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REVIEW **Open Access**

The roles of microglia in neural remodeling during retinal degeneration

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Summary. Retina remodeling is a consequence of many retinal degenerative diseases that are characterized by progressive photoreceptor death. Retina remodeling involves a series of complex pathological processes, consisting of photoreceptor degeneration and death, as well as retinal cell reprogramming and "rewiring". This rewiring alters retinal neural circuits that are centered on synaptic connections and lead to widespread death of retinal cells. Retinal remodeling, especially inner retinal remodeling, is the major factor that limits the effectiveness of various treatment strategies, including cell therapy; thus, it is important to elucidate the mechanisms involved in retinal remodeling during retinal degeneration. Microglia are the dominant immune cells in the retina. Microglia monitor the retinal microenvironment, are activated following retinal injury or degeneration, have powerful phagocytosis capabilities, and play a critical role in synaptic pruning during central neural system development. Analogously, microglia have been found to participate in the clearance of synaptic elements in a complement-dependent manner in the classic retinitis pigmentosa (RP) model, Royal College of Surgeons (RCS) rats, and retard the formation of ectopic neuritogenesis and the deterioration of visual function during retinal degeneration. Since previous research on microglia has rarely concentrated on synaptic remodeling during retinal degeneration, summarizing the microglial mechanisms involved in retinal remodeling is necessary in order to design compounds targeting microglia and retinal remodeling that might be promising therapeutic strategies for treating retinal degeneration.

Key words: Retinal degeneration, Microglia, Retinal remodeling, Synapse pruning

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Introduction

Retinal degenerative diseases, such as RP (Dias et al., 2018), age-related macular degeneration (AMD) (Mitchell et al., 2018), and Stargardt's disease, are complex diseases caused by both genetic defects and environmental factors, which are characterized by the dysfunction and death of photoreceptors (Marc et al., 2003; Pfeiffer et al., 2020). The vertebrate retina is a tandem device consisting of three order neurons. The first-order neurons, photoreceptors, absorb light, transform it into chemical signals, and transmit the signals. The second-order interneurons are mainly bipolar cells, which accept the signals via glutamatergic receptors and activate the third-neurons, and the retinal ganglion cells, which are responsible for retinal output (Hoon et al., 2014). Therefore, the loss of input signals resulting from the death of photoreceptors (known as deafferentation) triggers the migration, reprogramming, and death of inner neurons, as well as the disorder of retinal lamination and synaptic restructuring, thus aggravating the progression of retinal degeneration (RD) (Pfeiffer et al., 2020). In recent years, researchers have proposed many treatments for RD such as trophic support, cell therapy, gene therapy, and visual prostheses that promote partial restoration of visual function; however, retinal remodeling is a barrier to successful treatment of RD.

During the pathological progression of RD, the microglia may distress and/or protect the photoreceptors and inner neurons via monitoring, secretion, and phagocytosis (Arroba et al., 2011; Zhao et al., 2015). Furthermore, the microglia are the primary immune cells, and glia in the retina play important roles at different stages of life. During development and maturation, microglia support neuronal survival via microglia-neuron interactions, monitor the retinal microenvironment through the extension of microglial processes, regulate synaptic plasticity by phagocytosis, and maintain synaptic structural integrity and normal function (Silverman and Wong, 2018). Resting, also

called homeostatic microglia are activated under pathological conditions, but the fate as well as the function of activated microglia are unpredictable and confusing (Karlstetter et al., 2015). There is no doubt that microglia are a therapeutic target for RD; therefore, it is necessary to determine the precise functions of microglia in RD under different pathological conditions. Given the role of microglia in synaptic plasticity and the subtypes of microglia, in this review we discuss the effect of microglial subtype on retinal remodeling and provide novel insights into the role of microglia in RD in an effort to determine whether regulating microglia may optimize RD treatment.

In this review, retinal degeneration and remodeling are briefly introduced followed by a discussion focusing on the functions and advancement of microglia in healthy and diseased retinas, particularly synaptic formation and remodeling. Finally, promising therapeutic strategies for RD involving precise microglial regulation are examined.

