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Current Concepts in Gene Therapy of the Musculoskeletal System

Současné názory na genovou terapii pohybového systému

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ABSTRACT

The purpose of this article is to review the remarkable progress in the field of musculoskeletal system gene therapy. Since the introduction of this concept 15 years much of the preclinical and clinical data have emerged. The original target, rheumatoid arthritis, has been subjected to clinical phase II efficacy protocol, and osteoarthritis gene therapy efficacy is being thoroughly investigated in various animal models. The most promising area of research in this field however, is the tissue repair, because it doesn't require prolonged period of gene expression, local delivery is reasonably simple and it avoids substantial risk associated with systemic delivery, and levels of gene expression don't need to be so finely regulated. Gene transfer is successfully being used to aid the repair and regeneration of bone, cartilage, ligament tendon, meniscus and intervertebral disc. Other potential applications of gene therapy in musculoskeletal system include osteoporosis, aseptic loosening, genetic diseases and tumors. Highly encouraging data gained from these studies have confirmed that gene therapy is a promising therapeutic solution to treat various musculoskeletal system disorders.

Key words: gene therapy, regeneration, repair, musculoskeletal system, rheumatoid arthritis, bone, cartilage.

INTRODUCTION

The original premise of gene therapy concept is that defective gene in a specific disease can be replaced with a healthy one.

The first clinical trial with gene therapy was conducted in the United States in 1990 involving a patient who suffered from rare immune disorder called ADA-SCID, i.e. adenosine deaminase severe combined immunodeficiency. The idea behind this concept was to produce normal immune cells by replacing the defective gene. This approach could be effective in classic Mendelian disorders which result from single gene mutations such as Marfan syndrome, where defective fibrillin gene could be replaced with a healthy one. It should be noted that only few orthopaedic conditions result from single gene mutations, and this approach is not of much use in multifactorial disorders such as osteoarthritis or rheumatoid arthritis.

However, the concept of gene therapy has evolved to another level, especially in the field of musculoskeletal disorders. It is a logical consequence of the advances in orthopaedic application of specific biological factors which accelerate healthy tissue regeneration, which begun with bone morphogenetic protein (BMP) discovery in 1965 (86). However, those proteins have short half-lives and it is difficult to maintain adequate *in situ* concentrations necessary for their proper functioning. Furthermore, many proteins act intracellulary and because cells cannot normally import these proteins, they cannot be used in soluble forms. Gene therapy has become powerful alternative system for delivering therapeutic gene products to specific designated tissues of the musculoskeletal system (5). Moreover this method may provide higher and more constant concentrations of the protein at the therapeutical site. The purpose of this review is to describe general principles of gene therapy strategies, as well to present recent advances in their application to the orthopaedic surgery. Classic Mendelian disorders and cancer gene therapy will not be discussed.

GENERAL PRINCIPLES OF GENE THERAPY

Probably the most important question one should ask when dealing with gene therapy is: what protein(s) to use (Figure 1.)? There have been numerous preclinical and clinical trials conducted in order to determine true efficacy of biological factors on various tissues of the musculoskeletal system. Molecules of interest that are currently being used in orthopaedic surgery are bone morhogenetic proteins (BMPs), insulin-like growth factor-1 (IGF-1), basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF), etc. Various growth factors and their net effect on different musculoskeletal tissues are summarized in Table 1.

After the selection of protein that will be delivered, the next issue to resolve is where to deliver it, i.e. what tissues and what cells will be the targets. The aim of gene therapy is to convert certain population of cells at the appropriate site into local, product secreting cells. Up to date all clinical trials have involved only somatic cells, and not gamete cells (sperm or egg cells), and therefore no genetic change is passed to the patient's progeny. Target tissue and target cell are not necessarily the same, and there are numerous feasible combinations that can be used (40).

Exogenous, therapeutic DNA can not be efficiently taken up and expressed by cells and development of an efficient method for introducing a therapeutic gene into target cells is the key issue for feasible and efficient gene therapy. This is accomplished with the aid of vectors acting as a vehicle. Basically there are two main groups of vectors used in all gene therapy trials: viral and non-viral (23, 32).

