# 学 位 論 文 の 要 旨

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| 学 位 論 文 名 | Decoy Oligodeoxynucleotide Targeting Activator Protein-1 (AP-1) Attenuates Intestinal Inflammation in Murine Experimental Colitis |
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## INTRODUCTION

Inflammatory bowel diseases (IBD) are characterized by chronic intestinal immune-mediated disorders of unknown etiology. In sites of intestinal inflammation, granulocytes and macrophages produce high levels of pro-inflammatory cytokines, including interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α, which are directly involved in the pathogenesis of IBD. Although several kinds of therapeutic strategies are used for IBD, none have been found to be totally effective. The transcription factor-activated protein-1 (AP-1) is a dimeric complex of basic region-leucine zipper proteins, and consists of heterodimers or homodimers of the Jun, Fos, and ATF families. AP-1 is modulated by interactions with other transcriptional regulators and is further controlled by upstream kinases that link AP-1 to various signal transduction pathways. Recent studies have demonstrated that AP-1 is one of the key transcription factors that upregulate genes involved in immune as well as proinflammatory responses during the pathogenesis of IBD, suggesting that AP-1 may be an ideal target for the development of new therapeutic options for IBD. A decoy strategy that employs a synthetic double-stranded oligonucleotide (ODN) to competitively inhibit the binding of transcription factors to promoter regions of their target genes has emerged as a useful tool in a new class of anti-gene therapies presented in recent years. It has been reported that a decoy ODN targeted at the transcription factor nuclear factor (NF)-κB can exert potent immunosuppressive effects with certain inflammatory diseases. In the present study, we investigated the anti-inflammatory effects of

AP-1 decoy ODN in dextran sulfate sodium (DSS)-induced experimental murine colitis and compared its therapeutic potential to that of NF-κB decoy ODN.

## MATERIALS AND METHODS

### *Synthesis of Doubled-Strand ODN*

The double-strand decoy ODN was generated by annealing equimolar amounts of single-stranded sense and anti-sense phosphorothioate-modified ODN containing consensus AP-1 or NF-κB-binding sequences. Scrambled double-strand ODN samples were also generated as experimental controls for each decoy ODN.

### *Transfections of Decoy and Scrambled ODN and Transfection Efficiency*

The human colonic epithelial cell line HCT-15 was used for *in vitro* assays. HCT-15 cells were transfected with FITC-labeled decoy or scrambled ODN. Twenty-four hours after transfection, efficiency was assessed by fluorescence microscopy and flow cytometry.

### *Functional Efficiency of Decoy AP-1 and NF-κB ODNs*

The efficiency of the decoy ODNs to mediate the inhibition of AP-1 or NF-κB-induced transcription activity was initially evaluated *in vitro* using a reporter gene luciferase assay. HCT-15 cells were transfected with various concentrations of decoy or scrambled ODN, and reporter gene vectors. Twenty-four hours after transfection, cells were stimulated with salmonella flagellin for 12 h. The cell lysates were then used for measurement of firefly and renilla luciferase activities. In addition to the reporter gene assays, the functional efficacy of AP-1 or NF-κB decoy ODN on AP-1 or NF-κB-induced endogenous proinflammatory gene expression was evaluated by their effects on flagellin-induced IL-8 expression by HCT-15 cells. IL-8 expression was assessed by enzyme immuno assay (EIA) and real-time PCR.

### *Experimental colitis and Decoy ODN Therapy Protocol*

Seven-week-old specific pathogen-free male BALB/c mice were used. Experimental colitis was induced by administering 2.5% dextran sulfate sodium (DSS) solution in drinking water for 7 days. AP-1 or NF-κB decoy ODN or scrambled ODN was intraperitoneally injected once a day from days 2 to 5 during the DSS-administration period using a hemagglutinating virus of Japan (HVJ)-liposome method. Mice were euthanized at 1 day after the end of DSS administration, after which a segment of each distal colon was dissected. Colitis was assessed by weight (BW)

loss, colon length, histopathology, and detection of myeloperoxidase (MPO), IL-1β and TNF-α in colon tissue.

## RESULTS AND DISCUSSION

Flow cytometry revealed high positive rates (above 90%) of fluorescence activity in the cultured cells transfected with the FITC-labeled decoy or scrambled ODN. Transfection of decoy ODNs significantly inhibited the transcriptional activities of the AP-1 and NF-κB in reporter assays and flagellin-induced IL-8 production *in vitro*. In mice, AP-1 decoy ODN, but not scrambled ODN, significantly inhibited weight loss, colon shortening, and histological inflammation induced by DSS. Further, AP-1 decoy ODN decreased MPO, IL-1β and TNF-α in colonic tissue of mice with DSS-induced colitis. The AP-1 decoy therapeutic effect was comparable to that of NF-κB decoy ODN, which also significantly decreased intestinal inflammation.

Studies of treatments with decoy ODNs targeting several transcriptional factors have demonstrated regulation of a variety of biological responses including inflammation and tumorigenesis in various organs. Although AP-1 is a major immunoregulatory as well as proinflammatory transcription factor, nothing is known to have been reported regarding the effect of AP-1 inhibition by a decoy ODN on intestinal inflammation. In the present study, we designed a double-strand decoy ODN binding to AP-1-specific nucleotide sequences and investigated its efficacy to prevent murine experimental DSS-induced colitis. Our findings demonstrated that AP-1 decoy ODN markedly inhibited DSS-induced colonic inflammation, indicating that AP-1 might be one of the potent targets for IBD therapy. However, additional investigations addressing *in vivo* efficacy and safety should be carefully performed before ODNs can be considered for use in a clinical therapeutic strategy.

## CONCLUSION

The present study showed for the first time that AP-1 decoy ODN effectively attenuated intestinal inflammation, indicating the potential of targeting proinflammatory transcription factors in the development of new therapies for IBD.