Micro-RNAs as biomarkers and targets of herbal CAM in autoimmune arthritis.

Rheumatoid arthritis (RA) is a multifactorial disease that involves both a genetic and an environmental component. Approximately 0.5-1% of the population in the U.S. is afflicted with RA. Current treatments, including biologics such as anti-tumor necrosis factor-?, are effective, but only in about 50-60% of RA patients. In addition, their use is associated with severe adverse effects, such as immune suppression that renders RA patients more vulnerable to infections. In order to develop better therapeutics, a more comprehensive understanding of the pathogenesis of RA is required. Our lab has previously shown that there is a dynamic change in gene expression at different phases of adjuvant-induced arthritis (AA) in Lewis rats. Furthermore, a traditional Chinese medicine celastrus and its bioactive component celastrol can suppress AA. However, their mechanisms of action have not been fully defined. Micro-RNAs (miRNAs) are short (~19-22 nucleotides long), non-coding RNA sequences that repress the activity of specific genes. These short sequences either inhibit translation or cause degradation of the target messenger RNA (mRNA). We hypothesize that miRNAs play a vital role in the development and progression of autoimmune arthritis, and that specific miRNAs can also serve as biomarkers for this disease. Moreover, we suggest that celastrus/celastrol mediates its effect in part by regulating the levels of specific miRNAs. 

**Aim 1.** To define the miRNA profiles of arthritic Lewis rats at early and late phases of AA, and identify significantly altered and relevant miRNAs as well as their potential target genes that are involved in arthritis pathogenesis. 

**Aim 2.** To identify specific miRNAs whose expression is significantly altered following treatment of arthritic rats with celastrus or its bioactive component celastrol, and to define the role of those miRNAs in mediating the anti-arthritic activity of these herbal complementary and alternative medicine (CAM) products. Using total RNA purified from the draining lymph node cells and the synovial-infiltrating cells of Lewis rats following an arthritogenic challenge, with or without treatment with celastrus/celastrol, we plan to use miRNA microarray to identify miRNAs of interest. To further validate the selected miRNA, we will transfect a cell line with miRNA mimic/antagonist to over-/under-express, respectively the miRNA of interest. Then we will employ qRT-PCR, western blotting, and immunochemistry to determine their effects on target genes associated with arthritis pathogenesis as well as their control by herbal CAM therapy. We believe that our results would not only advance the understanding of arthritis pathogenesis, but also help develop novel therapeutics for RA.

Public Health Relevance

Rheumatoid arthritis (RA) is a debilitating, autoimmune disease affecting people worldwide, and availability of better diagnostic tools and drugs that are effective but safe would significantly improve the management of RA and other autoimmune diseases. MicroRNAs (miRNA) are a type of RNA that can regulate other genes, and therefore certain miRNAs can serve as biomarkers of disease and also be helpful in diagnosis of that disease. In addition, natural plant products can mediate their effect through miRNAs, and identifying such miRNAs would not only increase our understanding of how drugs work, but also facilitate new drug development to target those miRNAs.