Review

Jiang Li, Kaili Wang, Maria N. Starodubtseva, Eldar Nadyrov, Carolyn M. Kapron, Josephine Hoh and Ju Liu*

# Complement factor H in molecular regulation of angiogenesis

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**Abstract:** Angiogenesis, the process of formation of new capillaries from existing blood vessels, is required for multiple physiological and pathological processes. Complement factor H (CFH) is a plasma protein that inhibits the alternative pathway of the complement system. Loss of CFH enhances the alternative pathway and increases complement activation fragments with pro-angiogenic capacity, including complement 3a, complement 5a, and membrane attack complex. CFH protein contains binding sites for C-reactive protein, malondialdehyde, and endothelial heparan sulfates. Dysfunction of CFH prevents its interaction with these molecules and initiates pro-angiogenic events. Mutations in the *CFH* gene have been found in patients with age-related macular degeneration characterized by choroidal neovascularization. The *Cfh*-deficient mice show an increase in angiogenesis, which is decreased by administration of recombinant CFH protein. In this review, we summarize the molecular mechanisms of the anti-angiogenic effects of CFH and the regulatory mechanisms of CFH expression. The therapeutic potential of recombinant CFH protein in angiogenesis-related diseases has also been discussed.

**Keywords:** complement factor H; angiogenesis; mechanical properties; therapeutic target

Jiang Li and Kaili Wang contributed equally to this work.

***Corresponding author: Ju Liu**, Laboratory of Translational Medicine in Microvascular Regulation, Institute of Microvascular Medicine, Medical Research Center, The First Affiliated Hospital of Shandong First Medical University & Shandong Provincial Qianfoshan Hospital, Jinan, Shandong Province 250014, China; Shandong Provincial Key Medical and Health Laboratory of Translational Medicine in Microvascular Aging, Jinan, Shandong Province 250014, China; and Medical Research Center, Shandong Provincial Qianfoshan Hospital, Shandong University, Jinan, Shandong Province 250014, China, E-mail: ju.liu@sdu.edu.cn. https://orcid.org/0000-0001-9932-2613
**Jiang Li,** Laboratory of Translational Medicine in Microvascular Regulation, Institute of Microvascular Medicine, Medical Research Center, The First Affiliated Hospital of Shandong First Medical University & Shandong Provincial Qianfoshan Hospital, Jinan, Shandong Province, China; Shandong Provincial Key Medical and Health Laboratory of Translational Medicine in Microvascular Aging, Jinan, Shandong Province, China; and Medical Research Center, Shandong Provincial Qianfoshan Hospital, Shandong University, Jinan, Shandong Province, China
**Kaili Wang,** Laboratory of Translational Medicine in Microvascular Regulation, Institute of Microvascular Medicine, Medical Research Center, The First Affiliated Hospital of Shandong First Medical University & Shandong Provincial Qianfoshan Hospital, Jinan, Shandong Province, China; and Shandong Provincial Key Medical and Health Laboratory of Translational Medicine in Microvascular Aging, Jinan, Shandong Province, China
**Maria N. Starodubtseva,** Gomel State Medical University, Gomel, Belarus; and Institute of Radiobiology of NAS of Belarus, Gomel, Belarus
**Eldar Nadyrov,** Gomel State Medical University, Gomel, Belarus
**Carolyn M. Kapron,** Department of Biology, Trent University, Peterborough, ON, Canada
**Josephine Hoh,** Department of Ophthalmology, Yale School of Medicine, New Haven, CT, USA

## Introduction

Angiogenesis refers to the growth of new blood vessels sprouting from the pre-existing vasculature [1]. Following endothelial cell (EC) activation, angiogenesis comprises a series of events including basement membrane dissolution, EC migration and proliferation, EC differentiation and stabilization, and vessel maturation [2]. Physiologically, angiogenesis is tightly controlled. Under pathological conditions, an imbalance between stimulators and inhibitors leads to pathological angiogenesis in rheumatoid arthritis (RA), age-related macular degeneration (AMD) and malignant tumors [3–7]. Inhibition of angiogenesis became an efficient treatment for these diseases and thus, understanding the underlying mechanisms of angiogenesis may lead to novel strategies for the development of anti-angiogenesis therapy.

The complement system is involved in the regulation of angiogenesis [8]. The complement system modulates immune responses and related inflammatory responses [9]. It includes the classical, alternative, and mannose-binding lectin pathways, which are controlled by regulatory proteins that impede inappropriate complement components [9]. Complement

factor H (CFH) acts as a plasma regulator of the alternative complement pathway by interacting with the central C3b component [10]. CFH also binds with heparan sulfate (HS), C-reactive protein (CRP), malondialdehyde (MDA) and oxidized phospholipid, and prevents their pro-angiogenic functions [11–14]. Dysfunction of CFH may lead to excessive inflammation-induced angiogenesis [14]. In addition, the recombinant CFH downregulates expression of proangiogenic genes and upregulates expression of antiangiogenic genes [15]. This review describes the molecular mechanisms underlying the anti-angiogenic effects of CFH and its potential as a therapeutic target in pathological angiogenesis.

## CFH protein structure and its functions in complement system

CFH is a single polypeptide plasma glycoprotein synthesized constitutively by the liver. Identified as an inhibitor of the alternative complement pathway, CFH and several other complement regulators, including decay accelerating factor and C4b-binding protein, are encoded by closely linked genes within the regulator of complement activation (RCA) gene cluster located on human chromosome 1 [16]. The structure of the CFH protein was obtained using X-ray scattering and sedimentation coefficient modeling (Figure 1A) [17]. CFH protein consists of 1,213 amino acids, which are primarily distributed within 20 short consensus repeats (SCRs), also known as complement control protein modules (Figure 1B) [16, 18]. The SCRs are highly conserved repeating units of around 60 residues, joined by 19 linking sequences of 3–8 residues situated between the last cysteine of an SCR and the first cysteine of the next SCR [16, 18]. Four invariant cysteine residues become apparent upon alignment of all SCR sequences, and a near-invariant tryptophan residue is located between the third and fourth invariant cysteine [16, 18]. Other members of the RCA family also contain multiple copies of this consensus sequence, which regulates complement activation [19].

