

The relationship between intervention in the CD40 signal pathway and choroidal neovascularization

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Abstract: Age-related macular degeneration, pathologic myopia, ocular trauma, and other eye diseases can cause choroidal neovascularization (CNV). In recent years, photodynamic therapy (PDT), anti-vascular endothelial growth factor (anti-VEGF) medications, laser treatment, and other measures against CNV have been gradually applied in the clinical setting and in some cases have achieved good results. However, the pathogenesis of CNV has not been fully elucidated. The costimulatory system made up of cluster of differentiation 40 protein (CD40) and its ligand (CD40L) is an important signal transduction pathway among immune cells. The activation of CD40 can also stimulate the secretion of a variety of angiogenic growth factors (eg, VEGF) and basic fibroblast growth factors that might lead to CNV. The high level expression of CD40 and CD40L has been detected in CNV diseases. Interference with the CD40 signaling pathway may become a new target for CNV treatment. We review the relationship between CD40, CD40L, and CNV.

Keywords: choroidal neovascularization, NV, CD40, angiogenesis factor

Introduction

A variety of reasons including trauma, inflammation, and degeneration can lead to the production of choroidal neovascularization (CNV). Recently, its pathogenesis has been the focus of research in the ophthalmic community. Studies have shown that vascular endothelial growth factor (VEGF) is the central link in the formation of CNV. Some inflammatory cytokines such as monocyte chemoattractant protein-1, elastin, interleukin (IL)-1, IL-6, and tumor necrosis factor (TNF)- α also have an effect on VEGF.^{1,2} However, the specific pathogenesis of CNV has not been fully elucidated. Cluster of differentiation 40 protein (CD40) – inflammatory molecules which are expressed on the surface of B-cells, monocytes–macrophages, and endothelial cells (EC) – plays an important role in inflammation and the immune response. Reinders et al confirmed that the combination of CD40 and CD40 ligand (CD40L) could promote the expression of the proangiogenic factors of the vascular EC (eg, VEGF) and result in CNV.³

Structure, biological characteristics, and functions of the CD40 signal pathway

CD40 and CD40L are an important pair of costimulatory molecules involved in specific immune response systems in vivo, and are a pair of complementary transmembrane glycoproteins, which are involved in humoral and cellular immune response.

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Construction and distribution of the CD40 and CD40L costimulatory system

CD40, also known as leukocyte differentiation antigen (Bp50), is a kind of surface antigen associated with the function of T-cells and B-cells whose relative molecule mass is 45–50 kDa. CD40 is a type I transmembrane glycoprotein. It is composed of 227 amino acids where the ectodomain, transmembrane, and intramembrane areas are respectively made up of 193, 22, and 62 amino acids. The gene, located on chromosome 20q11-q13, belongs to the tumor necrosis factor receptor (TNFR) superfamily members. CD40 has a wide distribution of expression on different cell types, including B-cells, monocytes–macrophages, ECs, dendritic cells, and epithelial cells in vivo. Moreover, its expression is able to increase significantly in malignant B-cell tumors, atherosclerosis, and other pathological cases.

CD40L, also known as CD154, tumor necrosis factor-associated activation protein-1 (TRAP-1), or T-cell B-cell-activating molecule (TBAM), belongs to the type II transmembrane glycoprotein and has a molecular mass of 39 kDa. CD40L consists of 261 amino acids. Among them, there are 215 amino acids in the ectodomain, 24 in the transmembrane region, and 22 in the intramembrane. The *CD40L* gene is located on the X chromosome Xq26.3-q23.1 and belongs to the TNF superfamily members. CD40L is found mostly on activated T-cells, particularly activated CD4⁺ T-cells, natural killer cells (NK cells), monocytes–macrophages, B-cells, and platelet cells.⁴

