Diagn Interv Radiol 2005; 11:113-118

REVIEW

Gene therapy: applications in interventional radiology

Selen Biçeroğlu, Ahmet Memiş

ABSTRACT
Gene therapy is a new and rapidly developing area in medicine. Although it is a new therapy method, recent studies look promising. Since vascular wall is a good target for this treatment, interventional radiologists should become familiar with this new therapy and be involved in this multidisciplinary collaboration.

Key words: • *gene therapy* • *interventional radiology*

Gene therapy is a rapidly developing and a promising therapy modality. Research beginning with the advances in molecular biology by the 1960s has begun progressing in 1990s with the achievement of clinical gene therapy. Nowadays, more than 400 clinical trials are being carried out with more than 4,000 patients (1, 2). Interventional radiologists play an important role in transmission of genetic materials to the target cells and for this reason they must follow developments of terminology about gene therapy such as defining a gene, cloning, locating in a vector and detection of its expression. In this article, it has been tried to review our present knowledge of gene therapy modalities in interventional radiology.

Definition of gene therapy

Gene therapy is transferring recombinant genetic material (DNA or RNA) to the host cell in order to change the gene expression in the host cell to gain a therapeutic effect (Figure 1) (3). Even though cancers constitute the main area of gene therapy strategies' applications, monogenetic hereditary disorders, infectious diseases and vascular diseases constitute a large group of diseases for application as well (2). Genetic materials can be transported to target area either directly or indirectly.

In direct gene transfer, genetic material is transferred to the target cell (e.g., endothelial cell) *in vivo*. In indirect gene transfer, on the other hand, target cells are obtained from the patient, gene transfer occurs outside the body *(ex vivo)* and cells are returned to body either by transplantation or transfusion (Figure 2) (3).

Vectors for gene transfer

All gene therapy strategies aim the transfer of the gene or gene products to the target cells. Vectors are frequently used to insert the genetic materials in the cells. Gene transfer via the viral vectors is called transduction while transfer via the non-viral vectors is called transfection (4). Viral vectors (75%) are retroviral, adenoviral, and adeno-associated viral (AAV). Non-viral vectors (25%) are liposomes, plasmid, and synthetic oligonucleotides.

Ideal vector must be reliable, effective and stable. All these vectors identified have their own advantages and disadvantages, and none of them has the ideal vector properties yet (4).

Viral vectors

Adenoviruses

They are double stranded, linear DNA viruses that do not have an envelope. They infect proliferating and non-proliferating cells without integrating into target cell chromosome. They can be produced in high titers with *in vitro* culture systems. Mutation risk is low because they do

From the Department of Radiology (S.B. ✉ *selenb2000@hotmail.com*), Ege University School of Medicine, İzmir, Turkey.

Received 29 June 2004; revision requested 20 September 2004; revision received 21 November 2004; accepted 4 December 2004.

