

Mini Review

Application of Decoy Oligodeoxynucleotides for Arthritis

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Recent progress in DNA technologies has provided the strategies to regulate the transcription of disease-related genes *in vivo* using antisense oligodeoxynucleotide (ODN). Transfection of cis-element double-stranded oligodeoxynucleotides (decoy ODNs) has been reported as a new therapeutic tool of anti-gene strategies for gene therapy. In the field of arthritis, decoy ODNs strategies have been significant therapeutic potential. The concept of regulation the disease related gene expression at the level of transcriptional factor may be more therapeutic effects compared with monotherapy in arthritis.

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Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by synovial proliferation. Overexpression of inflammatory cytokines such as tumor necrosis factor α (TNF α), interleukin (IL)-1 and IL-6, are thought to play a critical role in pathogenesis of joint destruction in arthritic condition^{1,2}. Various kinds of cells migrating into synovium are the major source of these proinflammatory cytokines. Synovial macrophages are capable of differentiating into osteoclasts; the osteoclasts generated within the synovial membrane are probably involved in bone destruction *in vivo*³. Multinucleate tartrate-resistance acid phosphatase (TRAP) positive cells were also induced from CD14 positive cells in the synovial fluid from patients with RA⁴. Re-

cently biological agents targeting these cytokines have been successful both in experimental models and human trials⁵⁻⁹.

Recent progress in molecular biology has made a great impact on understanding of the pathological mechanisms, and has enabled the development of treatment strategies that exploit this increased knowledge of the structure and function of biomolecules in arthritis. A major focus of cellular and molecular research has been to develop means to regulate gene expression in an effort to treat and cure a variety of disease. These concepts have been applied in the field of arthritis.

Principles of the decoy approach

Correct regulation of gene expression is essential both to nor-

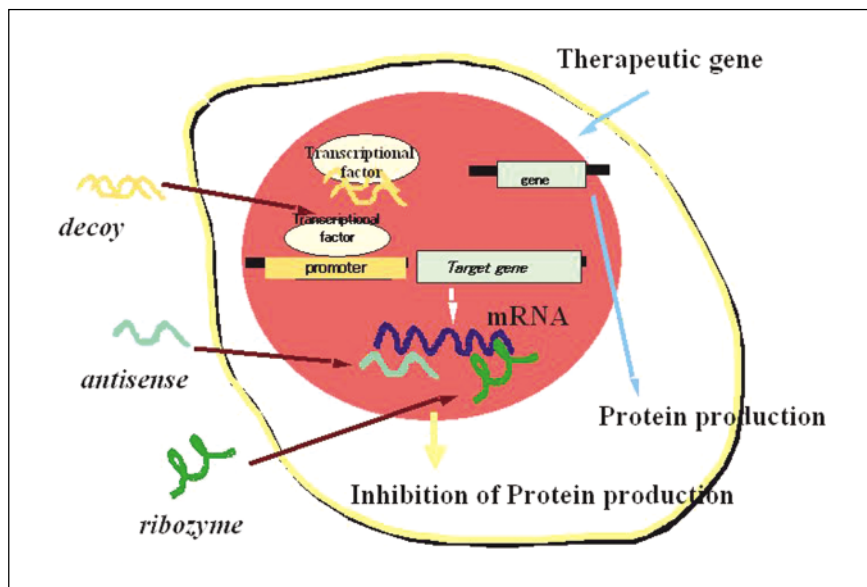


Fig.1 Target sites for decoy, antisense and ribozyme ODNs

mal development and to correct functioning of adult organism. Such regulation is usefully achieved at the level of DNA transcription, a process that controls which genes are transcribed into RNA, and through post-transcriptional regulation¹⁰. The transcription of a specific gene is controlled by transcriptional factors, which are grouped into families on the basis of shared DNA-binding motifs¹⁰. While some regions of transcriptional factors interact with DNA, others interact with RNA polymerase and its associated proteins to increase or decrease the rate of transcription. The vital role of transcriptional factors, together with the fact that a single transcriptional factor can affect the expression of many genes, suggests that the inactivation of a transcriptional factor as a result of an inherited mutation is incompatible with survival. Morishita R. invented the concept of therapeutic use of decoy ODNs and demonstrated its possibility in cardiovascular disease¹¹⁻¹⁵. Although the mechanisms of action of antisense ODNs are still unclear, the principle of the transcriptional factor decoy approach is simply the reduction promoter activity due to the inhibition of binding of a transcriptional factor to specific sequences in the promoter region (Fig.1). We have developed a therapeutic application of decoy ODNs for arthritis.

