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REVIEW

Progress in the Study of the Role and Mechanism of HTRA1 in Diseases Related to Vascular Abnormalities

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Abstract: High temperature requirement A1 (HTRA1) is a member of the serine protease family, comprising four structural domains: IGFBP domain, Kazal domain, protease domain and PDZ domain. HTRA1 encodes a serine protease, a secreted protein that is widely expressed in the vasculature. HTRA1 regulates a wide range of physiological processes through its proteolytic activity, and is also involved in a variety of vascular abnormalities-related diseases. This article reviews the role of HTRA1 in the development of vascular abnormalities-related hereditary cerebral small vessel disease (CSVD), age-related macular degeneration (AMD), tumors and other diseases. Through relevant research advances to understand the role of HTRA1 in regulating signaling pathways or refolding, translocation, degradation of extracellular matrix (ECM) proteins, thus directly or indirectly regulating angiogenesis, vascular remodeling, and playing an important role in vascular homeostasis, further understanding the mechanism of HTRA1's role in vascular abnormality-related diseases is important for HTRA1 to be used as a therapeutic target in related diseases.

Keywords: HTRA1, vascular anomalies, cerebral small vessel disease, AMD, tumor

Introduction

High temperature requirement A1 (HTRA1) is a family of highly conserved serine proteases involved in protein quality control. This protein is widely expressed from prokaryotes to eukaryotes and exists in humans as four homologues, HTRA1, HTRA2, HTRA3 and HTRA4.[1] Originally found in E. coli, HTRA1 is an important component of the heat shock response[2,3] and encodes a widely expressed serine protease that is significantly expressed in the vasculature,[1,4] which has both chaperone and serine protease activities and is involved in many physiological processes, particularly extracellular matrix (ECM) remodeling[5–7] and transforming growth factor-β (TGF-β) signaling.[1,8–11] HTRA1 plays an important role in cell proliferation, migration, and apoptosis in addition to controlling protein aggregation through refolding, translocation, or degradation.[12] HTRA1 is commonly expressed in normal human tissues, but at different levels in different tissues and organs, further suggesting that HTRA1 may play different roles in different cell types. *HTRA1* genes are associated with cerebral small vessel disease,[13,14] age-related macular degeneration (AMD),[15] multisite tumors,[16–19] arthritis,[20] pre-eclampsia[21] and other microvascular and macrovascular diseases. The pathogenesis is mainly due to impaired vascular function.[22] This review discusses the mechanisms of HTRA1's role in the vasculature and the progress of research on related diseases. Further understanding of the direct or indirect role of HTRA1 in the vasculature may help us to develop HTRA1 as a therapeutic target.

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HTRA1 Gene Abnormalities and Cerebrovascular Disease

HTRA1 and Hereditary Cerebral Small Vessel Disease (CSVD)

Cerebral small vessel disease is a common clinical cerebrovascular disease that can lead to stroke and dementia. 25% of strokes and 45% of dementia are caused by CSVD. Its harmful effects have recently received more and more attention. The clinical manifestations of cerebral small vessel disease are diverse, ranging from asymptomatic to various neurological symptoms, which can cause confusion in diagnosis and treatment.[23] Cerebral Autosomal Recessive Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CARASIL) is a relatively rare hereditary small vessel disease of the brain caused by a homozygous mutation in the *HTRA1* gene. The first patient with CARASIL was diagnosed in Japan in 1965, and the clinical manifestations of the disease are mainly cognitive impairment, recurrent strokes, low back pain and hair loss.[24] The pathological changes observed in patients with CARASIL broadly include marked thinning of the outer membrane of small cerebral arteries, massive loss of smooth muscle cells, intimal fibrosis and thickening, and centripetal narrowing of the lumen.[25–27] Dysfunction of smooth muscle cells is most prominent in small arteries.[28,29]

Japanese researcher Oka et al[1] found that HTRA1 can bind extensively to TGF-βfamily proteins, including bone morphogenetic protein 4 (BMP4), Growth and differentiation factor 5 (Gdf5) and TGF-βs. HTRA1 was found to inhibit TGF-βfamily protein-mediated signaling. Hara[30] first explored the possible molecular mechanism of CARASIL and found that HTRA1 could inhibit signaling by TGF-βfamily members, and that HTRA1 mutations led to a reduction in HTRA1 protease activity and thus failed to inhibit TGF-βsignaling, resulting in a decrease in TGF-βsignaling and increased in the intima of the affected small arteries. While the TGF-βsignaling pathway is closely linked to angiogenesis, remodeling and intravascular homeostasis,[31] increased TGF-βexpression could promote the formation and accumulation of ECM (fibronectin and versican) in small intracerebral arterioles.[32] This led to vascular fibrosis and dysregulation of the growth of small intracerebral blood vessels, ultimately leading to the pathogenesis of CARASIL.[30]