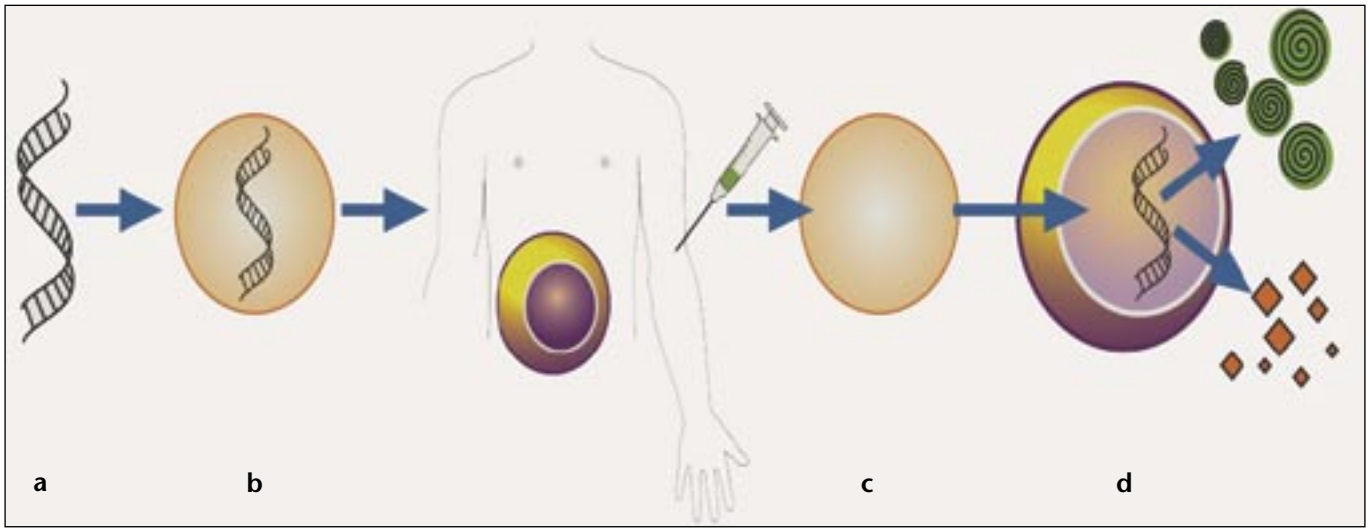


Figure 1. a-d. Scheme of gene therapy application. **a.** DNA (genetic material). **b.** Integration of gene into the virus. **c.** The virus releasing the gene within the target cell and gene starting the protein expression. **d.** Produced protein's effect on the involved cell or its paracrine or systemic effect when secreted. *(Obtained and modified from http://mededucation.bjmu.edu.cn/PPT/gene%20therapy.ppt)*

not integrate into target cell chromosome. Their disadvantage is that they do transient transgene expression and that they give rise to immune reactions. They are not tissue specific, therefore they affect many cell series. Adenoviruses are the most appropriate vectors for gene transfer to the vascular endothelium.

Retroviruses

They are single stranded enveloped RNA viruses. They enter the target cell via receptor-mediated endocytosis. Viral RNA is transformed into DNA within the cell by the help of the reverse transcriptase enzyme. It shows its effect by integrating its gene into the chromosome of the target cell and affects the replicating cells only. They are the most commonly used vectors in experimental and clinical studies. Their disadvantages are low transduction rate, unexpected effects due to their integration into the target cell chromosome and secondary diseases.

Adeno-associated viral vectors

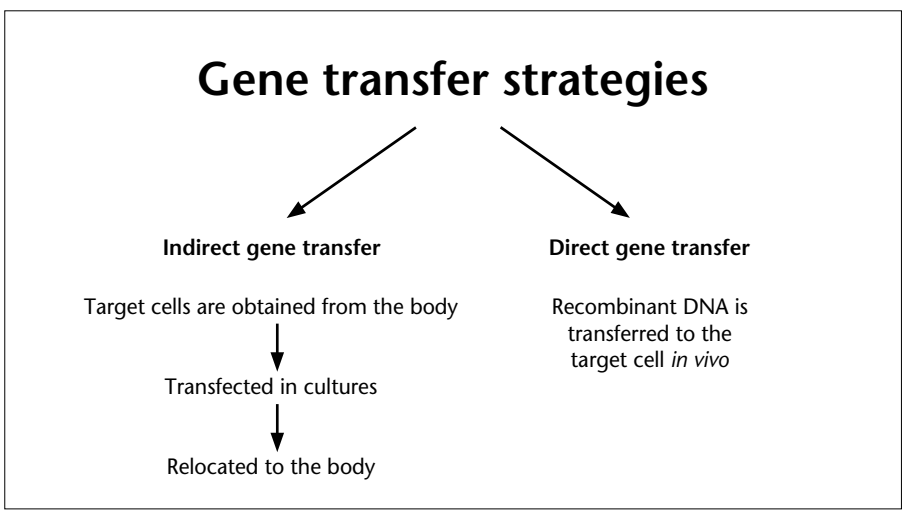
It is a defective parvovirus containing a single stranded linear DNA molecule and needs a helper virus like adenovirus to replicate. It affects both proliferating and non-proliferating cells. They can be produced in high titers. They are resistant to physical factors. They infect many cell series but their transduction rate is low (4-7).

