Targeting MAPK Signaling in Age-Related Macular Degeneration

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ABSTRACT: Age-related macular degeneration (AMD) is a major cause of irreversible blindness affecting elderly people in the world. AMD is a complex multifactorial disease associated with demographic, genetics, and environmental risk factors. It is well established that oxidative stress, inflammation, and apoptosis play critical roles in the pathogenesis of AMD. The mitogen-activated protein kinase (MAPK) signaling pathways are activated by diverse extracellular stimuli, including growth factors, mitogens, hormones, cytokines, and different cellular stressors such as oxidative stress. They regulate cell proliferation, differentiation, survival, and apoptosis. This review addresses the novel findings from human and animal studies on the relationship of MAPK signaling with AMD. The use of specific MAPK inhibitors may represent a potential therapeutic target for the treatment of this debilitating eye disease.

KEYWORDS: age-related macular degeneration, MAPK, ERK, JNK, p38, oxidative stress

Introduction

Age-related macular degeneration (AMD) is the leading cause of blindness in people 60 years and older in developed countries.[1] A recent systematic review and meta-analysis has shown that 8.7% of the worldwide population has AMD, and with the increase of lifespan the projected number of people with the disease will be at about 196 million in 2020, reaching 288 million in 2040.[2] About 1.75 million Americans are affected by AMD, and this number is expected to grow to almost 3 million by 2020.[3] AMD is a complex multifactorial disease that occurs over time and is characterized by degeneration of the retinal photoreceptors, retinal pigment epithelium (RPE), and choroidal neovascularization (CNV). AMD can be classified into two broad groups: dry (nonvascular or atrophic) affects 80%–90% of AMD patients and wet (neovascular or exudative) affects 10%–15%, but is responsible for approximately 90% of AMD-related vision loss.[4] There is no cure, but AMD treatments may prevent severe vision loss or slow the progression of the disease considerably. Several treatment options are available, including anti-VEGF therapy, laser surgery, photodynamic therapy, vitamins, and nutritional supplements.[5–8] The etiology of AMD is complex and includes both genetic and nongenetic factors. Genome-wide association studies (GWAS) have revealed common genetic variants at a number of loci. Alterations in genes of the complement system and inflammatory pathways, as well as variations in genes related to oxidative stress, have been associated with AMD.[9–12] Among the nongenetic factors, aging, smoking, hypertension, and diet significantly contribute to an increase in the AMD risk.[13] There is ample evidence that oxidative stress is involved in AMD pathogenesis and progression.[14,15] Oxidative stress and reactive oxygen species (ROS) have been implicated in the activation of various signaling pathways, including the mitogen-activated protein kinases (MAPKs).[16,17] MAPKs are important mediators of signal transduction and play a key role in the regulation of many cellular processes, such as cell growth and proliferation, differentiation, and apoptosis.[18] Several studies suggest that MAPKs are involved in oxidative stress-induced RPE degeneration,[19–22] which is described in more detail in the "AMD and MAPK signaling" section. Findings have revealed activation of the MAPK signaling pathways extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38 MAPK in model systems, including human RPE cell cultures and murine models of AMD.[23–28] In addition, linkage disequilibrium-independent genomic-enrichment analysis demonstrated association of AMD with genes encoding the MAPK signaling pathway (JNK, p38, ERK1/2, and ERK5).[29] Moreover, a new software "AMD Medicine" was used to compare the transcriptomes of normal human RPE-choroid and AMD-affected RPE-choroid samples.[30] The results demonstrated activation of several signaling pathways, including ERK in RPE-choroid AMD phenotypes. These data clearly suggest that MAPK signaling is involved in AMD pathogenesis, and

MAPK inhibitors could provide a novel therapeutic strategy for prevention or treatment of AMD.

AMD: Pathology, Genetics, and Current Treatments

AMD is the leading cause of blindness in the elderly that damages the central region of the retina (macula). Early stage of the disease is characterized by the presence of medium-sized drusen, which are extracellular deposits containing proteins, lipids, and inflammatory mediators.[31] As the disease progresses, it can develop into intermediate and late AMD. There are two types of late AMD: geographic atrophy and neovascular, or wet AMD. Early and intermediate, as well as geographic atrophy, are generally referred to as dry AMD. Dry AMD is characterized by RPE senescence and geographic RPE loss, while wet AMD is characterized by degeneration of RPE and abnormal growth of pathologic choroidal vessels. Dry AMD is a chronic disease that usually causes some degree of visual impairment, whereas wet AMD could rapidly progress to blindness if left untreated.

AMD is a complex, multifactorial disease of aging. In the aging retina, oxidative stress and ROS have been widely acknowledged to play a major role in the pathophysiology of disease.[14,32] The retina is highly susceptible to oxidative stress because of its high consumption of oxygen, high metabolic activity, and exposure to light. Excessive ROS levels can damage lipids, proteins, and nucleic acids. This process subsequently leads to cell death unless it is neutralized by the oxidant defense system. Retinas of patients with AMD show increased oxidative damage and drusen contain high amounts of oxidized proteins, as well as increased content of redox-sensitive proteins.[33–35] A growing body of evidence indicates that inflammation plays an important role in both dry and wet AMD.[11,36,37] This includes not only mild infiltration of macrophages and accumulation of microglia but also the presence of inflammatory components such as the complement pathway, cytokines, and chemokines. A longitudinal population study provides further support for the role of oxidative stress and inflammation in the pathogenesis of AMD.[38] Angiogenesis, the process of forming new blood vessels, is a hallmark in the pathology of wet AMD. Among the angiogenic factors investigated, the vascular endothelial growth factor (VEGF) has been shown to be a key factor in animal models and AMD patients. Animal models have provided evidence for a relationship between VEGF expression and the development of CNV.[39,40] Increased expression of VEGF was found in surgically excited CNV membranes[41,42] and increased levels of VEGF were detected in vitreous samples from AMD patients.[43] Although AMD risk involves many factors, it is well recognized that there is a strong genetic contribution, as evidenced by recent GWAS studies. This includes genes associated with the complementary pathway (*CFH, CFI, C2/CFB, C3*), lipoprotein metabolism (*APOE, CETP, LIPC*), angiogenesis (*VEGFA, TGFBR1*), extracellular matrix (*TIMP3, COL8A1/FILIP1L, COL10A1*), cell death