The most common and obvious way to insert DNA into host cells is by way of virus and the process is termed *transduction*. Viral vectors seem ideal for the purpose of gene therapy because their natural life cycles involve transfer of their own genes into the infected cell. However, before a viral vector is used in gene therapy, modifications are made to prevent it being pathogenic to the host. Some of the essential viral genes necessary for replication are deleted, so the virus is able for transduction but is unable to autonomously replicate within a host (41). The most important disadvantages of viral vectors are their toxicities, immunogenicity, potential viral recombination, and the risk of inducing mutagenesis through retrovirus integration (50).

Non-viral gene transfer is termed *transfection*, and it can be defined as process by which eukaryotic cells are able to take foreign DNA from the environment (see Table 2.) (32). Non-viral vectors posses several advantages over viral ones, they are less toxic, less immunogenic, and easier to prepare. On the other hand they have one important disadvantage – they are considerably less efficient in terms of the level and duration of transgene expression than viral vectors.

In the next step of gene therapy process one must decide how to deliver the vector to the host tissue, so the genetic material can be inserted into the cells (Figure 2.).

Table 1. The most commonly used growth factors in orthopaedics and their net effect on various tissues.

Growth factor	Bone	Articular structures	Skeletal muscles	Meniscus	Tendon and Ligament
TGF-β		+	+	+	+
BMP-2	+	+			+
BMP-4	+				
BMP-7 (OP-1)	+	+			+
BMP-12					+
IGF-1	+	+	+		+
Bfgf	+	+	+		+
NGF			+		
PDGF	+/-		+		+
EGF	-	+/-			+
Decorin	+				

TGF-β = transforming growth factor-beta, BMP-2 = bone morphogenetic protein-2, BMP-4 = bone morphogenetic protein-4, BMP-7 (OP-1) = bone morphogenetic protein-7 (osteogenic protein-1), BMP-12 = bone morphogenetic protein-12, IGF-1 = insulin-like growth factor-1, bFGF = basic fibroblast growth factor, NGF = nerve growth factor, PDGF = platelet-derived growth factor, EGF = epidermal growth factor

(Modified from *David Hannallah, Brett Peterson, Jay R. Lieberman, Freddie H. Fu, and Johnny Huard: Gene therapy in orthopaedic surgery. J. Bone Jt Surg., 2002; 84-A: 1046––1061.*)

Table 2. Common viral and non-viral vectors used in gene therapy of the musculoskeletal system.

VIRAL VECTORS	NON-VIRAL VECTORS
Adeno-associated virus	Naked DNA
Adenovirus	Gene gun
Lentivirus	Electroporation
Retrovirus	Liposomes
Herpes simplex virus	Synthetics

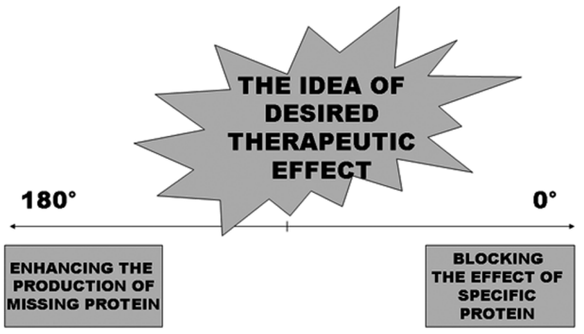


Figure 1. Gene therapy can be used in adverse fashion. Some disseases show lack of specific protein, while other show presence of undesired protein.