Non-viral vectors are developed as alternatives to viral vectors. They enable the transport of DNA to the target cells. Plasmids contain double stranded circular DNA. Plasmid DNA is pushed away by the negatively charged cellular membrane, thus decreasing the transfection rate of the naked plasmid DNA. To avoid this, plasmid DNA is covered with cationic liposomes (artificially produced positively charged lipid membranes) that enable it to pass through the membrane via receptor mediated endocytosis and carry its content to the intracellular area (6, 8).

Antisense oligonucleotides (ASO) are chemically synthesized, short (10-30 base pairs) molecules that are designed to complement the encoding sequence of the target RNA. ASOs form double stranded complexes with their complementary RNA within the cell and reduce the translation of RNA (protein synthesis from the mRNA). ASOs can be introduced to the cells simply by diffusion or as complexes with liposomes (3, 8).

Production of non-viral vectors is easy; they do not cause immune reactions; and they do not need an infectious agent. Mutagenesis probability is low because they do not integrate into the target cell chromosome. However, gene transduction and expression rate

Figure 2. In direct gene transfer, recombinant DNA is transferred to the target cell *in vivo.* In indirect gene transfer, target cells are obtained from the patient, gene transfer takes place in cultures and they are relocated to the body.



is low when compared with the other vectors. They cause transient gene expression (4-7).

Transfer of the genetic material to the cells

Although systemic intravenous route can be applied to deliver the genetic material to the cells, local delivery methods are more commonly used (surgical, percutaneous, US and CT guided and by means of catheters).

Percutaneous injection

If paracrine effect of the gene product is therapeutic, vector solution can be injected percutaneously into the tissue around the vessel. This simple and locally effective technique can be efficacious for increasing the number of capillaries within ischemic muscles (9).

Surgical method

This is the most common method used in the vascular gene therapy in animal models. In this procedure, the related vascular area is isolated by clamping from proximal and distal ends and its all side branches are ligated. The blood inside is drained and vector solution is injected into this isolated segment. After a certain time of incubation the vector solution is aspirated and the involved segment is washed unclamped, and ligations are removed. This technique has high transfection efficiency and only the isolated corresponding area is transfected. Its disadvantage is that it is an invasive procedure and the vessel is exposed to the straining effects of clamping and ligation of side branches (10, 11).

Catheter systems

New catheter systems are developed to increase the interaction between vectors and endothelial cells (4).

Pressure diffusion catheters are hydrogel-coated balloon, balloon in balloon, microporous balloon, double layer channelled perfusion balloon, and infusion sleeve catheter types.

Passive diffusion catheters are double occlusion balloon, and spiral balloon types.

Mechanical or electrically strengthened catheters are needle injection catheter, iontophoretic electric current-enhanced balloon, and stent based systems.

With the double occlusion balloon catheter, a vascular segment is isolated by two occlusion balloons and vector solution is given into this segment with a different port. It is a simple system enabling gene delivery to a local area but target vessel must have no branches. These catheters have low rate of transfection (4, 6, 12).

In order to make the gene transfer more effective, it is necessary to pass through the physical barrier made of internal elastic lamina and vascular endothelium. This necessity has led to development of pressure diffusion catheters and mechanically strengthened catheters (4).

Pressure diffusion catheters include two co-axial balloons. The vector solution is administered to the vessel by the pores on the outer balloon or the gene containing gel solution on the balloon surface (hydrogel coated balloon) during angioplasty (e.g., Wolinsky balloon; contains multiple 25 micron wide micropores on its surface; gene or vector solution reaches vessel wall via the jet flow through these pores). However, the transfection rate is low and there exists a risk of tissue damage (13, 14).

