Editorial

Cardiovascular Gene Therapy: Advances and Challenges

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Gene therapy is simply a procedure with the objective of replacing a defective disease-causing gene with a healthy normal one for the purpose of treatment or cure of the disorder. The idea of gene therapy emerged in the late 60's and early 70's under the name of "gene surgery". However, most scientists and clinicians in the field of gene therapy have reached the agreement that the birth of gene therapy was the September of 1990 when W. French Anderson and colleagues used gene therapy to treat a patient suffering from adenosine deaminase (ADA) deficiency, a disorder responsible for a poor and compromised immune system.[1]

Pioneers of gene therapy argued that the monogenic (single underlying genetic basis) and not polygenic (multiple underlying genetic basis) diseases are easier to treat and must be the starting target diseases for gene therapy. ADA deficiency is an example of monogenic and atherosclerosis is considered a polygenic disease. The view of achieving better disease outcomes when targeting the monogenic diseases is certainly a very logical and valid proposition. Nonetheless, gene therapy protocols have also been attempted for the treatment of polygenic diseases and the results have been promising.

The birth of cardiovascular gene therapy began with the treatment of human peripheral vascular disease using the gene for pro-angiogenic Vascular Endothelial Growth Factor (VEGF). In this pioneering clinical trial, Jeff Isner and his colleagues administered VEGF gene into a diabetic patient who would have undergone leg amputations due to advanced ischemia otherwise.[2] The rationale was that the excessive production of the VEGF protein from the administered VEGF gene would generate a vast number of new capillary networks within and around the ischemic and poorly circulated tissues leading to the reestablishment of sufficient blood circulation/perfusion in the patient's ischemic leg and thus avoiding the need for the limb amputation. Gene therapy approaches for the treatment of cardiovascular diseases (polygenic diseases) for which little effective drug therapy exist, such as peripheral artery disease, ischemic heart disease, restenosis following balloon angioplasty, vascular bypass graft occlusion, and transplant coronary vasculopathy, have been tested in preclinical studies as well as human clinical trials with various degrees of success.

Before we describe the experimental designs and the clinical outcomes of some of the seminal gene therapeutic approaches for the treatment of cardiovascular diseases, we would like to briefly define the concept of angiogenesis and list some of the well-recognized stimulators and inhibitors of angiogenesis.

Angiogenesis refers to the formation of new capillaries from a pre-existing capillary plexus. Angiogenesis primarily occurs in postcapillary venules. Physiological angiogenesis is restrained and limited in adults and lasts only a few days. Examples are wound healing and female ovulation. In contrast, pathological angiogenesis is excessive, unrestricted, and progressive as is observed in tumor angiogenesis and rheumatoid arthritis. The term angiogenesis was coined in 1794 by the British surgeon John Hunter to describe blood vessel growth in reindeer antlers as a result of long lasting exposure to cold, a condition that may be comparable to a response to vasoconstriction and, therefore, increased luminal shear stress.[3]

A diverse range of molecules, including a number of soluble polypeptide growth factors and proteolytic enzymes as well as insoluble cell surface receptors such as members of the integrin family and cadherin junctional proteins, play key roles in angiogenesis. The major sources of stimulators and inhibitors of angiogenesis include macrophages, fibroblasts, endothelial and vascular smooth muscle cells, and tumor cells. Extracellular matrix (ECM) also is a rich depot for angiogenic and antiangiogenic factors. A list of positive (stimulators) and negative (inhibitors) regulators of angiogenesis is shown in Tables 1 and 2.

Since the ultimate objective of angiogenesis gene therapy is to perfuse blood into an underperfused ischemic tissue through the induction of neovascularization, it is important to also briefly define arteriogenesis, an important yet different

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mechanism of neovascularization. Arteriogenesis refers to the formation of new small arterial vessels, serving as a detour route for the transport of blood, induced due to the stenosis of a major artery. Regardless of their mechanisms of formation, both angiogenesis and arteriogenesis act to improve/restore the flow of blood to the ischemic heart and limb tissues in order to repair/revive tissue damage.