(*IER3/DDR1, TNFRSF10A*), glucose and lactate transport (*SLC16A8, B3GALTL*), DNA repair (*RAD51B*), cleavage of proteoglycans and inhibition of angiogenesis (*ADAMTS9/MIR548A2*), and mitochondrial gene *ARMS2*, whose function remains unknown.[12] In addition to the 19 candidate genes based on GWAS, polymorphism in genes encoding chemokines and cytokines have been associated with the risk of AMD development and progression. For greater detail, the reader is referred to the excellent and comprehensive reviews on the genetic component of the disease.[9–12]

There is no cure for AMD, but several treatment options are available that can prevent severe vision loss or slow the progression of the disease considerably. Currently, three VEGF antagonists such as ranibizumab (Lucentis), bevacizumab (Avastin), and aflibercept (Eylea) are used as the standard treatment for wet AMD.[44] All agents are injected intravitreously and require proper treatment schedule. Another VEGF antagonist, pegaptanib (Macugen) was the first drug approved for wet AMD by the United States Food and Drug Administration in 2004. However, pegaptanib is hardly used anymore because of its limited efficacy compared to the other three drugs, and it is no longer available in some countries. Although the VEGF antagonists are the current standard therapy for wet AMD, they have limitations due to the costs of these drugs, their need for frequent injections, and possible systemic adverse events and ocular complications with repeated high dosages of anti-VEGF compounds.[45] Other treatments for wet AMD include photodynamic therapy, which is less common than anti-VEGF injections and is used mostly in combination with them for specific forms of wet AMD.[5,6,44] Thermal laser photocoagulation and macular surgery are less common than other treatments.[5,6] In addition, many potential therapeutic strategies focusing on inhibition of the complement pathway are in preclinical trials.[46] In contrast to wet AMD, there is no approved therapy for dry AMD, although a few are now in clinical trials. Current clinical trials are investigating multiple modalities, including drugs that decrease oxidative stress, treatments targeting complement pathway and inflammation, the visual cycle, neuroprotection, and cell replacement therapy.[47] Dry AMD management consists of lifestyle modifications such as quitting smoking and healthy diet supplemented by zinc and antioxidant vitamins (vitamin C, vitamin E, and beta-carotene).

MAPK Signaling

MAPKs are a family of evolutionary well-conserved protein kinases that are expressed in all eukaryotic cells. Members of the MAPK family play a critical role in many cellular processes, including proliferation, differentiation, apoptosis, and survival, among others. In mammals, the MAPK signaling pathways fall into four distinct groups: ERK1/2, JNK1/2/3, p38 (α, β, γ, and δ), and ERK5.[48] They are activated by diverse extracellular stimuli such as growth factors, cytokines, mitogens, hormones, and various cellular stresses including oxidative
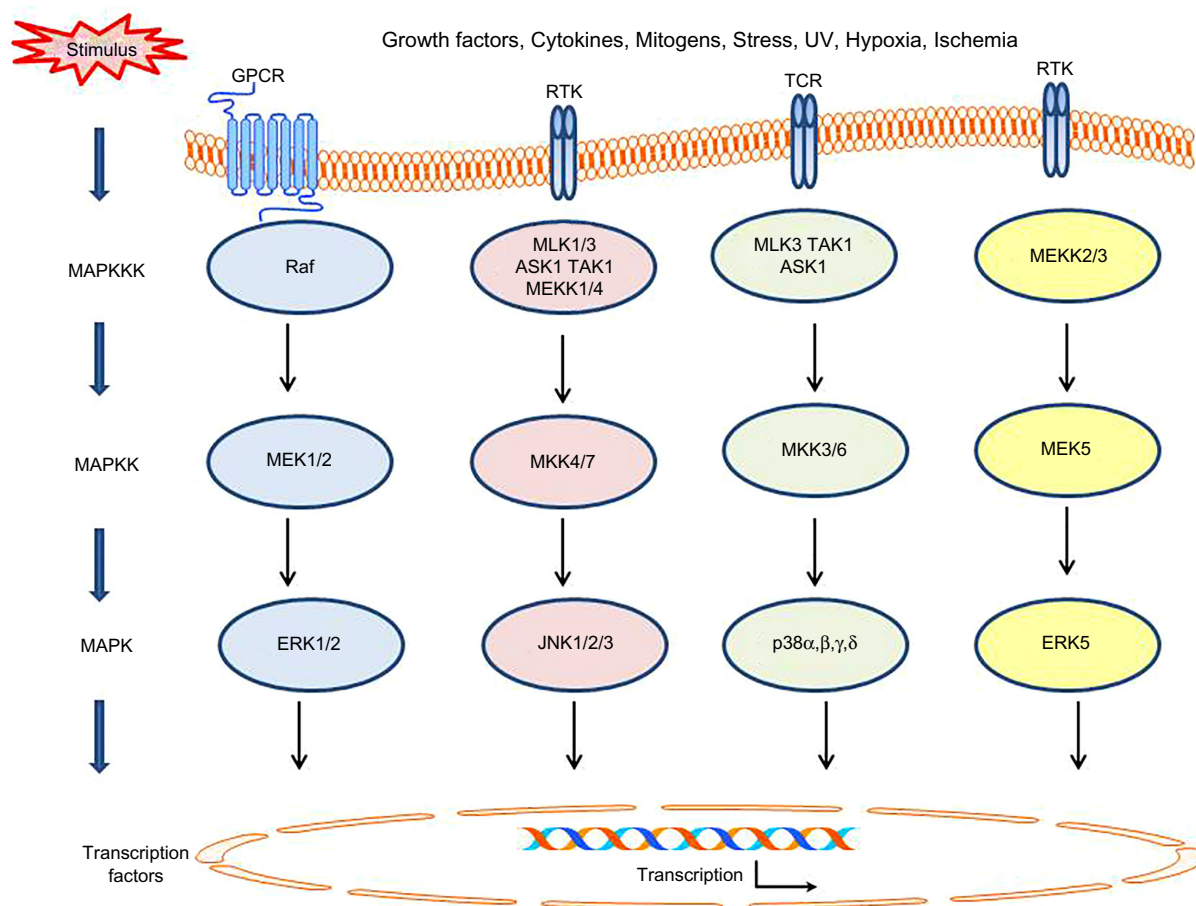
Figure 1. Simplified diagram depicting MAPK signaling. In mammals, the four major groups of MAPKs, ERK, JNK, p38, and ERK5 are activated by various extracellular stimuli. Once activated, MAPKs phosphorylate and activate an array of transcription factors.

stress, heat shock, ultraviolet (UV) irradiation, hypoxia, ischemia, and DNA-damaging agents via receptor-dependent and -independent mechanisms.[49] Each group of MAPKs contains a three-tiered kinase signaling cascade: MAPK kinase kinase (MAPKKK), MAPK kinase (MAPKK), and MAPK (Fig. 1). MAPKKKs are Ser/Thr protein kinases that are activated through phosphorylation, which, in turn, leads to phosphorylation and activation of MAPKKs, which then stimulate MAPK activity through dual phosphorylation on Thr and Tyr residues within a conserved Thr-X-Tyr motif located in the activation loop of the kinase domain.[50] For example, ERK1/2 and ERK5 have the dual phosphorylation motif Thr-Glu-Tyr, JNK has Thr-Pro-Tyr, and the Thr-Gly-Tyr motif is present in p38 MAPK.[51] Once activated, MAPKs phosphorylate and activate an array of transcription factors present in the cytoplasm and nucleus, leading to the expression of target genes and resulting in a biological response.