Application of decoy ODNs for arthritis

1) Nuclear factor κ B (NF κ B) decoy ODN

NF κ B plays a pivotal role in the coordinated transactivation of inflammatory cytokine genes, whose activation has been postulated to be involved in the destructive changes of articular car-

tilage and bone in arthritic joints. Synthetic double-strand DNA with high affinity for NF κ B could be introduced *in vivo* as decoy cis elements to bind transcription factors and block the activation of inflammatory mediator genes. *In vivo* transfection of NF κ B decoy ODN by intraarticular injection into rats with collagen-induced arthritis decreased the severity of hind-paw swelling¹⁶. Histologic and radiographic studies showed marked suppression of joint destruction in rats treated with NF κ B decoy ODN transfection (Fig.2). This treatment method also suppressed the production of TNF α and IL-1 in the synovium of arthritic joints. We have reported that *in vitro* transfection of NF κ B decoy ODN into synovial cells derived from RA patients resulted in suppressive effects on synovial cell proliferation and IL-1 β , IL-6, TNF α , ICAM-1 and matrix metalloproteinase-1 production from the synovium¹⁷. Based upon these results, we attempted to evaluate the therapeutic effects on joint destruction of NF κ B decoy ODN in a cynomolgus monkey CIA model which shares various features with human RA as a preclinical model of arthritis. Monkeys were given an intraarticular injection of NF κ B decoy ODN into bilateral wrists every 2 weeks from 1 week up to 11 weeks. Consecutive radiographic examinations showed marked suppression of joint destruction in the NF κ B decoy ODN injected group compared with the untreated group. Histologic examination showed hyperplasia of synovitis with severe destruction of articular cartilage and bone in the untreated groups. However, in the NF κ B decoy ODN injected group, slight synovitis was confirmed in the joints, and the damage of articular cartilage and subchondral bone was minimal in 70 % of joints. The levels of IL-1 and