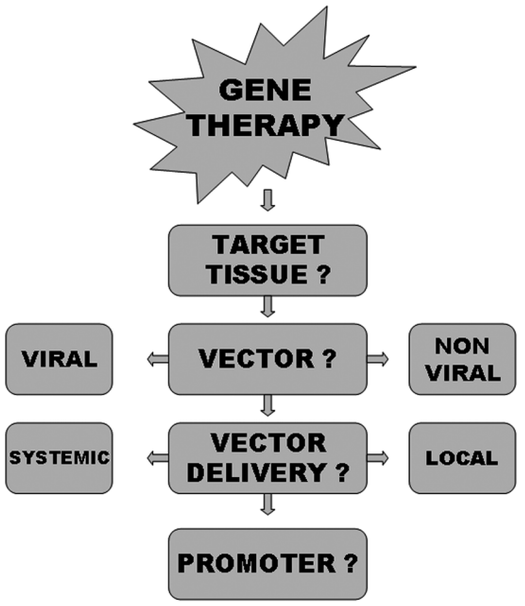


Figure 2. Basic questions that must be addressed when an investigator is designing a gene therapy experiment.

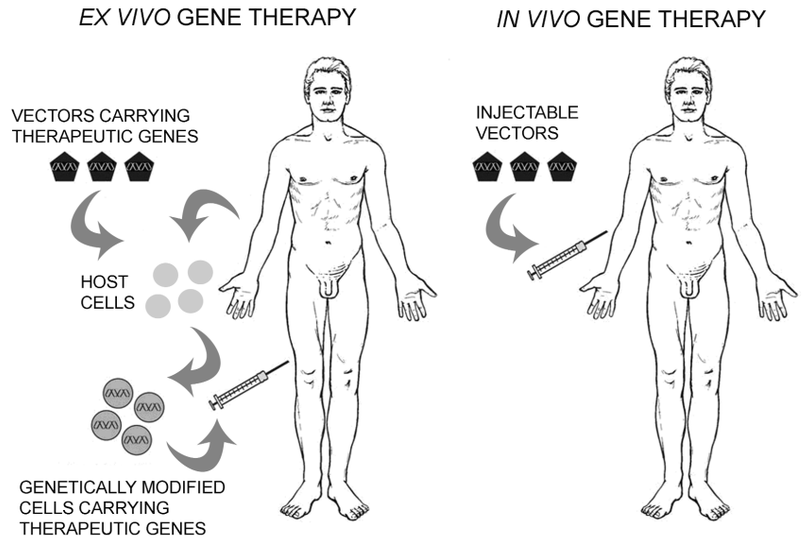


Figure 3. For ex vivo approach target cells are harvested and transduced with vector of interest. Genetically modified cells are then reinserted directly to the desired site. For in vivo approach vector is directly injected to the desired site (e.g. joint or fracture), enabling cells at the site to acquire desired gene and secrete locally the therapeutic product.

This can be accomplished either systemically or locally. Systemically administered vector (e.g. injection directly into the blood stream) may target all cells and are easily administered (16). Local administration of the vector is the most commonly used method in orthopaedic surgery, and the easiest way is to inject it into local tissue. Two main modalities of this approach exist: *in vivo* approach (direct) and *ex vivo* approach (indirect) (Figure 3.). *In vivo* gene therapy has been used to target bone (2), skeletal muscle (52), anterior cruciate ligament (28), spine (17), meniscus (27), tendons and ligaments (13, 35) and joints (47). Ex vivo gene therapy has been used to target articular cartilage (21), spine (22), bone (11).

