Mammalian Target of Rapamycin (mTOR) as a Potential Therapeutic Target in Pathological Ocular Angiogenesis

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Pathological ocular angiogenesis is a causative factor of retinopathy of prematurity, proliferative diabetic retinopathy, and wet age-related macular degeneration. Vascular endothelial growth factor (VEGF) plays an important role in pathological angiogenesis, and anti-VEGF agents have been used to treat the ocular diseases that are driven by pathological angiogenesis. However, adverse effects associated with the blockade of VEGF signaling, including impairments of normal retinal vascular growth and retinal function, were suggested. Therefore, the development of a safe, effective strategy to prevent pathological ocular angiogenesis is needed. Recent studies have demonstrated that inhibitors of the mammalian target of rapamycin (mTOR) target proliferating endothelial cells within the retinal vasculature. Here, we review the potential of targeting the mTOR pathway to treat pathological ocular angiogenesis.

Key words mammalian target of rapamycin; pathological angiogenesis; retina; vascular endothelial growth factor

1. INTRODUCTION

Pathological angiogenesis within the eye (pathological ocular angiogenesis) contributes to the pathogenesis of vision-threatening eye diseases, such as retinopathy of prematurity, proliferative diabetic retinopathy, and wet age-related macular degeneration. Pathological angiogenesis is not confined to the highly organized neuronal layers of the retina and frequently grows from the retina into the vitreous cavity. The abnormal growth of new blood vessels on the retina results in sight-threatening complications such as vitreous hemorrhage and tractional retinal detachment.

The system of the vascular endothelial growth factor (VEGF) and its receptors (VEGFRs) plays an important role in pathological ocular angiogenesis, and anti-VEGF agents, such as pegaptanib and ranibizumab, have been used to treat potentially blinding diseases that are driven by pathological angiogenesis. However, adverse effects associated with the blockade of VEGF signaling, including impairments of normal retinal vascular growth and retinal function, have been suggested. Therefore, the development of safe, effective drugs for pathological ocular angiogenesis has attracted increasing attention in recent years. An ideal strategy would target endothelial cells in a proliferative state but would not interfere with quiescent endothelial cells. Recent studies have demonstrated that inhibitors of the mammalian target of rapamycin (mTOR) display a narrow-spectrum effect on proliferating endothelial cells within the retinal vasculature. Therefore, one possible therapeutic target is the mTOR pathway. Here, we review the potential of targeting the mTOR pathway to treat pathological ocular angiogenesis.

2. mTOR PATHWAY AND ANGIOGENESIS

The mTOR is a serine/threonine kinase that belongs to the phosphatidylinositol-3 kinase-related kinase superfamily and regulates protein synthesis via the mRNA translation process and a variety of other functions associated with cell proliferation and cell-cycle control. mTOR forms two distinct functional complexes termed mTOR complexes 1 and 2 (mTORC1 and mTORC2): mTORC1 regulates protein synthesis, cell growth and proliferation, autophagy, cell metabolism, and stress responses, whereas mTORC2 regulates cell survival and polarity.

Rapamycin, a macrolide antibiotic that was originally developed as an antifungal agent, inhibits mTORC1 and it was later found to have potent immunosuppressive effects and antiproliferative effects on tumor cells. The effects of rapamycin are mediated via phosphorylation of at least two well-characterized effectors, the p70S6 kinases (S6K1 and S6K2) and eIF4E-binding proteins (4E-BPs). In addition to inhibiting tumor cell growth, rapamycin exhibits antiangiogenic effects. The antiangiogenic action of rapamycin could be mediated by decreasing VEGF production and/or by attenuating the response of endothelial cells to VEGF. Thus, mTORC1 contributes to angiogenesis by enhancing VEGF production and/or by acting as a downstream effector of VEGF signaling in endothelial cells. In the field of ophthalmology, the antiangiogenic effects of mTORC1 inhibitors have been demonstrated in rodent models of retinal, choroidal, and corneal neovascularization, but the mechanisms are not fully understood.

