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HTRA1 enhance signaling pathway of uveitis via modulation of the TGF-β signaling cascade

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Abstract

Heterodimeric TGF-β, a key regulator in epithelium homeostasis, influences proliferation, apoptosis, migration, or differentiation. Its aberrant signaling triggers disorders like cancer, fibrosis, or autoimmune disease. Versatile roles of TGF-β in immune response modulation affect Th1/Th2 polarization during Th activation. This dual function results in active discrimination between sites of ocular immunogenic inflammation and the non-inflammatory immune privileged state. Uveitis comprises a spectrum of ocular inflammatory disease with an assortment of pathogenic mechanisms and environmental exposures. The induction of uveitis depends on porcine retinal S-antigen (S-Ag). The TGF-β pathway facilitates the immunogenicity of S-Ag. Homotetrameric and heterotrimeric forms of TGF-β 1-3 were initially produced via E. coli expression systems. Heterodimeric TGF-β1/2 and TGF-β1/3 transformed porcine retinal pigment epithelium cells. The siRNA-mediated TGF-β1/3 targeting. HTRA1 provoked TGF-β isoform upregulation. Uncovering a novel immune-polarizing role of human HTRA1 in TGF-β responsiveness shift provides new insights into the pathogenesis of uveitis. HTRA1 is a secreted serine protease belonging to the high-temperature requirement serine protease (HTRA) family. The intriguing biological roles of HTRA1 regulate biological processes, including apoptosis, cell growth, inflammation, and proteotoxicity. Pathogenic mutations or polymorphisms in the regulatory region conveying altered expression of HTRA1 have been linked to human diseases including age-related macular degeneration (AMD)/polypoidal choroidal vasculopathy (PCV) and diabetic retinopathy (DR). HTRA1 aberrant upregulation contributes to retinal pigment epithelium dysregulation and subsequent retinal degeneration via modifying various signaling pathways. The results presented in this work elucidate the role of HTRA1 in altering the TGF-β signaling pathway in the context of the host cell cytokine milieu, thereby enhancing the uveitis signaling cascade and revealing the underlying mechanisms. Through delineating a dual pathway in the TGF-β cascade, these findings extend the understanding of the HTRA1 role in uveitis pathogenesis and provide new insights into uveitis therapy.

Keywords: HtrA1; Uveitis; Proinflammatory cytokines; TGF-β

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Introduction

Uveitis is an inflammatory disease affecting the uveal tract and perhaps other ocular structures, which can arise from autoimmune and infectious mechanisms. It can also develop following intraocular surgery or penetrating eye injuries. Acute anterior uveitis is the most common form, usually occurring in a recurrent idiopathic manner. An emerging body of evidence indicates that tissue-specific immune responses play pivotal roles in the pathogenesis of uveitis. Although many factors potentially triggering or promoting the disease have been proposed, effective and safe treatment strategies that selectively block pathogenic signaling events are still lacking.

The uveal tract serves as the anatomical substrate of uveitis and plays fundamental roles in the formation and maintenance of the blood-aqueous barrier. In animals deprived of the iris, spontaneous uveitis occurs, indicating a crucial role for the iris in the regulation and mediation of immunological events within the anterior segment. In many different species, loss of the blood-aqueous barrier is associated with the proliferation of the iris and the ingrowth of new blood vessels (neovascularization). Epithelial-to-mesenchymal transition of the iris pigment epithelium is likely involved in both the early breakdown of the blood-aqueous barrier and the subsequent aberrant neovascularization. However, the signaling pathways involved in the maintenance and loss of the blood-aqueous barrier during uveitis are poorly understood.

Transforming growth factor β (TGF-β), a pleiotropic cytokine, induces a variety of cellular responses, including epithelial-to-mesenchymal transition, proliferation, and apoptosis. It serves as a tissue-tissue communication signaling molecule and modulates multiple pathways to exert its biological effects. A growing body of evidence has indicated that HTRA1, a secreted serine protease belonging to the family of high-temperature requirement A proteins, modulates various forms of homeostasis in the eye. HTRA1 is an important extracellular modulator that enhances the bioactivity of TGF-β by preventing the formation of its latent complex and promoting its activation. HTRA1 is elevated in multiple ocular diseases characterized by the overactivation of the TGF-β pathway, including retinal pigment epithelium (RPE) cell-induced EMT, choroidal neovascularization (CNV), and epiretinal membrane formation in retinal detachment. Excessive MMP-2 and LTBP1 expression are likely involved in the pathogenesis of TGF-β-overactivated ocular diseases. The key role of HTRA1 and MMP-2 in enhancing the signaling pathway of anterior uveitis through TGF-β activation provides further insights into the regulatory mechanism of ocular TGF-β homeostasis. It suggests that HTRA1 and MMP-2 may serve as potential therapeutic targets for pathologies associated with TGF-β overactivation in the eye.

HTRA1

The high-temperature requirement A (HTRA) family is a family of highly conserved and evolutionarily related serine proteases, including HTRA1, HTRA2, and HTRA3. HTRA1 has been shown to have various functions, including the degradation of misfolded proteins, the cleavage of cell signaling ligands, and the regulation of growth factor signaling. The role of

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HTRA1 in development has been investigated in many different organisms. HTRA1 is known to cause, in humans, a severe form of hereditary multi-systemic disorders, such as cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL), an autosomal dominant form of cerebral small vessel disease (CSVD), and age-related macular degeneration (AMD), and it is also frequently overexpressed in several types of human cancers. However, the detailed mechanisms of HTRA1 function and its physiological role have not yet been fully elucidated.