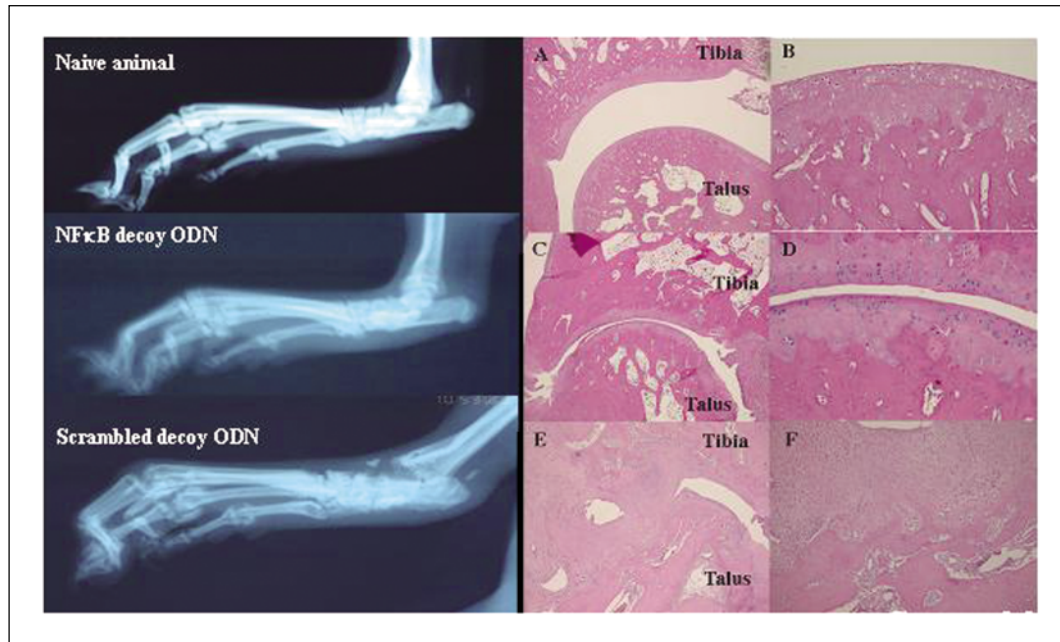


Fig.2 Radiographic and histologic examinations demonstrated the significant suppression of joint destruction treated with NF κ B decoy ODN

A,B: Naïve rats, C,D: NF κ B decoy treated rats, and E,F: Scrambled decoy treated rats.

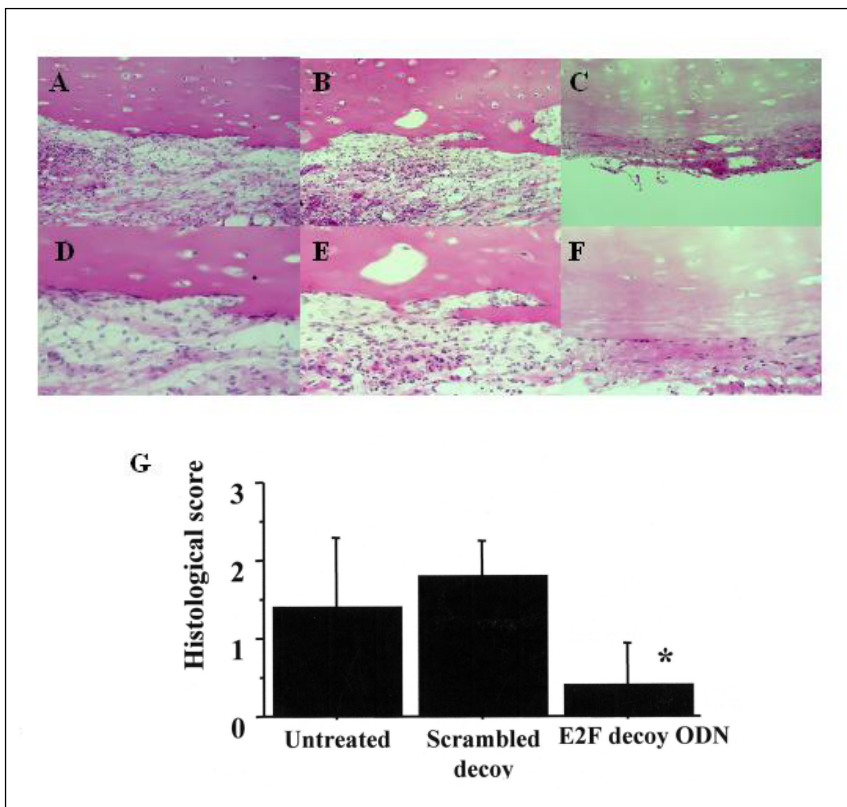


Fig.3 Histological analysis of the co-implanted synovial tissue and cartilage in SCID-HuRag mice

(A and D) H&E stained sections from untreated synovial tissue. (B and E) H&E stained sections from synovial tissue transfected with scrambled decoy ODN. (C and F) H&E stained sections from synovial tissue transfected with E2F decoy ODN. Untreated synovial tissue and synovial tissue transfected with scrambled decoy ODN showed stratification of synovial cells and infiltration of inflammatory cells. Marked invasion of co-implanted articular cartilage by synovium is apparent (A,B,D and E). Synovial tissue transfected with E2F decoy ODN showed less hyperplasia of synovial cells and infiltration of inflammatory cells. The sections in this group mainly maintained intact cartilage (C and F). There was a significant difference in histological score between E2F decoy ODN transfected group and untreated group or scrambled decoy ODN transfected group ($p < 0.05$) (G).

