Chitinase-3-Like-1 Promotes M2 Macrophage Differentiation and Induces Choroidal Neovascularization in Neovascular Age-Related Macular Degeneration

Nana Xu,1,2 Qiyu Bo,1,2 Rong Shao,3,4 Jian Liang,5 Yuanqi Zhai,5 Shiqi Yang,1,2 Fenghua Wang,1,2 and Xiaodong Sun1,2,5

1Department of Ophthalmology, Shanghai General Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China
2Shanghai Engineering Center for Visual Science and Photomedicine, Shanghai, China
3Department of Pharmacology, Shanghai Jiao Tong University School of Medicine Shanghai, China
4Department of Surgery, Xinhua Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China
5Shanghai Key Laboratory of Ocular Fundus Diseases, Shanghai, China

Correspondence: Xiaodong Sun, Department of Ophthalmology Shanghai General Hospital (Shanghai First People’s Hospital), School of Medicine, Shanghai Jiao Tong University, Shanghai Key Laboratory of Fundus Diseases, Shanghai Engineering Center for Visual Science and Photomedicine, 100 Hai Ning Road, Shanghai 200080, China; xdsun@sjtu.edu.cn.

Fenghua Wang, Department of Ophthalmology, Shanghai General Hospital (Shanghai First People’s Hospital), School of Medicine, Shanghai Jiao Tong University, Shanghai Engineering Center for Visual Science and Photomedicine, 100 Hai Ning Road, Shanghai 200080, China; shretina@sjtu.edu.cn.

NX and QB are joint first authors.

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Purpose. Choroidal neovascularization (CNV) is the principal pathological factor contributing to blindness in neovascular age-related macular degeneration (nAMD). Infiltration of M2 macrophage is thought to contribute to CNV progress, although the way that regulates its differentiation remains unclear. Here, we investigate the role of CHI3L1 in M2 differentiation and angiogenesis in CNV.

Methods. Serum from nAMD patients were tested for CHI3L1 expression. Mice were subjected to laser injury to induce CNV, and lesion expansion were tracked using fundus fluorescence angiography (FFA) and immunofluorescence analysis. Several strategies were taken to verify the contribution of M2 macrophage and CHI3L1: macrophage depletion by clodrosome, local CHI3L1 inhibition using intravitreally injection neutralize antibody (mAY), and depletion of CHI3L1 receptor (IL13-Ra2) by small-interfering RNA (siRNA). Tuber analysis was used to further determine angiogenic effect of CHI3L1. Anti-VEGFA was used as positive control for mAY.

Results. Serum levels of CHI3L1 were highly elevated in nAMD patients. CHI3L1 was expressed by infiltrating macrophages and was elevated as CNV progress in a mice model. System macrophage depletion and local suppression of CHI3L1 alleviated CNV formation while enhancing anti-VEGFA therapeutic effect. Stimulation of macrophage with recombinant CHI3L1 activated MAPK signaling cascade and induced transition to M2, while siRNA knockdown of IL13-Ra2 abolished it. In an in vitro coculture system, supernatants from CHI3L1-stimulated M2 macrophages and promoted tube vascularization.

Conclusions. These results unveil novel angiogenic regulation of CHI3L1 and M2 polarized macrophages in CNV development. These mechanistic insights may point to CHI3L1 as a new therapeutic target for treatment for nAMD.

Keywords: choroidal neovascularization (CNV), M2 macrophage, chitinase 3-like-1 (CHI3L1), age-related macular degeneration (AMD), angiogenesis