The ERK pathway is the first MAPK cascade elucidated and the best characterized.[52] ERK1/2 are stimulated in mammalian cells via tyrosine kinase receptors and G-protein-coupled receptors through both Ras-dependent and Ras-independent pathways.[53] They are also activated by growth factors, mitogens, cytokines, osmotic stress, and in response to insulin. Activated Ras-GTP phosphorylate Raf (isoforms A, B, and C), which in turn phosphorylates and activates MEK1/2 and ERK1/2. ERK pathway plays a central role mainly in the control of cell proliferation and differentiation, and also in neuronal plasticity, survival, and apoptosis.[54]

The first JNK family member, referred to as p54 MAP-2 kinase, was purified from cycloheximide-treated rat liver,[55] and subsequently cloned and named JNKs because of their ability to phosphorylate and activate the transcription factor c-Jun.[56] There are currently three mammalian JNKs with several isoforms, namely, JNK1, JNK2, and JNK3.[57] It has been shown that JNKs are activated in response to various cellular stresses such as heat shock, ionizing radiation, oxidative stress, DNA-damaging agents, cytokines through various receptors, including tyrosine kinase receptors, and to a lesser extent by some G-protein-coupled receptors.[58] JNKs are activated by the Rho family of small GTPases. These proteins in turn activate MEKK1/4, MKK4/7, and JNK1/2/3. In addition to MEKK1/4, apoptosis signal-regulated kinase (ASK1), mixed lineage kinases (MLK1/3), and TGF β-activated kinase (TAK1) regulate the JNK pathway.[59] JNK pathway has been shown to play important roles in the control of cell proliferation, differentiation, apoptosis, and inflammation.[60]

p38 MAPK was first identified in *Saccharomyces cerevisiae* as a protein kinase activated by hyperosmolarity, Hog1.[61] There are four isoformes of p38 MAPKs (α, β, γ, and δ) encoded from different genes.[48] Different isoformes are activated by inflammatory cytokines and various environmental stresses such as oxidative stress, UV radiation, hypoxia, ischemia, and others. Similar to JNKs, activation of p38 MAPKs through either stress or cell surface receptors involves members of the Rho family, which can activate and phosphorylate, MLKs, TAK1, ASK1, and MKK3/6.[48] In turn, MKK3/6 activates the four p38 isoformes. p38 pathway plays a critical role in normal immune and inflammatory responses, apoptosis, cell proliferation, and even survival.[62] The ERK5 pathway is one of the lesser studied and understood members of MAPK family. ERK5, also known as big MAPK (BMK1) because it is twice the size of other MAPKs, was initially found to be activated by oxidative stress and hyperosmolarity.[63] Subsequently, it was shown that ERK5 can be activated in response to serum, several growth factors, cytokines, and stress stimuli (reviewed by Drew et al).[64] The ERK5 signaling acts through sequential phosphorylation and activation of MEKK2/3, MEK5, and ERK5. The mechanism of activation of this pathway is still poorly elucidated; however, it is believed that several adaptor/scaffold proteins are involved, such as Lck-associated adapter[65] and Src.[66] ERK5 has been implicated in cell survival, differentiation, proliferation, and motility. In addition, several studies have suggested that ERK5 is involved in angiogenesis[67,68] and may potentially regulate VEGF-mediated neovascularization.[69]

AMD and MAPK Signaling

MAPKs have been implicated in many human pathologies, including neurodegenerative diseases (Alzheimer's, Parkinson's, and amyotrophic lateral sclerosis), diabetes, obesity, and different cancers. Given their pivotal role in key cellular processes, it is not surprising that alteration in expression and/or function of various intermediates of MAPK signaling is involved in the pathogenesis of AMD. Oxidative stress plays a central role in AMD. Commonly used experimental model to study the link between oxidative stress and AMD involves the use of cultured human RPE (ARPE19) cells. UV-induced damage is known to play a crucial role in eye diseases, including retinal degeneration. Studies have demonstrated that MAPKs ERK1/2, JNK, and p38 are activated in human RPE cells after UV exposure.[23,70] A recent study demonstrated the protective effect of resveratrol on RPE cells against UV-induced damages through inhibition of MAPK activation.[71] Based on these results, it is suggested that resveratrol may act as a suppressing agent for prevention of UV-induced ocular disorders.[71] Furthermore, RPE cells exposed to the oxidant *tert*-butyl hydroperoxide (*t*-BHP) showed activation of ERK1/2 and several downstream nuclear targets.[19] Inhibition of the ERK pathway was found to completely block *t*-BHP-induced apoptosis, while neither JNK nor p38 MAPK inhibition was able to prevent the *t*-BHP-induced apoptosis in RPE cells. ERK was postulated as a potential target for treating oxidative stress-induced RPE degeneration, such as AMD.[19] Cadmium, released from cigarette smoke and metal industrial activities, caused activation of ERK, JNK, and p38 MAPK in ARPE19 cells, suggesting that cadmium could be an important factor in RPE cell death.[72] Studies from this laboratory and others have shown that constitutive activation of ERK by cadmium induces authophagic cell death.[73] Autophagy, or *self-eating*, is a catabolic process by which cells degrade and recycle cellular components in order to maintain cellular metabolism and homeostasis.[74] There is now considerable evidence that autophagy plays a significant role in RPE, and this process is less effective with aging.[75] On the other hand, it was demonstrated that Ras/Raf/MEK/ERK signaling pathway is involved in serum-induced RPE cell proliferation.[76] After hydrogen peroxide stimulation, ERK1/2 does not seem to be involved in cell death, but to be associated with oxidative stress-induced proliferation,[77] while JNK and p38 activation is important for hydrogen peroxide-induced apoptosis in RPE cells.[78] Short-term activation of ERK1/2 is associated with cell proliferation,[79] whereas persistent activation of ERK1/2 leads to cell death.[80]