In mechanically strengthened catheters, needles surrounding the dilatation balloon penetrate the targeted vessel wall by inflation of the balloon containing the gene or vector solution. Transfection rate is high (low rate of clearance with the blood flow) (15). With the iontophoretic catheters, the movement of vectors to the vessel wall is tried to be increased with the aid of the electric current (16).

Gene transfer to the vascular bed has been used to investigate the pathophysiologies of vascular diseases and has aimed to develop new treatment modalities.

Gene treatment applications in peripheral vascular disease

Stenoses following balloon angioplasty or stenting (restenosis, stenosis in the stent), venous graft diseases and therapeutic angiogenesis are among the aims of gene therapy.

Prevention of restenosis after balloon angioplasty or stent placement is one of the primary goals of gene therapy because this problem leads to occlusion within 6 months in 20% of the patients that has undergone a balloon angioplasty procedure. Restenosis occurs slower in venous grafts but about 50% of those grafts occlude within 5 years (17-19). Many factors, such as proliferation and migration of smooth muscle cells at media layer, increased extracellular fluid production, thrombosis, thrombocyte activation and leukocyte adhesion play a role in formation of restenosis (20). Gene therapy strategies aim inhibition of smooth muscle cell proliferation and migration, inhibition of connective tissue formation and prevention of unwanted growth factor effects (9).

Another approach to the treatment of vascular diseases characterized with excess cellular proliferation is the expression of the thymidine kinase gene of the *Herpes simplex* virus (HSVtk). HSVtk encodes the thymidine kinase enzyme that phosphorilates nucleoside analogue gancyclovir or acyclovir and thus converts it to a metabolite that disturbs the DNA replication at the S phase of cell cycle. A by-product of this reaction diffuses to adjacent cells and enters into the proliferating cells to inhibit their replication. This effect is called the "bystander effect" which provides the inhibition of replication at a number of cells much more than the number of the transfected cells (Figure 3) (3, 21). This model was initially based on the vascular injury of the peripheral artery of a pig, and intimal hyperplasia was diminished by 50% (22). In the following studies on rats, rabbits and a transplant model, a 50% decrease in cell proliferation and lesion formation was achieved (23, 24).

Cytosine deaminase gene, p21 cycline dependant kinase inhibitor, and p53 gene were also used to inhibit the cell proliferation by cytostatic or cytotoxic products (25). Antisense oligonucleotides were also used to inhibit the gene expression necessary for smooth muscle cell proliferation. It was observed that the antisense oligonucleotides, developed against c-myb, c-myc, cdc-2, cdk-2, ras, bcl-x, E2F, NFkB and transforming growth factor beta (TGF beta) decreased intimal thickening in experimental restenoses (9, 25).

Another gene developed for inhibition of thrombosis and neointimal proliferation is the NO (nitric oxide) synthase gene. NO is an endogeneous inhibitor of vascular smooth muscle cell contraction and pre-inflammatory activities. NO loss occurs with endothelial cell injury due to vascular damage. Prevention of the neointimal proliferation by the transfer of NO synthase gene was tried in animal models.

