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REVIEW ARTICLE

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Gene therapy and spinal disorders

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Introduction

For many years, treatment of chronic lower back pain, intervertebral disc degeneration, spinal fusion, and spinal cord injury have been problematic. The advances in molecular biology have now made it possible to address these problems at a molecular level. By gene therapy, a defective gene is replaced with a normal or therapeutic gene. To be successful, the exact sequence and function of the specific gene must be understood, a vehicle for safe and efficient delivery of the gene into the cells must be located, and the expression of the gene should be well controlled. Until now, difficulties with efficient gene transfer and appropriate gene expression have still been an impediment. Besides, ethical problems of a carcinogenic and eugenic nature have arisen [15], but with gene transfer as drug delivery system, there is a great range of applications to acquired diseases. Research is also being conducted into various aspects of spinal disorders.

Vehicles for the gene delivery

The important step is to deliver the target gene into the patient's cells in a way that is safe, efficient, and specific. The vehicles that encapsulate therapeutic genes for delivery are called vectors (Fig. 1). Many vectors currently in use are modified or attenuated viruses. These modified viruses cannot replicate but can efficiently deliver genetic materials to the cells. Genes can be delivered in two ways: ex vivo or in vivo. With the former, the gene is transferred to a cell culture, which is later returned to the host, while with the latter, the gene is delivered directly into the host. In spinal research the viral vectors most commonly used are retroviruses, adeno-associated virus, and adenovirus. Other viral vectors being developed include herpes simplex, lentivirus, and papillomavirus.

Retroviruses

These were the first viral vectors used in human clinical trials [18]. The major advantage of retroviral vectors is their high efficiency of gene transfer into replicating cells. This precision and stability of gene transfer is not seen with other viruses. The disadvantages include the inability to infect non-dividing cells [19], and the inability to synthetically produce retroviral vectors, which must be produced by cultured cells.

Adeno-associated viruses

Like retroviruses, these viruses integrate their genetic material into the DNA of the cells they infect. They are non-pathogenic, with high titre, site-selective integration, stable expression and they are able to infect non-dividing cells. Their small size is a disadvantage because it limits their ability to carry extraneous DNA. Further disadvantages are caused by difficulties in production.

Adenovirus

This virus infects a wide spectrum of dividing and non-dividing cells without the mutagenic risks inherent with the insertion of DNA directly into the host chromosome. It can carry large segments of DNA, very high titre, and is suitable for infecting tissues in situ. The major disadvantages are the inclusion of many adenovirus genes in current vectors, which may stimulate immunity or have

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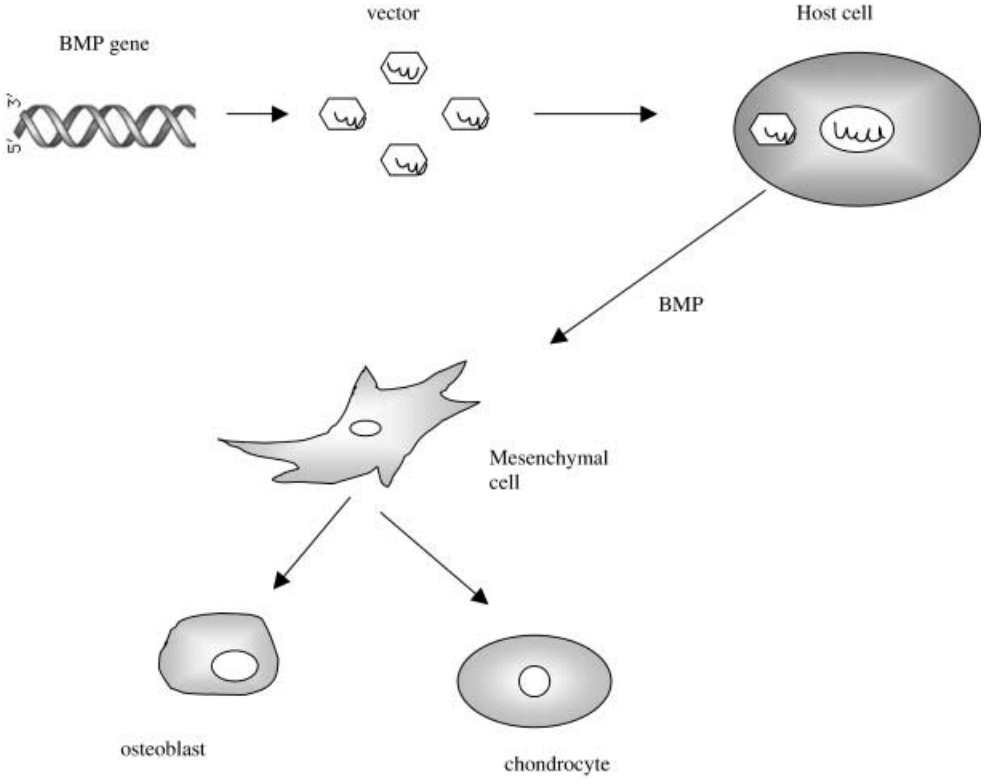

Fig. 1 Diagram showing how the genes are transducted to the host cell and stimulate the mesenchymal stem cells differentiation by expressing specific proteins

other adverse effects, and the potential instability of gene expression because the vector does not integrate into the chromosomal DNA.

