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Dysregulated co-stimulatory molecule expression in a Sjögren's syndrome mouse model with potential implications by microRNA-146a

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ABSTRACT

Sjögren's syndrome (SjS) is an autoimmune condition that primarily affects salivary and lacrimal glands, causing loss of secretion. We have previously shown that microRNA-146a (miR-146a) is over-expressed in the salivary glands and peripheral blood mononuclear cells (PBMC) of SjS-prone mice (C57BL/6.NOD-*Aec1Aec2*, B6DC) and in PBMC of SjS patients. The purpose of this research was to identify a target molecule of miR-146a and identify subpopulations of cells affected by altered miR-146a in the salivary glands of SjS-prone mice. *In silico* analyses identified costimulatory molecule CD80 as a potential target of miR-146a. Luciferase assay of the human CD80 3′untranslated region demonstrated miR-146a directly inhibited CD80 protein expression as indicated by reduced luciferase reporter expression and an examination of B6DC salivary glands revealed a reduction in CD80 protein. More interestingly, the specific reduction in CD80 protein was detected from the salivary gland epithelial cell population and in interstitial dendritic cells in the glands as well. The reduction in CD80 protein levels in salivary gland epithelial cells were negatively associated with elevated miR-146a expression. Therefore, this study provides the first indication that salivary gland epithelial cells may be critically involved in SjS progression by altering CD86:CD80 protein ratio in response to miR-146a upregulation.

Abbreviations: SjS, Sjögren's syndrome; miRNA, microRNA; PBMC, peripheral blood mononuclear cells; B6, C57BL/6J; B6DC, C57BL/6.NOD-*Aec1Aec2*; TLR, toll-like receptor; IRAK-1, interleukin-1 receptor-associated kinase-1; TNF, tumor necrosis factor; TRAF-6, TNF receptor-associated factor-6; LPS, lipopolysaccharide; Treg, regulatory T cells; Teff, effector T cells; MHC, major histocompatibility complex; CTLA-4, cytotoxic T lymphocyte antigen-4; ICAM-1, intercellular adhesion molecule-1; IFN-γ, interferon-gamma; UTR, untranslated region; NCBI, National Center for Biotechnology Information; MFE, minimum fold energy; PCR, polymerase chain reaction; qRT-PCR, quantitative real-time PCR; SEM, standard error of the mean; DC, dendritic cell; M, macrophage; APC, antigen presenting cell; AU, adenine–uridine; ARE, AU-rich elements; IDO, indoleamine 2,3-dioxygenase; DAPI, 4′,6-diamidino-2-phenylindole.

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1. Introduction

Sjögren's syndrome (SjS) is an autoimmune disorder that affects approximately four million adults in the United States, making it one of the most common autoimmune conditions (Sjogren's Syndrome Foundation, 2013). SjS manifests as loss of secretion in the eyes and/or mouth due to the autoimmune response to the lacrimal and/or salivary exocrine glands. In particular, loss of saliva secretion results in effects ranging from difficulty speaking and eating, oral candidiasis, and extensive tooth decay to chronic sialadenitis (Sjogren's Syndrome Foundation, 2013). The pathophysiology of SjS includes inflammatory cell aggregates, which are largely composed of CD4+ T and B cells. And these cell aggregates are closely associated with ductal epithelial structures of the exocrine glands (Tarpley et al., 1974). Current research has yet to identify the cause for formation of these immune cell foci in the exocrine glands. However, the trigger may involve how resident antigen presenting cells and exocrine epithelium participate in glandular inflammation (Manoussakis and Kapsogeorgou, 2010).

Recent studies have shown links between microRNA (miRNA) and autoimmune diseases such as with rheumatoid arthritis, systemic lupus erythematosus, and SjS (Chan et al., 2013; Kapsogeorgou et al., 2011; Pauley et al., 2008; Carlsen et al., 2013; Liang and Shen, 2012; Miao et al., 2013; Alevizos et al., 2011). MiRNAs are short endogenous non-coding RNAs capable of inhibiting the translation of targeted messenger RNA (mRNA) transcripts into protein. Our research on SjS pathogenesis in the SjS-prone mouse model (C57BL/6.NOD-*Aec1Aec2*, B6DC) has documented several disease characteristics related to SjS salivary and lacrimal gland pathophysiology (Bulosan et al., 2009; Cha et al., 2002a; Cha et al., 2002b; Killedar et al., 2006). Notably, our lab has identified significantly elevated miR-146a expression in the salivary glands of B6DC mice during the asymptomatic stage of SjS-like disease, which remains elevated following disease progression (Pauley et al., 2011). The observed increase in miR-146a prior to clinical onset could indicate underlying inflammation and may contribute to disease progression in the salivary glands. However, cell populations contributing to increased miR-146a and how miR-146a could affect the pathophysiology of autoimmune SjS remains to be elucidated.

