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Gene therapy: Myth or reality?

Thérapie génique : mythe ou réalité ?

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ABSTRACT

Gene therapy has become a reality, although still a fragile one. Clinical benefit has been achieved over the last 17 years in a limited number of medical conditions for which pathophysiological studies determined that they were favorable settings. They include inherited disorders of the immune system, leukodystrophies, possibly hemoglobinopathies, hemophilia B, and retinal dystrophies. Advances in the treatment of B-cell leukemias and lymphomas have also been achieved. Advances in vector development and possible usage of gene editing may lead to significant advances over the next years.

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Édition de gène

RÉSUMÉ

Bien qu'encore fragile, la thérapie génique est devenue une réalité. Un bénéfice clinique a été obtenu au cours des 17 dernières années dans un nombre limité de conditions médicales pour lesquelles des études physiopathologiques avaient déterminé qu'elles étaient favorables. Il s'agit des troubles héréditaires du système immunitaire, des leucodystrophies, éventuellement des hémoglobinopathies, d'hémophilie B et des dystrophies rétiniennes. Des progrès dans le traitement des leucémies à cellules B et de certains lymphomes ont également été accomplis. Les progrès dans le développement de vecteurs et la possibilité d'utiliser l'ingénierie génomique ciblée pourront conduire à des progrès importants au cours des prochaines années.

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1. Introduction

Gene therapy has attracted many scientists and clinicians for its potential to fix a disease based on genes. This was initially viewed as a way to correct genetic diseases, but further applications were rapidly considered since genes products can convey cells with characteristics of medical interest to fight cancer or protect from cell degeneration, for example. Ease in gene manipulation, addition of regulatory elements and usage of viruses mostly to drive entry into cells to target made this concept potentially feasible as envisaged in the early 1970s [1].

Nevertheless, it was rapidly perceived, despite a number of unsubstantiated claims, that gene transfer needs to be efficacious to overcome many hurdles. They include targeting of the appropriate cell lineage(s), obtaining a sufficient but not excessive level of gene expression, maintaining its expression over time, avoiding the genotoxic effects of the material if integrated into the cells' genome, limiting/eliminating immune reaction against the vectors and/or the gene's product... This explains, why for many years, the multiple attempts, notably in the area of cancer, failed, leading to skepticism about its future. In addition, the gene therapy strategy has to be based on an appropriate understanding of the pathophysiology of the disease to treat [2].

2. Strategy and vectors

Today, available technologies made feasible both ex vivo and in vivo gene therapy based on the persistence of the transgene either non-integrated or integrated in the genome. The former approach is appropriate to target post-mitotic cells, while the second is necessary to target mitotic cells (such as hematopoietic cells, for instance). Adeno-associated viral vectors (AAVs) are well matched for the first approach. They can infect virtually all cell types and permit to achieve stable transgene maintenance. Such vectors can relatively easily be engineered as non-replicative viruses and produced as very large batches. Inconveniencies rely on the relatively limited size of genetic material that can be delivered and on its immunogenicity. Indeed AAVs naturally infect humans who develop effective immune responses against its components, which can lead to neutralization of efficacy by destruction of transduced cells. Some AAV strains (such AAV8 or 9) do less frequently infect humans, leaving some opportunity for application (see below) [3].

Retroviruses offer the capacity of leading to integration into the genome. Gamma retroviruses, then lentiviruses have been designed to carry the genetic material into the cells and lead to stable integration into the genome(s) [4]. HIV-based lentiviral vectors are particularly attractive since their provirus can integrate both in dividing (like γ retroviral vectors) but also in non-dividing in the G1 phase of the cell cycle [5]. The major drawback of the utilization of retroviral vectors is the semi-random character of their integration, which can cause genotoxicity (see below).

Gene therapy can be considered as a way to add the copy of a gene, to modify a gene (for instance to skip a mutated exon), to inactivate a gene (for instance in the setting of a dominant mutation with a trans negative effect) or to correct a gene mutation based on recombinant technology.

3. Applications

3.1. The hematopoietic system

The first success of gene therapy were achieved around the year 2000 in the very specific field of treating severe inherited T-cell immune deficiencies by ex vivo gene transfer into hematopoietic stem (HS) or progenitor cells (C). Several reasons account for that. HSCs are accessible while the prospect of modifying HSCs should provide lasting benefit. In addition, T cells are known to be long lived and the analysis of rare revertant cases from severe combined immune deficiencies – sort of natural gene therapy – showed that precursor T cells can expand, providing demultiplication of gene transfer. Two diseases, severe combined immunodeficiency X1 (SCID X1) and adenosine deaminase deficiency (ADA), have thus been treated with success by using γ retroviral-mediated gene transfer into hematopoietic progenitor cells [6,7]. The efficacy has now been found sustained for 17 years since patients exhibit close to or normal T lymphocyte counts and function, enabling them to live normally. Nearly 100 patients have now been successfully treated world-wide [8–10]. Nevertheless, this success was tempered by the occurrence in the *SCID X1* gene therapy trials (five cases) and in other trials of cases of leukemia [11]. The latter originated from oncogene transactivation by the viral enhancer from the long terminal repeat (LTR) following proviral integration within the oncogene locus. These unanticipated genotoxic events led to halt clinical trials. Once the mechanism was understood, a new generation of vectors was produced, in which the viral LTR was deleted and instead an internal promoter was used, the so-called self-inactivated (SIN) vectors. These SIN vectors have now been used with success and safety with a follow up reaching more than eight years for several diseases including SCIDX1 [12,13].

The first successes of gene therapy were obtained in a favorable setting because of the selective advantage provided to the transduced cells. Utilization of vectors that are more potentially able to transduce HSCs, i.e. lentiviral vectors, have now been used with success to treat additional genetic diseases, including the Wiskott–Aldrich syndrome, another form of primary immunodeficiency [14,15] and then leukodystrophies, for which gene addition in the monocyte cell lineage was of interest [16,17]. The extension of indications to many more genetic diseases of hematopoiesis is being envisaged; it includes several primary immune deficiencies, but also Fanconi anemia or genetic disorders of hemoglobin (β-thalassemia and sickle cell disease).

The latter represents a formidable challenge, as transgene (β-globin) expression needs to be restricted to the erythropoietic lineage. Introduction of the regulatory locus control region makes it feasible. First trials have been initiated with promising preliminary results [18]. An

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