Biological effects of CD40 signal pathway

Because CD40 was found on B-cells for the first time, early researchers focused on the regulation of CD40 on B-cells' function. The study⁴ found that costimulation of CD40 and CD40L could not only mediate the activation, proliferation, and immunoglobulin (Ig) class switching of B-cells, but it also helps promote the generation of humoral immunity. In addition, the combination of CD40 and CD40L could facilitate T-cells' activation and bone marrow-derived antigen presenting cells (APC) maturity. Therefore, it is able to promote the cellular immune response.⁵ Subsequent studies have found that the combination could activate fibroblasts, induce the expression of CD54 and CD106, generate IL-6, and induce cells' proliferation, thus playing an important role in fibrosis progression. Furthermore, the combination could induce the expression of VEGF on EC and promote angiogenesis, indicating that CD40 has a significant effect on the occurrence and development of neovascularization.⁶

The CD40 signal pathway also plays an important role in the cellular immune response. Yang et al⁷ discovered that CD40L-deficient mice could not produce effective cytotoxic T-cells in transfected hepatocytes. Full reconstitution of cellular and humoral immunity was achieved in CD40L-deficient mice by administration of an activating antibody to CD40 that increased expression of B7.2 on spleen cells. This study suggested that CD40 primarily induced the activation of APC by raising the B7 molecule of APC. The costimulation of CD40 and CD40L played a role in the occurrence, development, and prognosis of the inflammation by raising the expression of adhesion molecules and promoting the generation of proinflammatory cytokines such as interferon (IFN), TNF, and IL.⁸

CD40 and angiogenesis

Angiogenesis

Angiogenesis is a complex process. Shear stress or various angiogenic proteins stimulate vessel permeability and EC activation, proliferation, migration, and differentiation into mature blood vessels as sprouts. These sprouts then form loops to become fully-fledged vessel lumens and build a net connecting neighboring vessels.⁹ It can happen not only in normal physiological processes but also in some diseases such as cancer, rheumatism, atherosclerosis, and blinding eye diseases (age-related macular degeneration [AMD], pathologic myopia, etc).^{10–12} A variety of cytokines contribute to the regulation of angiogenesis, especially the imbalance between angiogenic factors and antiangiogenesis factors; pathological angiogenesis comes from the overexpression of angiogenic factors or low expression of antiangiogenic factors. It has been confirmed that various cytokines such as VEGF, TNF- α , basic fibroblast growth factor- α , and transforming growth factor- β can promote angiogenesis; among them, VEGF is the strongest. Studies have demonstrated that in inflammatory response, lymphocytes and monocytes especially activated T-cells, as well as how mononuclear macrophages can secrete angiogenic factors and stimulate angiogenesis.^{13–18}

Relationship between CD40 and angiogenesis

Some studies have proven that because of the role of CD40 in immune response, the costimulation of it and CD40L can lead to the activation and proliferation of lymphocytes, monocytes, macrophages, and other inflammatory cells; these studies have also shown that activated lymphocytes and mononuclear macrophages can secrete a large number of

angiogenic factors including VEGF, inferring that CD40 has an effect on the regulation of angiogenesis.^{19,20}

For instance, after treating confluent cultures of EC with soluble CD40L (sCD40L) and by Western blot, Dormond et al²¹ found a marked increase in the phosphorylation of Akt, 4EBP-1, and S6K1 compared with untreated cells. EC were transfected with a full-length VEGF promoter-luciferase construct and cultured in the absence or presence of rapamycin and sCD40L. They found that rapamycin, which blocks mTORC1 and mTORC2 signaling, inhibited sCD40L-mediated transactivation of VEGF. In addition, by Western blot, they found that transfection of EC with small interfering ribonucleic acid (siRNA) to rictor (to inhibit mTORC2), and not raptor (to inhibit mTORC1), inhibited sCD40L-dependent protein expression of VEGF. In addition, they found that basal levels of both phosphorylated Akt and VEGF were increased in EC transfected with the raptor siRNA. Rapamycin also failed to inhibit VEGF promoter activation and VEGF protein expression in EC transfected with a constitutively active construct of Akt, further demonstrating that mTORC1 is not necessary for CD40- and Akt-induced expression of VEGF. Finally, they injected human CD40L-transfected fibroblasts or mock transfectants into human skin on severe combined immunodeficiency (SCID) mice. They found that the injection of CD40L transfectants – but not mock cells – resulted in VEGF expression and mediated a marked angiogenesis reaction; this response was reduced in mice treated with rapamycin. Together, these observations indicated that mTORC2 and Akt facilitate CD40-inducible expression of VEGF in EC.

