Gene Therapy in the Management of Cardiovascular Disease

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One of the most important scientific achievements in the past century was the rapid development of molecular biology and genetics. Researchers such as Miescher, Avery, Delbruck, Watson and Crick have contributed using brilliant experiments in the creation and progression of these novel sciences[1-3]. The relationship of DNA with protein synthesis was established after the initial purification, visualization and analysis of this molecule. Furthermore, DNA splicing and intracellular transfer were other important steps in this scientific progress[1-3]. The size and importance of these new discoveries lead Lewis Tomas to write in 1983 that "...almost every important experiment that moved the feet forward..., has come as a total surprise, most of all surprising to the investigators doing the work"[3]. Although the understanding of genetic basis and molecular mechanisms of human disease has unquestionable value, however this knowledge has not yet delivered the expected results in the field of disease treatment. On the other hand, all available scientific data suggest that clinical application of gene-based therapeutic approaches will soon be a reality and this therapeutic approach may contribute impressive results in the near future.

Gene therapy-definition

Gene therapy is the introduction of normal or modified genes into the somatic cells of a target organism to correct or prevent disease[1-4]. A wide range of different technologies are necessary for the achievement of this therapeutic approach, targeting the prevention or attenuation of major consequences of human diseases through the transfer and expression of specific genes[1-4]. The purification, identification and mapping of candidate genes, as well as the determination of respective coding protein product, are the foundation stones of this therapeutic approach of human disease[1-4].

Gene therapy-Historical Retrospection

By the time Watson and Crick discovered the structure of DNA and Chargaff formulated the theory of amino acid coding by a triplet of nucleotides, several decades of basic research have passed in order to make feasible the gene transfer in specific cells targeting the modification of their genetic properties. One of the pioneers of new sciences was the S. Benzer, who identified the genetic loci of two genes of a bacterial phage and showed that each gene is a long chain in chromosomes, which consisted of several hundreds of nucleotides[3-5].

During the same decade, biochemist F. Sanger, using the new technique of chromatography, determined the sequencing of amino acids within the insulin molecule[2-5]. The same researcher several years later significantly contributed to DNA structure analysis by exterminating the sequencing of DNA bases per pairs, thus, laying the foundations of gene mapping and localization[2-5]. Another important step contributed toward

the achievement of gene therapy, was the discovery of restriction enzymes, which are endonucleases which identify specific base sequences in the DNA double helix and degrade these sequences to fragments of specific molecular weight. The use of restriction enzymes in combination with a restriction fragment length polymorphism (RFLP) technique, rendered the isolation of genes and other DNA molecules possible[3-7]. This new technology has utilized the observation that various organisms use such enzymes as a defense mechanism for the degradation of exogenous DNA introduced into their cells. The ability of restrictive endonucleases to degrade the double helix of DNA in specific sites, affords them a uniqueness regarding their use in gene therapy field[3-8].

Several decades of basic research followed the discovery of restrictive enzymes. H.O. Smith in his Nobel lecture in 1979, describes how it began in the early 1950's with the observations by Luria and Human, and by Bertani and Weigle, who observed that phages growning on two different strains of bacteria, grew poorly on one strain (which was under the effects of restrictive enzymes) while it grew well on the other. They noticed also that some phages always escaped restriction and grew well on a new host[3-8]. The biochemical basis of this observation was discovered in 1966 by W. Arber, who showed that restriction was the result of degradation of the phage DNA. This was the first time that the action of restrictive enzyme was defined. Medelson and Yuan later showed that the restrictive enzymes cleave only unmodified DNA phage[3-8]. Later, H.O. Smith et al working in the Johns Hopkins University, discovered new and highly active enzymes which can selectively degrade duplex but not single-stranded DNA to fragments averaging 1000 base pairs[6-10]. They also found that DNA degradation is site-specific. A great boost to the knowledge of the action of restrictive enzymes came from Nathan's group who introduced gel electrophoresis for analysis of DNA restriction cleavage fragments and from Sharp's group who used ethidium bromide as a fluorescent stain for DNA[3,8,9]. In his Nobel lecture in 1979, H.O. Smith listed more than 25 restrictive enzymes together with their recognition sequence[3,8,9].