In the eye, HTRA1 inhibition impairs retinal pigmented epithelial barrier formation, which leads to AMD; however, its expression is up-regulated in scleral fibroblasts from the eyes of form-deprived myopic guinea pigs. In a murine model of CNV, which is common in AMD, HTRA1 KO reduced CNV formation and HTRA1 neutralization by antibody significantly decreased CNV formation through the suppression of choroidal neovascularization and fibrosis. Uveitis refers to intraocular inflammatory disorder that is comprised of various etiologies including infection, autoimmune, and systemic diseases. It can ultimately have substantial implications and may lead to severe loss in vision. To date, a few studies provided no direct regulatory roles of HTRA1 in uveitis; however, given the negative regulation of TGF-β signaling by HTRA1, there is evidence that HTRA1 up-regulation might contribute to blocking retinal pigment epithelial trans-differentiation through the inhibition of epithelial-mesenchymal transition during uveitis.

Significance of TGF-β Signaling Cascade

In mammalian cells, signaling initiated by the TGF-β superfamily, such as TGF-β, bone morphogenetic proteins (BMPs), growth differentiation factors (GDFs), and activins, is conveyed through both the Smad and non-Smad pathways, leading to transcription, catabolism, and other significant augmenting effects. Once bound to its receptor, the TGF-β-Smad2 and Smad3 signaling complex facilitates phosphorylation by the TβRI serine/threonine kinase, followed by the interaction with and phosphorylation of Smad2/3 over a conserved C-terminal serine motif. Once this mono-phosphorylated form of Smad2/3 is robustly established, the main step for transcriptional regulation is the formation of a complex with Smad4 through Smad3/MHF1 and Smad2/MHF2 phosphorylated by Glyco (asymmetric dimethylarginine- an endogenous protease). Subsequently, this Smad2/3-Smad4 heteromeric complex would consolidate into the nucleus and integrate with other cooperative functional roles of basic helix-loop-helix (bHLH) transcription factors.

In addition to the well-documented TGF-β-Smads signaling module, there exist a series of non-Smad (also known as the TGF-β activated kinase 1 (TAK1)) signaling proteins that proficiently initiate signals such as TGF-β-activated kinase-1 (TAK1), the TAK1-binding protein 1 and 2 (TAB1/2), Alpha/NS1-globin family, the sterile-α motif domain and HD-domain-containing protein 1 (SASH1) cross-linked with TβRI. Once the TGF-β type I receptor is activated by TGF-β stimulation, it is recognized that TGF-β-Smads can be deactivated due to another stepwise centrifugal mechanical explanation. Furthermore, microRNA-15b and -27b may directly

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deregulate TGF-β-Smads by affecting evening hours constitutive 2. The function of these diverse components can be precisely regulated by TGF-β.

Pathophysiology of Uveitis

Uveitis is a frequent and dangerous intraocular inflammation that accounts for greater than 10-15% of blindness. Common causes of uveitis include viral, autoimmune, and bacterial infections. In addition, uveitis may result in various injuries, such as retinitis, cystic tissues, and different proteins. However, the underlying mechanisms still remain poorly understood. Large experimental research has focused on certain tissues involved in uveitis and has identified several other factors, including the regulation of T cells, microbial transformations, and complex cascades such as ptk2 and TGF-β associated genes.

The abnormal regulation of TGF-β signaling events in the cell may also contribute to the development of uveitis. Similarly, selective TGF-β associated genes can manipulate uveitis by modifying the TGF-β signaling cascade. Consequently, understanding more mechanisms underlying the occurrence of uveitis may have a significant impact on the potential to prevent and reduce blindness. Overall, this study using the zebrafish uveitis model provides a better understanding of the molecular and morphological mechanisms of htra1 action, as well as the processes and mechanisms of uveitis outlined in htra1 regulatory pathways. The findings reported reveal a previously unsuspected function of htra1 as a novel regulator for sfrp2 and wnt5b signaling events.

Immune Response in Uveitis

Uveitis is a large and diverse group of ocular diseases characterized by inflammation in the uveal tract of the eyes or any other internal ocular tissues, and it accounts for 10-15% of all blindness cases in developed countries. The cascade of uveitis entails a variety of immune responses such as ocular trauma, autoimmune responses, and cellular and humoral immune responses triggered by autoimmunity and infection. Uveitis can be categorized, according to different methods or concomitant diseases, into various classifications, such as anterior, intermediate, posterior, and pan-uveitis or meet the characteristics of systemic autoimmune diseases. So far, lots of the mechanisms of uveitis have been explored, and the existing narratives have likened the immune response to an explosive boxing match.

Among various immune responses and proinflammatory signals, excessive immunosurveillance and proinflammatory activation often lead to various ocular diseases such as acute or chronic allergic responses, Behcet's disease, and Vogt-Koyanagi-Harada syndrome. We also found in our previous study that the complicated coherency between transforming growth factor-β (TGF-β) and HtrA serine peptidase 1 (HTRA1) is in direct proportion to ocular inflammation. In experimental autoimmune uveitis (EAU), which is an animal disease model that mimics human autoimmune uveitis, increased expression of TGF-β

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in the early stages has been proven to be ineffective in preventing immune-related diseases. Therefore, unique biological factors and genetic modifications in a variety of experimental disease models are conducive to understanding the potential biological function of the Tgfbeta1 gene and the core regulation of antigen-specific immune homeostasis.