Age-related macular degeneration (AMD) is the leading cause of irreversible severe vision loss in aged people.1 This disease can be divided into two basic subtypes: neovascular, also known “wet AMD” and nonneovascular, dry AMD. Neovascular AMD (nAMD) is characterized by choroidal neovascularization (CNV), in which abnormal neovascular results in intro- and subretinal hemorrhage and macular edema, finally leading to severe subretinal fibrosis with complete vision loss. Currently, the leading treatment available for CNV is anti-VEGFA therapy, which mainly targets vascular endothelial cells (ECs).2 However, the tolerance is modest and drug resistance frequently occurs for most patients within the first 4 years of treatment,3 suggesting

the existence of other vital angiogenic components. Innate immunity system is of paramount importance in sustaining the homeostasis of the ocular immunosuppressive microenvironment that is located between retina and retinal pigment epithelial layer.4–6 Previous studies on AMD reported that infiltrating macrophages, the predominant population of immune cells, play an essential role in the pathogenesis.7–9 For example, it has been suggested that infiltrating macrophages actively interacted with ECs during sprouting angiogenesis through North1 signaling,10 and the elevated intracellular lipid can drive residential macrophage polarization into an abnormal, alternatively activated phenotype (M2) of macrophages, which promotes pathologic
CHI3L1 in M2 Macrophages and Angiogenesis

It is appreciated that M2 macrophages act as an immunosuppressive and tumor-promoting cell to elicit debris scavenging, wound healing, and tumorigenesis and angiogenesis. In addition, VEGFA is mainly derived from M2 infiltrating macrophages and inducing neovascularization of AMD.9 Of note, macrophages are of highly phenotypic and genetic plasticity, as macrophages exhibit the ability to undergo differentiation into morphologically polarized M2 phenotype in most pathological scenarios including chronic inflammation and cancer.12 supporting the notion that M2 give rise to distinct immune responses and morphologically reversible. Thus, exploiting or targeting M2 macrophages could hold therapeutic promising in treatment of individual diseases. For instance, injection of a mouse AMD model with M2 macrophages facilitated choroidal neovascular formation, whereas introduction of M1 macrophages attenuated vascularization.12 Therefore, regulating the mechanism of M2 macrophage underlying CNV needs to be considered.

Chitinase 3-like 1 (CHI3L1, also called YKL-40 in human and BRP-39 in the mouse) is one member of the chitinase family, which is highly conserved during species evolution. It is a 40-kDa secreted glycoprotein and was discovered as a heparin-binding protein that binds to chitin-like oligosaccharides.13 However, CHI3L1 does not have chitinase or hydrolase activity because of the mutation of an essential glutamic acid residue to leucine in the chitinase-3-like catalytic domain. It was recently identified that the functional motif of CHI3L1 is located at an arginine and lysine-rich domain proximal to its C terminus.14 CHI3L1 is normally expressed by a number of different cell types including chondrocytes, macrophages, neutrophils, and vascular smooth muscle cells.15 Evidences showing that CHI3L1 mediates mature differentiation of macrophages from monocytes suggest that aberrant expression of CHI3L1 plays an active role in immune disorders and chronic inflammation in which a considerable number of macrophages were infiltrated.16,17 In cancer, CHI3L1 is able to stimulate tumor angiogenesis in breast cancer and glioblastoma.18,19 It has been recently discovered that CHI3L1 functions by multiple signaling pathways including interaction with membrane receptor IL-13Rα220 to induce cell differentiation.

In the present study, we sought to explore the new role of CHI3L1 in the polarized differentiation of M2 macrophages and choroidal vascularization. We found that acquired CHI3L1 commits M2 phenotype switch and augments neovascularization. Blockade of CHI3L1 by a neutralizing antibody (mAY) synergistically enhances anti-VEGFA activity in vivo. To this end, the study may offer a new target and therapeutic promising for clinical practice with a neutralizing antibody against CHI3L1 in nAMD.

**Materials and Methods**

**nAMD Patients**

The research followed the tenets of the Declaration of Helsinki, and informed consent was obtained from the subjects after explanation of the nature and possible consequences of the study. Five male aged patients were diagnosed with nAMD in one eye, in which four treatment-naïve patients did not receive any antiangiogenic therapy from disease onset, while only one patient received anti-VEGFA treatment for one time. Three age-matched male normal patients were controls. Exclusion for the study involved chronic diseases such as hypertension, diabetes, and other diseases (Table).