Many efforts have been made to establish animal models that mimic human AMD. The mouse is a genetically well-defined species and most of the retinal degeneration genes found first in mouse have been linked to a corresponding human retinal disease. The main drawback of using mice to study AMD is that the mouse retina has no macula. However, pathologic events seen in human AMD, such as drusen formation, thickened Bruch's membrane, various types of retinal degeneration, and CNV, have all been seen in various mouse strains.[81] It has been shown that deficient expression of the RNase III DICER1 leads to the accumulation of *Alu* RNA RPE of human eyes with geographic atrophy.[82] Studies revealed that *Alu* RNA overexpression or DICER1 knockdown increases the phosphorylation of ERK1/2 in mouse RPE *in vivo*, while JNK1/2 or p38 MAPK phosphorylation levels were unchanged.[26] Similar results were obtained when antisense oligonucleotide-mediated knockdown of DICER1 was used in primary human RPE cells.[26] PD98059, a potent and selective inhibitor of MEK, inhibits ERK1/2 phosphorylation and blocks RPE degeneration both in RPE cell culture and mice.[26] In addition, ERK1/2 pathway is also involved in experimental models of retinal and CNV.[83,84] In a specific AMD model and in particular for retinal angiomatous proliferation, a form of wet AMD, the *very low-density lipoprotein receptor* knockout mouse (*vldlr*−/−), we have reported that ERK1/2, JNK, and p38 are elevated and a single intravitreal injection of cerium oxide nanoparticles (nanoceria) for one week reduces the phosphorylation of the three MAP kinases to the control levels.[28] A recent study demonstrates that JNK1 deficiency or JNK inhibition leads to a decrease in apoptosis,

VEGF expression, and reduction of CNV in a mouse model of wet AMD (laser-induced CNV).[27]

Using data from a whole-genome genotyping microarray and a multilocus enrichment analysis, SanGiovanni and Lee have identified AMD-associated regions related to an ~30% change in the likelihood of having advanced AMD within six of 25 tested genes of JNK signaling pathway.[29] Makarev et al developed a new software AMD Medicine to evaluate the changes in functional pathway networks using changes in gene expression of single genes between AMD and normal eyes.[30] They discovered that several pathways including ERK are activated in RPE-choroid AMD phenotypes. The aforementioned findings indicate that MAPK pathways are undoubtedly involved in AMD pathology and are potentially attractive targets for both neovascular and atrophic AMD.

There is a crosstalk between MAPK signaling and several different pathways, such as VEGF, inflammasome, autophagy, and others. VEGF is known to be the major angiogenic factor involved in AMD.[39–43] MAPK pathways play an essential role in modulating VEGF. It has been demonstrated that constitutive activation of ERK1/2 leads to increased VEGF expression,[85] and overexpression of p38 and JNK leads to elevated VEGF expression.[86] Inflammasomes play a central role in innate immune system. ROS serves as an important inflammasome signal that activates MAPK signaling.[87] It has been shown that dysregulation of inflammasome plays a role in various pathological conditions including AMD.[88] It was reported that NLRP3 inflammasome activation by drusen induces interleukin-18 (IL-18) secretion, which, in turn downregulates VEGF, thus reducing the excessive neoangiogenesis associated with AMD.[89] Zhang et al demonstrated that the cytokine IL-17A induced the activation of ERK1/2 and p38 MAPK in RPE cells.[90]

Figure 2 shows a proposed model of MAPK signaling that leads to AMD.

MAPK Inhibitors

Although the application of anti-VEGF drugs for treatment of wet AMD has significantly improved the control of disease, not all patients benefited from these drugs. Undoubtedly, identifying additional or alternative therapies that can improve the current standard treatment is of great interest.

MAPKs have become the most intensively studied protein kinases in the past two decades with a number of pharmacological inhibitors developed to block MAPK signaling. Dudley et al discovered the first small-molecule inhibitor of MEK1/2, the PD98059 compound.[91] Subsequently, another potent inhibitor of MEK1/2, U0126, was identified.[92] However, because of their pharmaceutical limitations, none of these two compounds have moved to clinical trials. However, PD98059 and U0126 have proven to be invaluable research tools to investigate the role of the ERK1/2 pathway in normal cell physiology and disease process. To date, several MEK1/2 inhibitors have been tested clinically or undergoing clinical trials for treatment of various cancers.[93,94] These include trametinib (GSK1120212) for *BRAF*-mutated melanoma, selumetinib (AZD6244) for non-small cell lung cancer, binimetinib (MEK162, ARRY-162) for biliary tract cancer and melanoma, and PD0325901 for breast cancer, colon cancer, and melanoma. Raf inhibitors including vemurafenib, sorafenib, regorafenib, encorafenib (LGX818), and dabrafenib are in clinical trials for patients with tumor types harboring frequent mutations in *BRAF* gene and for treating renal, hepatocellular, and thyroid cancers.[95] However, adverse drug reactions including ophthalmologic complications occurred in patients treated with some MAPK inhibitors. For example, the incidence of retinal vein occlusion and retinal pigment epithelial detachments
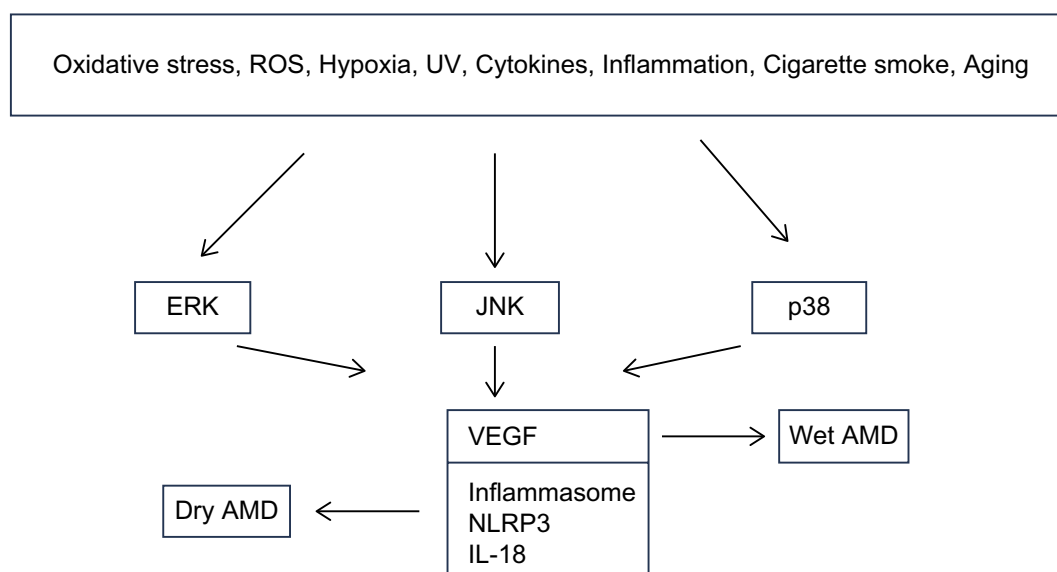

