췌장 베타세포에서의 포도당-지질 독성유발 관여 miRNA 검색 및 작용기전 규명을 통한 당뇨병 치료물질 개발

가톨릭대학교 내분비내과
윤건호

2008년 설원 연구비
Regional Estimates of Deaths Attributable to Diabetes (20~79 age group)

Composition of CV death in diabetes

Diabetes Atlas. 3rd ed. International Diabetes Federation; 2006,
Chan JCN, Yoon Kh et al: JAMA. 2009;301(20):2129-2140

Yoon KH et al: Lancet 2006;368:1681-88
Characteristics of Asian patients with T2DM

- **Type 2 Diabetes Mellitus**
  - “Asia’s big problem”
    - Epidemic in whole Asia
- **Characteristic findings**
  - Remarkable increase within short periods
  - Lesser degree of obesity
  - Early onset
  - High prevalence of diabetic chronic complications

Yoon KH et al: Lancet 2006; 368: 1681–88
Decreased \( \beta \)-cell mass in T2DM

Control 1: \( r = 0.64 \)

DM: \( r = 0.55 \)

BMI and \( \beta \)-cell mass were linearly correlated both in control group and DM group.

Yoon KH et al. J of Clin Endocrinol Metab, 2003
Progressive loss of β-cell mass in adults

- Hyperglycemia ("glucose toxicity")
- Pro-inflammatory cytokines, oxidative & ER stress
- Elevated Lipids ("lipotoxicity")
- Amyloid fibrillisation and fibrotic destruction

Increased β-cell apoptosis

β-cell dysfunction

- Reduced β-cell mass
- Impaired Insulin secretion
- Impaired Proinsulin processing

Establishment of glucolipotoxicity

![Diagram showing the establishment of glucolipotoxicity with timeline and images of cellular structures.]

- **SD rat isolation**
- **Glucolipotoxicity condition**

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<th>Glucolipotoxicity</th>
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<td><strong>Insulin</strong></td>
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<tr>
<td><strong>BETA2</strong></td>
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<td></td>
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<tr>
<td><strong>Pdx-1</strong></td>
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<td><strong>GAPDH</strong></td>
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**Insulin secretion**

- **5.5 G**
- **25 G**

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<th>GLTx</th>
<th>GLTx+AICAR</th>
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<tr>
<td>2 day</td>
<td></td>
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<tr>
<td>3 day</td>
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PGC-1α specifically suppresses BETA2/NeuroD gene transcription

miRNAs play important roles in cell proliferation, apoptosis and differentiation.
miRNAs in pancreas development

miR-290, miR-295
miR-12, miR-13, miR-14, miR-14
Dicer Ref: 34, 36, 37, 38

miR-9, miR-124a, miR-193, miR-18
miR-326 Ref: 45

miR-125a, -125b, miR-286, miR-23b, miR-18
Several miRNAs including miR-129a, miR-27a, miR-219, miR-29a, miR-182, miR-28 and miR-98
Ref: 46

miR-15a, -15b, miR-196, miR-18, miR-302b
Several miRNAs including miR-30a-5p, miR-30b, miR-224, miR-22 and miR-367
miR-503, miR-541, miR-214
Ref: 48, 39

miR-214
Several miRNAs including miR-30c, miR-133a
miR-342, miR-377, miR-136 and miR-182
miR-187, miR-365, miR-17, miR-193
miR-148a, miR-152, miR-195, miR-184, miR-130b
miR-296, miR-324-3p, miR-214, miR-133a,
miR-133b, miR-18a

miR-326, miR-150

miR-9, miR-375
Ref: 17, 16, 60

Trends in Endocrinology & Metabolism

Joglekar MV et al. 2007, Trends in Endocrinology & Metabolism
Aims...