CD40 and CNV Pathogenesis of CNV

The pathogenesis of CNV has not been fully elucidated. It is commonly believed that the production of CNV is related to a change in the integrity of the retinal pigment epithelium (RPE)-Bruch's membrane-choriocapillary complex. The new blood vessels of choriocapillaries grow through Bruch's membrane into the layer between Bruch's membrane and the RPE or the layer between the RPE and the retinal nerve sensory.²²

Some studies suggest that CNV is induced by inflammatory response. With aging, the phagocytosis function of RPE decreases, which causes inadequate degradation of the shedding on the outer end of photoreceptor cells and lipid deposition on Bruch's membrane and its vicinity. These sediments may be regarded as foreign antigens which lead to inflammation response and eventually give rise to CNV.^{23,24}

Important role of macrophages in the development of CNV

The exact pathogenesis of macrophages' participation in CNV formation is not clear, but it has been proven that the invasion of macrophages has a significant effect on the development of CNV.^{25,26}

After using CC chemokine receptor gene knockout (CCR2KO) mice and B6 mice and performing laser photocoagulation to induce choroid inflammation response, Tsutsumi et al¹ observed the role macrophages played in CNV through the analysis of reverse transcription polymerase chain reaction (RT-PCR) and histopathology. The experiment was divided into the experimental group (CCR2KO group) and the control group (B6 group). The study found a marked reduction in the number of ocular-infiltrating macrophages by flow cytometry. Additionally, they found that the area of CNV was significantly reduced by fundus fluorescence angiography in the CCR2KO mice compared with the control mice. The results confirmed that macrophage migration was closely associated with CCR2. As chemokines decreased in the CCR2KO mice, the gathering of macrophages in the inflammation response area was inhibited. The results showed a decrease in the number of macrophages and the area of CNV, thus implying that the infiltration of macrophages played an important role in the development of CNV. In addition, the study found that there were more B7.2, CD40 on macrophages than on the lung macrophages by flow cytometry, from which we inferred that T-cells and macrophages could promote the expression of VEGF through the interaction of CD40-CD40L, causing the generation of CNV. Thus it was confirmed that inflammation response played an important role in the occurrence of CNV.

Participation of CD40 in regulating the retina inflammation response

As stated above, the combination of CD40 and CD40L can lead to the activation and proliferation of inflammatory cells such as lymphocytes and monocytes, as well the secretion of inflammatory cytokines and chemokines that induce inflammation response. In addition, activated T-cells and monocytes can also lead to the secretion of proangiogenic factors, eventually causing angiogenesis. Thus, it is inferred that CD40 plays an important role in the generation of CNV.

Portillo et al²⁷ used one eye of male 57BL/6 (B6), CD40^{-/-}, and C57BL/6TgN (ACTbEGFP) 10sb (B6 EGFP, in which EGFP is enhanced green fluorescent protein) mice weighing 25–30 g to make a retinal ischemia model; the other eye of the same animals were set up as the control. The experiment was

divided into separate CD40^{-/-} and B6 EGFP groups with the purpose of examining the role of CD40 in the pathogenesis of retinal injury. The study found that compared with the B6 group, the retinal inflammation, loss of ganglion cells, and capillary degeneration were markedly attenuated in CD40^{-/-} mice. It showed that CD40 molecules could mediate retinal inflammation and neurovascular degeneration. It confirmed that CD40 activity could stimulate the expression of endothelial and Müller cell adhesion, that it could stimulate the production of chemokines, and that it promoted the recruitment of leukocytes which express nitric oxide synthase 2 (NOS2) and cyclooxygenase-2 (COX-2). However, retinal inflammation, loss of ganglion cells, and capillary degeneration were more severe in the mice expressing CD40 and in the B6 EGFP group.