One study found that HTRA1 cleaves proTGF-β1, leading to increased TGF-β1 and TGF-β signaling-induced proteins in the cerebral small vessels of CARASIL patients, further suggesting that HTRA1 can inhibit the TGF-βsignaling pathway.[10] However, contrary findings have also been made. By analysing brain tissues as well as fibroblasts from HTRA1-deficient mice and fibroblasts from CARASIL patients, it was found that HTRA1 gene mutation reduced expression of the HTRA1 protein, preventing the cleavage of latent TGF-β binding protein 1 (LTBP-1), an extracellular matrix protein that is a key regulator of TGF-β, and attenuating the TGF-βsignaling pathway transduction.[11] The different results of these studies may be due to different cell types, arterial models, differences in experimental conditions or experimental methods, and different perspectives in discovering experimental problems. The pathogenic mechanism of HTRA1 gene mutation has not been fully clarified so far. In terms of molecular structure, HTRA1 protease consists of a trimer, with neighbouring HTRA1 subunits activating each other through the linker region. HTRA1 mutations may result in impaired activation cascade or inability to form a stable trimer, which may lead to impaired HTRA1 protease activity.[33,34] It is currently believed that heterozygous mutations in the HTRA1 gene lead to reduced expression or loss of function of HTRA1, which in turn triggers abnormal expression of the TGFβ1/Smad signaling pathway, ultimately leading to the expression of downstream signaling molecules and target proteins in heterozygous HTRA1 mutations.

Cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) can be divided into 2 types, one is CADASIL type 1 (CADASIL1, OMIM 125310), caused by mutations in the NOTCH receptor 3 gene (NOTCH3), is the most common hereditary cerebral small vessel disease[35] and is broadly referred to collectively as CADASIL, while the other type, CADASIL type 2 (CADASIL2, OMIM 616779), also known as symptomatic carriers of HTRA1 gene mutations,[33,36] and studies have found that HTRA1 heterozygous mutations can cause cerebrovascular abnormalities.[37] The clinical manifestations of CARASIL and symptomatic carriers of HTRA1 mutations are approximately the same, but the latter have milder clinical symptoms[14,38] and later onset.[33,39,40] MRI of the head showed typical features of cerebral small-vessel disease, with pathological manifestations of intimal thickening, epicardial fibrosis, degeneration and loss of smooth muscle cells, and delamination and splitting of the internal elastic lamina of small vessels.[37] There were no osmiophilic granules on the surface of vascular smooth muscle on electron microscopy. It has been shown that mutations in the heterozygous HTRA1 gene result in a reduction in the protein

hydrolysis activity of HTRA1,[41] which is accompanied by an increase in the levels of TGF-β1/Smad proteins, with a similar molecular mechanism to CARASIL.[42]

Although CARASIL and CADASIL in the broad sense are two different diseases caused by mutations, there is a close association between HTRA1 and NOTCH, and HTRA1 is directly related to NOTCH3 signaling regulation.[28] CADASIL is caused by mutations in the NOTCH3 gene resulting in excessive accumulation of the NOTCH3 extracellular structural domain (NOTCH3-ECD) and vascular wall disruption.[43] Zellner et al[44] quantified the cerebrovascular proteome from CADASIL patients and control autopsy samples and obtained 95 proteins with significantly increased abundance and found that HTRA1 was highly enriched and colocalized with NOTCH3-ECD deposition in the patient's vasculature, which provides a basis for the development of CADASIL pathology during provides a key step in connecting the molecular mechanisms of two distinct cerebral small vessel diseases.

In HTRA1 associated cerebral small-vessel disease, researchers have focused more on the vascular smooth muscle cell (VSMC), and disturbances in VSMC function and integrity were the main causative agents of hereditary CSVD, which may lead to impaired blood perfusion in certain regions of the brain.[45] Further mechanistic studies on the phenotypic transformation of VSMCs by Klose et al[28] found that HTRA1 deletion increased NOTCH3 ligand JAG1 protein levels and NOTCH3 signaling activity in VSMCs, with activation of NOTCH3 signaling leading to increased transcription of HES and HEY repressors and contributing to the contractile phenotype of VSMC. The contractile phenotype VSMCs play an important role in the control of blood flow and blood pressure. Contractile phenotype VSMCs are characterised by slow proliferation and association with neurotransmitters.[46] The VSMC synthetic phenotype cell morphology is spindle-shaped and the main features are proliferation, migration and production of extracellular matrix.[46] In VSMC, HES and HEY proteins can inhibit transcription of VSMC contraction phenotype marker proteins such as SM22α, α-SMA, and Smoothelin expression, causing VMSC to turn into a synthetic phenotype, resulting in aberrant VMSC function.[47] JAG1 and NOTCH3 are the most abundantly expressed ligands and receptors on VSMC.[48,49] NOTCH3-ICD activates the transcription of contractile proteins, whereas Notch target genes of the HES and HEY families encode transcriptional repressor proteins that inhibit the expression of these contractile VSMC markers[28] (Figure 1). Cerebral arterial vascular abnormalities were

