BASIC SCIENCE LECTURE

GENE THERAPY AND GENOMIC STRATEGIES FOR CARDIOVASCULAR SURGERY: THE EMERGING FIELD OF SURGIOMICS

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Over the past decade, with the advent of molecular technologies, our understanding of the complex mechanisms behind cardiovascular diseases has expanded exponentially. From the identification of inherited single gene disorders to multigenic acquired pathology, the fundamental genetic contributions of these diseases are unquestionable. These developments have led to an enhanced interest in gene-based therapeutic strategies. The ability to alter patterns of gene expression or function in an effort to correct or prevent disease processes forms the basis for “gene therapy.” The delivery of genetic agents and successful manipulation of gene expression in living tissues has been crucial to the potential realization of this exciting technology.

The application of this technology not only will affect the overall approach to medical therapies, but likely will improve our ability to treat patients surgically. Complications that limit the long-term efficacy of traditional surgical cardiovascular therapies, such as transplantation and bypass grafting, will greatly benefit from advances in both our understanding and treatment of the genetic and molecular basis of these problems. In this review, we will present the state of gene therapy strategies and the range of methods for genetic intervention that currently exist. We will review the progress and application of this technology toward the improvement of surgical cardiac revascularization, as it applies to

molecular neovascularization of the myocardium and the enhancement of bypass graft patency. We will discuss the potential impact gene transfer and genetic engineering principles and technologies may have on cardiovascular surgery and on surgical outcomes. Finally, rapid progress in human genome sequencing and mapping is likely to affect cardiovascular therapeutics and surgery. We believe the integration of the disciplines of gene therapy and genomics and cardiovascular surgery should result in the emergence of a new field of therapy that we term *surgiomics*.

Gene therapy strategies and delivery systems

Gene therapy is defined as any manipulation of gene expression or function for the treatment of a specific disease. This manipulation may be achieved either via the introduction of foreign DNA into cells that encode a biologically active transgene or through “transfection” of short chains of nucleic acids known as oligodeoxynucleotides (ODNs) that modify endogenous gene expression in target cells (Fig 1). Gene transfer can result in the expression and replacement of a missing gene product or in the “overexpression” of a native or foreign gene whose product can prevent or reverse a disease process. Many therapeutic settings will demand some degree of control over the duration, location, and level of transgene expression. To this end, researchers have begun to develop gene promoter systems that allow regulation of the spatial or temporal pattern of gene expression. Gene blockade can be accomplished by transfection of cells with either complementary DNAs (cDNAs) encoding antisense or decoy sequences, or with antisense or decoy ODNs. Antisense ODNs are generally 15 to 20 bases in length and are designed to have a sequence that is complementary to a segment of the target gene messenger RNA (mRNA). By binding to the message, it renders the template unavailable for translation into its biologically active product.¹ Alternately, decoy ODNs are double-stranded chains designed to mimic the chromosomal binding sites of transcription factors (factors that regulate gene expression by binding to chromosomal DNA at specific promoter regions) and act as “decoys,” reducing the availability of transcription factors required for subsequent activation or suppression of target genes.² Another form of gene blockade is the use of

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Fig 1. Schematic representation of gene therapy strategies. **A,** Gene transfer. **B,** Gene blockade. *TF*, Transcription factor.

“ribozymes,” segments of RNA that can act like enzymes to destroy only specific sequences of target mRNA. Ribozymes contain both a catalytic region that can cleave other RNA molecules in a sequence-specific manner and an adjacent sequence that confers the specificity of the target.

Although many cells will naturally take up DNA from their extracellular environment in small amounts, such low levels are generally insufficient to achieve a physiologically significant influence on genetic function. Clinical gene therapies will therefore depend on the development of vector systems that enhance the efficiency of in vivo DNA transfer. The “ideal” vector would be capable of safe and highly efficient delivery to all cell types, both proliferating and quiescent, with the opportunity to select either short-term or indefinite gene expression. This ideal vector would also have the flexibility to accommodate genes of all sizes, incorporate control of the temporal pattern and degree of gene expression, and to recognize specific cell types for tailored delivery or expression. Although progress is being made on each of these fronts individually,

researchers remain far from possessing a single vector with all of these characteristics. Instead, a spectrum of vectors has evolved, each of which may find a niche in different early clinical gene therapy strategies.

