SHORT COMMUNICATION

Combined gene therapy: A new perspective in cancer therapeutics?

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Gene therapy is today a promising approach to treat many incurable diseases. However the success of such an approach has taken a long time to appear. The first clinical assay using gene therapy was launched in 1989, whereas the first success of gene therapy occurred in 2000 for children with X-linked severe immunodeficiency (1). Based on the principle of using genes as medication, gene therapy encountered many difficulties linked to gene transfer efficiency, transgene loss and adverse effects resulting from vector immunogenicity or proto-oncogene activation by integrated vector. Intensive research work was performed to solve these problems, and today several clinical assays of gene therapy have been successful for various pathologies, including rare diseases, neurodegenerative diseases and cancer (2). Cancer is the most frequent pathology targeted by gene therapy, as it represents 65% of the world clinical trials (http://abedia.com/wiley/indications.php). However there are only few drugs in the market which shows that therapeutic benefits need further improvement. Presently, the most promising therapy for cancer is immunotherapy. Several approaches exist, among which oncolytic viral therapy and genetically modified killer cells approach provide encouraging results in clinical trials (3, 4). The last generation oncolytic viruses have been engineered by insertion of genes coding for proteins able to stimulate the immune system, showing that in all approaches, combination with gene therapy brings a therapeutic benefit to treatments. In addition to immunotherapy, three other strategies of gene therapy have been developed to treat cancer, based on anti-angiogenesis, tumor suppressor and suicide therapies respectively. In spite of all these advances in cancer therapeutics, the remaining question is how to improve treatment efficiency. An important problem with cancer therapy is the development of resistance during the treatment by tumor cells. In such a context the concept of combined gene therapy brings interesting perspectives (5). Combination of several therapeutic molecules allows synergistic effect and/or multiple targeting, which is of great interest to improve the treatment of cancer as well as of other incurable diseases. In this purpose IRES-based vectors constitute powerful tools to co-express several genes of interest (5). Internal Ribosome Entry Sites (IRESs) are RNA elements which are naturally present in a few mRNAs. The classical mechanism of translation initiation requires ribosome recruitment at the mRNA 5'end followed by scanning and translation, whereas IRESs allow translation to start by direct ribosome recruitment at internal initiation codons. This feature has been used in biotechnology to design expression cassettes where several genes separated by IRESs are expressed from a single transcription unit. Co-expression from the same mRNA prevents competition between promoters and unexpected extinguishment of one of the transgenes, which is often observed when transgenes are expressed under the control of distinct promoters. Furthermore, IRESs are often activated by stress conditions including hypoxia, which is an advantage to obtain efficient protein synthesis in hypoxic conditions occurring in various pathologies (6–8). Thus the IRES-based vector-, also called multicistronic vector strategy, is particularly well suited for gene therapy of ischemic diseases as well as of cancer where cells are submitted to hypoxia. As regards side effects, combined gene therapy is advantageous: IRES-based vectors express lower doses of therapeutic molecules with superior therapeutic effects due to synergy, compared to vectors expressing single molecules (9). This increases safety and decreases the side effects. It was demonstrated in an approach of therapeutic angiogenesis of lower limb ischemia in mouse, where a monocistronic vector expressing Cyr61 increased tumor progression, the bicistronic vector co-expressing low doses of FGF2 and Cyr61 had no effect on tumor progression while generating a higher therapeutic benefit (9). A successful application of the multicistronic vector strategy has been recently published in Molecular Therapy by Renaud-Gabardos et al. (10). Here lentivectors have been designed for gene therapy of ischemic heart disease in a murine model of heart failure. This pathology is mainly characterized by coronary artery occlusion resulting from atherosclerosis. This generates several dysfunctions including decline in perfusion, cardiac fibrosis and cardiomyocyte death, which results in impaired contractile function. A previous study has suggested that angiogenic therapy could restore perfusion in myocardial tissue while transfer of genes able to restore cardiomyocyte function, could correct defaults of contractile function. Several clinical trials have been performed using angiogenic genes with a moderate success regarding therapeutic benefits (11). The most encouraging results have been observed in a 2a phase clinical trial (CUPID1) based on the use of the sarcoplasmic reticulum Ca^{2+}-ATPase SERCA2a gene, involved in calcium handling and able to restore contractile function (12). However the 2b phase (CUPID2) was really deceiving as it did not confirm the promising data of CUPID1, sending the researchers back to the bench to design new approaches for heart failure treatment (13, 14). The proposition of Renaud-Gabardos et al. was to combine SERCA2a with a strong angiogenic molecule, fibroblast growth factor 2 (FGF2), and a bioactive peptide, apelin, described for its features of fibrosis and cardiomyocyte apoptosis prevention (10). These authors designed a multicistronic lentivector co-expressing apelin, FGF2 and SERCA2a in the purpose of restoring several parameters of ischemic heart disease simultaneously. In the lentivector, the three genes of interest are separated by two copies of the FGF1 IRES, rather than the classically used Encephalomyocarditis Virus (EMCV) IRES. Indeed the FGF1 IRES is particularly suitable here as it is activated by hypoxia *in vivo* in ischemic heart. The lentivector was directly injected into infarcted myocardium. Results reveal a synergistical effect of FGF2 and apelin to stimulate angiogenesis, while the triplet apelin-FGF2-SERCA2a shows the best therapeutic effect to prevent fibrosis, heart hypertrophy and restore heart function. The authors looked at the impact of the treatment on the regulation of gene expression: they showed that combination of the three therapeutic genes is more efficient than treatment with the single genes, in restoring angiogenic balance as well as regulation of genes involved in heart remodeling and contractile function. Combining several therapeutic genes thus appears as a promising approach to restore the regulation of gene networks. A clinical assay is being elaborated to assess the apelin-FGF2-SERCA2a combined gene therapy on patients. The concept of combined gene therapy is applicable to many diseases, and in particular to cancer which is a very complex pathology. The first attempt of combined gene therapy of cancer was immunotherapy based on interleukin 12 (IL-12) and showed effective eradication of murine tumors using fibroblasts transduced by an IRES-based retroviral vector co-expressing the two IL-12 subunits with the neomycin resistance gene (15). Phase I clinical assay was launched in 1995 and provided encouraging results on patients with melanoma or head and neck cancer, without significant side effects (16). However the strong anti-tumoral effect observed in animals was not reproduced in cancer patients, due to an adaptative response downregulating IL-12 activity, as high doses of the cytokine expressed are toxic (17). IL-12 was also shown to cooperate with CD80 co-stimulation molecule in induction of effective antitumor immunity (18). In addition, association of molecules of co-stimulation has been assessed (19). These combination therapies remain clinically interesting as they can lower the threshold for IL-12 efficacy (20). Presently, IL-12 gene therapy is combined

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with suicide gene therapy using oncolytic adenovirus (21). Other interleukins are also assessed for cancer vaccine strategy, such as IL-18 combined to HSV-TK gene in colorectal cancer (22). In addition to immunotherapy, attempts have been performed to combine anti-angiogenic factors in the purpose of reducing the development of resistance to a single anti-angiogenic factor. These approaches were moderately successful. The study of Renaud-Gabardos et al. provides a new perspective with regards to cancer gene therapy, for example by combining a gene of immune stimulation with an anti-angiogenic factor and a tumor suppressor. Such a perspective is illustrated by a very recent study combining DESI2 and endostatin to induce apoptosis and it inhibit angiogenesis, showing improved antitumor efficacy in mouse colon and lung cancer models (23). Targeting several parameters of this pathology in which many genes are deregulated appears, nowadays, as a promising way in the field of cancer therapeutics.

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