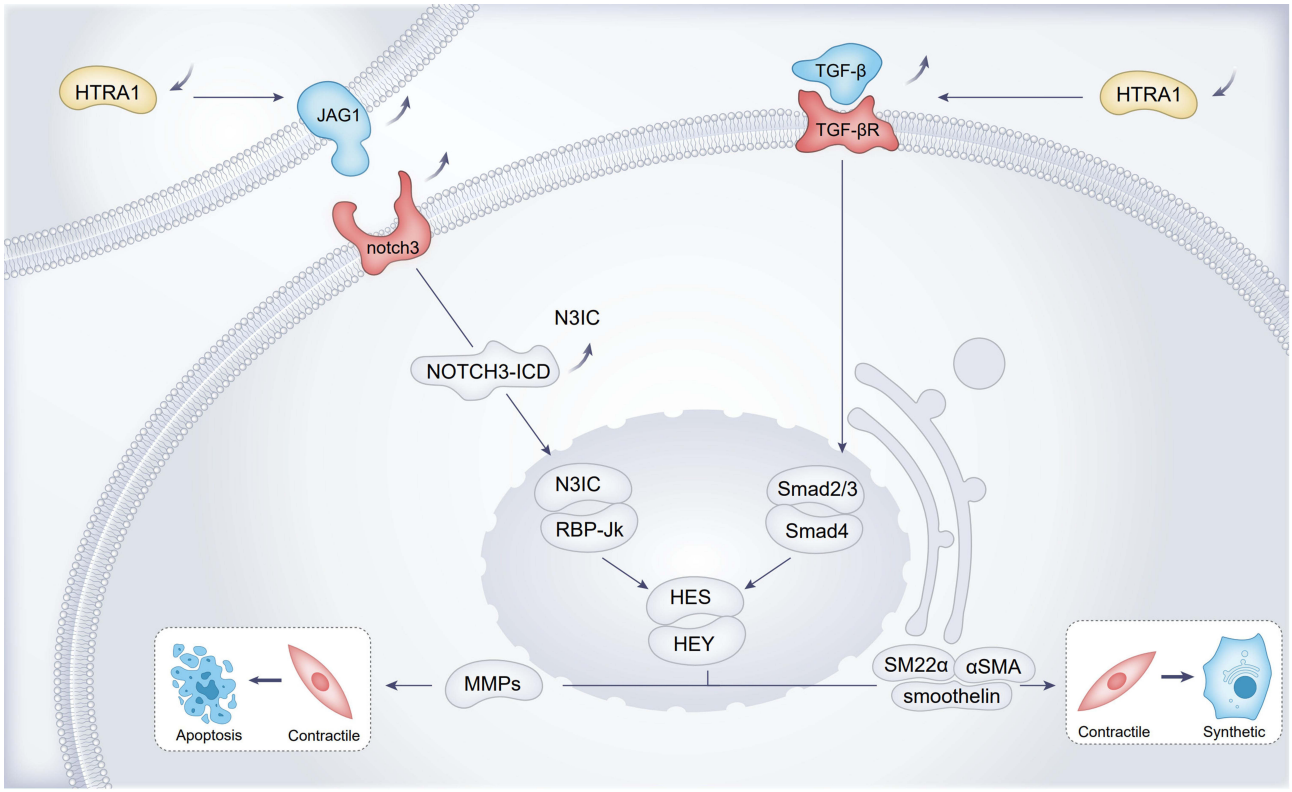

Figure 1 Loss of function of HTRA1 induces an increased VMSC synthetic phenotype and VMSC apoptosis.

observed in HTRA1-/- mice, from which aortic VMSCs were isolated that were predominantly of the synthetic phenotype and showed enhanced proliferation and migration.[29] The synthetic phenotype of VMSCs induces elevated expression of matrix metalloproteinases (MMPs), which degrade the extracellular matrix and may lead to the increased VSMC death. Abnormal synthetic regulation may be one of the mechanisms by which HTRA1 deficiency causes vascular abnormalities[29] (Figure 1).

Kato et al[50] found that HTRA1-/- mice exhibited pathological features of CARASIL-associated arteriopathy, and that reduced HTRA1 expression could lead to the accumulation of matrisome proteins such as hub protein fibronectin (FN) and latent TGF-β binding protein 4 (LTBP-4). Candesartan treatment, however, attenuated matrisome proteins accumulation and normalised vasodilation and cerebral blood flow. Candesartan, an inhibitor of TGF-β signaling,[51–53] amend arteriopathy in HTRA1-/- mice, thus suggesting that drugs with HTRA1 activity may be new therapeutic candidates for CARASIL, and that these approaches may open new pathways for preventing the progression of CSVD.

In summary, in HTRA1-associated cerebral small vessel disease, downregulation of HTRA1 protein expression can affect TGF-β signaling pathway transduction, which indirectly regulates angiogenesis, influences the phenotypic changes of VSMCs in the vasculature, and induces an increase in the expression of MMP9, which leads to the death of VMSCs. Meanwhile, FN, LTBP-1, LTBP-4 and other related matrisome proteins were increased in the vascular endothelium, leading to changes in vascular structure. In conclusion, reduced HTRA1 protease activity can lead to growth dysregulation of small blood vessels in the brain, and more studies are needed to differentiate the pathology, clinical features, and mechanisms of action between CARASIL and heterozygous mutation carriers.

