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TRANSLATIONAL MEDICINE: BENCH TO BEDSIDE

Harnessing the Power of Posttranscriptional Gene Silencing in Crohn's Disease

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Introduction

Posttranscriptional gene silencing or RNA interference is a mechanism by which the expression of one or more genes is partially or fully suppressed by noncoding RNAs, particularly small RNAs. In the laboratory this concept has been utilized to introduce synthetic anti-microRNA (miRNA) oligonucleotides to downregulate or completely abrogate gene expression. MiRNAs are a group of small (~22 nucleotide), noncoding RNAs that confer such posttranscriptional regulation of gene expression.[1] They have emerged as key regulators of a wide variety of biological processes and also as candidate therapeutic targets. MiRNAs silence genes by binding to target sites found within the 3′untranslated region (UTR) of the targeted mitochondrial RNA. This results in the suppression of protein synthesis and/or degradation of the transcript. A number of miRNAs have already been identified as regulators of pathways that underlie the pathogenesis of Crohn's Disease (CD).[2] For example, miR-192, miR-122, miR-29, and miR-146a have been shown to target and repress NOD2, which has been implicated in CD.[3–6] Using small RNA-sequencing a suite of miRNAs were identified in colon tissue that stratify CD patients according to disease behavior independent of the effect of inflammation. Furthermore, levels of specific miRNAs in these patients could predict progression to penetrating and fistulizing CD.[7] The purpose of this short review is to highlight the advances in using posttranscriptional gene silencing in understanding and treating CD.[8,9]

Elucidating the Role of Posttranscriptional Gene Silencing in the Pathogenesis of CD

Enteric microbes are key instigators and perpetuators of chronic inflammation in a genetically susceptible host. Adherent-invasive *E. coli* (AIEC) is an invasive *E. coli* strain highly prevalent in the ileal mucosa of CD patients.[10] AIEC is able to penetrate and survive in human intestinal epithelial cells and replicate within macrophages leading to a profound inflammatory response. Autophagy is a homeostatic process that allows for the elimination of damaged cellular components under deprived and/or inflammatory conditions via the lysosomal pathway. Nguyen *et al.*[8] identified miR-30c and miR-130a as regulators of autophagy via targeting the expression of key autophagy genes, ATG5 and ATG16L1, during AIEC infection of T84 cells and in mouse enterocytes. Using a luciferase reporter assay, the authors demonstrated the binding of miR-30c and miR-130a to the 3′-UTR of ATG5 and ATG16L1, leading to their downregulation. These *in vitro* findings were supported by an inverse correlation of miR-30c/miR-130a and ATG5/ATG16L1 levels in ileal tissues of patients with CD. Mechanistically, chromatin immunoprecipitation demonstrated the direct binding of transcription factor nuclear factor-κB to the miR-30c and miR-130a gene promoters upon AIEC stimulation. Using AIEC-infected transgenic mice they suppressed miR-30c and miR-130a using antisense oligonucleotides to miR-30c and miR-130a resulting in an enhanced autophagy response, clearance of the bacteria, and reduction of ileal production of inflammatory cytokines. Defects in autophagy-mediated handling of the CD-associated bacteria are well described, but until now the role of posttranscriptional gene silencing in this process was unknown. This study highlights an understudied molecular mechanism in CD, which is likely to lead to future insights into disease pathogenesis, and treatment.

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Posttranscriptional gene Silencing Using Antisense Oligonucleotide in Patients With CD

Defects in the anti-inflammatory cytokine pathway of transforming growth factor (TGF)-β1 has been implicated in the inappropriate immune response to the enteric microbiota observed in patients with CD. SMAD7, an intracellular protein that binds to the TGF-β1 receptor and prevents TGF-β1-driven signaling, is elevated in immune cells isolated from CD tissue.[11] The use of a specific antisense oligonucleotide to posttranscriptionally inhibit SMAD7 can restore TGF-β1 activity and inhibit inflammatory cytokine production.[12]

Preclinical and phase 1 studies have shown that Mongersen, an antisense oligonucleotide-containing compound, can inhibit SMAD7 in the ileum and colon.[12,13] Monteleone *et al*.[9] conducted a double blind, placebo-controlled phase 2 trial to evaluate the clinical efficacy of Mongersen in treating patients with active CD. A total of 166 patients with moderate–severe disease (Crohn's disease activity index (CDAI) score 220–400) were randomized to receive either placebo or Mongersen at doses of 10, 40, or 160 mg daily for 2 weeks. The primary outcome was the percentages of patients who were in clinical remission at day 15 (CDAI score <150) and remained in clinical remission for at least 2 weeks. This was achieved in 65% and 55% of the patients dosed with 160 and 40 mg of drug respectively; both were significantly higher than the 10 mg and placebo groups, 12% and 10%, respectively ($P<0.001$). In addition, rates of clinical response, defined as a decrease in the CDAI score of 100 points, were significantly higher in the 160 and 40 mg groups compared to the 10 mg and placebo groups at days 15 and 28. Mongersen-treated patients had significant decrease in the mean concentrations of the inflammatory cytokines, interleukin-8 and TNF-α, compared to placebo. Interestingly, in the 102 patients with elevated C-reactive protein (CRP) levels at baseline, neither the placebo nor the active therapy at any dose significantly reduced the median CRP levels at days 15, 28, and 84. The clinical response rates observed in this study were the highest documented in recent clinical trials, evaluating the efficacy of biologics in the treatment of CD. There are several limitations to the study. There is a disconnect between the high rates of clinical remission and lack of normalizations of CRP levels, something observed routinely in prior clinical trials of patients with similar disease severity. No endoscopic determination was made to validate the clinical response with mucosal healing. Despite these caveats, use of Mongersen demonstrates the incorporation of an agent with a novel mechanism of action to the ever-growing armamentarium of therapeutic agents for CD.

Conclusion

Posttranscriptional gene silencing with miRNAs has advanced our understanding of chronic inflammation in patients with inflammatory bowel diseases, both in terms of pathogenesis and prognosis. Furthermore, using specific antisense oligonucleotides to posttranscriptionally inhibit key genes driving the inflammatory response have emerged as potentially powerful therapeutic agents. Future studies will determine how we can practically and safely apply this technology to clinical practice.

CONFLICT OF INTEREST

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