SPECIFIC ORTHOPAEDIC APPLICATION OF GENE THERAPY

Chronic (nonmendelian) diseases

Rheumatoid arthritis

Rheumatoid arthritis (RA) is inflammatory autoimmune reaction to an unknown antigenic stimulus, against the background of genetic predisposition (6). Although etiology of this debilitating disease remains unknown, the cascade of immunological and inflammatory reactions has been elucidated. There are numerous proinflammatory cytokines such as IL-1, IL-6, IL-8, tumor necrosis factor-á (TNF-á), and granulocyte-macrophage colony-stimulating factor (GM-CSF) that are found to be involved in the mentioned cascade, and these cytokines induce release of metalloproteinases (MMPs) from neutrophils, fibroblasts and chondrocytes. Because traditional treatment options fail to provide long-lasting control of symptoms, recent accumulation of knowledge concerning the pathophysiology of RA have led to the development of novel biological treatments. Most of the research work has been directed at inhibiting the actions of IL-1 and TNF-á. They are interesting because they not only induce leukocyte infiltration and joint inflammation, articular cartilage erosion and matrix breakdown, but also have natural antagonists – interleukin-1 antagonist protein (ILRa1) and tumor necrosis factor receptor fusion protein (TNFR:Fc) (39). These proteins compete with IL-1 and TNF-á for cell surface receptors and reduce their biological activity. Conducted preclinical research focused on appropriate method of gene delivery (local or systemic), vectors and antiarthritic genes (14). Gene delivery of ILRa1, TNFR:Fc, IL-4, IL-10, IL-13 and tissue inhibitors of metalloproteinases (TIMPs) proved to be effective in various animals models (46). All the data collected from preclinical research led to the development of phase I gene therapy protocols for RA, and the first such protocol was conducted in 1996. by Evans et al. (6). They delivered ILRa1 transgene in ex vivo fashion using a retrovirus, to the second through fifth metacarpophalangeal joints of postmenopausal women 1 week before these joints were removed during total joint replacement surgery. Similar trial has been conducted in Germany, and both trials proved that it is possible to transfer genes to human joints and to maintain transgene expression within the joint (8). These findings enabled phase II efficacy protocol to be launched.

Osteoarthritis

Osteoarthritis (OA) is characterized by focal erosions of the articular cartilage, depletion of matrix proteoglycans and abnormal chondrocyte metabolism. Unlike RA, OA is not a systemic disease but pertains to the articular cartilage itself, and is therefore even more suitable for local gene therapy. Changes in cartilage metabolism are primarily attributed to IL-1 and it is a key target in pending research (7). Among many delivery strategies, local gene transfer to synovium is in the most advanced stage of development. Results from preclinical studies

on dog, rabbit and equine osteoarthritic model confirm that delivery of IL-1Ra cDNA were shown to slow cartilage loss (10). Even more, there are convincing evidence that gene therapy combining anabolic growth factors to stimulate matrix synthesis and catabolic blockers to prevent matrix degradation by IL-1, protects and causes partial restoration of cartilage matrix, and suggest a potential benefit of combination gene therapy for cartilage healing (54).

Aseptic loosening

Aseptic loosening of prosthetic implants and the associated bone loss remains a serious orthopaedic problem. The current theory that explains periprosthetic osteolysis is that particles of wear debris (ultrahigh-molecular-weight polyethylene or polymethylmethacrylate) generated from the prosthesis and/or the cement are phagocytosed by macrophages, leading to a localized inflammatory reaction in the surrounding bone. Cytokines released during this process induce osteoclastic destruction of bone. Currently, there is no approved drug therapy to prevent or inhibit periprosthetic osteolysis, but gene therapy similar to that described for RA offers novel possibilities in the treatment of aseptic loosening. Animal model studies showed that direct retroviral delivery of genes for IL-1Ra and IL-10 strongly reduced the inflammatory cellular reaction to particles of ultrahigh-molecular-weight polyethylene or polymethylmethacrylate (53). Similar studies on air pouch murine model showed that gene therapy using a recombinant adeno-associated viral vector that expresses osteoprotegerin (OPG) can inhibit wear debris-induced osteolysis. OPG is a natural decoy protein that inhibits osteoclast activation and bone resorption (44). Other studies showed that genes encoding OPG, IL-10 or TNF-R were able to inhibit osteolysis in response to titanium particles (3).

