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Impaired anti-inflammatory activity of PPARγ in the salivary epithelia of Sjögren's syndrome patients imposed by intrinsic NF-κB activation

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ABSTRACT

Sjögren's syndrome (SS) patients manifest inflammation in the salivary glands (SG) and evidence of persistent intrinsic activation of ductal SG epithelial cells (SGEC), demonstrable in non-neoplastic SGEC lines derived from patients (SS-SGEC). The peroxisome-proliferator-activated receptor-γ (PPARγ) mediates important anti-inflammatory activities in epithelial cells. Herein, the comparative analysis of SG biopsies and SGEC lines obtained from SS patients and controls had revealed constitutively reduced PPARγ expression, transcriptional activity and anti-inflammatory function in the ductal epithelia of SS patients that were associated with cell-autonomously activated NF-κB and IL-1β pathways. Transcriptome profiling analysis revealed several differentially expressed proinflammatory and metabolism-related gene sets in SS-SGEC lines. These aberrations largely correlated with the severity of histopathologic lesions, the disease activity and the occurrence of adverse manifestations in SS patients studied, a fact which corroborates the key role of the persistently-activated epithelia in the pathogenesis of both local and systemic features of this disease. The treatment of control SGEC lines with PPARγ agonists was found to diminish the NF-κB activation and apoptosis induced by proinflammatory agents. In addition, the in-vitro application of PPARγ agonists and pharmacologic inhibitors of IL-1β and NF-κB had significant beneficial effects on SS-SGEC lines, such as the restoration of PPARγ functions and the reduction of their intrinsic activation, a fact which may advocate the future clinical study of the above agents as therapeutic modalities for SS.

1. Introduction

Primary Sjögren's syndrome (SS or autoimmune epithelitis) is a relatively common autoimmune disorder that is primarily characterized by chronic lymphoepithelial inflammatory reactions in the exocrine glands, mainly the salivary and lachrymal glands [1]. SS may extend from disease confined to the exocrine glands (organ-specific exocrinopathy) to various extraglandular manifestations (systemic disease) and the development of non-Hodgkin B-cell lymphoma (NHL) [2]. Several lines of evidence presented previously from our group and others indicate that the salivary gland epithelial cells (SGEC) of SS patients have an important role in the development of inflammatory reactions in salivary gland (SG) tissues, as indicated by the presence of an intrinsic (cell-autonomous) activation phenotype in these cells and various functional aberrations thereof [3,4].

Peroxisome proliferator-activated receptor-gamma (PPARγ) is a ligand-activated transcription factor that is widely expressed in mammalian tissues, where it has a broad range of key regulatory effects on adipogenesis, metabolism, inflammation, cellular differentiation, proliferation and survival [5]. PPARγ is activated by diverse ligands, including natural (such as prostaglandins and polyunsaturated fatty acids) and synthetic substances (such as thiazolidinediones) [6]. Upon ligand binding, PPARγ heterodimerizes with retinoid X receptors (RXRs) and positively or

Abbreviations: SS, Sjögren's syndrome; SG, salivary glands; SGEC, ductal salivary gland epithelial cell lines; PPARγ, peroxisome-proliferator-activated receptor-γ; Rosi, Rosiglitazone; Cig, Ciglitazone; DEG, differentially expressed genes.

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negatively regulates the transcription of target genes through binding to PPAR-responsive elements in the promoter regions [7]. In addition to such direct target gene regulation, the direct antagonistic interference of PPARγ with the transcription factors NF-κB, AP1, STAT1 and NFAT has been also proposed [5,8,9]. Importantly, the activation of PPARγ has been associated with the inhibition of inflammatory cell responses, such as the production of inflammatory chemokines, cytokines and iNOS by various types of cells, including epithelial cells [10–13]. Impaired epithelial PPARγ expression and signaling has been previously observed in several human inflammatory diseases whose pathogenesis involves the dysfunction of epithelia [14,15]. Nevertheless, the role of PPARγ has not been previously addressed in the context of SS. Therefore, herein we have comparatively investigated the expression and anti-inflammatory function of PPARγ in salivary epithelia, using SG-tissue biopsy specimens and cultured non-neoplastic ductal SGEC lines obtained from SS patients and non-SS control individuals. Our findings indicate that a significant proportion of SS patients, especially those that manifest severe disease manifestations, display significantly down-regulated in-situ expression of PPARγ in the ductal salivary tissue epithelia. The cultured SGEC lines of SS patients (SS-SGEC) were also found to display constitutively impaired expression and anti-inflammatory function of PPARγ, which were associated with cell-autonomous NF-κB activation and autocrine interleukin-1β (IL-1β) production. The pharmacologic stimulation of PPARγ, as well as the inhibition of basal NF-κB and IL-1β signaling was found capable of ameliorating the cell-autonomously activated phenotype of SS-SGEC lines, a fact which may advocate further evaluation of these pathways as novel treatment targets for SS.