HTRA1 and Neurodegenerative Lesions, Cerebral Microhemorrhages and Cerebral Amyloidosis

HTRA1 is associated with neurodegenerative lesions, cerebral microhemorrhages, cerebral amyloidosis, and in a Finnish cohort study, investigators performed whole-exome sequencing (WES) in patients with cerebrovascular cognitive impairment (VCI), supporting a pathogenic role for HTRA1 in cerebrovascular cognitive impairment and cerebral small vessel disease, considering vasculogenic mechanisms associated with neurodegenerative diseases[54,55] and providing new insights into the molecular mechanisms of cerebrovascular cognitive disorders diseases.

Mild to moderate cerebral microhemorrhages are common in CARASIL patients, but diffuse cerebral microhemorrhages are rare. Wen et al[56] reported a 43-year-old woman whose imaging showed multiple cerebral microhemorrhages in all lobes, the brainstem and the cerebellum. After sequencing, it was found to be a novel mutation in HTRA1, so HTRA1 gene mutation was considered to be associated with cerebral microbleeds.

Cerebral amyloid angiopathy (CAA) is an age-related disease that is a major cause of cerebral hemorrhage and cognitive decline. In CAA patients, amyloid-β (Aβ) deposits in the vasculature and leads to disruption of the structural integrity of the vasculature, resulting in the development of the disease.[57] Various fragments of amyloid precursor proteins, involved in the amyloid pathway, are co-localized with Aβ deposition in human brain samples.[58] Zellner et al using proteomics techniques, also similarly found that HTRA1 co-localized with Aβ deposition in brain capillaries of CAA type 1 patient's vascular. The upregulation of HTRA1 levels may reflect extracellular matrix remodeling in the vasculature as a compensatory response to vascular Aβ accumulation.[59] These findings suggest that abnormal HTRA1-mediated protein degradation plays an important role in CAA type 1 microvasculature and has a molecular link between multiple types of cerebral microvascular disease.[60]

HTRA1 is Associated with Age-Related Macular Degeneration (AMD)

Age-related macular degeneration is a chronic progressive disease characterized by chronic progressive degeneration of the macular photoreceptors, retinal pigment epithelium, bruch's membrane (BrM) and choroidal capillaries.[61] In the clinic, age-related macular degeneration initially causes damage to the central area of the retina (the macula) and is divided into early and late stages (advanced age-related macular degeneration). The advanced stages of AMD are divided into non-exudative or atrophic AMD (dry AMD) and exudative or neovascularised AMD (wet AMD).[62] In dry AMD, drusen deposits are observed between the retinal pigment epithelium and the BrM membrane with progressive

development of geographic atrophy, whereas in wet AMD, vision loss occurs due to macular choroidal neovascularization (CNV) or polypoidal choroidal vasculopathy (PCV).[63] PCV and CNV are considered as subtypes of wet AMD. Patients with wet AMD have more rapid vision loss than those with dry AMD.

Most studies have shown that HTRA1 gene variants are significantly associated with wet AMD disease.[64–68] The HTRA1 single nucleotide polymorphism (SNP) locus rs11200638, located on human chromosome 10q26, was significantly associated with AMD. This SNP increased the expression of HTRA1 protein in the retinal pigment epithelium of AMD patients.[69] It was shown that HTRA1 rs11200638 has a stronger pathogenic risk for wet AMD than for dry AMD.[70] Because of the clear correlation between wet AMD and vascularity, here we focus on wet AMD. The results of the exploration of the role of HTRA1 in the pathogenesis of PCV can help guide the corresponding clinical treatment.

HTRA1 and Wet AMD

HTRA1 overexpression is associated with choroidal neovascularisation in the retina and macula.[12,71,72] The main manifestations include abnormal enlargement of choroidal vessels, loss of the elastic lamina inside as well as outside the choroidal arteries, with severe regression or absence of the middle layer.[73–75] In a recent study, HTRA1 modulated subclinical inflammation of retina and choroid, and it has been confirmed by in vivo and in vitro experiments that HTRA1 had a pro-angiogenic function, and the inflammatory environment induced by HTRA1 overexpression may increase angiogenic response.[76]

