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# Tissue factor with age-related macular degeneration

*Guan-Feng Wang*[1,2], *Xiu-Lan Zou*[1]

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[1]Department of Ophthalmology, Guangzhou General Hospital of Guangzhou Military Command, Guangzhou 510010, Guangdong Province, China
[2]Jinan University, Guangzhou 510630, Guangdong Province, China
**Correspondence to:** Xiu-Lan Zou. Department of Ophthalmology, Guangzhou General Hospital of Guangzhou Military Command, Guangzhou 510010, Guangdong Province, China.xlzou2003@yahoo.com.cn

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## Abstract

• Wet age-related macular degeneration which incidence increases year by year is a blinding eye disease, but current clinical methods of treatment on this disease are limited and the outcome is not ideal. Recent studies have found abnormally high expression of tissue factors which are targets for the treatment of wet age-related macular degeneration to achieve a certain effect in the choroidal neovascularization. Related literatures are reviewed as following.

• KEYWORDS: wet age-related macular degeneration; choroidal neovascularzation; tissue factor; photodynamic therapy; immunotherapy
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## INTRODUCTION

Age-related macular degeneration (AMD) is a blinding eye disease caused by progressive degeneration of retinal pigment epithelial cells and neural retina. AMD is the leading cause for irreversible damage of the vision of people over the age of fifty. The incidence of AMD also shows a growing tendency in Asia [1]. Two types of AMD in pathological classification are dry (atrophic) and wet (neovascular). Wet AMD is more serious than dry AMD in vision damgae. It is reported that 90% clinical AMD patients with serve vision loss are caused by choroidal neovascularization (CNV) and the dry one can transform into the wet one [2].Therefore, currently mainstream treatments are around how to eliminate or inhibit CNV, but the overall efficacy of these measures are not satisfactory. Visual acuity improved only 20% -40% even with comprehensive treatments in clinical. Recent years many scholars not only find a high expression of tissue factor (TF) in tumor angiogenesis, but also find an abnormally high expression of TF in CNV. Because of the superiority of TF targeted therapy in tumor, some scholars do some related researches in the TF targeted therapy for wet AMD. Related literatures are as follows.

## TF AND THE PATHOGENESIS OF AMD

### TF with Inflammation and AMD

**TF with inflammation** Cells do not express TF in direct contact with blood under the physiological condictions. However, vascular endothelial cells, monocytes and macrophages are able to express TF which leads to the inflammation in toxic shock in the stimulation of a variety of inflammatory mediators, endotoxin such as bacterial lipopolysaccharide (LPS),inflammatory cytokines (eg TNF-a, IL-1, IL-6) and other factors. In turn, the high expression of TF expression can induce the expression of pro-inflammatory cytokines sucn as IL-1, IL-6, IL-8 and macrophage inflammatory protein-2a.

Up regulation of TF expression caused by inflammatory factors could cause coagulation cascade and induce thrombosis and disseminated intravascular coagulation (DIC). Therefore, Croce believes that the network of coagulation and inflammation play an important role in the development of atherosclerosis. This network is constituted of many materials which participate in the formation of vicious circle between the coagulation system and inflammatory response system and play a different role in the process of connecting the coagulation and inflammation. TF is the most critical among these materials [3]. In addition, Randolph's study provided a direct evidence for the participation of TF in the inflammatory response. During the initial stage of inflammation, most of the monocyte-macrophage cells penetrate endothelial cells into connective cells, but these cells migrate from the base of lymphatic

endothelial cells to the cavity surface in reverse migration and re-enter the lymphatic drainage when the inflammation subsided [4]. TF mediated the adhesion between the monocyte-macrophage cells and endothelial cells in this migration process.

**Inflammation and AMD** Complement factor H (CFH)is a complement inhibitor which strictly control the complement and maintain homeostasis by reducing the proinflammatory effects of complement activation on normal circumstances[5,6]. In recent years, many studies show that the polymorphism of CFH gene is closely related to AMD which provides genetic evidence for the immunity doctrine of AMD pathogenesis [7]. Immunohistochemical method of choroidal neovascularization show that macrophages with high expression of tumor necrosis factor-a increase the incidence of choroidal neovascularization [8]. One or more antigens of RPE cells in the inferior vena can induce immune response which leave residual cell debris as a series of package targets of inflammatory mediators. Subsequently, other factors or substances make it an initial nuclear which progressive develop the drusen, chemoattractant and activate inflammatory cells, secrete cytokines which could promote the development of AMD. In addition, stimulated RPE cells express monocyte chemoattractant protein-1(MCP-1) which can migrate from inflammation sites to the subretinal space mediated by G protein-coupled receptor Ccr-2. High concentrations of lipids also have the role of chemokines on macrophages which secrete vascular endothelial growth factor (VEGF) to promote angiogenesis in drusen.