2. Results

2.1. The salivary gland epithelial cells of SS patients manifest significantly reduced in-situ and in-vitro expression of PPARγ that correlate with the occurrence of severe disease manifestations

In all SG biopsies of non-SS controls, the immunohistochemical analysis revealed particularly strong and diffuse cytoplasmic expression of PPARγ protein in the striated and intercalated duct epithelial cells, with only weak expression detected in the nucleus, whereas no expression was observed in the acinar and stromal cells (Fig. 1Aa-b). In the SG biopsy specimens from SS patients (SS-SG biopsies), PPARγ protein expression was also found exclusively on ductal epithelial cells; however it was significantly decreased ($p = 0.0012$) both in the cytoplasm and nuclei of cells, compared to controls (Fig. 1Ac-f and 1B). Such reduced PPARγ expression was consistently observed throughout tissues, both adjacent to immune cell infiltrations (Fig. 1Ac-e) and in areas without evident inflammation (Fig. 1Af). Notably, the expression of PPARγ protein by the ductal epithelial cells of SS-SG tissue specimens was found to strongly and inversely correlate with the severity of the histopathologic lesions ($r = -0.703$, $p = 0.0006$, Fig. 1C). Finally, weak PPARγ expression was also detected in the infiltrating mononuclear cells of SS-SG biopsy specimens, as well as in the occasional resident mononuclear cells in the non-SS control tissues (Supplementary Fig. 1 A-B).

The comparative study of cultured SGEC lines derived from SS patients and controls (SS-SGEC and normal-SGEC, respectively) also revealed significantly lower constitutive PPARγ mRNA and protein expression in SS-SGEC (for $p < 0.0001$ and $p < 0.005$, respectively, Fig. 1D–E), as well as reduced PPARγ binding activity to cognate DNA response elements ($p < 0.05$, Fig. 1F). Among the 36 SS-SGEC lines studied, more than half manifested significantly low PPARγ mRNA expression (Supplementary Table-1). The occurrence of low PPARγ mRNA expression in SS-SGEC lines correlated significantly with several manifestations of severe SS, including heavier mononuclear cell infiltrates ($p = 0.015$), higher disease activity scores (ESSDAI; $p = 0.030$) and higher occurrence of NHL ($p = 0.006$), purpura ($p = 0.019$), type-I disease ($p = 0.039$), serum anti-Ro (SSA) antibodies ($p = 0.017$) and rheumatoid factor ($p = 0.030$) (Supplementary Table-1). In contrast to SGEC lines, no significant difference was observed in the PPARγ expressed by the PBMC of SS patients and non-SS controls (Supplementary Fig. 1C).

2.2. The activation of normal-SGEC by proinflammatory agents is associated with down-regulation of PPARγ expression

The impaired expression of PPARγ in SS-SGEC lines may represent a feature of the intrinsic activation phenotype of these cells [3]. Therefore, we sought to examine whether such aberration can be reproduced in normal-SGEC by in-vitro stimulation with proinflammatory agents. To address such influence of external stimuli on PPARγ expression, normal-SGEC lines were either mock-treated or treated with several cytokines or with TLR3 (PolyI:C) and TLR4 (LPS) ligands and monitored for PPARγ expression in various time points. These experiments indicated that the mRNA and protein expression of PPARγ is temporarily but significantly suppressed in SGEC following treatment with PolyI:C, LPS, IFNγ and IL-1β, whereas it is induced by treatment with IL-4 (Supplementary Figs. 2A and 2B).

2.3. SS-SGEC manifest significant constitutive activation of IL-1β that correlates with decreased PPARγ expression

The down-regulatory effect of proinflammatory agents on PPARγ expressed by normal-SGEC likely indicates a similar effect of autocrine factor(s) produced in-vitro by cultured SS-SGEC. In this context, we assessed the expression of IL-1β by epithelial cells in both SG tissues and SGEC lines, since this cytokine, apart from its suppressive effect on PPARγ expression, is also a well-known autocrine agent of epithelia. In fact, immunohistochemical analyses revealed that compared to controls, the SG biopsy specimens of SS patients manifested significantly increased IL-1β protein expression, that was mainly detected in ductal epithelial cells ($p = 0.023$), as well as in mononuclear infiltrates (Fig. 2Aa-b). Notably, there was a significant inverse correlation ($r = -0.745$, $p = 0.0014$) between the protein expression of IL-1β and that of PPARγ in ductal epithelial cells of the respective SG tissue biopsies tested (Fig. 2Ac). SS-SGEC lines were also found to manifest constitutively significantly increased levels of pro-IL-1β mRNA (Fig. 2B), as well as of IL-1β protein in the cytosols and in culture supernatants (Fig. 2C–D). We have also evaluated the cytosolic extracts of SGEC lines for the expression of active 20kD form of caspase-1, which is known to mediate the cleavage of pro-IL-1β and the extracellular release thereof of the biologically active IL-1β. SS-SGEC lines exhibited higher baseline levels of constitutively active caspase-1, compared to control (Fig. 2E), further corroborating the constitutive activation of IL-1β in the cells of SS patients. Similarly to in-situ observations in ductal SGEC, there was also a linear and inverse correlation between the constitutive expressions of IL-1β and PPARγ in the SGEC lines studied ($r = -0.510$, $p = 0.0013$, Fig. 2F–G).

2.4. PPARγ activation in normal-SGEC dampens cellular responses to proinflammatory stimuli and is protective against cell death induced by TLR3 triggering

To investigate the immunoregulatory function of PPARγ expressed by the SGEC, we questioned whether the activation of

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