Review

Role of TLR7 in the pathogenesis of primary Sjögren's syndrome

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Received on September 30, 2023; accepted in revised form on January 8, 2024.

Clin Exp Rheumatol 2024; 42: 2513-2519.

Key words: primary Sjögren's syndrome, TLR7, pathway, X-chromosome inactivation, pathogenesis

Competing interests: none declared.

ABSTRACT

Primary Sjögren's syndrome (pSS) is an autoimmune disorder characterised by immune-driven damage to the exocrine glands, leading to diminished salivary and tear production. While the pathogenesis of pSS remains incompletely understood, its clinical presentations vary widely, and no specific treatments are currently available. Toll-like receptor 7 (TLR7) belongs to the Toll-like receptor family and is crucial for the innate immune response, notably in recognising pathogenic patterns. TLR7 is predominantly found in the endoplasmic reticulum (ER) and endosomes, where it identifies single-stranded RNA (ssRNA). Upon ligand binding, TLR7 activates the Myd88-dependent signalling cascade, eliciting an immune response. Dysregulation and variations in TLR7 expression are implicated in several autoimmune disorders. In genetically predisposed individuals, factors such as infections, endocrinological abnormality and metabolic abnormalities can cause TLR7 dysregulation, aggravating pSS symptoms and progression. While studies on TLR7 in pSS are limited, they offer insights into the disease's pathophysiological processes, vital for the treatment and prognosis. This article explores the mechanisms of TLR7 dysregulation, its involvement in pSS pathogenesis, and prospective therapeutic significance.

Introduction

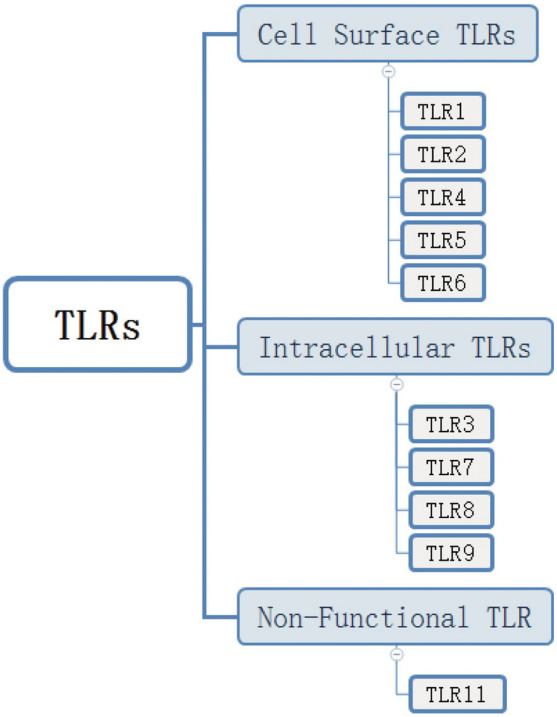
Primary Sjögren's syndrome (pSS) is a chronic autoimmune disorder characterised by mononuclear cell infiltration of exocrine glands, mainly the salivary and lacrimal glands, leading to decreased salivary and tear production (1). Clinically, pSS presents with considerable heterogeneity. While dry eyes and mouth are common symptoms, nearly all organs can be affected. Roughly one-third of patients manifest extraglandular symptoms, including severe fatigue, musculoskeletal pain, polyarthritis, myalgia, vasculitis, interstitial nephritis, and pulmonary complications (2). In China, the disease's estimated prevalence ranges from 0.1% to 0.77%, witnessing an upward trend recently. As per the American College of Rheumatology (ACR) data, females represent approximately 90% of pSS cases, with males constituting a mere 10% (3), this gender distribution is similar in China (4). At present, no specific treatments exist; clinical approaches focus on local and systemic therapies to mitigate symptoms and enhance patient comfort.

The exact pathogenesis of pSS remains elusive. While genetic predispositions contribute to its onset (5, 6), in genetically predisposed individuals, factors such as infections, endocrinological abnormality and metabolic abnormalities can induce aberrant immune responses. This encompasses the atypical activation of T lymphocytes, notably Th1 (7), and upregulated expression of Th17 in affected salivary glands (8). B lymphocyte activation, followed by dendritic cell (DC) activation, culminates in anomalous B lymphocyte activity (9). This process, coupled with the release of I-IFN, establishes a self-reinforcing feedback loop. Various viruses, including Epstein-Barr virus (EBV) (10), coxsackie virus (11), hepatitis C virus (12), cytomegalovirus (CMV) (13), and retrovirus (14) are proposed as potential disease triggers. TLR7, found in plasmacytoid dendritic cells (pDCs) and other immune cells, is an immune receptor that detects viral nucleic acids (15), playing a pivotal role in the initiation and progression of pSS.