Acting as a co-factor for factor I, CFH renders C3b vulnerable to proteolytic inactivation and yields iC3b (Figure 2). CFH causes the dissociation of Bb from C3b in C3bBb (the alternative C3 convertase) and accelerates the decay of the formed alternative C3 convertase, which is termed decay accelerating activity (Figure 2). In the CFH molecule, SCRs 1–4 (the N-terminus), SCR 6–8, and SCRs 19–20 (the C-terminus) are responsible for interaction with C3b [20]. The binding of C3b to SCRs 1–4 mediates the cofactor activity and decay accelerating activity of CFH. In addition, C3 and C5 activation is prevented by CFH competing with Bb for C3b binding and thus reducing the formation of the alternative C3 convertase and C5 convertase (C3bBb3b) (Figure 2). These characteristics provide CFH with the ability to regulate the alternative pathway and to protect the host cell from inappropriate complement activation

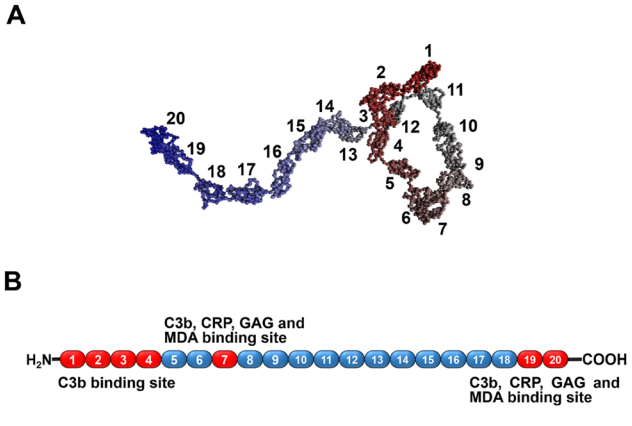


**Figure 1:** Structure of CFH protein. (A) The image of the best-fit model of CFH in 137 mM NaCl was generated using Visual Molecular Dynamics (VMD) (University of Illinois at Urbana-Champaign). The short consensus repeats (SCRs) are numbered from 1 to 20 to indicate the positions of the SCR domains. (B) Major binding sites for C3b, CRP, GAG and MDA were highlighted in red. CFH, complement factor H; CRP, C-reactive protein; GAG, glycosaminoglycan; MDA, malondialdehyde.

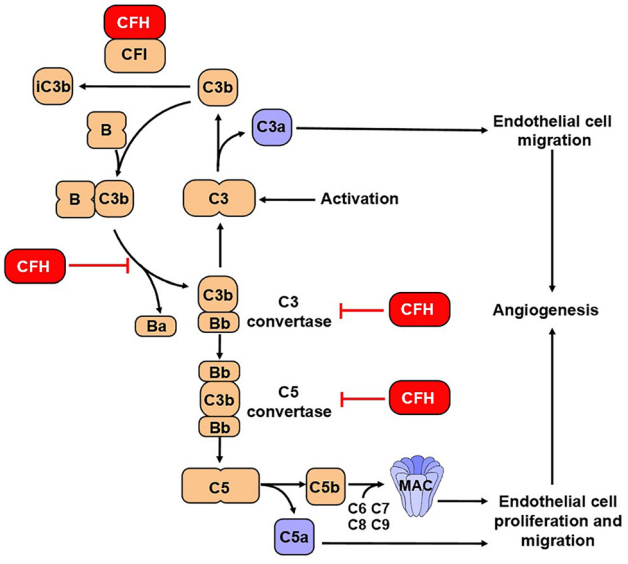


**Figure 2:** The function of CFH in alternative pathway of the complement system. The alternative pathway C3 convertase (C3bBb) cleavage C3, which leads to release bioactive fragments, C3a and C3b. The generation of C3b form more C3bBb with Bb, resulting in positive feedback. C3bBb binding C3b also form C5 convertase to cleavage C5, which leads to the common terminal pathway, and finally produces the lytic membrane attack complex (MAC). CFH inhibits the alternative pathway by inactivating C3b, reducing the formation of C3 convertase and accelerating decay of the formed C3 convertase and C5 convertase. C3a, C5a and MAC contribute to endothelial cell migration and proliferation to promote angiogenesis. Thus, CFH decreases the generation of three pro-angiogenic effectors, C3a, C5a and MAC, to inhibit angiogenesis. CFH, complement factor H; CFI, complement factor I.

**Table 1:** Interactions of complement factors and CFH.

| Complement factors | Roles in complement system | Interaction with CFH | Effects on angiogenesis |
|---|---|---|---|
| C3a | A cleavage product of C3 that binds to C3aR and promotes leukocyte-induced pro-inflammatory activities [25] | Accelerates decay of C3 convertase to inhibit the formation of C3a | Increases vascular permeability and promotes EC migration [25, 26] |
| C3b | Acts as opsonin and forms C3 convertase to amplify the activation of complement | Assists complement factor I (CFI) to inactivate C3b and accelerates decay of C3 convertase to inhibit the formation of C3b | Promotes HUVEC migration and tube formation induced by complement activation [27] |
| C5a | A cleavage product of C5 that binds to C5aR and attracts leukocytes [27] | Accelerates decay of C5 convertase to inhibit the formation of C5a | Enhances vascular permeability and promotes EC proliferation and migration [25, 26] |
| C5b | Forms MAC | Accelerates decay of C5 convertase to inhibit the formation of C5b | Forms MAC |
| Factor B | Forms C3 convertase | Competes with Bb binding to C3b to inhibit the information of C3 convertase | Amplifies the activation of angiogenic complement |
| Factor P | Stabilizes C3 convertase and C5 convertase [22] | Reduces $CD4^+$ T cell proliferation but Factor P enhances $CD4^+$ T cell proliferation [22] | Promotes proliferation of $CD4^+$ T cell with the ability to regulate EC migration and proliferation [22, 23] |
| CFI | Inactivates C3b | Assists CFI to inactivate C3b | Inhibits angiogenesis by blocking complement activation |
| MAC | Lyses cell | Inactivates C3b and accelerates decay of C3 convertase and C5 convertase to inhibit the formation of MAC | Induces EC proliferation and migration and upregulates proangiogenic factors, including VEGF [28, 29] |

CFH, complement factor H; EC, endothelial cell; HUVEC, human umbilical vein endothelial cell; MAC, membrane attack complex; CFI, complement factor I; VEGF, vascular endothelial growth factor.

