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Radionuclide reporter gene imaging for cardiac gene therapy

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Abstract
Introduction In the field of cardiac gene therapy, angiogenic gene therapy has been most extensively investigated. The first clinical trial of cardiac angiogenic gene therapy was reported in 1998, and at the peak, more than 20 clinical trial protocols were under evaluation. However, most trials have ceased owing to the lack of decisive proof of therapeutic effects and the potential risks of viral vectors. In order to further advance cardiac angiogenic gene therapy, remaining open issues need to be resolved: there needs to be improvement of gene transfer methods, regulation of gene expression, development of much safer vectors and optimisation of therapeutic genes. For these purposes, imaging of gene expression in living organisms is of great importance. In radionuclide reporter gene imaging, "reporter genes" transferred into cell nuclei encode for a protein that retains a complementary "reporter probe" of a positron or single-photon emitter; thus expression of the reporter genes can be imaged with positron emission tomography or single-photon emission computed tomography. Accordingly, in the setting of gene therapy, the location, magnitude and duration of the therapeutic gene co-expression with the reporter genes can be monitored non-invasively. In the near future, gene therapy may evolve into combination therapy with stem/progenitor cell transplantation, so-called cell-based gene therapy or gene-modified cell therapy.

Conclusion Radionuclide reporter gene imaging is now expected to contribute in providing evidence on the usefulness of this novel therapeutic approach, as well as in investigating the molecular mechanisms underlying neovascularisation and safety issues relevant to further progress in conventional gene therapy.

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Introduction

Reporter genes such as LacZ, green fluorescent protein (GFP) and firefly luciferase (F-Luc) have long been used in vitro to measure genetic transcription activity. Recently, the approach has been transferred to radionuclide imaging for monitoring of transgene expression in vivo, primarily for external determination of the location, magnitude and duration of gene expression in gene therapies [1, 2]. Among various gene therapy strategies in the field of cardiology (Table 1) [3–5], angiogenic gene therapy has been most extensively investigated [6–8].

In this article, we first comment upon the current status of cardiac angiogenic gene therapies, then provide an overview of radionuclide reporter gene imaging for the evaluation of such therapies, and finally discuss new related research directions.

Cardiac angiogenic gene therapy

Current status of clinical trials

During the 1990s a lot of promising results on therapeutic angiogenesis were reported from animal studies [9, 10]. In

Table 1 Representative gene therapy strategies proposed for cardiac disease

Target condition	Mechanism	Therapeutic genes
Ischaemic heart disease	Facilitation of neovascularisation	VEGF, FGF, HGF, HIF-1α
Post-PTCA	Limitation of restenosis by endothelial repair	VEGF
	Limitation of restenosis by cytotoxic effect on SMC	HSV1-tk
	Limitation of restenosis through cell cycle regulation	NO synthase, VEGF, PDGF-P receptor
Heart failure	Correction of calcium handling	SERCA-2a
	Restoration of β-adrenergic receptor signalling	β-adrenergic receptor, β-ARK1 inhibitor
	Modulation of apoptosis	*Bcl*-2, IGF-1, PI 3-kinase, *Akt*, p38α
Atrial fibrillation	Modification of atrioventricular nodal conduction	Inhibitory G protein α subunit
	Increase in automaticity	Kir 2.1, HCN2

PTCA percutaneous transluminal coronary angioplasty, *VEGF* vascular endothelial growth factor, *FGF* fibroblast growth factors, *HGF* hepatocyte growth factor, *HIF* hypoxia-inducible factor, *SMC* smooth muscle cells, *HSV1-tk* herpes simplex virus type 1 thymidine kinase, *NO* nitric oxide, *PDGF* platelet-derived growth factor, *SERCA* sarcoendoplasmic reticulum Ca^{2+}-ATPase, *ARK* adrenergic receptor kinase, *IGF* insulin-like growth factor, *PI* phosphatidylinositol, *HCN* hyperpolarisation-activated cyclic-nucleotide-gated

1998 the first clinical trial of cardiac angiogenic gene therapy was published [11], and at the peak, more than 20 clinical trial protocols were under evaluation (Table 2) [12, 13]. However, most have ceased owing to lack of decisive proof of therapeutic effects and the potential risk of viral vectors. According to aNational Institutes of Health (NIH) internet resource, as of the end of January 2007, just one clinical trial is currently recruiting patients for angiogenic gene therapy, the primary objective being to test the gene transfer method using the NOGA navigational catheter [14].