Viral vectors. Viruses are the most common vehicles for exogenous gene delivery into mammalian cells. Although they take on many distinct forms, all viruses consist of a genetic nucleic acid code encapsulated in a machinery that facilitates gene transfer and, in many cases, gene expression. Recombinant viral particles used as gene transfer vectors are distinguished from their naturally occurring derivative viruses, most importantly by their inability to replicate. This is achieved by deletion or mutation of various genetic elements required for completion of the normal viral life cycle. Instead, therapeutic genes are cloned into the recombinant genome and coupled to the necessary regulatory elements.

Just as viral vectors appear to be nature’s solution to the problem of efficient gene transfer, the true realization of their technologic potential has been confounded by the biologic barriers that have evolved to protect

cells and organisms from viral infection. Not only has the host immunologic response limited the efficacy of viral gene transfer, particularly when repeated administrations are considered, but the inflammatory response to viral antigens has impeded and, at times, negated the therapeutic benefits of transgene expression.³ Engineering of viral genomes does not always preclude residual cytotoxicity in infected cells, and the possibility for regression to replication proficiency or for further mutation and recombination with other virulent viruses in the environment pose biologic hazards that are difficult to quantitate or predict.

Retroviruses encode RNA-dependent DNA-polymerases, known as reverse transcriptases, that convert a viral RNA genome to double-stranded DNA.⁴ The DNA is then inserted into a host chromosome, where stable transgene expression may be possible. For this integration to take place, the infected cell must undergo cell division within a short time after infection, thus limiting the delivery of the DNA to replicating cells. Lentiviruses are another class of retrovirus that can integrate into the host genome in the absence of replication. Although this is an attractive alternative for gene transfer into quiescent cardiovascular cells, safety concerns remain concerning the use of these members of the human immunodeficiency virus family; some concern also exists over mutation into its pathogenic phenotype. Unlike retroviruses, recombinant adenoviruses are capable of infecting nondividing cells and have therefore become the most widely used viral vector for experimental *in vivo* gene transfer in animal models of cardiovascular disease.⁵ The use of adenoviral vectors has been associated with significant immunologic and cytotoxic complications. The immune response and the absence of gene integration significantly limit gene expression, which rarely lasts beyond 2 weeks after adenoviral gene transfer. Researchers are therefore exploring removal of nearly all adenoviral genes, creating "gutless" adenoviruses, both to reduce the immunogenicity of the vector and to increase the size of possible transgene insertions. Adeno-associated virus is a human parvovirus that is not able to replicate unless a helper virus, such as adenovirus or herpes virus, is present in the same cell. Adeno-associated virus has not been linked to human disease and can infect a wide range of target cells, establishing a latent infection by integration into the genome of the cell, thereby yielding stable gene transfer. Unlike retroviruses, adeno-associated viruses can infect nonreplicating cells. However, adeno-associated virus is limited by its small size and hence the size of transgene DNA that can be inserted. In addition, the

efficiency of adeno-associated virus vectors for *in vivo* cardiovascular gene therapy remains to be determined.⁶

Nonviral vectors. Plasmids are circular chains of DNA that were originally discovered as a natural means of gene transfer between bacteria. Naked plasmids can also be used to transfer DNA into mammalian cells. The direct injection of plasmid DNA into tissues *in vivo* can result in transgene expression, although being limited to a few millimeters surrounding the injection site. Numerous nonviral methods are available to enhance the delivery of plasmid or oligonucleotide DNA into cells *in vitro*, including calcium phosphate, electroporation, and particle bombardment, but have shown only limited efficiency *in vivo*. The encapsulation of DNA in artificial lipid membranes (liposomes), primarily cationic liposomes, can facilitate its uptake and cellular transport and provides flexibility in substituting different transgene constructs in comparison with the relatively complex process of constructing recombinant viral vectors. Other substances, such as lipopolyamines and cationic polypeptides, are now being investigated as potential vehicles for enhanced DNA delivery for both gene transfer and gene blockade strategies with ODN. Our collaborators have developed a novel fusigenic-liposome-mediated gene transfer that uses a combination of fusigenic proteins of the Sendai virus (hemagglutinating virus of Japan; HVJ) in conjunction with neutral liposomes.⁷ We⁸ have recently reported that the application of a controlled pressurized environment to cardiovascular tissue in a nondistending manner can enhance both ODN and plasmid uptake and nuclear localization. This method has been used for the efficient, *ex vivo* delivery of DNA to both experimental and human vein grafts and in heart transplantation models.