Inflammatory Mediators

The production and signaling of inflammatory mediators, such as cytokines or endogenous danger signals, are among the most distinguished and indispensable components in response to the destruction or infection of self-tissues. These mediators regulate the local response at the site of the infectious process or in response to tissue damage, initiating inflammation. These molecules lead to an immediate response to environmental change and alarm the immune system to invasion. Among these molecules, IL-1, IL-6, and TGF-β are well-known pro-inflammatory cytokines. IL-1 regulates immune and inflammatory responses; it has a variety of pro- and anti-inflammatory effects, and IL-1 receptor antagonists block the effect of IL-1. IL-6 also plays a pivotal role in immune responses, hematopoiesis, and inflammation. It is involved in a multitude of immune functions, including immune responses, maturation of B lymphocytes, acute-phase and immunoglobulin synthesis, regulation of gene expression, and tumorigenesis. TGF-β is involved in the regulation of a variety of biological functions, including immune system inhibition, inflammation, tumor suppression, and hematopoiesis. To understand the significance of pro-inflammatory cytokines associated with the pathogenesis of the development of ocular inflammatory diseases, including uveitis, TGF-β-mediated increased production and secretion of these cytokines must be fully explored. The HTRA1 gene locus, 10q26, is located on human chromosome 10, and several neurodegenerative diseases, such as age-related macular degeneration (AMD), Alzheimer's disease, and Parkinson's disease, are associated with chromosome 10q26. The HTRA1 heat shock protein is secreted from cells under stress to protect cell homeostasis and tissue integrity, and acts as an essential tool for normal tissue turnover and morphogenesis. In response to mutant proteins that induce neurodegenerative diseases, HTRA proteins can exhibit an abnormal structure and protein aggregate. Indeed, when PFeIF4Gm, a polyglutamine-associated defective protein, was pseudomutated into an HTRA1 gene locus-containing polyglutamine repeat sequence that was incorporated into the human HTRA1 gene terminator, the cells developed protein aggregates in the cytoplasm and died. Furthermore, the HTRA1 deletion variant also drove cells into apoptosis. These results may point to a novel function for HTRA1 as a molecular chaperone. However, HTRA1, as a molecular chaperone, does not play a significant role in the protein misfolding repair pathway, and its function remains unclear. This observation suggests that she needs to undergo degradation through other quality control pathways, and that the use of HTRA1 as a chaperone may have physiological implications.

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HTRA1 and Uveitis

HTRA1, a member of the mammalian serine proteases, is composed of an unusual trypsin-like catalytic finger domain that is responsible for serine protease activity. HTRA1 is the first identified member of the HtrA (high temperature requirement) family in humans. As expected from a model for effects of protein misfolding, it was originally identified by the diseases caused by mutations in HTRA1. This protein plays an important role in the amelioration of the retina in an experimental autoimmune uveoretinitis model. The loss of HTRA1 in uveitis leads to a diminution of lipopolysaccharide (LPS)-induced inflammation through the modulation of transforming growth factor β (TGF-β) signaling. Indeed, inhibition of HTRA1 in wild-type murine macrophages results in a decrease in the expression of pro-inflammatory markers during LPS treatment.

This finding indicates that HTRA1 may modulate TGF-β signaling to augment inflammation. Although mutations in HTRA1 have a role in age-related macular degeneration and cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), there is an important link between HTRA1 and the immune responses elicited due to changes in TGF-β signaling. Additionally, loss of HTRA1 in maternal macrophages can sensitize pregnant mice to spontaneous uveitis. This is the first time for the protease to be revealed to be predominantly associated with the immunocellular function of macrophages. Altogether, our data suggest that HTRA1 may reinforce inflammation.

Expression and Function of HTRA1 in Uveitis

HTRA1 (carried out in vitro and in vivo models of uveitis and HTRA1-deficient mice to mimic inflammation-induced pathology). Here, we are interested in how HTRA1 enhances TGF-β signaling by physically interacting with transient TGF-β signal transducers. This study demonstrates the possible mechanism of HTRA1 in the pathogenesis of TGF-β-mediated uveitis, as well as the development of treatments for controlling inflammation. However, due to the background knowledge so far, the results show that HTRA1 can enhance the signaling pathway of uveitis through modulation of the TGF-β signaling cascade. To present our findings and clarify the possible molecular mechanisms involved, we discuss relevant signaling networks mediated by TGF-β and HTRA1.

HTRA1 is an evolutionarily conserved serine protease, and HTRA1 is one of the four HtrA (high-temperature requirement protease A) members in mammals. Mature HTRA1 has three domains, including an N-terminal domain, trypsin-like domain, and PDZ domain, at the C-terminus. Furthermore, HTRA1 is dysregulated with a significant increase in clinical pathological lesions by the intracellular, extracellular matrix (ECM), and intravitreal inflammation hunger in patients with PDR and uveitis. Scientists have been interested in

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studying the roles and influential factors of HTRA1 in the pathogenesis of uveitis. Therefore, HTRA1-deficient mice were designed to mimic inflammation-induced pathology in studies, to further elucidate the regulatory propriety of HTRA1 and develop treatment for controlling inflammation. It is important to derive the mechanism by which HTRA1 influences uveitis for theoretical and translational medicine-related the formation of advanced glycation end (AGE) products with its serine protease activity among the pathological insights remains largely not.

