Gene therapy: State of the art

- Ramon Gomis MD, PhD, MAE.
- Hospital Clínic. IDIBAPS. University of Barcelona.
Gene Therapy: First Steps
Type 1 diabetes: An islet disease

Pancreatic Islet

Alpha cell

Beta cell
Beta cell destruction

Insulitis

Amyloid deposits
Insulin analogs.

Short acting

Long acting
Inhaled insulin
Cell Therapy for Diabetes Mellitus: An Opportunity for Stem Cells?

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aCABIMER (Andalusian Center for Molecular Biology and Regenerative Medicine), and bVirgen de Macarena Hospital, Seville, Spain; cStem Cell Technologies (I), and dDepartment of Orthopaedics and Traumatology, NUH, Singapore, Singapore
Stem cells making insulin

Gene Therapy in diabetes

1. Design of genes construction.
2. Tissue target
3. Protein expression
4. Functionality
5. Effectiveness
The four barriers to successful gene therapy

1.- Uptake, transport and uncoating.

2.- Vector genome persistence

3.- Transcriptional activity

3.- The immune response can limit the viability of the transduced cells
Kay MA, Nature Reviews Genetics, 2011
Plasmid-based gene therapy for diabetes

1.- Therapy with cytokine inhibitors in autoimmune disease.

2.- DNA vaccination against type 1 diabetes

3.- Insulin delivered by gene therapeutic approaches.

4.- Leptin gene therapy in models of obesity and diabetes
Pivotal role of leptin-hypothalamus signaling in the ethiology of diabetes uncovered by gene therapy: a new therapeutic intervention?

Kalra SP Gene Therapy (2011) 18, 319-325
Reinstatement of central leptin sufficiency by single systemic injection of recombinant adenovirus vector encoding leptin gene suppressed hyperglycemia in rodent models of type 1 diabetes.

Stable restoration of leptin sufficiency, solely in the hypothalamus, with biologically active leptin imposed euglycemia by stimulating glucose disposal in the periphery in models of diabetes type 1.
Leptin gene therapy for Diabetes

I Diabetes Type 1
- Hypothalamic Network
- Neural Signal

II Diabetes Type 2

a

LEPTINOPENIA
WAT
INSULIN DEFICIT

Leptin Insufficiency

(-)

(-)

(-)

Leptin Insufficiency

Hyperleptinemia
Obesity

WAT
LEPTIN

(-)

(-)

(-)

Pancreas
Liver
Muscle
BAT

Glucose Disposal

Diabetes
Hyperglycemia

Glucose Disposal

DIABETES

b

Leptin Gene Therapy

LEPTINOPENIA
WAT
INSULIN DEFICIT

Leptin Sufficiency

(+)

(+)

(+)

Leptin Gene Therapy

(+)

(+)

(+)

Pancreas
Liver
Muscle
BAT

Glucose Disposal

Euglycemia

Euglycemia

Kalra SP, Gene Therapy, 2011
Non-viral-mediated hepatic expression of IGF-1 suppresses autoimmune diabetes in mice.

Gene Therapy in diabetes

1.- Design of genes construction.

2.- Tissue target

3.- Protein expression

4.- Functionality

5.- Effectiveness
RNA interference (RNAi) is a powerful approach for reducing the expression of endogenously expressed proteins. It is used to silence mRNAs encoding pathogenic proteins for therapy.

Recently, RNAi-based gene silencing approaches have been demonstrated in humans, and ongoing clinical trials hold promise for treating fatal disorders or providing alternatives to traditional small molecule therapies.

