SIRT1 inhibits proliferation of smooth muscle cells and neointimal lesion formation by deacetylation of STAT3

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Smooth muscle cells in vasculo-proliferative diseases

Dzau VJ et al., Nat Med., 2002 Nov;8(11), 1249-56
Sirt1 – a class III histone deacetylase


Sirt1

- histone deacetylation and methylation via SUV39H1
- inhibition of histone acetyltransferases (CLOCK, p300)
- Inhibition of transcription factors (p53, FoxO3a, E2F1, NFκB, MEF2, MyoD, YY1)

- heterochromatin formation
- euchromatin formation

Abdellatif M Circulation Research 2012;111:642-656
Sirt1 and its various functions

- Diabetes mellitus
- Neurodegenerative diseases
- Vascular Diseases
- Cancer

Finkel T et al., Nature, 2009, 460;587-59
Function of Sirt1 in smooth muscle cells of the vasculature and during vascular remodeling processes?
Expression of Sirt1 in vascular SMC

→ Expression of Sirt1 in the murine femoral artery

→ Expression of Sirt1 in human coronary SMC
Regulation of Sirt1 in vascular remodeling

Non dilated
1 week
3 weeks

Relative SIRT1 mRNA expression

0
0.6
1.2

Non
dilated
1 week

*
Knock-down of Sirt1 augments SMC proliferation

siRNA-mediated knock-down of Sirt1

SMC proliferation Absorbance 450nm

WT

SIRT1 KO

MEF proliferation Absorbance 450nm

48h FCS

Sirt1 knock-down augments SMC proliferation.
Over-expression of Sirt1 prevents SMC proliferation

Adenovirus construction

**Sirt1 WT**

- H355
- Ac

**Sirt1 H355A**

- A355
- Deacetylase inactive mutant

Adenovirus production:
- pAd-Sirt1; pAd-Sirt1(H355A)

Deacetylase activity is important for Sirt1’s antiproliferative effect!

**Graph:**
- pAd control
- Sirt1WT
- Sirt1H355A

<table>
<thead>
<tr>
<th>SMC Proliferation (Absorbance 450nm)</th>
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- * indicates significant difference.
Identification of Sirt1 regulated genes

Cytokine-cytokine receptor interaction

Survivin expression

Survivin expression

Dilated femoral artery

Upregulation is essential for cell cycle progression and proliferation
Sirt1 negatively regulates survivin

- Relative survivin mRNA expression in siControl vs. siSIRT1
- Western blot showing survivin and tubulin
- Relative survivin mRNA expression in WT and SIRT1-/-
- Bar graph showing relative survivin mRNA expression in different conditions
Direct interaction of Sirt1 and Stat3

**SIRT1**
- IP: acetyl Stat3

**Stat3**
- IP: acetyl Stat3

**Donor (Cy3)**
- Acetyl STAT3

**Acceptor (Cy5)**
- SIRT 1

**Experiment stimulation**
- acetSTAT3 Cy3/
  - H300B Cy5

**Negative control**
- Cy3/
  - H300B Cy5

**Negative control**
- acet STAT3 Cy3/
  - Cy5

**ΔIF**
- n=4
  - n=42
  - n=44
  - n=43
  - n=42
  - n=42
  - n=40

**Statistical Analysis**
- ***p<0.001**
Stat3 deacetylation by Sirt1 prevents transactivation of survivin

Stat3 is deacetylated by Sirt1

Sirt1-dependent deacetylation attenuates Stat3 promoter-binding
Conditional KO of Sirt1 in SMC augments neointima formation.
Overexpression/reconstitution of Sirt1 prevents neointima formation

Neointima/media ratio

- control
- Sirt1 WT
- Sirt1 H355A

* indicates significant difference compared to control.
Sirt1 activation or reconstitution: Novel approach to prevent vascular proliferative disease?

VSMC proliferation neointima formation
Thank you!

Molecular Cardiology

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