In the 80's, the cloning of many genes contributed to the better understanding of growth and functions of human organism systems. This fact led to the first effort of gene therapy in humans by Anderson et al in the early 90's[10]. This therapeutic approach targeted the repairing of a genetic deficit which caused adenosine deaminase insufficiency and immunosuppression in two children and the preliminary results were not encouraging. Many approaches of gene therapy in human disease followed with controversial results[7,11,12]. In the field of cardiovascular diseases, special emphasis has been placed on the treatment of atherosclerosis, hyperlipidemias, restenosis following angioplasty and congestive heart failure[13-16].

One essential problem for the application of gene therapy has yet to be solved: the introduction of a candidate gene into the somatic cells. This has been attempted by both *ex vivo* and *in vivo* methods[13-17]. In the past, transfection of the cells was accomplished *in vitro* in cell cultures, while *in vivo*, gene transfection or transduction (when intermediate viral hosts were used) took place in the organism. Both non-infectious and infectious approaches have been used[14-17]. The non-infectious approaches were mainly based on the use of cationic liposomes, while infectious approaches used viruses as vectors (viruses which have incorporated and transferred the candidate gene into cellular targets), such as retroviruses, adenoviruses or other relevant viruses[14-17]. The most widely used approach has been that of transduction with adenoviral vectors. In the non-infectious approach, liposomes (positively charged artificial lipid vesicles that incorporate negatively charged DNA) deliver nucleic acid to the cells through fusion with the cell membrane and receptor-mediated endocytosis[15-19]. Major vector systems and cellular targets of gene therapy into cardiovascular system are summarized in table 1.

Table 1. Vectors and cellular targets of gene transfer into the cardiovascular system.

I) Vectors
– retroviruses
– adenoviruses
– adenovirus like viruses
– DNA plasmids
– synthetic oligonucleotides
– liposomes
– cell transplantation
II) Cellular targets
– cardiomyocytes
– vascular smooth muscle cells
– endothelial cells
– hepatic cells
– skeletal muscle cells

Gene therapy - applications to cardiovascular disease

Gene therapy in cardiovascular diseases is another field of research and controversies. The main cardiovascular disorders, which are potential targets for gene therapy, are summarized in table 2. Although the initial efforts focused on the use of gene therapy in patients with homozygous familiar hypercholesterolemia[14], most researchers today consider that gene therapy is a very promising therapeutic strategy in the management of widespread cardiovascular diseases[15,17,20]. Figure 1 describes potential therapeutic steps for gene interventions targeting the inhibition of coronary atherosclerotic disease progression. This kind of therapeutic strategy includes genetic interventions in the field of risk factor modification and chronic myocardial ischemia, as well as in the final expressions of coronary artery disease such as congestive heart failure and malignant arrhythmias[15,17,20,21]. The approach of gene transfer and therapy has been also used for the inhibition of coronary artery restenosis after angioplasty, the treatment of hyperlipidemias in general and the prevention or attenuation of post-infarction cardiac remodelling[15,17].

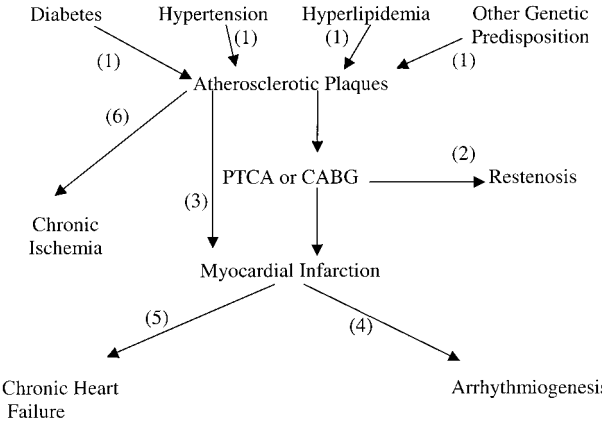
Various genes which regulate the proliferation and migration of vascular smooth muscle cells, and the formation of neo-intima into vascular wall, are involved in the restenosis process[15,17,22,23]. Various therapeutic approaches of gene transfer include the use of anti-sense oligonucleotides against the oncogenes c-myc, c-myb, cdc-2, cdk-2, ras, bcl-x, NF-κB,

Table 2. Cardiovascular diseases - Potential targets for gene therapy.