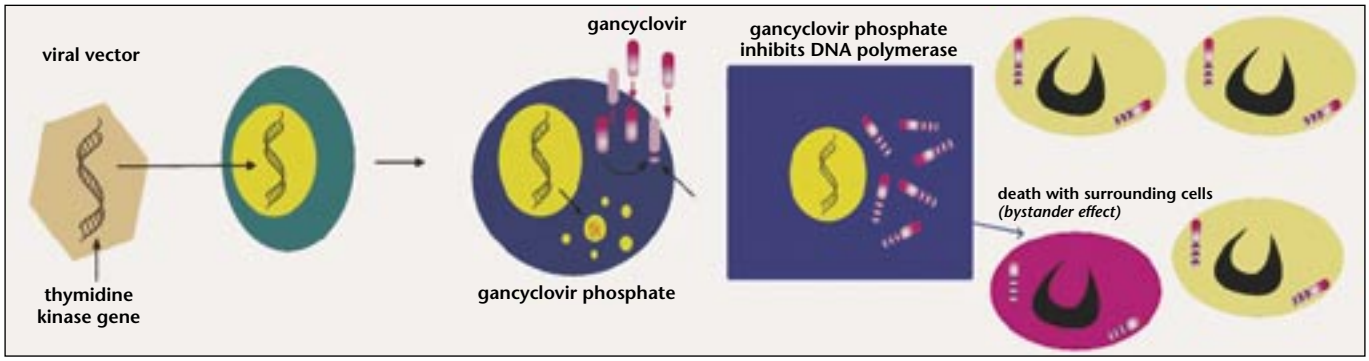


Figure 3. HSVtk encodes for thymidine kinase enzyme that phosphorylate nucleoside analogue gancyclovir to a metabolite that demolishes DNA replication at S phase of the cell cycle. A by-product of this reaction diffuses to surrounding cells and can inhibit cellular replication after entering into the proliferating cell. This effect is called the "bystander effect" and provides inhibition of replication of a greater population of cells than transfected cells. *(Obtained and modified from website http://mededucation.bjmu.cn/PPT/gene%20therapy.ppt)*

It was found that the endothelial cell nitric oxide synthase (ec-NOS) inhibits the neointimal formation after balloon damage (3, 21, 25, 26).

Gene therapy with the angiogenetic growth factors may be useful in increasing the collateral artery development in ischemia of the myocard or the extremities. Known endothelial cell specific growth factors and their receptors are vascular endothelial growth factor (VEGF) and angiopoetin family. Another potent stimulant of angiogenesis is fibroblast growth factor (FGF). FGF also has receptors at other cells like fibroblasts and vascular smooth muscle cells (25,27). The studies, performed in surgically produced ischemic rabbit posterior extremities, with intramuscular injection of angiogenetic proteins consisting of FGF showed that capillary densities and blood flows increase (28, 29). Increased collateral perfusion was also observed following genetic transfer of VEGF by hydrogel balloon in the rabbit model with lower extremity ischemia (30). Our knowledge about the processes that angiogenetic factors use in providing blood vessel formation is not exact. It should be kept in mind that therapeutic angiogenesis may have some other effects like stimulation of tumoral angiogenesis and hemangioma formation (31-33).

Gene therapy in malignancies

Developments in molecular oncology and tumor immunology gave rise to development of gene therapy for treatment of cancers. Present therapy strategies aim the stimulation of immune response to tumor (tumor vaccines, cytokine gene therapy), reducing oncogenic expression, correcting defects in tumour suppressor gene functions, increasing susceptibility to chemotherapeutics via gene transfer to proliferating tumor cells and modulation of angiogenesis (5, 7, 8, 34).

p53 mutations are present in many of the cancers and these mutations trigger the complex molecular events leading to tumor formation. For this reason, another method in the gene therapy of tumors is exchanging the mutated or lost p53 with its new copy. Inhibition of the tumor cell development by injecting the adenoviral vector encoding p53 tumor suppressor gene to the lung cancer nidus by a biopsy needle has been tried (1, 7). Antitumoral activity can be provided with this method but small amount of tumor cells can be transducted by intratumoral injection and remaining cells proliferate rapidly therefore limiting antitumoral effect (7).

In order to overcome this problem, methods of by-stander effect, meaning a method aiming to destroy the tumor cells at distant locations or in the neighborhood of but not in direct contact with the gene therapy vector, are being developed. Most common example of this method is using the HSVtk in combination with gancyclovir. HSVtk/gancyclovir combination is still tested on some malignancies like malignant mesothelioma, brain and liver tumours by a mechanism similar to the mechanism used in vascular diseases (5, 7, 34-36).

In pathologic conditions characterized by angiogenesis, proliferating endothelial cells can be targeted by a retrovirus. This strategy was tested in malignant glioma models, in which tumour endothelium was transfected with genes susceptible to the drug. This transfection was shown to cause extensive hemorrhagic necrosis in glioma (37).