Structure and neural circuit network in healthy and RD retinas

Normal retinal structure and synaptic connection

The retina is a highly specialized sensory organ and intricate neural network that is in charge of processing of visual information in parallel and serial. It is a layered tissue consisting of six types of neurons and three types of glia, which produces visual output. Retinal cell types, histological structure, and physiological function are highly conserved from low vertebrates to primate species (Grünert and Martin, 2020). The somas of retinal cells occupy three nuclear layers, and the cell processes form the two plexiform layers, which separate the three nuclear layers. The nuclei of rod and cone photoreceptors are in the outer nuclear layer (ONL), and their processes contact the dendrites of bipolar cells and horizontal cells and form synapses in the outer plexiform (OPL). The nuclei of interneurons, including bipolar cells, horizontal cells, and amacrine cells, as well as the Müller glia, which make up most of the retinal glia, are housed in the inner nuclear layer (INL). In the inner plexiform layer, bipolar cells send nerve impulses to ganglion cells contained in the ganglion cell layer (GCL) via ribbon synapses. Finally, ganglion cell axons in the nerve fiber layer (NFL) project into the brain via the optic nerve and transfer coded visual formation to the visual center (Hoon et al., 2014).

In the OPL, the rod photoreceptor terminals form chemical synaptic triads, called ribbon synapses, with ON-bipolar cells and horizontal cells to transfer information by releasing the neurotransmitter glutamate (Tom Dieck and Brandstätter, 2006). The ribbon synapse is a horseshoe-shaped structure, around 2 μm in length, which is formed by the invagination of bipolar cells and horizontal cells into the rod photoreceptors (Rao-Mirotznik et al., 1995). The core element of the ribbon synapse, the synaptic ribbon, is in a specialized depression of the rod terminal membrane called the synaptic ridge, which is a plate-like, electron-dense structure with a large surface area that tethers hundreds of synaptic vesicles via slender proteinaceous strands to continuously release neurotransmitters (Heidelberger et al., 2005). The presynaptic elements, represented by extracellular leucine-rich repeats and fibronectin type III domain 1 (ELFN1), directly interact with the metabolic glutamate receptor 6 (mGluR6) located in the dendritic terminal of ON-bipolar cells to mediate the glutamate flux, while the horizontal cells modulate synaptic transmission via a mechanism of inhibitory feedback, which is consistent with our results (He et al., 2019; Moser et al., 2019; Furukawa et al., 2020). Under dark conditions, the "dark current," caused by the opening of the K^+ channel and cGMP-dependent Na^+ channel, maintains the resting potential and the opening of voltage-gated L-type Ca^+ channel that triggers the influx of Ca^+ in rod photoreceptors. As with conventional synapses, the influx of Ca^+ results in the elevation of the intracellular Ca^{2+} concentration and the subsequent fusion of the synaptic vesicle and presynaptic membrane to release glutamate into the synaptic cleft. Then, the binding of glutamate and mGluR6 causes the activation of G_0, a heterotrimeric G protein, which closes the transient receptor potential cation channel subfamily M member 1 (TRPM1) cation channel followed by the hyperpolarization of ON-bipolar cells. Once a light stimulus is sensed by the rod photoreceptors, the release of glutamate from the rod photoreceptors decreases, causing the inactivation of mGluR6 and the depolarization of the ON-bipolar cells (Daw et al., 1990; Heidelberger et al., 2005; Zanazzi and Matthews, 2009; Moser et al., 2019; Furukawa et al., 2020). The bipolar cells subsequently conduct the next level of information transmission via the ribbon synapse established with the ganglion cell in the IPL to produce the retinal output (Moser et al., 2019). The ribbon synapse is the core element in visual information transduction. Normal function of the ribbon synapse requires good structural integrity and that all kinds of synaptic proteins are correctly located.

Retinal remodeling under RD

The retinal structure and neural circuits are gradually destroyed during RD followed by the occurrence of retinal remodeling. Retinal remodeling can be described in three phases: Phase 1 is defined by the primary stress and insult of photoreceptors with neural reprogramming and glial response, including glial hypertrophy and metabolic dysfunction. Phase 2 is the progressive loss of photoreceptors and initial degeneration of cones resulting in partial deafferentation that triggers more serious retinal remodeling, including the continuous exacerbation of Phase 1 phenotypes and the translocation of neurons and glia. This causes a disorder of the retinal laminar structure, excessive growth of

resident photoreceptor neurites, retraction of bipolar and horizontal cell dendrites from the ONL, and restructuring of the synaptic circuits. Phase 3 occurs with the progression of RD and is characterized by complete loss of photoreceptors, formation of the glial scars, destruction of neural circuits, and deafferentation, followed by the widespread death of inner neurons.