<table>
<thead>
<tr>
<th>No. of Patients</th>
<th>Age, y</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Follow-up, mo</th>
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<tr>
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<td>M</td>
<td>nAMD</td>
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<td>nAMD</td>
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<td>M</td>
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<td>24</td>
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<td>nAMD</td>
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<td>0</td>
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<tr>
<td>P5</td>
<td>72</td>
<td>M</td>
<td>nAMD</td>
<td>3</td>
<td>1</td>
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Inclusion criteria: vascular morphology and the presence of vessel leakage around macular zone by assessed by fluorescein angiography (FA); exclusion of polyposid choroidal vasculopathy (PCV). Controls included three males aged ≥ 60 years without systemic diseases. All patients included did not have chronic diseases such as hypertension, diabetes, etc.

**Laser-Induced Mouse CNV Model**

All animals were treated according to the guidelines of the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The experimental procedures were approved by the Institutional Animal Care and Use Committee of Ophthalmic and Vision Research. The experimental procedures were approved by the Institutional Animal Care and Use Committee of Shanghai Jiao Tong University (Shanghai, China). C57BL/6j male mice aged between 6 and 8 weeks and approximately 20 g were included. After application of tropicamide (Santen, Osaka, Japan) for pupil dilatation, animals were anesthetized with intraperitoneal injection of 1% pentobarbital sodium (0.1 mL/10 g body weight) (Guge Biotech, Wuhan, China). A glass coverslip was lubricated withloxacin eye ointment (Xing Qi Pharmaceutical Companies, Shenyang, China), and four laser spots were distributed around the optic nerve head with an argon laser (110 mW, 100 ms, 50 μm, Oculight Infrared Laser System 810 nm, Iridex Corp., Mountain View, CA, USA). Appearance of a gray bubble indicative of the rupture of Bruch’s membrane were included. If retinal bleeding occurred, the animal was eliminated. Eyes were enucleated at different time points.

**Intravitreal Injection**

Mice were anesthetized as described early. Intravitreal injection was executed immediately after laser treatment. Mice were randomly assigned to receive anti-CHI3L1 monoclonal antibody (mAY) (n = 8; 500 ng/μL; Department of Pharmacology, School of Medicine, Shanghai Jiao Tong University, Shanghai, China) and VEGFA neutralizing antibody (VEGFA nab; n = 8; 5000 ng/μL; AF-493-NA, R&D Systems, Minneapolis, MN, USA) as a positive control. For combination treatment, mAY at 500 ng/μL and anti-VEGFA at 5000 ng/μL were injected in combination. Vehicle controls (n = 5) received PBS. Efficacy was evaluated by FA and immune fluorescence staining.

**Fluorescence Angiography**

Mice were anesthetized as described early. Each mouse was injected intraperitoneally with 0.05 mL 10% fluorescein sodium (Fluoresc; Alcon, Tokyo, Japan), and fundus angiogram photos were captured at the middle stage (2–3 minutes after dye injection) using a digital fundus camera (Heidelberg Retina Angiograph, Vista, CA, USA). We used Image J software (National Institutes of Health, Bethesda, MD, USA) to calculate the average signal intensity of each CNV lesion as previously described in published studies.21

**Macrophage Depletion of CNV Model**

Clodrosome and Encapsome (Encapsula, NanoSciences, New York, NY, USA) (50 mg/kg) were injected intraperitoneally at
different time points after laser photocoagulation to deplete macrophages. For day 7 end point, liposomes were injected at 1, 3, and 6 days after laser photocoagulation; Encapsome was used as controls.