Osteoporosis

Osteoporosis is typically treated by the long-term repeated administration of antiresorptive agents. IL-1 has been identified as one of the osteoclastic cytokines involved in the development of osteoporosis, and therefore it was logical to assume that blocking the actions of IL-1 may have therapeutic potential. Indeed, a cDNA encoding the human interleukin-1 receptor antagonist (IL-1Ra) was injected intramedullary to ovariectomized mice (surgically induced animal model of osteoporosis) (1). Quantitative histomorphometric analysis showed that IL-1Ra not just arrested bone loss, but in some cases resulted in supranormal bone density. Furthermore, the protective effect of IL-1Ra gene was not restricted to bones receiving intramedullary injection of the vector, but occurred in all bones that were evaluated. Other study showed that gene therapy with human recombinant osteoprotegerin reverses established osteopenia in ovariectomized mice by inhibiting osteoclast formation, function, and survival (24).

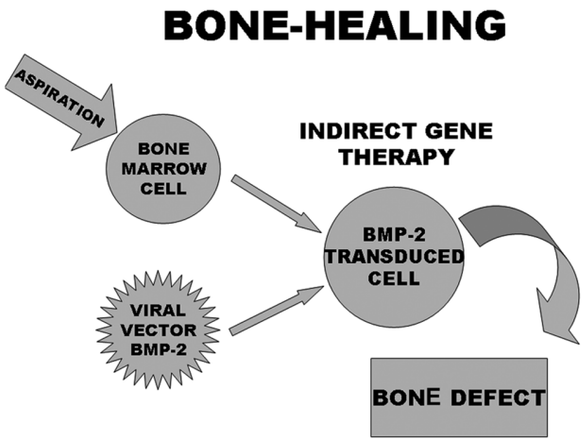


Figure 4. Possible approach in treating bone-defect with bone marrow aspirate transduced with BMP-2 (26).