MiR-146a is a well-known modulator of differentiation and function in a variety of innate and adaptive immune cells (Labbaye and Testa, 2012; Nakasa et al., 2011; Taganov et al., 2006; Yang et al., 2012; Zhao et al., 2013). Research has demonstrated that miR-146a is highly up-regulated following toll-like receptor (TLR) or inflammatory cytokine signaling, as well as during the maturation of immune cells into an active state (Labbaye and Testa, 2012; Taganov et al., 2006; Yang et al., 2012; Zhao et al., 2013; Quinn et al., 2013). MiR-146a is generally believed to be up-regulated to dampen inflammatory processes by down-regulating target cytokine expression (Lindsay, 2008). Although current lines of research suggest that miRNAs provide different functional outcomes in a cell depending on the existing transcriptome and accessibility of the mRNA target sequences. One well-studied outcome of miR-146a expression is the attenuation of innate immune signaling cascades through the down-regulation of adaptor molecules interleukin-1 receptor-associated kinase (IRAK)-1 and tumor necrosis factor (TNF) receptor-associated factor (TRAF)-6. Our research on miR-146a's innate immune functions using the human THP-1 monocyte cell line have shown miR-146a mimics decreased cytokine production in response to lipopolysaccharide (LPS) stimulation of TLR-4 and also showed enhanced phagocytosis of *Escherichia coli* bacteria (Pauley et al., 2011).

In addition, some of the most critical aspects of the innate immune response are the processes of antigen presentation and costimulation of antigen-specific T-cells, typically in response to a pathogen. Autoimmunity is associated with antigen presentation and costimulation of self-reactive T-cells without proper down-regulation by regulatory T-cells (Tregs) (Buckner, 2010). The initiation and maintenance of any naïve T-cell response requires at least two signals. The first signal is delivered through binding of the antigen-specific T-cell receptor to its major histocompatibility complex (MHC) ligand displaying the cognate antigen. And the second signal is delivered through binding of CD28 T-cell receptor to costimulatory molecules (either CD86 or CD80) on the surface of the antigen-presenting cell. Research suggests that CD86 mediates the initiation phase of T cell responses. In contrast, CD80 contributes more towards maintenance through CD28 receptor activation or termination of the ongoing T-cell response through cytotoxic T lymphocyte antigen (CTLA)-4 (CD152) signaling (Sansom and Walker, 2013; Zheng et al., 2004; Walker and Sansom, 2011; Manzotti et al., 2002; Manzotti et al., 2006; Sansom et al., 2003). Other research supports a more regulatory function of CD80 by showing that interaction with programmed death-ligand 1 (PD-L1) can directly suppress T-cell activation (Butte et al., 2007; Dilek et al., 2013).

Interestingly, our previous research indicates that overexpression of miR-146a in human THP-1 monocytes did not inhibit MHC class I or II molecules, CD86, or intercellular adhesion molecule (ICAM)-1 (CD54) surface expression in response to interferon-γ (IFNγ) (Pauley et al., 2011). As a continuation of the study focusing on the impact of miR-146a on innate immunity involving antigen presentation and costimulation, we investigated in this study a potential target of miR-146 in the salivary glands of SjS-prone mice, which may contribute to immune dysregulation. In addition, we narrowed down the subset of cells in the glands responsible for the altered expression of miR-146a and its target molecule.

2. Materials and methods

2.1. Bioinformatics

TargetScan predicts biological targets of miRNAs by searching for the presence of conserved target sites from annotated human and orthologous mRNA UTRs that match the seed region of a particular miRNA. We utilized TargetScan 5.1 (http://www.targetscan.com) analysis to initially identify potential innate immune targets of miR-146a. Human CD80 3′UTR was selected for further analyses based on favorable context score. The potential CD80 3′UTR sequences were identified from both TargetScan analysis as well as from the National Center for Biotechnology Institute (NCBI) nBLAST® alignments to the human CD80 3′UTR. NCBI BLAST® search of the published nucleotide collection (www.ncbi.nlm.nih.gov/Blast) was also used to obtain homologue information for human CD80 3′UTR. The MAFFT 7.110 (http://mafft.cbrc.jp/alignment/server/) multiple sequence alignment program, which utilizes L-INS-I alignment method, was then used for sequence comparison among species. Visualized consensus sequence and percentage identity were created from the JalView 2.0 program applet (http://www.jalview.org/). AREsite prediction in human CD80 3′UTR was carried out by searching the website (http://rna.tbi.univie.ac.at/AREsite/). RNAhybrid 2.2 (http://bibiserv.techfak.uni-bielefeld.de/rnahybrid/) was also used to predict base pairing interactions and minimum fold energy (MFE) between the mature miR-146a target sequence and CD80 mRNA 3′UTR target sequence for mice and humans.

2.2. Cell culture

Human cell lines were obtained from the American Type Culture Collection. HEK293 cell line was grown in Dulbecco's mod-