Table 1. Stimulators of Angiogenesis

Peptides/growth Factors/ Cytokines	FGF-2 (also known as basic FGF)
	VEGF (also known as VPF)
	FGF-1 (acidic FGF)
	PD-ECGF
	TGF-α
	TGF-ß
	TNF-α
	Angiogenin
	Interleukins (IL)-1, -6, -8
	GM-/G-CSF
	IGF-I
	Angiopoietins (Ang)-1 and -2
	Substance P
	EGF
	HGF
Proteases	MMP-1, -2, 3, and -9
	u-PA
	Cathepsins
Small molecules	Adenosine
	Nicotinamide
	Nitric Oxide (NO)
	Trace elements:
	Copper
Oncogenes	c-myc
	ras
	c-src
	v-raf
	c-jun
Signal transduction enzymes	Thymidine phosphorylase
	Farnesyl transferase
	Geranyl geranyl transferase

FGF-2, Basic fibroblast growth factor; VEGF, Vascular endothelial growth factor; VPF, Vascular permeability factor; FGF-1, Acidic fibroblast growth factor; PD-ECGF, Platelet derived-endothelial cell growth factor; TGF-α, Transforming growth factor alpha; TGF-ß, Transforming growth factor beta; TNF-α, Tumor necrosis factor alpha; GM-/G-CSF, Granulocyte macrophage/granulocyte-colony stimulating factor; IGF-I, Insulin-like growth factor-I; EGF, Epidermal growth factor; HGF, Hepatocyte growth factor; MMP, Matrix metalloproteinase; u-PA, Urokinase-plasminogen activator; c-myc, Myelocytomatosis proto oncogene; ras, Rat sarcoma gene; c-src, Rous sarcoma virus protooncogene; v-raf, Ras-activated factor oncogene; c-jun, Protooncogene of avian sarcoma virus 17

Table 2. Inhibitors of Angiogenesis

Various sources	TIMP-1, -2
	PAI-1
	Thrombospondin-1
	Interferon-γ
	P53, Rb
	Platelet factor-4
	Angiostatin
	Endostatin
	Tumstatin
	Decorin
	Zinc

TIMP, Tissue inhibitor of metalloproteinase; PAI, Plasminogen activator inhibitor; P53, 53 kilodalton tumor suppressor; Rb, Retinoblastoma

Cell therapy giving helping hands to gene therapy

Stem cell therapy

We discussed the setbacks and challenges that have prevented gene therapy from becoming a mainstream method of treatment in clinical settings in spite of an abundance of scientific investments and international publicities. In other words, the routine treatment interventions for the classical cardiovascular diseases such as myocardial infarction (MI) and peripheral artery disease (PAD) remain limited to the established methods of angioplasty and bypass grafts. With the emergence of new advances in the stem cell technology (both adult and embryonic), the idea of using a combination of gene and cell therapy as the new magic drug for the revascularization of the occlusive artery diseases, hence treating these diseases with better recovery outcomes, is gaining increasing attention. Therefore, it is important to describe those attributes of the stem cells that most likely provide helping hands for a more effective gene therapy.

Adult stem cells

The current thinking is that the bone marrow is the richest source for adult stem cells. Broadly speaking, two types of precursor cells are generated in the bone marrow, hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs). HSCs are the precursors to red and white blood cells, whereas MSCs have the ability to differentiate into different types of solid tissues, including vascular endothelial cells and cardiomyocytes. A subtype of MSCs is called endothelial progenitor cells (EPCs) with the ability to mobilize from the bone marrow, enter the circulation, lodge and integrate into the injured vessel wall, and differentiate into mature ECs as part of the repair process for the injured vessel.[4-6] Cell surface protein markers (biomarkers) are the main molecules

used to distinguish and isolate various precursor stem cells. For example, biomarkers for the endothelial progenitor cells in human are AC133 (also known as CD133, and Prominin-like protein-1; disappears upon EPCs differentiation into mature ECs.) and vascular endothelial growth factor receptor 2 (VEGFR2).