Tissue repair

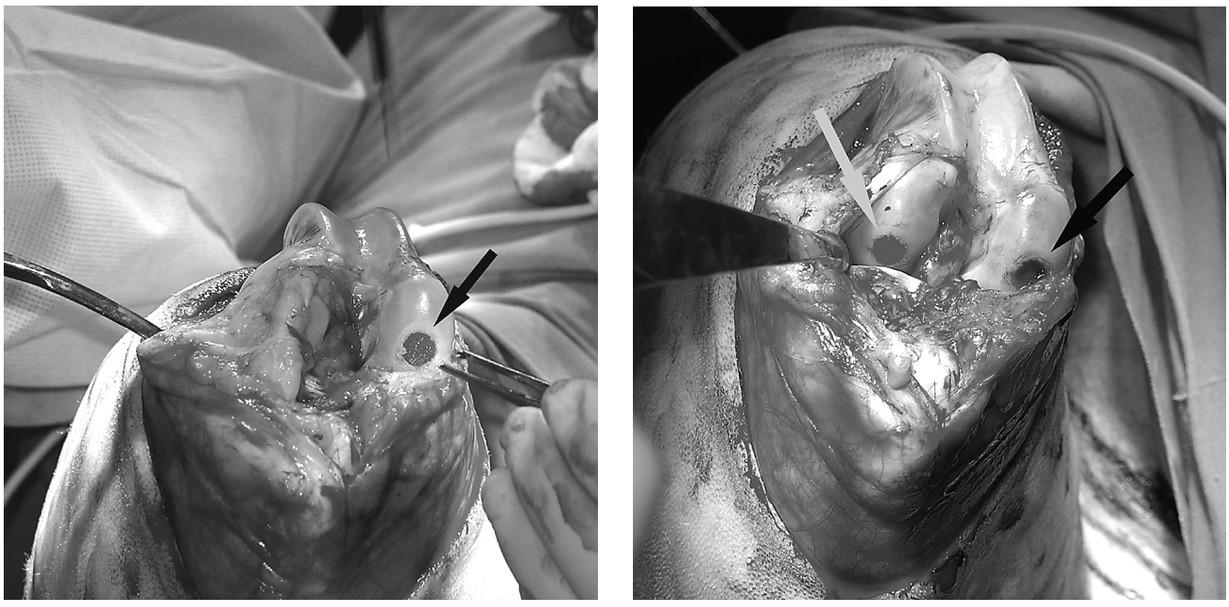
Bone

Bone and liver are the only two organs in the human body with impressive ability to regenerate itself, i.e. to heal spontaneously without any scarring. Nevertheless, there are many cases in the clinical practice where this ability is not sufficient and the process fails. Examples include delayed unions, nonunions and critical-sized defects, as well as need for sufficient amount of bone for spongioplasty in many cases of spine fusions or reconstructive bone surgery. Recent advances in understanding the biology of bone healing have led to identification of many growth factors, such as bone morphogenetic proteins (BMPs), acidic and basic fibroblast growth factor (aFGF and bFGF) insulin-like growth factor I and II (IGF-I and -II), transforming growth factor β (TGF-β) and platelet derived growth factor (PDGF), that are involved in the bone development and regeneration process (4, 48).

BMPs are are potent stimulators of osteoblast differentiation and bone formation *in vivo*, and two of them, BMP-2 and BMP-7 (OP-1) have been approved for limited clinical use (38). Viral-based gene therapy is a promising solution for BMP delivery to the bone tissue, and has been successfully implemented in many preclinical studies, which showed that both, *in vivo* and *ex vivo* strategies can induce bone formation.

Ex vivo approach uses genetically modified osteoprogenitor cells derived from periosteum, muscle, fat and skin, or bone marrow stromal cells. The very first *ex vivo* trial was conducted by Lieberman et al. and they used bone marrow stromal cells transduced with BMP-2 cDNA to heal critical-sized femoral defects in rats (Figure 4.). Their results showed that healing achieved by gene transfer was superior to that achieved with recombinant protein alone (26). Similar results were obtained with AdCMV-BMP7 transduced fibroblast and mesenchymal cells (11).

In vivo approach is less expensive and much easier to perform, and many investigators have focused on this



A B

Figure 5. Animal model used for ex vivo cartilage repair approach. A) osteochondral defect prepared for "gene plug" application (black arrow); B) chondral (gray arrow) and osteochondral (black arrow) defects filled with "gene plug".

approach. In this approach vector carrying transgene is directly injected into the lesion. Alternative strategy involves use of prefabricated matrices impregnated with DNA.

Although each one of the proposed BMPs 2, 4 and 7 can induce bone ectopic formation, studies show that certain advantages may be obtained by using combination of these proteins.

Articular cartilage

There is no natural mechanism to regenerate damaged or diseased cartilage, and all the present treatment modalities (surgery, pharmacological agents, cell-based therapy) may provide temporary relief of the symptoms, but do not restore the normal cartilage. Accumulating knowledge about signaling molecules and their role in skeletal development together with the growing armamentarium of therapeutic genes and versatile gene transfer methods has opened new perspectives on cartilage repair.

Experimental studies suggested that several known growth factors such as IGF-1, TGF-β, bFGF, BMP-2 and -7 influence the healing of cartilage *in vivo* (19, 37). On the other hand many of the known cytokines such as IL-4, IL-10, IL-1Ra, IL-1sR and TNFsR have anti-catabolic/anti-inflammatory effect, and are particularly useful in the treatment of chronic degenerative and inflammatory joint disease. Basically, there are three different strategies that can be used to heal the cartilage: (1) induction of chondrogenesis (2) induction of mitosis and the synthesis and deposition of cartilage extracellular matrix components by chondrocytes, or (3) inhibition of cellular responses to inflammatory stimuli (43). Gene delivery to treat cartilage defects can be accomplished by *in vivo* or *ex vivo* approaches.

In vivo strategies use direct injection of the vectors into the joint. Adenovirus-mediated transfer of TGF- β1 and IGF-1 to the synovium of the rabbit knees increased matrix synthesis by chondrocytes, but didn't increase the cellularity of the lesion (29). Moreover, intra-articular TGF-β1 expression resulted in significant pathological changes in the rabbit knee as well as in adjacent muscle tissue. As demonstrated by several authors direct intraarticular injection of vectors carrying TGF-β1 and BMP-2 cDNA led to the ectopic chondrogenesis in the ligaments and paraarticular tissues, as well as osteophyte formation at the joint margins (12). Such side effects were not observed with diffused intraarticular distribution of IGF-1, which makes it a good candidate for direct gene therapy with synovial lining as a potential target. Other drawbacks of the direct approach include the fact that the direct intraarticular injection of adenoviral vectors has been found to lead to vector spread into numerous organs far away from the injection site, as well as strong immune response which limits the duration of transgene expression and interferes with the desired restorative processes (33, 51).