Figure 2. Schematic presentation depicting the role of ERK, JNK, and p38 in dry and wet AMD.

in patients treated with trametinib in clinical trials is 0.2% and 0.8%, respectively.[96] Uveitis occurred in 1% of patients receiving dabrafenib[97] and in 2.1% of patients treated with vemurafenib.[98] Therefore, these MAPK inhibitors cannot be used for treatment of AMD because of their ocular toxicity. The two broad spectrum inhibitors sorafenib and regorafenib are the most attractive drugs to target MAPK signaling in AMD. Both inhibitors target multiple kinases, including Raf, VEGF receptors 1–3, fibroblast growth factor receptor 1, and platelet-derived growth factor receptor, thereby inhibiting tumor growth and angiogenesis.[99,100] No ocular toxicities were reported for sorafenib except one case of retinal tear possibly associated with the use of this drug.[101] Regorafenib (Stivarga; Bayer HealthCare) eye drops have been developed to inhibit VEGF activity in a small group of patients with neovascular (wet) AMD and a phase II trial has been recently completed (ClinicalTrials.gov Identifier: NCT02222207), pending results to evaluate the safety and tolerability of these eye drops. Because regorafenib is a multikinase inhibitor that inhibits VEGF, to what extent the inhibition of Raf/MEK/ERK signaling contributes to the clinical activity of this inhibitor is yet to be determined. Further understanding of the effects of sorafenib and regorafenib to target MAPK pathways in AMD is an area for research exploration. According to two new studies, presented as posters at the Association for Research in Vision and Ophthalmology 2015 Annual Meeting, regorafenib showed positive results as a potential topical therapy in the nonhuman primate laser-induced CNV model and in two different rodent models of ocular neovascularization.[102,103] Because currently available treatments require intravitreal injections, regorafenib eye drops offer an innovative and noninvasive potential treatment option for wet AMD. However, local side effects are possible for theoretically every drug used in ophthalmology, which include toxicity related to the compound itself.

Additional pharmacological inhibitors that target p38 MAPK have been one of the most intensively studied classes of therapies for the treatment of inflammation. P38 MAPK inhibitors that are in phase II clinical trials for patients with autoimmune diseases and inflammatory processes include pamapimod, losmapimod, dilmapimod, doramapimod, VF-702, BMS-582949, ARRY-797, and PH-797804.[104] No ocular toxicities have been associated with the use of these inhibitors. Research into the role of p38 in AMD is an exciting area in the future that will be important to determine how best to make use of the therapeutic potential of targeting this signaling pathway.

Although there are several small-molecule JNK inhibitors such as SP600125, JNK-IN-8, and tanzisertib (CC-930), none of them proved to be effective in human tests yet.

Conclusion

In recent years, we have witnessed a mounting body of data suggesting that MAPKs are implicated in the pathogenesis of many human diseases. The evidence presented in this review indicates that MAPK pathways are involved in the development of AMD. Given the role of MAPK signaling in key cellular processes, interest in protein kinases as drug targets has exploded in the past few years. This may shift current research and clinical practice toward the use of MAPK inhibitors, alone or in combination with other therapeutics for treatment of AMD.

Author Contributions

Conceived the concepts: SVK. Analyzed the data: SVK. Wrote the first draft of the manuscript: SVK. Developed the structure and arguments for the paper: SVK. Made critical revisions: SVK. The author reviewed and approved the final manuscript.

REFERENCES

1. Lim LS, Mitchell P, Seddon JM, Holz FG, Wong TY. Age-related macular degeneration. *Lancet*. 2012;379(9827):1728–38.
2. Wong WL, Su X, Li X, et al. Global prevalence of age-related macular degeneration and disease burden projection for 2020 and 2040: a systematic review and meta-analysis. *Lancet Glob Health*. 2014;2(2):e106–16.
3. Friedman DS, O'Colmain BJ, Munoz B, et al. Prevalence of age-related macular degeneration in the United States. *Arch Ophthalmol*. 2004;122(4):564–72.
4. Fine SL, Berger JW, Maguire MG, Ho AC. Age-related macular degeneration. *N Engl J Med*. 2000;342(7):483–92.
5. Barakat MR, Kaiser PK. VEGF inhibitors for the treatment of neovascular age-related macular degeneration. *Expert Opin Investig Drugs*. 2009;18:637–46.
6. Holz FG, Schmitz-Valckenbergs S, Fleckenstein M. Recent development in the treatment of age-related macular degeneration. *J Clin Invest*. 2014;12(4):1430–8.
7. Yonekawa Y, Kim IK. Clinical characteristics and current treatment of age-related macular degeneration. *Cold Spring Harb Perspect Med*. 2015;5:a017178.
8. Hobbs RP, Bernstein PS. Nutrient supplementation for age-related macular degeneration, cataract, and dry eye. *J Ophthalmic Vis Res*. 2014;9(4):487–93.
9. Deangelis MM, Silveira AC, Carr EA, Kim IK. Genetics of age-related macular degeneration: current concepts, future directions. *Semin Ophthalmol*. 2011;26(3):77–93.
10. Patnapriya R, Chew EY. Age-related macular degeneration – clinical review and genetic update. *Clin Genet*. 2013;84:160–6.
11. Cascella R, Ragazzo M, Strafella C, Strafella C, Missiroli F, Borgiani P. Age-related macular degenerations: insights into inflammatory genes. *J Ophthalmol*. 2014;2014:582842.
12. Black JR, Clark SJ. Age-related macular degeneration: genome-wide association studies to translation. *Genet Med*. 2016;18(4):283–9.
13. Klein R. Overview of progress in the epidemiology of age-related macular degeneration. *Ophthalmic Epidemiol*. 2007;14(4):184–7.
14. Winkler BS, Boulton ME, Gottsch JD, Sternberg P. Oxidative damage and age-related macular degeneration. *Mol Vis*. 1999;5:32.
15. Hollyfield JG. Age-related macular degeneration: the molecular link between oxidative damage, tissue-specific inflammation and outer retinal disease: the Proctor lecture. *Invest Ophthalmol Vis Sci*. 2010;51:1275–81.
16. Torres M, Forman HJ. Redox signaling and the MAPK signaling pathways. *Biofactors*. 2003;17(1–4):287–96.
17. McCubrey JA, Lahair MM, Franklin RA. Reactive-oxygen species-induced activation of the MAP kinase signaling pathways. *Antioxid Redox Signal*. 2006;8(9–10):1775–89.
18. Kyosseva SV. Mitogen-activated protein kinase signaling. *Int Rev Neurobiol*. 2004;59:201–20.
19. Glotin AL, Calipel A, Brossas JY, Faussat AM, Treton J, Mascarelli F. Sustained versus transient ERK1/2 signaling underlies the anti- and proapoptotic effects of oxidative stress in human RPE cells. *Invest Ophthalmol Vis Sci*. 2006;47(10):4614–23.
20. Klettner A, Roider J. Constitutive and oxidative-stress-induced expression of VEGF in the RPE are differently regulated by different Mitogen-activated protein kinases. *Graefes Arch Clin Exp Ophthalmol*. 2009;247(11):1487–92.
21. Wang Z, Bartosh TJ, Ding M, Roque RS. MAPKs modulate RPE response to oxidative stress. *J Med Biol Eng*. 2014;3(1):67–73.
22. Koinzer S, Reinecke K, Herdegen T, Roider J, Klettner A. Oxidative stress induces biphasic ERK1/2 activation in the RPE with distinct effects on cell survival at early and late activation. *Curr Eye Res*. 2015;40(8):853–7.