TNF α in the synovium of arthritic joints were significantly lower in the NF κ B decoy ODN injected group compared with the untreated group. At this point, the principle of regulation at the level of transcriptional factor should be better compared with monotherapy to increase the therapeutic effects. During the observation period, other than inflammatory parameters, no obvious abnormal change in biochemical parameters suggesting adverse events due to administration of ODN was recognized.

2) E2F decoy ODN

The transcription factor E2F regulates the expression of multi-cell-cycle regulatory genes that are critical to cell growth and proliferation. The upregulation in E2F-binding activity in synovial fibroblasts derived from patients with RA was confirmed¹⁸⁾. The effect of E2F decoy ODN on cartilage invasion by RA synovium in a murine model of human RA was investigated. E2F decoy ODN were introduced into synovial tissue and synovial fibroblasts derived from patients with RA using hemagglutinating virus of Japan (HVJ)-liposomes. The effect of E2F decoy ODN on synovial fibroblast proliferation was evaluated by MTT assay and by RT-PCR for the cell cycle regulatory genes proliferating-cell nuclear antigen (PCNA) and cyclin-dependent kinase 2 (cdk2). Changes in production of inflammatory mediators by RA synovial tissue following transfection with E2F decoy ODN were assessed by ELISA. Human cartilage and RA synovial tissue transfected with E2F decoy ODN were co-transplanted in severe combined immunodeficient (SCID) mice. After 4 weeks, the mice were sacrificed and the implants histologically examined for inhibition of cartilage damage by E2F decoy ODNs. E2F decoy ODN resulted in significant inhibition of synovial fibroblast proliferation, corresponding with reduced expression of PCNA and cdk2 mRNA in synovial fibroblasts. The production of interleukin-1 β (IL-1 β), IL-6 and matrix metalloproteinase (MMP)-1 by synovial tissue was also significantly inhibited by the introduction of E2F decoy ODN. Further, in an *in vivo* model, cartilage that was co-implanted with RA synovial tissue transfected with E2F decoy ODN exhibited no invasive and progressive cartilage degradation (Fig.3). These data demonstrate that transfection of E2F decoy ODN prevents cartilage destruction by inhibition of synovial cell proliferation, and suggest that transfection of E2F decoy ODN may provide a useful therapeutic approach for the treatment of joint destruction in arthritis.

Development of next generation decoy ODN

One of the major limitations of the decoy ODN strategies is rapid degradation of phosphodiester ODN by intracellular nu-

cleases. Circular dumbbell double-strand decoy ODNs (we call these ribbon-type decoy ODN) were developed to resolve these issues¹⁹⁾ (Fig.4). Ribbon-type NF κ B ODN showed high resistance to exonuclease III and observed as a major band in gel electrophoresis. Compared to RNODN, phosphorothionated NF κ B decoy ODN (PNODN) was degraded after incubation in the presence of exonuclease III²⁰⁾.

Effect on osteoclastogenesis of Ribbon-type NF κ B ODN

Osteoclasts are multinucleated giant cells formed by the fusion of hematopoietic cells of the monocyte/macrophage lineage. NF κ B is associated with the activation of osteoclasts and is important for both the differentiation of osteoclast precursors. Synovial macrophages are capable of differentiating into osteoclasts; the osteoclasts generated within the synovial membrane are probably involved in bone destruction *in vivo*. To regulate the osteoclast differentiation and bone resorbing function is one of the most important issues in therapeutic point of view in arthritis.

In vitro examination using osteoclast differentiation system with rat bone marrow cells induced by M-CSF and RANKL, osteoclastogenesis was inhibited by incubation with RNODN (Fig.5). Inhibitory effect was not observed when cells were incubated with RSODN. In addition, we examined the effect of RNODN on mature osteoclasts activity for bone resorption using pit formation assay. Results showed that calcified matrix resorption by RANKL-induced osteoclast-like cells were significantly inhibited by incubation with RNODN. Inhibitory effect was not observed when cells were incubated with RSODN²⁰⁾.