(Table 1). In addition, the binding sites of CFH also interact with molecules unrelated to the complement system. The SCRs 7 and 19–20 carry two binding sites for glycosaminoglycans (GAGs) and CRP [20, 21]. The SCRs 7 and 20 facilitate the binding of CFH to MDA [13]. By interacting with these ligands, CFH modulates inflammation and oxidative stress. In addition, CFH interacts with a variety of cells and regulates cellular immune responses. For example, CFH produced by dendritic cells (DCs) reduces allogenic $CD4^+$ T cell proliferation and promotes the generation of adaptive regulatory T cells (Tregs) from $CD4^+$ T cells, therefore it decreases the number of $CD4^+$ T cells which promotes angiogenesis by stimulating endothelial sprouting [5, 22, 23]. CFH also induces a tolerogenic and anti-inflammatory phenotype in monocyte-derived DCs, which appears to depend on SCR 19–20 [5]. The C-terminal domains of CFH induce the differentiation of $CD14^+$ human monocytes into immunosuppressive macrophages [24].

## CFH in AMD

AMD is one of the leading causes of visual loss in the elder population [30]. Advanced AMD is clinically classified into two types: dry AMD and wet AMD. Dry AMD is known as geographic atrophy, whereas wet AMD is characterized by choroidal neovascularization (CNV). In patients of wet AMD, blood vessels extend abnormally from the choroidal vasculature, through the damaged Bruch's membrane and the retinal pigment epithelium (RPE), and grow into the subretinal space beneath the photoreceptors (Figure 3B). Fundus examination in wet AMD patients shows edema, subretinal fluid, and subretinal hemorrhage. To date, at least 34 loci was found to a significant association with the risk of AMD [31]. A significantly associated genetic risk factor is a single nucleotide polymorphism (SNP) that histidine (H) is substituted for the normally expressed tyrosine (Y) at codon 402 in SCR 7 of CFH (Figure 3A) [32–34]. This rs1061170 polymorphism significantly impairs the ability of CFH to bind to coagulation factor XI, HS, CRP, MDA and oxidized phospholipid [13, 14, 35–37]. In addition, rs800292, rs3753394 and rs1329428, rs3766405, rs412852 polymorphism of gene for *CFH* is strongly associated with wet AMD [38–40].

To investigate wet AMD, the mouse models have been established to clarify the effects of CFH on angiogenesis (Table 2). These mice manifest pathological angiogenesis in the eye as a result of rupture of the RPE and Bruch's membrane by

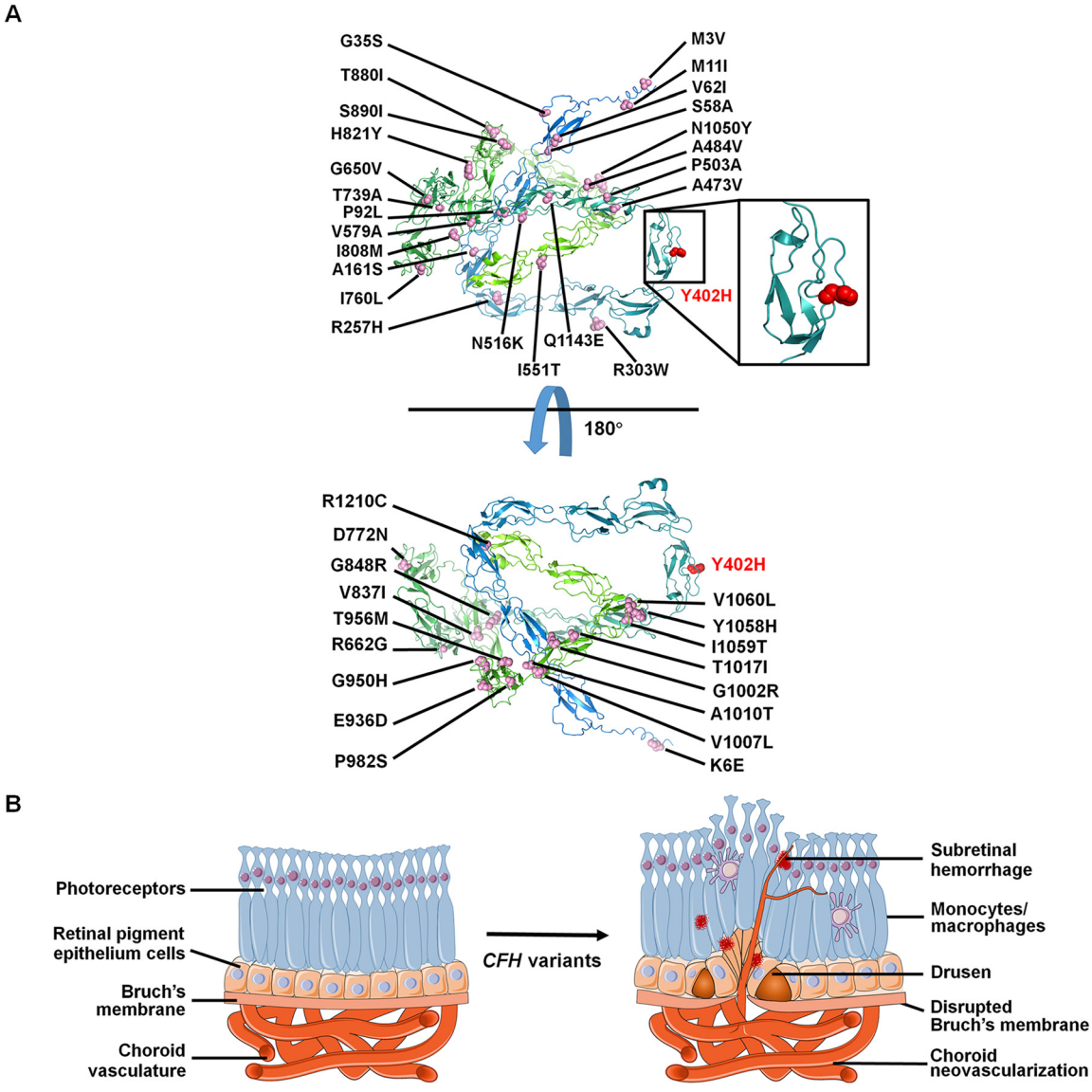


**Figure 3:** Schematic cartoon of wet AMD in the presence of CFH variants. (A) CFH variants related to AMD were highlighted. The protein structure was predicted by AlphaFold (DeepMind). (B) CFH variants promote proangiogenic events. The left panel is the physiological condition in the presence of CFH. The right panel is wet AMD under dysfunction of CFH. Blood vessels extend from the choroidal vessel into the subretinal space beneath the photoreceptors through the damaged Bruch's membrane and retinal pigment epithelium. CFH, complement factor H; AMD, age-related macular degeneration.