Advances in vector technology, along with the identification of critical pathogenic gene expression, has led to the application of gene therapy technology in animal models of disease in an effort to improve existing cardiovascular surgical therapies. Improved patency of bypass grafts, myocardial neovascularization, and immunomodulation of cardiac grafts are direct examples of investigators' attempts to improve current surgical therapies. Additionally, the application of these technologies may lead to the introduction of cell-based or genetic therapies for the treatment of heart failure as an adjuvant therapy during surgical treatments.

Gene therapy for vein graft failure

The long-term success of surgical revascularization in the lower extremity and coronary circulations has been limited by significant rates of autologous vein

graft failure. No pharmacologic approach has been successful at preventing long-term graft diseases such as neointimal hyperplasia or graft atherosclerosis. Gene therapy offers a new avenue for the modification of vein graft biology that might lead to a reduction in clinical morbidity from graft failures. Intraoperative transfection of the vein graft also offers an opportunity to combine intact tissue DNA transfer techniques with the increased safety of *ex vivo* transfection. A number of studies have documented the feasibility of *ex vivo* gene transfer into vein grafts using a variety of vector systems.

The vast majority of vein graft failures have been linked to the neointimal disease that is part of graft remodeling after surgery. Although neointimal hyperplasia contributes to the reduction of wall stress in vein grafts after bypass, this process can also lead to luminal narrowing of the graft conduit during the first years after operation. Furthermore, the abnormal neointimal layer, with its production of proinflammatory proteins, is believed to form the basis for an accelerated form of atherosclerosis that causes late graft failure.

Given the proliferative basis of neointimal hyperplasia, our group chose to target the cell cycle as a means of limiting vein graft disease. Preliminary studies indicated that blockade of at least two cell cycle genes succeeded in preventing significant neointima formation in experimental grafts. We therefore tested a single, intraoperative treatment of vein grafts with a decoy ODN targeting the transcriptional factor E2F, known to be critical in the up-regulation of cell cycle proteins. These genetically engineered vein grafts resisted neointimal hyperplasia for at least 6 months in the rabbit model. Furthermore, these conduits were able to stabilize wall stress in the absence of a neointima via a process of medial hypertrophy and proved resistant to diet-induced graft atherosclerosis. Abnormal endothelial cell function, a significant contributor to graft failure, was also shown to be improved by ODN inhibition of the neointimal disease process.

A small-scale prospectively randomized double-blind trial of human vein graft treatment with E2F decoy ODN was conducted in patients undergoing peripheral bypass surgery.⁹ Efficient delivery of the ODN was accomplished within 15 minutes during the operation by placement of the graft after harvest in a device that exposes the vessel to ODN in physiologic solution and allows simultaneous application of a pressure of 300 mm Hg to all sides of the vessel, avoiding any potential distention injury. This approach resulted in ODN delivery to greater than 80% of graft cells and effectively blocked target cell cycle gene expression, as well as

vascular cell proliferation among samples brought back to the laboratory for organ culture analysis. Primary graft failure was defined in this study as graft occlusion, graft revision, or evidence on ultrasonography of a stenosis of greater than 75% at 12 months after surgery in those patients who were not candidates for revision. Although this study was not designed to detect a statistically significant reduction in primary failures, the enrollment of a large proportion of high-risk grafts using poor-quality vein conduits led to an overall event rate that allowed comparison of E2F decoy grafts to untreated controls. Fewer failures were observed in E2F decoy-treated grafts, and, unlike controls, failures were not observed beyond the first 6 months after the operation (Fig 2). Although the efficacy of this approach requires further validation in large-scale multicenter trials, this study demonstrates the safety and feasibility of *ex vivo* gene therapy of bypass grafts and suggests a possible stabilization of human graft biology similar to that seen in experimental grafts. The success of this study may have broader implications for other forms of native arterial atherosclerosis, such as coronary artery disease, and offers an encouraging glimpse into the future applicability of this therapeutic approach.

With the development of virus-mediated gene delivery methods, some investigators have begun to explore the possibility of using these systems *ex vivo* in autologous vein grafts. Chen and colleagues¹⁰ demonstrated the expression of the marker gene β -galactosidase along the luminal surface and in the adventitia of 3-day porcine vein grafts infected with a replication-deficient adenoviral vector at the time of surgery. The vein segments were incubated in a high viral titer suspension for approximately 2 hours before implantation. Others have explored the use of a novel adenovirus-based transduction system, in which adenoviral particles were linked to plasmid DNA via biotin/streptavidin-transferrin/polylysine complexes. β -Galactosidase expression was documented 3 and 7 days after surgery in rabbit vein grafts that had been incubated for 1 hour with complexes before grafting. Expression was again greatest on the luminal surfaces of the grafts, although the presence of transfected cells in the medial and adventitial layers was also reported.