Gene therapy in central nervous system (CNS) diseases

CNS is an organ system with rare or no cell turnover, thus gene therapy is a controversial method in CNS system (38). Application areas of gene therapy in CNS consist of monogenetic enzyme deficiencies, metabolic disorders, neuro-oncology and cerebral vascular diseases (8).

Huntington's disease, Parkinson's disease, Tay-Sachs disease, adrenoleukodystrophy, neurofibromatosis and some metabolic disorders are among the goals of gene therapy. Genetic targets are defective gene itself, genes encoding in production, or genes playing role in product metabolism. For example, Parkinson's disease is characterized with local dopamine deficiency in corpus striatum. Cells arranged to increase this neurotransmitter and secrete dopamine can be applied to striatum (39).

There are investigations suggesting that the prevention of vasospasm following subarachnoid hemorrhage can be possible by two ways: 1) with vector aided administration of the gene which is encoding the "vasospasm preventing vasoactive protein"; or 2) via the administration of the gene which is inhibiting the effects of the "spasm inducing substance (e.g., endothelin 1)" (40).

Gene therapy in cerebral ischemia aims induction of angiogenesis in cerebral vessels, as in peripheral vasculature, by transferring cDNA via vectors that encodes the substances like FGF,

VEGF that stimulate vascular proliferation in ischemic conditions (40).

Therapeutic agents may also be introduced to CNS by mechanical devices (stents containing genetically modified endothelial cells, coils, etc.). Coating the surface of platinum GDC (Guglielmi detachable coils) with bioactive substances increases the endothelization as well as organization of the clot and this effect is experimentally used in treatment of aneurysms (41, 42).

Gene therapy in hepatic diseases

Among the diseases that are candidates for gene therapy are some metabolic disorders (Crigler–Najjar type I, alpha-1 antitrypsin deficiency, hemophilia, hereditary thyrosinemia, ornitine transcarbamylase deficiency), hepatocellular cancer and familial hypercholesterolemia (35).

Familial hypercholesterolemia (FH) is an autosomal dominant disease that occurs due to a defect in function or expression of LDL (low density lipoprotein) receptor. As a result of this, cholesterol levels and accompanying coronary artery disease risk increase in the patients. In various animal models with hypercholesterolemia, hepatocytes which express LDL receptor were delivered to the liver and this had provided improvement (43, 44).

In another clinical research consisting of five FH patients, hepatocyte cultures were produced from the resected left lateral segment of the liver and LDL receptor genes were placed in these hepatocytes with recombinant retrovirus. This genetically modified material was administered to the patients via a portal venous catheter; in three of the five patients LDL cholesterol levels significantly decreased in the long-term (45).

Gene therapy in lung diseases

Cystic fibrosis is the main lung disease considered for gene therapy. It is thought that cystic fibrosis is an ideal candidate for gene therapy since the disease is due to a single gene mutation (CFTR-cystic fibrosis transmembrane conductance regulatory gene), and the main target is the respiratory epithelium (46).

Other lung diseases among the targets of gene therapy are alpha-1 antitrypsin deficiency which is due to a single gene mutation, pulmonary hypertension, lung cancer, and lung transplantation (46).

Monogenetic hereditary disorders and cancers are the primary goals in treatment of diseases with gene therapy. Clinical trials in cardiovascular diseases have shown progress in proliferative diseases and angiogenesis.

In conclusion, gene therapy is a promising technique that still has important difficulties to overcome and multi-disciplinary (medical doctors, biologists, genetic engineers, physics engineers) approach is mandatory for improvements. Interventional radiologists play a role in the safe and selective delivery of the therapeutic material to the target area.