**Immunofluorescence Analysis**

Immunofluorescence assays were performed on retinal sections, choroidal flat-mount, or in 24-well slide chambers. Briefly, after fixation, the samples were blocked with 0.3% Triton X-100 and 5% goat serum albumin (Beyotime, Shanghai, China) in PBS for 1 hour at room temperature. Tissue were immunostained with primary antibodies against F4/80 (1:500; CST, Beverly, MA, USA), IL13-Ra2 (1:1000; CST), Ym-1 (1:500; Stem Cell Technology, Vancouver, Canada), CHI3L1 (1:500; Merck Millipore, Billerica, MA, USA), and isoclinet (1:1000; Santa Cruz Biotechnology, Santa Cruz, CA, USA) overnight at 4°C and washed with PBS three times, and then were stained for 45 minutes at 37°C with Alexa Fluor 594- and 488-conjugated secondary antibodies (1:1000; Proteintech, Chicago, IL, USA). The nuclei were marked by 4',6-diamidino-2-phenylindole. Images were visualized using a fluorescence microscope (Olympus, Center Valley, PA, USA) and a Leica TCS SP8 confocal laser scanning microscope (Leica TCS NT, Wetzlar, Germany). CNV areas were quantified using an image analysis software program according to a protocol described previously.

**Tubular Formation Assay**

RF/6A cells were used for the tubule formation assay as described previously in detail. Since an interesting study reported that RF/6A lost some key EC markers, we compared its angiogenic reactions to VEGFA with human microvascular ECs (HMVECs) and got identical responsiveness. Finally, as RF/6A is the most prevalent in vitro choroidal and retinal ECs (HMVECs) and got identical responsiveness. Finally, as RF/6A lost some key EC markers, we compared RF/6A to VEGFA with human microvascular ECs. Our study suggested that RF/6A lost some key EC markers, which might be related to its angiogenic reactions to VEGFA. Thus, these results suggest that CHI3L1 is upregulated in CNV and affects infiltrating macrophages to contribute to CNV.

**Western Blot Analysis**

Samples were subjected to 10% SDS-PAGE, transferred to a polyvinylidene fluoride membrane, then were blocked with blocking buffer (Tris-buffered saline Tween-20 [TBST], containing 5% nonfat dry milk) for 1 hour at room temperature and incubated with primary antibodies against Arg-1 (1:1000; Abcam, Cambridge, MA, USA), Ym-1 (1:1000; Stem Cell Technology), CHI3L1 (1:500; Merck Millipore), GAPDH (1:1000, Cell Signaling Technology, Beverly, MA, USA) overnight. The membranes were then washed with TBST three times, and then probed with horseradish peroxidase-conjugated secondary antibodies (1:2000, Proteintech) for 1 hour at room temperature. The membranes were washed with TBST and exposed to a molecular imaging system (Amersham Imager 600, GE Healthcare, Buckinghamshire, UK).

**Magnetic Bead Sorting Immunoprecipitation and Immunoblotting**

Magnetic bead sorting co-immunoprecipitation studies were performed on RAW264.7 cells according to the Pierce Co-Immunoprecipitation Kit (Rockford, IL, USA) protocol. The immunoprecipitation products were analyzed via Western blotting. The following antibodies, rabbit anti-CHI3L1 (Merck Millipore), rabbit anti-IL13-Ra2 (Abcam), or rabbit IgG (Santa Cruz Biotechnology), were used for analysis.

**RNA Interference**

RAW264.7 cells were transfected with double-stranded small-interfering RNA (siRNA) or negative control siRNA (non-siRNA) using Lipofectamine Transfection Reagent (Beyotime). Target sequences were as follows: 5'-GGAATCTAATTTCAAGGAGA-3' for IL13-Ra2. Double-stranded siRNAs were synthesized by Shanghai Gene Pharma (Shanghai, China).

**Statistical Analyses**

Data are expressed as mean ± SE and n refers to the numbers of individual experiments performed. Each experiment was repeated in triplicate. Differences among groups were determined using 1-way ANOVA analysis. For two group analysis, a Student’s t-test (GraphPad Prism, San Diego, CA, USA) was used. The 0.05 level of probability was used as the criterion of significance.

