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REVIEW ARTICLE

Strategies And Vectors For Gene Therapy: Its Prospective Therapeutic Attributes Against Restenosis

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ABSTRACT

Gene therapy is seen as one of the upcoming technologies not only against diseases which have monogenetic etiology, but also against complex diseases such as cancer and cardiovascular disorders. Amongst the cardiovascular disorders, restenosis is one of many disorders which has seen a major increase in the clinical trials, using gene therapy, in recent years. Restenosis, which is simply reoccurrence of stenosis, is seen mainly post surgically in an artery or blood vessel which had been unblocked. Importantly, even though stents have been introduced to prevent restenosis to occur post surgically, the effect seems to be limited to decreasing the statistical rate , and restenosis still persists as a problem for which a definite solution or remedy, acting on the very roots of its pathogenesis, is the need of the hour. Gene therapy, transfer of a healthy gene for curing a disorder, seems to a promising modality for the purpose. To meet this end a definite strategy, an appropriate vector and target for efficient and persistent expression of the healthy gene in the desired or localized area, is what will make gene therapy against restenosis more effective.

Key Words: Restenosis, Gene therapy, Vector, Remodeling

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Introduction

About 40-50% of vessels undergo Restenosis after Coronary Artery Bypass Graft (CABG) or Percutaneous Coronary Angioplasty (PTCA), and in occasions where traditional stents are used (.i.e. when the diameter of the vessel is > 3mm in diameter) this rate is reduced to 20% -30% [1]. This loss of lumen in a previously operated / dilated artery which results in poor vascular patency is due to increase in number of intimal (inner layer of the vessel) cells, known as neointimal hyperplasia. Neointimal hyperplasia along with constrictive remodeling are the two phenomenons responsible for restenosis, and in both, extra cellular matrix (ECM) accumulation is the causative factor (90% of the bulk of neointima comprises of ECM). Constrictive remodeling is said to be the major cause for this luminal loss, especially vessels which have been dilated due to atherosclerosis as the primary cause [1]. This remodeling can be prevented by transferring a healthy gene, into the patient's body, which is thought to be playing a pivotal role in its formation. One of the key challenges at present is finding the appropriate vector for delivering a healthy gene or a cocktail of genes (multigenic approach) in the target tissue. Another aspect that needs to be

considered is the duration of the gene expression, post gene delivery, by the vector.

Strategies For Gene Delivery

Cardiovascular diseases can either be inherited or acquired, and each type needs to be dealt with a different strategy [2] [Table/Fig 1] .There are basically two strategies used for the gene delivery, namely in-vivo and ex-vivo gene delivery. When therapeutic or desired genes are delivered inside the body then it is known as *in-vivo* gene delivery, if the cells are removed from the body and the therapeutic genes then transferred into the cells, it is termed as *ex-vivo* gene delivery. To achieve delivery of the desired gene or product in the target tissue certain steps need to be considered. Firstly, DNA (desired genes) must be delivered to the nucleus and secondly, the central dogma (DNA→RNA→ Protein (functional)) should follow. The first step can occur either in in-vivo or ex-vivo, but the second step always occurs within the body (in-vivo).

Ex-Vivo Gene Delivery

Ex-vivo gene delivery is a relatively simple method mainly used in vein graft failure. A good demonstration of its use was shown against familial hypercholesterolemia. The Kupffer cells (cells were taken out by partial hepatectomy) were cultured ex-vivo and then transduced with retrovirus containing the gene for LDL receptor, as a result there was decrease in cholesterol levels [3][4]. There are certain advantages with the ex-vivo gene delivery, for example, it has a high efficiency for gene transfer into the targeted cells, its specificity can be restricted to the desired cell type by careful optimization and designing and also the immune response to the vector transferring the gene is minimized as it is performed outside the host. The disadvantages for ex-vivo gene delivery may be due to the procedure involved, for example, the patient may have to undergo two invasive procedures one for the cell harvest and the other for the cell reintroduction after the transfer (like in the case of hepatocytes. [Table/Fig 2]

In-Vivo Gene Delivery

In In-vivo gene delivery there is only one procedure required, i.e., injection of the gene vector and there is no need of cell harvesting and reimplantation. Also any cell of the body organ is the potential target for the gene transfer. But there are some drawbacks with this method as well like, it will be difficult to reach to remote tissues like that of the myocardium or a narrow artery in which the vector may be washed away or the pathogenic mechanisms (e.g. ischemia) may occur before the transfer takes place. Also the systemic release of the vector would really be unavoidable and so optimization of the gene expression (localization) will also be hard to control. Also the vector may produce an immune response and result in a rejection to it, especially if the immune system has had a prior exposure to it. Keeping in mind the fact about the diversity of the cells as targets in our body, the vector system needs to be developed for individual applications [5] , and has a long way still to go to be able to give an efficient gene transfer at the same time meeting all the safety concerns.

