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REVIEW ARTICLE

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Gene therapies for joint diseases

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Introduction

New treatments have been proposed for such joint diseases as rheumatoid arthritis (RA), osteoarthritis (OA), and cartilage injuries. Among these, gene therapy has attracted the attention of researchers as a useful drug delivery system which provides long-term local expression of therapeutically effective substances. The first clinical trial was conducted in 1996 by Evans et al.[1] using an ex vivo method. They delivered the interleukin-1 receptor antagonist (IL-1Ra) gene into synovial cells in vitro using a retrovirus vector, and then transferred the cells into the joints of RA patients. The aim of gene therapy at that time was to deliver genes which encode therapeutically effective proteins into the cells within the joints, and to make continuous expressions of these proteins. Since then, several new vectors as well as new strategies for gene therapy have developed. Nowadays, gene therapy is designed not only to introduce anti-inflammatory substances into the joints, but also to modify the phenotype and to regulate cell death in order to obtain therapeutic efficacy. During the past few years, the number of research papers reporting a gene delivery technique has increased sharply. This article reviews the progress of gene therapy in treatments for RA and OA.

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Gene delivery systems

The development of optimal delivery systems is the most important theme in gene therapy, because the simple exposure of target cells to naked DNA cannot achieve a sufficiently high gene delivery rate. The major techniques at present utilize diethylaminoethyl (DEAE)-dextran,[2] calcium phosphate precipitation,[3] electroporation,[4] virus vectors,[5] and cationic liposomes.[6] These techniques, except the ones using virus vectors and cationic liposomes, could damage target cells and are strongly toxic, and therefore they are not appropriate for gene therapy. For this reason, most studies utilize either virus vectors or cationic liposomes. The technique using a virus as the vector for transferring genes utilizes the infectivity of the virus to host cells, and has achieved a good delivery rate. The majority of gene therapy trials to date have used this technique, and there are two delivery system. One is the ex vivo method, in which target cells obtained from the body receive a gene transfer in vitro, and are then returned to the body. The other is the in vivo method, in which gene transfer is done inside the body.

Retrovirus vectors

Research into this technique is the most advanced in the field, and has been used in a clinical trial with RA joints in humans.[1] Theoretically, the decrease in genes due to cell division is small because the gene is transferred into a chromosome of the target cell. The virus itself has no cytotoxicity, but an insertion mutation due to the random insertion of the gene into the host chromosome has been suspected. This technique also has some other disadvantages. For example, the delivery rate is relatively low, and an in vivo method into the joints is not applicable.[7,8] The protein expression period, in comparison to vectors which do not have the gene inserted into a chromosome, was not as long as was theoretically expected. In addition, when virus vectors are used, the target cells must be dividing cells. Therefore, this technique is regarded as being ineffective except in the ex vivo method where the gene is transferred into cultured

cells, and the cells are selected with antibiotics or by other means and then returned to the body. This ex vivo method requires considerable time, is very expensive, and needs specialized equipment. Therefore, the chance of this becoming a common therapy for such joint diseases as RA and OA seems to be slim, although it could be useful to supplement cells in traumatic cartilage injuries.

Some recent studies have suggested the future applicability of retrovirus vectors. In studies on direct gene transfer (the in vivo method), using a high titer retroviral vector ($\geq 10^8$ CFU), to arthritis models of rabbits[9] and rats,[10,11] the gene was transferred to synovial tissue when the knee joints had active synovitis. In addition, the newly developed lentivirus vector is transferable to nondividing cells, and is expected to have great potential.[12]