The role of CD40 and CD40L in abnormal antigen commission

IFN- γ -activated human retinal pigment epithelial (hRPE) cells express human leukocyte antigens DR (HLA-DR), intercellular adhesion molecule-1, and IL-1; they also enhance the secretion of a variety cytokines and chemokines such as IL-1, IL-6, granulocyte-macrophage colony stimulating factor (GM-CSF), and monocyte chemotactic proteins. All these activated immune factors can further facilitate the expression and secretion of growth regulating gene 1 (GRO-A) and IL-8 on T-cells, thereby causing the gathering of lymphocytes. Therefore, IFN γ -activated hRPE cells, as a kind of antigen-presenting cell, can cause retinal inflammatory diseases and retinal transplant rejection response.²⁸

Willermain et al²⁹ analyzed the expression of CD40 costimulatory (CD40, B7.1, cell B7.2, CD54, and CD58) molecules on activated adult hRPE cells by flow cytometry and evaluated their effect as potential antigen presentation cells by measuring the secretion of IL-6, IL-8, IL-10, and IL-12 using an enzyme-linked immunosorbent assay (Figure 4). The study found that IFN γ -activated hRPE cells expressed CD40, but not B7.1 or B7.2. Although IFN γ promoted the production of IL-6 and IL-8, it did not induce the secretion of IL-12. hRPE cells did not activate allogeneic T-cells but did inhibit the proliferation of T-cells partly through induction of apoptosis (Figure 5). In addition, the study found that RPE was closely associated with the expression of CD40 in the process of antigen presentation; the binding reaction of CD40 and CD40L on hPRE cells inhibited the proliferation of T lymphocytes, which might be related to the role of hPRE cells in antigen presentation. The exact molecular mechanisms are still unclear mainly because of the interaction of CD40 and CD40L in the process.

CD40 and CD40L plays a role in abnormal antigen commission by inducing hRPE cells to increase the yield of IL-6 and IL-8, thus causing inflammation of the retina and inducing angiogenesis.

Conclusion

The pathogenesis of CNV is a complex process involving a variety of signal-mediated pathways. A variety of cytokines participate in the occurrence and development of CNV. The occurrence of CNV involves the rupture of Bruch's membrane, the proliferation and migration of capillary EC, and angiogenesis. CD40, an important inflammatory marker on the cell surface, plays an important role in inflammation and immune response, as well as angiogenesis. However, there are some studies suggesting that the high expression of CD40 in RPE and the resulting signal transmission can inhibit the proliferation of T lymphocytes.³⁰ It is also reported that RPE has the function of inhibiting the activation and proliferation of T-cells and B-cells.^{31,32} However, the RPE these researchers studied were from healthy animals; they did not study the role the expression of CD40 in RPE has in animal models of CNV nor the role of the inflammation reaction.

Although there are reports that the occurrence of CNV is related to lipid deposits on Bruch's membrane and its vicinity, it needs to be further confirmed. Increasingly with age, there is a change in the number of lipid deposits around Bruch's membrane, but this alone does not mean that CNV will occur. In short, although CD40 is indeed found and highly expressed in CNV cases, the reason and the pathogenesis mechanism require further study.