The feasibility of gene transfer in vein grafts has therefore led to the investigation of potential therapeutic end points such as inhibition of neointimal hyperplasia. In a rabbit vein graft model, intraoperative transfection of the senescent cell-derived inhibitor gene, a downstream mediator of the tumor suppressor gene p53, using the HVJ-liposome sys-

Fig 2. Kaplan-Meier comparison of time to graft failure between E2F-decoy ODN and untreated groups. Human saphenous vein grafts treated with E2F decoy ODN demonstrated a reduction in primary failure rates when compared with untreated grafts.

tem, was found to partially inhibit neointima formation. In an alternative approach, George and colleagues,^{11,12} using a replication-deficient adenovirus expressing tissue inhibitor of metalloproteinase-1 or -2, were able to demonstrate decreased neointimal changes in a human saphenous vein organ culture model. In a porcine carotid interposition vein graft model, this same group demonstrated that overexpression of tissue inhibitor of metalloproteinase-3, using a replication-deficient adenovirus, was able to limit neointimal disease, further validating the therapeutic potential of this strategy.¹³ Finally, adenovirus overexpression of a nonphosphorylatable, constitutively active Rb gene was also able to demonstrate a reduction in neointima formation.¹⁴ Beyond affecting local graft biology, the overexpression of a secreted therapeutic protein by a transduced graft may lead to the treatment of disease in tissues downstream to the location of graft implantation, further expanding the versatility of this bypass conduit.

Gene therapy for neovascularization

The identification and characterization of “angiogenic” growth factors have created an opportunity to attempt therapeutic “neovascularization” of tissue rendered ischemic by occlusive disease in the native arterial bed. Angiogenesis refers more strictly to the sprouting of new capillary networks from pre-existing vascular structures, whereas vasculogenesis is the de novo development of both simple and complex

vessels during embryonic development. Although molecular factors can stimulate angiogenesis *in vivo*, as clearly established in a number of animal models, it is less certain that these molecules can induce the development of larger, more complex vessels of “neovasclogenesis” in adult tissues that would be capable of carrying significantly increased bulk blood flow. Nevertheless, the possibility of enhancing even microvascular collateralization as a “biologic” approach to the treatment of tissue ischemia has sparked the beginning of human clinical trials in neovascularization therapy.

After the first description of the angiogenic effect of fibroblast growth factors (FGFs), an abundance of “pro-angiogenic” factors was discovered to stimulate endothelial cell proliferation, enhanced endothelial cell migration, or both. Vascular endothelial growth factor (VEGF) and two members of the FGF family, acidic FGF (FGF-1) and basic FGF (FGF-2), have received the most attention as potential therapeutic agents for neovascularization. Much debate persists regarding the preferred agent and the optimal route of delivery for angiogenic therapy in the ischemic human myocardium or lower extremity. VEGF may be the most selective agent for stimulating endothelial cell proliferation, although VEGF receptors are also expressed on a number of inflammatory cells including members of the monocyte-macrophage lineage. This selectivity has been viewed as an advantage, because the unwanted stimulation of fibroblasts and vascular smooth muscle cells in native arteries might exacerbate the growth of neointimal or atherosclerotic lesions. Despite this theoretical selectivity, however, the experimental use of VEGF in animal models has been associated not only with capillary growth, but also with the apparent stimulation of vascular smooth muscle cell proliferation and an exacerbation of neointimal hyperplasia after vascular injury. Furthermore, increases in local myocardial VEGF levels, through either direct gene delivery or implantation of genetically engineered myoblasts, has been shown to result in the formation of angiomas rather than organized capillary or vascular networks. The FGFs are believed to be even more potent stimulators of endothelial cell proliferation, but, as their name implies, are much less selective in their pro-proliferative action as well.