The dysregulation of Toll-like receptors (TLRs) has been implicated in

various autoimmune diseases, including pSS. The TLR family is pivotal in recognising pathogens and initiating innate immunity. Highly conserved from drosophila to humans, TLRs share structural and functional similarities. With 11 members in humans, they recognise pathogen-associated molecular patterns (PAMPs) on infectious agents, triggering cytokine production for effective immunity. TLRs exhibit distinct expression patterns for recognising structural components in microorganisms; for instance, TLR-3 and -8 are crucial for single-stranded RNA virus recognition. This variation highlights their specialised roles in host defence. In humans, the TLR family comprises 10 functional members (TLR1-10) and one non-functional pseudogene (TLR11) (Fig. 1). Among them, TLR7, primarily expressed in immune cells, belongs to the Toll-like receptor family. It detects viral structures and cellular nucleic acids, initiating an immune response by binding to single-stranded RNA (ssRNA) molecules and activating immune cells (15). TLR7's role is pivotal in the immune system, particularly regarding viral infections, autoimmune disorders, tumours, and immune regulation. Recent studies link the onset of pSS to TLR7 overactivation, resulting in atypical immune reactions. This article offers a concise review of the research exploring the association between TLR7 and pSS pathogenesis.

Fig. 1. The classification of all the family of TLRs.



Toll-like receptor 7 (TLR7)

TLR7, a member of the Toll-like receptor family, is situated within the intracellular ER and is classified as a type I transmembrane glycoprotein receptor. Its structure comprises a signal peptide sequence, extracellular, transmembrane, and intracellular regions. The signal peptide sequence directs the translated protein to the membrane surface (16). The extracellular region harbours multiple leucine-rich repeat sequences (LRRs) for pathogen recognition and binding. Within this structure, the Z-loop, positioned between LRR14 and LRR15, is integral to the TLR7 ligand recognition mechanism (17). Research by Zhang *et al*. (18) identified two binding sites within this region. The first pertains to small or chemical ligands, inducing TLR7 conformational shifts and activation. The latter associates with ssRNA, bolstering TLR7 aggregation and activity. The transmembrane section has a singular α-helix that anchors TLR7 to the cellular membrane. The intracellular section houses the Toll/IL-1 receptor (TIR) domain, responsible for signal relay and downstream immune activation (19). Within this domain, TRAM, a crucial protein, amplifies the TLR7 signalling pathway's transmission (20). MyD88, a component of the TRAM proteins, dictates the bifurcation of the TLR signalling pathway into MyD88-dependent and independent routes. Notably, TLR7 employs the MyD88-dependent path (21). TLR7 is found in an array of immune cells such as monocytes, macrophages, pDCs, B cells, microglia, and dendritic cells (22, 23), with a notably high expression in human pDCs. Its expression is also profuse in the heart, spleen, bone marrow, and lymph nodes (24).

TLR7 primarily recognises ssRNA rich in guanosine and uridine, encompassing a range of viral and bacterial RNAs (25, 26). Certain chemically synthesised small molecules, like imiquimod and R848, can emulate the structure of natural RNA, consequently activating the TLR7 pathway (24). Known inhibitors of TLR7 encompass hydroxychloroquine (27), small molecules AT791 and E6446 (28), and Toll-like receptor dual agonists (29).

TLR7 activates the immune system through the MyD88-dependent pathway. Upon ligand binding, TLR7 forms a MyD88-associated complex (Myddosome) involving IRAK-4, IRAK-1, and TRAF6, initiating downstream events (31). TRAF6 engages with TAK1, TAB1, TAB2, and TAB3, forming a complex with UBC13 and UEV1A. TAK1, a MAPK kinase, modulates various pathways, including NF-κB. TAB1 orchestrates protein kinase activation, and TAB2 activates NF-κB and JNK effectors via multi-ubiquitin chains (32, 33). UBC13, a ubiquitin-conjugating enzyme, and UEV1A, an E2 variant, trigger TAK1 activation, phosphorylating the IKK complex and MAP kinase. The IKK complex (IKKα, IKKβ, NEMO/IKKγ) then initiates NF-κB translation, and phosphorylated MAP kinase promotes AP-1 transcription factor translation (34-40). These pathways culminate in cytokine synthesis (I-IFN, IL-12, TNF-α), contributing to pSS pathogenesis (Fig. 2).

