Comparative Analysis of Bevacizumab and Sorafenib on the Survival of Retinal Ganglion Cells in the Treatment of Retinal Diseases

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Directed by Professor Chan Yun Kim

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ABSTRACT

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(Directed by Professor Chan Yun Kim)

Purpose: This study examines the effects of bevacizumab, a common vascular endothelial growth factor (VEGF) inhibitor, in ocular neovascular disorder treatment. Bevacizumab has been reported to be associated with adverse effects on retinal ganglion cell (RGC) survival. Our research validates these reports, seeks an alternative VEGF inhibitor with similar antiangiogenic efficacy but no effects on RGC, and elucidates the underlying mechanisms responsible for these distinctions.

Methods: The efficacy of bevacizumab in promoting the survival of primary RGCs extracted from neonatal rats was compared with that of other VEGF inhibitors. Human umbilical vascular endothelial cells (HUVECs) were used to evaluate the apoptotic effects of the VEGF inhibitors. The cell survival pathways influenced by the VEGF-inhibitor treatment were also investigated. Similarly, the genes affected by the treatment were identified using RNA sequencing.

Results: Sorafenib and bevacizumab exhibited potent VEGF-inhibitory effects in HUVECs. Notably, 2 mg/mL bevacizumab demonstrated a comparable VEGF inhibitory effect to 0.5 μM sorafenib. However, the RGC survival rate was higher following treatment with 0.5 μM sorafenib than 2 mg/mL bevacizumab. Western blot analysis indicated lower Akt levels after bevacizumab than sorafenib treatments. RNA sequencing revealed that the PI3K/AKT,

Ras, and MAPK signaling pathways were involved in RGC viability, whereas the JAK/STAT pathway did not play a pivotal role.

Conclusions: Our results suggest that sorafenib may be a more effective and safer treatment option than bevacizumab for various retinal diseases. This study identified novel genes implicated in this process and highlighted the intricate involvement of multiple signaling pathways. These insights will help facilitate the development of safer therapeutic approaches for various retinal diseases, particularly those associated with glaucoma.

Keywords: retinal ganglion cell; vascular endothelial growth Factor; bevacizumab; sorafenib; glaucoma

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I. INTRODUCTION

Excessive expression of vascular endothelial growth factor (VEGF) contributes to several sight-threatening eye conditions, including age-related macular degeneration and diabetic retinopathy, both of which are leading causes of blindness.[1-6] Intravitreal VEGF-inhibitor injections have shown effectiveness in the management of these debilitating diseases.[6-8]

In the realm of VEGF-inhibitor treatments, bevacizumab has been employed by ophthalmologists in an off-label capacity as an intravitreal agent for the treatment of proliferative (neovascular) eye diseases.[9] Administration of 1.25–2.5 mg bevacizumab into the vitreous cavity has long been a common practice.[9-11]

However, the indiscriminate inhibition of VEGF throughout the retina, as typically achieved with VEGF-inhibitor therapy, may cause potential adverse effects.[12] Previous research has highlighted the multifaceted role of VEGF as a pro-survival factor in various cell types.[13-15]

Glaucoma, a chronic eye disease characterized by optic nerve damage primarily associated with elevated intraocular pressure (IOP), affects over 64 million individuals worldwide.[16-18] This condition remains incurable, necessitating continuous treatment upon diagnosis. While the reduction of IOP remains the primary therapeutic approach,

interventions that effectively halt the progression of glaucoma are currently unavailable. Notably, the pathology of glaucoma persists even when IOP is adequately controlled.[19-21] Addressing this enigma requires the identification of neuroprotective mechanisms capable of safeguarding retinal ganglion cells (RGCs), which constitute the pivotal pathophysiological substrate of glaucoma.

Given the increased vulnerability of RGCs to various stressors, promoting the survival of these cells is an effective therapeutic approach. Key factors regulating survival mechanisms under stressful conditions include hypoxia-inducible factor-1α, VEGF, and nitric oxide synthase.[21-23] Specifically, the expression levels of these factors increase in response to hypoxia and glaucoma.[21-23]

We have previously demonstrated the potential detrimental impact of VEGF-inhibitor therapy on RGC survival, particularly under stressful conditions.[23] Consequently, multiple rounds of VEGF-inhibitor treatments may inadvertently exacerbate glaucoma owing to excessive RGC loss.

The objectives of this study are to explore the effects of bevacizumab, a VEGF-inhibitor agent, on RGC survival and identify enhanced treatment strategies to promote RGC survival with similar antiangiogenic efficacy. The results of this study will help facilitate the development of novel therapeutic approaches for various retinal diseases, particularly those associated with glaucoma.

II. MATERIALS AND METHODS

1. Animals

Sixty pregnant Sprague–Dawley rats were acquired from Orientbio Inc. (Seongnam, Korea). A total of 840 newborn rat pups were humanely euthanized by decapitation to obtain sufficient RGC samples. Ethical approval for the study was obtained from the Institutional Animal Care and Use Committee of Yonsei University College of Medicine, Seoul, Korea (Approval Number: 2022-0053). All procedures involving rats were performed in accordance with the ethical guidelines outlined by the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research. Extensive measures were taken to minimize the number of animals used and to alleviate potential suffering.

2. Preparation of RGCs

RGCs were isolated using a two-step immunopanning method as previously described.[23-25] Briefly, retinal tissues were extracted from 1- to 4-day-old newborn Sprague–Dawley rats and combined to form a mixed suspension of retinal cells. The retinal cell suspension was incubated with a rabbit anti-rat macrophage antibody (dilution 1:50; Fitzgerald Industries International, Acton, MA, USA) for 5 min. Subsequently, the suspension was placed in a 10 cm Petri dish coated with goat anti-rabbit immunoglobulin G antibody (dilution 1:200; Southern Biotechnology Associates, Birmingham, AL, USA) for 30 min.

Non-adherent cells were subsequently transferred to a second 10 cm Petri dish coated with mouse anti-rat thymocyte differentiation antigen (Thy) 1.1 antibody (dilution 1:50; Bio-Rad, Hercules, CA, USA) for 1 h. The cells were then incubated with anti-biotin magnetic microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany). Finally, the magnetically labeled RGCs were collected using a magnetic separation unit. All the procedures were performed simultaneously at room temperature (20–25 ℃) in a laminar flow hood.

The isolated cells were cultured in Dulbecco’s modified Eagle’s medium/nutrient mixture

F-12 (DMEM/F-12; catalog no. SH30023.01; HyClone Laboratories, Logan, UT, USA) supplemented with 10% fetal bovine serum (Life Technologies, Grand Island, NY, USA), 100 U/mL penicillin, and 100 μg/mL streptomycin (Life Technologies). These cells were seeded onto 12 mm glass coverslips pre-coated with poly-L-ornithine and laminin (Sigma-Aldrich, St. Louis, MO, USA). The cultures were maintained at 37 ℃ in a humidified atmosphere containing 5% CO_2 and 95% air.

The authenticity of the cultured RGCs was validated through Brn3a (dilution 1:1,000, SC-8429, Santa Cruz Biotechnology, CA, USA) immunostaining (Figure 1).

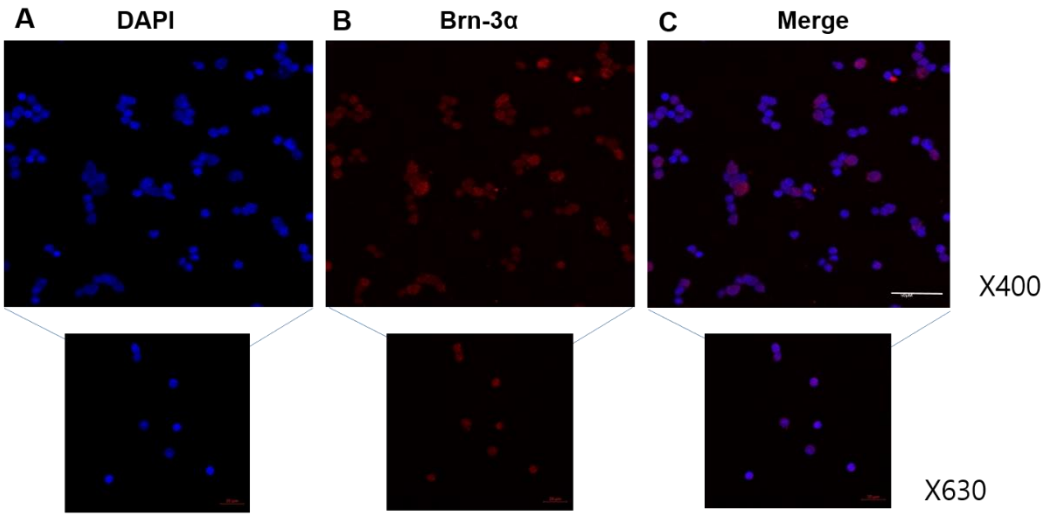


Figure 1. Identification of retinal ganglion cells.