I) Hyperlipidemias
– Familiar hypercholesterolemia
– Apo-E deficiency
– Reduced HDL

II) Vascular diseases
– Early stages of atherosclerotic disease
– Restenosis after angioplasty
– Vascular thrombosis
– Atherosclerotic plaque rupture
– Atherosclerosis of transplants

III) Cardiac diseases
– Cardiomyopathies
– Congestive heart failure
  i. Prevention of cardiac remodeling, anti-remodeling process
  ii. Enhancement of cardiac contractility
  iii. Regeneration of cardiomyocytes or fibroblast conversion to cardiomyocytes
  iv. Myocyte transplantation
– Chronic ischemia, angiogenesis



(1) Modification of risk factors
(2) Treatment of restenosis
(3) Atherosclerotic plaque stabilization
(4) Treatment of arrhythmiogenesis
(5) Prevention of cardiac remodeling
(6) Angiogenesis

Figure 1. Potential targets for gene modification in the development and progression of atherosclerotic disease.

E2F and PCNA, which directly or indirectly prompt the vascular smooth muscle cell to begin the process of mitosis[15,17,22-24]. The inhibition of expression of these genes may lead to decrease of vessel restenosis thickness after angioplasty. Reduction of restenosis may also be achieved with the transfer of onco-suppressor genes such as p53, p21 and retinoblastoma gene, which prevent the vascular smooth muscle cell entering the cellular cycle and its subsequent proliferation[15,17,25,26].

In the treatment of restenosis of coronary arteries following angioplasty, two technical steps are involved: the delivery system and vectors[15,17,26,27]. Most delivery systems use catheters. A number of vectors have also been used. For example, *ex vivo* gene transfer of porcine endothelial cells expressing a beta galactosidase gene from a murine amphotropic retroviral vector has been successfully accomplished by directly introducing a catheter into the denuded arteries to deliver the recombinant virally infected cells[28,29]. This strategy aimed at direct formation of new endothelial intima into angioplasty-induced injured vessel wall, as well as at restriction of smooth muscle cell exposure to growth factors of flowing blood and reduction of cellular migration and proliferation within the potential restenosis lesions[30,31]. Subsequent studies have employed adenoviral vectors, herpes simplex virus, anti-sense oligonucleotide technology and introduction of cytostatic proteins,

in order to stop the hyperplasia and proliferation of vascular smooth muscle cells in the restenosis lesions[15,17,28-31].

The genetic treatment of hyperlipidemias has shown some encouraging developments but to date reproducible clinical success has not been achieved. Several methods have been attempted to reduce the hyperlipidemia by *in vivo* and *ex vivo* manipulation, using recombinant adenoviruses containing the low-density lipoprotein (LDL) receptor gene through hepatic delivery[14,32]. Autologous hepatocytes, genetically corrected *ex vivo* with recombinant retroviruses, have also been used. Other genetic approaches, used properly modified viral vectors aiming at the increase of high-density lipoprotein (HDL) or the reduction of Lp(a),[14,33,34].

Another wide field of research about gene therapy is based on the use of adenoviruses harbouring genes, which code angiogenic factors such as VEGF (Vascular Endothelial Growth Factor) and FGF (Fibroblast Growth Factor)[35,36]. Theoretically, these factors can regulate the reperfusion of ischemic myocardial areas through the creation of new vessels in the ischemic tissues. The infusion of these factors into the vessels associated with hypoperfused regions did not give positive results in randomized clinical studies[36,37]. The use of gene therapy offers some advantages because of selective gene transfer in specific regions of ischemic myocardium and continuous production of these angiogenic factors[38,39]. Blind placebo-controlled studies in animals have shown that intra-coronary or intra-myocardial administration of adenoviral vectors expressed angiogenic factors, improve the perfusion of ischemic myocardial regions[35-37]. Novel angiogenic factors such as HGF (Hepatocyte Growth Factor), PDGF (Platelet Derived Growth Factor) and MCP -1 (Macrophage Chemoattractant Protein-1), are under investigation[35-37]. Potential side effects associated with neoangiogenesis, such as the stimulation of non-functional vessels and haemangiomas or the excitation of undesirable angionesis in hypoclinical neoplasmatic regions, should be estimated during the evaluation of the cost-benefit relationship[35-37].