Punnanitinont *et al*. (41) employed the NOD.B10Sn-H2b mouse model

to determine the effects of the TLR7 agonist, Imiquimod. They discovered it promotes T-bet+ B cells' proliferation, subsequently hastening the progression of both local and systemic autoimmune reaction in pSS. Another investigation (42) utilising TLR8-deficient (TLR8ko) mice upon stimulation with TLR7 agonists unveiled symptoms of pSS, such as lymphocytic inflammation in exocrine glands, production of anti-SSA and anti-SSB autoantibodies, abnormal level of multiple cytokines, immune complex deposition, and frequent lung inflammation. Additionally, in TLR8ko mice, the exocrine glands displayed ectopic lymphoid structures characterised by B/T cell clustering regions. However, such manifestations were absent in the double TLR7/8 deficient mice, underscoring TLR7's significant influence on pSS development. In another study, Savarese *et al.* (43) observed that introducing a small nucleolar ribonucleic acid, U1snRNA, and oligonucleotides from U1snRNA to pDCs directly induces TLR7-mediated I-IFN release in specific C57BL/6 mouse models. These animal models collectively highlight the pivotal role TLR7 plays in the initiation and advancement of pSS. Research comparing TLR7 deficient and wild-type mice underscored TLR7's significance in autoimmune disease evolution, revealing that its absence slows autoimmune disease progression in mice. Notably, TLR7 exhibits high expression in the lacrimal gland of wild-type mice (20).

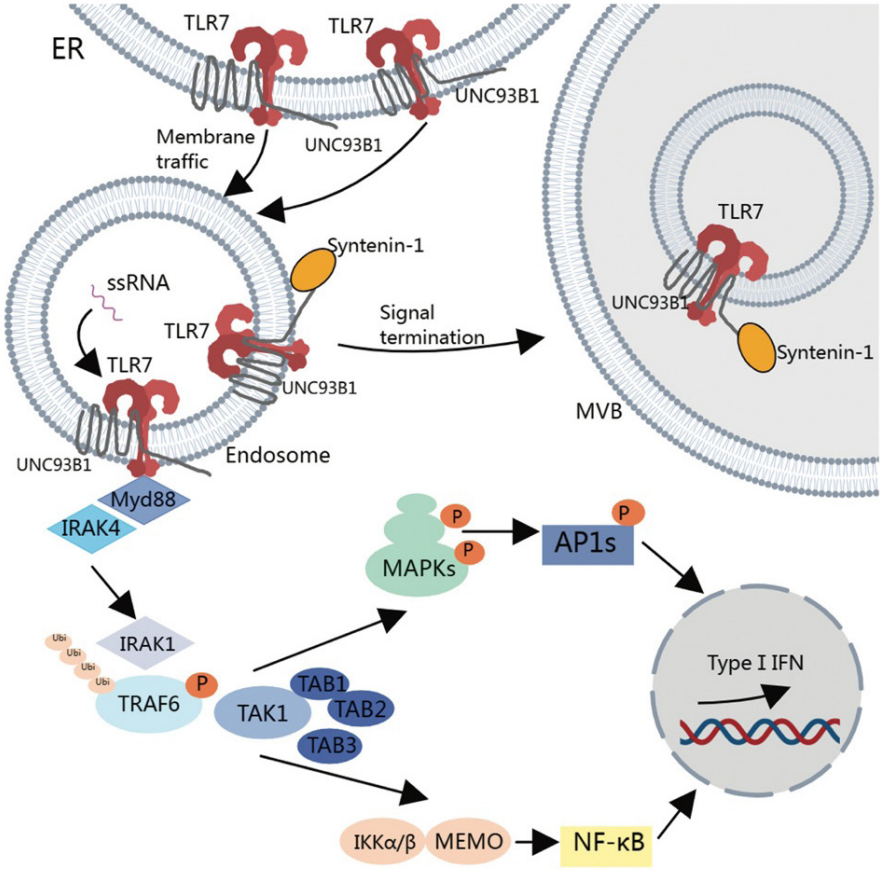


Fig. 2. Intracellular TLR7 trafficking and signalling.
Upon TLR7 activation, the cytoplasmic adapter protein MyD88 forms a complex (Myddosome) with IRAK-4, IRAK-1, and TRAF6. Downstream signalling involves TRAF6 interacting with TAK1, TAB1, TAB2, and TAB3, along with UBC13 and UEV1A. TAK1, a MAPK kinase, modulates pathways including NF-κB activation, while TAB1 and TAB2 contribute to protein kinase activation and NF-κB/JNK effector activation through multi-ubiquitin chains, respectively. UBC13 and UEV1A play roles in non-canonical ubiquitination and multi-ubiquitin chain synthesis. The assembled complex activates TAK1, leading to the phosphorylation of the IKK complex and MAP kinase. This results in the translation of NF-κB and AP-1 transcription factors, culminating in the synthesis of cytokines (I-IFN, IL-12, and TNF-α) implicated in pSS pathogenesis.

