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New therapeutics for pulmonary arterial hypertension: do gene therapies have translational values?

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Pulmonary arterial hypertension (PAH) is a devastating disease characterized by a marked and sustained elevation of pulmonary vascular resistance (PVR) increasing pulmonary arterial pressure. Progressively, patients will develop right ventricular hypertrophy (RVH) and then right ventricular failure. The pathogenesis of PAH is multifactorial. Endothelial cell (EC) dysfunction is well recognized as an early feature of the disease, leading to an imbalance in production of vasodilatator and vasoconstrictor factors (a decrement in vasodilatators such as prostacyclin and nitric oxide [NO], and a concomitant increase in vasoconstrictors such as thromboxane and endothelin-1) [1]. Over the past several years, therapy has focused on re-establishing this imbalance by, for example, exogenous delivery of vasodilatating prostaglandins and inhaled NO, or by blocking the endothelin axis, which leads to vasodilatation and improves the pulmonary circulation. Unfortunately, some of these treatments have undesired side effects, principally systemic hypotension (epoprostenol) and liver toxicity (sitaxentan, withdrawn from the market in 2010). Moreover, some patients are resistant to these therapies, and even for those who show responses, they failed to totally reverse PAH, unless the patients also received a lung/heart transplant. In fact, the major cause of the elevated PVR is the obstructive vascular remodeling due to an imbalance in the proliferation and apoptosis rates of the pulmonary artery smooth muscle cells (PASMCs) [1], which show a cancer-like behavior. Thus, the scientific community has started to focus more and more on the cellular and molecular mechanisms implicated in pulmonary artery remodeling and have started to develop therapies aimed at reversing the proliferative phenotype of PASMCs in the vascular wall [2]. Genomic approaches such as gene expression profiling and sequencing have demonstrated that many genes are aberrantly expressed in PAH [3] and are often involved in the activation of pathways responsible for the pro-proliferative and anti-apoptotic phenotype of PASMCs. Moreover, advances in gene-transfer technologies made the development of 'gene therapy' the modification of choice for therapeutic proposes. First designed to restore a genetic disorder or a mutation by transferring a normal copy of the gene, it soon became apparent that the range of targeted diseases could be extended to those showing aberrant gene expression. Therefore, in the last decade, several researches explored gene therapy for PAH.

Major recent advances

Compared with other thoracic malignancies, such as lung cancer, asthma, emphysema and cystic fibrosis, PAH appears to be late in gene therapy applications. In fact, the first clinical trial implicating gene transfer in PAH only began in 2010 [4]. Autologous endothelial progenitor cells (EPCs) programmed to overexpress the endothelial NO synthase (eNOS) were administered to patients with iPAH and

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PAH associated with systemic sclerosis. Preliminary data showed modest but significant improvements in 6-min walk test and mean pulmonary arterial pressure. Given the fact that EPC therapy only (nonassociated with gene transfer) in two small-randomized trials in humans showed improvement in 6-min walk test and PVR [5], it is difficult to evaluate the real benefit of this additional gene therapy.

Antiproliferative candidates for gene therapy in PAH

Gene therapy targeting endothelial dysfunction is currently being studied, but targeting the pro-proliferative phenotype of PASMCs is still to be achieved. Several experiments restoring mRNA levels of genes implicated in the aberrant activation of proliferative pathways in PAH have been performed in the last 5 years in animal models of PAH, and results are really promising.

■ *BMPRII* gene therapy

Heterozygous mutations in *BMPRII*, a member of the TGF family of receptors, have been identified in many cases of familial and sporadic PAH [6]. In the case where *BMPRII* is not mutated, its downregulation is often observed in the PAH patients. Adenoviral delivery of vector containing *BMPRII* gene in pulmonary vascular endothelium of chronic hypoxia-induced PAH (CH-PAH) rats reduced the pulmonary hypertensive responses [7]; whereas *BMPRII* intratracheal nebulization of adenoviral gene therapy in the monocrotaline rat model of PAH did not improve pulmonary hypertension despite a good distribution of the gene in the arteriolar network [8]. The heterogeneity of these results suggests that *BMPRII* may not be the best candidate for gene therapy. Nevertheless, even if *BMPRII* gene therapy could not be universally applied for PAH patients, it may be beneficial in some cases, or could be effectively coupled with other genes therapies.