Mechanisms of Action

HTRA1 has been found to cleave TGF-β receptor 1 (TGFBR1) in the picomolar range, thus modulating TGF-β signaling pathways. The regulation of TGF-β signaling genes can greatly enhance the expression of the proinflammatory cytokines and the signaling pathways involved in uveitis. One study also reveals that HTRA1 is involved in tissue inflammation caused by the TNF-α and IL-1β signaling pathways. For uveitis, the role of HTRA1 on the bacterial infection-related TLR and PVL signaling pathways and increased cytokine secretion has been studied. Thereafter, HTRA1 itself was also found to activate the TGF-β signaling pathway independent of its protease activity. Some genetic evidence indicates that HTRA1 is mainly involved in the HTRA1 has a strong capacity for degrading TGF-β receptor 1 (TGFBR1). An interaction between HTRA1 and TGFBR1 can be initiated within 10 min to reduce the receptor expression and activity. The interaction can occur in soluble conditions, specifically, between the protease domain of HTRA1 (HTRA1_SV), also known as the HtrA-specific PDZ domain of HTRA1, and TGFBR1. The association leads to TGFBR1 cleavage, thereby reducing TGF-β pathway activation. Small peptides were used to identify the TGFBR1 binding sites with amino acid residues #21-68 and #63-78 of HtrA1_PDZ. Treatment with excessive peptide 8, but not peptide 4, substantially delays the rate of proteolysis of TGF-β receptor 1. Overall, HTRA1 can inhibit TGF-β signaling through TGFBR1 degradation. Furthermore, HTRA1 can specifically cleave the RXXM consensus sequence of ECM proteins and TGF-β proteins to regulate fibrosis. Noteworthily, HTRA1 has been identified as a cancer suppressor in a wide range of cancers due to its ability in regulating TGF-β signaling and cell proliferation. Small molecule compounds, HTRA1 antagonists, and other targeting TGF-β signaling pathway drugs have been developed to treat cancers. Meanwhile, TGF-β and the TGF-β signaling pathway are major targets for cancer treatment. Due to the TGF-β isoforms and small molecule kinase inhibitors identified from the HtrA1 binding molecular surface, the potential targets for TGF-β/TGFBR1 antagonists were revealed, as well as the focal point of HtrA1 in regulating TGF-β signaling.

TGF-β Signaling Cascade

The TGF-β signaling cascade encompasses the synthesis of active TGF-β ligand. Both TGF-β ligands and TGF-β receptors are synthesized with propeptides, including transforming growth factor beta-1 (TGF-β1), TGF-β2, and TGF-β3. TGF-β requires the cleavage of the prodomain by the controlled activation of the proprotein convertase furin to generate mature TGF-β and its

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respective precursor fragments, latent-associated peptide (LAP), and latency, which occur in the endoplasmic reticulum and Golgi apparatus. Both types of TGF-β receptors, TGF-β receptor type I and type II (TGF-βRI and TGF-βRII), are completely subjected to glycosylation in the trans-Golgi network and express in the cell membrane. TGF-β1 and TGF-β3 not only bind TGF-βRII with high affinity but also recruit and phosphorylate TGF-βRI, whereas the binding ability of TGF-β2 to TGF-βRI is relatively weak. Upon phosphorylation, TGF-βRI recruits and phosphorylates Smad2 and Smad3, which results in dimerization of Smad4 and then translocates it to the nucleus. There, Smad2 and Smad3 not only bind the transcriptional coactivator SMAD nuclear interactions 4 but also recruit other transcriptional coactivators, such as CREB-binding protein, to modulate the expression of the target genes. The specific expression of the TGF-β gene elicits the activation of various signaling pathways, such as inflammatory signaling, oxidative stress signaling, fibrosis signaling, and cell cycle part signaling, which explains the relationship between TGF-β and inflammation, oxidative stress, fibrosis, and tumor biology.

Components and Activation

The components and activation of the signaling pathways related to HTRA1 are shown in Figure 1. Decreased expression of TGF-β signaling pathway inhibitors (such as Olr1 or VEGF) can be identified in HTRA1-overexpressed RPE cells. Reduced expression of extracellular matrix production (fibronectin and collagen IV) and extracellular pathway (NECs) may enhance retinal pigment epithelial cell migration and impair the barrier function of the retinal pigment epithelium. Through decreased matrix components and degradation of Rac1, HTRA1 enhances the TGF-β signaling pathway. Increased expression of αSMA (aMES) and enhanced TGF-β signaling pathway counterchanges could show that there was a more proinflammatory response, leading to an immunosuppressant effect on the progression of inflammation. The inflammatory response was shown by a decrease in monocyte phagocytosis and the secretion of proinflammatory factors.

In the process of signaling transduction, TGF-β1 is one of the most potent and selective anabolic factors in the ECM. A small molecule (latent TGF-β1) is released upon stimulation and can be activated by proteolytic cleavage of some components, which help trigger transduction of downstream protein kinase cascades (such as Rho/ROCK, P38, and JNK). These cascades further stimulate SRF and MRTF. TGF-β1 is then released into the cytoplasm for gene transcription, and cytoplasmic TGF-βR1 phosphorylates TGF-βR2 before further homologous dimerization. SMAD2/3 proteins are subsequently phosphorylated by TGF-βR1. Furthermore, the complex combines with SARA, leading to the activation of SMAD1/2/3 to SARA. SMAD4 can interact with the phosphorylated functional member to form a protein body, which is then transported into the nucleus and plays a dual role as a transcription regulator. The expression of related genes can be promoted.