23. Roduit R, Schorderet DF. MAP kinase pathways in UV-induced apoptosis of retinal pigment epithelium ARPE19 cells. *Apotosis*. 2008;13(3):343–53.

24. Weng CY, Kothary PC, Verkade AJ, Reed DM, Del Monte MA. MAP kinase pathway is involved in IGF-1-stimulated proliferation of human retinal pigment epithelial cells (hRPE). *Curr Eye Res*. 2009;34(10):867–76.

25. Li C, Huang Z, Kingsley R, et al. Biochemical alterations in the retinas of very low-density lipoprotein receptor knockout mice: an animal model of retinal angiomatous proliferation. *Arch Ophthalmol*. 2007;125(6):795–803.

26. Dridi S, Hirano Y, Tarallo V, et al. ERK1/2 activation is a therapeutic target in age-related macular degeneration. *Proc Natl Acad Sci U S A*. 2012;109(34):13781–6.

27. Du H, Sun X, Guma M, et al. JNK inhibitions induces apoptosis and neovascularization in a murine model of age-related macular degeneration. *Proc Natl Acad Sci U S A*. 2013;110(6):2377–82.

28. Kyosseva SV, Chen L, Seal S, McGinnis JF. Nanoceria inhibit expression of genes associated with inflammation and angiogenesis in the retina of Vldlr null mice. *Exp Eye Res*. 2013;116:63–74.

29. SanGiovanni JP, Lee PH. AMD-associated genes encoding stress-activated MAPK pathway constituents are identified by interval-based enrichment analysis. *PLoS One*. 2013;8(8):e71239.

30. Makarev E, Cantor C, Zhavoronkov A, Buzdin A, Aliper A, Csoka AB. Pathway activation profiling reveals new insights into age-related macular degeneration and provides avenues for therapeutic interventions. *Aging (Albany NY)*. 2014;6(12):1064–75.

31. Mullins RF, Russell SR, Anderson DH, Hageman GS. Drusen associated with aging and age-related macular degeneration contain proteins common to extracellular deposits associated with atherosclerosis, elastosis, amyloidosis, and dense deposit disease. *FASEB J*. 2000;14:835–46.

32. Beatty S, Koh H, Phil M, Henson D, Boulton M. The role of oxidative stress in the pathogenesis of age-related macular degeneration. *Surv Ophthalmol*. 2000;45(2):115–34.

33. Shen J, Dong A, Hackett S, Bell R, Green R, Campochiaro P. Oxidative damage in age-related macular degeneration. *Histol Histophatol*. 2007;22:1301–8.

34. Crabb JW, Miyagi M, Gu X, et al. Drusen proteome analysis: an approach to the etiology of age-related macular degeneration. *Proc Natl Acad Sci U S A*. 2002;99(23):14682–7.

35. Decanini A, Nordgaard CL, Feng X, Ferrington DA, Olsen TW. Changes in select redox proteins of the retinal pigment epithelium in age-related macular degeneration. *Am J Ophthalmol*. 2007;143(4):607–15.

36. Ambati J, Atkinson JP, Gelfand BD. Immunology of age-related macular degeneration. *Nat Rev Immunol*. 2013;13(6):438–51.

37. Bowes Rickman C, Farsiu S, Toth CA, Klingeborn M. Dry age-related macular degeneration: mechanisms, therapeutic targets, and imaging. *Invest Ophthalmol Vis Sci*. 2013;54(14):ORSF68–80.

38. Klein R, Myers CE, Cruickshanks KJ, et al. Markers of inflammation, oxidative stress, and endothelial dysfunction and the 20-year cumulative incidence of early age-related macular degeneration: the beaver dam eye study. *JAMA Ophthalmol*. 2014;132(4):446–55.

39. Kwak N, Okamoto N, Wood JM, Campochiaro PA. VEGF is major stimulator in model of choroidal neovascularization. *Invest Ophthalmol Vis Sci*. 2000;41(10):3158–64.

40. Heckenlively JR, Hawes NL, Friedlander M, et al. Mouse model of subretinal neovascularization with choroidal anastomosis. *Retina*. 2003;23:518–22.

41. Lopez PF, Sippy BD, Lambert HM, Trach AB, Hinton DR. Transdifferentiated retinal pigment epithelial cells are immunoreactive for vascular endothelial growth factor in surgically excised age-related macular degeneration-related choroidal neovascular membranes. *Invest Ophthalmol Vis Sci*. 1996;37(5):855–68.

42. Bhutto IA, McLeod DS, Hasegawa T, et al. Pigment epithelium-derived factor (PEDF) and vascular endothelial growth factor (VEGF) in aged human choroid and eyes with age-related macular degeneration. *Exp Eye Res*. 2006;82(1):99–110.