Vectors For Gene Therapy

Vectors can be either non-viral or viral. At the moment, out of the two, non viral vectors are suggested to meet the properties of an ideal vector, simply because of it being nonpathogenic, more efficient in gene delivery and less immunogenic. Additionally, because the mechanisms, by which viral vectors work and can be controlled, requires a lot more research and better comprehension for them to be used therapeutically. But limitation of sustained gene expression by non-viral vectors needs to be addressed for it to make it to the clinical practice.

Non-Viral Vectors

This group of vectors consists of naked DNA (plasmid), liposomes, ribozymes, oligodeoxynucleotides (ODN) [Table/Fig3], protein-polylysine complexes and bombardment of micro particles[6].The main advantage of these vectors is the minimal toxicity to the body due to it being less

immunogenic, though local inflammation and edema is seen with plasmid DNA and liposomal complexes [7]. Additionally, their failure of showing sustained effect (gene expression) and inefficient gene delivery limits their use at present in the clinical scenario. This is due to their poor nuclear targeting and also due to their increased intracellular degradation. However, the efficiency of plasmid DNA (Transfection) can be increased with ultrasound. Studies have shown an increase of 300 fold in transmission with usage of ultrasound with microbubble contrast agents which create multiple small holes in the cell membrane (increasing its permeability) thereby enabling the naked DNA to be rapidly translocated [8]. This increased efficiency (Ultrasound and microbubble contrast mediated) has also been demonstrated in human vascular smooth muscle and human aortic endothelial cells without any toxic effects [9]. Similarly, cationic liposomes (positively charged fatty spheres which have negatively charged DNA to be transferred incorporated in them) working as a vector is facilitated by the bi-lipid structure of the cell membrane, which allows these fatty vesicles to simply roll through it thus transferring the DNA in the heart of the cell. But the efficiency of this method is quite low at present. Various methods have been tried to increase their efficiency, one of them is by transfer of the cell specific proteins from the viruses onto their surface (hybrid vector) [10]. Another method that has been implemented is by changing the properties of the cationic polymers. Moreover, liposome vectors (with hemagglutinating virus of Japan (HVJ)) have shown to induce angiogenesis in both non-infarcted and infarcted myocardium [7]. HVJ-Liposome is one of the most efficient non-viral vectors available, but again it has a short duration of gene expression [6]. Liposome vectors which are capable of carrying large DNA inserts are commercially available and have the potential to target a wide variety of cells making them a good choice in certain applications like their use in gene transfer to vascular smooth muscle cells [3].

Viral Vectors

Viral vectors can be classified in two groups on the basis of the fate of their genome inside the host (cell nucleus), i.e., the first group consists of adenoviruses, adeno-associative viruses (AAV) and herpes simplex virus (HSV), whose genome lies in the cell nucleus extra-chromosomally in the form of episomes. Whereas, the second group consists of oncoretroviruses and lentiviruses, whose genome integrates with that of the host's cellular chromatin. This categorization is done simply to determine the suitable vector for a particular application.

Adenoviruses

This virus is one of the most common vectors being used in clinical trials to date [Table/Fig 4], and this is simply because of it being easy to produce in large scale, shows reasonable infection efficiency, and also expresses itself in non-proliferating cells. It transduces the mammalian cells by attaching itself to the Coxsackie Adenovirus Receptor (CAR) and then entering through receptor mediated endocytosis [8].

Adeno Associated Viruses (Aavs)

Adeno- associated virus vectors are a class of small non-enveloped parvoviruses (single stranded DNA) which are emerging as important vector systems for gene therapy. This vector generally exists episomally in DNA (>80%) and has the ability to integrate with the genome (< 2%). This may prove beneficial for long term gene expression. Most of the recombinant (rAAVs) has been AAV serotype 2. Pseudotyping the rAAV2 vector genome with capsids of other serotypes to improve the efficiency of gene transfer into the cell is also under trials. The conversion of its single stranded genome into a double stranded one has increased its efficiency as a vector [11]. Lack of immunogenicity of this vector gives it a significant edge over the other choices. This

vector uses heparin sulphate proteoglycans as a cellular receptor.