References

1. Weissleder R, Mahmood U. Molecular imaging. Radiology 2001; 219:316-333.
2. Romano G, Pacilio C, Giordano A . Gene transfer technology in therapy: current applications and future goals. Oncologist 1998; 3:225-236.
3. Nabel EG, Dzau VJ. Cardiovascular tissue modification by genetic approaches. In: Fuster V, Alexander RW, O'Rourke RA, eds. Hurst's The Heart. 10th ed. McGraw-Hill, 2002; 157-166.
4. Yang X. Imaging of vascular gene therapy. Radiology 2003; 228:36-49.
5. Voss S, Kruskal J. Gene therapy: a primer for radiologists. Radiographics 1998; 18: 1343-1372.
6. Thomas J, Kuo M, Chawla M, et al. Vascular gene therapy. Radiographics 1998; 18:1373-1394.
7. Albelda SM, Wiewrodt R, Sterman DH. Gene therapy for lung neoplasms. Clin Chest Med 2002; 23:265-277.
8. Amar A, Zlokovic BV, Apuzzo ML. Endovascular restorative neurosurgery: a novel concept for molecular and cellular therapy of the nervous system. Neurosurgery 2003; 52:402-412.
9. Yla-Herttuala S, Martin JF. Cardiovascular gene therapy. Lancet 2000; 355: 213-222.
10. Willard JE, Landau C, Glamann DB, et al. Genetic modification of the vessel wall: comparison of surgical and catheter-based techniques for delivery of recombinant adenovirus. Circulation 1994; 89:2190-2197.
11. Lim CS, Chapman GD, Gammon RS, et al. Direct in vivo gene transfer into the coronary and peripheral vasculatures of the intact dog. Circulation 1991; 83:2007-2011.
12. Rome JJ, Shayani V, Newman KD, et al. Adenoviral vector-mediated gene transfer into sheep arteries using a double-balloon catheter. Hum Gene Ther 1994; 5:1249-1258.
13. Feldman LJ, Steg GP, Zheng L, et al. Low-efficancy of percutaneous adenovirus-mediated arterial gene transfer in atherosclerotic rabbit. J Clin Invest 1995; 95: 2662-2671.
14. Flugelman MY, Jaklitsch M, Newman KD, Cassels W, Bratthuer G, Dichek DA. Low level in vivo gene transfer into the arterial wall through a perforated balloon catheter. Circulation 1992; 85:1110-1117.
15. Barath P, Popov A, Dillehay GL, Matos G, McKiernan T. Infiltrator angioplasty balloon catheter: a device for combined angioplasty intramural site-specific treatment. Cathet Cardiovasc Diagn 1997; 41: 333-341.
16. Fernandez-Ortiz A, Meyer BJ, Mailhac A, et al. A new approach for local intravascular drug delivery: iontophoretic balloon. Circulation 1994; 89:1518-1522.
17. Narins CR, Holmes DR, Topol EJ. A call for provisional stenting: the balloon is back. Circulation 1998; 97:1298-1305.
18. Bittl JA. Advances in coronary angioplasty. N Engl J Med 1996; 335:1290-1302.
19. Grondin CM, Campeau L, Lespérance J, Enjalbert M, Bourassa MG. Comparison of late changes in internal mammary artery and saphenous vein grafts in two consecutive series of patients 10 years after operation. Circulation 1984; 70:208-212.
20. Kullo IJ, Simari RD, Schwartz RS. Vascular gene transfer: from bench to bedside. Arterioscler Thromb Vasc Biol 1999; 19: 196-207.
21. Nabel EG. Gene therapy for cardiovascular disease. Circulation 1995; 91:541-548.
22. Ohno T, Gordon D, San H, et al. Gene therapy for vascular smooth muscle cell proliferation after arterial injury. Science 1994; 265:781-784.
23. Chang MW, Ohno T, Gopdon D, 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 of the rat carotid artery. Mol Med 1995; 1:172-181.
24. Guzman RJ, Hirschowitz EA, Brody SL, Crystal RG, Epstein SE, Finkel T. In vivo suppression of injury-induced vascular smooth muscle cell accumulation using adenovirus- mediated transfer of the herpes simplex virus thymidine kinase gene. Proc Natl Acad Sci USA 1994; 91:10732-10736.
25. Manninen HI, Makinen K. Gene therapy techniques for peripheral arterial disease. Cardiovasc Intervent Radiol 2002; 25:98-108.
26. Baek S, March KL. Gene therapy for restenosis: getting nearer the heart of the matter. Circ Res 1998; 82:295-305.
27. Henry TD. Therapeutic angiogenesis. BMJ 1999; 318:1536-1539.
28. Pu LQ, Sniderman AD, Brassard R, et al. Enhanced revascularization of ischemic limb by angiogenic therapy. Circulation 1993; 88:208-215.
29. Unger EF, Banai S, Shou M, et al. Basic fibroblast growth factor enhances myocardial collateral flow in a canine model. Am J Physiol 1994; 266:H1588-H1595.
30. Takeshita S, Weir L, Chen D, et al. Therapeutic angiogenesis following arterial gene transfer of vascular endothelial growth factor in a rabbit model of hind limb ischemia. Biochem Biophs Res Commun 1996; 227:628-635.
31. Nicosia RF. What is the role of vascular endothelial growth factor-related molecules in tumor angiogenesis? Am J Pathol 1998; 153:11-16.
32. Ferrara N, Davis-Smyth T. The biology of vascular endothelial growth factor. Endocr Rev 1997 ;18:4-25.