Before photoreceptor death, the connectivity of the neural circuit changes (Fig. 1). Rhodopsin is transferred into the rod inner segment from the rod outer segment accompanied by the sprouting and retraction of neurites in the rod photoreceptor (Sanyal et al., 1992). Stress activates the excessive outgrowth of rod neurites. Rhodopsin-positive neurites then extend into the GCL of the inner retina. This may be a way that the rod avoids death due to survival stress in the ONL and sub-retinal space (SRS) (Fariss et al., 2000). This phenomenon may represent a failure of homeostasis rather than plasticity, which is also indicated by the fact that sprouting neurites mislocate targets and cannot transfer visual information. The shift of interneuron metabolism and phenotype along with aberrant rewiring then occurs. (Peng et al., 2000; Puthussery et al., 2009; Phillips et al., 2010). We and other groups have found that mGluR6 changes into iGluR6 on bipolar cell dendrites accompanied by the ectopic expression of mGluR6 and disconnection with the rod, leading to the growth of aberrant processes that are not functional (Bayley and Morgans, 2007). We further studied changes at the protein level and observed the loss of synaptic proteins, especially mGluR6 (He et al., 2019). In addition, the levels of other synaptic components, including synaptophysin, syntaxin-I, and synapsin-I, increased in the rd1 mice, indicating the compensatory mechanism response to the loss of the ribbon synapse (Dagar et al., 2014; He et al., 2019). Ultrastructurally, the normal synaptic structure decreased, and the free synaptic components increased, indicating mislocation of synaptic proteins and disintegration of the postsynaptic triad (He et al., 2019). Electroretinogram and patch-clamp techniques also demonstrated impairment of synaptic connectivity and the non-functional aberrant synapses (Strettoi et al., 2002, 2003; Puthussery et al., 2009; He et al., 2019). The above, along with neural reprogramming and rewiring, do not represent plasticity characterizing regeneration; rather they lead to further destruction of the retinal