Open questions on strategies for therapeutic angiogenesis

In order for cardiac angiogenic gene therapy to evolve further, a number of open issues need to be resolved: there needs to be improvement of gene transfer methods, regulation of gene expression, development of much safer vectors and, especially, optimisation of therapeutic genes [15].

As shown in Table 2, the published clinical trials of cardiac gene therapy have to date exclusively employed isoforms of vascular endothelial growth factor (VEGF) or fibroblast growth factors (FGFs), while various kinds of angiogenic growth factors (AGFs) have been identified (Table 3) [16, 17]. Their roles have been analysed principally in the context of tumour vessel growth, and they have not been well studied in myocardial ischaemia. Elucidation of their roles and of the interactions between them in cardiac angiogenesis is essential in order to optimise therapeutic genes. Taking advantage of a “master gene”, the expression of which activates various other genes in an angiogenic cascade, may induce broader angiogenesis [18]. Otherwise, using multiple therapeutic genes carried together (a “gene cocktail”) may be useful to exert synergistic angiogenic effects, with consequent reduction in the dose and risk of each angiogenic factor [19]. Furthermore, utilisation of an “angiogenesis inhibitor” as a regulator of angiogenic processes in combination with angiogenesis stimulators (AGFs) may allow angiogenesis to proceed more physiologically, given that it is widely accepted that angiogenic processes are tightly and dynamically regulated by a local balance between the levels of angiogenesis stimulators and inhibitors [20]. To date, various angiogenesis inhibitors have been reported, and some of them have been confirmed to contribute to tumour angiogenesis as regulators, but little is known about their contribution in cardiac angiogenesis (Table 3) [16, 17]. If a direct negative feedback mechanism involved in cardiac angiogenesis could be identified and reproduced with these inhibitors, well-controlled new vessel development might be induced.

General safety issues regarding gene transfer with viral vectors

In addition to the disappointing results of the clinical trials, two cases of serious adverse events after gene transfer with viral vectors also had a negative influence generally on gene therapy. Fortunately, such serious complications have never been reported in cardiac gene therapy, but all researchers in gene therapy should keep the aforementioned tragedies firmly in mind.

The first case was the death of an 18-year-old male with partial ornithine transcarbamylase (OTC) deficiency who participated in a pilot study of gene therapy [21]. This experience pointed to the limitations of animal studies in predicting human responses, the steep toxicity curve for replication defective adenoviral vectors, substantial subject-to-subject variation in host responses to systemically administered vectors, and the need for further study of the immune response to adenoviral vectors [22].

The second case involved the onset of leukaemia in three children almost 3 years after successful gene therapy for X-linked severe combined immunodeficiency (SCID-X1) [23]. In all patients, analysis of the leukaemia cells showed