laser treatment [41]. During the course of experimentally-induced CNV progression, the Cfh expression is down-regulated [41]. Local knock-down of *Cfh* gene expression in the mouse eye accelerated the formation of CNV and caused more severe CNV compared to that in the control [42]. In addition, CFH-deficient mice showed larger CNV lesions compared with the control mice [43]. Administration of human CFH into the vitreum suppresses CNV formation and induces regression of established CNV in a CNV rat model [44]. Huang et al. investigated the role of CFH using complement receptor 2 (CR2)-fH, a recombinant complement inhibitor that contains the N-terminus (SCR 1–5) of mouse CFH (mCFH) linked to CR2 [45]. This recombinant protein possesses greater complement inhibitory activity than the endogenous serum mCFH [45]. Intravenous injection of CR2-fH reduces laser-induced CNV size in C57BL/6 mice [46]. In another report, intraocular human plasma and recombinant CFH were as effective at reducing CNV on a murine model as a currently used therapy for wet AMD, anti-vascular endothelial growth factor (VEGF) antibody [15]. In addition, GEM103, a recombinant full CFH protein, shows similar complement regulation function of native CFH and protects erythrocytes from hemolysis [47]. Hence, delivery of recombinant CFH by intravitreal injection may be considered as a potential therapy for AMD patients with *CFH* gene

**Table 2:** Rodent models for functional studies of CFH.

| Genotype | Genetic background | Treatment | Phenotype | Mechanism |
|---|---|---|---|---|
| *Cfh*$^{-/-}$ | C57BL/6 mice | | Increased laser-induced neovascularized area in aged *Cfh*$^{-/-}$ mice | Complement deposition VEGF production and angiopoetin-2 release [43] |
| *Cfh*$^{flox/flox}$ mice crossed with *Rosa26-Cre* mice | C57BL/6 mice | | Vessel growth in *Cfh*$^{-/-}$ mice is similar to WT mice in the aorta ring assay; Increased capillaries penetrated into matrigel plugs in *Cfh*$^{-/-}$ mice than in the WT mice | Inhibition of EC migration via interaction of *Cfh* in blood plasma with EC [48] |
| WT | C57BL/6 mice | CFH gene locally knocked down by injection of siRNA | Increased MAC deposition and laser-induced CNV size | Alternative pathway activation and MAC-induced angiogenic factor [42] |
| WT | C57BL/6 mice | Administration of recombinant CFH | Reduced laser-induced CNV size | Inhibition of VEGF expression and C3 deposition [46] |
| WT | Brown Norway rats | Administration of purified human CFH | Decreased MAC deposition and laser-induced CNV size | Inhibition the formation of MAC [44] |
| WT | Long Evans rats | Administration of recombinant CFH | Reduced laser-induced CNV size and choroidal neovascular leakage | Restriction of complement activation and upregulation of proangiogenic factors [15] |

CFH, complement factor H; VEGF, vascular endothelial growth factor; WT, wild-type; EC, endothelial cell; MAC, membrane attack complex; CNV, choroidal neovascularization.

**Table 3:** Diseases or disorders associated with CFH deficiency.

| Diseases or disorders | Pathogenic mechanisms related to CFH deficiency |
|---|---|
| AMD | CFH deficiency induces activation of AP of complement in choroid and C3 accumulation in the RPE [49]<br>Activation of the AP increases CNV lesion size [42]<br>C3a and C5a recruit monocytes and increases VEGF expression to promote CNV [50] |
| Neisseria meningitidis infection | Increases C3 convertase activity leads to consumption of C3 in the circulation and subsequent low C3 plasma levels [51] |
| MPGN II | Uncontrolled activation of the AP of complement and deposition of complement product in the GBM [51, 52] |
| Haemolytic uremic syndrome | Uncontrolled complement activation induces the release of complement cleavage products including C3a and C5a [53]<br>C3 deposits in glomeruli and serum C3 is remarkably reduced [54] |
| aHUS | Inappropriate activation or insufficient inhibition of the AP of complement [55]<br>Production of autoantibodies against CFH [56, 57] |
| MGRS | Production of autoantibodies against CFH [58] |
| Chronic hypocomplementemic renal disease | Blockage of CFH secretion and intracellular catabolism [59] |
| Primary IgA nephropathy | CFH deficiency increases C3 deposition with IgA in the glomerular mesangium [60, 61] |
| Collagen type III glomerulopathy | CFH deficiency facilitates glomerular deposition of type III collagen [62] |
| C3 glomerulonephritis | Mesangial C3 deposits [63]<br>Production of autoantibodies against CFH [55] |
| Endocapillary glomerulonephritis | C3, C4, C1q, IgG and IgM deposit in the capillary wall in a coarse granular pattern [51] |
| SLE | Production of autoantibodies against CFH [64]<br>ADAMTS7 directly interacts with and degrades CFH [65] |
| RA | Production of autoantibodies against CFH [64] |
| Asthma | The circulating level of pro-inflammatory C5a increases [66] |

CFH, complement factor H; AMD, age-related macular degeneration; RPE, retinal pigment epithelium; VEGF, vascular endothelial growth factor; CNV, choroidal neovascularization; MPGN II, membranoproliferative glomerulonephritis type II; AP, alternative pathway; GBM, glomerular basement membrane; aHUS, atypical hemolytic uremic syndrome; MGRS, monoclonal gammopathy of renal significance; SLE, systemic lupus erythematosus; RA, rheumatoid arthritis.

polymorphisms [47]. In addition, we summarized diseases associated with CFH deficiency and the underlying mechanisms (Table 3). Among these diseases, AMD is directly caused by abnormal angiogenesis.

## CFH and angiogenesis

Accumulated evidence suggests that plasma CFH inhibits angiogenesis. Conditioned medium from RPEs with reduced levels of CFH promotes migration and matrigel tube formation of human umbilical vein endothelial cell (HUVEC) [67]. *Cfh*$^{-/-}$ mice display a proangiogenic phenotype in a matrigel assay, in which the number of capillaries that penetrated into the matrigel plugs injected subcutaneously was significantly higher in *Cfh*$^{-/-}$ mice than in those in wild-type (WT) mice [48]. Subsequently, the plasma from *Cfh*$^{-/-}$ mice does not affect the proliferation of ECs, but significantly increases EC migration [48]. Recent study indicated that isolated kidney ECs from *Cfh*$^{-/-}$ mice demonstrated an increased angiogenic potential suggesting that EC-intrinsic CFH may also have an inhibitory effect on angiogenesis [68].