Immunofluorescence staining was conducted on RGCs at day 2 of culture, revealing positive expression of the Brn3a marker (Panel B). DAPI nuclear staining is depicted in Panel A, while the merged image is presented in Panel C, demonstrating the co-localization of Brn3a-positive RGCs with nuclear staining.

3. Preparation of human umbilical vascular endothelial cells

Human umbilical vein endothelial cells (HUVECs) (C2519A, LONZA, Walkersville, MD, USA) were cultured in a 10 cm dish containing endothelial growth medium (EGM)-2 (CC-3162, LONZA, Walkersville, MD, USA) with EGM™-2 Single-Quots ™ Supplements (CC-4176, LONZA, Walkersville, MD, USA) and incubated at 37 ℃ with 5% CO_2 following the manufacturer's instructions and as previously described.[26] The medium was changed every 3 days. The cells were harvested upon reaching 70–85% confluence.

4. Cell viability assay

Cell viability was evaluated using the cell counting kit-8 (CCK-8, Dojindo Laboratories, Kumamoto, Japan). RGCs and HUVECs were seeded onto a 96-well plate and incubated in the culture medium at 37 ℃ in 5% CO_2 for 24 h. After the treatment period, 10 μl of CCK-8 solution was added to each well, and the plate was incubated for 2–4 h. Absorbance was measured using a spectrophotometer at a wavelength of 450 nm, within a spectral range of 420–480 nm, with a reference wavelength set at 600 nm.

5. Western blot

For western blot, total cell lysates were first obtained using a cell lysis buffer (Cell Signaling Technology, MA, USA). The lysates were incubated on ice for 5 min. Subsequently, the lysates were sonicated, and the resulting cell homogenates were centrifuged at 15,000 × g for 15 min at 4 ℃.

Following centrifugation, the protein concentration in the supernatants was quantified using the Pierce™ Bicinchoninic Acid Protein Assay Kit (Thermo Fisher Scientific, Foster City, CA, USA). Soluble proteins (10 μg per sample) were boiled for 5 min and separated using 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Proteins were subsequently electrotransferred onto polyvinylidene fluoride membranes with a pore size of 0.45 μm. To prevent nonspecific binding, the membranes were blocked with 5% skim milk in Tris-buffered saline containing 0.1% Tween 20 (TBS-T) and incubated overnight

with primary anti-Akt (9272S, Cell Signaling Technology) and anti-α-tubulin (T6199-100, MillporeSigma, Darmstadt, Germany) antibodies. These primary antibodies were diluted in a solution containing 0.1% bovine serum albumin and 0.01% sodium azide in TBS-T. The blots were washed three times with TBS-T and incubated with horseradish peroxidase-conjugated secondary antibodies (Cell Signaling Technology) for 1 h at room temperature (20–25 ℃). After another three washes with TBS-T, immunoreactive bands were visualized using enhanced chemiluminescence. The relative intensities of the immunoreactive bands were measured after normalization against α-tubulin levels.

6. Flow cytometry

To detect apoptosis, we utilized an Annexin V-fluorescein isothiocyanate (FITC) apoptosis kit from BioVision (Milpitas, CA, USA) following the manufacturer's instructions. Briefly, after collagenase digestion, isolated cells were prepared for analysis. The cells were stained with Annexin V-FITC and propidium iodide (BioVision). Flow cytometry was conducted using a FACS LSR II instrument (BD Biosciences, San Jose, CA, USA). The percentage of viable cells was determined based on the proportion of Annexin V- and propidium iodide-positive cells in the samples. Flow cytometric analysis enabled the identification and quantification of apoptotic cells within the population of interest.

7. Lactate dehydrogenase assay

A lactate dehydrogenase (LDH) cell cytotoxicity kit, specifically the CytoTox 96® Non-Radioactive Cytotoxicity Assay Kit from Promega Corporation (Madison, WI, USA), was utilized to quantitatively assess cell survival. Briefly, RGCs were cultured with various VEGF inhibitors. The cells were incubated with LDH detection buffer for 30 min at room temperature in the dark. A stop solution was added to terminate the reaction. Absorbance was measured at 490 nm using a microplate reader (Bio-Rad, Hercules, CA, USA). LDH release, which indicates cytotoxicity, was calculated by dividing the experimental time point values by the maximum LDH release values and multiplying by 100. Maximum LDH

release values were obtained by subjecting each culture to a freeze–thaw process, inducing nearly complete cell damage.

8. Cell counting

RGC death was induced by treatment with various VEGF inhibitors. The cells were fixed with 4% paraformaldehyde for 30 min and thoroughly washed with phosphate-buffered saline (PBS). At room temperature (20–25 ℃), the RGCs were stained with DAPI solution for visualization, incubated for 5 min, and washed three times with PBS. For each sample, five images of DAPI-stained RGCs were captured using a light microscope (Olympus IX73; Olympus Corp., Tokyo, Japan) at 100× magnification. The number of RGCs in the presence or absence of oxidative stress was counted manually in each image.

9. RNA sequencing

RNA library preparation and sequencing were performed by LAS Inc. (Gimpo, Korea; http://www.lascience.co.kr/) using the SMARTER Stranded Total RNA-seq kit-v2—Pico Input Mammalian (Takara Bio, Mountain View, CA, USA) in accordance with the manufacturer's protocol. This process involved ligating RNAs with 3′ and 5′ adaptors and subsequently reverse transcribing them into cDNA. Polymerase chain reaction (PCR) was performed using different Illumina index primers to distinguish multiple time points after injury in both the proximal and distal segments. All libraries with 75 bp paired-end reads were sequenced on a NextSeq 500 System (Illumina, San Diego, CA, USA).

Quality control of the reads was conducted using FastQC v0.11.5, and any sequencing adapters and low-quality bases in the raw reads were trimmed using Skewer version 0.2.2. The resulting high-quality reads were mapped to the reference genome using STAR version 2.6 software.

The mapped reads were quantified as gene expression values relative to the reference genome using Cuffquant in Cufflinks version 2.2.1.

Gene annotation from the reference genome rn6 (UCSC genome,

https://genome.ucsc.edu) in GTF format was used for gene models, and expression values were calculated as fragments per kilobase of transcripts per million mapped reads (FPKM). Differential gene expression analysis among the four selected biological conditions (RGCs versus RGCs with VEGF treatment versus RGCs with Bevacizumab treatment versus RGCs with Sorafenib treatment) was conducted using Cuffdiff within the Cufflinks package. Genes with a fold change cutoff of 2 and a p-value cutoff of 0.05 were identified as differentially expressed. The normalized expression values of a selected few hundred differentially expressed genes (DEGs) were subjected to unsupervised clustering using R scripts provided by LAS Inc. to compare the expression profiles among samples. Scatter plots for gene expression values, volcano plots for expression fold changes, and p-values between two selected samples were also generated.

A gene set overlapping test between the analyzed DEGs and functionally categorized genes, encompassing the biological processes of Gene Ontology (GO), KEGG pathways, and other functional gene sets, was performed using g:Profiler2 version 0.2.0 to gain insights into the biological functional roles of the DEGs.

10. Network analysis and visualization

Functional analysis was conducted using the Database for Annotation, Visualization, and Integrated Discovery (DAVID) bioinformatics platform (http://david.abcc.ncifcrf.gov), in addition to Ingenuity Pathway Analysis. GO enrichment analysis was employed to explore the associations between significantly expressed genes and their respective cellular compartments, biological processes, and molecular functions. Statistical significance was determined within DAVID using LPEseq (Seoul, Korea)[27] to generate corrected q-values. Differential gene expression analysis was performed using the criteria of $|\log2FC| > 1$ and q-value < 0.05. Only terms with a corrected q-value < 0.05 were considered statistically significant. The top list of DEGs was input into the STRING database to identify protein–protein interactions (PPIs) using a medium confidence threshold of 0.400. These interactions were subsequently visualized using Cytoscape 2.8.3

(http://www.cytoscape.org). Candidate genes were identified using MCODE to establish clusters, and key biological processes and pathways associated with the clusters of genes within a functionally grouped network were visualized using ClueGo/CluePedia and KEGGscape plugins.

11. Quantitative reverse-transcription polymerase chain reaction (RT-qPCR) and RNA-Seq data validation

RNA was isolated using an RNeasy Micro Kit (Qiagen, Hilden, Germany) and reverse transcribed into complementary DNA (cDNA) using EcoDry™ Premix (Takara Bio). Real-time PCR was conducted utilizing SYBR® Premix Ex Taq™ (Takara Bio) and predesigned primers (Table 1) on the StepOnePlus™ Real-Time PCR System (Applied Biosystems/Thermo Fisher Scientific). Gene expression levels were quantified using the comparative cycle threshold method and normalized to β-actin as an internal control within the same sample.