HTRA1 acts on many key components of the BrM, and it hydrolyses ECM such as aggrecan, decorin, type II collagen,[77] fibronectin, fibromodulin, and vitronectin,[5] as well as being involved in degradation of elastin fibres in the choroidal vasculature and the BrM membrane.[78] Degradation of proteins in the BrM membrane makes it easier for blood vessels to grow from the choroid across the BrM membrane to the neural retina, leading to the development of AMD. Notably, the ECM fragments produced by HTRA1 hydrolysis of these ECMs may themselves promote angiogenesis, further exacerbating choroidal and RPE disruption.[79] Transgenic mice overexpressing equal amounts of human HTRA1 in the RPE were found to have elevated expression of fibronectin fragments in the REP and choroidal layers and reduced levels of fibulin-5 and tropoelastin protein expression.[7] Fibulin-5 can bind to tropoelastin, promote elastin maturation, accelerate elastic fibre synthesis, and play a role in promoting fibre synthesis.[80] At the same time, the researchers found that the BrM membranes of this mouse were fragmented and the continuity was deteriorated. (Figure 2)

In induced pluripotent stem (iPS)-RPE cells at high risk for the ARMS2/HTRA1 allele, expression of the HTRA1 substrate EFEMP1 is elevated. EFEMP1 is an extracellular matrix protein that causes drusen deposition, activates the complement pathway, induces an inflammatory response, and regulates the TGF-β signaling pathway thereby affecting angiogenesis.[81] Thrombospondin (TSP1) was also found to be elevated.[81,82] The possible reason for the increase in ECMs was that HTRA1 cleavage activity is elevated to a feedback loop that produces more HTRA1 cleavage substrates. TSP1 is a well known inhibitor of angiogenesis and mediates differential effects through interaction with CD47.[83,84] Although TSP1 itself is anti-neovascular, hydrolysis produces fragments that promote endothelial cell lumen formation and hence neovascularization.[81] (Figure 2)

HTRA1 interacts with a variety of receptor-binding factors of the TGF-β family to mediate the regulation of angiogenesis, of which growth differentiation factor 6 (GDF6) is a member of the TGF-β family.[85] Mutations in the GDF6 gene are associated with phenotypes such as microphthalmia and anophthalmia, which control the development of blood vessels and nerves in the eyes.[86] The interaction between GDF6 and HTRA1 is crucial for TGF-β signal transduction and ocular vascular and nerve development. HTRA1-associated PCV risk genes (rs10490924T, rs6982567) were associated with elevated HTRA1 levels and reduced GDF6 levels, leading to an increased risk of AMD.[85–87] In addition, AMD-associated synonymous variants in HTRA1 downregulate the expression of TGF-β1 downstream proteins such as phosphorylated SMAD2 and PAI-1, which leads to impaired TGF-β signaling regulation, thus further affecting vascular and neuromodulation[88] (Figure 2). HTRA1 inhibits transforming growth factor-β2 (TGF-β2) signaling, an important regulator of angiogenesis.[89] TGF-β2 knockout mice exhibit abnormal ocular morphogenesis phenotypes in the retina.[90] Because of the important role of TGF-β2/activin receptor-like kinase 5 (ALK5) / SMAD2/3 signaling in neovascularisation,[91] researchers[92] used the CAG-promoter transgenic mice overexpressing HtrA1, found that HTRA1 cleaves TGF-β RII and inhibits SMAD2/3 phosphorylation, resulting in reduced protein expression of the downstream TGF-β- ALK5 -SMAD2/3 signaling pathway (Figure 2).