TLR7 and pSS pathogenesis

The impact of TLR7 gene polymorphisms on pSS susceptibility

Gene polymorphism denotes the presence of multiple variants of a single gene among diverse individuals, potentially influencing the onset and progression of specific diseases. In the context of autoimmune diseases like pSS, research has highlighted a strong association between splicing variations and single nucleotide polymorphisms (SNPs) of the TLR7 gene and the disease's onset and progression. Additionally, polymorphisms within certain human leukocyte antigen (HLA) genes have been linked to the emergence of pSS. Such genetic variations can impact the immune system's capacity for antigen recognition and processing, thereby elevating the risk of pSS development.

It is noted that TLR7 is implicated in the CMV immune response (44). In a study conducted by Arav-Boger *et al.* (5), during CMV infection, of the four TLR7 SNPs, those homozygous for the minor allele displayed an elevated antibody response compared to either heterozygotes or homozygotes carrying the common allele. Notably, rs179008 and rs179009 in TLR7 were significantly correlated with this antibody response, along with the association of CMV infection and the onset of pSS (5) suggesting that TLR7 gene polymorphism may influence disease development during viral infections.

Researchers from various countries conducted gene sequencing and comparative analyses on patients with systemic lupus erythematosus (SLE) *versus* the healthy controls of Egypt (45), Denmark (46), East Asia (Korea, China, Japan) (47). Their findings consistently indicated that, relative to the general population, the CG genotype of TLR7- rs3853839 and the G-allele of TLR7- rs3853839 are more prevalent among SLE patients, they are also associated with an increased risk of SLE in African Americans, Native Americans, and European Americans/Hispanic Americans (48). This mutation induces an overactive TLR7 signalling pathway, prompting the immune system to target its own tissues. Furthermore, in Japanese women, the TLR7 SNPs rs179019 and rs179010 are

linked to the onset of SLE and remain unaffected by TLR7- rs3853839 (49). These studies offer potential insights into the effects of TLR7 gene polymorphism on pSS vulnerability.

The multichannel transmembrane protein, UNC93B1, resident in the ER, plays a pivotal role in determining the localisation and functional activity of TLR7. UNC93B1 facilitates the transit of TLR7 from the ER to the endosomes, where TLR7 exerts its role (74). Concurrently, the C-terminal tail of UNC93B1 interacts with syntenin-1, forming a complex (Fig. 1). This complex aids the translocation of activated TLR7 into intraluminal vesicles for subsequent degradation or sequestration (45), a mechanism that curtails TLR7 signal transduction and thwarts autoimmune reactions (46). Additionally, UNC93B1 modulates and dampens the overactivation of TLR7 via TLR9 (52). Research by Majer *et al*. (51) identified multiple mutations in UNC93B1 that can amplify TLR7 signalling in macrophages, such as PRQ(524-526)/AAA, PKP(530-532)/AAA, DNS(545-547)/AAA, and DES(548-550)/AAA.

Impact of abnormal TLR7 expression on the pathogenesis of pSS

In the progression of pSS, there is a prominent involvement of the overproduction of Type I interferon (IFN-I). The Type I IFN signature, comprising a set of Type I interferon-inducible genes (IFIGs), encapsulates the overarching effects of IFN, allowing for the assessment of its impact on various immune cells and responses (53). A significant proportion, over half of pSS patients, exhibit elevated expression levels of this signature, correlating with intensified disease activity and increased autoantibody concentrations (54).

Research by Maria *et al*. (55) demonstrated that TLR7 is markedly upregulated in IFIGs-positive pSS patients, along with the RNA sensors RIG-I and MDA5, a finding echoed by Zheng *et al*. (56). Additionally, in the salivary glands of pSS patients, TLR7-positive cells are found in not only ductal epithelial cells but also epithelial islands and lymphocytes, which is different from the control group where they are confined to the ductal epithelial cells (56). Beyond mRNA analysis, Karlsen *et al*. (57) identified enhanced TLR7 expression in pSS patients' peripheral blood mononuclear cells through Western blotting. Japanese research pinpointed the predominant expression in pSS patients' lip glands to be TLR-7, which concurrently expresses with MyD88, TRAF6, and Interferon regulator factor 7 (IRF7) (58). Cumulatively, these investigations suggest that aberrant TLR7 expression potentially instigates irregular immune system activation, intensifying the manifestation and progression of pSS.