**TF with inflammation and AMD** Ranged from inflammation with TF relations and the important role of inflammation in AMD pathogenesis, many foreign experts simulate the AMD disease microenvironment to investigate the relationship of TF expression and AMD in vitro test. LPS induces abnormal expression of TF in mononuclear cells, platelets and human umbilical vein endothelial cells by Norris, Meszaros, Stephens and so on [9-11]. Cho *et al* [12] find that LPS can persistently enhance the transcription and protein expression of TF in ARPE-19 cells. This indicates that TF is abnormally expressed in a pro-inflammatory environment of ARPE cells which could better understanding the enhancement of TF expression by AMD.

Although there are a few researches on the TF-inflammation and AMD, the TF of AMD pathology can be analyzed on the basis of TF expression in many other human diseases. The increase of TF expression is a sign of many inflammatory diseases, such as sepsis, arterial atherosclerosis, antiospholipid syndrome. Redecha *et al* [13]found that anaphylatoxin C5a can induce abnormal expression of TF in the mouse neutrophil cell surface treated by antiphospholipid antibodies.TF can promote the generation of reactive oxygen species (ROS) in human macrophages. In addition, the expression of TF in the inflammatory active area of people CNV is more active than that in non-inflammatory active area [14-16]. So, up-regulation of TF in AMD can damage the RPE and photoreceptors via increasing the inflammation.

**TF with Oxidative Stress and AMD** Many age-related diseases are related to the accumulation of reactive oxygen intermediates (ROI), such as hydrogen peroxide, singlet oxygen, superoxide anion, and hydroxyl radical. Excessive oxygen free radicals or slowly remove which could have a range of damages to the organism. Due to a variety of reasons for the particularly sensitivity of ROI to the retina, such as high oxygen tension of photoreceptors, long-term exposure to light radiation and high proportion of polyunsaturated fatty acids for photoreceptor outer segments, a large number of chromophores as lipofuscin, melanin, cytochrome C are containing in retina and RPE cells. In recent years, a large number of studies have shown that lipofuscin plays an important role in oxidative damage of AMD.

Basic research has found that stimulated H202 induced increased mRNA and protein levels of TF, compared with the inflammation treatments of LPS. Recent evidence suggests that many transcription factors such as nuclear factor KB(NF-KB) and activator protein-1(AP-1) play a role of signal transduction pathways of oxidative stress in a variety cells[17-19]. But CD40L reaction original are containing in -278bp to +121bp region of TF start promoter including AP-1,NF-KB and early growth response gene-1 (Egr-1) [20]. $H_2O_2$ also induces the translocation of NF-KB and combination of AP-1. But Penn observed that levels of TF mRNA or TF antigen of cell surface did not significantly increase in the smooth muscle cells of $H_2O_2$, enhance the activity of pre-existing and potential TF. It is noteworthy that apoptosis is also an important factor in the formation of plaque thrombosis and co-localization of apoptotic cells within and outside the TF expression is found in the lipid core of atherosclerotic plaque [21]. In AMD, oxidative stress induces apoptosis of RPE and photoreceptors to enhance the expression of TF.

**TF with Aging and AMD** Aging is the most important risk factors of AMD. Incidence of risk is nearly doubled as each additional 5 years of age [22]. As age increased, accumulation role of external harmful factors with decline

of metabolism and repair capacity lead to irreversible retinal damage[23].

At present, foreign scholars potential study TF-aging relationship with AMD by Ccl-2-/-/Cx3cr1-/- (double knockout, DKO) mouse retinopathy model [13]. Accompany with the growth of the age, TF expression is also increased in the wild-type (WT) and DKO-type of neural retinal tissue. TF expression of young group in DKO-type does not differ significantly compared with that in WT-type. However, TF expression of elderly group in DKO-type differs significantly compared with that in WT-type. Young group of DKO starts to appear symptoms at 4-6 weeks, and the old group of DKO performs integration of disease and photoreceptor damage[24,25].

**TF AND CNV**

**TF and Angiogenesis** In addition to the important role in angiogenesis during embryonic development, TF is still involved in adult angiogenesis, especially in tumor blood vessels and choroidal neovascularization. Belting and colleagues find that phosphorylation of TF is related to the pathological neovascularization, but not related to the normal blood vessels of diabetic retinopathy which suggests the potential role of TF in CNV formation[26]. Basic research finds that high expression of TF in CNV abundantly formed and growed faster. The opposite is the low expression one. They find that TF is highly expressed in RPE cells and macrophages by immunostaining of CNV Biopsy[27].

