control</th>
<th>UUO</th>
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<tbody>
<tr>
<td>SHP</td>
<td></td>
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<tr>
<td>TGF-β</td>
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<td>PAI-1</td>
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<td>Collagen (type 1)</td>
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<tr>
<td>Fibronectin</td>
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<tr>
<td>β-actin</td>
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</tbody>
</table>

B

- **SHP**
  - Control: 10.0
  - UUO: 2.5
  - Change: -7.5
  - Significance: *

- **TGF-β**
  - Control: 0.5
  - UUO: 3.0
  - Change: 2.5
  - Significance: **

- **PAI-1**
  - Control: 7.5
  - UUO: 2.5
  - Change: -5.0
  - Significance: *

- **Collagen (type 1)**
  - Control: 4.0
  - UUO: 2.0
  - Change: -2.0
  - Significance: *

- **Fibronectin**
  - Control: 5.0
  - UUO: 4.0
  - Change: 1.0
  - Significance: **

UUO → SHP ? → TGF-β → Renal fibrosis
The effect of loss of SHP on fibrotic genes and renal fibrosis

A

<table>
<thead>
<tr>
<th>Gene</th>
<th>SHP +/-</th>
<th>SHP +/-</th>
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<tbody>
<tr>
<td>SHP</td>
<td>![Image]</td>
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<tr>
<td>PAI-1</td>
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![Bar Graphs]

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<thead>
<tr>
<th>Gene</th>
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<td>PAI-1</td>
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<td>α-SMA</td>
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<tr>
<td>β-actin</td>
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</table>
Loss of SHP accelerated renal fibrosis after UUO

SHP plays an important role in ECM accumulation in obstructive nephropathy.

Masson’s trichrome staining
Does SHP Inhibit TGF-β-stimulated PAI-1 and ECM Protein Expression?
The effect of SHP on the TGF-β induced PAI-1

A table showing the effect of SHP on fibrotic gene expression:

<table>
<thead>
<tr>
<th>Ad-SHP (moi)</th>
<th>TGF-β</th>
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<tr>
<td>-</td>
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<td>10</td>
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<td>GFP</td>
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<th>PAI-1</th>
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<th>Collagen (type 1)</th>
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<th>Fibronectin</th>
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SHP inhibits fibrotic gene expression through the downregulation of TGF-beta-stimulated transcription factor activity.

A bar graph showing the effect of TGF-β and SHP on PAI-1/Luc gene expression:

- TGF-β: -
- SHP (ng): -
- 100
- 300
- 500

Fold activity: 4

* PAI-1/Luc

A bar graph showing the effect of TGF-β and SHP on PAI-1/Luc gene expression:

- TGF-β: -
- SHP (ng): -
- 100
- 300
- 500

Fold activity: 3

* PAI-1/Luc
What is the mechanism by which SHP inhibits PAI-1 expression?
TGF-beta-Smad signaling

Table 1  Smad-interacting DNA-binding transcription factors in mammalian cells

<table>
<thead>
<tr>
<th>Smad-binding partners</th>
<th>Interacting Smad and domains</th>
<th>Features/mechanisms of action</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nuclear receptor family</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Androgen receptor (AR)</td>
<td>Smad3 (MH2)</td>
<td>Reciprocal inhibition of Smad3 DNA-binding activity and of AR activity</td>
</tr>
<tr>
<td>Estrogen receptor</td>
<td>Smad1/3/4(MH2)</td>
<td>Repression of Smad target genes</td>
</tr>
<tr>
<td>Glucocorticoid receptor</td>
<td>Smad3 (MH2)</td>
<td>Inhibition of Smad3 transactivation activity</td>
</tr>
<tr>
<td>IFN4</td>
<td>Smad3/4</td>
<td>Cooperative activation</td>
</tr>
<tr>
<td>RXR</td>
<td>Smad3 (MH2)</td>
<td></td>
</tr>
<tr>
<td>Vitamin D3 receptor</td>
<td>Smad3 (MH1)</td>
<td>Coactivation of ligand-induced transactivation of vitamin D receptor</td>
</tr>
</tbody>
</table>

Blobe GC et al. NEJM 2000

Feng XH et al. Annu. Rev. cell. Dev. Biol 2005

Cellular effects
- Differentiation
- Growth inhibition
- Deposition of extracellular matrix
- Apoptosis
Orphan Nuclear Receptor Small Heterodimer Partner Inhibits Transforming Growth Factor-β Signaling by Repressing SMAD3 Transactivation

A. 

B. 

C. 