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3. ROLE OF THE mTOR IN RETINAL VASCULAR DEVELOPMENT

The neonatal mouse retina provides a useful model system for studying the mechanisms of angiogenesis and vascular network formation, because the superficial plexus forms after birth and migrates out toward the periphery of the retina during the first postnatal week. This process could be...
impaired by treatment with agents that block the VEGF signaling pathway.\(^9,21-25\) When the inhibition of VEGF signaling was started on the day of birth, retinal vasculature formation was completely suppressed.\(^9,25\) Thus, the VEGF/VEGFR system plays a crucial role in the initiation of vascular formation and the development of the vascular bed in the retina.\(^9,23-27\)

The mTORC1 pathway also contributes to retinal vascular development.\(^9,28\) However, unlike the blockade of VEGF signaling, the inhibition of mTORC1 immediately after birth cannot completely prevent the expansion of the retinal vascular bed.\(^9\) Although mTORC1 inhibitors decrease the rate of expansion and the capillary density of the vascular bed in the retina, the effects on retinal blood vessels are less than those of VEGFR inhibitors.\(^9\) These results suggest that a VEGF-dependent but mTORC1-independent mechanism also contributes to retinal vascular development.

mTORC1 in endothelial cells is partly activated by VEGF, and mTORC1 inhibitors were shown to prevent VEGF-driven angiogenesis.\(^7\) Alternatively, mTORC1 increases the cellular level of hypoxia inducible factor-\(\alpha\), thereby increasing VEGF expression levels.\(^29\) Thus, mTORC1 not only acts as a downstream effector of VEGF signaling but also as a stimulator of VEGF production. However, the \textit{in situ} hybridization and immunohistochemical analyses of retinal flat-mount preparations revealed that rapamycin does not reduce the VEGF expression levels on the retinal surface.\(^9\) Similar observations were made in ocular pathological angiogenesis model mice treated with rapamycin.\(^18\) These results suggest that mTORC1 acts as a downstream effector of VEGF signaling in retinal endothelial cells, and mTORC1 inhibitors prevent the VEGF-driven retinal vascular formation.

The status of the mTORC1 pathway is evaluated by determining the levels of phosphorylation of ribosomal protein S6 (S6), a downstream target of mTORC1.\(^30\) Immunohistochemical analyses of retinal flat-mounts revealed that immunoreactivities to phosphorylated S6 (pS6) are detected in numerous cells on the retinal surface of neonatal mice.\(^9\) In addition to nonvascular cells in the retinal parenchyma, endothelial cells located at the vascular front exhibited strong pS6 immunoreactivity, whereas no pS6 immunoreactivity was observed in quiescent and/or mature endothelial cells.\(^9\) High-magnification images revealed that strong pS6 immunoreactivities are detected in some endothelial tip cells, which are leading cells located at the angiogenic front, and in stalk cells, which are endothelial cells following the migrating tip cells (Fig. 1A). Stalk cells proliferate in response to VEGF, create a blood vessel lumen, and bridge the gap between the tip cell and the parent vasculature.

Retinal vascular phenotypes of apelin-deficient mice resemble those of mice treated with rapamycin, and rapamycin shows no significant effect on retinal angiogenesis in apelin-deficient mice.\(^20\) Apelin produced by tip cells may act on stalk cells in a paracrine manner through its receptor, APJ, to stimulate endothelial proliferation.\(^28,31\) The presence of strong pS6 immunoreactivity in stalk cells supports the idea that mTORC1 acts downstream of the apelin–APJ system in endothelial cells at the vascular front.\(^32\)

Tip cells primarily migrate but proliferate only minimally, in contrast to stalk cells. However, the cells located at the angiogenic front could be attractive targets for preventing retinal pathological angiogenesis.\(^24,33\) The mTORC1 pathway is activated in growing endothelial cells at the vascular front, and mTORC1 inhibitors exhibit a more potent inhibitory effect on endothelial cell in these particular regions.\(^9\) Therefore, the mTORC1 pathway may be an attractive target for preventing retinal pathological angiogenesis.

The pS6 immunoreactivity in endothelial cells is no longer detectable in adult mice.\(^9\) Consistently, the effects of mTORC1 inhibitors on retinal blood vessels decrease in a postnatal age-dependent manner.\(^9\) This observation is similar to observations made with VEGF inhibition\(^23\) and is associated with the decreased proportion of growing and/or immature blood vessels within the vasculature. As described above, the mTORC1 pathway appears to play an important role in proliferating endothelial cells of growing and/or immature blood vessels, and the contribution of this pathway to the survival and maintenance of retinal endothelial cells is decreased in a postnatal age-dependent manner. Taken together, mTORC1 inhibitors target endothelial cells in a proliferative state but do not interfere with quiescent endothelial cells.