Adenovirus vectors

Adenovirus vectors have a high gene delivery efficiency to dividing cells, resting cells, and nondividing cells. In vivo delivery is also possible because high-titer viruses can be prepared. The gene is delivered outside the host chromosome, and therefore the possibility of insertion mutation is low. However, gene numbers decrease with the mitotic growth of the cells. The disadvantages of adenovirus vectors are immunogenicity and local inflammatory reactions. The adenovirus stimulates the intracellular signaling pathway when it binds to synoviocytes by using integrin on the cellular membrane, and this induces expressions of cyclooxygenase-2 (COX-2) and matrix metalloproteinase.[13] On the other hand, many recent gene therapy studies for arthritis have used an in vivo method with an adenovirus vector. This shows that the simple procedures of an in vivo method are highly desirable. These studies examined a wide range of genes, e.g., genes of anti-inflammatory cytokines,[14–16] a gene which can change intracellular signaling,[17] and a gene which induces apoptosis,[18] and inflammatory reactions due to the adenovirus were rarely reported. This is probably because the side effects of the adenovirus itself are minor when it is used in order to modify immune reactions in joints with synovitis. At present, the cytotoxicity and immune reactions due to the adenovirus should be carefully considered when an adenovirus vector is applied to such diseases as OA, or to cartilage injuries where synovitis is not the major symptom.

Herpes simplex virus (HSV) vectors

The herpes simplex virus (HSV) vector is a large DNA virus with high infectivity which is latent in nerve cells. Because of its large genome size and strong cytotoxicity, its development as a vector was not pursued. However, Oligino et al.[19] recently delivered an IL-1Ra gene and a tumor necrosis factor (TNF)-α soluble receptor gene to a rabbit arthritis model using a second generation, low-toxicity HSV vector and the in vivo method, and succeeded in suppressing arthritis with the IL-1Ra.

Adeno-associated virus (AAV) vectors

The adeno-associated virus (AAV) vector is derived from a nonpathological virus, and has attracted attention as a new vector in gene therapy. The AAV vector is not cytotoxic, is very safe, can deliver a gene even to nondividing cells, can be inserted specifically into the 19th chromosome, provides a long and stable expression of the gene, and can be prepared in a high titer which is applicable with the in vivo method. Its main disadvantage is the complicated preparation procedure, and this delayed its clinical application. The authors previously demonstrated in vitro gene delivery to chondrocytes which are surrounded with extracellular matrix and where delivery is impossible with the other virus vectors.[20] Goater et al.[21] administered marker genes to arthritic knees of mice which were overexpressing TNF-α by using the AAV vector with the in vivo method, and found that the gene was delivered to synovial cells, chondrocytes, and meniscus cells, but not to healthy knees. In addition, they confirmed that in vitro pretreatment of cells with ultraviolet radiation, γ-irradiation, or TNF-α increased the delivery rate of AAV vectors. Pan et al.[22] reported that the gene was delivered to the synovial tissues of rats using the AAV vector when the tissues were inflammed, and the expression of the gene lasted 30 days. AAV has been proven to be an excellent vector, and is expected to be used in a wider range of clinical treatments.

Nonviral vectors

Direct administration of plasmid DNA to a target organ is a simple, safe, and economical method. Song et al.[23] administered the plasmid DNA encoding transforming growth factor (TGF)-β1 directly and intramuscularly to rats with streptococcal cell-wall-induced arthritis, and reported remarkable suppressive effects on the chronic disease, i.e., suppression of inflammation at the peak of the acute phase, and suppression of cartilage damage, bone damage, and pannus formation in the chronic phase. However, in general, naked DNA alone has rather a low delivery rate, and should be accompanied by an appropriate vector. Fellowes et al.[24] administered IL-10 plasmid with cationic liposomes to the abdominal cavity of a mouse with collagen-induced arthritis, and observed amelioration of the disease. In RA, macrophageo take an important role, and they phagocyte liposomes.[25] Tomita et al.[26] developed a hemagglutinating virus of Japan (HVJ, Sendai virus)–liposome suspension in order to improve the delivery rate without increasing the cytotoxicity, and succeeded in delivering genes by the in vivo method to synovial and cartilage tissues. The authors expect the future development of two cationic polymers, i.e., degraded polyamidoamine (PAMAM) dendrimer and linear polyethyleneimine (PEI), which have relatively high delivery rates to various cells. In our experiments, the gene was delivered to synovial tissue by directly administering a complex of Epstein–Barr virus (EBV) episomal vector and PEI to the joints (Kubo et al., unpublished data). The structure of the EBV episomal vector is derived from the latent infection system of the EBV, and a higher delivery rate was expected.