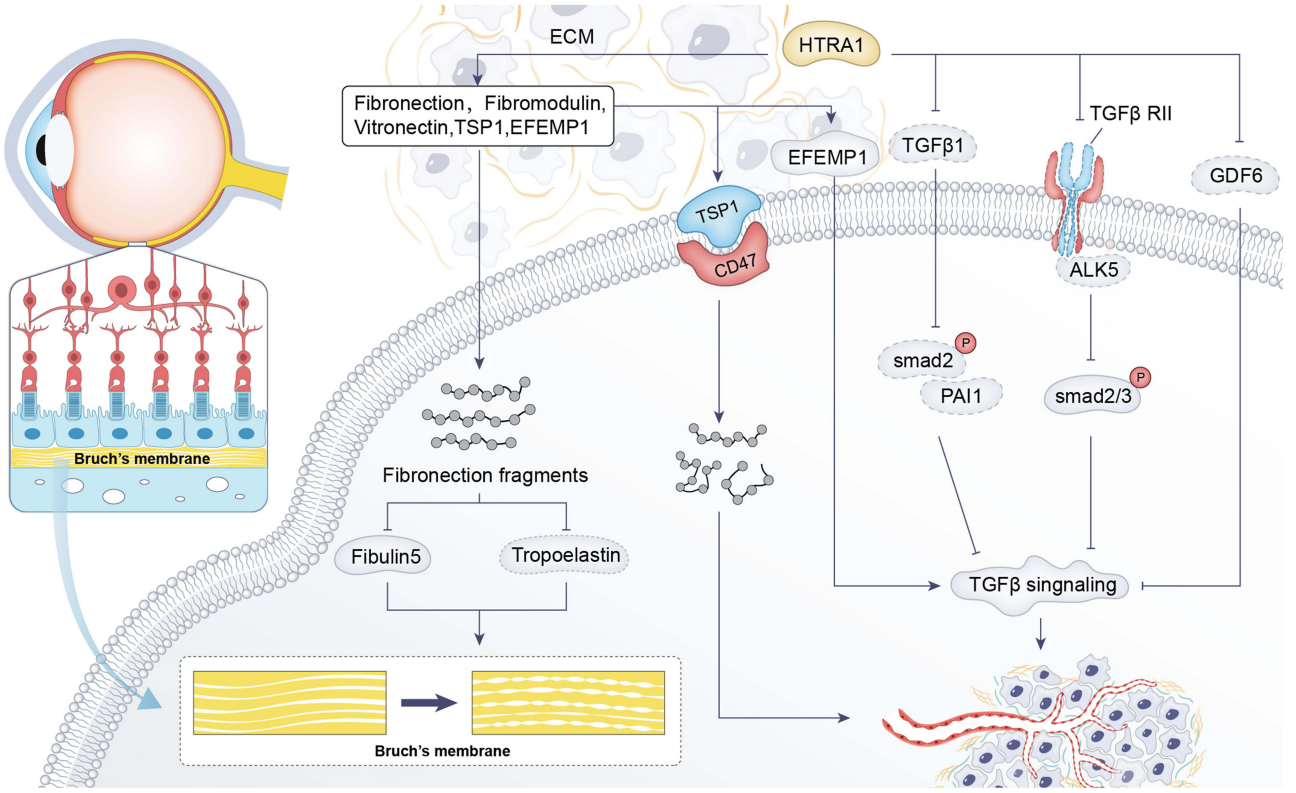

Figure 2 HTRA1 can affect BrM morphology through cleavage of ECM (Fibronection). HTRA1 affects angiogenesis through ECM (TSP1, EFEMP1) and TGF-β signaling pathways (TGF-β, TGF-βRII, GDF6)-related proteins and receptors in AMD.

In vivo and in vitro experiments, pathology and genetics have helped to provide a deeper understanding of the pathogenesis of AMD. Structurally, HTRA1 disrupts the integrity of the choroidal vasculature and Bruch's membrane; genetically, the HTRA1 locus has been shown to be one of the risk loci most strongly associated with wet AMD; Molecularly and biologically, HTRA1 can regulate ocular neovasculogenesis through the effects of cleavage of ECM as well as modulation of signaling pathways, which are involved in the pathogenesis of wet AMD.

HTRA1 and VEGF-Related Content

Wet AMD is characterized by vascular lesions leading to plasmacytic exudation and hemorrhage, and studies have identified an association with abnormal angiogenesis mediated by vascular endothelial growth factor (VEGF).[93,94] VEGF is a highly conserved homodimeric glycoprotein that regulates physiological and pathological angiogenesis. It is also a potent vascular permeability factor, increasing the permeability of the vessel wall.[95] Studies have confirmed that VEGF can induce neovascularization and that anti-VEGF treatment can significantly improve patients' visual acuity.[96,97] A meta-analysis showed a significant association between the HTRA1rs11200638 gene and response to anti-VEGF treatment, and blocking the angiogenic pathway may be partly responsible for the effect of HRTA1 on response to anti-VEGF treatment.[98] In vitro and in vivo experimental studies have shown that HTRA1 can synergize with oxidized phospholipids to promote inflammation and macrophage infiltration, which are essential for ocular VEGF expression, and that HTRA1 may mediate VEGF expression in RPE cells through the Wnt signaling pathway, further suggesting that, inhibition of HTRA1 expression reduces choroidal neovascularization in mouse disease models.[99]

Binding of the Gtf2i-β/δ transcription factor to the ARMS2 gene leads to elevated HTRA1-secreting proteins in transfected cells and AMD-derived induced pluripotent stem cells (iPSCs), overexpression of HTRA1 in mice using the CAG-promoter, and increased blood levels of HTRA1 protein, causing VEGF upregulation and a CNV-like phenotype.[92] Tom et al[75] developed a Fab fragment to block HTRA1, using Dick-kopf-related protein 3 (DKK3) as a biomarker, and demonstrated that in AMD patients treated with an HTRA1-blocking Fab fragment, HTRA1 activity was inhibited in

AMD patients treated with HTRA1 blocking Fab fragment. Thus the anti-HTRA1 approach offers a promising alternative treatment option for wet AMD that is complementary to anti-VEGF therapy.