Table 1. Primers for quantitative reverse-transcription polymerase chain reaction

Gene	Sequence (5`-3`)	Primers
rCar4	ATCTGCCCACCCAGTACAAG	F
	AGCCCTCGTTTACCTCGTTT	R
rLama2	TCACGCTGTCAAGGATTCAG	F
	TTGACTTCTGGCCTTGCTTT	R
rHgf	CGAGCTATCGCGGTAAAGAC	F
	TGTAGCTTTCACCGTTGCAG	R
rVegfc	AGCAGCCACAAACACCTTCT	F
	TGCTGAGGTAACCTGTGCTG	R
rFgf2	GAACCGGTACCTGGCTATGA	F
	CCGTTTTGGATCCGAGTTTA	R
rFgf4	AGGCTGCGGAGACTCTACTG	F
	ACTCCGAAGATGCTCACCAC	R

rFgf7	GCTCTACAGACCGTGCTTCC	F
	CCCCTCCTTCCATGTAGTCA	R
rScx	ACATTTCTCACCTGGGCAAC	F
	CGTCTCTGGCCAGTGGTAGT	R
rPak2	GCTGTGCTGGATGTCTTGAA	F
	CTGACCCCTTGGTGTTCAGT	R
rGfap	AGAAAACCGCATCACCATTC	F
	TCCTTAATGACCTCGCCATC	R
rRtn4r	GGCTGCCAGTGACTTAGAGG	F
	TGAGTGCATTTCCAGCAGAC	R
rCdh17	CAAAGCAGAAAACCCTGAGC	F
	GGGGAAATACAGGCACTTCA	R
rWt1	GCCTTCACCTTGCACTTCTC	F
	GACCGTGCTGTATCCTTGGT	R
rEwsr1	ACTTCGCCTGGAGAACAGAA	F
	TCCATGAGTCCACCTCTTCC	R

12. siRNA transfection

Cells were transiently transfected with 200 nM siRNA targeting Akt and non-targeting siRNA using Lipofectamine 2000 in accordance with the manufacturer's guidelines. Akt knockdown efficacy following siRNA transfection was assessed using a western blot. After a 48-h transfection period, the cells were harvested in six-well plates to evaluate Akt protein levels and their functional impact.

13. Statistical analysis

In adherence to rigorous scientific standards, all experiments were conducted in triplicate to ensure data accuracy and reliability, and their results are presented as the mean ± standard deviation. A robust statistical approach was employed to assess the significance of the observed differences between groups. Student's *t*-test and one-way analysis of

variance were used for initial group comparisons. A linear mixed model was employed to show statistically significant trends. All statistical analyses were performed using GraphPad Prism version 9.0 for Windows (GraphPad Software, San Diego, CA, USA), SPSS V22.0 software (SPSS, Chicago, IL, USA), SAS version 9.4 (SAS Institute Inc., Cary, NC, USA), and the R package (version 4.3.0, packages; survival; The R Project for Statistical Computing, Vienna, Austria). Statistical significance was considered at $p < 0.05$, ensuring the utmost reliability in our findings, in line with the highest standards of medical research.

III. RESULTS

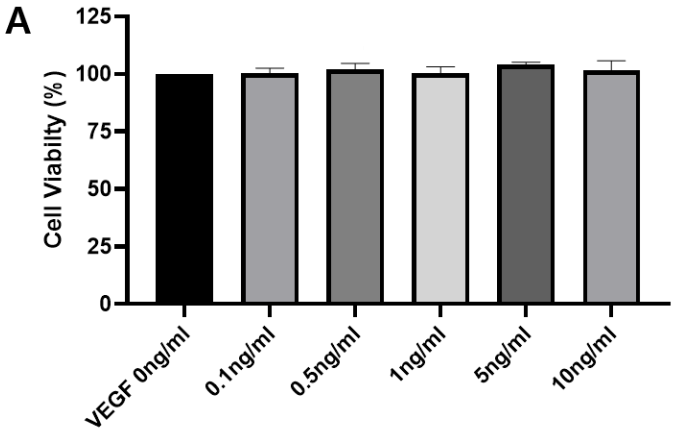
1. Effects of VEGF and bevacizumab on RGC survival

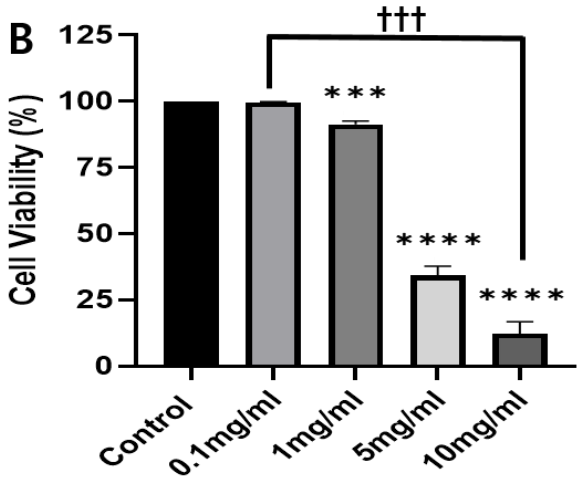
An *in vitro* experiment was conducted to assess the effects of VEGF and bevacizumab on RGCs. RGCs were cultured with different concentrations of VEGF (0.1, 0.5, 1, 5, and 10 ng/mL) and bevacizumab (0.1, 1, 5, and 10 mg/mL) for 24 h, and their viability was subsequently compared with that of RGCs cultured without these agents (control group).

The viability of RGCs exposed to the various VEGF concentrations did not significantly differ from that of the control group ($p > 0.05$, Figure 2A).

Although the viability of RGCs cultured with 0.1 mg/mL bevacizumab was not significantly altered compared with that of the control group, the viability of the cells cultured with 1, 5, and 10 mg/mL bevacizumab was significantly lower than that of the control group ($p < 0.05$; Figure 2B). To analyze the dose effect of bevacizumab, a linear mixed model was employed, revealing a statistically significant trend ($p < 0.001$; Figure 2B).

These findings were corroborated through immunostaining for Brn3a, which revealed a similar trend. Compared with the control group, RGCs cultured with 1 and 2 mg/mL of bevacizumab had a lower cell count (Figure 2C).





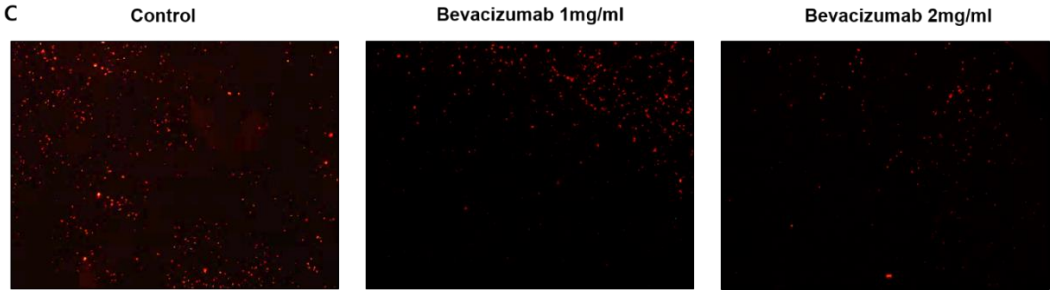


Figure 2. Effects of VEGF and bevacizumab on RGC survival.

RGCs were cultured with VEGF and bevacizumab for 24 h, and their viability was subsequently compared with that of RGCs cultured without these agents (control group). (A) The viability of RGCs cultured with various concentrations (0.1, 0.5, 1, 5, and 10 mg/mL) of VEGF did not significantly differ from that of the control group ($p > 0.05$). (B) The viability of RGCs cultured with 1, 2, 5, and 10 mg/mL of bevacizumab was significantly lower than that of the control group ($p < 0.05$). A linear mixed model was employed to analyze the dose effect of bevacizumab, revealing a statistically significant trend ($p < 0.001$). (C) Immunostaining for Brn3a revealed that RGCs cultured with 1 and 2 mg/mL bevacizumab exhibited significantly lower cell counts than the control group (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, †††$p < 0.001$, ****$p < 0.0001$).

2. Effect of bevacizumab on the Akt pathway

To elucidate the mechanisms of action of bevacizumab on RGC survival, we examined the effects of 0.5, 1.0, and 2.0 mg/mL of bevacizumab on signaling molecules involved in pro-survival pathways, such as Akt. The Akt expression level was measured 24 h after bevacizumab treatment. Western blot analysis revealed that Akt levels gradually decreased with increasing bevacizumab concentrations (Figure 3).