Spinal applications

Spinal fusion

Spinal fusion is a common surgical procedure. With various instruments and grafts the fusion rate is still around 80%–85% [25, 34]. Besides, the morbidity associated with harvesting autogenous iliac bone grafts may approach 30% [8, 27, 32, 33]. To improve the results, focus has been centred on improvement of healing and reduction of donor site morbidity. Osteogenic proteins such as bone morphogenetic protein (BMP) have been promising and may be an attractive alternative to autogenous bone grafting. Various BMPs have been used to promote spinal fusion in dogs, rabbits, mice, and non-human primates [3, 9, 12, 24]. Because of the short biological half-lives, they are often used in combination with a biodegradable scaffold that acts as a slow release device. Many scaffolds or carriers have been tried, including poly-lactic acid, demineralized bone matrix, hydroxyapatite, and hydroxyapatite tricalcium phosphate [17, 23, 26, 35]. However, all these exhibit problems of biodegradability, inflammatory reactions, immunological rejection, disease transmission, and an inability to provide sustained levels of growth factors over time. With the techniques of gene therapy as a delivery system, a sustained high concentration of growth factors could however be achieved locally. Furthermore, this endogenously synthesized protein, unlike the exogenous recombinant protein, may have a greater biological responsiveness.

In experimental spinal fusion, the hBMP-2 gene carried by an adenoviral vector is commonly used, since hBMP-2 has been synthesized commercially by DNA recombination technology [30]. In a rabbit model, Riew et al. [22] found that mesenchymal stem cells transduced with BMP-2 gene could produce the BMP-2 protein in vitro, and result in transformation into an osteoprogenitor line capable of producing bone in vivo. This was actually an ex vivo study, as the transduction of the mesenchymal stem cells was done in culture. Alden et al. [1], in a more recent in vivo study, injected adenovirus carried BMP-2 gene percutaneously and paraspinally at the lumbosacral junction, resulting in endochondral bone formation.

Furthermore, in a rat model, a novel LMP-1 (LIM mineralization protein-1) cDNA also showed effective results on posterior lateral spine fusion when transfected into bone marrow cells and secondly reimplanted at the fusion site [4].

Although gene therapy has shown promising results in small animals, problems still exist regarding the proper dosage of osteogenic protein in primates to achieve spinal fusion. Boden et al. [2] showed that, in comparison with the rabbit, an eightfold increase in the bovine-derived osteoinductive growth factor concentration was needed to induce adequate bone formation in the rhesus monkey spine. Too much bone formation or bone formed outside the intended region could have deleterious effects. Miyamoto et al. [20] reported ossification of the

ligament flavum and secondary spinal cord compression in mice, while implanting BMP in the lumbar extradural space. Furthermore, the bone-inductive properties of BMPs vary in different species and it is unclear which BMPs will be the proper ones in primates. Detailed studies should also be carried out on how gene therapy is controlled, i.e., the proper transduction rate, the required duration of expression, and the minimal immune response against vectors.

Intervertebral disc degeneration

Intervertebral disc degeneration (IVD) and the related lower back pain or radicular pain may still be major social and economic issues even in the next century. So far, treatments are seldom based on the pathogenesis. Few approaches are available for the prevention, and the cause of degeneration is still not completely elucidated. Evidence shows that loss of proteoglycan in the nucleus pulposus may directly affect the biomechanical function of the intervertebral disc, which may alter the loading upon the facet joints and other structures, causing degenerative changes [5]. Thus, factors that influence the proteoglycan metabolism may be of great interest. Thompson et al. [28] demonstrated that the addition of recombinant human transforming growth factor (TGF)-beta1 to canine disc tissue in culture, stimulated in vitro proteoglycan synthesis, suggesting that this growth factor might be useful in the treatment of disc degeneration. With the development of gene therapy as a superior drug delivery system, several investigations have been made on treating the underlying pathogenesis of disc degeneration. Wehling et al. [31] have successfully transferred interleukin-1 receptor antagonist complementary DNA, which has the potential to attenuate disc degeneration, into the chondrocytic end plate cells. Nishida et al. [21] have, by adenovirus vector, transducted a marker gene *lacZ* into the nucleus pulposus cells both in vitro and in vivo. Candidate genes for gene therapy of the IVD degeneration, include tumour necrosis factor α antagonists, and inhibitors of matrix metalloproteinases.

Other disorders

Muscular disorders such as Duchenne muscular dystrophy, which results in scoliosis, are now being studied for gene therapy. The gene *dystrophin* has been identified and cloned [10, 11]. Animal study has shown that transgenic mice that carry a gene which corrects mutation in the dystrophin gene, show considerable correction of the muscular abnormality [7].

Spinal cord injury is a serious complication after spinal trauma. Neuron regeneration is the key to treatment. Various nerve growth factors are crucial in development and maintenance of the central nervous system [16]. Axonal regrowth, which spontaneously occurs within days after trauma, must be supported by trophic factors on the one hand, and by blocking of the glial scar formation on the other. Gene therapy can be used to deliver neurotrophic factors to promote axonal regeneration. Neurotrophic factors have been transducted into fibroblasts and Schwann cells, and have induced robust neuritic growth in which differential growth of sensory, motor, and noradrenergic neurites were also observed [29]. Besides NGF, other factors like brain derived neurotrophic factor (BDNF) [14], platelet-derived growth factor (PDGF) [13] have also been used in transduction for gene therapy.

Osteogenesis imperfecta, the inherited disease with abnormal collagen production, has a high incidence of scoliosis. Because the specific genetic defect is precisely known, and useful murine models for the disease are available [6], gene therapy may offer a better choice of treatment.

In general, the spinal disorders that we face now will still be prevalent in the next century. With the rapid understanding of the molecular basis of these disorders, gene therapy is certain to play a major role in overcoming such problems in the future.

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