ANIMAL MODELS FOR GENE THERAPY OF GLYCOGENOSIS

Mireia Ramos Muntada | Genetics Degree

Since the first human trial in 1990, gene therapy has generated great expectations in society. After over 20 years, there are a lot of gene therapy protocols that have reached the clinical stage. In the near future, gene therapy will be an effective alternative to pharmacological efforts, and enable treatment of many diseases that are refractory or not suitable for pharmacological treatment alone. Thus, gene therapy is a therapeutic tool that gives us virtually unlimited possibilities to develop better and more effective therapies for previously incurable diseases.

Before applying gene therapy in humans it is necessary to do preclinical studies. The aim of these is to protect humans of toxic effects that the studied drug may have.

An important element in preclinical studies are animal models. First, tests are made with small animals like mice. If they are successful, then tests are made with larger animals, like dogs. Finally, if these studies give good results then they are passed to higher animals: primate or humans.

OBJECTIVES

- To gain insight on the role of gene therapy in the field and on the different approaches that are being studied.
- To catch up with the preclinical phases of gene therapy.
- To know glycogen storage diseases and their types.
- To know the assays with animal models in gene therapy.

GLYCOGEN STORAGE DISEASES (GSD)

<table>
<thead>
<tr>
<th>Clinical disorder</th>
<th>Gene</th>
<th>Affection</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSD-1</td>
<td>GY52</td>
<td>Hepatic</td>
</tr>
<tr>
<td>GSD-IIa/b (Non Gaucher)</td>
<td>G6PC/G6PT1</td>
<td>Hepatic</td>
</tr>
<tr>
<td>GSD-IIb (Pompe/Danon)</td>
<td>GAA/LAMP2</td>
<td>Multigorgan</td>
</tr>
<tr>
<td>GSD-III (Ceri-Forbes)</td>
<td>AGL</td>
<td>Multigorgan</td>
</tr>
<tr>
<td>GSD-IV (Andersen)</td>
<td>GBE1</td>
<td>Multigorgan</td>
</tr>
<tr>
<td>GSD-V (McArdle)</td>
<td>PYGM</td>
<td>Muscle</td>
</tr>
<tr>
<td>GSD-VI (Hers)</td>
<td>PYNL</td>
<td>Hepatic</td>
</tr>
<tr>
<td>GSD-VII (Tarui)</td>
<td>PFKM</td>
<td>Muscle</td>
</tr>
<tr>
<td>GSD-VIIa/d</td>
<td>PHKA1</td>
<td>Muscle</td>
</tr>
<tr>
<td>GSD-VIIa/k2/x2</td>
<td>PHKA2/PHKG2</td>
<td>Hepatic</td>
</tr>
<tr>
<td>GSD-Ixb</td>
<td>PHKB</td>
<td>Multigorgan</td>
</tr>
<tr>
<td>Lafora</td>
<td>EPM2A</td>
<td>Multigorgan</td>
</tr>
</tbody>
</table>

GSD are hereditary disorders, usually autosomal recessive, affecting the glycogen metabolism (Figure 2). Almost all proteins involved in the synthesis or degradation of glycogen and its regulation have been discovered for some types of GSD.

Depending on the organ that glycogenosis affect we have three types of glycogenosis (Table 1).

The frequency of all types of GSD in Europe is about 1/20,000-25,000 live births. The most common, 90% of the total, are: GSD-I, II, III and VI[2].

HEPATIC GSD: GSD-la

Deficiency in the G6Pase-catalytic unit that hydrolyses G6P into glucose and phosphate in the terminal step of glycogenolysis and glycogenesis.

MOUSE MODEL

Creation a G6Pase-knockout mouse

Introduction Ad-mG6Pase at 2-week-old G6Pase−/− mice

A single administration of Ad-mG6Pase improved the growth rate and completely corrected the metabolic abnormalities in these mice [3].

DOG MODEL

Naturally p.M121I G6PC mutation in Maltese Breed

Crossbreed Maltese and Beagle dogs

Administration AAV2/8 vector to affected dog on day 3 or 4 of life

PAS staining revealed decreased liver glycogen (a) 11 weeks following AAV vector administration compared with an untreated (b), affected control liver [4].

SKELETAL MUSCLE GSD: GSD-VII

Mutations in the muscle 6-phosphofructokinase (PFK).

- Exercise intolerance
- Muscle weakness
- Cramping
- Mild myopathy
- Myoglobinuria
- Compensated hemolisys

MOUSE MODEL

Obtaining transgenic mice without PFK-1 activity

Using the technique of homologous recombination in pluripotent embryonic stem cell and microinjection of blastocysts

CONCLUSIONS

- Gene therapy represents a promising tool to cure some of those diseases that conventional drug therapies cannot.
- The availability of animal models is key to preclinical phases.
- In GSD-la, adenoviral therapy produces only short term corrections and only impacts liver expression of the gene; while AAV-mediated therapy achieves longer term correction and the transgene arrives to both the liver and kidney.
- In GSD-VII, the availability of the murine model allows determination of the role of such metabolic alterations in different tissues and organs together with their interactions, and, importantly, allows the study of GSD-VII as a systemic disorder.

REFERENCES