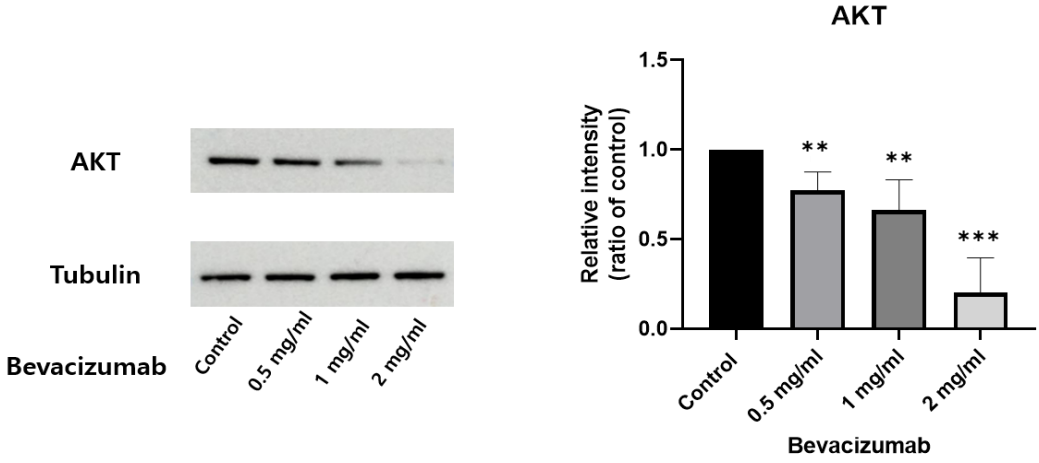


Figure 3. Effect of bevacizumab on Akt

To elucidate the mechanisms of action of bevacizumab on RGC survival, we investigated the effects of different concentrations (0.5, 1.0, and 2.0 mg/mL) of bevacizumab on pivotal signaling molecules associated with pro-survival pathways, notably Akt. Western blot analysis revealed a progressive decline in Akt levels as the concentration of bevacizumab increased (**$p < 0.01$, ***$p < 0.001$).

3. Effects of various VEGF inhibitors on RGC survival

To determine an alternative approach to inhibiting VEGF, we assessed the efficacy of various VEGF inhibitors, including sorafenib, regorafenib, trametinib, vemurafenib, selumetinib, and pazopanib, in promoting the survival of RGCs. We compared the apoptotic effects of these VEGF inhibitors on HUVECs with those of 2 mg/mL bevacizumab to identify their optimal concentrations. Subsequently, we used the following concentrations of the VEGF inhibitors for HUVEC experiments: 0.5 μM sorafenib, 2 μM regorafenib, 0.5 μM trametinib, 1 μM vemurafenib, 10 μM selumetinib, and 10 μM pazopanib (data not shown). In the RGC viability experiment, sorafenib induced the highest RGC survival rate among the tested VEGF inhibitors and significantly surpassed bevacizumab (Figure 4). Therefore, sorafenib was used in all subsequent comparative analyses with bevacizumab.

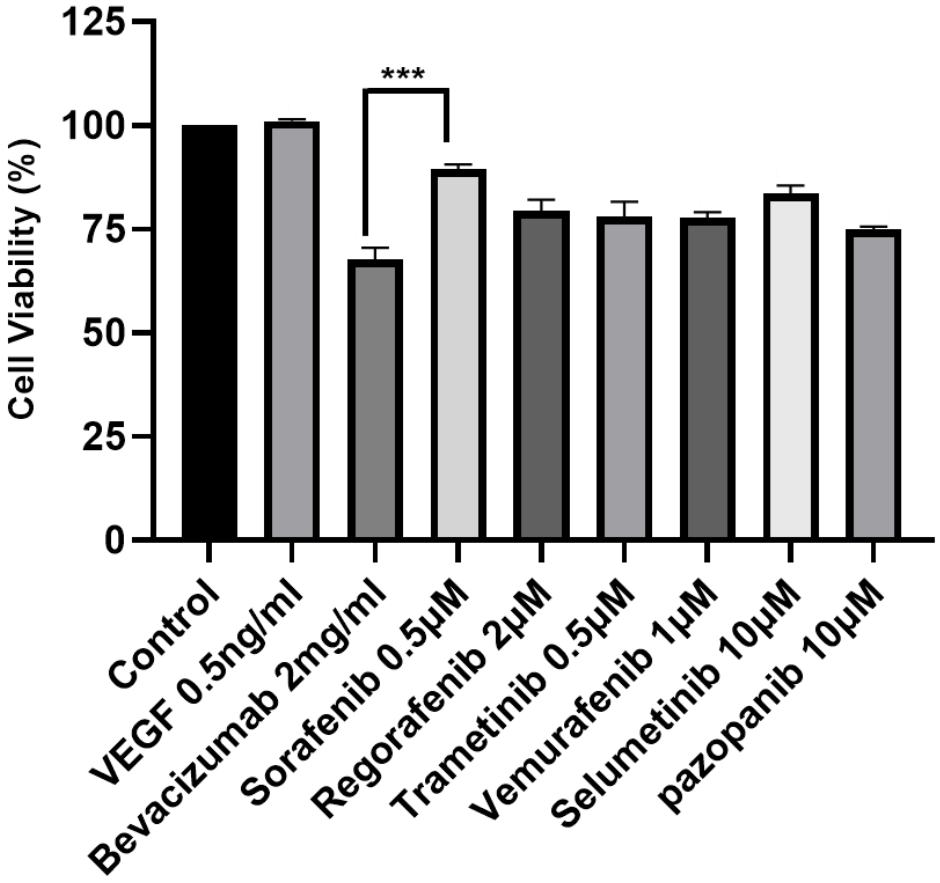


Figure 4. Effects of various VEGF inhibitors on RGC survival.

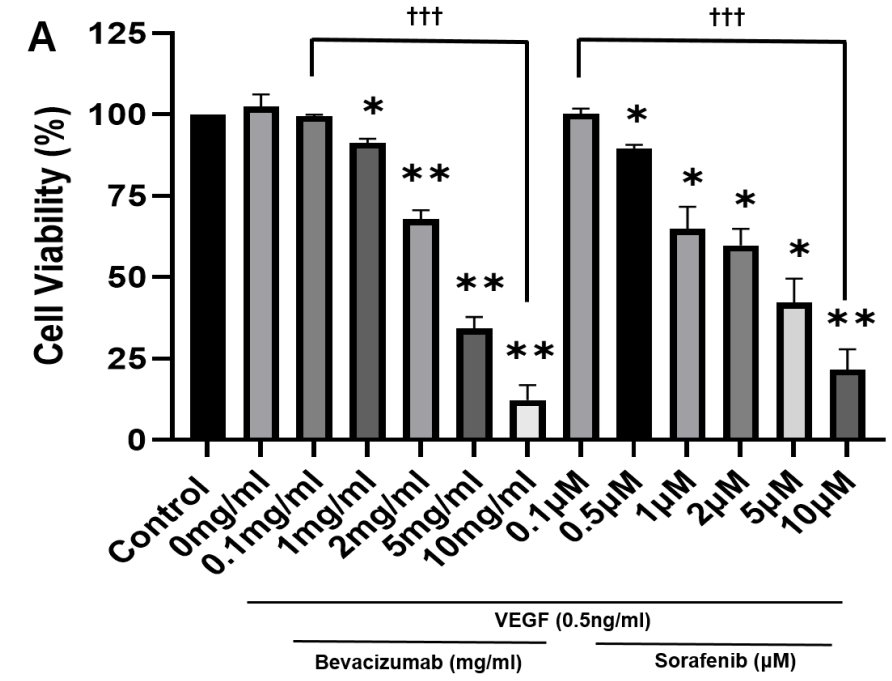
We evaluated the effectiveness of various VEGF inhibitors, namely sorafenib, regorafenib, trametinib, vemurafenib, selumetinib, and pazopanib, in promoting the survival of RGCs. To establish the optimal concentrations for each VEGF inhibitor, we conducted comparative analyses of their apoptotic effects on HUVECs, with 2 mg/mL bevacizumab used as a reference. Consequently, we determined the following concentrations for HUVEC experiments: 0.5 μM sorafenib, 2 μM regorafenib, 0.5 μM

trametinib, 1 μM vemurafenib, 10 μM selumetinib, and 10 μM pazopanib. In our subsequent RGC viability experiment, sorafenib exhibited the highest RGC survival rate among the tested VEGF inhibitors and significantly surpassed bevacizumab. (***$p < 0.001$).