### Effects of CFH on endothelial cell properties

As a plasma protein, CFH directly interacts with endothelial glycocalyx enriched with proteoglycans and glycoproteins [69]. Heparan sulfate proteoglycans (HSPGs), the major constituent of endothelial glycocalyx, are anchored to the EC surface by transmembrane or membrane-bound proteoglycan protein core attached with multiple HS chains [70, 71]. Cyclosporine, calcineurin inhibitor, decreases endothelium glycocalyx through shedding of HS side chains [69]. Reduction of glycocalyx by cyclosporine prevents CFH surface binding, leading to complement-induced endothelial injury [69]. Inside ECs, syndecan-4 proteoglycan, a transmembrane HSPG, is linked to cytoskeletal elements including actin and vinculin [72]. Loss of syndecan-4 proteoglycan disrupts the arrangement of the cytoskeleton network in ECs and increases cell migration and proliferation [72]. CFH interacts with HS chains of HSPGs to protect them from complement attack [69]. CFH deficiency results in a disorder of actin cytoskeleton arrangement, possibly due to the loss of HSPGs. CFH contains HS binding sites at SCR 6–8 and SCR 19–20 [68, 73]. HS has a high affinity for full-length CFH but the relatively lower affinity for fragments of SCR 6–8 or SCR 16–20, suggesting the co-operativity of the two sites [74]. Notably, the SCR 7 and 20 in CFH showed the most net charge densities, which may promote optimal interactions of CFH with anionic HS [17]. The HS chains are composed of three types of regions characterized by high sulfation (NS-domain), low sulfation (NA domain), or intermediate sulfation (NA/NS domain) [75]. CFH interacts strongly with the NS-domains of HS but less so with the NA domains, indicating the importance of the maximally sulfated regions of HS in CFH binding [74]. HS also binds to fibroblast growth factors (FGFs) and VEGFs, both of which potently promote proliferation, migration, and differentiation of ECs [76, 77]. HS forms a ternary structure with $VEGF_{165}$/VEGFR2 or bFGF/FGFR1 to enhance growth factor-receptor binding in a high-affinity complex (Figure 4) [76, 77]. The binding of growth factors to HS requires sulfated regions [75]. As mentioned above, CFH binds to with NS domains

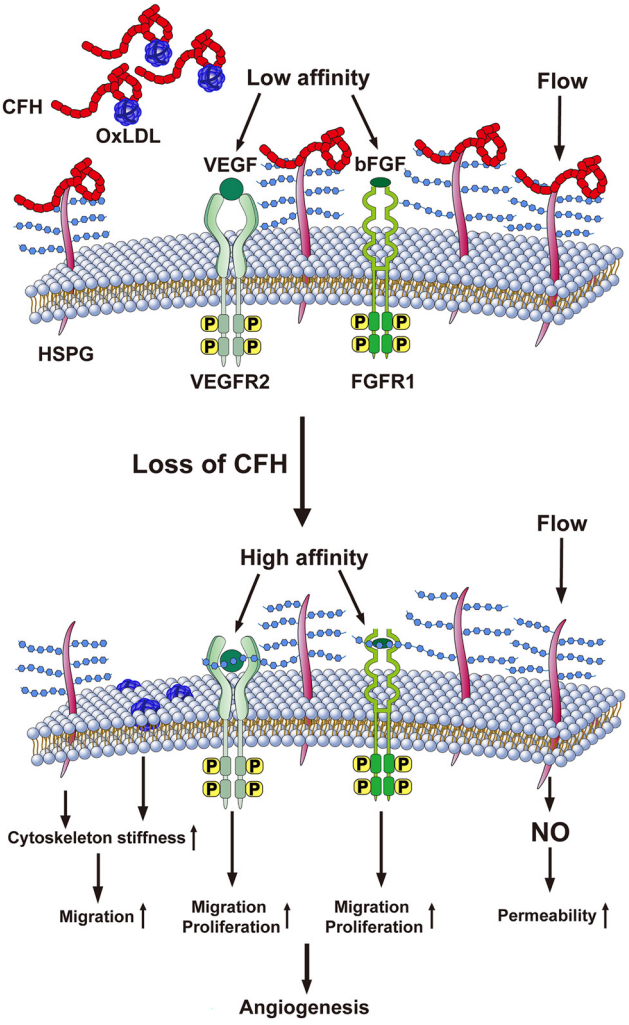


**Figure 4:** The potential effects of CFH on HS and the stiffness of ECs. CFH may bind HS structures and CFH-HS complexes reduce the binding of VEGF and bFGF to their receptors. Loss of CFH may increase of stiffness of cytoskeleton and shear stress induced NO, which increase EC migration and permeability. CFH, complement factor H; OxLDL, oxidized low-density lipoprotein; VEGF, vascular endothelial growth factor; bFGF, basic fibroblast growth factor; HSPG, heparan sulfate proteoglycan; VEGFR2, vascular endothelial growth factor receptor 2; FGFR1, fibroblast growth factor receptor 1; NO, nitric oxide; HS, heparan sulfate; EC, endothelial cell.

of HS with a strong affinity, thus it may compete for HS binding sites with growth factors to prevent the formation of the HS-growth factor complexes and the activation of pro-angiogenic signal (Figure 4) [74]. However, the Y402H variant of CFH requires highly sulfated structures for binding and recognizes fewer HS regions compared to the normal form, which interacts with a wider range of HS structures [78]. Fewer CFH Y402H interactions with HS may lead to an increase of formation of HS-growth factor complexes which promote angiogenesis. In addition, through the extracellular HS chain for shear sensing and the cytoplasmic domain linked with signaling elements, HSPGs mediate blood flow-induced mechanotransduction to produce nitric oxide (NO), an endothelial survival factor [79, 80]. Endothelium-derived NO is a mediator of angiogenesis. Inhibition of NO production reduces endothelial migration and tube formation [81]. The binding of CFH to HSPGs may prevent the transmission of the stress-induced signals (Figure 4) and NO production, which subsequently inhibits angiogenesis.