Optimizing the route of drug delivery depends heavily on the pharmacokinetic properties of the agent. Angiogenesis, however, is a very complex biologic process involving multiple cell types engaged in multiple activities, including extracellular tissue dissolution and remodeling, cell proliferation, cell migration, cell

recruitment, and programmed cell death. The role of any single agent must be understood within the complicated orchestration of multiple signaling agents and effectors. Despite the large amount of data that has become available in the past two decades, details of the cellular and molecular mechanisms of angiogenesis remain poorly understood. Still, it is believed that many of the known angiogenic factors, including VEGF and the FGFs, are exquisitely potent and would not, therefore, require large or prolonged dosing regimens. These conclusions are partly based on the results of *in vivo* experiments in which a broad range of dosing strategies, from implantation of sustained release formulations to single intra-arterial boluses, have been reported to induce similarly successful increases in tissue perfusion.¹⁵

Preclinical studies of angiogenic gene therapy have used a number of models of chronic ischemia. An increase in capillary density was reported in an ischemic rabbit hind limb model after VEGF administration. These results did not differ significantly, regardless of whether VEGF was delivered as a single intra-arterial bolus of protein, plasmid DNA applied to the surface of an upstream arterial wall, or direct injection of the plasmid into the ischemic limb. Direct injection of an adenoviral vector encoding VEGF also succeeded in improving regional myocardial perfusion and ventricular fractional wall thickening at stress in a model of chronic myocardial ischemia induced via placement of a slowly occluding Ameroid constrictor around the circumflex coronary artery in pigs.¹⁶ A number of studies have demonstrated increased regional blood flow and improved myocardial contractility after perivascular or intravascular delivery of FGF gene transfer agents.¹⁵

A number of phase I safety studies have already been reported in which angiogenic factors or the genes encoding these factors have been administered to a small number of patients.¹⁷ These studies have involved either the use of angiogenic factors in patients with peripheral vascular or coronary artery disease who were not candidates for conventional revascularization therapies, or the application of pro-angiogenic factors as an adjunct to conventional revascularization. The modest doses of either protein factors or genetic material delivered in these studies were not clearly associated with any acute toxicities. Concerns remain, however, regarding the safety of potential systemic exposure to molecules that are known to enhance the growth of possible occult neoplasms or that can enhance diabetic retinopathy and potentially even occlusive arterial disease itself. Despite early enthusiasm, there is also little experience with the administration of live viral vectors

in extremely large numbers to a large number of patients, and it is uncertain whether potential biologic hazards of reversion to replication competent states or mutation and recombination will eventually become manifest. The results of two phase II studies investigating the intravascular delivery of either VEGF or FGF-4 have been reported and have failed to demonstrate a statistically significant improvement in exercise tolerance over placebo. These studies may underscore the critical nature of targeting effective delivery of these agents in a human clinical setting.

The elucidation of neovascularization as an adaptive and possibly therapeutic phenomenon in the setting of ischemic disease has coincided with the development of what at first seemed to be a simpler approach to revascularization. Transmyocardial laser revascularization (TMR) involves the application of a high-energy laser beam to the epicardial surface of the heart so that tissue vaporization leads to formation of a transmural channel. A percutaneous approach has more recently been developed in which an intraventricular catheter delivers laser energy to the endocardial surface for the creation of channels that pass most but not all of the way through the heart wall. It was originally hoped that these channels would remain patent and provide a source of "direct" revascularization of the myocardium from the ventricular chamber. Although improved myocardial perfusion and function have been demonstrated after TMR in animal models of chronic ischemia, histologic analysis of animal and human specimens has now established that these channels are rapidly occluded via thrombosis and fibrosis. Instead, an increased capillary network has been documented to develop, and it is now widely believed that TMR stimulates an angiogenic response. It is most likely that the effect is part of a generalized "response to injury," in which inflammatory and healing processes stimulate the growth of new vessels that may ameliorate the underlying ischemic nature of the injured tissue.¹⁸ Prospective randomized clinical studies assessing the effects of human TMR have reported outcomes ranging from complete relief of anginal symptoms, along with improvement in functional status and myocardial perfusion, to short-term reduction in anginal symptoms without changes in myocardial function or perfusion 1 year after treatment.^{19,20}

In light of the observations that TMR may stimulate multiple elements of the multifactorial machinery for tissue neovascularization and that single angiogenic factor therapy can successfully enhance blood flow to ischemic tissues, we have hypothesized that a combination of single factor gene transfer with

Fig 3. Combined gene transfer and TMR. Schematic representation of chronic ischemia induced by placement of an Ameroid constrictor around the circumflex coronary artery in pigs. Ischemic hearts that underwent TMR followed by injection of plasmid-encoding VEGF demonstrated better normalization of myocardial function than either therapy alone.