Research Progress of HTRA1 Gene Abnormalities Associated with Tumors

Tumor angiogenesis is closely related to tumor invasion, infiltration, and metastasis, which directly affects the prognosis of tumor patients, and HTRA1 may be involved in tumorigenesis and tumor angiogenesis. The expression of HTRA1 was found to be downregulated in most tumors.[100] Overexpression of HTRA1 in highly aggressive melanoma, oesophageal squamous cell carcinoma and pancreatic cancer cells inhibits the proliferation and migration of tumor cells and also causes apoptosis.[18,101,102] HTRA1 can promote cancer cell death through apoptosis and oxidative stress pathways.[103,104] Another study reported that HTRA1 overexpression in ovarian cancer cells inhibited epidermal growth factor receptor (EGFR) activation, leading to a decrease in AKT/MAPK phosphorylation levels and a significant increase in cell death.[105] Certain anticancer drugs, such as cisplatin and paclitaxel, can upregulate HTRA1, which was found to initiate programmed cell death through autocatalytic activation.[104] Thus HTRA1 is considered to be a tumor suppressor gene in some cancers that promotes cell death. However, it has also been shown that HTRA1 is an oncogene that promotes tumor cell metastasis.[106,107]

The current studies on HTRA1 in tumor vascular related mechanisms of action are as follows, in terms of tumor growth inhibition, Klose et al[22] investigated the effect of HTRA1 on tumor angiogenesis. NOTCH1 signaling regulates endothelial cell proliferation, migration and tube formation. Experiments further confirmed that HTRA1 plays a key role in regulating NOTCH1 signaling by cleaving JAG1.[22] JAG1 antagonises the NOTCH ligand DLL4 (Notch ligand Delta-like 4), which has anti-angiogenic effects.[108] Inhibition of NOTCH1 signaling can lead to increased VEGFR2 expression, which further affects angiogenesis.[109] Moreover, inhibition of the DLL4 can also lead to elevated VEGFR2 expression.[110] This ultimately leads to abnormal functioning of the vascular network as well as increased hypoxia in the tumor tissue, thereby inhibiting tumor growth (Figure 3).

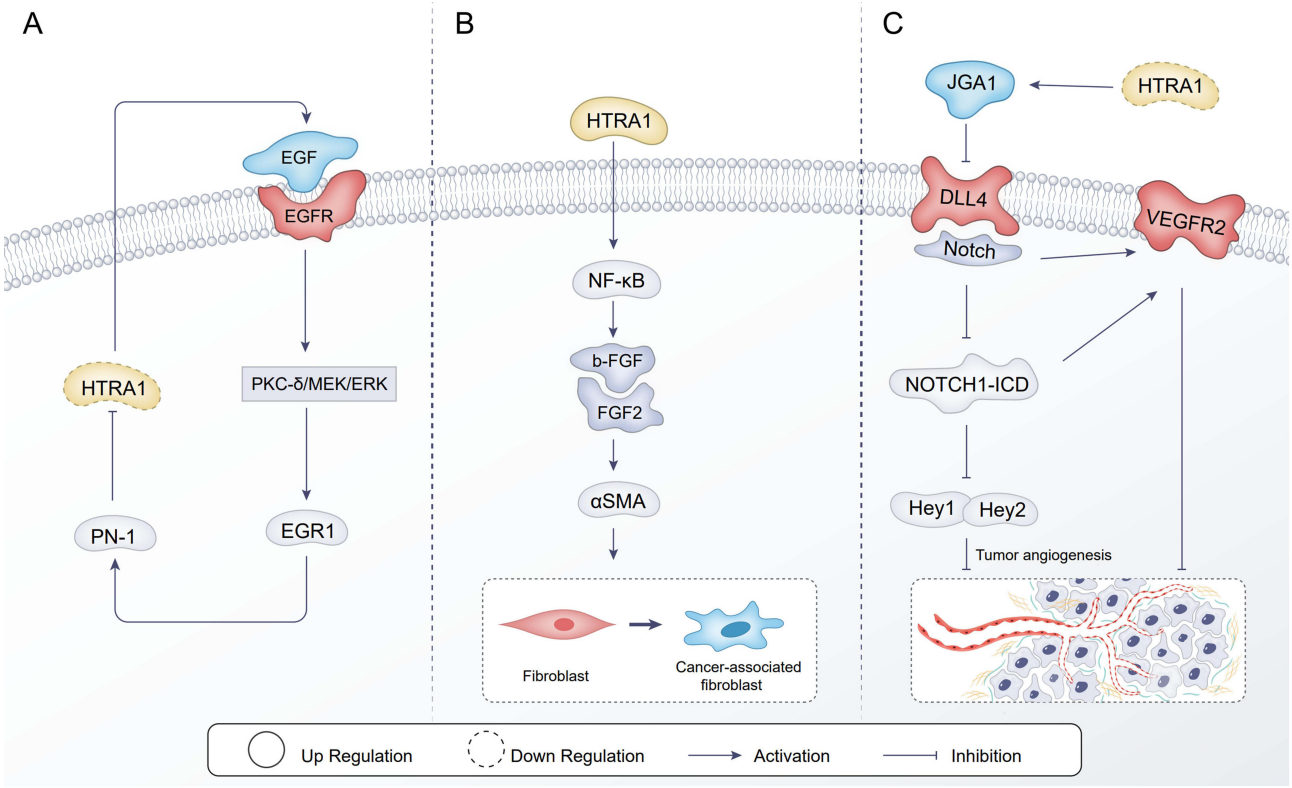

Figure 3 Mechanistic studies of HTRA1 associated with the tumor microenvironment. (**A**) PN-1 expression is regulated through the EGF /PKC-δ/MEK/ERK/EGR1 signaling pathway to inhibit the effect of the HTRA1 protein on tumors; (**B**) HTRA1 promotes the transdifferentiation of normal fibroblasts to CAF by activating the bFGF/FGF2 signaling axis secreted by gastric cancer cells through activation of the NF-kB signaling pathway; (**C**) in vascular endothelial cells, HTRA1 affects angiogenesis through the NOTCH signaling pathway.