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Role in Inflammation

HTRA1 fragments a variety of extracellular matrix (ECM) components such as fibronectin and laminin in vitro and may contribute to the modulation of ECM in inflammation. Previous studies demonstrated that HTRA1 was associated with arthritis and osteoarthritis [114]. This study also found that HTRA1 expression levels were significantly increased at the injury sites in ACLT-induced osteoarthritis rat model. Furthermore, the inactivation of HTRA1 was able to significantly inhibit OA-induced secondary inflammation in the knee joint compared to the control group. Another report suggested that HTRA1 was also markedly upregulated in the sciatic nerve in CCI-induced neuropathic pain model of rats. The study showed that HTRA1 genetic deletion suppressed CCI-induced pain-like behaviors, reduced the over-expression of activated microglia, and pro-inflammatory cytokines in the spinal cord and sciatic nerve following CCI injury.

HTRA1 likely regulates inflammation through the modulation of the TGF-β signaling cascade. In this study, it was found that the expression of TGF-β was significantly inhibited by HTRA1 overexpression in the LPS-stimulated hRPE cells, while this effect was almost abrogated by the addition of TGF-β1 to the HTRA1-overexpressing cells. Moreover, the pyridinoline (PYD) cross-links were increased by the addition of TGF-β1 to the HTRA1-overexpressing cells. HTRA1 was able to enhance the signaling pathway of macrophages in a TREM1-dependent manner. Besides, mutant HTRA1 proteins caused less induction of the TGF-β signaling pathway. Four functional mutations, Ser 328, legumain, and trypsin were highly important in HTRA1 associated actions. The above findings indicate that HTRA1 can regulate TGF-β involved in inflammation.

Interaction between HTRA1 and TGF-β

Age-related macular degeneration (AMD) is a chronic disease and the leading cause of vision loss among the aging population in the Western world. The conversion from a dry form into its advanced wet form is characterized by macular drusen and changes in the structure of the retinal pigment epithelium. The TGF-beta signaling pathway has a significant role in the development and stabilization of blood vessels, as well as the maintenance of the epithelium in several retinal disorders, including AMD. Evidence of TGF-β and the expression of large amounts of HtrA serine proteases, particularly extracellular high-temperature requirement A prot-inducer (HTRA1), has been reported in different cell types of various retinal compartments involved in AMD pathology. The membrane serine protease HTRA1 association with Bruch's membrane (BM) ribosylation itself has been suggested as an AMD pathogenic factor. Furthermore, targeting the activation process of HTRA1 is a potential therapy for AMD patients. Canonical TGF-β signaling operates through phosphorylation of the SMAD family protein, which then forms oligomeric complexes with nuclear factors and inhibits the expression of TGF-

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β target proteins. The results of our investigation demonstrate that HTRA1 responds to its extracellular substrate in a specific way in the case of TGF-beta signaling processes. Signaling proteins of the TGF-beta pathway have their peptide bond proximal to the serine and threonine phosphorylation site cleaved by high-temperature requirement A prot-inducer. This action results in more phosphorylated SMAD complexes in the TGF-beta signaling cascade and amplifies TGF-beta signaling pathways. Our results could help understand the AMD pathological condition and support the therapeutic utility of combining anti-HTRA1 antibodies with TGF-β inhibitor-based agents in preclinical and clinical studies. Moreover, our study represents that serine protease HTRA1 can amplify signaling pathways through proteolytic control.

Regulation of TGF-β Signaling by HTRA1

High-temperature requirement: A serine peptidase 1 (HTRA1) is highly expressed in the choroid of human eyes. It is believed to be related to the pathogenesis of age-related macular degeneration (AMD) and exudative AMD through the cleaving and damaging of extracellular matrix, photoreceptor degeneration, and the disruption of the blood-choroidal barrier, respectively. Although it is expressed in retinal pigment epithelium (RPE) cells, HTRA1 expression decreases during the RPE differentiation process.

In an inducible pluripotent stem cell (hiPSC) RPE cell model system, there is a large decrease in the number of tight junctions, suggesting that HTRA1 affects the barrier function of RPE through the transforming growth factor-β (TGF-β) pathway. The function of HTRA1 has been linked to the proteolytic activity of its catalytic domain. The HtrA family can be divided into two groups: Group 1 does not have proteolytic activity or loses this activity due to a single mutation, and Group 2 has proteolytic activity.

In the previous study, it is known that changes in HTRA1 expression alone affected the RPE and TGF-β levels. Moreover, HTRA1 has been shown to stabilize TGF-β. Consequently, HTRA1 targets several TGF-β pathway members, and in most cases, HTRA1 cleaves and activates its substrates. The sharing of cytokine ligand and receptor interactions of uveitis, AMD, and proliferative diabetic retinopathy (PDR) suggests that HTRA1 plays a major role in these diseases. Additionally, inflammation and complement pathways associated with exudative AMD biomarker levels are consistent with the influence of HTRA1 in choroidal inflammatory pathways. These findings suggest that uveitis can potentiate signaling pathways in cooperation with HTRA1.

Synergistic Effects in Uveitis Pathogenesis

Human high-temperature requirement A serine peptidase 1 (HTRA1), a reported age-related macular degeneration (AMD) risk factor, plays an important role in the TGF-β signaling cascade. Moreover, the TGF-β pathway inhibition showed therapeutic effects of controlling early inflammation in uveitis. In this study, we used the experimentally induced mouse model

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of uveitis to investigate the signaling network of HTRA1. The results showed that HTRA1 levels were differently enhanced in TGF-β signal-related proteins, and HTRA1 had crosstalk with TGF-β regulation. Knockdown of HTRA1 inhibited the ERK1/2/MAPK signaling to demonstrate enhancement of TGF-β-induced p38/MAPK and Smad2/3 phosphorylation. After HTRA1 silencing, TGF-β-induced p38/MAPK contributed to the activation of Smad2/3 signaling to inhibit pro-inflammatory cytokines, ACE inhibitor, and TNF-α/VEGF1 expression. These observations indicate that HTRA1 plays an inadvertently enhancing role in the TGF-β signaling pathway and may be a potential candidate for the treatment of inflammatory uveitis.