Herpes Simplex Viruses (Hsv)-1

These viruses are used as vectors after the deletion of all or a combination of the early viral genes (ICP0, ICP4, ICP22, ICP27, ICP44) . But the early gene ICP0 is not only essential for the long and sustained gene expression but also the one which causes toxicity. However to overcome the adversities, a HSV-1 protein which is activated during latency, replaced ICP0, resulting in a sustained and toxic free gene expression in non-neural cells [12].

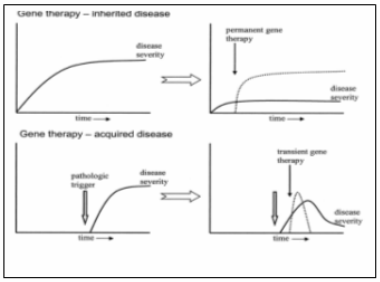
Retroviruses

These viruses along with adenoviruses have been the most commonly used vectors in clinical trials for gene transfer so far [Table/Fig 4]. They were the first class to be developed. These vectors have been used basically for ex-vivo transduction of haemopoetic stem cells. The main limitation of these vectors is its inability to penetrate the nuclear membrane on its own, thereby gaining access to the nucleus only in dividing cells. Although work with Spleen Necrosis Virus (SNV) in the matrix of C-type retroviruses has shown successful gene transfer in non-dividing cells [12].

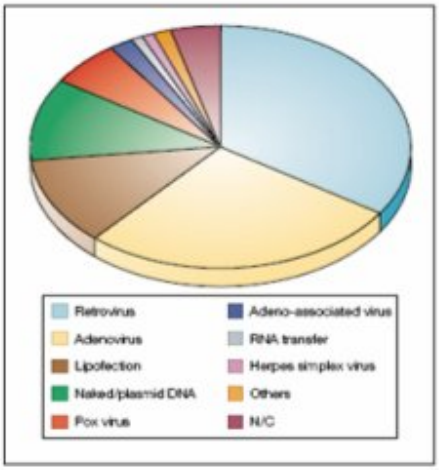
Lentiviruses

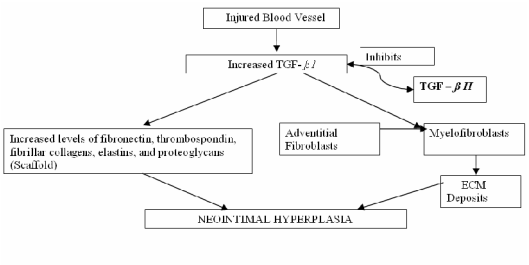
These vectors are basically retrovirus derived but possesses in them the capability to penetrate the nuclear membrane and thus affect the non-dividing cells as well. Pseudotyping with the coated proteins increased its biosafety profile and thus makes it a obvious choice for replacing retroviral vector. Longer expression of the transgene is another vital advantage for using these viruses [12] .

[Table/Fig 1] Shows schematic presentation of different gene therapy strategies (TOP) set of figures shows approach towards inherited type of cardiovascular disorders at an early stage of life in the form of permanent gene therapy (BOTTOM) set of figures show the approach towards an acquired cardiovascular disorder which requires a transient expression of the therapeutic gene in later part of life to alter the physiological response as a result of the disease or insult [2].

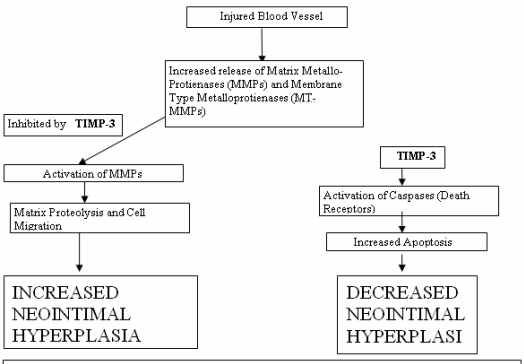


[Table/Fig 4] Shows the results of the survey of Gene Transfer Clinical Trials, from The Journal of Gene Medicine Clinical Trial Database. Shows Retroviruses and Adenoviruses are the commonest vectors under trial [12], [14].