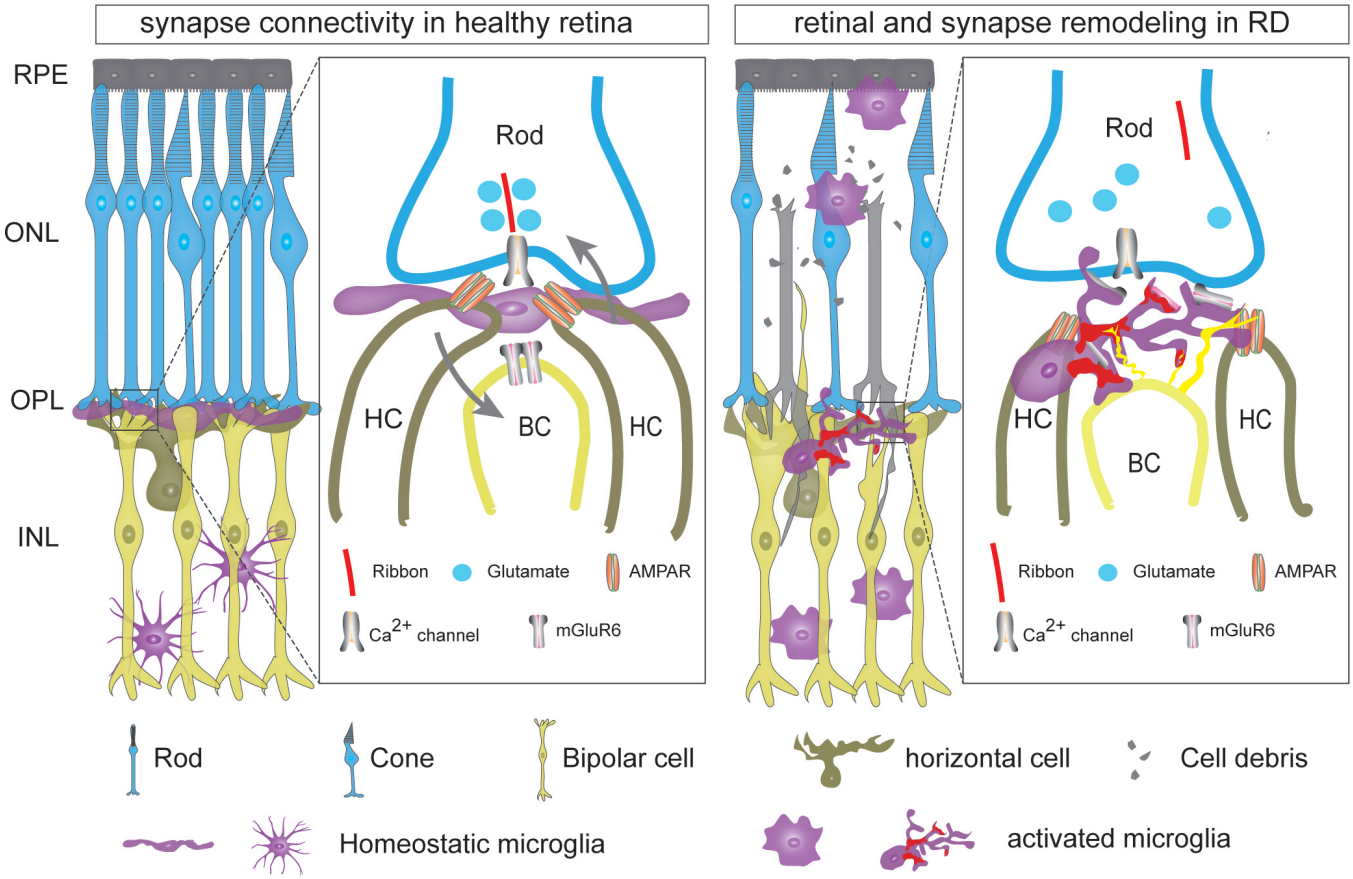

Fig. 1. The illustration of microglia regulating the retinal remodeling. Following synapse disintegration, activated microglia can engulf the postsynaptic protein mGluR6 in a complement-dependent manner to retard the formation of aberrant processes of bipolar cells and improve retinal remodeling during retinal degeneration. HC: horizontal cells; BC: bipolar cells.

structure. In advanced RD, the fragments of aberrant synapses participate in the formation of many microneuromas, which is extremely serious (Marc et al., 2008). Photoreceptor impairment and loss are hard to avoid in RD; however, if the normal structure of the inner retina can be preserved, a preferred microenvironment for photoreceptor transplantation could be provided. During photoreceptor transplantation for advanced RD, the grafted cells first need to survive in the diseased microenvironment and then make synaptic connections with host bipolar cells, though this may be partially prevented by the retinal remodeling (Soto and Kerschensteiner, 2015). However, as the proverb says, every coin has two sides, the retinal remodeling prefers to be like the "Janus." Although retinal remodeling exacerbates impairment of visual function, the surviving inner neurons extend processes to establish the putative synaptic connections, which contributes to restoring the disruption of cellular Ca^{2+} homeostasis caused by deafferentation and cell survival. From an ultrastructural and metabolic standpoint, these surviving inner neurons are largely healthy and can support photoreceptor transplantation and other treatments for RD (Pfeiffer et al., 2020). However, determining the modulatory mechanism of retinal synaptic remodeling is necessary to retard RD progression and optimize RD treatment.

The role of microglia in the healthy and diseased retina

Microglia, as the main immune cells in retina, have a strong ability to engulf and prune synapses and are important candidates for modulating retinal remodeling.

The microglia in healthy retina

It is commonly believed that retinal residual microglia are of mesodermal lineage and originate from primitive macrophages in the yolk sac during embryonic development (Hanisch and Kettenmann, 2007; Kierdorf et al., 2013). During the formation of blood circulation, microglia progenitors filtrate into the retina, leading to the generation of a microglia pool that can maintain the population via self-renewal in adults (Bruttger et al., 2015). In recent years, it has been suggested that bone marrow-derived cells may be another source of microglia. Our group has summarized the origin, turnover, and function of both residual microglia and bone marrow-derived cells extensively in a previous review (Jin et al., 2017).