In myocardial infarction, one goal has been to replace dead cardiac myocytes with viable, contracting myocytes. Adult cardiomyocytes can not regenerate after injury and new viable cardiomyocytes have to be introduced into the still viable heart muscle or into the infracted sclerotic area[40,41]. This has been attempted by means of engrafting viable myocytes into the infracted area. Engrafting has also been attempted by means of the use of fetal cells[40,42]. Another approach is transplantation of fibroblasts[40,43]. Apparently cardiac fibroblasts can be converted into skeletal muscle by forced expression of the MyoD gene. Fibroblasts isolated from rat hearts were infected with retrovirus carrying the MyoD gene. Histochemical analysis identified successful transfection in several hearts. Another effort was transplantation of skeletal muscle cells into heart muscle with the hope that transplanted skeletal muscle cells can undergo transformation to myocytes. Transplantation of hemopoietic stem cells derived from bone marrow was also another effort targeting the potential transformation of these cells to viable cardiomyocytes[40,45].

However, apart from the attempts of cardiac tissue regeneration, great importance has been attributed by several researchers to the suppression of some factors which promote the further loss of cardiomyocytes (via the necrosis and apoptosis), cardiac fibrosis, oxidative stress and generally, maladaptive left ventricular remodeling following acute myocardial infarction or progression of chronic heart failure[9,16,46-48]. Finally, gene transfer in animal models via adenoviruses expressed intracellular calcium handling proteins, such as SERCA2α, improves diastolic and systolic function of failing hearts, by restoring abnormal calcium homeostasis into SR of cardiomyocytes[49]. The main genetic interventions targeting cardiac anti-remodeling process are summarized in table 3.

Table 3. Cardiac anti-remodeling process. Approaches of gene therapy.

A) Methods
– Gene modification-deletions or insertions of genetic material
– Anti-sense oligonucleotides
– Viral vectors
– Targeted mutagenesis
– Myocyte transplantation
B) Targets
– contractile proteins (myocin)
– SR proteins (SERCA, phospholamban)
– β_2-adrenergic receptor
– transcriptional factors (NF-κB)
– growth factors (TGF-β)
– angiogenetic factors (VEGF)
– constitutive NO synthase (cNOS)
– anti-apoptotic genes (bcl-2)
– anti-oxidative proteins (superoxide dismutase)
– metalloproteinases (MMP-1,MMP-9)
– collagen genes (collagen type I)
– heat shock proteins (Hsp 70)
– cardiomyocyte regeneration

Summary - perspectives

From the beginning of the 80 's until today, significant progress has been accomplished in the field of somatic gene therapy. The development and improvement of suitable vector systems and cloning of several genes associated with the disease origin, have impressively increased the number of diseases which can be treated by gene therapy. The use of new animal models derived from transgenic technology for the control of gene therapy efficacy, is expected to improve the effectiveness of such approaches. Already, the application of gene therapy in experimental models of cardiovascular disease, such as hyperlipidemias, restenosis following angioplasty, chronic heart failure and chronic myocardial or peripheral ischemia, has given impressive results. Factors which will encourage the application of gene therapy in human cardiovascular disease, are the use of more improved vectors with selective expression in various cells of cardiovascular system, the construction of more versatile delivery systems (catheters, etc.) of vectors into the cardiovascular network and peripheral organs, as well as the better understanding of molecular mechanisms associated with cardiovascular system functions under both normal and abnormal conditions[5,7]. Finally, large scale clinical trials must be conducted for the documentation of safety and efficacy of each genetic intervention in human cardiovascular disease.

In the present review, we have only focused on some of the discoveries, which constitute the basis of gene therapy in the cardiovascular field, an approach still in the early stages of its development. Thus, we should agree with the opinion of R. Bing that " ...we recognize the reality, that we are only at the beginning and not at the end of the path which leads to clinical success and application"[3].