- To search the candidate miRNAs related to glucolipotoxicity-induced β-cell dysfunction
- To clarify the intracellular action mechanism of newly discovered miRNA
Glucolipotoxicity in pancreatic islets

Kim et al. 2009, Endocrinology
miRNAs in glucolipotoxicity-induced rat islets

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miRNA | Target gene names
miRNA-26 | GSK 3 beta, IGF 1 (somatomedin C), CEBPb, myotrophin, PGC-1 b
miR-22 | EIA binding protein p300, PGC-1 alpha
miR 30 5p | IRS 1, 2, ngn1, Vimentin, Insulin like growth factor 2R, FOXO1, 3A, insulin induced gene 2
miR-183 | IRS 1, insulin induced gene 1
miR-16/155/424/497 | BH4H domain, activin A receptor, synaptophysin-like 1, CREB protein, PGC-1
miR-103/107 | IRS 2, PGC-1 alpha
miR-24 | Activin A receptor, CBP/p300, Follistatin, IGF binding protein, insulin induced gene1, NeuroD1
miR-182 | FOXO3, Activin A receptor, CEBPb, BH4H domain, CBP/p300, insulin induced gene 1, Synaptophysin
miR-185 | IGF receptor, NeuroD 2, Activin A receptor
miR-375 | Insulin induced gene 2, Insulinoma-associated 1, myotrophin, Synaptophysin

Array

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<thead>
<tr>
<th>miRNA</th>
<th>Low</th>
<th>Glucolipotoxicity</th>
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<tbody>
<tr>
<td>miR375</td>
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<td>Down: 11</td>
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Effects of miRNAs on expression of pancreatic specific gene by inhibition or overexpression of miRNA
Ad-si30a-5p using an AdEasy™ Adenoviral Vector System

Cloning Gene of Interest

Homologous Recombination in vivo in Bacteria

Virus Production in AD-293 Cells

GLTx + Ad-si30a-5p

Gluclolipotoxicity

Ad-si30a-5p - +

miR30a-5p
Effects of miRNAs on expression of pancreatic specific gene by inhibition of miRNA

**Insulin 3’UTR**

**BETA2/NeuroD 3’UTR**

**PGC-1α 3’UTR**

- **Conserved sites for miRNA families broadly conserved among vertebrates**
  - Conserved sites for conserved miRNA families
  - Conserved sites for conserved miRNA families
  - Conserved sites for conserved miRNA families
Effect of miR30a-5p on insulin secretion and insulin contents
miR30a-5p directly targets the BETA2/NeuroD 3' UTR

(a) Diagram showing the target sites of miR30a-5p on the BETA2/NeuroD 3' UTR.

(b) Bar graph showing relative protein levels (GFP) with and without anti-miR30a-5p and anti-miR30a-5p MUT treatments.

(c) Bar graph showing relative protein levels (BETA2) with and without anti-miR30a-5p and anti-miR30a-5p MUT treatments in low and glucolipotoxicity conditions.
In vivo study

Ad-si30a-5p injection into celiac artery

Harvest

0

18 days

Body weight
Non-fasting glucose
IP-GTT
Insulin staining
Real-time PCR
(insulin & BETA2/NeuroD)

Ad-si30a-5p → 1.0 x 10^9 pfu / 300ul into celiac artery
Function of miR30a-5p in type 2 diabetes animal model

(a) Non-fasting glucose concentration over time for Ad-si30a-5p (n=11) and Ad-GFP (n=10).

(b) Blood glucose level over time for Ad-si30a-5p (n=11) and Ad-GFP (n=10).

(c) Area Under the Glucose Curve for Ad-GFP and Ad-si30a-5p.

(d) Body weight over time for Ad-si30a-5p (n=11) and Ad-GFP (n=10).

(e, f) Histological images comparing Ad-GFP and Ad-si30a-5p.

(g, h) Additional histological images comparing Ad-GFP and Ad-si30a-5p.

(i) mRNA expression levels of Insulin and BET2/NeuroD for Ad-GFP and Ad-si30a-5p.
Expression of miR-30a-5p increased in glucolipotoxicity-exposed islets.

We find that miR-30a-5p is regulated by glucolipotoxicity and alters insulin gene expression by affecting the expression of BETA2/NeuroD.

Our data provide evidence that as inhibition of miR-30a-5p prevented deterioration of glucose tolerance, it may constitute a novel pharmacological target for the treatment of diabetes.
Acknowledgments

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