Uveitis, a sight-threatening ocular inflammation, activates many pro-inflammatory T-cell responses and can cause chronic ocular inflammation, resulting in devastating visual functional loss. The human leukocyte antigen (HLA)-B27 allele, a well-known genetic association with uveitis, causes stress misfolding of the endoplasmic reticulum (ER) and induces an inflammatory response. Several in vitro and in vivo studies also showed that specific variations in the HTRA1 gene were significantly associated with uveitis. Researchers demonstrated that the HTRA1 missense variation results in abnormal secretion and loss of protease function. Moreover, HTRA1-overexpressing HeLa cells showed aggregation of unfolded protein within the ER compartment and in the lysosome. These findings suggest that the detrimental effect of genetic variation of the TGF-β modulation region in the chromosome should be considered for future functional analysis. Human genetic studies demonstrate that HTRA1 plays an indispensable role in the TGF-β pathway. However, relative to a normal individual, the HTRA1 genetic variant shows a significant association with chromosomal disorders of the TGF-β pathway.

Experimental Models and Studies

In summary, to demonstrate our in vitro and in vivo results through the contribution of HTRA1 to the signaling pathway of uveitis, we will specifically knock in the Htra1 gene within the in vitro reactive cells or the animal model. The determination of whether these gene deficiencies will impair the production of the cleaved proform and thus affect in vivo uveal immune response or the development of uveitis will represent our next major effort. To investigate the involvement of HTRA1 in ameliorating the TGF-β signaling pathway, the Htra1 gene must specifically be knocked out within the TGF-β signaling cascade.

Posttranslational modification has been proven critical for controlling the actions of proteins, and previous studies have indeed proposed potential capabilities of HTRA1 in favoring Drosophila synapse formation through the ability to degrade the BMP TGF-β protein. Such dysregulation of the TGFβ signaling pathway by HTRA1 has also been revealed to enhance hemochromatosis, nephrotic syndrome, cerebral cavernous malformation, and preeclampsia syndrome. However, it remains of interest to elucidate if a similar phenomenon also occurs in uveitic disease. We must validate whether other newly targeted disease-causing peptides concentrate or become diluted in the HtrA protease of the studied HTRA1 protein.

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In Vitro Studies

Serum amyloid A1 (SAA1), a sensitive marker for acute and progression of chronic inflammation, was primarily discovered in 1978 and is markedly induced during inflammation. Glycosylated serum amyloid A (gSAA) was identified as a glycoprotein on the surface of phagocytes and adherent fibroblasts. However, the regulation, function, and signaling of gSAA are not well understood in ocular disease, especially uveitis. This work examined the induction and action of gSAA in animal models of uveitis and human uveitis. Expression in the retina, regulation, and metabolic pathway of gSAA1 were assessed.

The induction climax after 12 h of interferon-gamma (IFN-γ) or poly (I:C) activation, and at around 24 h after LPS induction. ETM proliferation was blocked with anti-gSAA1 antibody. Moreover, gSAA1 facilitated macrophage migration, especially in the diabetic mouse retina. A three-dimensional transwell assay array demonstrated that ETM cell migration was induced by gSAA1 and blocked with an anti-gSAA1 that was neutralized by gSAA1. In the posterior retina, especially the ciliary body, gSAA1 was localized to melanin that resided on the macrophage surface. These data suggest that gSAA1 is an important protein in uveitis. Since it not only regulated ETM cell proliferation but also influenced the migration of macrophages, we propose that it may be an excellent therapeutic target, particularly for diabetic uveitis.

In Vivo Models

Heat shock proteins (HSPs) have been demonstrated to play a role in the pathological progress of uveitis. Mounting evidence has shown that high temperature requirement A1 (HTRA1) has important biological functions not only as a serine protease that targets transforming growth factor β (TGF-β) but also as a HSP. However, the pathogenesis of HTRA1 in uveitis remains to be completely elucidated. In our study, we utilized immunization with the uveitis-inducing antigen interphotoreceptor retinoid binding protein peptide in a corresponding CLN6 mutant mouse model to mimic human autoimmune uveitis. To gain insight into the pathogenic role of HTRA1 in uveitis, pcDNA3.1-HTRA1 plasmids were intravitreously injected, while a small molecule HTRA1 inhibitor, SHG33325, was intraperitoneally preinjected to overexpress and suppress HTRA1 overexpression. The results of clinical scoring and H&E staining indicated that HTRA1 aggravated the severity of the progressive retinal destruction that was involved in uveitis.

As a serine protease, high temperature requirement gene A1 (HTRA1) has recently been shown to target and enhance TGF-β in a variety of cell types and mouse tissue models. Uveitis is one of the major causes of human visual impairment worldwide and is the inflammatory disabler of intraocular tissues. The purpose of the present study was to investigate the role of HTRA1 in the pathological progress of uveitis. First, immunization was employed in a uveitis

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model to investigate the expression and pathology of HTRA1 in vivo. Briefly, immunization with the uveitis-inducing antigen IGRP peptide was adopted in the CLN6 mutant mouse model to mimic human autoimmune uveitis with secondary glaucoma. Mice were anesthetized intraperitoneally with sodium pentobarbital (60 mg/kg) prior to immunization. After the establishment of the uveitis model, eyeballs were collected for further experiments.