Prospect for decoy ODN strategy for arthritis

The efficacy of decoy ODN therapy has been demonstrated in several diseases including arthritis. In cardiovascular and dermatitis lesion, clinical trials have already started in USA and Japan. As far, no serious adverse event has reported. Arthritis is non-fetal disease and the safety is one of the most important issues in consideration of application of decoy ODN therapy. In this point, the administration of decoy ODN is local injection into the joint cavity of the affected joint, and it helps to decrease adverse events, due to its short half-life in the systemic circulation. We believe it is time to take a hard look at practical issues that will determine its real clinical potential for arthritis. Osteoarthritis (OA) is another major joint disease and the number of the patients with OA is rapidly increasing with the aging of society. We have already started the basic examination to confirm the

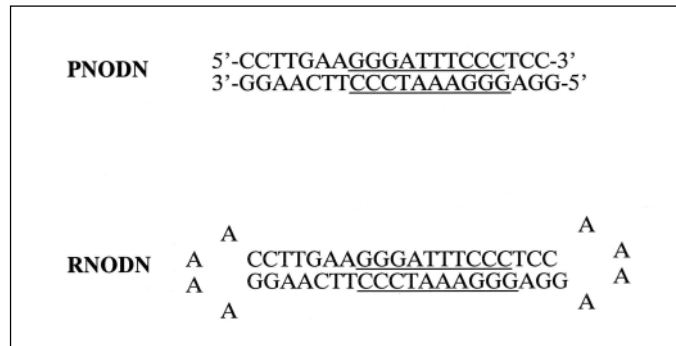


Fig.4 Structures and sequences of the decoy oligodeoxynucleotides for NF κ B. PNODN (phosphorothionated decoy oligodeoxynucleotides) and RNODN (ribbon type decoy ODN) contain the NF- κ B-binding site in its double-stranded lesion (consensus sequences are underlined).

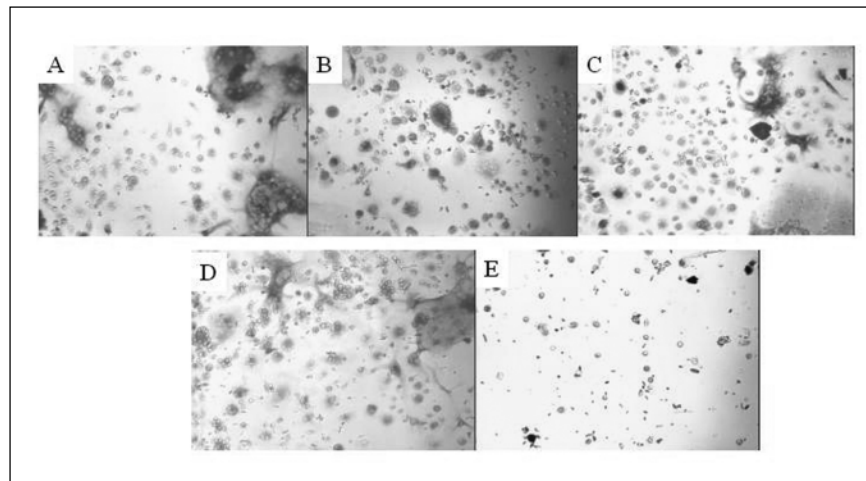


Fig.5 Osteoclast differentiation induced *in vitro* by macrophage colony-stimulating factor (M-CSF) and soluble receptor activator of nuclear factor κ B ligand (RANKL)

Cells were transiently transfected with B: phosphorothionated scrambled decoy ODNs C: phosphorothionated NF κ B decoy ODNs D: ribbon-type scrambled decoy ODNs E: ribbon-type NF κ B decoy ODNs or A: untreated alone.

effect of decoy ODN strategy for OA. Continuous endeavor to elucidate the pathogenesis of arthritis will contribute to develop more efficient and less invasive therapeutic strategies.

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