However, many hurdles remain for using these technologies for therapy.
Time course of Type 2 Diabetes pathogenesis

- **Normal**
  - Circulating FFA
  - Fasting Serum Insulin
  - Fasting Plasma Glucose
  - Pancreatic β-Cell Mass

- **Adaptation**
  - INCREASING AGE AND/OR DEGREE OF OBESITY
  - Increasing peripheral insulin resistance

- **Glucose Intolerant**
  - Circulating FFA
  - Fasting Serum Insulin
  - Fasting Plasma Glucose
  - Pancreatic β-Cell Mass

- **Type-2 Diabetes**
  - Circulating FFA
  - Fasting Serum Insulin
  - Fasting Plasma Glucose
  - Pancreatic β-Cell Mass

*INCREASING AGE AND/OR DEGREE OF OBESITY* (Increasing peripheral insulin resistance)
Genes expressed differentially between obese and control rats

**Obese (CAF) rats**
- 5 arrays

**Control (STD) rats**
- 5 arrays

**Islets**
- (10, 30 days)

**Affymetrix GeneChip RAE 230 2.0**

**Statistical analysis**

**Genes expressed differentially between obese and control rats**
## Microarray analysis

<table>
<thead>
<tr>
<th>Affymetrix ID</th>
<th>Gene Title</th>
<th>Gene Symbol</th>
<th>Fold Change CAF vs STD</th>
<th>CAF vs STD</th>
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<tbody>
<tr>
<td>1378097_at</td>
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<td>---</td>
<td>+2.16</td>
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<tr>
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<td>period homolog 2 (Drosophila)</td>
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<td>1370812_at</td>
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<td>Bcl2l1</td>
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<td>Apoptosis</td>
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<td>1377418_at</td>
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<td>---</td>
<td>-1.55</td>
<td>---</td>
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<td>1394913_at</td>
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<td>---</td>
<td>-1.95</td>
<td>---</td>
</tr>
<tr>
<td>1393069_at</td>
<td>secreted frizzled-related sequence protein 5</td>
<td>Sfrp5</td>
<td>-2.30</td>
<td>Development, Proliferation</td>
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</tbody>
</table>

* n=6, p<0.01

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![Graph showing mRNA relative expression for Per2, Bcl2l1, and sFRP5 between STD and CAF conditions.](image-url)
sFRP5 and Wnt pathway

Results of PCR Arrays in CAF islets:

<table>
<thead>
<tr>
<th></th>
<th>Down-regulated genes</th>
<th>Up-regulated genes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fold change (CAF vs STD)</td>
<td></td>
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<tr>
<td>APC</td>
<td>-1.45</td>
<td>Dishevelled 2</td>
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<tr>
<td>Casein kinase 1, alpha 1</td>
<td>-2.25</td>
<td>Wisp1</td>
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<tr>
<td>E1A binding protein p300</td>
<td>-1.35</td>
<td>Tcf7</td>
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<tr>
<td>Wif1</td>
<td>-1.50</td>
<td>Cyclin D1</td>
</tr>
<tr>
<td>Sfrp5</td>
<td>-2.17 *</td>
<td>Myc</td>
</tr>
</tbody>
</table>

|                   |                     |
| Fzd1              | 1.77                |
| Fzd2              | 1.45                |
| Wnt1              | 1.56                |
| Wnt11             | 2.02                |
| Wnt2b             | 2.32                |
| Wnt5a             | 1.33                |
| Wnt5b             | 1.62                |
| Wnt7a             | 1.34                |
| Wnt7b             | 1.36                |
| Wnt9a             | 2.05                |

n=4
*
, p<0.02
Knock down of sFRP5 in INS1E cells

The decrease of sFRP5 expression in INS1E leads to an increase in proliferation.
To confirm the implication of sFRP5 in beta cell plasticity

Knockdown of sFRP5 in dispersed cells from pancreatic islets (Wistar rats).

![Image: Islets → Dispersed cells from islets → IF: Dispersed cells (Insulin)]

![Graph: Strp5 mRNA relative expression]

- si control
- si SFRP5

* Significant difference
Knockdown of sFRP5 in dispersed cells from islets

The decrease in sFRP5 expression in dispersed cells from islets promotes the increase in proliferation without effect on apoptosis.
Rosa Gasa
Anna Novials
Rita Malpique
Sandra Rebuffat