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ORIGINAL ARTICLE

Identification of key genes involved in Down's syndrome pathogenesis by gene therapy

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Abstract
Viruses have evolved ways of encapsulating and delivering their genes into human cells. Gene therapy takes advantage of this capability to manipulate the viral genome and convert an infectious agent into an efficient vector that delivers therapeutic genes. In the current work we have applied gene therapy approaches based on adeno-associated virus and lentivirus delivery to identify candidate genes (protein-coding or miRNAs) involved in the cognitive deficits in Down Syndrome. We show that the hippocampal injection of the adeno-associated virus AAV2/1-shDyrk1A normalized Dyrk1A expression in the trisomic Ts65Dn mice. As a consequence the regulation of key molecular players in memory and learning processes was rescued and mice showed an attenuation of synaptic plasticity defects and improved efficacy in learning strategies. All together these results reinforce the role of Dyrk1A in cognition. On the other hand, with the lentiviral strategy developed to specifically inhibit miR-155 and miR-802 (Lv-anti-miR155/802), we were able to show a tight control of the miRNAs target Mecp2 suggesting that the downregulation of MeCP2 in Down syndrome could be a contributing factor to the cognitive defects.

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PALABRAS CLAVE
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Identificación de genes clave implicados en el síndrome de Down mediante terapia génica

Resumen
La terapia génica nos ofrece la posibilidad de manipular los virus para convertir un agente infeccioso en un vehículo que transporta en su genoma secuencias de DNA con potencial terapéutico. En este trabajo hemos aprovechado la metodología que nos proporciona la terapia génica para desarrollar vectores virales derivados de virus adenoasociados y lentivirus con el objetivo de identificar genes clave (proteínas o microRNA [miRNA]) implicados en las alteraciones cognitivas presentes en el síndrome de Down (SD). Hemos demostrado que en un contexto de trisomía, como es el modelo de ratón Ts65Dn, la normalización de la expresión de Dyrk1A a través de la administración de los virus adenoasociados AAV2/1-shDyrk1A contribuye a restablecer la regulación de moléculas clave en los procesos de memoria y aprendizaje. Ello permite una atenuación de los defectos en plasticidad sináptica y facilita el desarrollo de una estrategia de aprendizaje visuoespacial más eficiente. Estos estudios refuerzan el papel destacado de Dyrk1A en los procesos cognitivos. Por otro lado, la estrategia de control de la expresión de miRNA desarrollada mediante los lentivirus Lv-anti-miR155-802 nos permite proponer a *MeCP2* como un gen cuya desregulación en el síndrome de Down puede tener un papel clave en el deterioro cognitivo.

Chromosome 21

The presence of a complete or partial copy of chromosome 21 (Hsa21) is the cause of Down's syndrome (DS). This excess of genetic material leads to a dysregulated expression of certain genes. The functional impact of these changes could be a direct result of the action of the proteins expressed in excess by the Hsa21 genes, or indirectly, through the proteins that they regulate. In any case the effect will be different according to the protein involved.

In some cases it may be that the excess of a particular protein is innocuous, while small changes in the expression of key proteins may be sufficient to alter fundamental cell processes and functions. In the last few years it has been discovered that the genome contains genes that do not code for proteins, but that they are transcribed to give rise to small RNAs, microRNAs (miRNAs), which exert a negative regulation of gene expression. Each miRNA can act on a large number of proteins, thus, the changes in a single miRNA can control the expression of a large number of proteins and thus, have a very notable impact on certain cell functions. Several miRNAs have been described in chromosome 21, five of which (miR-99a, miR-125b-2, miR-155, miR-802 and let7-c) have been detected in excess in some tissues[1]. The presence of these miRNAs in excess suggests that, in trisomy conditions, some of those proteins regulated by these miRNAs could be under-expressed. The lack of these could cause a change in the physiological processes in which they participate.

The imbalance of the proteins in the trisomic cells, whether mediated by coding or non-coding genes (such as miRNAs) is significantly gene-dependent. While for certain genes the compensatory effects may lessen the impact of the genetic imbalance, for other genes these changes could have a high functional impact. This demonstrates the critical role of certain dose-dependent genes. It is important to identify these dosage-sensitive elements of the genome to be able to better understand their effects and their pathogenic impact.

Gene therapy as a strategy to study the effects of dosage-sensitive genes

Gene therapy consists of the transfer of genetic material to the cells of an individual, with the aim of correcting genetic defects and to be able to change the course of the disease or relieve some of its symptoms. Its development has arisen mainly, but not exclusively, for the treatment of hereditary diseases in which the causal alteration resides in mutations in a known gene that prevents the correct expression of the protein, and thus its function. There are currently a significant number of gene therapy trials, particularly for the treatment of some rare diseases[2-4]. It should be mentioned that in November 2012, the European Commission approved the first gene therapy drug, and it was for the treatment of a lipoprotein lipase deficiency[5]. Gene therapy clinical trials are also being conducted for the treatment of complex diseases such as Alzheimer's disease (registry number at clinicaltrials.gov: NCT00876863). Thus, in the future it may be possible that individuals with DS could enter gene therapy clinical trials, either for the treatment of the Alzheimer-type anomalies associated with DS, or other clinical manifestations present in individuals with DS. However, at the moment nobody with DS has been included in any clinical trial.

Furthermore, gene therapy has, to a great extent, led to the development of numerous tools and strategies to approach the treatment of diseases, advances that are of interest for many other applications, not necessarily therapeutic. One of the key elements has been the development of viral vectors. The ability of a virus to infect cells is exploited in order to transform them into recombinant, replication-defective, viruses capable of transporting genetic elements that will be expressed within the target cell.

In this work we demonstrate that gene therapy provides a new opportunity to identify key genes in the pathophysiology of Down's syndrome. We have developed a strategy based on the use of viral vectors: *a)* modulating the expression of *Dyrk1A*, a gene for which there is previous