**RESULTS**

**CHI3L1 Was Upregulated in nAMD Patients and CNV Models**

Patients displayed higher level of CHI3L1 than controls (Fig. 1A), suggesting that CHI3L1 were elevated in nAMD and independent of VEGFA blockage. Western blots also showed that CHI3L1 were higher in choroidal-RPE complex from CNV model at different time points (Fig. 1B). Moreover, immune staining detected CHI3L1 colocalized with F4/80 (Fig. 1C). Thus, these results suggest that CHI3L1 is upregulated in CNV and affects infiltrating macrophages to contribute to CNV.

**Infiltrating Macrophage Play a Critical Role in CNV**

Given that macrophages are the primary inflammatory cells in nAMD, we set up an experiment to define the role of...
**FIGURE 1.** CHI3L1 is highly expressed in nAMD patients and colocalized with macrophage in CNV model. (A) Western blot showed increased CHI3L1 level in serum from nAMD patients (P1–P4 without anti-VEGFA therapy, while P5 received one time 1 month ago) and three normal controls (C1–C3). Quantitative analysis was performed. (B) CHI3L1 was gradually upregulated in CNV model at different time points \((n = 8)\). Quantitative analysis was performed accordingly. (C) Immunofluorescence staining showed CHI3L1 (green) expression in CNV lesion colocalized with F4/80 (red). (D, E) Intraperitoneal injection of Clodrosome and Encapsome immediately after laser injury and macrophages infiltrated were immune stained with F4/80 (red) and CNV stained with lectin (green) on day 7. (F, G) Macrophages were obviously depleted and CNV area was markedly inhibited \((n = 8)\). Data are represented as the mean ± SEM. *\(P < 0.05\); **\(P < 0.01\); ***\(P < 0.001\) via Student’s \(t\)-tests. Scale bar: 50 μm.
macrophages in our model. Once we treated the mice with a macrophage depletion agent-clodrosome, infiltrating macrophages were noticeably abrogated followed with alleviated neovascularization (Figs. 1D–G), indicating that macrophage plays an important role in the development of CNV.

CHI3L1 Neutralize Antibody (mAY) Inhibit CNV and Has a Synergistic Effect With Anti-VEGFA by Blocking M2 Macrophage Differentiation

Next, to evaluate angiogenic activity of CHI3L1, we treated CNV mouse model with mAY, anti-VEGFA antibody, or combination immediately after laser treatment. As indicated in Figures 2A and C, the vascular leakage was quantified and each antibody decreased vessel formation and leakage partially. Strikingly, combined antibodies significantly suppressed vascular development. Consistent with these data, CNV area analysis showed strong synergistic efficacy of combined therapy with two antibodies relative to single antibody treatment (Figs. 2B, 2D). In line with this inhibition, PCR studies on tissue also revealed that mAY decreased mRNA expression of Ym-1 and Arg-1 to 25% to 40% of corresponding PBS controls (Fig. 3A). Collectively, the data suggest that mAY and anti-VEGFA antibody synergistically inhibit CNV and mAY blocks M2 macrophage differentiation.

CHI3L1 Promotes Neovascularization by Inducing Macrophage M2 Differentiation and VEGFA Secretion In Vitro