Joint diseases are not lethal, and therefore absolute safety and high therapeutic efficacy are required in gene therapy in this field. At present, nonviral vectors have a lower delivery rate than virus vectors, but they do not have any side effects. If their delivery rates can be improved, nonviral vectors will become a major delivery method.

Strategies for gene therapy for RA

Gene therapy for RA has progressed remarkably with increased knowledge of RA pathology. There are two main types of strategy in gene therapy for RA. One is the delivery of secreted protein genes, e.g., genes of cytokines and their receptors which suppress inflammation and modify immune reactions, and which have been well studied. The other is the delivery of genes which stimulate the signaling pathways in synovial cells, suppress cell division, or induce apoptosis.

Cytokines, cytokine receptors, or cytokine antagonists

One of the purposes of gene therapy for joint diseases is the continuous expression of therapeutically efficacious proteins by delivering their genes into the joints. None of the conventional drug delivery systems meets both the desired local concentration and an effective expression time of the drugs. For example, no therapeutic effects were obtained when IL-1Ra, an antagonist of IL-1 which has an important role in the development of RA pathology, was administered intravenously or subcutaneously to a rabbit RA model.[27] Direct administration to the joint with experimental arthritis requires quite a large volume of IL-1Ra protein in order to be efficacious.[28] In gene delivery, on the other hand, each cell becomes the production base of the drug, and an efficacious concentration can be maintained in the target area for a long time with minimum systemic side effects. In addition, the proteins generated by the cells after gene delivery may be modified posttranslationally, and could have a greater biological activity than recombinant proteins. Most of the genes which are reported to be effective for RA are those of anti-inflammatory cytokines, or of receptors of inflammatory cytokines, or of their antagonists.

IL-1Ra

IL-1Ra has been regarded as a target gene in gene therapy, and has been studied most intensively.[29–31] IL-1Ra has also been used in a clinical trial.[1] Its anti-inflammatory effect on synovitis was not as strong as expected,[32] but it is known to have strong cartilage protection.[33] These findings support recent reports that TNF-α has an important role in synovitis, while IL-1 has an important role in cartilage degeneration.[34]

Anti-TNF-α

In clinical studies on human RA, the efficacy of soluble receptors for TNF (sTNFR)[35] and antibodies to TNF[36] were confirmed, and this led the studies on gene therapy targeted on TNF-related proteins.[37–39] However, most of these studies used a mouse model with severe combined immunodeficiency disorders (SCID) to which RA tissues were grafted, and the direct effects of the gene therapy on the RA were not clear. In the study by Quattrocchi et al.,[40] the gene of the p55TNFR-IgG fusion protein was systemically administered to collagen-induced arthritis (CIA) mice and arthritis was suppressed for 10 days, but severe arthritis occurred again after that time.

IL-10

The clinical efficacy of recombinant IL-10 for RA has been confirmed in animals,[41,42] and has also been examined in a clinical trial.[43] IL-10 suppresses T helper-1 (Th1) but stimulates Th2. Overall, IL-10 stimulates an immune reaction, and its clinical application is doubtful. However, the viral homologue of IL-10 (vIL-10) which is derived from EBV does not stimulate an immune reaction, and has been actively investigated.[24,44] Hirsch and co-workers[15,46] reported that systemic, intravenous administration of the vIL-10 gene did not have any clinical efficacy on established RA, but did have efficacy on RA before disease onset. Evans and Robbins and co-workers considered the finding of Whalen et al.[14] that the intraarticular injection of the vIL-10 gene with an adenovirus vector to CIA mice suppressed arthritis not only of the injected joints, but also of other joints. They used an antigen-induced rabbit arthritis model, and reported the possible traffic of delivered cells to the contralateral control knees.[46] Hirsch and co-workers[47] recently conducted a study using CIA mice, and reported that the increased serum vIL-10 protein concentration following intraarticular gene administration is an important factor which induces clinical efficacy in the other joints, and that circulating virus vectors of vIL-10 are also making a contribution.