Table 2 Published clinical trials of cardiac gene therapy

First author	Publication	No. of pts.	Therapeutic gene	Vector	Route	Concomitant therapy	Randomised, double-blind, placebo-controlled?
Losordo DW	*Circulation*, 1998	5	$VEGF_{165}$	Plasmid	Intramyocardial	None	No
Rosengart TK	*Circulation*, 1999	21	$VEGF_{121}$	Adenovirus	Intramyocardial	CABG (n=15) or none (n=6)	No
Symes JF	*Ann Thorac Surg*, 1999	20	$VEGF_{165}$	Plasmid	Intramyocardial	None	No
Esakof DD	*Hum Gene Ther*, 1999	17	$VEGF_{165}$	Plasmid	Intramyocardial	None	No
Rosengart TK	*Ann Surg*, 1999	21	$VEGF_{121}$	Adenovirus	Intramyocardial	CABG (n=15) or none (n=6)	No
Laitinen M	*Hum Gene Ther*, 2000	10	Mouse VEGF	Plasmid	Intracoronary	PTCA or PTCA+stenting	Randomised, double-blind, placebo-controlled
Vale PR	*Circulation*, 2000	13	$VEGF_{165}$	Plasmid	Intramyocardial	None	No
Lathi KG	*Anesth Analg*, 2001	30	$VEGF_{165}$	Plasmid	Intramyocardial	None	No
Vale PR	*Circulation*, 2001	6	VEGF-2	Plasmid	Intramyocardial	None	Randomised, single-blind, placebo-controlled
Sarkar N	*J Intern Med*, 2001	39	$VEGF_{165}$	Plasmid	Intramyocardial	None	No
Harvey BG	*Hum Gene Ther*, 2002	31	$VEGF_{121}$	Adenovirus	Intramyocardial	CABG (n=15) or none (n=16)	No
Crystal RG	*Hum Gene Ther*, 2002	2	$VEGF_{121}$	Adenovirus	Intramyocardial	CABG (n=1) or none (n=1)	No
Grines CL	*Circulation*, 2002	60	FGF-4	Adenovirus	Intracoronary	None	Randomised, double-blind, placebo-controlled
Losordo DW	*Circulation*, 2002	19	VEGF-2	Plasmid	Intramyocardial	None	Randomised, double-blind, placebo-controlled
Ben-Gary H	*Mol Ther*, 2002	31	$VEGF_{121}$	Adenovirus	Intramyocardial	CABG (n=15) or none (n=16)	No
Hedman M	*Circulation*, 2003	65	$VEGF_{165}$	Adenovirus (n=37) or plasmid (n=28)	Intracoronary	PTCA or PTCA+stenting	Randomised, double-blind, placebo-controlled
Fortuin FD	*Am J Cardiol*, 2003	30	VEGF-2	Plasmid	Intramyocardial	None	No
Grines CL	*J Am Coll Cardiol*, 2003	35	FGF-4	Adenovirus	Intracoronary	None	Randomised, double-blind, placebo-controlled
Kolsut P	*Kardiol Pol*, 2003	22	$VEGF_{165}$	Plasmid	Intramyocardial	CABG (n=14) or none (n=8)	No
Tio RA	*J Nucl Med*, 2004	10	$VEGF_{165}$	Plasmid	Intramyocardial	None	Randomised
Kastrup J	*J Am Coll Cardiol*, 2005	40	$VEGF_{165}$	Plasmid	Intramyocardial	None	Randomised, double-blind, placebo-controlled
Gyongyosi M	*Circulation*, 2005	40	$VEGF_{165}$	Plasmid	Intramyocardial	None	Randomised, double-blind, placebo-controlled
Bokeriya LA	*Bull Exp Biol Med*, 2005	29	$VEGF_{165}$	Plasmid	Intramyocardial	CABG, TMLR, CABG+TMLR	No

VEGF vascular endothelial growth factor, *FGF* fibroblast growth factors, *CABG* coronary artery bypass grafting, *PTCA* percutaneous transluminal coronary angioplasty, *TMLR* transmyocardial laser revascularisation