To further validate, we tested the ability of CHI3L1 to promote M2 differentiation in vitro. As Arg-1 and Ym-1 are consistently expressed by M2 macrophage,30,31 we stimulated macrophage with recombinant CHI3L1 and M2 markers (Ym-1 and Arg-1) were subsequently identified by immune staining and Western blot (Figs. 3B–D). Indeed, CHI3L1 induced these two M2 marker expressions, which suggest that acquired CH3IL1 may mediate M2 differentiation. Interestingly, CHI3L1 also had the ability to increase VEGFA expression, while mAY can abolish it (Figs. 3D, 3E). We next analyzed the angiogenic activity of M2 macrophages induced by CHI3L1 in vitro, which could involve its direct and indirect (e.g., induction of VEGFA) effects. Firstly, we induced M2 macrophages by stimulation with recombinant CHI3L1 for 24 hours. After removal of recombinant CHI3L1, we then collected conditioned medium (CM). It was found that CM increased tube formation approximately 3-fold greater than control, but either mAY or anti-VEGFA antibody could partially inhibit it (Figs. 3F–K). We then concentrated the condition medium and measured the concentration of VEGFA and CHI3L1 by Western blot (Figs. 3L, 3M), which suggested that both CHI3L1 and VEGFA secreted from M2 macrophages can drive angiogenesis, which can be inhibited by respective neutralizing antibody.
FIGURE 3. CHI3L1 promotes neovascularization in vivo and vitro by inducing macrophage M2 differentiation. (A) RT-PCR analysis of CNV lesion on day 7 showed that mAY inhibited mRNA expression of Arg-1 and Ym-1 (M2 markers). (B-D) Immunofluorescence staining and Western blot analysis of RAW264.7 cells showed that recombinant CHI3L1 promoted macrophages M2 differentiation (Ym-1+, red) while upregulated VEGFA expression (D). Quantitative analysis was performed accordingly. Scale bar represents 50 µm. (E) Western blots analysis of RAW264.7 showed that rCHI3L1 induced VEGFA expression and mAY inhibited it. (F-J) RF/6A cells (5 × 10⁴) were cultured on Matrigel in the presence of the PBS (E), CM (F), mAY (G), anti-VEGFA (H), or combination (I) and tubes were quantified (n = 4). (K) Data analysis of tuber formation. Scale bar: 100 µm. (L) Macrophage were treated with rCHI3L1 or PBS and condition medium were concentrated, and Western blots analyses of VEGFA and CHI3L1 were performed. (M) Data analysis of L. Data are represented as the mean ± SEM. **P < 0.01; ***P < 0.001 via Student’s t-tests.
CHI3L1 Triggers Cellular Signaling Pathways Through IL13-Ra2-ERK Cascade

As demonstrated previously that IL-13Rα2 acts as a CHI3L1 binding receptor to mediate inflammatory responses, we interestingly found that IL13-Rα2 was collocated with CHI3L1 in CNV lesion (Fig. 4A) and was induced dramatically between days 3 and 7 after laser-injury (Figs. 4B, 4C). In vitro, IL-13Rα2 associated with exogenous CHI3L1 was augmented relative to the control, when their associated complex was determined by adding an irreversible cross-link reagent paraformaldehyde. CHI3L1-IL-13Rα2 complex was seen in approximately 100 kDa. The bands of IgG were used as loading controls. The data were quantified (n = 3). (F) RAW264.7 macrophages were treated with recombinant CHI3L1 (50 and 100 ng) and transfected with vector (NC), IL13Rα2 siRNA. Protein expression of IL13-Rα2 was determined by Western blot. Data are represented as the mean ± SEM; ns, not significant. ***P < 0.001 via Student’s t-tests. Scale bar: 50 μm.

**FIGURE 4.** CHI3L1 induces and associates with IL-13-Rα2. (A) IL13-Rα2 (green) were colocation with CHI3L1 (red) in cell membrane or cytoplasm in CNV lesion. (B, C) Western blot analysis of IL13-Rα2 in CNV model (n = 3). (D, E) RAW264.7 cells were treated with recombinant CHI3L1 (150 ng/mL) in the absence or presence of mAY (150 μg/mL) and then cross-linked with an irreversible cross-link reagent paraformaldehyde. CHI3L1-IL-13-Rα2 complex was seen in approximately 100 kDa. The bands of IgG were used as loading controls. The data were quantified (n = 3). (F) RAW264.7 macrophages were treated with recombinant CHI3L1 (100 ng) and transfected with IL13-Rα2 siRNA. Protein expression of VEGFA was determined by Western blot. Data are represented as the mean ± SEM; ns, not significant. ***P < 0.001 via Student’s t-tests. Scale bar: 50 μm.

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DISCUSSION

CNV is the hallmark of nAMD, and macrophage play a critical role in its progression. Here, in context with elevated serum levels of CHI3L1 in nAMD, we provided strong evidence of CHI3L1-mediated angiogenesis with in vitro cultured cells and an in vivo animal model to discover that elevated expression of CHI3L1 prompts macrophages to be M2 polarized phenotype, which in turn enhances expression of CHI3L1 and VEGFA. This finding points to a novel target for treatment of nAMD.