43. Tong JP, Chan WM, Liu DT, et al. Aqueous humor levels of vascular endothelial growth factor and pigment epithelium-derived factor in polypoidal choroidal vasculopathy and choroidal neovascularization. *Am J Ophthalmol*. 2006;141(3):456–66.

44. Haller JA. Current anti-vascular endothelial growth factor dosing regimens: benefits and burden. *Ophthalmology*. 2013;120(5 suppl):S3–7.

45. Falavarjani KG, Nguyen QD. Adverse events and complications associated with intravitreal injection of anti-VEGF agents: a review of literature. *Eye (Lond)*. 2013;27(7):787–94.

46. Troutbeck R, Al-Qureshi S, Guymer RH. Therapeutic targeting of the complement system in age-related macular degeneration: a review. *Clin Exp Ophthalmol*. 2012;40(1):18–26.

47. Buschini E, Fea AM, Lavia CA, et al. Recent developments in the management of dry age-related macular degeneration. *Clin Ophthalmol*. 2015;9:563–74.

48. Kyriakis JM, Avruch J. Mammalian MAPK signal transduction pathways activated by stress and inflammation: a 10-year update. *Physiol Rev*. 2012;92:689–737.

49. Cargnello M, Roux PP. Activation and function of the MAPKs and their substrates, the MAPK-activated protein kinases. *Microbiol Mol Biol Reviews*. 2011;75(1):50–83.

50. Plotnikov A, Zehorai E, Procaccia S, Seger R. The MAPK cascades: signaling components, nuclear roles and mechanisms of nuclear translocation. *Biochim Biophys Acta*. 2011;1813(9):1619–33.

51. Widmann C, Gibson S, Jarpe MB, Johnson GL. Mitogen-activated protein kinase: conservation of a three-kinase module from yeast to human. *Physiol Rev*. 1999;79(1):143–80.

52. Seger R, Krebs EG. The MAP kinase signaling cascade. *FACEB J*. 1995;9:726–35.

53. Lewis TS, Shapiro PS, Ahn NG. Signal transduction through MAP kinase cascades. *Adv Cancer Res*. 1998;74:49–139.

54. Yoon S, Seger R. The extracellular signal-regulated kinase: multiple substrates regulate diverse cellular functions. *Growth Factors*. 2006;24(1):21–44.

55. Kyriakis JM, Avruch J. pp54 Microtubule-associated protein 2 kinase: a novel serine/threonine kinase regulated by phosphorylation and stimulated by poly-L-lysine. *J Biol Chem*. 1990;265(28):17355–63.

56. Derijard B, Hibi M, Wu IH, et al. JNK-1: a protein kinase stimulated by UV light and Ha-Ras that binds and phosphorylates the c-jun activation domain. *Cell*. 1994;76(6):1025–37.

57. Kyriakis JM, Banerjee P, Nikolakaki E, et al. The stress-activated protein kinase subfamily of c-Jun kinases. *Nature*. 1994;369(6476):156–60.

58. Gupta S, Barrett T, Whitmarsh AJ, et al. Selective interaction of JNK protein kinase isoforms with transcription factors. *EMBO J*. 1996;15:2760–70.

59. Bogoyevitch MA, Kobe B. Uses for JNK: the many and varied substrates of the c-Jun N-terminal kinases. *Microbiol Mol Biol Rev*. 2006;70:1061–95.

60. Sabapathy K. Role of the JNK pathway in human diseases. *Prog Mol Biol Transl Sci*. 2012;106:145–69.

61. Han J, Lee JD, Bibbs L, Ulevitch R. A MAP kinase targeted by endotoxin and hyperosmolarity. *Science*. 1994;265(5173):808–11.

62. Cuenda A, Rousseau S. p38 MAP kinase pathway regulation, function and role in human diseases. *Biochim Biophys Acta*. 2007;1773(8):1358–75.

63. Abe J, Kusuhara M, Ulevitch RJ, Berk BC, Lee JD. Big mitogen-activated protein kinase 1 (BMK1) is a redox-sensitive kinase. *J Biol Chem*. 1996;271(28):16586–90.

64. Drew BA, Burrow ME, Beckman BS. MEK5/ERK5 pathway: the first fifteen years. *Biochim Biophys Acta*. 2012;18259(1):37–48.

65. Sun W, Kesavan K, Schaefer BC, et al. MEKK2 associates with the adapter protein Lad/RIBP and regulates the MEK5-BMK1/ERK5 pathway. *J Biol Chem*. 2001;276(7):5093–100.

66. Scapoli L, Ramos-Nino ME, Martinelli M, Mossman BT. Src-dependent ERK5 and Src/EGFR-dependent ERK1/2 activation is required for cell proliferation by asbestos. *Oncogene*. 2004;23(3):805–13.

67. Hayashi M, Kim SW, Imanaka-Yoshida K, et al. Targeted deletion of *BMK1/ERK5* in adult mice perturbs vascular integrity and leads to endothelial failure. *J Invest*. 2004;113(8):1138–48.

68. Pi X, Garin G, Xie L, et al. BMK1/ERK5 is a novel regulator of angiogenesis by destabilizing hypoxia inducible factor 1α. *Circ Res*. 2005;96(1):1145–51.

69. Wu Y, Zuo Y, Chakrabarti R, Feng B, Chen S, Chakrabarti S. ERK5 contributes to VEGF alteration in diabetic retinopathy. *J Ophthalmol*. 2010;2010:465824.

70. Chan CM, Huang JH, Lin HH, et al. Protective effects of (-)-epigallocatechin galate on UVA-induced damage in ARPE19 cells. *Mol Vis*. 2008;14:2528–34.

71. Chan CM, Huang CH, Li HJ, et al. Protective effects of resveratrol against UVA-induced damage in ARPE19 cell. *In J Mol Sci*. 2015;16:5789–802.

72. Chu YK, Lee SC, Byeon SH. VEGF rescues cigarette smoking-induced human RPE cell death by increasing autophagic flux: implications of the role of autophagy in advanced age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2013;54(12):7329–37.

73. Cagnal S, Chambard JC. ERK and cell death: mechanisms of ERK-induced cell death – apoptosis, autophagy and senescence. *FEBS J*. 2010;277(1):2–21.

74. Ryter SW, Cloonan SM, Choi AM. Autophagy: a critical regulator of cellular metabolism and homeostasis. *Mol Cells*. 2013;36(1):7–16.