During development, the distribution of microglia is highly dynamic due to continuous migration. Homeostatic microglia control programmed neuron death, support neuron survival via the trophic effect, and engulf cell debris (Jin et al., 2017; Silverman and Wong, 2018). Microglia-mediated developmental removal of astrocytes via phagocytosis is critical for the formation of the retinal vascular system, as dysfunction of this system leads to retinal hemorrhage (Puñal et al., 2019). In the mature retina, microglia are mainly distributed in three retinal layers, the NFL, IPL, and OPL, and display a branched morphology with extending protrusions. The microglia move and stretch their processes constantly to interact with neurons and comprehensively monitor the entire retinal microenvironment, which is dependent on physical cell-cell contact, complement system and a large number of cytokines and receptors, such as transforming growth factor β (TGFβ), and CX3CL1-CX3CR1 (Damani et al., 2011; Karlstetter et al., 2015).

However, the importance of microglia in maintaining the synaptic structure and function of the adult retina is unclear. Following depletion of microglia using a tamoxifen diet in $CX3CR1^{CreER}$; $Rosa26^{LSL\text{-}DTA}$ mice, the ribbon synapses in the OPL degenerated and broke down, and there was a progressive decrease in responses to light, although the retinal lamina, the survival of neurons, and the growth of processes were not influenced (Wang et al., 2016). Repopulating microglia may restore the integral synaptic structure and visual function. This seems to suggest that microglia are required to maintain retinal synaptic structure and synaptic transmission to preserve visual function in the adult retina (Zhang et al., 2018). However, we observed that pharmacological depletion of microglia via Colony Stimulating Factor 1 Receptor (CSF1R) inhibitor PLX3397 did not alter synaptic structure and visual function in the healthy rat retina (He et al., 2019). Similarly, in the adult brain, the ablation of microglia via tamoxifen in a genetic model resulted in learning and cognitive function deficits, but the microglia-depletion in mice via PLX3397 did not lead to dysfunction in behavior or cognition, indicating that different experimental methods to delete microglia may account for the discrepancies in the conclusions regarding microglia function (Parkhurst et al., 2013; Elmore et al., 2014). Given that tamoxifen itself can influence visual function in rd10 mice (Wang et al., 2017), we think pharmacological depletion via CSF1R inhibitor is a more suitable approach for studying microglial function. Therefore, further research on the role of microglia in retinal structure and function in adults is needed.

The microglia in RD

Under pathological conditions, homeostatic microglia are activated by many triggers related to stress and degeneration of retinal neurons, which is the common feature of RD (Xu et al., 2009; Silverman and Wong, 2018). The conversion from branched morphology into amoebic morphology is the key sign of microglial activation, along with acquiring the ability to proliferate and migrate, leading to the accumulation and infiltration of microglia in the ONL (Zou et al., 2019). Microglia are closely associated with the pathological progression of RD by protecting or impairing the retina. Microglia migrate towards the SRS in a CX3CR1-

dependent manner, and this migration is related to the appearance of drusen and exacerbation of choroidal neovascularization. Microglia also cause excessive activation of neuroinflammation and develop a neurotoxic phenotype, which leads to AMD towards advanced phase (Combadière et al., 2007; Indaram et al., 2015). Although the expression of CX3CR1 on microglia is consistent with the progression of RD, the activation of CX3CR1 signaling may play a beneficial role in RD. Infiltration into the ONL, secretion of inflammatory cytokines, and phagocytosis of microglia in CX3CR1-deficient mice are significantly increased, compared with normal rd10 mice, accelerating photoreceptor degeneration and death. Interestingly, compensation of CX3CL1-CX3CR1 signaling recovers the phenotype (Zabel et al., 2016). In the early stage of degeneration in rd10 mice, infiltrating microglia enhance the phagocytic capacity and interact with living photoreceptors dynamically, leading to the engulfment of living photoreceptors. The functional inhibition or ablation of microglia prevents the exacerbation of degeneration in rd10 mice, morphologically and functionally (Zhao et al., 2015). The inhibition of microglial activation by tamoxifen decreases the inflammatory cytokine release and microglia-mediated toxicity to the photoreceptors, thus significantly improving the retinal structure and visual function in RD mice. This is consistent with the results of research done by our group using metformin, stem cell, or stem cell-derived exosome therapy to inhibit microglial activation and retard the progression of RD (Wang et al., 2017; A et al., 2019; Zou et al., 2019; Bian et al., 2020).