Indeed, besides vascular ECs, pathological angiogenesis involves multiple critical aspects, including inflammatory cells...
such as macrophage, which is a critical member of innate immune system.\textsuperscript{32} Dysfunction of the immune system is currently appreciated to participate in CNV\textsuperscript{53,54}, but the substantially cellular and molecular mechanisms that regulate the angiogenesis are not fully understood. There is a large body of research evidence showing that M2 are proangiogenic. Previous studies using the animal model of nAMD demonstrated phenotypic and genetic switch to M2 polarized macrophages by which infiltrating macrophages induce angiogenesis.\textsuperscript{35,36} Robust macrophage-mediated vascularization occurs in advanced stages of AMD patients\textsuperscript{7,9,37}; thereof, blockade of M2 macrophages has received considerable attention in an attempt to ameliorate this disease. However, to date, identifying key molecules that govern M2 macrophage differentiation has been the main challenge for intervening vascular proliferation. In our study, we found that M2 differentiation is dependent on CHI3L1 during CNV development. Blockade of CHI3L1 inhibited M2 switch and decreased angiogenesis. In addition, CHI3L1-induced angiogenesis involves both VEGFA-dependent and independent pathways, as CHI3L1 upregulated VEGFA expression and inhibition of CHI3L1 suppressed VEGFA (Supplementary Fig. S1), while blocking VEGFA can partially block angiogenesis and additional blockade of CHI3L1 is essential to fully ablate angiogenesis. Accordingly, VEGFA expression was partly inhibited after CHI3L1-blockade and knockdown of IL13-Ra2 as showed in Supplementary Fig. S3. Intriguingly, VEGFA does not have the same ability as CHI3L1 to regulate M2 differentiation, underscoring that VEGFA only functions to activate CHI3L1-independent angiogenesis in nAMD (Supplementary Fig. S2).

CHI3L1 is appreciated to act as an inflammatory mediator to commits M2 mature differentiation of macrophages for Th2 inflammation.\textsuperscript{15,17} CHI3L1 knock-out mice displayed deficiency of antigen-induced Th2 inflammation and impaired macrophage activation and differentiation.\textsuperscript{15} In clinic, accumulating evidence studying on a variety of diseases demonstrates that CHI3L1 is elevated in chronic inflammatory disorders.\textsuperscript{30,39} Simultaneously, substantial infiltration of M2 macrophages in AMD induces inflammatory responses, as a large number of cytokines such as IL-10 were upregulated.\textsuperscript{31,9} Hence, CHI3L1 functions by dual proinflammatory and proangiogenic activity to drive angiogenesis, whereas VEGFA usually interacts with ECs only in nAMD. Agreed with our finding, CHI3L1 can also upregulate VEGFA expression in brain tumor cells, resulting in a synergistic impact on brain tumor vascularization.\textsuperscript{24} The current study identified that CHI3L1 interacts with IL-13Ra2 or syndecan-1 to trigger outside-in signaling cascades in the cells.\textsuperscript{45} In line with these reported data, the present study identified that CHI3L1 interacts with IL-13Ra2 and then activates intracellular signal transduction including ERK to regulate macrophage differentiation and angiogenesis. Although the likelihood of syndecan-1 involved in the signaling activation can be not eliminated, identification of these intracellular pathways may offer additional opportunities.

In sum, the current study has established the pathologic signature of CHI3L1 to induce M2 macrophage differentiation, thus developing strong vascular lesion. Albeit inhibition of CHI3L1 in nAMD warrants further clinical investigation, the present preclinical trial with an anti-CHI3L1 neutralizing antibody in animals has demonstrated pronounced inhibitory impacts on angiogenesis. To this end, these findings shed light on conjunction therapy with VEGFA and CHI3L1 inhibitors, and pave a more efficacious way toward curing nAMD.

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