4. Effects of sorafenib and bevacizumab on RGC survival

We comprehensively assessed the effects of different concentrations of sorafenib (0.1, 0.5, 1.0, 2.0, 5.0, and 10.0 μM) and bevacizumab (0.1, 1.0, 2.0, 5.0, and 10.0 mg/mL) on RGC survival using cell viability and LDH assays.

The viability of RGCs was significantly decreased following treatment with bevacizumab and sorafenib in a dose-dependent manner, starting at 1.0 mg/mL and 0.5 μM, respectively ($p < 0.001$; Figure 5A). Consistently, LDH assay results revealed that LDH release was notably increased following treatment with bevacizumab and sorafenib in a dose-dependent manner, starting at 1.0 mg/mL and 2.0 μM, respectively ($p < 0.001$; Figure 5B).



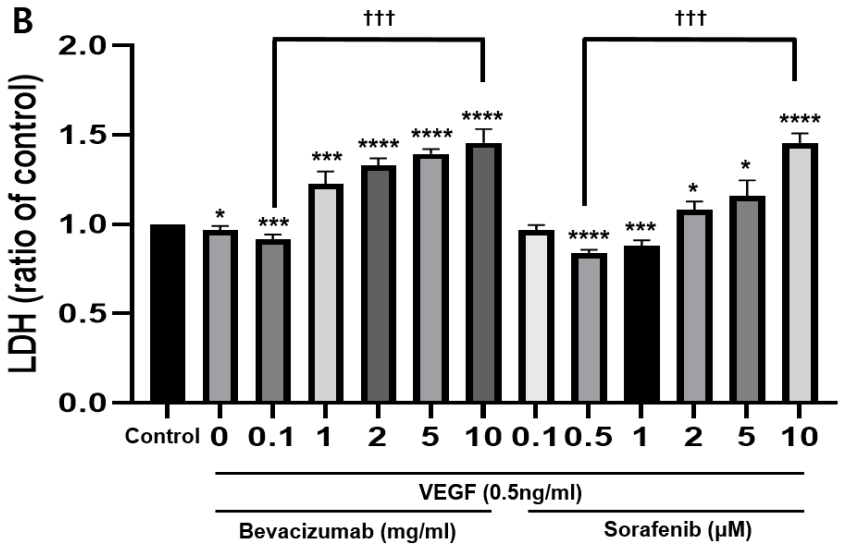


Figure 5. Effects of sorafenib and bevacizumab on RGC survival.

We comprehensively assessed the effects of different concentrations of sorafenib (0.1, 1.0, 2.0, 5.0, and 10.0 μM) and bevacizumab (0.1, 1.0, 2.0, 5.0, and 10.0 mg/mL) on RGC survival using cell viability and LDH assays. The viability of RGCs was significantly decreased following treatment with bevacizumab and sorafenib in a dose-dependent manner, starting at 1.0 mg/mL and 0.5 μM, respectively (Figure 5A). Concurrently, LDH assay results demonstrated that LDH release was dose-dependently increased following treatment with bevacizumab and sorafenib, starting at 1.0 mg/mL and 2.0 μM, respectively (Figure 5B). A linear mixed model was employed to analyze the dose effect of bevacizumab and sorafenib, revealing a statistically significant trend (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, †††$p < 0.001$, ****$p < 0.0001$).

5. HUVEC survival

To investigate the effects of VEGF inhibitors on vascular cells, we assessed the effects of various concentrations of sorafenib (0.1–5.0 μM) and bevacizumab (0.1–10.0 mg/mL) on the viability of HUVECs.

Cell viability assay results showed that the viability of HUVECs was significantly decreased by bevacizumab and sorafenib in a dose-dependent manner, starting at 2.0 mg/mL and 0.5 μM, respectively (Figure 6A).

To further validate these findings, we assessed live and dead cells through flow cytometry. The results of flow cytometry confirmed those of the cell viability assay, and 0.5 μM sorafenib exhibited a similar or even superior apoptotic effect to 2.0 mg/mL bevacizumab on HUVECs (Figure 6B). These results were also corroborated through immunostaining. Specifically, the cell count was lower after treatment with 0.5 μM sorafenib than with 2.0 mg/mL bevacizumab (Figure 6C).

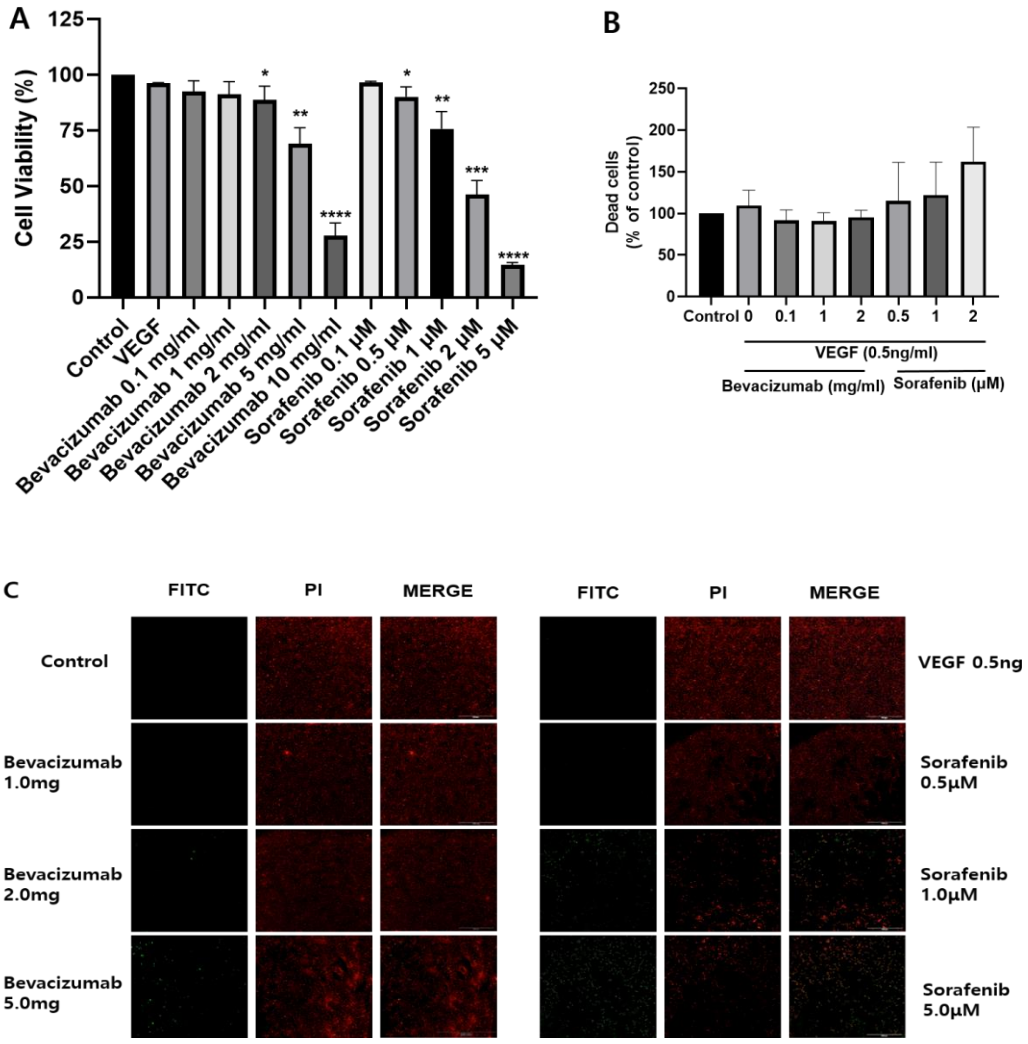


Figure 6. Human umbilical vein endothelial cell (HUVEC) survival.

In the cell viability assay, various concentrations of bevacizumab (0.1–10.0 mg/mL) and sorafenib (0.1–5.0 μM) were used. The viability of HUVECs was significantly decreased by bevacizumab and sorafenib in a dose-dependent manner, starting at 2.0 mg/mL and 0.5 μM, respectively (Figure 6A). Flow cytometry analysis demonstrated that 0.5 μM sorafenib exhibited a similar or even superior apoptotic effect to 2.0 mg/mL bevacizumab on

HUVECs (Figure 6B). Consistently, immunostaining indicated a lower cell count after treatment with 0.5 μM sorafenib than with 2.0 mg/mL bevacizumab (Figure 6C) (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$).

6. Effects of sorafenib and bevacizumab on RGC Akt

We investigated the effects of 2.0 mg/mL bevacizumab and 0.5 μM sorafenib on signaling molecules associated with pro-survival pathways, specifically Akt, in RGCs. Western blot analysis revealed that Akt levels significantly increased after VEGF treatment. However, Akt levels were markedly lower after treatment with 2.0 mg/mL bevacizumab than with 0.5 μM sorafenib, indicating the differential effects of these agents on Akt signaling (Figure 7A). To corroborate these findings, we used the siRNA method and observed consistent results (Figure 7B).