TMR might synergistically yield an even stronger therapeutic response than either therapy alone. To test this theory of complimentary pro-angiogenic mechanisms, we conducted a series of experiments in collaboration with the cardiac surgery laboratory of Lawrence Cohn at Brigham and Women's Hospital.²¹ Among 35 pigs that had undergone placement of an Ameroid constrictor on the circumflex artery, those that subsequently received VEGF₁₆₅ gene therapy via plasmid injection combined with TMR displayed a greater normalization of load-dependent and -independent contractility and of ventricular wall motion than those that were treated with either VEGF gene therapy or TMR alone (Fig 3).

In addition to issues of safety, it is also unclear whether the clinical success of conventional revascularization, which has involved the resumption of lost bulk blood flow through larger conduits, will be reproduced via biologic strategies that primarily increase microscopic collateral networks. Neovascularization is a naturally occurring process, and the addition of a sin-

gle factor may not overcome the causes of an inadequate endogenous neovascularization response in patients with myocardial and lower limb ischemia. Despite these limitations, angiogenic gene therapy may provide an alternative not currently available to a significant number of patients with untreatable disease and may offer an adjunct to traditional therapies that improves their long-term outcomes.

Cell and genetic engineering of bioprosthetic grafts

Prosthetic materials, such as polytetrafluoroethylene or Dacron fabric, have been limited in their long-term use as small-caliber grafts due to their thrombogenic surfaces. A bioengineering, cell-based strategy for decreasing or eliminating this thrombogenicity may therefore yield a small-caliber bioprosthetic graft capable of maintaining normal flow. Successful isolation of endothelial cells and their seeding onto prosthetic grafts in animal models has been well characterized. Furthermore, it has been hypothesized that the function of these endothelial cells can be enhanced via the trans-

fer of biologically active genes before implantation onto the graft surface. The first indication for the possible use of this strategy was presented by Wilson and colleagues,²² who demonstrated successful endothelialization of a prosthetic vascular graft with autologous endothelial cells transduced with a recombinant retrovirus encoding the *LacZ* gene. Additionally, seeding of transduced vascular smooth muscle cells into the interstices of a polytetrafluoroethylene graft then lumenally seeded with untreated endothelial cells revealed stable expression of the reporter gene after 5 weeks.

For therapeutic purposes, the use of a biologically active transgene in these systems has led to mixed results. Given that thrombus formation is a primary pathobiologic process responsible for the failure of these grafts, genes expressing antithrombotic proteins have been tested. Dunn and colleagues²³ seeded 4-mm Dacron grafts with retrovirally transduced endothelial cells encoding the gene for human tissue plasminogen activator and implanted them into the femoral and carotid circulation of sheep. The proteolytic action of tissue plasminogen activator resulted in a decrease in seeded endothelial cell adherence, with no improvement in surface thrombogenicity. In a primate model of an arteriovenous graft, however, seeding of endothelial cells retrovirally transduced to secrete hirudin onto a 4-mm polytetrafluoroethylene graft resulted in both decreased thrombus formation and neointimal changes at the venous anastomosis. The use of VEGF in this context also has potentially significant therapeutic applications. VEGF, when transduced into a limited number of endothelial cells and placed on the graft surface, may promote endothelial survival and replication and yield improved and more rapid graft coverage with a non-thrombogenic endothelial layer. Additionally, secretion of VEGF could lead to angiogenesis distal to the grafted area in what is likely to be an ischemic tissue bed. Further studies are needed to determine the local effect of VEGF secretion on endothelial cell proliferation along with distant angiogenic stimuli.

Until recently, the need to harvest autologous donor tissue for target cells, coupled with the increased costs and complexity of tissue culture, has largely limited this strategy to experimental models. Recent reports have demonstrated the feasibility of obtaining autologous progenitor endothelial cells from the peripheral circulation that, when placed in proper culture conditions, can be stimulated to differentiate into functional endothelial cells.²⁴ With the advent of this new technology, it may become feasible one day to develop functional bioprostheses capable of use in patients undergoing coronary or

peripheral revascularization. In addition, such a cell and genetically engineered bioprosthesis could be useful for delivering factors that would enhance graft function and survival and, furthermore, provide an avenue for intravascular drug delivery.