Table 3 Representative stimulators and inhibitors of angiogenesis

Angiogenesis stimulators (angiogenic growth factors, AGFs)	Angiogenesis inhibitors
Angiogenin transforming growth factors	Angiostatin
Angiopoietin	Basic fibroblast growth factor receptor (bFGF-R)
Angiotropin	Endostatin
Fibroblast growth factors (FGFs)	Interferon-α (IFN-α)
Granulocyte-colony stimulating factor (G-CSF)	Interleukin-1, -6, -12 (IL-1, -6, -12)
Hepatocyte growth factor (HGF)	Placental proliferin-related protein
Interleukin-8 (IL-8)	Platelet factor-4 (PF-4)
Matrix metalloproteinases (MMPs)	Prolactin (PRL)
Placental growth factor	Thrombospondin (TSP)
Platelet-derived growth factor (PDGF)	Tissue inhibitors of metalloproteinases (TIMPs)
Proliferin	Transforming growth factor-β (TGF-β)
Tumour necrosis factors (TNFs)	
Vascular endothelial growth factor (VEGF)	

retroviral vector integration in proximity to the LIM domain only-2 (LMO2) proto-oncogene promoter, leading to aberrant transcription and expression of LMO2. That is, activation of an oncogene by “insertional mutagenesis” occurred, this having long been one of the apprehensions about gene therapy. Importantly, these results indicate that the retroviral integration site is not selected at random, as used to be believed; rather, there is a preference for particular targets such as proto-oncogenes with activated chromatin structures [24].

On the other hand, there is no ideal non-viral vector. Naked plasmids are essentially non-toxic. Furthermore, they can be expressed efficiently in striated muscles compared with cancer cells [25] and in ischaemic tissues compared with non-ischaemic tissues [26], but their transfection efficiency is poor. Transfer of plasmids can be enhanced by the use of cationic liposomes, but their gene transfer efficiency is still clearly lower than that of adenoviruses [27]. Ultrasound- and electroporation-facilitated deliveries of plasmid DNA have been reported to offer great improvements in transfection efficiency, but further assessment is needed [28, 29].

Given these considerations, much safer vectors with high expression efficiency are urgently required for future gene therapy. Various viral and non-viral vectors are currently being improved or newly developed [30].