IL-4

IL-4, as well as IL-10, is a Th2-derived cytokine. Lubberts et al.[16] directly administered the IL-4 gene with an adenovirus vector to arthritic joints of CIA mice, and reported that direct administration did not suppress inflammation, but did suppress bone changes. They also demonstrated that IL-4 gene administration suppressed the production of the other cytokines and metalloproteinases, and that it also protected the cartilage.[48] In vivo gene administration using a high-titer retrovirus vector also showed suppression of joint swelling and bone erosion.[11]

Apoptosis and the signaling pathway

In the development of RA pathology, not only inflammation but also the activation of synovial cells play an important role.[49] Gene therapy to regulate the signaling pathway of cells has been actively studied in order to suppress hyperplasia of synovial cells and their invasion of articular cartilage. Studies on gene therapy using the above-

mentioned cytokine genes are usually started only after the therapeutic efficaciousness of the proteins have been confirmed. However, in the regulation of the signaling pathway, gene delivery itself is, in many cases, the only means to confirm its efficacy. Needless to say, safety must be a great concern in this field, but many original studies have been reported. Takayanagi et al.[17] delivered the *csk* gene in order to suppress the Src family tyrosine kinase, and succeeded in suppressing synovial cell proliferation and IL-6 production, and in a rat adjuvant arthritis model, their gene therapy significantly suppressed bone destruction and inflammation. Taniguchi et al.[50] delivered the $p16^{INK4a}$ senescence gene, an inhibitor of cyclin-dependent kinase (CDK) which regulates the cell cycle, to synovial cells, and succeeded in obtaining therapeutic efficacy in adjuvant arthritis. On the other hand, nuclear factor (NF)-κB, a transcriptional factor, was reported to suppress the expressions of various genes, and to take an important role in the inflammatory reaction, the proliferation of synovia, and the suppression of apoptosis in RA.[51,52] The NF-κB decoy oligonucleotide as well as the cytoplasmic inhibitor of NF-κB, i.e., IκBα, are considered to be the targets in gene therapy for RA.[53,54]

There are also many gene therapy studies which aim at nonoperative synovectomy by inducing apoptosis in inflammatory cells and fibroblasts within synovial tissue. Following the study of Nakajima et al.,[55] which revealed that Fas-mediated apoptosis of synoviocytes has a very important role in the development of RA pathology, the therapeutic efficacy of Fas-ligand gene transfer to RA models was confirmed using an ex vivo method[56] and an in vivo method.[57] In the in vivo method using an adenovirus vector, this therapy had no undesirable effects on chondrocytes.[57] In addition to the progress in studies of the signaling pathway, the range of studies of apoptosis has been widened, and nowadays is not limited to the Fas-ligand. Some examples are the studies on Fas-mediated apoptosis of synoviocytes using a Fas-associated death domain protein (FADD),[18] and the apoptosis of activated T cells using Galectin-1 (GAL-1).[58]