The above-mentioned results indicate that microglia activation impairs retinal structure and function; however, some groups have demonstrated the beneficial role of microglia activation. IGF-I treatment decreases the apoptosis of photoreceptors in a microglia-dependent manner, and microglia ablation or inhibition diminishes this effect, which suggests that IGF-I-mediated neuroprotection requires microglia (Arroba et al., 2011; Ferrer-Martín et al., 2015). The complement system is an important part of the retinal immune system, and increased expression of C3 signaling is localized in activated microglia during RD. C3-CR3 signaling modulates the microglial phagocytosis of photoreceptors and maintains the expression pattern of inflammatory genes, whereas a deficiency in C3-CR3 signaling accelerates the degeneration of retinal structure and function in RD (Silverman et al., 2019).

Diverse subtypes of microglia

How can these different, even opposite results, be explained? The fact that these studies were conducted under different conditions and with different species and diseases may account for the heterogeneous effects of microglia. However, even using the same RP model of RCS rat, we and another group reached opposite conclusions. We demonstrated that microglia contribute to improving the retinal structure and function in RCS and that microglia depletion by a CSF1R blocker leads to further deterioration of RP (He et al., 2019), but the result from Lew et al. indicated that microglia were activated into a pro-inflammatory state and participated in the apoptosis of photoreceptors in the early stage of RD, which was not driven by the phagocytic dysfunction of the retinal pigmented epithelium (RPE). Inhibition of microglia via the tamoxifen treatment or a combination of liposomal clodronate and tamoxifen preserves the viability and function of the photoreceptors (Lew et al., 2020). It is not clear why suppression of microglia via different treatments produced opposite results in the same RD model. Despite the heterogeneity of species and diseases, we are committed to finding the role of microglia in RD and to treating RD by targeting microglia. The identification of microglial subtypes may be an effective strategy. Traditionally, activated microglia polarize into two phenotypes: an M1-pro-inflammatory phenotype and an M2-anti-inflammatory phenotype, though this classification method has been questioned and impedes research into microglia (Martinez and Gordon, 2014; Ransohoff, 2016). In recent years, with the advent of technologies, such as single-cell RNA sequencing, researchers have demonstrated that microglia consist of various cell subtypes rather than a homogeneous population, which provides a basis for studying the role of microglia (Keren-Shaul et al., 2017; Masuda et al., 2020). Microglia have a strong plasticity, which is fundamental for them to convert into different subtypes and hinges on the different stages of life, CNS region, and conditions of health or disease (Masuda and Prinz, 2016). Microglia can be defined as various subtypes by differential gene expression pattern, special markers, and differential structural/ultrastructural properties including TREM2-microglia, CD11c-microglia, Hox8b-microglia, satellite microglia, and “dark” microglia (Stratoulias et al., 2019; Masuda et al., 2020). Under defined pathological conditions, unique microglial subtypes with distinct molecular hallmarks, cellular kinetics and responses to diverse stimuli have been found in the brains of both mice and humans by single-cell analysis (Masuda et al., 2019). As with the brain, the microglia in the retina also have spatial and temporal heterogeneity. The dependency on interleukin-34 and contribution to visual function of the two distinct niches of microglial subtypes is different. These two subtypes migrate into the SRS and play a role in protecting RPE integrity during RD, which also demonstrates the influence of the retinal niche on microglial fate (O'Koren et al., 2019).

It is worth mentioning that Keren-Shaul et al. identified and described the transcriptional features of all microglia populations. Subsequently, they defined the novel disease-associated microglia (DAM) using spatial location, molecular features, and functions that retarded neurodegeneration in an Alzheimer’s Disease model (Keren-Shaul et al., 2017). Similarly, our group identified the DAM in the RP model via comprehensive

analysis of the morphology and transcriptome and by staining for specific markers. We also found that DAM in the retina protects the diseased retina (unpublished data). Many paradoxical conclusions about the role of microglia in RD can be explained from the perspective of microglial subtypes, which contributes to the understanding of the RD pathological process. In addition, the identification and analysis of microglial subtypes contribute to understanding the precise role of every microglial subtype in the pathogenesis, treatment, and prognosis of RD, thus providing novel therapeutic targets for RD.