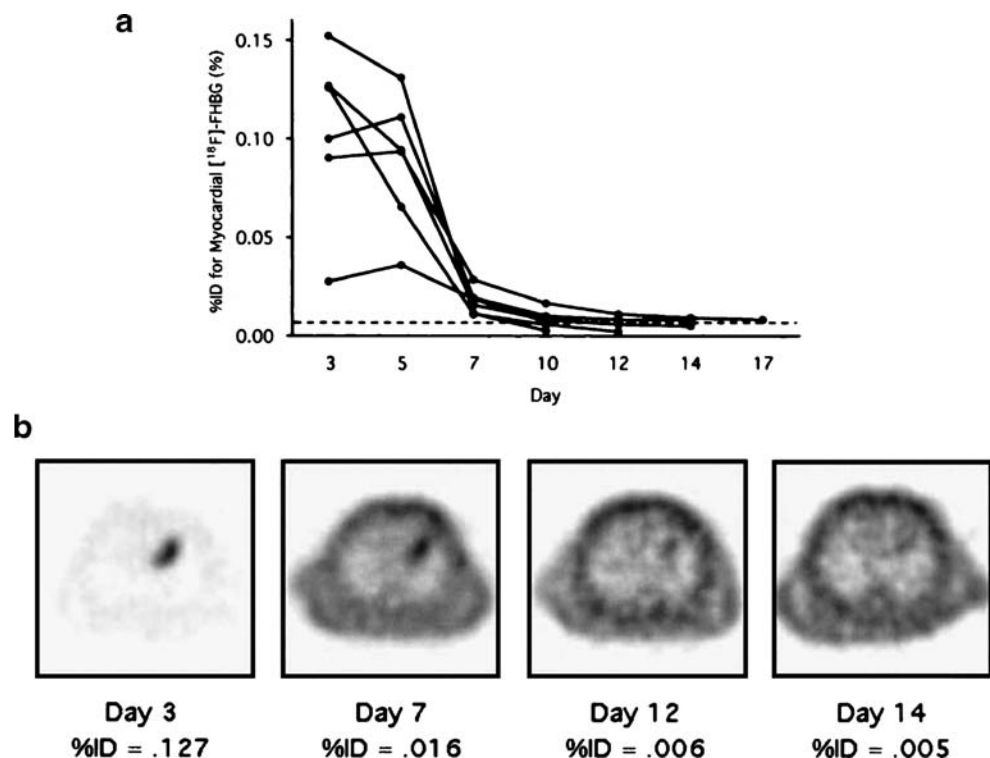

Fig. 1 An example of radionuclide reporter gene imaging with a mutant herpes simplex virus type 1 thymidine kinase (HSV1-sr39tk) reporter gene and 9-(4-[^{18}F]fluoro-3-hydroxymethylbutyl)-guanine ([^{18}F]FHBG) reporter probe. **a** Time course of myocardial accumulation of the reporter probe (percent injected dose, %ID) calculated from serial microPET images in six rats. **b** Transaxial [^{18}F]FHBG microPET images at similar slice levels of a representative rat scanned serially. Grey scale is normalised to the individual peak activity of each image. (Reproduced from reference [34])

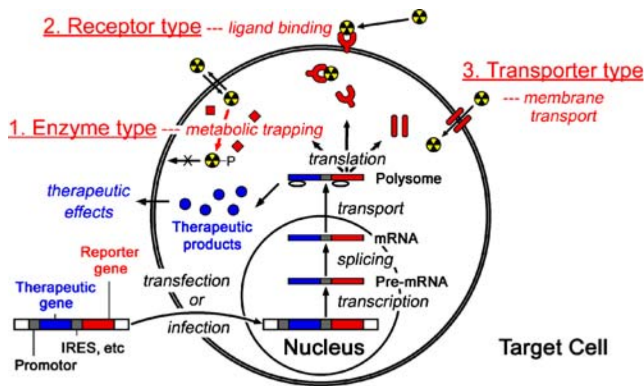

Fig. 2 Three types of strategy for radionuclide reporter gene imaging: enzyme based, receptor based and transporter based

Radionuclide reporter gene imaging

Basics

Imaging of gene expression in living organisms is of great importance both for investigation of the molecular mechanisms underlying neovascularisation and for assessment of the safety and efficiency of novel vectors. Most current imaging strategies for gene expression are indirect methods using “reporter genes” combined with complementary “reporter probes”. Among several molecular imaging modalities (e.g. magnetic resonance, optical, ultrasound) for reporter gene imaging, radionuclide imaging has enjoyed exceptional growth because it is sensitive, objective, quantitative, and widely applicable to subjects from mice to humans, and also can employ various biological tracers for functional assessment [15, 31–33]. In radionuclide reporter gene imaging, reporter genes transferred into cell nuclei encode for a protein that retains a complementary reporter probe of a positron or single-photon emitter, and thus expression of the reporter genes can be imaged in vivo with positron emission tomography (PET) or single-photon emission computed tomography (SPECT). Accordingly, in the setting of gene therapy, if one connects the reporter gene to a therapeutic gene prior to administration, the location, magnitude and duration of the therapeutic gene co-expression with the reporter gene can be monitored non-invasively (Fig. 1) [34]. The strategies for radionuclide reporter gene imaging are divided into three types in general: enzyme based, receptor based and transporter based (Fig. 2).

Recent advances

The proof of principle of radionuclide reporter gene imaging was established through early experimental studies using a herpes simplex virus type 1 thymidine kinase (HSV1-tk) reporter gene and its mutant. However, HSV1-tk and the derivative genes are also suicide genes when combined with certain antiviral agents, and thus are suitable for molecular targeted therapy and imaging for cancers rather than for myocardium, where the cells should survive. Therefore, a great variety of alternative combinations of