Impact of abnormal TLR7 activation on the pathogenesis of pSS

The distinction pDCs draw between viral and self-cellular nucleic acids is mediated through the intracellular localisation of TLR7 and other associated TLRs. Unlike viral nucleic acids, those released by self-cells degrade rapidly in the extracellular environment and usually cannot penetrate pDCs under typical conditions (59). In the context of autoimmune diseases, nucleic acids released from self-cells, when internalised by pDCs, can activate these cells through TLR7. Specifically, when self-RNA complexes with the endogenous antimicrobial peptide LL37, it gains access to the pDC's ER, subsequently triggering TLR7 (60). A study by Salvi *et al*. (61) revealed that exosomes derived from the serum of SLE patients can activate pDCs *ex vivo*, culminating in I-IFN secretion. This stimulatory effect can also be achieved using microRNA extracted from exosomes, indicating that microRNA might serve as an intrinsic ligand for TLR7 activation in autoimmune scenarios (43).

Factors influencing TLR7 expression and function

Several factors, including genetics, sex hormones, infections, and endogenous elements, influence TLR7 expression and function. Collectively, these elements elevate TLR7 expression, contributing to the emergence of pSS.

Notably, the prevalence of pSS is significantly higher in women than in men, with a ratio of approximately 9:1 (3). Recent research has shed light on the potential roles of oestrogen and X chromosome dosage in the occurrence of pSS.

Interferon-α (IFN-α), mediated by TLR7, activates both IRF7 and IRF5/NF-κB pathways, which in turn stimulate the synthesis of pro-inflammatory cytokines. This establishes the significant immunoregulatory function of TLR7-mediated IFN-α within pDCs (62). In mouse models, Panchanathan *et al*. (63, 64) observed that oestrogen signalling elevates the expression levels of the TLR7 transmembrane protein Unc93b1 and the IRF5 gene in immune cells. Comparative studies indicate a diminished TLR7-mediated immune response in postmenopausal women's pDCs, relative to their premenopausal counterparts (65). This intimates a potential analogous function of oestrogen in human immune cells, warranting further investigation.

Laffont *et al*. (65) utilised quantitative PCR flow cytometry to determine that oestradiol activates the oestrogen receptor α (ERα) in mouse pDCs, augmenting their capability to produce IFN-α and pro-inflammatory cytokines upon TLR7 and TLR9-mediated stimulation. Moreover, the team investigated the ER gene's expression in human pDCs to discern potential gender-related disparities. Notably, while ERα expression in female pDCs marginally exceeded that in males, the variance was not statistically significant. Hence, alternate mechanisms might mediate the influence of oestrogen receptor signalling on TLR7 expression and IFN-α synthesis.

X-chromosome inactivation (XCI) in female mammals is a process by which one X chromosome is randomly inactivated to maintain gene dosage equilibrium (59). In human cells, however, XCI is incomplete. Using single-cell transcriptomics and genomic sequencing, Tukiainen *et al*. (66) determined that nearly one-third of the X-chromosome genes in female cells exhibit biallelic expression. This variable expression across individuals is known as escape from XCI. Notably, the TLR7 gene, situated on the X chromosome's short arm, belongs to a non-homologous segment (67) and may display this escape phenomenon. Through single-cell RNA

sequencing, Souyris *et al*. (68) found that TLR7 in female immune cells evades XCI, resulting in expression levels double that of males. Similarly, TLR7 in Klinefelter patients (46, XXY) also shows biallelic expression, twice the rate seen in typical males. Interestingly, Scofield *et al*. (69) recorded an uptick in autoimmune diseases among the Klinefelter population. The gene Chromosome X open reading frame 21 (CXorf21), encoded on the X chromosome, sees enhanced expression when TLR7 binds to its ligand. Disrupting CXorf21 hampers the TLR7-mediated production of IFN-α (70). Recent findings indicate that females express CXorf21 at higher levels in single nucleated cells and B cells than males, suggesting a possible escape from XCI (71). Collectively, the data insinuate that variations in X chromosome dosage and the phenomenon of gene escape from XCI might influence the prevalence of pSS.