A major challenge is that the population of EPCs is too low in the circulation and therefore difficult to isolate and propagate. ACC133+ and VEGFR2+ cells typically comprise less than 0.01% of circulating mononuclear cells in the peripheral circulation.[7] However, vascular injury is a trigger for an increase in the number of EPC appearance in the blood plasma. It is believed that injury-induced cytokines, monokines, and growth factors play key roles in the mobilization of progenitor cells from the bone marrow and their further recruitments from the blood circulation into the damaged tissues for repair purposes.[8, 9] For example, granulocyte colony stimulating factor (GCSF) enhances the recruitment of EPCs to the ischemic limbs. Up until now, the common stem cell therapy trials have involved the sole use of autologous bone marrow transplantations with the hope that the minute adult progenitor cells present in the infused bone marrow will come into contact with the appropriate factors, get directly targeted to the injured sites, and proceed to differentiate into healthy tissue for the purpose of repairing the injury. For example, in a recent study, PAD patients who were injected with their autologous bone marrow and were subsequently placed on antioxidant supplements of vitamins C and E as well as the amino acid L-arginine, a precursor for the nitric oxide, (NO) showed significant improvement in circulation in their ischemic tissues.[10] An example of combined cell/gene therapy is the collection of the patient's own bone marrow cells, introduction of the angiogenic growth factor VEGF gene into the cultured marrow cells harvested from the patient, and finally the injection of the propagated genetically-modified autologous bone marrow cells into the patient. Those patients who underwent the described gene/cell therapy protocol showed improved left ventricular (LV) function. A select number of completed clinical trials using cells alone or combined cell/gene therapy are cited in references.[11-34] The readers are also encouraged to visit the NIH-sponsored site (http://www.clinicaltrials.gov) for an up-to-date list of the ongoing and recruiting clinical trials.

Future prospects

Although gene therapy has not delivered as a mainstream therapeutic modality yet, its future is bright. Our optimism for the future of gene therapy is based on recent reports by Yamanaka's group[35, 36] and Melton and colleagues.[37] Interestingly, both of these seminal studies do not directly address the field of cardiovascular gene therapy but the result of their findings will have great implication on the future of gene therapy. Yamanaka and colleagues reported generation of inducible pluripotent stem (iPS) cells from adult mouse and human somatic cells by viral transduction[35] or non-viral DNA transfections[36] of 2 oncogenic (Klf4, c-myc.) and 2 non-oncogenic (Oct3/4, Sox2) transcription factors: this remarkable achievement in reversing the fate of a cell provides a new scientific tool to generate precursor stem-like cells with the ability to redifferentiate into a whole cast of desirable cells. In other words, one can foresee the development of future clinical gene/cell therapies where the defective cells can be turned into an undifferentiated (stem-like) intermediate state, prior to undergoing a second set of gene therapy interventions aimed at a deliberate and defined redifferentiation into desired healthy and functional cells.

In another seminal paper, Melton and colleagues employed the tools of gene therapy and were able to transform one type of mouse pancreatic cells (exocrine cells) responsible for making digestive enzymes into a different type (β cells) capable of making insulin in a diabetic mouse model. In this study, generation of an intermediate cell state was not needed. They then went on to show that the induction of pluripotent stem cells from primary human fibroblasts was facilitated only by a small molecule inhibitor of histone deacetylase inhibitor (valproic acid) with just two transcription factors, Oct4 and Sox2, without the need for the oncogenes c-Myc or Klf4.[38] We would emphasize that although these two seminal studies are not directly related to the topic of this review article, they will open many doors in the future and make the case for the use of gene therapy as the appropriate method/tool for changing the identity of an existing adult cell type for the purpose of cardiovascular treatment. In other words, these two sets of findings will pave the way for the repair of the infarcted cardiac tissues through the regeneration of the healthy myocardial cells and the feeding of the neuronal system, a concept contrary to the existing dogma stating that the damage/death to the cardiac myocytes is irreversible with absolutely no expected recovery/renewal of the damaged tissues.

Conclusion

Accumulating evidence suggests that the future is bright for the cardiovascular treatment using gene therapeutic interventions. Most likely, the ideal therapy will consist of a series of hybrid protocols of gene and cell (patch implantation or stem cell) deliveries. The major current challenges in developing an optimal gene/cell therapy for the treatment of cardiovascular diseases are overcoming difficulties in the gene targeting specificities in order to replace the defective genes, inducing the proliferation (amplification) and differentiation of the healthy implanted cells into functional and synchronized cardiac tissues, as well as overcoming the

host immunological barriers in order to maintain healthy and long-lasting repairs. It is our prediction that the next 10 years will be the golden age for the development of the routine protocols for the treatment of cardiovascular diseases using gene/cell therapy.