Table 4 Reporter genes and reporter probes for radionuclide imaging

Type of reporter	Reporter gene	Radiolabelled reporter probe
Enzyme type	Herpes simplex virus type-1 thymidine kinase (HSV1-tk)	[^{14}C/^{123}I/^{124}I/^{125}I/^{131}I]FIAU, [^{11}C/^{14}C/^{18}F]FMAU, [^{18}F/^{76}Br]FBAU, [^{18}F]FCAU, [^{3}H]FEAU, [^{3}H/^{18}F]FFAU, [^{18}F]FFEAU, [^{18}F]FPAU, [^{18}F]FBrVAU, [^{18}F]FTMAU, [^{123}I/^{125}I]FIRU, [^{123}I/^{125}I/^{131}I]IVFRU, [^{125}I]IVDU, [^{3}H]IUdR, [^{18}F]FUdR, [^{3}H]TdR, [^{3}H]ACV, [^{3}H/^{14}C]GCV, [^{18}F]FGCV, [^{3}H]PCV, [^{18}F]FPCV, [^{18}F]FHPG, [^{18}F]FHBG, [^{11}C]ABE
	HSV1-sr39tk (mutant HSV1-tk)	[^{14}C]FIAU, [^{3}H]PCV, [^{18}F]FPCV, [^{18}F]FHBG
	Cytosine deaminase (CD)	[^{18}F]fluorocytosine
	LacZ	[^{125}I]PETG
Receptor type	Dopamine type-2 receptor (D2R)	[^{18}F]FESP, [^{3}H]spiperone, [^{123}I]iodobenzamine, [^{11}C]isoremoxipride (FLB457)
	Somatostatin receptor subtype-2 (SSTr2)	^{18}F/^{64}Cu/^{67}Ga/^{68}Ga/^{86}Y/^{111}In/^{123}I-octreotide, ^{99m}Tc-depreotide (P829), ^{99m}Tc-vapreotide (RC160), ^{99m}Tc-P2045, ^{99m}Tc-sandostatin, ^{94m}Tc-demotate 1, ^{111}In-pentetreotide
	Oestrogen receptor ligand (ERL)	[^{18}F]FES
Transporter type	Sodium/iodide symporter (NIS)	^{123}I, ^{124}I, ^{125}I, ^{131}I, ^{99m}TcO$_4^-$, ^{76}Br$^-$
	Norepinephrine transporter (NET)	[^{131}I]MIBG, [^{11}C]mHED
	Dopamine transporter (DAT)	^{99m}Tc-TRODAT-1

FIAU 2′-fluoro-2′-deoxy-1-β-D-arabinofuranosyl-5-iodouracil, *FMAU* 1-(2′-fluoro-2′-deoxy-D-arabinofuranosyl)-5-methyluracil, *FBAU* 2′-deoxy-2′-fluoro-5-bromo-1-β-D-arabinofuranosyluracil, *FCAU* 2′-deoxy-2′-fluoro-5-chloro-1-β-D-arabinofuranosyl-uracil, *FEAU* 2′-fluoro-2′-deoxyarabinofuranosyl-5-ethyluracil, *FFAU* 2′-deoxy-2′-fluoro-5-fluoro-1-β-D-arabinofuranosyluracil, *FFEAU* 2′-fluoro-2′-deoxy-1-β-D-arabinofuranosyl-5-(2-fluoroethyl)-uracil, *FPAU* 2′-fluoro-2′-deoxy-5-propyl-1-β-D-arabinofuranosyluracil, *FBrVAU* 2′-fluoro-2′-deoxy-5-bromovinyl-1-β-D-arabinofuranosyluracil, *FTMAU* 2′-fluoro-2′-deoxy-5-trifluoromethyl-1-β-D-arabinofuranosyluracil, *FIRU* 1-(2-fluoro-2-deoxy-D-ribofuranosyl)-5-iodouracil, *IVFRU* (*E*)-5-(2-iodovinyl)-2′-fluoro-2′-deoxyuridine, *IVDU* (*E*)-5-(2-iodovinyl)-2′-deoxyuridine, *IUdR* 5-iodo-2′-deoxyuridine, *FUdR* 5-fluoro-2′-deoxyuridine, *TdR* thymidine, *ACV* acyclovir, *GCV* ganciclovir, *FGCV* 8-fluoroganciclovir, *PCV* penciclovir, *FPCV* 8-fluoropenciclovir, *FHPG* 9-[(3-fluoro-1-hydroxy-2-propoxy)methyl]-guanine, *FHBG* 9-(4-fluoro-3-hydroxymethyl-butyl)guanine, *ABE* 2-amino-6-(4-methoxyphenyl-thio)-9-[2-(phosphonomethoxy)ethyl]purine bis(2,2,2-trifluoroethyl) ester, *PETG* phenylethyl-β-D-thiogalactopyranoside, *FESP* fluoroethylspiperone, *FES* fluoro-oestradiol, *MIBG* metaiodobenzylguanidine, *mHED* meta-hydroxyephedrine