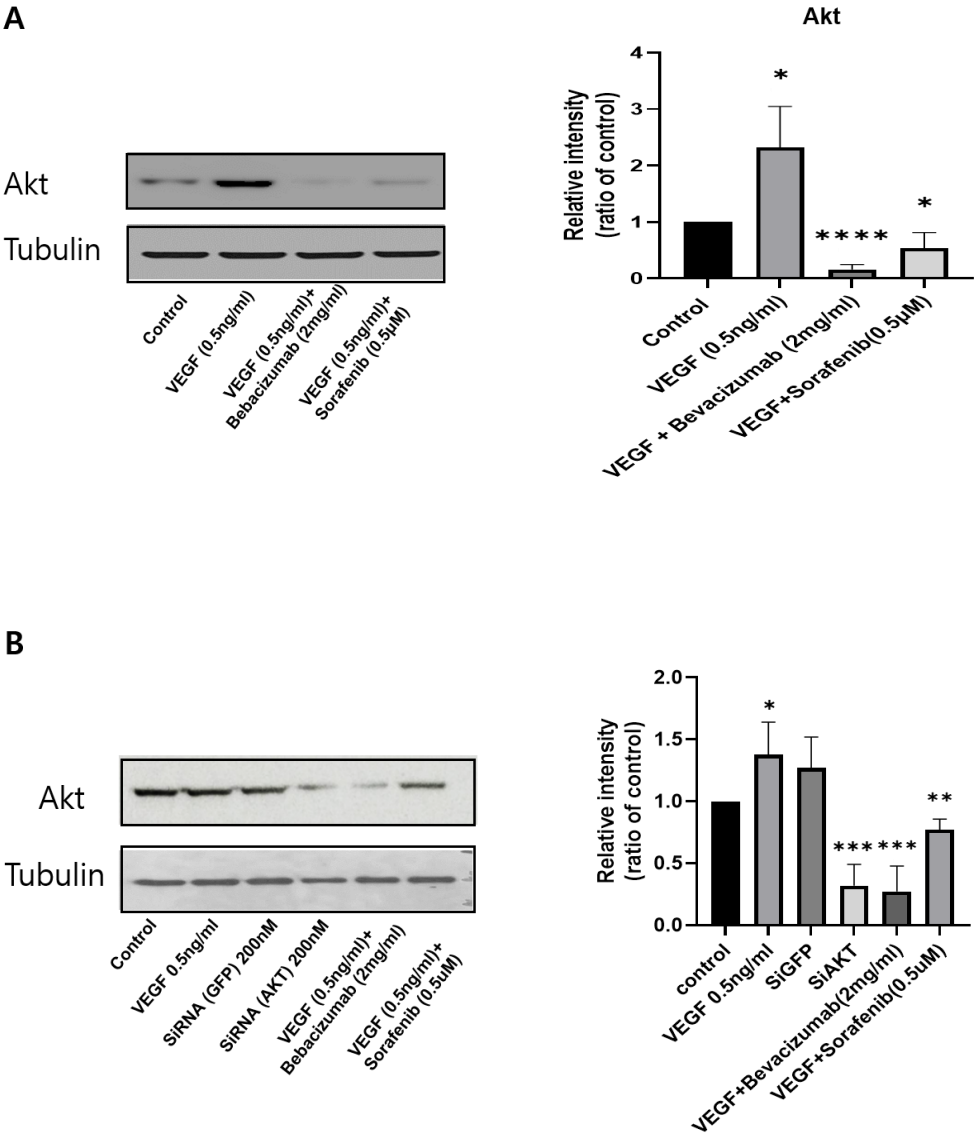


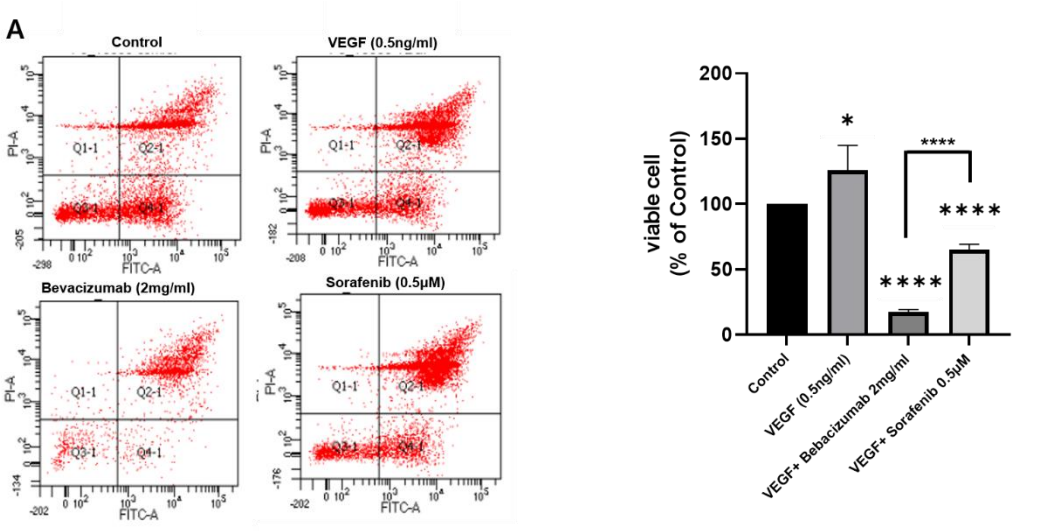
Figure 7. Effects of sorafenib and bevacizumab on RGC Akt.

We examined the effects of 2.0 mg/mL bevacizumab and 0.5 μM sorafenib on Akt signaling in RGCs. Akt was measured 24 h after bevacizumab treatment. Western blot analysis revealed that Akt levels significantly increased after VEGF treatment. Notably,

Akt levels were markedly lower after treatment with 2.0 mg/mL bevacizumab than with 0.5 μM sorafenib, indicating the differing impacts of the two VEGF inhibitors on Akt signaling (Figure 7A). To validate these findings, the siRNA method was used and yielded consistent results (Figure 7B) (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$).

7. Effects of treatment with 0.5 μM sorafenib and 2 mg/mL bevacizumab on RGC survival

The effects of 2.0 mg/mL bevacizumab and 0.5 μM sorafenib on the survival of RGCs were evaluated. Results of advanced flow cytometry showed that the survival rate of RGCs substantially increased after VEGF treatment. However, the survival rate of RGCs was significantly lower after treatment with 2.0 mg/mL bevacizumab than with 0.5 μM sorafenib, indicating the distinct effects of these agents on RGC viability (Figure 8A). The observed results were confirmed through a tunnel assay (Figure 8B).



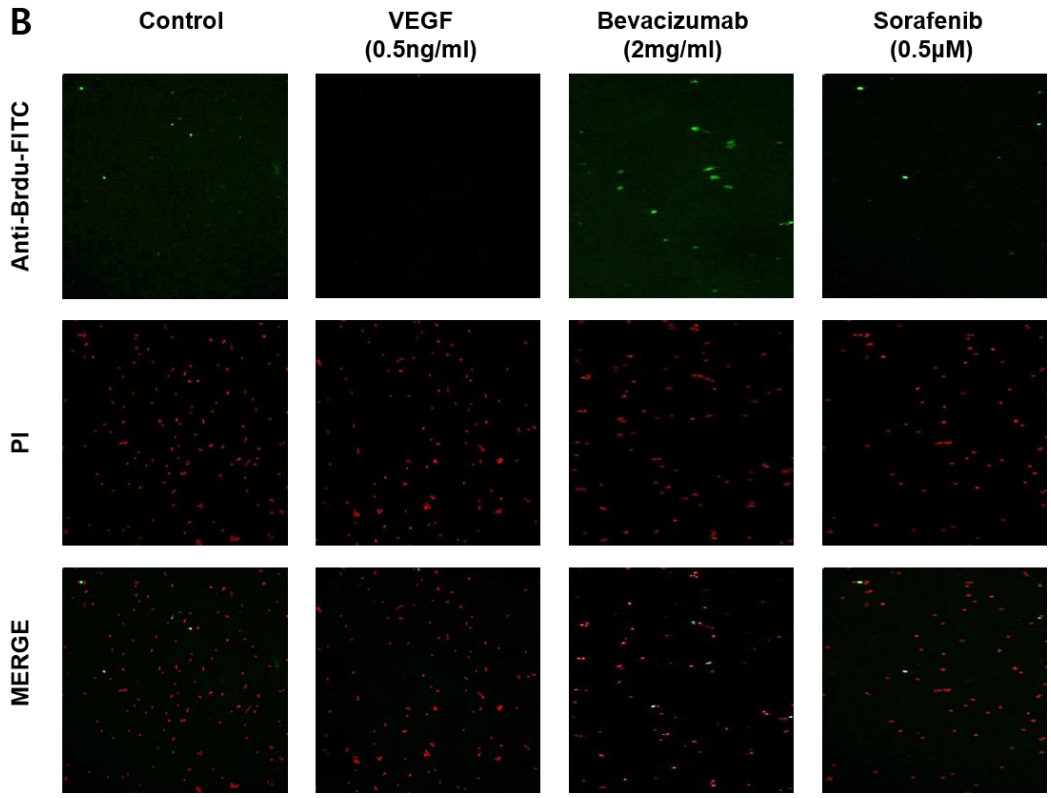


Figure 8. Effects of treatment with 0.5 μM sorafenib and 2 mg/mL bevacizumab on RGC survival.