Due to the mentioned obstacles, a cell-mediated gene transfer approach combining the supply of appropriate precursor cells with the stimulatory effects of transgenic growth or differentiation factors is the most common approach for gene delivery to the cartilage defects. In this approach genetically modified cells can be administered by simple injection or by topical transplantation.

Present studies included transduction of autoulogous chondrocytes in horses and periosteal cells in rabbits with cDNA BMP-7 (15). Other investigators used bFGF and TGF-β1 gene-transduced chondrocytes to treat cartilage defects in rabbits and mice respectively (25).

Topical transplantation of genetically modified cells assumes strict adherence to the defects. It certainly minimizes the risk of previously mentioned unwanted side effects of potent growth and differentiation factors such as BMP-2 or TGF- β. Pascher et al. (34) developed a novel ex vivo method by using coagulated bone marrow aspirate as a mean of gene delivery to cartilage (Figure 5). Vector-seeded and cell-seeded bone marrow clots ("gene plugs") were found to maintain their structural integrity following extensive culture and maintained transgenic expression for several weeks.

Meniscus

Lesions in the avascular two-thirds of the meniscus do not heal well and are usually surgically treated. It is known fact that various growth factors promote the synthesis of matrix by meniscal cells and thus have the potential to augment healing. Gene therapy represents attractive alternative to a surgical procedure and is currently being investigated.

Studies that investigated the feasibility of gene transfer to the meniscus, confirmed that it is possible to transfer genes to sites of meniscal damage and to express them locally within the lesion for several weeks (27).

Ligament and tendon

Various growth factors have the potential to enhance the native repair processes in ligamentous lesions, and gene therapy provides interesting delivery options for these growth factors. Five growth factors have been best characterized during tendon healing: IGF-1, TGF-β, VEGF, PDGF, and bFGF. All of them are significantly up-regulated following tendon injury and are active at multiple stages of the healing process. Feasibility studies showed that it is possible to transfer marker genes to tendons and ligaments by various *in vivo* and *ex vivo* methods using various vectors and functional studies followed shortly after (13, 18).

Anterior cruciate ligament (ACL) lesions have been in the focus of many gene therapy trials. As demonstrated by Murray et al. it is possible to stimulate healing of the human anterior cruciate ligament (ACL) after rupture by the implantation of a biodegradable scaffold which the host cells invade, populate and remodel. This process never happens *in vivo* because natural scaffold, which is a blood clot, does not form in the injured ACL (9). Further studies enhanced this approach by incorporation adenovirus vectors with collagen sponge to form gene activated matrix (GAM) (35). Transplant integration is one of the main concerns in the ACL reconstructive surgery, and it was demonstrated by Mihelic et al. (30) that integration can be improved with use of BMPs. Martinek et al. (28) demonstrated improved integration of a double-bundle semitendinosus graft that had been genetically engineered to express BMP-2.