References

1. Friedmann T: A brief history of gene therapy. Nat Genet 1992; 2: 93-98.
2. Mulligan R: The basic science of gene therapy. Science 1993; 260: 926-932.
3. Bing RJ: Molecular biology: the beginnings. Heart News and Views 1998; 6: 1-2.
4. Miller: Human gene therapy comes of age. Nature 1992; 357: 455-460.
5. Nabel E, Leiden JM: Gene transfer approaches for cardiovascular disease. In K.R.Chien (ed): Molecular basis of cardiovascular disease 1999; 86-112.
6. Chien KR, Grace AA: Principles of cardiovascular molecular and cellular biology. In Braunwald E (ed): Heart Diseases (Philadelphia WB Saunders) 1996; 1626-1650.
7. Leiden JM: Gene therapy - Promise, pitfalls and prognosis. N Engl J Med 1995; 333: 871-873.
8. Vogel F, Motulsky AG: Human Genetics: Problems and approaches, 3rd edition. Berlin, Springer 1997; 10-39.
9. Leiden JM, Barr E: In vivo gene transfer into the heart. In Wolf JA (ed): Gene therapeutics: Methods and Applications of Direct Gene Transfer. Boston, Birkhauser 1994; 363-380.
10. Anderson WF: Human gene therapy. Science 1992; 256: 808-813.
11. Crystal RG: Transfer of genes to humans: Early lessons and obstacles to success. Science 1995; 270: 404-410.
12. Rosenfeld MA: In vivo transfer of the human cystic fibrosis transmembrane conductance regulator gene to the airway epithelium. Cell 1992; 68: 143-155.
13. Muller DW: Gene therapy for cardiovascular disease. Br Heart J 1994; 72: 309-312.
14. Kozarsky KF, Wilson JM: Gene therapy of hypercholesterolaemic disorders. Trends Cardiovasc Med 1995; 5: 205-209.
15. Yiä-Herttualla S, Martin JF: Cardiovascular gene therapy. Lancet 2000; 355: 21322-21332.
16. Chang MW, Leiden JM: Gene and cell-based therapies for myocardial dysfunction. In Edelman ER (ed): Molecular Interventions and Local Drug Delivery in Cardiovascular Disease. Philadelphia, WB Saunders, 1995; 313-326.
17. Channon KM, Blazing MA, George SE: Vascular gene transfer. Heart 1996; 75: 215-217.
18. Muzyczka N: Adeno-associated virus (AAV) vectors: Will they work? J Clin Invest 1994; 94: 1351-1356.
19. Lasic D, Papahadjopoulos D: Liposomes revisited. Science 1995; 267: 1275-1280.
20. Nabel E: Gene therapy for cardiovascular disease. Circulation 1995; 91: 541-548.
21. Spandidos D, Flouris G, Kalantzi M, Parissis J, Arvanitis D: Genetic basis of atherosclerosis. Proceedings of 2nd International Meeting of the Onassis Cardiac Surgery Center 2000; 168-171.
22. Bennett M, Anglin S, McEwan J, et al: Inhibition of vascular smooth muscle cell proliferation in vitro and in vivo by c-myc antisense oligonucleotides. J Clin Invest 1994; 93: 820-828.
23. Simons M, Edelman ER, Dekeyser J, et al: Anti-sense c-myb ologonucleotides inhibit intimal arterial smooth muscle cell accumulation in vivo. Nature 1992; 359: 67-70.
24. Epstein SE, Speir G, Finkel T: Do anti-sense approaches to the problem of restenosis make sense? Circulation 1993; 88: 3151-1353.
25. Chang MW, Barr E, Seltzer J, et al: Cytostatic gene therapy for vascular proliferative disorders using a constitutively active form of retinoblastoma product. Science 1994; 267: 518-522.
26. Ohno T, Gordon D, San H, et al: Gene therapy for vascular smooth muscle cell proliferation after arterial injury. Science 1994; 265: 781-784.
27. Steg P, Feldman L, Scoazec JY, et al: Arterial gene transfer to rabbit endothelial and smooth muscle cells using percutaneus delivery to an adenoviral vector. Circulation 1994; 90: 1648-1656.
28. Vonder Leyen HE, Gibbons GH, Morishita R, et al: Gene therapy inhibiting neointimal vascular lesion: In vivo transfer of endothelial cell nitric oxide synthase gene. Proc Natl Acad Sci USA 1995; 92: 1137-1141.