We assessed the effects of 2.0 mg/mL bevacizumab and 0.5 μM sorafenib on RGC survival. Results of advanced flow cytometry showed that the survival rate of RGCs substantially increased after VEGF treatment. However, the survival rates of RGCs were significantly lower after treatment with 2.0 mg/mL bevacizumab than with 0.5 μM sorafenib, indicating the distinct effects of these agents on RGC viability (Figure 8A). These results were further validated using a tunnel assay (Figure 8B) (*$p < 0.05$, ****$p < 0.0001$).

8. RNA sequencing results

To unravel gene expression discrepancies and delineate the specific cellular pathways influenced by VEGF-inhibitor treatment, we conducted comprehensive RNA sequencing. After a 24-h stabilization period, isolated RGCs were subjected to four distinct conditions: control (RGCs only), VEGF treatment, 2.0 mg/mL bevacizumab treatment, and 0.5 μM sorafenib treatment. RNA sequencing was then performed. The results of the initial analysis showed that VEGF-inhibitor treatment modified the gene expression profiles of RGCs (Figure 9A and B).

We focused on genes that were upregulated by VEGF treatment but downregulated by VEGF-inhibitor treatment compared with the control group, or genes that were downregulated by VEGF treatment but upregulated by VEGF-inhibitor treatment compared with the control group (Figure 9C). This stringent selection process yielded 14 genes with a greater than two-fold change and an adjusted p-value of less than 0.05 (Table 2). This value was confirmed with RT-qPCR (Figure 9D).

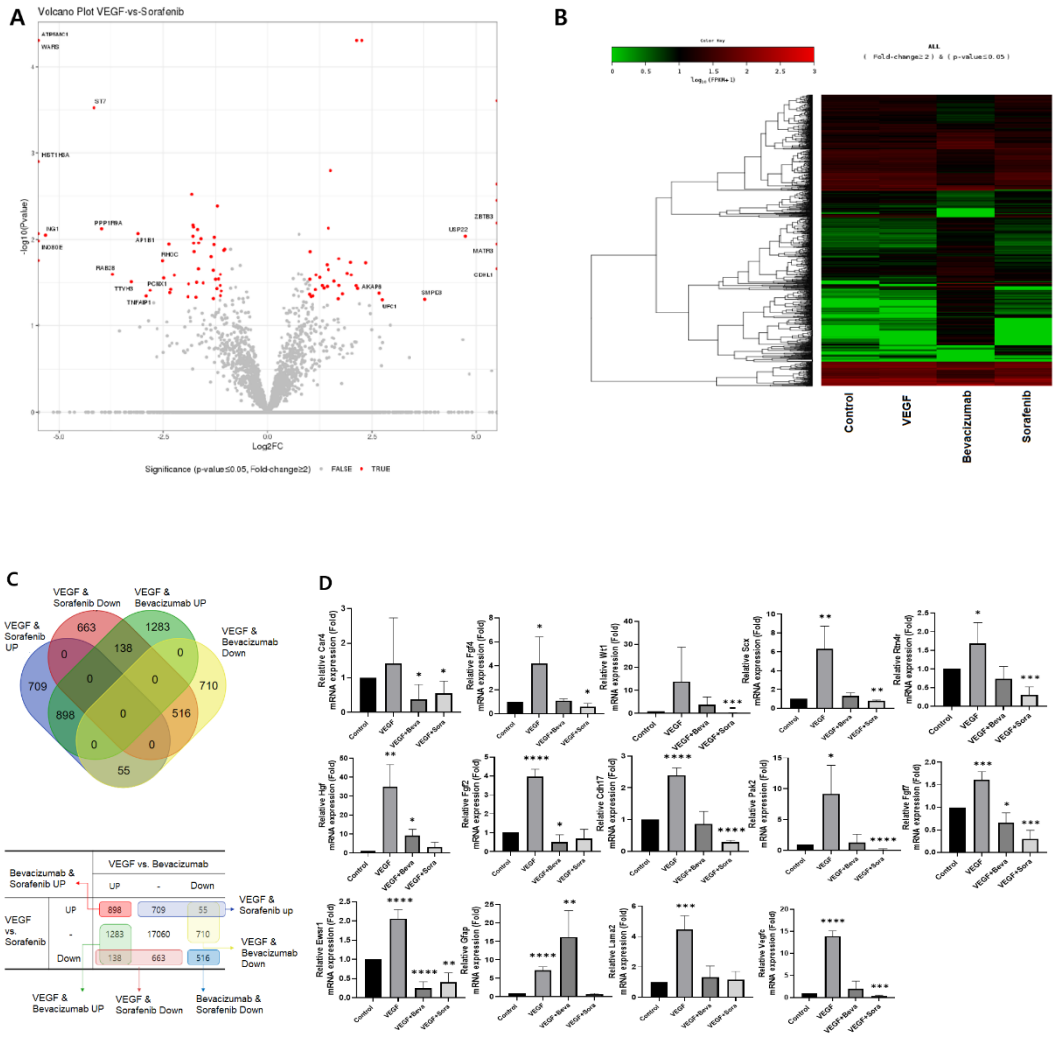


Figure 9. RNA sequencing results.

We conducted an RNA sequencing study after a 24-h stabilization period for isolated RGCs. The cells were subjected to four distinct conditions: control (RGCs only), VEGF treatment, 2.0 mg/mL bevacizumab treatment, and 0.5 μM sorafenib treatment. (A) Volcano plot showing the log2-fold change in gene expression after 0.5 μM sorafenib

treatment. Each dot represents a single gene. (B) Heat map of gene expression in RGCs. (C) Our analytical approach focused on genes that were upregulated after VEGF treatment but downregulated after VEGF-inhibitor treatment when compared with the control group or genes that were downregulated after VEGF treatment but upregulated after VEGF-inhibitor treatment when compared with the control group. (D) Fourteen genes displayed a greater than two-fold change with an adjusted p-value of less than 0.05. This value was confirmed with RT-qPCR. (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001).

Table 2. Genes that displayed a greater than two-fold change with an adjusted p-value of less than 0.05 in RNA sequencing

Gene symbol	Log2(FC)			Full descriptions of the gene
	VEGF	Bevacizumab	Sorafenib	
Gfap	7.314	4.170	3.937	Glial fibrillary acidic protein [Source:RGD Symbol;Acc:2679]
Vegfc	6.093	2.309	1.913	Vascular endothelial growth factor C [Source:RGD Symbol;Acc:619800]
Rtn4r	5.759	2.964	2.645	Reticulon 4 receptor [Source:RGD Symbol;Acc:620810]
Pak2	4.515	3.194	1.650	P21 (RAC1) activated kinase 2 [Source:RGD Symbol;Acc:61953]
Ewsr1	3.971	1.687	1.603	EWS RNA-binding protein 1 [Source:RGD Symbol;Acc:1307258]
Fgf4	3.196	0.000	0.000	Fibroblast growth factor 4 [Source:RGD Symbol;Acc:620127]
Wt1	3.101	1.228	1.153	WT1 transcription factor [Source:RGD Symbol;Acc:3974]
Fgf2	2.145	0.000	0.779	Fibroblast growth factor 2 [Source:RGD Symbol;Acc:2609]
Fgf7	1.501	1.375	0.772	Fibroblast growth factor 7 [Source:RGD Symbol;Acc:61805]
Scx	1.313	2.319	0.000	Scleraxis bHLH transcription factor [Source:RGD Symbol;Acc:1588254]
Car4	1.170	0.523	0.233	Carbonic anhydrase 4 [Source:RGD Symbol;Acc:2242]
Cdh17	1.141	2.436	0.525	Cadherin 17 [Source:RGD Symbol;Acc:619748]

Lama2	0.994	0.380	0.236	Laminin subunit alpha 2 [Source:RGD Symbol;Acc:1308889]
Hgf	0.947	2.313	0.390	Hepatocyte growth factor [Source:RGD Symbol;Acc:2794]

9. Pathway analysis

Functional analysis was conducted using the DAVID bioinformatics package to gain deeper insights into the biological relevance of the identified genes. GO enrichment analysis was employed to explore the associations between significantly expressed genes and their cellular compartments, biological processes, and molecular functions.