Strategies for gene therapy of OA

Studies on gene therapy for OA have not yet progressed as much as those for RA. There have been only two experimental OA studies: one in which IL-1Ra was administered to a rabbit anterior cruciate ligament (ACL) transection model using the ex vivo method,[59] and one using a rabbit partial menisectomy model.[60] OA usually occurs in a single joint, while RA is a systemic immunological disease. Therefore, local administration is appropriate in gene therapy for OA. However, chondrocytes are covered with a rich extracellular matrix, and this makes gene delivery using an in vivo method almost impossible. In fact, many gene delivery studies for OA have been targeted on synovial cells.[28,32,61–63] This is mainly because (i) the synovia has a large area facing a joint cavity, (ii) it does not have a basal membrane, which would be an obvious barrier, and it can therefore be the base for drug delivery, (iii) its cell density is high, and (iv) gene delivery is relatively easy using either the in vivo or the ex vivo method. In RA, synovia could be an appropriate target because it is a major part of the disease. However, it does not seem reasonable to target synovia when it is the cartilage which needs treatment. If synovial cells could produce therapeutic substances to fill the joint cavity at an effective level, then therapeutic effects on the cartilage could be expected, but at the same time, this might be associated with side effects to other tissues, e.g., bone. In addition, the maintenance of an effective level within the cartilage might be difficult. The ideal regimen is gene delivery to the chondrocytes in an OA lesion. Genes can be delivered to degenerated cartilage tissue by using an adenovirus vector,[64] an AAV vector,[21,21] or HVJ-liposomes.[26] Resurfacing of the articular cartilage is possible by transplanting transduced chondrocytes.[65,66] Gene therapy to cartilage tissue is expected to advance rapidly in the near future.

There are two major treatment strategies for OA. One is to regulate the cartilage metabolism in order to prevent degeneration of the cartilage matrix. The other is to maintain or prevent the decrease in the number of chondrocytes. To regulate the cartilage metabolism in order to achieve therapeutic efficacy, the synthesis of the cartilage matrix should be accelerated, or cartilage degeneration should be suppressed. The synthesis could be accelerated by growth factors, e.g., TGF-β or bone morphologic proteins (BMPs), and cartilage degeneration could be suppressed by growth factors,[67] IL-1Ra,[59,60] or tissue inhibitors of metalloproteinases (TIMPs).

Recent studies have shown that chondrocyte apoptosis could be involved in the progression of OA pathology,[68] that chondrocyte apoptosis would be induced by nitric oxide (NO),[69] and that an inhibitor of NO synthase reduced the progression of OA in a canine ACL transection model.[70] In addition, it has been confirmed that hyaluronan, which has frequently been used in OA treatment, does suppress chondrocyte apoptosis in a rabbit ACL transection model.[71] These could all be target substances in gene therapy for OA.

Regulation of delivered gene expression

If expression of delivered genes can be controlled according to the disease stage, the clinical effects will be improved. Inducible promoters can regulate gene expression according to external stimuli, and promoters such as progesterone antagonists (RU 486)[72] and tetracycline[73] have been actively studied. In ideal conditions, the ligands should not stimulate other genes and should not be toxic. Thymidinekinase (tK) metabolizes ganciclovir (GCV) and produces monophosphorylated products which prohibit DNA polymerase. Goosens et al.[74] delivered the tK gene with an adenovirus vector to a CIA monkey, intravenously injected GCV for the next 14 days, and found an increase in apoptotic cell death in the synovial tissue. The application of a heat-shock

protein promoter has also been examined in order to control the delivered gene by heat stress.[75] The current disadvantages in this approach are the weak expression of the inducible promoter and no tissue specificity.

Future prospects

Gene therapy for joint diseases could be very useful in the future. However, because these diseases are not lethal, gene therapy must be completely safe and efficacious. In order to meet these requirements, it is important to pursue painstaking basic studies, e.g., a better delivery rate by using new-generation vectors, and cell-specific delivery, expression, and regulation. At present, combinations of different genes are being actively studied, because it would be difficult to treat the various pathological aspects of a disease with only one gene.[15,76,77] In the future, gene therapy could be prescribed in conjunction with other medication. Genes would be delivered to target tissues using an in vivo method and a nonviral vector, and the administration of various gene combinations could be selected at each stage of joint diseases.

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