With regard to the promotion of tumor growth by HTRA1, the main focus has been on the effects on the tumor stroma, the main component of which is the tumor vasculature. HTRA1 can promote tumor growth by inducing cancer-associated fibroblasts (CAF).[111] CAFs secrete a range of cytokines to regulate the tumor vascular network, and CAFs in the tumor microenvironment contribute to tumourigenesis.[111] α-SMA is a surface marker for CAFs.[107] Wu et al[19] found that HTRA1 was positively correlated with α-SMA expression in gastric cancer tissues, and HTRA1 promoted the bFGF/FGF2 signaling axis secreted by gastric cancer cells through activation of the NF-kB signaling pathway, which in turn facilitated the transdifferentiation of normal fibroblasts to CAFs (Figure 3). Knockdown of HTRA1 inhibits migration and invasion of breast epithelial cells in vitro.[112] In the tumor microenvironment, Protease nexin-1 (PN-1) could increase breast cancer cell migration and invasion through the epidermal growth factor (EGF) / protein kinase (PKC)/ Mitogen-activated protein kinase (MAPK)/ early growth response protein 1 (EGR1) axis.[113] PN-1 promoted cancer cell metastasis mainly by remodeling the tumor stroma. EGF expression was elevated in the breast tumor microenvironment, which upregulated the expression of PN-1 by binding to epidermal growth factor receptor (EGFR), activating the downstream protein kinase C-δ (PKC-δ), extracellular signal-regulated kinase (ERK), MEK and its transcription factor EGR1. PN-1 blocks the function of HtrA1, which disrupts the cleavage of EGF, leading to further activation of EGF signaling, acting as a feedback signal to increase PN-1 expression[113] (Figure 3).

In addition, it has been shown that HTRA1 bind to the tumor promoter lysyl oxidase (LOX), which inhibits TGF-β1 signaling pathway transduction and increases the expression of Matrilin2 (MATN2), further increasing the expression of the EGFR to promote tumor progression and metastasis.[114] TGF-β is associated with HTRA1 during tumorigenesis via the Wnt/β-catenin signaling pathway, EMT and EGFR.[105,115–117] In the early stages of cancer, the TGF-β signaling pathway can act as a tumor suppressor to induce cell death.[118] In advanced stages of cancer, the TGF-β signaling pathway can act as a tumor promoter in the tumor microenvironment.[119] Further studies are needed to unravel the complex interactions between HTRA1 and TGF-β in a cell type-dependent manner, which is necessary to understand HTRA1 as a multifunctional regulator in cancer. Therefore, in the tumor mesenchyme, further investigation of the effects of HTRA1 on the tumor mesenchyme, especially the tumor vasculature, could enhance our understanding of the role of HTRA1 in normal and malignant cells.

Problems and Prospects

The molecular mechanism of human HTRA1 action is a promising study, and current research on HTRA1 is still in its early stages, with increasing evidence that HTRA1 plays a role in the regulation of development, cell growth, differentiation, and apoptosis. HTRA1 is associated with diseases such as cerebrovascular disease, AMD and tumors, mainly in terms of vascular changes in the associated diseases, suggesting that whether it is the endothelial cells, smooth muscle cells, fibroblasts, and other structures related to the vasculature of the blood vessels, HTRA1 has an impact on their functions and physiological processes. HTRA1 plays an important role in vascular homeostasis by regulating signaling pathways or refolding, translocation, and degradation of extracellular matrix proteins, thereby directly or indirectly regulating angiogenesis and vascular remodeling, and vascular and related structural dysfunction caused by abnormal function of the HTRA1 gene is an important initiator and high risk factor. This article suggests that more research on vascular diseases involving cardiovascular,[120] renal,[121] and hepatic diseases[122] should include HTRA1 as an important research target, and even in the process of tissue repair and regeneration,[123] scarring,[124] senescence,[125,126] and pregnancy complications[87] should include HTRA1 as an indispensable research component.

To fully understand the role of HTRA1 in disease, in particular, further study of the mechanisms of HTRA1 action in the vasculature could provide a theoretical basis for clinical practice, which is essential to understand their relevance in cellular physiology and pathogenesis and their use as targets in the future treatment of diseases. Future indepth studies on HTRA1 and vascular-related signaling pathway transduction are believed to lead to more discoveries about HTRA1 and provide new ideas for clinical diagnosis and treatment.

Data Sharing Statement

All data supporting the findings of this study appear in the submitted manuscript or are available from the corresponding author upon reasonable request.

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Disclosure

The authors report no conflicts of interest in this work.

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