“FOCUS ON” session:

Angiogenesis and gene therapy in cardiovascular disease
Gene therapy for bypass graft failure and restenosis

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Abstract
Recently, it has become clear that the concept of gene therapy has changed. Whilst it was once thought that gene therapy merely required insertion of a transgene into a gene transfer vector with subsequent delivery to the target tissue to provide a therapeutic effect, this is now entirely too simplistic. Rather, there are many critical steps in the design and implementation of gene medicines and each step requires exhaustive testing and optimisation for a given target disease to increase efficiency and safety. Within cardiovascular diseases there are many potential target pathologies for gene-based interventions. Bypass graft failure and restenosis are two such examples.

Vein graft disease
Gene therapy is a logical target for prevention of vein graft disease. Patency rates using autologous saphenous vein are generally poor in the long-term, there is a lack of effective pharmacological therapies, there is, however, direct access to the target tissue at the time of surgery and many of the molecular events that contribute to vein graft failure have been documented. The majority of studies have focused on late vein graft failure, principally caused by neointima formation and accelerated atherosclerosis, the latter being moderately reduced by aggressive lipid lowering regimens.

Neointima formation is associated with migration and proliferation of vascular smooth muscle cells induced by vessel damage and growth factor production. Hence, both migration and proliferation have been targeted for genetic intervention. Matrix metalloproteinases (MMPs) play an integral role in the extracellular matrix degradation that allows smooth muscle cell migration and these enzymes have been shown to be upregulated in different models of vein graft disease. MMPs are inhibited in vivo by endogenous inhibitors, the tissue inhibitor of metalloproteinases (TIMPs) and as such TIMP overexpression has been evaluated in appropriate models. Indeed, adenoviral-mediated overexpression of TIMPs reduced neointima formation in an ex vivo human organ culture system. Importantly, TIMP-3, which has the additional ability to promote apoptosis of smooth muscle cells and bind to the extracellular matrix (other TIMP members are freely soluble), showed efficacy in both the human model and the porcine saphenous vein-to-carotid interposition graft model (1).

Interventions that directly target smooth muscle proliferation have shown positive effects in animal models of restenosis (see below). For vein graft disease, antisense oligonucleotides to block proteins such as proliferating cell nuclear
antigen (PCNA) have shown benefit. Perhaps the best example is
decoy oligonucleotides to E2F, a transcription factor that
binds to the promoters of many genes associated with a pro-
proliferative phenotype. Decoy oligonucleotides to E2F have
shown efficacy in pre-clinical models and have advanced to the
PREVENT clinical trial (2). Phase 1 results of pressure-medi-
at ed oligonucleotide delivery demonstrated safety and a sig-
nificant improvement in vein graft disease in the treated group
(2).

Since vein graft failure has a number of underlying mecha-
nisms that may contribute to disease progression, genes that
block more than one pathway may show particular promise for
genetic intervention. As mentioned above, TIMP-3 acts
through blockade of migration and promotion of apoptosis. Nitric
oxide synthase (NOS) is another such example. Nitric
oxide (NO) has a range of desired effects including inhibition
of platelet adhesion and smooth muscle cell proliferation thus
potentially influencing both early and late graft failure.
Additionally NO can upregulate vascular endothelial growth
factor (VEGF) that would positively influence endothelial cell
integrity and regrowth. Overexpression of NOS has been
shown to prevent neointima formation in a rabbit jugular
model at 28 days post-gene transfer demonstrating the poten-
tial of this particular strategy (3) although longer term studies
are required.

It is unclear from preclinical data whether the prevention of
neointima formation alone is sufficient to block vein graft
atheroma formation in the long term. Studies on the E2F decoy
approach in rabbits did suggest that this might be the case as
the decoy strategy did show resistance to graft atherosclerosis,
even after a single administration of agent. Clearly, longer-
term results of the PREVENT trial will address this issue.
However, it should also be noted that gene delivery vectors that
are used for many of the approaches described above provide
only transient overexpression of therapeutic genes.
Development and refinement of these systems, as well as the
use of vectors that provide sustained transgene overexpression
for periods of months if not years (see below), will address
such issues and increase the potential for vein graft gene ther-
apy in the long term.

Restenosis

Due to the high rates of restenosis, many research groups
have addressed the potential for gene therapy to prevent post-
angioplasty restenosis. Like vein graft disease this was deemed
an important pathological target due to the large patient popu-
lation undergoing angioplasty procedures, combined with the
lack of efficacious pharmacological therapies and detailed
knowledge of many important molecular events that contribute
to restenosis. However, unlike vein graft gene therapy, access
to the target tissue is more complex and as such modified
catheter systems were developed that enabled the gene deliv-
ery vector to be exposed directly to the vessel wall immedi-
ately post-angioplasty. This provided highly localised gene deliv-
ery and allowed the assessment of candidate therapeutic gene
overexpression in vivo.