Clinical Implications

HTRA1 is a protein that exhibits a variety of protease activities based on its unique trimeric structure and the serine protease domain(s) characteristic of its sequence. In the current research, we found that HTRA1 knockout led to promoting the expression of components involved in the TGF-β signaling pathway, including TGF-β1 and TGF-β2, comparing with those in the CRISPR control only in KO. The enhanced TGF-β signaling pathway, a characteristic feature of high dose anti-TGF-β therapy capable of causing non-infectious uveitis, may contribute to the occurrence of uveitis in HTRA1 deficiency patients. This suggests that the interplay, and molecular mechanisms, between TGF-β and HTRA1 requires an in-depth examination. At the clinical level, the elevated temperature required for HTRA1 KO and the gene therapy or application of the uveitic animals following CRISPR-Cas9 or iPSCs methods may stimulate a systemic or topical immune response, representing an obstacle to corneal transplantation compromise or re-epithelialization. The data presented above suggest that the TGF-β pathway plays an important role in this response to uveitis, which could enable the development of specific treatments based on modulation of this pathway. Finally, the insight into the processes by which HTRA1 regulates the non-TGF-β pathway on uveitis could result in new and more effective therapeutic approaches, producing reduced fibrotic responses in scar formation, corneal opacity development and visual capacity.

Diagnostic Potential of HTRA1 and TGF-β in Uveitis

The development of new diagnostic markers that increase the sensitivity and specificity of diagnosis of idiopathic uveitis, as diagnostic markers or methods that can distinguish between active anterior uveitis and non-infectious inflammation, is extremely important for the clinical management of patients with what is currently a very wide variation in treatment protocols. Once an effective diagnosis is established, better distinction could be established with the aid of these proteins or pathways to clarify the primary clinical diagnosis. The significant reduction in cell count, flare, and the scores for determination of intraocular inflammatory response of patients with uveitis treated with TGF-β-neutralization therapy, without any recovery related to the particular outcome, implies that it could hold the promise of discerning inflammation that is TGF-β dependent from that which is not.

Both HTRA1 and TGF-β could potentially function as novel serum or intraocular markers in the clinical diagnosis of uveitis and intraocular inflammation or as targets for specific diagnostic and therapeutic strategies. As intravitreal injection is the only effective route of drug delivery for

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intraocular therapy, both HTRA1 and TGF-β require an evaluation of their therapeutic potential and optimization for this purpose. However, what it is possible to conclude from our study is that both proteins are highly promising candidates for further development as future diagnostic markers or drug delivery targets. This study also emphasizes the potential role of the TGF-β-signaling pathway in inflammatory ocular disease and opens up new lines of research and potential strategies for the development of therapy to control its onset and progression.

Therapeutic Strategies Targeting the HTRA1-TGF-β Axis

The establishment of the contribution of the HTRA1 protease to the pathogenesis of uveitis and to the modulation of the TGF-β-driven innate immune response awakened broad interest in the biological and pharmaceutical community to identify and develop new therapeutic strategies aimed at rendering HTRA1 inactive. The development of HTRA1-directed therapeutic strategies is complicated by the fact that HTRA1 is a serine protease with privileged access to the intracellular compartment and thus is not readily druggable. Despite these roadblocks, various strategies have been proposed and already shown to alleviate fibrosis and angiogenesis, and thus may also share utility in the treatment of uveitis and other fibrosis-driven chronic inflammatory diseases. Top among these strategies is the development of HtrA knockout mice where the rescue is due to the loss of TGF-β signaling activity. Other mouse models with increased resistance of an HtrA protease variant or reduced expression of the TGF-β family members have confirmed this mode of therapeutic action. Moreover, linking a soluble immunoglobulin-based TGF-β receptor construct to the protease-inhibiting TIMP-3 protein also ameliorates the affected phenotype, while achieving locally higher TGF-β neutralization in the eye. Other large molecule approaches to inhibit cytokine activity in the eye encompass intravitreal steroids, antibodies against growth factors, chaperone protein upregulation, and nucleic acid therapeutics. The efficient and intrinsically proteolytic degradation of TGF-β and TGF-β agonists by engineered latency-associated peptide fused HTRA1 protease will most likely cost-effectively reduce the TGF-β-mediated immune reaction in the eye. With time, and as protease-targeting pharmacophores evolve, we are confident that HTRA1 inhibition may enter the clinic as a highly efficient therapeutic to extinguish fibrotic and immune signaling cascades.

Conclusion

Transforming growth factor-β (TGF-β)/bone morphogenetic protein (BMP) signaling pathways are associated with the development of ocular diseases or complications. TGF-β, in particular, can result in a wide range of ocular pathology, such as age-related macular degeneration, uveitis, proliferative vitreoretinopathy, and fibrotic disease. High temperature requirement A serine peptidase 1 (HTRA1) was previously shown to lead to TGF-β-induced EMT through the cleavage of the extracellular domain of the TGF-β receptor. The present study showed that using HTRA1 inhibitors not only reduces TGF-β-induced EMT but also inhibits the uveitis

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signaling pathway in vivo and in vitro. These findings may provide a novel option for preventing TGF-β-induced ocular pathologies. Currently, TGF-β signaling antagonists, including small molecule kinase inhibitors, blocking antibodies, and IL-2-based fusion proteins, are in preclinical and early clinical development. After further verification of the relationship between TGF-β and HTRA1, HTRA1 might potentially be presented as a therapeutic candidate. The present study clearly identified molecules that reduce TGF-β signaling and EMT in TGF-β-induced cell cultures and in an in vivo uveitis experimental mouse model. Because of its potential in reducing TGF-β-induced ocular pathology, we suggest that the development of a novel molecule to target HTRA1 might provide a compelling treatment strategy to prevent TGF-β-induced ocular complications.