reporter gene and reporter probe have been proposed for radionuclide imaging (Table 4) [35, 36]. Among them, a human sodium/iodide symporter (hNIS) gene has recently been observed to be a safe and convenient reporter gene [37]. NIS is an intrinsic transmembrane glycoprotein that mediates the uptake of sodium and iodide ions, and is naturally expressed on the membrane of mammalian thyroid cells. So, when NIS is used as a reporter gene, various radioactive iodides (^{123}I, ^{124}I, ^{125}I, ^{131}I) and pertechnetate ($^{99m}TcO_4^-$) are available as its reporter probe; this avoids the troublesome problems of tracer synthesis, labelling stability and metabolites. For future applications of radionuclide reporter gene imaging in humans, such non-toxic and non-immunogenic methods using human-oriented genes instead of viral genes will be essential.

Future prospects

Stem/progenitor cells and AGF are respectively sometimes likened to "seeds" as the origin of vessels and "soil" which supports the vessel growth, and both are indispensable for vascular regeneration. Therefore, gene therapy may be expected to evolve into combination therapy with stem/progenitor cell transplantation, so-called cell-based gene therapy [38] or gene (genetically)-modified cell therapy [39]. In other words, by transferring therapeutic genes transduced into stem/progenitor cells into ischaemic myocardium, a synergic effect of concurrent myogenesis and angiogenesis can be anticipated. In fact, an in vivo mouse study reported that the dose of endothelial progenitor cells (EPCs) transduced with adenovirus encoding VEGF was 30 times less than that required in ordinary EPC transplantation to achieve neovascularisation and flow recovery [40]. This virtue may overcome the difficulty of securing sufficient autologous stem/progenitor cells for cell therapy. The combination has more advantages for gene therapy, including specifically localised, regulatable expression and reduced immunogenicity. Since tracking transplant cells by transducing reporter genes in advance can also be seen in a different light, as a simulation of the combined cell and gene therapy, radionuclide reporter gene imaging is expected to contribute in providing evidence on the usefulness of this novel therapeutic approach.

Conclusion

The clinical trials of cardiac angiogenic gene therapy which were started immediately after the procurement of favourable results in animal studies produced somewhat disappointing results, and serious adverse events occurred in some patients. In retrospect, the initiation of these trials seems to have been excessively hasty. The enthusiastic expectations for cardiac gene therapy have now moderated, and much attention is being directed towards stem/progenitor cell transplantation for the purpose of therapeutic angiogenesis. However, while such cell-based gene therapy has also reached the stage of clinical trials, many questions remain to be answered. Thus while investigations into the various therapeutic approaches, including gene therapies, cell therapies and combinations thereof, are to be welcomed, in order for any of these approaches to become a routine clinical strategy the following prerequisites will have to be met: (a) an in-depth understanding of the molecular mechanisms of disease development, (b) assurances of safety in vectors, cells, gene constructs and procedures, and (c) accumulation of evidence of therapeutic effectiveness. We hope that radionuclide reporter gene imaging will contribute in further advancing such research.

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