As VEGF and CD40 are both involved in angiogenesis, it can be inferred that CD40 is essential to the formation of retinal neovascularization and CNV. Currently, the application of antiCD40 antibody to interfere with the CD40 signaling pathway has been successfully used in the treatment of tumor, atherosclerosis, transplant rejection reaction, and autoimmune disease;³³ it has achieved a certain degree of effect. Thus, the interference of CD40 signaling pathway is expected to be a new method for the early prevention and treatment of CNV. Perhaps it can provide a new therapeutic approach for immune therapy of neovascular eye diseases.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Tsutsumi C, Sonoda KH, Egashira K, et al. The critical role of ocular-infiltrating macrophages in the development of choroidal neovascularization. *J Leukoc Biol*. 2003;74(1):25–32.

2. Skeie JM, Mullins RF. Elastin-mediated choroidal endothelial cell migration: possible role in age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2008;49(12):5574–5580.
3. Reinders ME, Sho M, Robertson SW, et al. Proangiogenic function of CD40 ligand-CD40 interactions. *J Immunol*. 2003;171(3):1534–1541.
4. Phipps RP. Atherosclerosis: the emerging role of inflammation and the role CD40-CD40 ligand system. *Proc Natl Acad Sci U S A*. 2000;97(13):6930–6932.
5. Uckun FM, Gajl-Peczalska K, Myers DE, Jaszcz W, Haissig S, Ledbetter JA. Temporal association of CD40 antigen expression with discrete stages of human B-cell ontogeny and the efficacy of anti-CD40 immunotoxins against clonogenic B-lineage acute lymphoblastic leukemia as well as B-lineage non-Hodgkin's lymphoma cells. *Blood*. 1990;76(12):2449–2456.
6. Flaxenburg JA, Melter M, Lapchak PH, Briscoe DM, Pal S. The CD40-induced signaling pathway in endothelial cells resulting in the overexpression of vascular endothelial growth factor involves Ras and phosphatidylinositol 3-kinase. *J Immunol*. 2004;172(12):7503–7509.
7. Yang Y, Wilson JM. CD40 ligand-dependent T cell activation: requirement of B7-D28 signaling through CD40. *Science*. 1996;273(5283):1862–1864.
8. Li Qiu, Yang Xiqiang. CD40/CD40L inflammatory reaction in the role of interleukin.1. *Chinese Journal of Pediatrics*. 1999;37(2):123–125.
9. Brown LF, Detmar M, Claffey K, et al. Vascular permeability factor/vascular endothelial growth factor: a multifunctional angiogenic cytokine. *EXS*. 1997;79:233–269.
10. Carmeliet P, Jain RK. Angiogenesis in cancer and other diseases. *Nature*. 2000;407(6801):249–257.
11. Xie F, Shi Q, Wang Q, et al. CD40 is a regulator for vascular endothelial growth factor in the tumor microenvironment of glioma. *J Neuroimmunol*. 2010;222(1–2):62–69.
12. Chakrabarti S, Rizvi M, Morin K, et al. The role of CD40L and VEGF in the modulation of angiogenesis and inflammation. *Vascul Pharmacol*. 2010;5(3–4):130–137.
13. Sidky YA, Auerbach R. Lymphocyte-induced angiogenesis: a quantitative and sensitive assay of the graft-vs-host reaction. *J Exp Med*. 1975;141(5):1084–1100.
14. Polverini PJ, Cotran PS, Gimbrone MA Jr, Unanue ER. Activated macrophages induce vascular proliferation. *Nature*. 1977;269(5631):804–806.
15. Auerbach R, Sidky YA. Nature of the stimulus leading to lymphocyte-induced angiogenesis. *J Immunol*. 1979;123(2):751–754.
16. Moulton KS, Melder RJ, Dharnidharka VR, Hardin-Young J, Jain RK, Briscoe DM. Angiogenesis in the huPBL-SCID model of human transplant rejection. *Transplantation*. 1999;67(12):1626–1631.