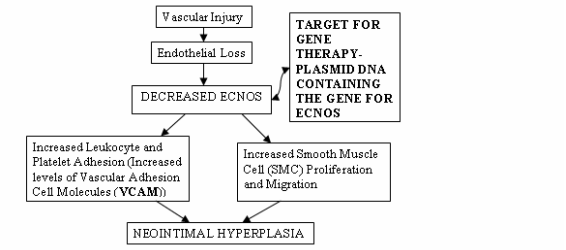




[Table/Fig 5] shows the pathogenesis of neointimal hyperplasia through TGF-β1 whose action is inhibited by TGF – β II thus presenting the gene for TGF – β II receptor, as potential target against restenosis [16]

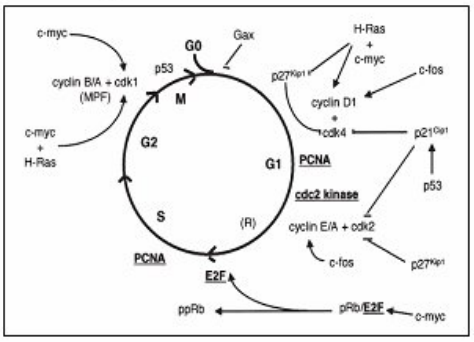


[Table/Fig 6] Shows the dual mechanism of TIMP-3 which includes inhibition of cell migration and increased apoptosis thus eventually resulting in decrease in neointimal hyperplasia. thereby making the gene for TIMP-3 a potential therapeutic target against restenosis [5].

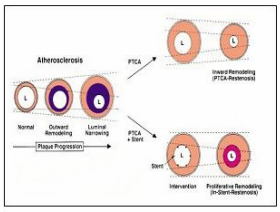


[Table/Fig 7] Shows the role of endothelial released Nitric oxide in neointimal hyperplasia and the targets for gene therapy. VCAMs are also potential target for prevention against restenosis [5].

[Table/Fig 8] Shows phases of a cell cycle, G0-quiescent G1 phase, G1-Early growth, G2-Late growth, S-DNA synthesis, M-Mitotic phase, R-Resting phase. It also shows the cell cycle regulatory proteins which can be targeted for prevention of VSMC proliferation [2].



[Table/Fig 9] Shows the difference made by the usage of a stent after a PTCA, as seen in the above diagram, is not very significant against restenosis [17].



Pathogenesis and Targets for Restenosis

Risk factors like diabetes, hypertension, hyperlipidemia, genetic predispositions, Vascular surgeries lead to formation of atherosclerotic plaques for which CABG / PTCA is performed which results in constrictive remodeling and neointimal hyperplasia resulting in restenosis. Various molecular mechanisms have been implicated in its genesis and thus they have been used as targets for gene therapy against its prevention [13]. There are quite a few candidate targets (corresponding genes), which can be used against restenosis. The effectiveness of each target is yet to be compared, but most of these fit into the picture and are shown to be able to play a major role in the prevention of restenosis.

TARGETS FOR GENE THERAPY AGAINST RESTENOSIS:

Target 1 –Transforming Growth Factor (TGF) – ß Type II Receptor.

TGF ß proteins play a significant role in the scaffold formation in a blood vessel on injury (which could be surgery or placement of a stent). TGF ß Type II Receptor has inhibitory effect on TGF ß Type I Receptor whose main role is to increase the formation of the fibronectin, elastin, collagen and proteoglycans like materials which together forms a scaffold in the vessel, giving rise to neointimal hyperplasia of the intima wall of the vessel [16]. Thereby, Type II agonists and Type I antagonists may have a therapeutic potential against restenosis [Table/Fig 5].

Target 2 – Tissue Inhibitors Of Metallo-Protienase-3 (TIMP-3)

Injury to the blood vessel stimulates the release of both Matrix Metallo Proteinases (MMPs) and Membrane Type Mettalo Proteinases (MT-MMPs) which in turn cause matrix proteolysis and cell migration which results in neointimal hyperplasia. TIMP-3 inhibits this proteolysis and cell migration and also activates the Caspases which results in increased apoptosis, the net result being decrease in the neointimal hyperplasia. These properties of TIMP-3 make it a potential target against restenosis [Table/Fig 6]

Target 3 – Endothelial Cell Nitric Oxide Synthase (ECNOS) And Vascular Cell Adhesion Molecule (VCAM)

Another event which triggers post injury to a blood vessel is that of endothelial loss which results in decrease in the ECNOS levels which in turn contributes toward neointimal hyperplasia by increasing Smooth Muscle Cell (SMC) migration and proliferation and increasing the levels of Vascular Cell Adhesions Molecules (VCAM) .Therefore a plasmid DNA containing the ECNOS gene is also a potential tool against restenosis [Table/Fig 7]

Target 4 – Cell Cycle Regulatory Proteins Using The AntisenseOligodeoxynucleotides (ODN) Approach.