The Y-linked autoimmune accelerator (Yaa) locus is a potent allele associated with autoimmunity, causing aberrations in TLR7-mediated innate immune reactions (72). Through transcriptomic analysis, Subramania *et al*. (73) studied the expression patterns of the X chromosome gene cluster in B cells from male mice harbouring the Yaa. They found that the translocation of the TLR7 gene to the Yaa chromosome resulted in its twofold overexpression, which is sufficient to perturb the innate immune response mediated by TLR7.

The transportation mechanism of TLR7 can influence its expression and functionality within the ER. Petes *et al*. (74) suggest that agents such as chloroquine or hydroxychloroquine can potentially modify the folding and transportation of TLR7 within the ER, leading to diminished expression levels. Additionally, stress within the ER may prompt TLR7 aggregation and subsequent degradation.

Furthermore, TLR7 sensitivity contributes to the development of pSS. Research by Bekeredjian-Ding *et al*. (75) indicates that pDCs and I-IFN secretion modulate the responsiveness of juvenile B cells to TLR7 ligands. These factors notably amplify the TLR7 sensitivity in both juvenile B cells and memory B cells.

TLR7 and pSS treatment

Recent research indicates that TLR7 plays a role in the pathogenesis of pSS by facilitating the production of autoantibodies and instigating inflammatory responses. Such insights could be pivotal for advancing pSS treatment.

Recent studies offer promising directions for the development of innovative therapeutic approaches.

Modulating the upstream signals of TLR7 can alter the TLR7 signalling pathway, potentially offering a therapeutic avenue for disease treatment. Bekeredjian-Ding *et al*. (75) emphasised the potential benefits of adjusting TLR7 sensitivity in immature B cells. They proposed that targeting pDCs and I-IFN signalling pathways might effectively regulate TLR7-mediated B-cell activation, consequently lowering the risk of pSS. Additionally, there exists a reciprocal amplification between TLR7 and IFN; the activation of TLR7 stimulates I-IFN production, which in turn boosts TLR7 signalling and immune response. This suggests that future therapeutic interventions could involve drugs targeting TLR7 and agents that modulate IFN production (76). Given the roles of UNC93B1 and syntenin-1 in the TLR7 signalling process (Fig. 1), strategies that regulate these entities present plausible therapeutic options (50-52). Furthermore, the transport mechanism of TLR7 can influence its expression and function within the ER. Agents like chloroquine or hydroxychloroquine might modulate the folding and transport of TLR7 in the ER, subsequently reducing its expression and fulfilling therapeutic goals (74).

The activation of TLR7 can be modulated for therapeutic purposes. In their research, Salvi *et al*. (61) employed synthesised microRNA to pinpoint an IFN-inducible motif that is vital for TLR7-dependent activation, maturation, and survival of human pDCs. Their findings suggest that exosome-delivered microRNA serves as an endogenous ligand of TLR7, contributing to pSS onset. This underscores the potential role of microRNA as a novel pathogenic factor and a potential therapeutic target for IFN-mediated diseases.

Modulating the downstream signalling pathway of TLR7 presents a potential therapeutic approach for pSS. Upon ligand binding to TLR7, its TIR domain interacts with various proteins, initiating downstream signalling. This TIR domain is therefore a critical target for drug development aimed at TLR7 signalling. Decoy peptide inhibitors, derived from functional protein interactions, maintain the binding affinity of the original protein to the target protein's binding site, thereby inhibiting signal transmission. Given their ability to traverse cell membranes and operate intracellularly, cell-penetrating decoy peptide inhibitors hold promise for drug development and pSS treatment (77).

Continued research is imperative to validate the efficacy of these prospective therapeutic targets and to formulate associated treatment strategies.

Conclusion

In conclusion, our comprehensive review sheds light on the multifaceted impact of TLR7 in the pathogenesis of pSS. The intricate interplay between TLR7 gene polymorphisms, abnormal TLR7 expression, and activation underscores its pivotal role in shaping the immune dysregulation observed in pSS patients. Genetic variations within TLR7, particularly those associated with splicing variations and single nucleotide polymorphisms, contribute to the susceptibility and progression of pSS. While current research on TLR7 in pSS patients remains limited, the correlation between TLR7 and pSS is not fully elucidated. Nonetheless, these investigations provide insights into the underlying pathophysiological mechanisms of pSS, holding potential significance for diagnosis, disease activity monitoring, innovative treatments, and prognosis of pSS.

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