29. Jia L, Bonaventura C, Bonaventura J: A dynamic activity of blood involved in vascular control. Nature 1996; 380: 221-226.
30. Chang MW, Ohno T, Gordon T, et al: Adenovirus-mediated transfer of the herpes simplex virus thymidine kinase gene inhibits vascular smooth muscle cell proliferation and neointima formation following balloon angioplasty. Mol Med 1995; 1: 172-181.
31. Simari R, San H, Rekhter M, et al: Regulation of cellular proliferation and intimal formation following balloon injury in atherosclerotic rabbit arteries. J Clin Invest 1996; 98: 225-235.
32. Ishibashi S, Brown MS, Goldstein JL, et al: Hypercholesterolemia in low density lipoprotein receptor knockout mice and its reversal by adenovirus-mediated gene delivery. J Clin Invest 1993; 92: 883-893.
33. Leiden JM: Adenovirus-mediated gene transfer as an in vivo probe of lipoprotein metabolism. Circulation 1996; 94: 2046-2051.
34. Rubin GM, Krauss RM, Spangler EA, et al: Inhibition of early atherogenesis in transgenic mice by human apolipoprotein AI. Nature 1991; 353: 265-267.
35. Patterson C, Runge MS: Therapeutic myocardial angiogenesis via Vascular Endothelial Growth Factor gene therapy: Moving on down the road. Circulation 2000; 102: 940-942.
36. Hammond HK, McKirnan MD: Angiogenic gene therapy for heart disease: a review of animal studies and clinical trials. Cardiovasc Res 2001; 49: 561-7.
37. Takeshita S, Zheng LP, Brogi E, et al: Therapeutic angiogenesis. J Clin Invest 1994; 93: 662-670.
38. Isner JM, Rieczek A, Schainfeld R, et al: Clinical evidence of angiogenesis following arterial gene transfer of phVEGF. Lancet 1996; 348: 370-374.
39. Vale PR, Losordo DW, Milliken CE, et al: Percutaneous myocardial gene transfer of phVEGF-2. Circulation 1999; 100: 2462-2463.
40. Williams RS: Cell cycle in the terminally differentiated myocyte: A platform for myocardial repair? Cardiology Clinics 1998; 16: 739-754.
41. Soonpaa MH, Dand AI, Koh GY, et al: Potential approaches for myocardial regeneration. Ann NY Acad Sci 1995; 752: 446-454.
42. Koh GY, Soonpaa MH, Klug MG, et al: Stable fetal cardiomyocyte grafts in the heart of dystrophic mice and dogs. J Clin Invest 1995; 96: 2034-2042.
43. Lenfant C: Fixing the failing heart. Circulation 1997; 95: 771-772.
44. Megeney LA, Kablar B, Garrett K, et al: Myo D is required for myogenic stem cell function in adult skeletal muscle. Genes Dev 1996; 10: 1173-1183.
45. Chiu RC, Libaitis A, Kao RL: Cellular cardiomyoplasty: Myocardial regeneration with satellite cell implantation. Ann Thorac Surg 1995; 60: 12-18.
46. Mann DL: Mechanisms and models in heart failure: A combinatorial approach. Circulation 1999; 999-1008.
47. Dzan VJ, Von der Leyen HG, Morishita R: The concept and potentials of cardiovascular gene therapy. Dialogues Cardiovasc. Med 1997; 2: 3-17.
48. Weber KT, Sun Y, Guntaka RV: Rebuilding and remodeling following myocardial infarction: The good, the bad and the ugly of tissue repair. Dialogues Cardiovasc Med 1999; 4: 3-19.
49. Del Monte F, Williams E, Lebeche D, et al: Improvement in survival and cardiac metabolism after gene transfer of sarcoplasmic reticulum Ca^{++}-ATPase in a rat model of heart failure. Circulation 2001; 104: 1424-1429.