17. Koch AE, Polverini PJ, Leibovich SJ. Induction of neovascularization by activated human monocytes. *J Leukoc Biol*. 1986;39(2):233–238.
18. Wiseman DM, Polverini PJ, Kamp DW, Leibovich SJ. Transforming growth factor-beta (TGF beta) is chemotactic for human monocytes and induces their expression of angiogenic activity. *Biochem Biophys Res Commun*. 1988;157(2):793–800.
19. Karimi MH, Pourfathollah AA. CD40 and tolerance induction. *Iran J Allergy Asthma Immunol*. 2012;11(1):1–13.
20. Kawabe T, Matsushima M, Hashimoto N, Imaizumi K, Hasegawa Y. CD40/CD40 ligand interactions in immune responses and pulmonary immunity. *Nagoya J Med Sci*. 2011;73(3–4):69–78.
21. Dormond O, Contreras AG, Meijer E, et al. CD40-induced signaling in human endothelial cells results in mTORC2- and Akt-dependent expression of vascular endothelial growth factor in vitro and in vivo.1. *J Immunol*. 2008;181(11):8088–8095.
22. Hoerster R, Muether PS, Vierkotten S, Schröder S, Kirchhof B, Fauser S. In-vivo and ex-vivo characterization of laser-induced choroidal neovascularization variability in mice. *Graefes Arch Clin Exp Ophthalmol*. 2012;50(11):1579–1586.
23. Grossniklaus HE, Ling JX, Wallace TM, et al. Macrophage and retinal pigment epithelium expression of angiogenic cytokines in choroidal neovascularization. *Mol Vis*. 2002;8:119–126.
24. Shi YY, Wang YS, Zhang ZX, et al. Monocyte/macrophages promote vasculogenesis in choroidal neovascularization in mice by stimulating SDF-1 expression in RPE cells. *Graefes Arch Clin Exp Ophthalmol*. 2011;49(11):1667–1679.
25. Huang H, Parlier R, Shen JK, Luttly GA, Viores SA. VEGF receptor blockade markedly reduces retinal microglia/macrophage infiltration into laser-induced CNV. *PloS One*. 2013;8(8):e71808.
26. Zhao H, Roychoudhury J, Doggett TA, Apte RS, Ferguson TA. Age-dependent changes in FasL (CD95L) modulate macrophage function in a model of age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2013;54(8):5321–5331.
27. Portillo JA, Van Grol J, Zheng L, et al. CD40 mediates retinal inflammation and neurovascular degeneration. *J Immunol*. 2008;181(12):8719–8726.
28. Grisanti S, Ishioka M, Kosiewicz M, Jiang LQ. Immunity and immunoprivilege elicited by cultured retinal pigment epithelial celltransplant. *Invest Ophthalmol Vis Sci*. 1997;38(8):1619–1626.
29. Willermain F, Caspers-Velu L, Baudson N, et al. Role and expression of CD40 on human retinal pigment epithelial cells. *Invest Ophthalmol Vis Sci*. 2000;41(11):3485–3491.
30. Curcio CA, Millican CL. Basal linear deposit and large drusen are specific for early age-related maculopathy. *Arch Ophthalmol*. 1999;117(3):329–339.
31. Ishida K, Panjwani N, Cao Z, Streilein JW. Participation of pigment epithelium in ocular immune privilege. 3. Epithelia cultured from iris, ciliary body, and retina suppress T-cell activation by partially non-overlapping mechanisms. *Ocul Immunol Inflam*. 2003;11(2):91–105.
32. Sugita S, Horie S, Yamada Y, Mochizuki M. Inhibition of B cell activation by retina pigment epithelium. *Invest Ophthalmol Vis Sci*. 2010;51(11):5783–5788.
33. Kelley SK, Gelzleichter T, Xie D, et al. Preclinical pharmacokinetics, pharmacodynamics, and activity of a humanized anti-CD40 antibody (SGN40) in rodents and non-human primates. *Cancer Res*. 2006;148(8):1116–1123.

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