75. Mitter DK, Rao HV, Qi X, et al. Autophagy in the retina: a potential role in age-related macular degeneration. *Adv Exp Med Biol*. 2012;723:83–90.

76. Heequet C, Lefevre G, Valtink M, Engelmann K, Mascarelli F. Activation and role of MAP kinase-dependent pathways in retinal pigment epithelial cells: ERK and RPE proliferation. *Inest Ophthalmol Vis Sci*. 2002;43(9):3091–8.

77. Tsao YP, Ho TS, Chen LS, Cheng HC. Pigment epithelium-derived factor inhibits oxidative stress-induced cell death by activation of extracellular signal-regulated kinases in cultured retinal pigment epithelial cells. *Life Sci*. 2006;79(6):545–50.

78. Ho TC, Yang YC, Cheng HC, et al. Activation of mitogen-activated protein kinases is essential for hydrogen peroxide-induced apoptosis in retinal pigment epithelial cells. *Apoptosis*. 2006;11(11):1899–908.

79. Fukunaga K, Miyamoto E. Role of MAP kinase in neurons. *Mol Neurobiol*. 1998;16(1):79–95.

80. Stanciu M, Wang Y, Kentor R, et al. Persistent activation of ERK contributes to glutamate-induced oxidative toxicity in a neuronal cell line and primary cortical neuron cultures. *J Biol Chem*. 2000;276(16):12200–6.

81. Rakoczy PE, Yu MJT, Nusinowitz S, Chang B, Heckenlinely JR. Mouse models of age-related macular degeneration. *Exp Eye Res*. 2006;82(5):741–52.

82. Kaneko H, Dridi S, Tarallo V, et al. DICER1 deficit induces Alu RNA toxicity in age-related macular degeneration. *Nature*. 2011;471(7338):325–30.

83. Hua J, Guerin KI, Chen J, et al. Resveratrol inhibits pathologic retinal neovascularization in Vldlr(-/-) mice. *Invest Ophthalmol Vis Sci*. 2011;52(5):2809–16.

84. Xie P, Kamei M, Suzuki M, et al. Suppression and regression of choroidal neovascularization in mice by a novel CCR2 antagonist, INCB3344. *PLoS One*. 2011;6(12):e28933.

85. Milanini J, Vinals F, Pouyssegur J, Pages G. p42/44 MAP kinase module plays a key role in the transcriptional regulation of the vascular endothelial growth factor gene in fibroblasts. *J Biol Chem*. 1998;273(29):18165–72.

86. Pages G, Berra E, Milanini J, Levy AP, Pouysseger J. Stress-activated protein kinases (JNK and p38/HOG) are essential for vascular endothelial growth factor mRNA stability. *J Biol Chem*. 2000;275(34):2684–91.

87. Harijith A, Ebenezer DL, Natarajan V. Reactive oxygen species at the crossroad of inflammasome and inflammation. *Front Physiol*. 2014;5:352.

88. Celkova L, Doyle SL, Campbell M. NLRP3 inflammasome and pathology in AMD. *J Clin Med*. 2015;4:172–94.

89. Doyle SL, Campbell M, Ozaki E, et al. NLRP# has a protective role in age related macular degeneration through the induction of IL-18 druzen components. *Nat Med*. 2012;18(5):791–8.

90. Zhang S, Yu N, Zhang R, Zhang S, Wu J. Interleukin-17A induces IL-1β secretion from RPE cells via the NLRP3 inflammasome. *Invest Ophthalmol Vis Sci*. 2016;57(1):218–26.

91. Dudley DT, Pang L, Decker SJ, Bridges AJ, Saltiel AR. A synthetic inhibitor of the mitogen-activated protein kinase cascade. *Proc Natl Acad Sci USA*. 1995;92(17):7686–9.

92. Favata MF, Horiuchi KY, Manos EJ, et al. Identification of a novel inhibitor of mitogen-activated protein kinase kinase. *J Biol Chem*. 1998;273(29):18623–32.

93. Frémin C, Meloche S. From basic research to clinical development of MEK1/2 inhibitors for cancer therapy. *J Hematol Oncol*. 2010;3:8.

94. Uehling DE, Harris PA. Recent progress on MAP kinase pathway inhibitors. *Bioorg Med Chem Lett*. 2015;25(19):4047–56.

95. Mandal R, Becker S, Strebhardt K. Stamping out RAF and MEK1/2 to inhibit the ERK1/2 pathway: an emerging treat to anticancer therapy. *Oncogene*. 2016;35(20):2547–61.

96. Infante JR, Fecher LA, Falchook GS, et al. Safety, pharmacokinetic, pharmacodynamic, and efficacy data for the oral MEK inhibitor trametinib; a phase 1 dose-escalation trial. *Lancet Oncol*. 2012;13(8):773–81.

97. Chapman PB, Hauschild A, Robert C, et al. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. *N Engl J Med*. 2011;364(26):2507–16.

98. Hauschild A, Grob JJ, Demidov LV, et al. Dabrafenib in BRAF-mutated metastatic melanoma: a multicentre, open-label phase 3 randomised controlled trial. *Lancet*. 2012;380(9839):358–65.

99. Adnane L, Trail PA, Taylor I, Wilhelm SM. Sorafenib (BAY 43-9006, Nexavar), a dual-action inhibitors that targets RAF/MEK/ERK pathway in tumor cells and tyrosine kinases VEGFR/PDGFR in tumor vasculature. *Methods Enzymol*. 2006;407:597–612.

100. Wilhelm SM, Dumas J, Adnane L, et al. Regorafenib (BAY 73-4506): a new oral multikinase inhibitor of angiogenic, stromal and oncogenic receptor kinases with potent preclinical antitumor activity. *Int J Cancer*. 2011;129:245–55.

101. Gaertner KM, Caldwell SH, Rahma OE. A case of retinal tear associated with use of sorafenib. *Front Oncol*. 2014;4:196.

102. Boettger MK, Klar J, Richter A, von Degenfeld G. Topically administered regorafenib eye drops inhibit grade IV lesions in the non-human primate laser CNV model. *Invest Ophthalmol Vis Sci*. 2015;56(7):2294.

103. Klar J, Boettger MK, Freundlieb J, Keldenich J, Elena PP, von Degenfeld G. Effects of the multi-kinase inhibitor regorafenib on ocular neovascularization. *Invest Ophthalmol Vis Sci*. 2015;56(7):246.92.

104. Arthur JS, Ley SC. Mitogen-activated protein kinases in immunity. *Nat Rev Immunol*. 2013;13(9):679–92.