Gene therapy for immunomodulation

Genetic manipulation of donor tissues offers the opportunity to design organ-specific immunosuppression during cardiac transplantation. Although transgenic animals are being explored as potential sources for immunologically protected xenografts, the delivery of genes for immunosuppressive proteins, or the blockade of certain genes in human donor grafts, may allow site-specific, localized immunosuppression and a reduction or elimination of the need for toxic systemic immunosuppressive regimens. Gene activity has been documented in transplanted mouse hearts for at least 2 weeks after intraoperative injection of the tissue with either plasmid DNA or retroviral or adenoviral vectors. The transfer of a gene for either transforming growth factor- β or interleukin 10 in a small area of the heart via direct injection in this model succeeded in inhibiting cell-mediated immunity and delaying acute rejection. It has been shown that the systemic administration of antisense ODN directed against intercellular adhesion molecule-1 (ICAM-1), when combined with a monoclonal antibody against the ligand for ICAM-1, leukocyte function antigen-1 (LFA-1), also prolonged graft survival and induced long-term graft tolerance. This ICAM-1/LFA-1 blockade strategy was also successful at inducing long-term tolerance and donor-specific suppressor T-cell activity via a single *ex vivo*, pressure-mediated delivery of ICAM-1 antisense ODN to the donor heart combined with a 6-day postoperative course of systemic LFA-1 monoclonal antibody treatment of host immune cells, providing support for an organ-specific gene manipulation approach.²⁵

Gene and cell therapy of heart failure

Failure of the myocardium due to insults such as ischemia, infection, metabolic disorders, or substance abuse afflicts millions annually. Functionally, a principal defect of the failed myocardium is its ineffective or weakened contractility. To this end, investigators have explored the possibility of improving or reversing this derangement by introducing directly and overexpressing transgenes that can improve ventricular function or alter the molecular process of heart failure. Another approach is the implantation of myocardial cells that may be genetically engineered to enhance myocardial function. These may be intro-

duced at the time of surgery and may be an adjunct therapy to cardiac surgery.

The β -adrenergic receptor is known to be a critical player in mediating the inotropic state of the heart and has shown to be down-regulated in failed myocardium. As a result, it has received significant attention as a target for genetic therapeutic intervention in congestive heart failure. Milano and colleagues,²⁶ using transgenic mice overexpressing the β_2 -adrenergic receptor exclusively in the myocardium, demonstrated an approximately 200-fold increase in the level of β_2 -adrenergic receptor along with highly enhanced contractility and increased heart rates in the absence of exogenous β -agonists. This genetic manipulation of the myocardium has generated considerable interest in the transfer of the β -adrenergic receptor gene into the ailing myocardium as a means of therapeutic intervention. Akhter and colleagues²⁷ have demonstrated improved contractility after adenovirus-mediated gene transfer of the human β_2 -adrenergic receptor of rabbit ventricular myocytes that had been chronically paced to produce hemodynamic failure. Furthermore, they were able to demonstrate improvement in myocardial function after adenovirus-mediated overexpression of human β_2 -adrenergic receptor delivered via the coronary arteries in a rabbit heart failure model.²⁸

There has also been recent interest in the enhancement of contractility through the manipulation of intracellular calcium levels. Sarcoplasmic reticulum Ca^{2+} -adenosinetriphosphatase (SERCA2a) transporting enzyme, which regulates Ca^{2+} sequestration into the sarcoplasmic reticulum, has also been shown to be decreased in a variety of human and experimental cardiomyopathies. Using adenovirus-mediated gene transfer, Hajjar and colleagues²⁹ were able to overexpress the SERCA2a protein in neonatal rat cardiomyocytes. This led to an increase in the peak $[\text{Ca}^{2+}]_i$ release, a decrease in resting $[\text{Ca}^{2+}]_i$ levels, and more importantly to enhanced contraction of the myocardial cells as detected by shortening measurements. Furthermore, using a replication-deficient adenovirus, overexpression of SERCA2a in the myocardium of a rat heart failure model led to restoration of both systolic and diastolic function.³⁰ The success of this approach to improving myocardial contractility has yet to be documented in vivo but once again provides a novel and potentially exciting means by which to treat the failed heart.