33. Hiltunen MO, Turunen MP, Laitinen M, Yla-Herttuala S. Insights into the molecular pathogenesis of atherosclerosis and therapeutic strategies using gene transfer. Vasc Med 2000; 5:41-48.
34. Jiao LR, Havlik R, Nicholls J, Jensen SL, Habib NA. Suicide gene therapy in liver tumors. Methods Mol Med 2004; 90:433-450.
35. Nunes FA, Raper SE. Liver directed gene therapy. Med Clin North Am 1996; 80: 1201-1213.
36. LeMay DR, Kittaka M, Gordon EM, et al.Intravenous RMP-7 increases delivery of ganciclovir into rat brain tumors and enhances the effects of herpes simplex virus thymidine kinase gene therapy. Hum Gene Ther 1998; 9:989-995.
37. Cohen ZR, Duvdevani R, Nass D, Hadani M, Ram Z. Intraarterial delivery of genetic vectors for the treatment of malignant brain tumors. Isr Med Assoc J 2001; 3:117-120.
38. Seidenwurm DJ, Rowley HA. Imaging-guided gene therapy in the central nervous system. Radiology 1998; 208:17-18.
39. Freese A. Restorative gene therapy approaches to Parkinson's disease. Med Clin North Am 1999; 83:537-548.
40. Heistad DD, Faraci FM. Gene therapy for cerebral vascular disease. Stroke 1996; 27: 1688-1693.
41. Abrahams JM, Forman MS, Grady MS, Diamond SL. Delivery of human vascular endothelial growth factor with platinum coils enhances wall thickening and coil impregnation in a rat aneurysm model. AJNR 2001; 22:1410-1417.
42. Murayama Y, Vinuela F, Tateshima S, Song JK, Gonzales NR,Wallace MP. Bioabsorbable polymeric material coils for embolisation of intracranial aneurysms: a preliminary experimental study. J Neurosurg 2001; 94:454-463.
43. Chowdhury JR, Grossman M, Gupta S, Chowdhury NR, Baker JR, Wilson JM. Long-term improvement of hypercholesterolemia after ex vivo gene therapy in LDLR-deficient rabbits. Science 1991; 254: 1802-1805.
44. Wilson JM, Chowdhury NR, Grossman M et al. Temporary amelioration of hyperlipidemia in low density lipoprotein receptor-deficient rabbits transplanted with genetically modified hepatocytes. Proc Natl Acad Sci USA 1990; 87:8437-8441.
45. Grossman M, Rader DJ, Muller DN, et al. A pilot study of ex vivo gene therapy for homozygous familial hypercholesterolaemia. Nat Med 1995; 1:1148-1154.
46. West J, Rodman DM. Gene Therapy for Pulmonary Diseases. Chest 2001; 119:613-617.