## CFH-inhibited pro-angiogenic factors

The complement system regulates angiogenesis [8, 27]. Bora et al. demonstrated that the complement activation contributes to the development of laser-induced CNV in C57BL/6 mice via the generation of anaphylatoxins and membrane attack complex (MAC) [28]. Complement anaphylatoxin peptides C3a and C5a promote angiogenesis through leukocyte recruitment and VEGF production [50]. In addition, C3a and C5a enhances vascular permeability [25]. C3a increases VEGF mRNA levels in human RPEs, and knock-down of C3a significantly reduces VEGF transcription [82]. The deficiency of C3 in mice reduces the expression of the mouse VEGF-$A_{164}$ isoform [83]. In addition, C3a and C5a increase C3aR and C5aR1 expression on HUVECs [26]. The $C3^{-/-}$ mice fail to develop laser-induced CNV [28]. Anti-C5 antibody effectively inhibits laser-induced CNV in mice and decrease C5a-induced monocyte chemoattractant protein-1 (MCP-1) and VEGF secretion [84]. Decreased MCP-1 prevents infiltration of pro-angiogenic M2 macrophages [84]. Mice treated with C5aR1 inhibitor PMX53 or $C5aR1^{-/-}$ mice display reduced lung vascular density, suggesting that C5a promotes angiogenesis through its receptor C5aR1 [85]. C5a increases the proliferation and migration of cultured human microvascular ECs and promotes the formation of microvascular-like structures in a matrigel plug assay [86]. MAC, also known as C5b-9, is the end-product of the complement terminal pathway that assembles on the plasma membrane of target cells and forms lytic pores [9]. Suppression of CFH in mice causes higher MAC deposition [42]. Individuals with the CFH Y402H variant have nearly 70 % more MAC in the choroid compared to individuals that have a low-risk genotype [87]. The formation of MAC at the site of injury is required for the development of laser-induced CNV [28]. The choroidal ECs exposed to MAC showed an increase in expression of angiogenic genes including matrix metalloproteinase-3 (MMP-3) and MMP-9, and VEGF-A [88]. MAC directly induces EC proliferation and migration, and this effect is dependent on the inactivation of forkhead box transcription factor O1 (FOXO1) via the PI3K/Akt pathway [29]. FOXO1, as a suppressor of angiogenesis, reduces the proliferative and metabolic activity of ECs [89]. For non-ECs, MAC increases the release of proangiogenic growth factors such as VEGF and b-FGF [28]. As a negative regulator of the alternative pathway, CFH has an inhibitory effect on angiogenesis by decreasing C3a, C5a, and MAC formation.

CRP, one of the acute-phase reaction proteins, triggers the classical pathway [90]. In addition, CRP promotes proliferation, migration, and tube-like structure formation of ECs via phosphorylation of extracellular signal-regulated kinases 1 and 2 (ERK1/2) [91, 92]. CRP treatment results in a rapid increase in EC monolayer permeability [92]. Human choroid organ treated with CRP exhibits an increased expression of pro-inflammatory genes, including intercellular cell adhesion molecule-1 (ICAM-1) that promotes leukocyte recruitment [93]. In RPES, CRP induces the expression of interleukin-8 (IL-8) and MCP-1, both of which promote the proliferation of ECs and the formation of CNV [94–97]. CFH binds strongly to CRP [37]. The high-risk CFH genotype binds CRP less efficiently and CRP is elevated in individuals with the high-risk CFH genotype, which may induce pathological angiogenesis [37, 92, 98].

MDA is a lipid peroxidation product [99]. MDA increases VEGF expression in RPEs, and intravitreal MDA injection in mice enlarges laser-induced CNV sizes [100]. CFH has been recognized as a major MDA-binding protein [13]. The binding of CFH to MDA at SCR 7 and 20 inhibits complement activation and the MDA-induced expression of IL-8 in leukocytes [13]. The CFH Y402H variant impairs the ability of CFH to bind MDA [13]. Mice expressing a chimeric human *CFH* transgene with a variant SCR 6–8 show higher amounts of MDA adduct and an increased expression of NOD-like receptor thermal protein domain associated protein 3 (NLRP3), which facilitates the tube formation of retinal microvascular ECs [101, 102]. Impaired binding of CFH to MDA may increase complement activation and production of proangiogenic cytokines in RPEs.

Oxidized low-density lipoproteins (OxLDLs) in blood plasma induce the reorganization of the EC cytoskeleton through disruption of the integration of lipid rafts, the cholesterol-rich membrane domains directly link to cytoskeletal proteins [103]. OxLDL-induced cytoskeleton remodeling leads to an increase in EC stiffness and EC mechanical force

generation, facilitating the ability of ECs to form the vascular network [103, 104]. OxLDL induces EC stiffening through the CD36/RhoA/ROCK/MLCP/MLC2 pathway and promotes capillary formation [105]. Dyslipidemia, with high OxLDLs levels in plasma, alters elastic properties of EC and induces EC stiffness [106]. CFH interacts with OxLDLs and prevents the binding of OxLDLs to cell surface, thereby inhibiting angiogenesis [14]. The Y402H polymorphism impairs the ability of CFH to interact with OxLDLs, thus contributes to endothelial stiffness [14].

In addition, the extracellular matrix (ECM) and the mechanical properties of ECM regulate cell migration, invasion, proliferation, and survival [107]. The increased ECM stiffness upregulates VEGFR2 expression and promotes angiogenic sprouting in ECs [108, 109]. ECM stiffness is enhanced by the increased composition of collagen and fibronectin [110]. CFH deficiency increases the expression of collagen and fibronectin in ECs [111]. Thus, CFH deficiency may increase composition of collagen and fibronectin induced-ECM stiffness to promote angiogenesis. CFH also binds directly to ECM component fibromodulin, which facilitates angiogenic processes by enhancing EC adhesion, spreading, and tube formation [112, 113]. The binding of CFH to fibromodulin may also prevent its pro-angiogenic effects.

# Regulation of CFH expression

In addition to hepatocytes, CFH is expressed in DCs, RPEs, ECs, fibroblasts, platelets, podocyte. The expression of CFH is regulated by a variety of stimulators and inhibitors [22, 67, 114–117].

## Upregulation of CFH expression

VEGF, a robust angiogenic growth factor, increases production of CFH in human RPEs. Genetic ablation and or pharmacological inhibition of the *VEGFA* gene in mice results in a reduction of CFH in plasma along with complement activation [118]. VEGFR2/PKC-α/cAMP response element binding protein (CREB) signaling was observed to increase local CFH expression [118]. The putative binding site for the transcription factor CREB was found in the human *CFH* promoter, and activation of VEGF/VEGFR2 signaling increases CFH expression through phosphorylation of CREB (Figure 5A) [119]. HUVECs treated with placental growth factor (PlGF) secretes more CFH protein than that of the control or HUVECs treated with both PlGF and soluble fms-like tyrosine kinase receptor 1 (sFlt1), suggesting that angiogenic imbalance may upregulate CFH expression [120].