At the cellular level, heart failure due to myocardial infarction results from the loss of functional cardiomyocytes that are subsequently replaced by a fibroblast-rich scar tissue. Because cardiomyocytes are terminal-

ly differentiated, there is no regeneration of myocytes to repopulate the area of injury after infarction. Researchers have therefore pursued the possibility of genetically converting cardiac fibroblasts into functional cardiomyocytes. The feasibility of this notion gained support after in vitro expression of *MyoD*, a skeletal muscle lineage-determining gene, converted cardiac fibroblasts into cells resembling skeletal myocytes using retrovirus-mediated gene transfer. Fibroblasts expressing the *MyoD* gene were observed to develop multinucleated myotubes, similar to those seen in striated muscle, which expressed major histocompatibility complex and myocyte-specific enhancer factor-2. Additionally, Murry and colleagues³¹ also showed expression of myogenin and embryonic skeletal major histocompatibility complex after transfection of rat hearts injured by freeze-thaw with an adenovirus-containing the *MyoD* gene. At this time, however, functional cardiomyocytes have not yet been identified in regions of myocardial scarring treated with in vivo gene transfer. More recently, researchers have reported that bone marrow stromal cells may contain cardiac stem cells that can differentiate into adult cardiomyocyte.³² These cells may be potentially harvested, cultured, and implanted into the myocardium for therapeutic purposes.

Genomics and surgery

Research of the human genome including the Human Genome Project and many other studies will have a major and transforming impact on the understanding and conduct of biology, clinical medicine, and therapeutics, including surgery.³³ Expressed sequence tags representing all sequences expressed in humans will be determined and their genomic positions will be defined (sequence tagged sites). The discovery of all the variants in the human genome that contribute to the genetic diversity of the human population will result in the construction of dense polymorphic maps. The rapid growth of databases of expressed sequence tags, sequence tagged sites, and single-nucleotide polymorphism, coupled with impressive technologic advances, will surely have a dramatic effect on biomedical research and clinical medicine. An understanding of the genetic diversity and how this diversity contributes to variations in normal and abnormal physiology will have a powerful effect on medicine in the coming decades. It will be technically feasible to genotype and analyze these variants on an individual basis. It will be possible to genotype at several thousand single-nucleotide polymorphic regions. The ability to rapidly genotype indi-

viduals at high density will greatly enhance the ability to determine genes playing causal roles in the development of disease on a population basis and possibly to predict which individuals will be susceptible to disease and to better diagnose and treat disease.

The knowledge of the location of genes in the genome will greatly enhance the ability of investigators to identify disease genes. Genetic analysis results in the definition of genomic regions linked to disease. With the accumulations of mapped genes, the likelihood that these regions will contain genes is growing. Indeed, the likelihood of the disease gene of interest being listed among these candidate expressed sequence tags in a chromosomal region is rapidly escalating. The ability to identify genes (even unknown genes) residing within an interval linked to a particular disease, especially if the tissue patterns of expression of these genes are known or can be determined, will dramatically increase the power of genetic analysis and more rapidly yield candidate genes for further analysis. The analysis of genetic variations will be useful in the prediction, diagnosis, and prognosis of disease. For example, the prognosis of a complex disease that may be treated by surgery can be determined with a high degree of accuracy through the genotyping of a handful of genes. This will enable a surgeon to determine the most appropriate time to perform surgery and to identify the subset of patients who will respond positively to surgical intervention. The power of this approach will be enhanced by the recent technical advances that increase the ability to assess genetic variants, for example, use of DNA microarrays. In the context of cardiovascular disease, it may become possible to identify patients more susceptible to stroke, myocardial infarction, or renal disease and to treat these individuals more aggressively. Moreover, it may become possible to find subsets of patients who are more susceptible to end-organ damage and who respond differentially to surgery with a reduction in risk of end-organ damage. Furthermore, genomics may be useful in predicting and thus avoiding complications of surgery, including tissue reactions, incompatibility, and rejection. Thus, genetic/genomic profiling may identify individuals at increased risk for surgical complications and assist the surgeon in preoperative, intraoperative, and postoperative management.

Finally, genomic studies will also enable the discovery of new gene targets for therapy. As discussed earlier, the application of gene therapy and genetic engineering approaches has targeted known candidate genes whose inhibition or overexpression yields therapeutic effects. However, these known candidate genes are currently limited in number. In the future,

genomic research will discover many new genes that can be manipulated in vivo or ex vivo to improve surgical outcome.

Summary

The introduction of gene transfer, genetic engineering, and genomic principles and technologies may one day enhance the tissue compatibility, durability, and performance of surgical therapies for cardiovascular disease. Furthermore, surgical implants may potentially be used as sources for local or targeted therapeutic gene products. These potential applications of genomics promise to expand the horizons of current surgical therapies and extend the surgeons' reach to the level of cellular and molecular physiology. This synergistic evolution of surgical and molecular science, however, will require the close collaboration of experts from a wide variety of biologic and medical sciences and will help transform the practice of both medicine and surgery in the 21st century.

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