■ *Kv* gene therapy

Contractility and proliferation of PASMCs is controlled by cytosolic Ca^{2+} levels, which are largely determined by membrane potential (Em). In fact, Em is depolarized in human and experimental PAH cells due to, at least in part, the decreased expression and function of voltage-gated K^+ channels (Kv1.5 and Kv2.1). This 'K^+-channelopathy' leads to PASMCs depolarization and Ca^{2+} overload, thus promoting vasoconstriction and PASMCs proliferation. Therefore, targeting and improving expression and function of these channels is thought to be promising. Restoration of Kv channel expression in PAH by aerosol gene therapy using an adenovirus expressing Kv2.1 might actually be beneficial [9]. Furthermore, Kv1.5 expression in established CH-PAH in rats reduces PVR and restores RVH [10], and *KCNA5* gene transfer in human PASMCs increases K^+ currents and enhances apoptosis [11], demonstrating that Kv1.5 may serve as an important strategy for preventing the progression of PAH.

■ *Survivin* gene therapy

Pulmonary arterial remodeling in PAH might be explained by a pro-proliferative and antiapoptotic phenotype of the PASMCs. Survivin, a member of the inhibitor of apoptosis protein family, was first discovered in cancer. It is normally undetectable in healthy adult differentiated tissues, but it is expressed in remodeled PAs from patients and rats with PAH, highlighting once again this proliferative phenotype. Adenovirus-mediated survivin overexpression induces PAH in rats, underlying an implication of this oncoprotein in PAH, whereas inhalation of a *survivin*-dominant negative adenovirus reverses established monocrotaline-induced PAH (MCT-PAH) [12] by avoiding PASMC proliferation and, thus, the subsequent PAH.

■ *VIP* gene therapy

Among its actions, vasoactive intestinal peptide inhibits proliferation of vascular smooth muscle cells. PASMCs treated with adenovirus expressing the *VIP* gene are less proliferative [13]. It could be interesting to see *VIP*-based gene therapy trials on animal models.

■ *CGRP* gene therapy

Calcitonin gene-related peptide has also been described to have an antiproliferative effect. Intratracheal injection of adenovirus carrying the pre-pro*CGRP* gene into the lung of hypoxia-induced PAH mice attenuates the increase in PVR, RVH, remodeling and pulmonary pressure [14].

■ Growth factor gene therapy

VEGF-A overexpression in MCT rats using cell-based gene transfer prevents PAH [15]. In the same way, *HGF* gene transfection in MCT rats prevents media wall thickening [16]. These findings imply that VEFA-A and HGF have protective effects against remodeling and could be good candidates for gene therapy.

Limitations of gene therapy

Gene therapies are hopeful treatments that might in the future help PAH patients. The aim of PAH treatment is to reduce pulmonary hypertension without affecting systemic circulation. Gene delivery into the lung via aerosol is one of these strategies. This could avoid many problems associated with systemic delivery by intravenous injection, such as immediate nuclease degradation and difficulty penetrating the endothelial barrier. Successful gene delivery via inhalation strongly

depends on the development of advanced gene vectors. They must be able to protect the plasmid or sequence, provide a specific targeting site and effectively release these plasmids for the desired pharmacological effect. In the past two decades, numerous preclinical and clinical trials have been performed for thoracic malignancies, and lessons must be learned from these experiments.

First trials with adenovirus and adeno-associated viral vectors appeared to be ineffective and associated with detrimental immunologic responses and toxicity [17]. Recent work show some ingenious constructions, such as antibiotic coupling adenovirus or 'helper-dependent adenovirus' in which the proinflammatory virus sequences are missing. This permits a decreased inflammation associated with gene therapy. Lentiviruses are more efficient than other viral vectors but they need to be frequently readministered and lead to the same inflammatory problems.