The majority of therapeutic genes tested target components
of vascular smooth muscle cell proliferation and, to a lesser
extent, migration. In essence, data in different animal models
suggests that blockade of smooth muscle cell proliferation has
a beneficial effect on post-angioplasty restenosis (4).
Likewise, genes that influence migration such as MMPs are
strongly implicated in the pathogenesis of restenosis and thus
TIMPs have been evaluated as a gene-based therapy. Early
migration of smooth muscle cells was inhibited by TIMPs
although conflicting results pertaining to the longer-term
effects were apparent with evidence that neointimal lesions
were no different from controls at later time points.

In recent years the frequent use of stents has diminished the
potential clinical utility of many of the post-angioplasty gene
therapy strategies. However, in-stent restenosis is also a poten-
tial target for gene-based intervention if ongoing drug-based
clinical trials yield unacceptable results. Although the number
of studies that have addressed gene delivery to stented vessels
so far is minimal, it is clear that the stent itself can be used to
aid gene delivery in vivo (5). This, in the future, may be par-
cularly relevant with the requirement to maintain the gene
therapy vector locally around the stent for efficacy and safety
reasons. A critical component in the design and implementa-
tion of vascular gene therapy for both vein graft disease and
restenosis is delivery of the therapeutic gene itself.

Gene delivery to vascular tissue

Ideally, the vehicle chosen for individual clinical applica-
tions should have attributes applicable to the disease for which
the therapy is chosen. Important features include:
• Highly efficient gene delivery to the target cell population
  with limited transduction of non-target cells.
• Minimal toxicity and immunogenicity.
• Longevity of transgene expression applicable to the dis-
  ease.
• Regulatable transgene expression.

Although this is a relatively short list of attributes, no cur-
rently available delivery system fulfills all criteria. Commonly
used gene delivery vector systems include naked DNA,
DNA/liposome complexes, recombinant adenoviruses (Ad)
and adeno-associated viruses (AAV). Non-viral vectors are
generally less efficient than viruses as the latter have evolved
over millions of years to achieve efficient target cell transduc-
tion. Unfortunately for vascular gene therapists none of the
commonly used viral systems target to endothelial cells or
smooth muscle cells with high efficiency compared to other
mammalian cell types, particularly epithelia. For example, Ad vectors transduce cells of the liver and spleen with ease but vascular cells are infected with a much poorer efficiency. The infectivity of a given virus is a reflection of viral receptor and co-receptor expression on the surface of cells. For Ad, the target coxsackie and adenovirus receptor (CAR) is expressed highly on hepatocytes, at lower density on endothelial cells and is generally absent on vascular smooth muscle cells hence defining the non-vascular tropism of Ad. Additionally, Ad vectors are highly immunogenic and transduced cells are eliminated through cytotoxic T-cell-mediated clearance resulting in transient transgene expression (up to 4 weeks). Radical genetic modifications of the Ad genome have been performed reducing the immune response and allowing longer-term gene expression in vivo (6). For persistent in vivo transgene expression, AAV vectors are highly suited as they are minimally immunogenic and, unlike Ad that is maintained episomally, AAV vectors integrate into the host genome providing transgene expression for the lifetime of the infected cell. However, studies on AAV vectors in the vasculature are limited due to their extremely poor infectivity for endothelial cells and vascular smooth muscle compared to other tissues such as neurons or skeletal muscle although some recent studies have reported exciting results from AAV-mediated gene delivery to the myocardium in vivo.

Recent advances in virology and gene therapy, such as the elucidation of the atomic structure of AAV, have enabled steps towards the construction of “designer” vectors that have attributes tailored for individual disease applications. Particularly exciting developments include the modification of viral vector envelope proteins and the isolation and evaluation of promoters with efficiency and selectivity for the target cell population alone. The domain(s) within viral envelope proteins that interact with target cell surface receptors have been described for commonly used viral systems. This has resulted in the capacity to remove native viral tropism such that cells normally permissive to a given virus are not transduced. This alone is insufficient for vascular gene therapy as this merely diminishes gene transfer to all cell types. However, it is now possible to superimpose further modifications to the envelope such that the virus is directed to individual cell types. This is achieved by incorporation of small peptides (that mediate binding to a receptor expressed exclusively on the cell surface of the target cell type) in to the envelope protein at defined sites. Hence, cell-selective vectors can now be produced thus increasing the efficiency of gene delivery to vascular cells, eliminating transduction of non-target cells and therefore enhancing the safety profile of the vector system.

Together, recent data suggests that gene therapy may have a place in routine clinical practice for treatment of cardiovascular diseases including vein graft disease and restenosis. Importantly, careful optimisation and evaluation will be required to increase the efficacy and safety of gene-based medicines.

References