The basis of gene therapy: from the molecule to the treatment

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Introduction and Aims

Gene therapy raised recently as one the most promising techniques in the field of medicine. Although it is still in preclinical phase in most diseases up-to-date successes attest the enormous possibilities. Hovewer, the majority of the population barely knows about the potential of gene therapy, even those that have relatives suffering from diseases that can be treated through this technique. The aim of this project is to provide a comprehensive guide of the main aspects of the actuality of gene therapy regarding to the most recent literature. Questions of variable scope are treated, including sociological, ethical and biological issues. Finally, an analysis of current situation of treatment of some genetic diseases of blood is presented to provide an example of current situation of research in different areas

Methods

The information was gathered through a critical revision of the current literature. The informatic support for the project was Office Power Point, because it visually enhance understanding of the concepts.

Structure

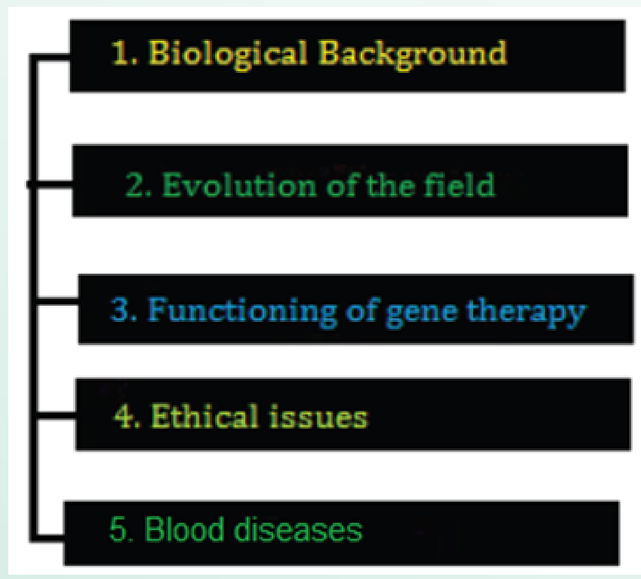

Figure 2: Representation of the structure of the work divided in the relevant sections

1. Biological Background.

Some of the most important terms to understand gene therapy:

Cells > DNA> Mutations>Genetic diseases

Figure 1: Alipogene tiparvovec, first approved drug for gene therapy for LPL deficiency

2. Evolution of the field

SCID was the first disease to present full or partial correction of a defective gene.
2 individuals died related to gene therapy protocols.
First protocol for gene therapy approved last year

3. Functioning of gene therapy

Necessary steps to carry out a gene therapy protocol:
Cloning DNA>Vector insertion>Target cells insertion

5 main factors to take account of:

- Therapeutic gene
- Vectors
- Targeted cells
- Route of administration
- Animal models

Viral methods

Method	Main Advantages	Main Limitations
Retrovirus	Vector integrates in genome, simple to engineer, cellular tropism, low immunogenic and existing immunity, vector particles at high titers	Transduce only replicative cells, difficult to target specific cells, random integration in the genome, high risk of insertional mutagenesis, low stability
Lentivirus	Transduce cells independently of the divinding state, prolongued expression of transgene, integrattion-defective vectors available	Posible insertional
Herpes virus	Cellular tropism, carry up to 50 kb, viable as oncolytic vector, vector particles at high titers, tropism for neuronal or B-lymphoid cells	Possible cytotoxicity, temporal expression of the transgene, risk of recombinantion with latented herpex infected cells, vector does not integrate into genome, high immunity
Proxvirus	Multiple insertion sites, good as recombinant vaccine, low levels of immunity, carry up to 30 kb, viable as oncolytic vector	Potentially citotxic, difficult to generate recombinants, temporal expression of the transgene, immunogenic, use of heterologous promoters complicated
Adenovirus	Tranduce cells independently of the dividing state, high levels of transgene expression, viable as oncolytic vector, vector particles at high titers	Highly immunogenic, vector does not integrate into genome, temporal expression of the transgene, high immunity
Adeno-associated virus	Transduce cells independently of the dividing state, cellular tropism, potential site-specific integration, low immunogenic	Only carry up to 5kb of DNA, high vector titers difficult to achieve, need co-infection with other virus

Non viral methods

Method	Main advantages	Main limitations
Microinjection	Simple, efective, reproducible, non toxic, able to transfer large size DNA	Non suitable for transfecion of large number of cells
Needle injection	Simple, safe	Low efficiency
Gene gun	Safe, efective	Tissue damage
Electroporation	Highly efective, reproducible, potential for localized gene delivery, able to transfer large size DNA	Limited working range restricts gene delivery to large area of tissue. Surgical procedure is required to transfer genes into internal organs. The stability of genomic DNA might be influenced by high voltage.
Sonoporation	Safe, non invasive, able to transfer genes into internal organs without surgical procedure, potential to disrupt blood-brain barrier	Low efficiency
Hydrodynamic gene transfer	Simple, reproducible, highly efective, (especially for liver gene delivery)	Large injection volume is required which restricts its clinical application
Mechanical massage	Simple, safe	Low efficiency. Only has been reported for liver gene delivery
Cationic lipids	Easy to be prepared, low cost, highly efective in vitro	Toxicity, low eficiency in vivo
Cationic polymers	Easy to be prepared and chemically modified, low cost, effective	Toxicity. Some polymers are non-biodegradable

Figure 3: Comparison of the main characteristics of the currently most used viral/non viral vectors
Extracted from: WANG, W., LI, W., MA, N. and STEINHOFF, G., 2013
VANNUCCI, L., LAI, M., CHIUPPESI, F., CECCHERINI-NELLI, L. and PISTELLO, M., 2013

4. Ethical and social issues

Human trials only for therapeutic purposes
Germ-line modifications are forbidden by the NIH
Genetic enhancement may become available for rich

Figure 4: Germ-line modifications may lead to control of the genome of future generations

5. Blood diseases

SCID: First disease to show paliative efects by gene therapy
Haemophilia: First clinical trials in haemophilia B show great success
Thalassemia: Succesful preclinical trials support initiation of studies in human
LPL deficiency: The clinical protocol has been released already
Leukemia: Modified T-cells atack cancer cells, this approach shows promising potential to treat other cancers

Conclusions

This work provides a global perspective of the field of gene therapy and its main developments thus far.

Figure 5: Image of the front page of the work

References

(1) NIENHUIS, A.W., 2008. Development of gene therapy for blood disorders. Blood, 111(9), pp. 4431-4444.
(2) KLOSS, C.C., CONDOMINES, M., CARTELLIERI, M., BACHMANN, M. and SADELAIN, M., 2013. Combinatorial antigen recognition with balanced signaling promotes selective tumor eradication by engineered T cells. Nature biotechnology, 31(1), pp. 71-75.