Nonviral gene transfers are generally less efficient. In fact, plasmids as well as vectors used, require improvements. Some nucleotide sequences such as CpG motifs have been demonstrated to enhance proinflammatory responses [18] and have to be avoided in plasmid construct. Promoters, which regulate, in part, duration and tissue-specific localization of gene expression, have to be carefully chosen, particularly in the case of a desired restricted area of gene delivery (e.g., the vascular bed).

With the development of nanotechnologies, the emergence of new kinds of construction can be hoped for. Some nonviral vectors, for example liposomal ones, seem to cause less immunologic lung responses [19], which could worsen PAH. This is also true with nanomicelles. Formulations such as polyethylenimine (25 kDa, Sigma), cationic lipid 67 (GL67A, Genzyme corporation) or DNA nanoparticles (Copernicus), were shown to increase efficiency and duration of transgene expression.

Physical methods have also opened a new window in lung gene transfer improvement. Magnetic particles linked to DNA enhance a DNA response to magnetic fields (magnetofaction) and increase transfection rates *in vitro*. Unfortunately, application of this method *in vivo* is unsuccessful. Ultrasound (sonoporation) and electroporation have been shown to improve gene transfer in various tissues, but are not applicable *in vivo* because they are associated with lung damage such as hemorrhage.

Future perspective

The increased knowledge and understanding of stem cells has provided the scientific community hope to find cell therapies for PAH. Some are already used for genetic pathologies, mainly embryonic stem cells. The concept of using the patients' own stem cells is strongly emerging, avoiding ethical and immune issues. EPCs appear to be promising candidates, as described previously. Mesenchymal stem cells are also very promising [20]. The understanding of smooth muscle cell differentiation mechanisms are increasing and could be very useful for all vascular diseases, including atherosclerosis, heart failure and PAH.

The principle of coupling gene and cell therapies is also coming to the fore: isolate stem cells from a patient, make them express a gene of interest and then re-inject them into the patient. Coupling gene and cell therapy is already in trials using eNOS-transformed EPCs to treat PAH [4], and it could be speculated that targeting genes involved in proliferation and antiapoptotic mechanisms would be even more efficient. Furthermore, using mesenchymal stem cell-coupled gene therapy could be very promising considering their multipotency and their ability to accumulate at the site of tissue/organ damage and inflammation *in vivo* [21].

Targeting genes involved in the pro-proliferative and antiapoptotic phenotype by RNAi or siRNA could be one other possibility. As RNAi is increasly studied and better understood its use as a therapeutic is slowly emerging. Aerosol coupling siRNA and transfecting agent would theoretically be able to silence the pathologically overexpressed gene(s) observed in PAH PASMCs. This is undoubtedly a promising concept to work on, even though technical issues remain to be resolved. siRNA lifespan, application modes and frequencies have to be characterized. The specificity, structural role and off-target effects also remain to be determined. Regarding the emerging concept of microRNA, which have a natural RNAi action on genes and whose aberrant expression is implicated in human diseases and in PAH, we can also speculate that progress will continue in the next years. Administered mimics or antagomir in order to restore aberrant microRNA expressions could be another therapeutic intervention, but one that also requires improvements in transfectant agents.

Conclusion

Treating PAH by gene therapy is very promising and targets and methods are being extensively studied. Treatments based on inhaled siRNA targeting different genes once a week or durable gene/cell therapies restoring one or few gene expression are achievable. Some technical issues remain to be solved and time is needed to realize the trials. Nevertheless, these therapeutics are associated with high technical costs. In this way, it will be difficult for these new treatments to be competitive against cheaper agents, such as dehydroepiandrosterone, dichloroacetic acid and trimetazidine [22]. These agents, already in trials, need much less expensive manufacturing costs for the relative same efficiency.

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