This paper is dedicated in memory of our friend and colleague Dr. Goodarz Goodarzi who lost his battle to cancer.

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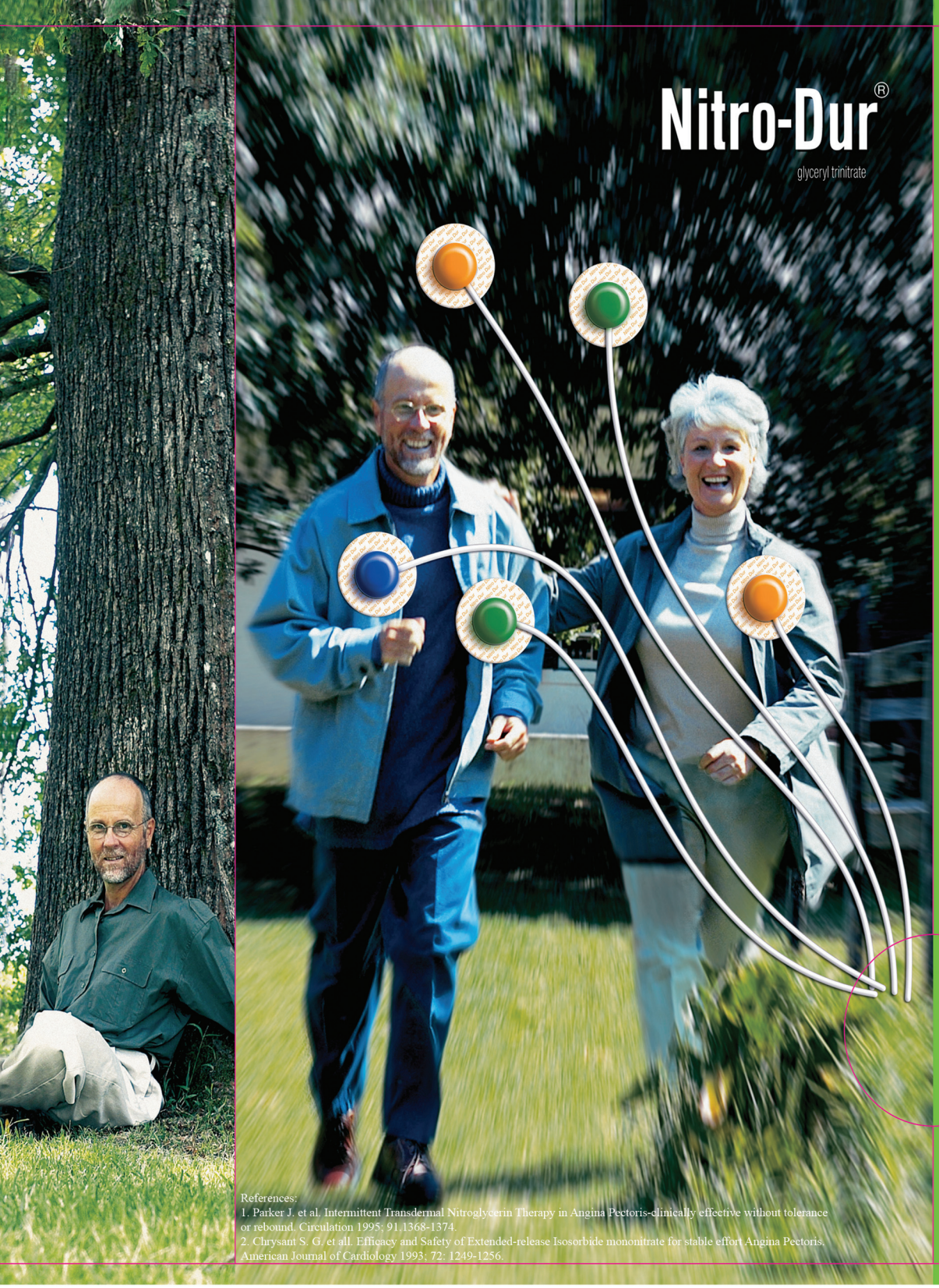

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