The role of microglia on synaptic pruning and neural remodeling

Microglia contribute to synaptogenesis during development

The synapse pruning and synaptogenesis functions of microglia during development are well known. Development is an extremely delicate and complex process, requiring precise microglia-mediated modification and sculpting via the phagocytic or non-phagocytic mechanism to establish correct neural circuits (Wright-Jin and Gutmann, 2019; Dixon et al., 2021). During development, microglia infiltrate into the CNS, then their numbers increase significantly during the postnatal first week, which coincides with the intense period of synaptogenesis. Microglia express thrombospondins that can induce synaptogenesis and various cytokines to regulate synaptic function (Bessis et al., 2007). Microglia can promote the formation of learning-associated synapses through brain-derived neurotrophic factor (BDNF) signaling. Microglia depletion or BDNF removal caused deficits in learning task and the learning-dependent synaptogenesis is reduced significantly (Parkhurst et al., 2013). In the developing cortex, activated microglia induce the formation of filopodia by contacting with dendrites directly during the period of intense synaptogenesis. Microglial ablation decreases the spine density and destroys the neural connections (Miyamoto et al., 2016).

Microglia contribute to synapse pruning in a non-complement-dependent manner during development and diseases

In addition to promoting synaptogenesis, microglia mediate the clearance of synaptic material. Loss or excessive activation of synaptic clearance causes the corresponding disease; therefore, regulating the balance of synaptic clearance is important (Raiders et al., 2021). Stimulated emission depletion microscopy and three-dimensional reconstruction demonstrate microglia engulfed presynaptic and postsynaptic material in a CX3CR1-dependent manner during synapse maturation (Paolicelli et al., 2011). In the whisker lesioning brain, CX3CR1 signaling is also required for microglia-mediated synaptic elimination. Considering the importance of CX3CL1-CX3CR1 signaling in RD, determining whether this signal influences the progression of RD by synapse pruning is necessary (Zabel et al., 2016; Gunner et al., 2019). Triggering receptor expressed on myeloid cells 2 (TREM2) is expressed in microglia in the CNS and is necessary for synapse pruning during the early stages of development. The dysfunction of TREM2 results in the deficits of synapse elimination, enhanced excitatory neurotransmission, and autistic behavior in mice (Filipello et al., 2018). Exposed phosphatidylserine is also an important signal that says "eat me," which is localized in the synaptic surface and is involved in microglia-mediated synapse elimination (Sapar et al., 2018; Shacham-Silverberg et al., 2018; Scott-Hewitt et al., 2020). The synaptic plasticity driven by the binding of interleukin-33 (IL-33) expressed in neurons and IL-33 receptors expressed in microglia is important for the integration of newborn neurons, the connectivity of neural circuits, and the formation of memory by instructing microglia to engulf the extracellular matrix (Nguyen et al., 2020; Zaki and Cai, 2020). In addition to the IL-33 expressed in neurons, IL-33 released by the astrocytes can recruit microglia to clear excess synapses via phagocytosis during development. The absence of IL-33 led to the excessive formation of excitatory synapses and overexcited neural circuits (Vainchtein et al., 2018). Interestingly, the CD47 expressed in neurons can send inhibitory signals to microglia to prevent the neurons from being engulfed. This suggests the balanced mechanism of microglial phagocytosis (Lehrman et al., 2018).

Microglia contribute to synapse pruning in a complement-dependent manner during development and diseases

The complement system is a classic regulator for synapse elimination and is required for synaptic pruning to refine visual system connections (Schafer et al., 2012). C1q, a component of the classical component pathway, is localized to the retinal synapses and is necessary for synapse elimination during retinal development. A deficiency in C1q or the downstream complement component C3 causes the dysfunction of synapse elimination, leading to defects in the visual pathway neural connection (Stevens et al., 2007). C1q is also found in microglia and instructs the horizontal cell axons and dendrites growth towards the outer retina during the maturation of outer retina synapses. The deficiency of C1q causes the reduction of microglial activation and phagocytosis, ectopic neurite growth, and the maturation disorders of retinal neural circuits (Burger et al., 2020). In the early stage of glaucoma, C1q is upregulated and is accompanied by synaptic relocalization. This enhanced signaling increases the loss of presynaptic elements engulfed by microglia, leading to visual loss (Stevens et al., 2007). Similarly, C1q is

increased and localized to infected neurons and presynaptic material, leading to dysfunctions of memory and cognition. The deficiency of C3 rescues this diseased phenotype to some extent in patients who are infected by the West Nile virus (Vasek et al., 2016). In the early stage of Alzheimer's disease, C1q is upregulated and is associated with synaptic material, leading to the pathological loss of synapses (Hong et al., 2016).