Intervertebral disc

Intervertebral disc degeneration (IDD) is characterized by progressive loss of the proteoglycan matrix due to an imbalance in anabolic and catabolic activity within the disc, whereby synthesis decreases and degradation increases, respectively. Restoring the balance between these two processes is therefore an amenable option to treat IDD. Exogenous growth factors such as IGF-1, TGF-β1, BMP-2, BMP-12 and OP-1 have been shown to transiently increase matrix synthesis (42). Studies also confirmed elevated levels of matrix metalloproteinases (MMPs) and inflammatory cytokines in degenerated discs (20). Gene therapy offers new possibilities in transfer of these factors to the disc. Both, *in vitro* and *in vivo* feasibility studies showed that it is possible to transfer marker genes to the disc cells via an adenoviral vector (31). Further functional studies demonstrated that successful transfer of therapeutic gene for TGF-β1 up-regulated synthesis of proteoglycans *in vivo* and *in vitro* (31). Tissue inhibitors of metalloproteinase (TIMP) are powerful endogenous inhibitors of MMPs and Wallach et al demonstrated that gene transfer of TIMP-1 increased proteoglycan synthesis as much as 5-fold compared to controls (49). Other approaches explore use of transcription factors which are key mediators in the natural production of growth factors. Sox9 is the most important transcription factor in chondrogenesis, and Paul et al. (36) successfully transduced human disc cells and demonstrated increase in concentration of type II collagen. LIM mineralization protein-1 (LMP-1) is very important regulator of osteoblast differentiation and for synthesis of several BMPs. When delivered to rat intervertebral disc cells LMP-1 exhibited a chondrogenic effect and increased proteoglycan synthesis.

Conclusion

By 2005, over 1 000 gene therapy clinical protocols had been initiated worldwide. Although majority of these protocols are related to cancer, monogenic, vascular and infectious diseases, orthopaedic surgery unexpectedly became one of the most promising fields for gene therapy application.

Major breakthrough was the discovery of growth factors and their importance in development and healing, and their potential role as therapeutic agents in various musculoskeletal diseases. Short half-life of these proteins limits their use, because many orthopaedic conditions (especially chronic ones) require sustained concentration of the factors within the tissue for a certain period of time. Gene therapy provided an answer to this limitation and became powerful technology for the delivery of these proteins to specific tissues. Fracture healing, repair/regeneration of cartilage, meniscus, ligaments, and tendons are obvious candidates for gene therapy, but it's application in rheumatoid arthritis, osteoarthritis, orthopaedic tumors and genetic disorders are being thoroughly investigated as well.

Tissue repair is probably the most convenient area of orthopaedic surgery for gene therapy approaches. It doesn't require prolonged period of gene expression, local delivery is reasonably simple and it avoids substantial risk associated with systemic delivery, and levels of gene expression don't need to be so finely regulated.

Safety is an important issue since the treatment of non-lethal diseases should not endanger the life of the patient. There are several important issues that must be considered: humoral and cellular immune response, local or systemic pathologic responses to the transgene protein, and the replicative potential of the viral vector. Nevertheless, efforts to construct safer viral and more efficient non-viral vectors continues.

Major advances in molecular genetics and tissue engineering approaches have initiated a new biologic era in orthopaedic surgery. Functional and feasibility studies proved that it is possible to deliver genes to the specific tissues, and future research will determine safety and true efficacy of gene therapy approaches in the treatment of various musculoskeletal conditions.

ZÁVĚR

Cílem této práce je podat přehled o pozoruhodném pokroku v genové terapii pohybového systému. Od uvedení této tematiky před 15 lety bylo publikováno mnoho klinických i preklinických výsledků. Původní cíl výzkumu, revmatoidní artritida, prošel druhou klinickou fází protokolu ke zjišťování účinnosti. Účinnost genové terapie při osteoartritidě se pečlivě zkoumá v různých modelových experimentech na zvířatech. Nejnadějnější oblastí pro výzkum na tomto poli je náhrada tkání, protože exprese genů nevyžaduje dlouhou dobu, místní podání je dostatečně jednoduché a je prosto rizika spojeného se systémovou aplikací a úrovně genové exprese nemusí být řízeny do jemných detailů. V současné době je přenos genů úspěšně používán při podpoře náhrady tkání a regenerace kostí a chrupavek, úponů šlach, menisků a meziobratlových plotének. Mezi další možná použití genové terapie v pohybovém systému patří osteoporóza, aseptické uvolňování, genetická a nádorová onemocnění. Údaje získané z těchto studií jsou velice povzbudivé a potvrzují, že genová terapie je slibným řešením v léčení poruch pohybového systému.

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