Interferon response elements are located on the *CFH* promoter [121]. Interferon-gamma (IFN-γ) upregulates the expression of CFH in ECs and podocytes (Figure 5A) [114, 117]. Activation of the IFN-γ pathway increases phosphorylation of signal transducer and activator of transcription 1 (STAT1), which increases *CFH* transcription through upregulation of interferon regulatory factor-1 (IRF-1) and IRF-8 [122]. Interleukin-27 (IL-27) also increases the expression of CFH in RPEs [122, 123]. Similar to the IFN-γ, IL-27 increases IRF-1 and IRF-8 through STAT1 activation. However, a recent study demonstrated that CFH expression is more significantly upregulated by STAT4 than by STAT1 in lung adenocarcinoma cells [124]. As a result of suppressors of cytokine signaling-1 (SOCS-1) and SOCS-3 silencing in these cells, STAT4 is activated and subsequently increases CFH expression.

Cadmium (Cd) is a toxic metal that induces CFH expression in human renal glomerular ECs via activation of the c-Jun N-terminal kinase (JNK) pathway [125]. c-Jun and c-Fos, known as activator protein-1 (AP-1) transcriptional factor, are downstream targets of JNK. These transcription factors bind to the murine *Cfh* promoter in mouse astrocytes, and a mutation at −295 AP-1 motif reduces the promoter activity by 90 % [126]. The −1635 AP-1 motif on human *CFH* promoter was determined to be the corresponding site to regulate *CFH* gene transcription (Figure 5A) [125].

## Inhibition of CFH expression

Oxidative stress downregulates CFH expression in RPE cells [127]. In RPE cells pretreated with blue light-induced photo-oxidative stress, IFN-γ-induced CFH expression is decreased but restored by vitamin C, a reducing agent [128]. The observations suggest that IFN-γ-mediated increase in CFH expression are reduced by oxidative stress in RPE cells [129]. Since STAT1 mediates IFN-γ-induced CFH expression, oxidative stress-acetylated FOXO3 enhances the binding of the *CFH* promoter and reduces the binding of STAT1 that is displaced from its site in the *CFH* promoter, leading to the decrease of *CFH* transcription (Figure 5B) [129, 130].

Nuclear transcription factor-κB (NF-κB) -sensitive microRNA (miRNAs) including miRNA-9, miRNA-125b, miRNA-146a and miRNA-155 recognize complementary sequences in CFH mRNA in the 3′-untranslated regions (3′-UTRs), leading to inhibition of CFH expression in glial cells (Figure 5B) [131, 132]. miRNA-146a and miRNA-155 have partial overlapping binding sites in the CFH mRNA 3′-UTR [132]. Upregulation of miRNA-146a coupled to downregulation of CFH was observed in IL-1β and amyloidβ-42-treated primary human neural cells [133]. In addition, TNF-α inhibits CFH expression by upregulation of miRNA-155, and incubation of antisense oligonucleotides to