In addition to the classical component pathway, C3-CR3 signaling is necessary for microglial synaptic pruning in the visual system, disruption of which results in the failure of synaptic connectivity (Schafer et al., 2012). Under diseased conditions, CR3-mediated microglial phagocytosis results in the early loss of synapses, leading to the exacerbation of Alzheimer's disease. The inhibition of complement decreases the synapse loss and rescues the function (Hong et al., 2016). The microglia-mediated synapse elimination triggered by the excessive activation of complement components is found in schizophrenia patients and in forgetting (Sellgren et al., 2019; Wang et al., 2020). Likewise, we found that the expression of C3 upregulated and labeled the free postsynaptic protein, mGluR6, located in the dendrite terminals of bipolar cells in the OPL of RCS rats. Furthermore, homeostatic microglia were activated with enhanced phagocytosis ability and engulfed free synaptic proteins via the binding of C3 and CR3 on activated microglia. This was demonstrated by the reduction in the mGluR6 protein, the increase in mGluR6 RNA, and ultrastructural evidence. The above contributed to maintaining the functional stability of bipolar cells and inhibited the formation of anomalous processes, thus improving retinal remodeling and visual function (Fig. 1) (He et al., 2019). The CSF1R inhibitor diet eliminated activated microglia, which increased the number and length of bipolar cell anomalous processes, which progressed retinal remodeling into the advanced phase more quickly. Deficiency of C3 or CR3 decreased the microglial phagocytosis of apoptotic photoreceptors and increased photoreceptor toxicity, thus accelerating the degeneration of visual function in the RD model (Silverman et al., 2019).

Microglia contribute to synapse pruning via a non-phagocytic mechanism

Remarkably, we focused on the microglial phagocytosis-mediated synapse elimination in the past. However, the role of the non-phagocytic mechanism on synapse elimination exists. During retinogeniculate circuitry maturation, microglia that are proximal to Fn14 (fibroblast growth factor-inducible protein, 14 kDa)-expressed neurons and release TNF-associated weak inducer of apoptosis (TWEAK) to clear excessive synapses via the binding of Fn14 and TWEAK. This defines a new role of microglia in synapse elimination that is independent of phagocytosis (Cheadle et al., 2020).

In short, the microglial phagocytosis of synapse proteins plays an important role in the developing and diseased retina. The inhibition or excessive activation of synapse elimination results in the occurrence of disease; therefore, controlling the balance between synthesis and synapse elimination is vital. We should be aware that in various RD diseases, microglia-mediated synapse reconstruction is not only a concomitant event of degenerative disease but may also be the initiating or predisposing factor. Although we have made a series of advancements in understanding the modulatory mechanisms of synapse elimination, more precise and comprehensive research is required to improve retinal remodeling and provide a more appropriate environment for RD treatment.

Conclusions and prospects

As the age of the population increases, the incidence of RD is constantly rising, which not only affects the mental and physical well-being of patients but also causes severe economic burdens in society. Scientists are committed to slowing or stopping the progression of degeneration in early RD and to restoring visual function in advanced RD via stem cell transplantation. Although we have achieved a lot in preclinical and clinical trials, the therapeutic effect of stem cell transplantation has not been as satisfactory as expected, and only some patients have demonstrated limited improvement in visual function (Jones et al., 2017; Jin et al., 2019; Singh et al., 2020). The destruction of the inner retinal structure caused by retinal remodeling and the disruption of the retinal microenvironment caused by microglia impede stem cell survival and integration and may be the main obstacles limiting the therapeutic effect of stem cell transplantation.

The results above indicate that microglia can significantly alter the pathological progression of RD by influencing retinal remodeling, especially synapse reconstruction, and diverse activated states of microglia can either protect or impair retinal structure and function. Studying the role of microglia as a homogeneous population in RD is not appropriate as this may result in contradictory conclusions and does not represent the true role of microglia in RD. We must recognize and analyze the microglial heterogeneity in diverse species, disease models, regions, and disease phases using new technologies, such as cytometry, time-of-flight mass spectrometry, and single-cell RNA sequencing. A unified standard for the definition of microglial subtypes that considers function, such as DAM, is required. Microglia should be precisely manipulated at the subtype level to treat and improve RD. In other words, targeting microglia and retinal remodeling is a promising strategy in the treatment for RD.

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