**TF and the Invasion of Nevascularization** The level of TF abnormally highly expressed in the endothelial cells of choroidal neovascularization and tumor vascular endothelial cells of pancreatic, breast , colon and so on. In recent years, foreign clinical observations and experiments *in vivo* and vitro confirmed that FVIIa/TF complex could promote the growth, invasion and metastasis of choroidal neovascularization. High expression of TF in neovascularization has a strong transfer capacity. Sub-line TF established by the metastatic lesions is more expression than matriarchal. Clinical studies also have confirmed that the formation density of CNV is related to the expression level of TF and VEGF on vascular endothelial cell surface.

**Relationship Between TF and VEGF** The intensity of TF expression in the CNV is much higher than that of VEGF. TF mRNA expression in the vascular endothelial cells of wet AMD patients is 32 times more than that in the vascular endothelial cells of normal macular region, but mRNA of VEGF is only 4-6 times. Many foreign scholars have found a close link between TF and VEGF. There is a positive feedback loop condition between VEGF and TF in tumor and inflammation, TF raises the increase of VEGF to induce the formation of angiogenesis [14,28]. In turn, up-regulation of VEGF can increase the expression of TF. Makin detected the levels of TF, VEGF and sFlt-1 in the plasma of 234 cases of patients with peripheral vascular disease and 50 healthy patients. The test results have found that levels of TF and VEGF significantly increased in the plasma of patients with peripheral vascular diseases compared with normal healthy, $P<0.05$. Correlation analysis revealed that content of VEGF was positively correlated to TF($r=0.351$, $P<0.001$), sFlt-1 was positive correlated to TF and VEGF ($r=0.268$, $P<0.001$; $r=0.449$, $P<0.001$), respectively. The results confirmed that the increase of TF was one of the most important factors which caused peripheral vascular diseases. And they also found that the main mechanism of angiogenesis was the high expression of VEGF caused by TF.

At present, the research mechanism of promoting angiogenesis by TF may summarized as follows: (1) FXa dependent on coagulation factor FVIIa hydrolyzes and activates a large number of thrombin which combines PAR21 and PAR22 to activate a different biological effect. (2)Activated FVIIa makes signal transduction and amplification in TF cytoplasmic. (3) Independence of the thrombin-activated cytokines bind with mitosis protease-activated receptor. (4) Direct ligand is interacted with the one that not depend on cytoplasmic. Therefore, the important role of TF in angiogenesis is not different from that in a single thrombus formation. On one hand, it can activate coagulation factors to achieve the physiological or pathological effect of blood; on the other hand, it can also be served as a signa molecule passing different types of cells to play a biological effect intracellular and extracellular .

**TF TARGETED TREATMENT for CNV**

Abnormal expression of TF plays an important role in AMD which could be as retinal neovascularization treatment for AMD.Therefore, Lu *et al*[29] modified and filtered out ICON a polypeptide high affinity with TF which was as nature ligand of Factor VII. They combined ICON with Verteporfin to synthesis TF targeted new photosensitizer. To the non-targeted verteporfin photoreceptor therapy for rat model with CNV, FFA showed that only 25% CNV were occlusion after 7 days of treatment. After treatment of 14 days, FFA showed that not only the remaining CNV was still leakage, but also found the new CNV. To the TF-targeted verteporfin photoreceptor therapy for rat model with CNV, FFA showed that 67% CNV were occlusion after 7 days of treatment, remaining 64% CNV were still occlusion after 14 days of treatments. Pathological

examination showed that the TF targeted PDT therapy was less harmful to normal tissue. Therefore, TF targeted PDT treatment has more significant effect for wet AMD compared with non-targeted verteporfin. In addition, TF targeted immunotherapy, FVII-Fc chimeric antibody, can selectively eliminate the rats and guinea pigs CNV caused by laser, and reduce CNV migration without side effects[30,31]. However, TF targeted drugs showed more specific, higher sensitivity, less vascular toxic effects to VEGF targeted drugs. Combination of Factor VII and TF easily leads to the potential spread of bleeding. These macromolecular complexes are not conductive to drug absorption coupled with short half-life which may influence the efficacy of targeted drugs. Now there is less study of TF targeted therapy for CNV which is still in animal experiments. We need a large number of reasonable designs, high quality randomized controlled clinical cases to evaluate and study its efficacy.

## SUMMARY AND OUTLOOK

In summary, incidence of wet-AMD increases year by year. The formation of wet-AMD is a complex process. Although its pathogenesis has not yet been fully elucidated with complex etiology and lacking of effective treatment, many studies have found that TF are abnormally efficient expressed in the endothelial cells of CNV which could be as the targeted therapy for CNV. Future research is focused on the screening of short peptides with high-affinity of TF. It could reduce the vascular toxic effects and the side effects of high-affinity macromolecular complexes. Detect the combination of drugs and high-affinity peptide. Evaluate TF targeted drugs of metabolism, effects and adverse reactions. In addition, we still need a large number of clinical cases to continuously improve the treatment effect of TF-targeted drugs on AMD-CNV, to set up specific, efficacy, less side effects and new therapeutic approaches.

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