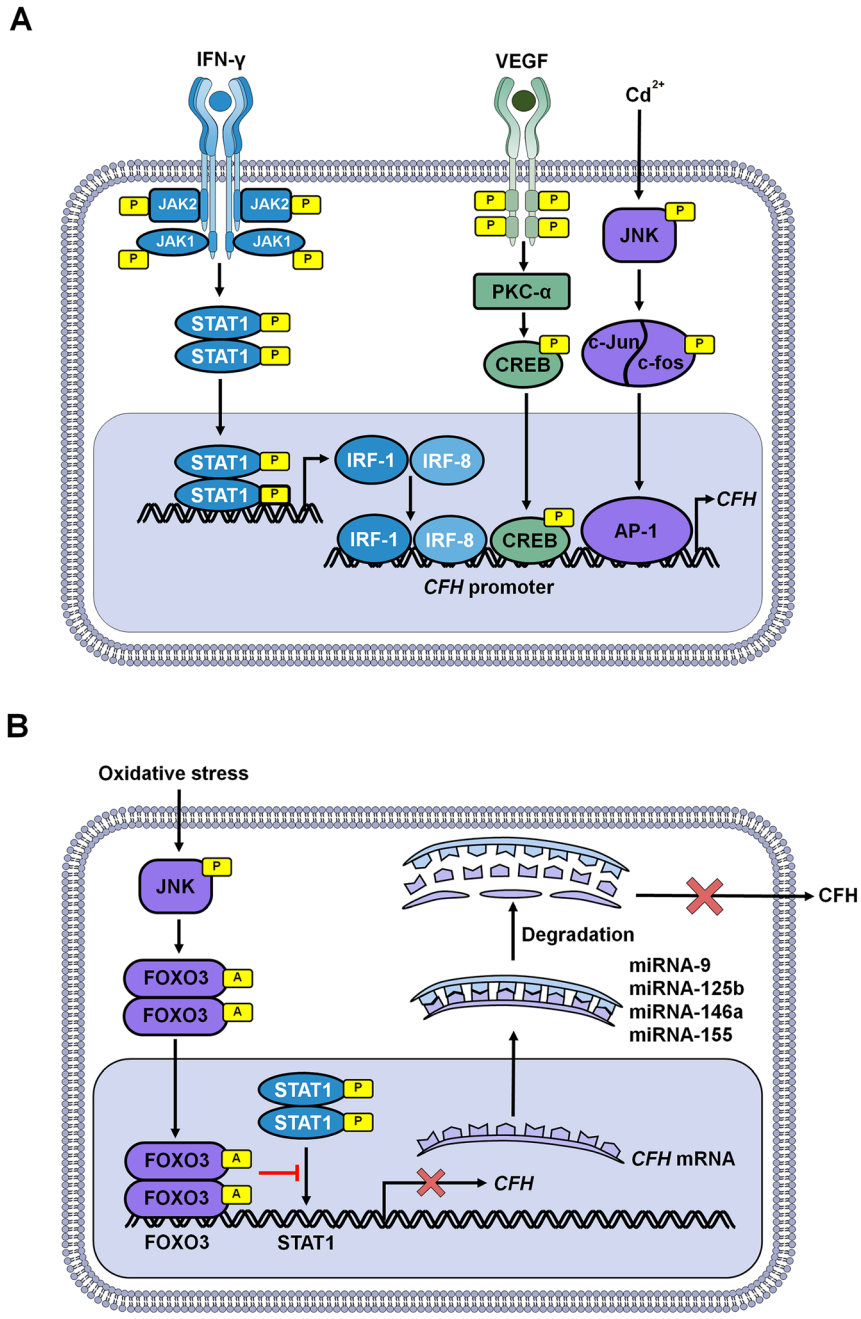


**Figure 5:** Regulatory mechanism of CFH expression. (A) Upregulation of CFH expression. VEGF stimulates phosphorylation of CREB by PKC-α to increase CFH expression. Cadmium activates the −1635 AP-1 binding element on the *CFH* promoter. IFN-γ stimulates the nuclear translocation of STAT1, leading to up-regulation of CFH expression mediated by IRF-1 and IRF-8. (B) Downregulation of CFH expression. Oxidative stress induces acetylation of FOXO3 which binds competitively to the *CFH* promoter, to suppress CFH expression. The miRNAs (miRNA-9, miRNA-125b, miRNA-146a, and miRNA-155) bind to CFH mRNA and induce its degradation. IFN-γ, interferon-gamma; VEGF, vascular endothelial growth factor; JAK1, Janus kinase 1; JAK2, Janus kinase 2; STAT1, signal transducer and activator of transcription 1; JNK, c-Jun N-terminal kinase; PKC-α, protein kinase C alpha; CREB, cAMP response element binding protein; IRF-1, interferon regulatory factor-1; IRF-8, interferon regulatory factor-8; FOXO3, forkhead box transcription factor O3; AP-1, activator protein-1; miRNA, microRNA.

miRNA-146a and miRNA-155 restores CFH expression in neurons and glial cells [133, 134].

## Therapeutic potential for angiogenesis-related diseases by CFH

In addition to wet AMD, targeting CFH may have therapeutic potential for other angiogenesis-related diseases like cancer. As the immunosuppressive role of CFH, it is particularly useful for autoimmune diseases accompanied by angiogenesis like RA [5, 24].

### Rheumatoid arthritis

RA is an autoimmune disease in which leukocytes migrate through the vessel wall into the synovium and eventually induce joint destruction [3]. Angiogenesis facilitates transendothelial migration of leukocytes, and promotes the formation of the highly vascularized pannus, which directly destroys cartilage and bone [3]. Blockade of angiogenesis

reduces synovial inflammation and joint destruction in murine antigen-induced arthritis [135]. CFH inhibits the recruitment of macrophages that contribute to synovial angiogenesis [3, 15]. In response to inflammatory stimuli, fibroblasts in synovial tissue produce pro-inflammatory cytokines which modulate expression of adhesion molecules and MMPs to promote angiogenesis [136]. Thus, CFH protects synovial fibroblasts from inflammatory damage to prevent angiogenesis [115].

Complement activation fragments are elevated in the synovial fluid and synovial tissue in RA patients [137]. Established animal models of RA, such as collagen antibody-induced arthritis, show a dependency on the alternative pathway for induction and progression of joint injury [138]. CFH inhibits the alternative pathway and may reduce synovial inflammation. In mice, administration of the recombinant mouse CR2-fH protein inhibits the alternative pathway, and decreases pannus formation, and cartilage damage in knee joints [139]. CFH may have advantages over other complement inhibitors in reduction of the risk of infection as it specifically inhibits the alternative pathway.

Induction of self-tolerance would be an ideal therapeutic strategy for RA with loss of tolerance to self-antigens. Several potentially tolerogenic cellular therapies in patients with arthritis, including tolerogenic DCs and Tregs, have been developed [140, 141]. Direct targeting of CFH on tolerogenic cell types *in vivo* is an alternative. As mentioned above, CFH creates an immunosuppressive state via induction of immunosuppressive macrophages and tolerogenic monocyte-derived DCs [5, 24]. The administration of recombinant CFH into joints may provide a powerful tolerance for RA. The anti-angiogenic, anti-inflammatory and tolerogenic effects of CFH make it a potential therapeutic target in RA.

### Tumors

It has been reported that the *Cfh*$^{-/-}$ mice develop spontaneous hepatic tumors with leukocyte infiltration and complement deposition [142]. Leukocyte-induced chronic inflammatory and complement-mediated injury increases risk of liver carcinogenesis [142]. CFH inhibits complement activation and decreases complement fragments to recruit leukocytes. Therefore, it may prevent tumor growth and metastasis. However, tumor cells are able to exploit CFH as a complement evasion strategy for protection against complement-mediated lysis, like B cell chronic lymphocytic leukemia cells [6]. In breast cancer, CFH induces an immunosuppressive microenvironment, which also promotes immune evasion [5]. In these tumors, the development of anti-CFH antibodies may be considered as an anti-tumor strategy [7]. The role of CFH in tumor growth may depend on the type of cancer and the stage of tumor progression.

## Conclusions

CFH acts as an inhibitor for the alternative complement pathway. Dysfunction of CFH causes complement disorders, leading to C3a, C5a and MAC-induced angiogenesis. In addition, the binding of CFH to CRP, MDA and endothelial HS inhibits their angiogenic activities. As a plasma protein, CFH may directly interact with ECs and alter their mechanical properties to inhibit angiogenesis. Mutations of the *CFH* gene have been detected in wet AMD patients. In a wet AMD mouse model, intraocular human recombinant CFH reduces CNV, with a potency comparable to currently used anti-VEGF treatment. To date, therapeutic strategies that target CFH have not been developed. Plasma therapy, as a source of secreted CFH, may be a feasible option for the treatment of pathological angiogenesis. However, this therapeutic approach is restricted by the risks of transfusion-associated circulatory overload. By contrast, supplementation with plasma purified or recombinant CFH would reduce these risks. Intravitreal injection of human purified CFH inhibits the formation of new CNV and contributes to the regression of established laser-induced CNV. While purified CFH ensures delivery of the appropriate amount of CFH without the risk of circulatory overload and immune response, it may introduce a risk of transinfection. Recombinant technology with high expression yields and homogeneity can reduce the risk compared with the native protein. In addition, the use of fragments of CFH that contain the major functional domains might be promising, since intravenously-administered recombinant CR2-fH also reduces the size of CNV. Further investigation of molecular mechanisms underlying the effects of CFH on neovascular events may help develop a new therapeutic approach for angiogenesis-related diseases.

## Highlights

- Complement factor H (CFH), an inhibitor of the alternative pathway of the complement system, demonstrate anti-angiogenic properties.
- Loss of CFH increases complement activation fragments with pro-angiogenic capacity, including complement 3a, complement 5a, and membrane attack complex.
- CFH maintains vascular homeostasis by binding with C-reactive protein, malondialdehyde, and endothelial heparan sulfates.

- Mutations in the *CFH* gene have been found in patients with age-related macular degeneration characterized by choroidal neovascularization.
- Recombinant CFH protein demonstrates therapeutic potential in angiogenesis-related diseases.

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