4. ROLE OF THE mTOR IN RETINAL PATHOLOGICAL ANGIOGENESIS

Several \textit{in vivo} experimental models have been developed to study the mechanisms of pathological ocular angiogenesis.\(^22,34-36\) One of the major models is oxygen-induced retinopathy (OIR) in mice.\(^22,34\) In this model, neovascular tufts are formed on superficial blood vessels at the border between the vascularized and the central avascular regions in the retina.\(^34\) The neovascular tufts consist of activated endothelial cells and resemble pathological features observed in patients with proliferative retinopathy. Strong pS6 immunoreactivities are observed in some neovascular tufts (Fig. 1B). Inhibitors of mTORC1 reduced the extent of retinal neovascular tufts and pS6 immunoreactivity.\(^30,36\) However, some neovascular tufts were resistant to mTORC1 inhibitors,\(^10\) consistent with the findings that some neovascular tufts were negative for pS6. On the other hand, the formation of neovascular tufts was almost completely blocked by the inhibition of VEGF signaling.\(^30\) These results suggest that VEGF-driven retinal angiogenesis is partially, but not completely, mediated by the mTORC1 pathway, and activation of this pathway in endothelial cells contributes to retinal pathological angiogenesis.\(^10\)

Apelin expression dramatically increases during the hypoxic phase in OIR mice, and APJ is colocalized in proliferative endothelial cells.\(^32\) The contribution of the apelin–APJ system to retinal angiogenesis is also enhanced in pathological conditions of the retina.\(^32\)

The antiangiogenic effects of mTORC1 inhibitors are less than those of VEGFR inhibitors. However, vascular defects, such as impairment of vascularization and loss of capillaries, can lead to severe hypoxia. The key driving force for aggressive angiogenesis is pronounced hypoxia, leading to a marked enhanced VEGF production. The severity of vascular abnormalities observed following treatment with VEGFR inhibitors is related to that of vascular defects.\(^37\) Therefore, when mTORC1 inhibitors are used instead of VEGF inhibitors for antiangiogenic treatment, the risk of appearance of aggressive angiogenesis following cessation of the treatment may be reduced.
activity may indirectly affect endothelial cell growth. Moreover, it was reported that pS6 immunoreactivity is enhanced in Müller cells in the injured retina, and mTORC1 inhibitors markedly reduced pS6 immunoreactivity in these nonvascular cells as well as in endothelial cells; therefore, it will be important to determine the effects of mTORC1 inhibitors on such nonvascular cells in future studies.

5. ROLE OF THE mTOR IN NONVASCULAR CELLS IN THE RETINA

In addition to proliferating endothelial cells, nonvascular cells, such as Müller cells, microglia, and retinal ganglion cells, also show pS6 immunoreactivity in the retina. Thus, the mTORC1 pathway is apparently activated in several retinal cell types. Both microglia and ganglion cells may participate in retinal angiogenesis at the early stage of vascular development and therefore the decreased mTORC1 activity may indirectly affect endothelial cell growth. Moreover, it was reported that pS6 immunoreactivity is enhanced in Müller cells in the injured retina, and mTORC1 inhibitors exhibit protective effects against retinal neuronal damage. Rapamycin markedly reduced pS6 immunoreactivity in these nonvascular cells as well as in endothelial cells; therefore, it will be important to determine the effects of mTORC1 inhibitors on such nonvascular cells in future studies.

6. CONCLUSION

The inhibition of VEGF and its receptors represents a promising therapeutic strategy for treating pathological ocular angiogenesis. Currently, anti-VEGF agents (ranibizumab, pegaptanib, and aflibercept) are in clinical use to slow the progression of neovascular or “wet” macular degeneration in Japan. However, adverse effects associated with the inhibition of VEGF signaling were reported. One of them is attributable to impaired physiological function of VEGF, such as normal retinal vascular growth and retinal function. The mTORC1 pathway is activated in proliferating endothelial cells at the developing vascular front and is inactivated in quiescent endothelial cells within the retinal vasculature (Fig. 2). Therefore, mTORC1 inhibitors display a narrow-spectrum effect on endothelial cells in a region- and status-dependent manner. Although the precise role of mTORC1 in nonvascular cells remains to be elucidated, the ability of mTORC1 inhibitors to target endothelial cells in a proliferative state is a favorable trait for safe, effective antiangiogenic therapy in retinal diseases that are driven by pathological angiogenesis.

Conflict of Interest

The authors declare no conflict of interest.

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