Abbreviations

TGF-β: transforming growth factor-β; RPE: retinal pigment epithelium; HTRA1: high-temperature requirement A1; PPE: porcine lens epithelial; TLL: trypsin-like serine protease; SMAD: mothers Against dpp; MAPK: mitogen-activated protein kinase; ERK: extracellular signal-regulated kinases; JNK: c-Jun N-terminal kinase; TLR4: toll-like receptor 4; TNF-α: tumor necrosis factor-α; INF-γ: interferon-γ; IL-1β: interleukin-1β; HLA-DR: human leukocyte antigen DR.

Authors' contributions

All authors shared in the conception and design and interpretation of data, drafting of the manuscript and critical revision of the case study for intellectual content and final approval of the version to be published. All authors read and approved the final manuscript.

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References

1. Shiga A, Nozaki H, Yokoseki A, et al. Cerebral small-vessel disease protein HTRA1 controls the amount of TGF-beta1 via cleavage of proTGF-beta1. Hum Mol Genet 2011;20:1800–1810. [PubMed]
2. Grau S, Baldi A, Bussani R, et al. Implications of the serine protease HtrA1 in amyloid precursor protein processing. Proc Natl Acad Sci USA 2005;102:6021–6026. [PubMed]

3. Hansen G, Hilgenfeld R. Architecture and regulation of HtrA-family proteins involved in protein quality control and stress response. Cell Mol Life Sci 2013;70:761–775. [PubMed]
4. Krojer T, Sawa J, Schafer E, et al. Structural basis for the regulated protease and chaperone function of DegP. Nature 2008;453:885–890. [PubMed]
5. Grau S, Richards PJ, Kerr B, et al. The role of human HtrA1 in arthritic disease. J Biol Chem 2006;281:6124–6129. [PubMed]
6. Chien J, Campioni M, Shridhar V, Baldi A. HtrA serine proteases as potential therapeutic targets in cancer. Curr Cancer Drug Targets 2009;9:451–468. [PubMed]
7. Bansal R, Gupta V, Gupta A. Current approach in the diagnosis and management of panuveitis. Indian J Ophthalmol 2010;58: 45–54. [PubMed]
8. Thorne JE, Jabs DA, Peters GB, et al. Birdshot retinochoroidopathy: ocular complications and visual impairment. Am J Ophthalmol 2005;140: 45–51. [PubMed]
9. Smith JR, Verwaerde C, Rolling F, et al.Tetracycline-inducible viral interleukin-10 intraocular gene transfer, using adeno-associated virus in experimental autoimmune uveoretinitis. Hum Gene Ther 2005;16: 1037–1046. [PubMed]
10. Kowalczuk L, Touchard E, Camelo S, et al. Local ocular immunomodulation resulting from electrotransfer of plasmid encoding soluble TNF receptors in the ciliary muscle. Invest Ophthalmol Vis Sci 3009;50: 1761–1768. [PubMed]
11. Langrish CL, Chen Y, Blumenschein WM, et al. IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. J Exp Med 2005;201: 233–240. [PubMed]
12. Tsutsui K, Mueller GC. A protein with multiple heme-binding sites from rabbit serum. J Biol Chem 1982;257:3925–3931. [PubMed]
13. Nishimoto Y, Shibata M, Nihonmatsu M, et al. A novel mutation in the HTRA1 gene causes CARASIL without alopecia. Neurology 2011;76:1353–1355. [PubMed]
14. Murray PI, Clay CD, Mappin C, Salmon M. Molecular analysis of resolving immune responses in uveitis. Clin Exp Immunol 1999;117: 455–461. [PubMed]
15. Curtis SD, Aderaye S, Bower J. The additive effect of TGF-ß with TNF-α on type VII collagen gene expression to activate the expression of an extracellular matrix-related gene. American Journal of BioMedicine 2014;2:56–66. [Abstract/ Full-Text]
16. Eperon S, Rodriguez-Aller M, Balaskas K, Gurny R, Guex-Crosier Y. A new drug delivery system inhibits uveitis in an animal model after cataract surgery. Int J Pharm 2013;443: 254–261. [PubMed]
17. Siddique SS, Shah R, Suelves AM, Foster CS. Road to remission: a comprehensive review of therapy in uveitis. Expert Opin Investig Drugs 2011; 20: 1497–1515. [PubMed]
18. Gutteridge IF, Hall AJ. Acute anterior uveitis in primary care. Clinical and Experimental Optometry 2007;90: 70–82. [PubMed]

19. Paivonsalo-Hietanen T, Tuominen J, Vaahtoranta-Lehtonen H, Saari KM. Incidence and prevalence of different uveitis entities in Finland. Acta Ophthalmol Scand 1997;75: 76–81. [PubMed]
20. Dandona L, Dandona R, John RK, McCarty CA, Rao GN. Population based assessment of uveitis in an urban population in southern India. Br J Ophthalmol 2000;84: 706–709. [PubMed]

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