Does SHP inhibit Smad3 activity?
SHP inhibits fibrotic gene expression through down regulation of the TGF-β/Smad3 and it repressed Smad3 transactivation by inhibition of DNA binding
The effect of SHP on UUO-induced renal interstitial fibrosis

A

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Ad-GFP</th>
<th>Ad-SHP</th>
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<tbody>
<tr>
<td>PAI-1</td>
<td><img src="PAI-1_control.png" alt="Image" /></td>
<td><img src="PAI-1_Ad-GFP.png" alt="Image" /></td>
<td><img src="PAI-1_Ad-SHP.png" alt="Image" /></td>
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<tr>
<td>Collagen</td>
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<td><img src="Collagen_Ad-GFP.png" alt="Image" /></td>
<td><img src="Collagen_Ad-SHP.png" alt="Image" /></td>
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<tr>
<td>α-SMA</td>
<td><img src="%CE%B1-SMA_control.png" alt="Image" /></td>
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<td><img src="%CE%B1-SMA_Ad-SHP.png" alt="Image" /></td>
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</table>

**Bar Graphs**

- **PAI-1**
  - Control
  - Ad-GFP
  - Ad-SHP

- **Collagen (type 1)**
  - Control
  - Ad-GFP
  - Ad-SHP

- **α-SMA**
  - Control
  - Ad-GFP
  - Ad-SHP
The effect of SHP on UUO-Induced renal interstitial fibrosis
The effect of SHP on fibrotic gene expression in the UUO model

A

UUO

<table>
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<th>Control</th>
<th>Ad-GFP</th>
<th>Ad-SHP</th>
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<tr>
<td>SHP</td>
<td><img src="image1" alt="Image of SHP" /></td>
<td><img src="image2" alt="Image of SHP" /></td>
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<tr>
<td>PAI-1</td>
<td><img src="image4" alt="Image of PAI-1" /></td>
<td><img src="image5" alt="Image of PAI-1" /></td>
<td><img src="image6" alt="Image of PAI-1" /></td>
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<td>Collagen (type 1)</td>
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<tr>
<td>Fibronectin</td>
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<td><img src="image14" alt="Image of 18S" /></td>
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B

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Ad-GFP</th>
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<tbody>
<tr>
<td>Relative mRNA expression</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHP</td>
<td><img src="image16" alt="Graph of SHP" /></td>
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<tr>
<td>PAI-1</td>
<td><img src="image19" alt="Graph of PAI-1" /></td>
<td><img src="image20" alt="Graph of PAI-1" /></td>
<td><img src="image21" alt="Graph of PAI-1" /></td>
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<td>Collagen (type 1)</td>
<td><img src="image22" alt="Graph of Collagen" /></td>
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<tr>
<td>Fibronectin</td>
<td><img src="image25" alt="Graph of Fibronectin" /></td>
<td><img src="image26" alt="Graph of Fibronectin" /></td>
<td><img src="image27" alt="Graph of Fibronectin" /></td>
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</table>

C

UUO

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Ad-GFP</th>
<th>Ad-SHP</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAI-1</td>
<td><img src="image28" alt="Image of PAI-1" /></td>
<td><img src="image29" alt="Image of PAI-1" /></td>
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<td>Fibronectin</td>
<td><img src="image31" alt="Image of Fibronectin" /></td>
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<tr>
<td>β-actin</td>
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<td><img src="image35" alt="Image of β-actin" /></td>
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D

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Ad-GFP</th>
<th>Ad-SHP</th>
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</thead>
<tbody>
<tr>
<td>Relative protein expression</td>
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<tr>
<td>PAI-1</td>
<td><img src="image37" alt="Graph of PAI-1" /></td>
<td><img src="image38" alt="Graph of PAI-1" /></td>
<td><img src="image39" alt="Graph of PAI-1" /></td>
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<tr>
<td>Fibronectin</td>
<td><img src="image40" alt="Graph of Fibronectin" /></td>
<td><img src="image41" alt="Graph of Fibronectin" /></td>
<td><img src="image42" alt="Graph of Fibronectin" /></td>
</tr>
</tbody>
</table>
UOO markedly increased the expression of PAI-1, type I collagen, and fibronectin but decreased SHP gene expression.

Moreover, in kidneys of SHP-/- mice, the expression levels of PAI-1, type I collagen, fibronectin and α-smooth muscle actin (α-SMA) were increased compared with those in kidneys of wild-type mice.

In addition, loss of SHP accelerated renal fibrosis after UOO.
Summary of Results (2)

- Adenovirus-mediated overexpression of SHP in cultured rat mesangial cells and renal tubular epithelial cells inhibited TGF-β-stimulated PAI-1, type I collagen, and fibronectin expression.

- SHP inhibited TGF-β- and Smad3-stimulated PAI-1 promoter activities and TGF-β-stimulated Smad3 binding to its response consensus element on the PAI-1 promoter.

- Moreover, up-regulation of SHP expression in the kidney by adenovirus expressing SHP inhibited the expression of UUO-induced PAI-1, type I collagen, fibronectin, and α-SMA.
Conclusion

Fibrotic kidney disease
Diabetic nephropathy
Glomerulonephritis

TGF-β

Smad

SHP

AP-1
Sp1
PAI-1

Kidney
ACKNOWLEDGEMENTS

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Ye-Jin Suh MS student

Kyungpook Natl. University School of Medicine
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Chonnam Natl. University, Hormone Research Center
Hueng-Sik Choi, PhD

University of Ulsan College of Medicine
Ki-Up Lee, MD, PhD