The Vascular Smooth Cell Proliferation (VSMC) is responsible for the neointimal hyperplasia (restenosis) and it depends on the increased expression of certain cell cycle regulatory genes like Proliferating Cell Nuclear Antigen (PCNA), cell division cycle 2 (cdc2) kinase, cyclins, cell dependent kinases (cdk). Other candidate target genes for which Antisense ODN or siRNA approach (Figure 3) may be effective are E2F, c-Myc, Ras, Rb, p53, Bcl-x, cdc2 kinase where it causes inhibition of cell division (mitosis) of cells [Table/Fig 8]

Delivery Of The Gene Therapy For Restenosis

The most efficient method of delivery of the targeted genes is by local transmission of these genes, intravascularly, at the time when there is access to the vessels of the heart (i.e. at the time of operation like PTCA and CABG). But to achieve this, is a bit difficult, because after the local transmission of the gene, the cellular uptake for it is very limited because of the impermeability of the atheroma plaques (lipid rich) and connective tissue, which contribute to the pathogenesis of restenosis [17] [Table/Fig 9]. While needle catheters do enable us to achieve the intraluminal gene transfer, there is a lot still under research. Moreover there is always a chance of the needle it self causing aggravation of the vascular injury. Coated stent technology may be a more practical way for local (intraluminal) gene delivery. However, based on the recent BASKET-LATE trials, coated stents pose a greater risk for late and sudden restenosis [18]. The ideal vector which is non-immunogenic and efficient gene carrier as well is still under research. Various non-viral vectors have been tried like fusogenic liposome, plasmids, and pressure but the biggest disadvantage with them is their reduced efficiency , which outshines their being non immunogenic and the potential to act as carriers for ODNs which can be used as an alternative form of genetic manipulation [Table/Fig 3][15]. Retroviruses have been also tried but with them the fear of transformation (immunogenic) is always there. Adenoviruses are one of the most efficient vectors and the recombinant forms are one of the most suitable candidates for gene delivery against restenosis, but second generation adenoviruses have also been implicated with local inflammatory response. The gutless adenoviruses or helper-dependent adenoviruses (HD-Ad) mediated gene therapy (removal of all the viral elements – with capability of prolonged gene expression) using the previously mentioned targets, may turn to be an invaluable tool for the prevention of the neointimal hyperplasia after angioplasty.

Discussion

The therapeutic attribute of gene therapy against various disorders, will require a well planned and systematic approach to be most effective. Many factors like etiology, choice of vector, mode of delivery of vector, choice of targets etc. all have to be carefully planned based on the merits of each case. Gene fingerprinting and pharmacogenomics may further accentuate its effectiveness.

At present optimization of various available vectors and search for new potential vectors is the area of focus in the field of gene therapy. More and more clinical trials are being initiated in this sector and many new strategies being tested. Many safety concerns and ethical issues have arisen with this methodology of treatment, and adverse effects like neoplasms, edema, immune responses, etc. have acted as a rate limiting step in the advancement of research in this field. But at the same time researches addressing these concerns have been very promising. Recent example being the discovery of a novel mechanism involving protein Hexon and a blood clotting enzyme ,Factor X by Dr. Baker's group (Waddington et al) at the University of Glasgow. Mutations in the Hexon protein and pharmacological blockade of the interactions of these proteins blocked the gene transfer, suggesting the mechanism by which gene transfer takes place in case of fibre modified viral vectors [19].

This new fact can be used to design safer fiber modified vectors for gene delivery. Gene therapy could be the answer to many diseases, especially against Restenosis, for prevention of which today the most common tool are the stents. The incidence of Restenosis when no stent is used in 25-40% , but when a medicated stent is used this incidence can be brought down to 10-20%, which still is quite a considerable rate considering the number of CABGs carried out [20].

Gene therapy is one promising modality which can be combined with present modalities like coated stents (which may no longer pose any threat of late and sudden occurrence of restenosis associated with it) to fill this vacuum and act at the root level against restenosis .

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