The top DEGs were then subjected to the STRING database to elucidate PPIs, employing a medium confidence threshold of 0.400. These interactions were subsequently visualized using Cytoscape software, wherein the genes were organized into functionally grouped networks.

In this network, the inner circles serve as indicators of the effects of the VEGF inhibitors on specific genes, with blue representing the effect of sorafenib, purple that of bevacizumab, and yellow that of both inhibitors. (Figure 10)

The outer circles denote common survival pathways, with orange highlighting the PI3-Akt signaling pathway, purple the Ras signaling pathway, red the MAPK signaling pathway, and green the JAK-STAT signaling pathway. Interestingly, our analysis revealed significant involvement of the PI3-Akt, Ras, and MAPK signaling pathways in the context of our study. However, genes associated with the JAK-STAT pathway showed no significant involvement in our study.

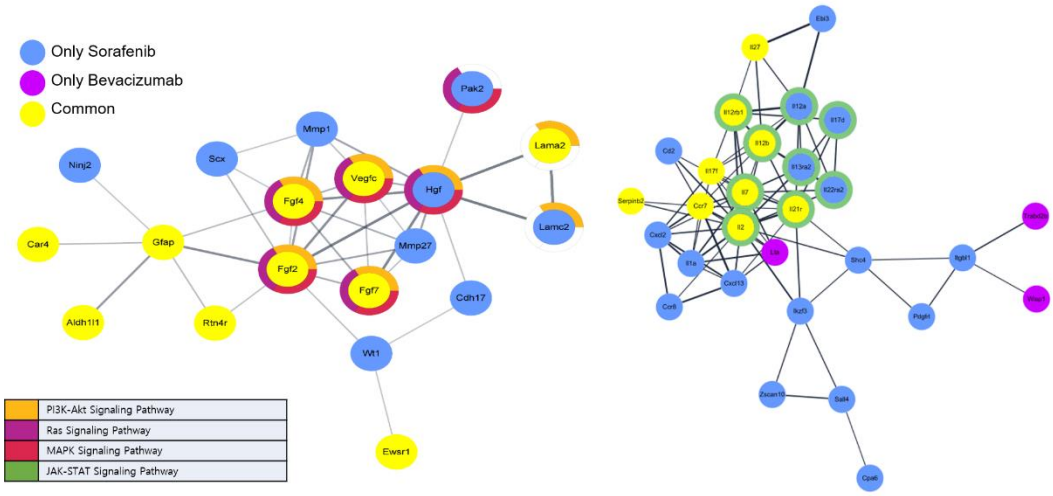


Figure 10. Pathway analysis

Inner circles indicate the effects of specific genes related to VEGF-inhibitor treatment, with blue representing the effect of sorafenib, purple that of bevacizumab, and yellow that of both sorafenib and bevacizumab. Outer circles represent well-established survival pathways, with orange highlighting the PI3-Akt signaling pathway, purple the Ras signaling pathway, red the MAPK signaling pathway, and green the JAK-STAT signaling pathway. Our analysis unveiled the significant involvement of the PI3-Akt, Ras, and MAPK signaling pathways in the context of our study. However, genes associated with the JAK-STAT pathway showed no significant involvement in our investigation.

IV. DISCUSSION

The present study investigated the effects of VEGF inhibitors, particularly bevacizumab and sorafenib, on the survival of RGCs and their mechanisms of action. First, our findings revealed that bevacizumab exhibited RGC toxicity, necessitating careful consideration of its use. Second, administration of bevacizumab to RGCs disrupted the PI3-Akt pathway. Third, compared with bevacizumab, sorafenib exerted a milder effect on the PI3-Akt pathway and less toxicity to RGCs while maintaining a similar apoptotic effect on vascular endothelial cells. These results suggest the possibility of an alternative approach that preserves the effects of VEGF inhibitors on the retina while protecting RGCs.

Bevacizumab, a monoclonal antibody specifically designed to target VEGF, has attracted significant attention as a potential treatment modality for retinal diseases characterized by abnormal blood vessel growth.[9,11] These conditions include age-related macular degeneration (AMD), diabetic retinopathy, and retinal vein occlusion, all of which are characterized by the pathological formation of blood vessels within the retina. One critical aspect of bevacizumab use in the context of retinal diseases is its potential effect on RGCs, which play a fundamental role in visual function. RGCs are responsible for transmitting visual information from the retina to the brain, making their health and survival pivotal for maintaining normal vision.[28,29] Several issues have been associated with the use of bevacizumab in relation to RGCs. For instance, the potential toxicity of bevacizumab to RGCs could jeopardize the health and proper functioning of RGCs, resulting in visual impairment or exacerbation of existing visual deficits.[30-33] This may be even more dangerous for patients with glaucoma.[34,35]

Glaucoma encompasses a group of eye diseases characterized by their detrimental effects on the optic nerve, specifically targeting the RGCs responsible for transmitting crucial visual information from the eye to the brain.[36] The genesis of this damage is often attributed to elevated IOP, which exerts deleterious pressure on these RGCs, leading to their degeneration and consequential harm to the optic nerve. This process is typically insidious

and progressive, initially manifesting as peripheral (side) vision impairment. If left untreated, it may culminate in complete blindness. Glaucoma is a chronic condition and a leading cause of blindness globally.[18,37] Regular eye examinations are indispensable, especially for individuals at risk or those with a family history of glaucoma, as they facilitate early detection and intervention. Timely management is crucial since it can effectively slow down or prevent vision loss resulting from damage to the RGCs and optic nerve. Glaucoma is a non-reversible condition, making the preservation of RGCs a paramount concern in the management of this disease.[37] Evidence suggests that injecting VEGF inhibitors, particularly bevacizumab, can contribute to RGC damage in patients with glaucoma.[31,33-35] The vulnerability of RGCs in glaucoma is well established since these cells play a pivotal role in transmitting visual information from the eye to the brain. Elevated IOP, a hallmark of glaucoma, places significant stress on RGCs, ultimately leading to their degeneration and consequent damage to the optic nerve. VEGF-inhibitor therapy, while effective in controlling abnormal blood vessel growth in various eye conditions, may inadvertently exert adverse effects on RGCs. The potential RGC damage due to anti-VEGF injections, including bevacizumab, underscores the need for careful consideration when selecting treatment options for patients with glaucoma. Balancing the benefits of VEGF-inhibitor therapy in managing glaucoma with its potential impact on RGCs remains a critical aspect of glaucoma care and requires ongoing research and clinical vigilance.

RGC damage associated with anti-VEGF injections, particularly bevacizumab, appears to stem from multiple factors. First, IOP increases after VEGF-inhibitor injections.[38,39] This elevated IOP can place additional stress on RGCs, exacerbating their vulnerability and contributing to damage. Second, bevacizumab exhibits RGC toxicity.[30,31] The specific mechanisms underlying this toxicity are an area of ongoing research, but the direct impact of the drug on RGCs can lead to their impairment and potential degeneration. Collectively, these factors highlight the complex relationship between VEGF-inhibitor therapy and RGC damage. Although VEGF-inhibitor injections have shown efficacy in managing various eye conditions, their potential adverse effects on RGCs underscore the importance of

careful patient monitoring and individualized treatment approaches to mitigate the risk of further harm to these critical retinal cells.

Numerous studies have investigated the molecules involved in promoting the survival of RGCs. Among these factors are nerve growth factor, brain-derived neurotrophic factor, ciliary neurotrophic factor, VEGF, and insulin-like growth factors.[23,40-42] VEGF is a glycoprotein with a molecular weight of 46 kDa that binds to receptors on the surface of vascular endothelial cells, stimulating their proliferation and increasing capillary permeability.[43,44] This factor promotes the development and maturation of neural tissues, including the retina.[4,45] During development, VEGF is expressed by various cell types in the retina, such as astrocytes in the RGC layer, inner nuclear layer cells, Müller cells, and retinal pigment epithelial cells.[4,46,47] Even in the mature retina, VEGF is expressed without active neovascularization and is implicated in the maintenance and function of adult retinal neuronal cells.[47] Moreover, VEGF exerts neuroprotective effects, particularly in safeguarding injured RGCs and slowing down their degeneration post-axotomy.[48] Our previous research confirmed that VEGF promotes the survival of RGCs under hypoxic conditions.[23] In this study, when VEGF activation was hindered with bevacizumab after 4 h of hypoxia, the RGC survival rate dose-dependently decreased.[23] Collectively, these findings emphasize the critical role of VEGF in supporting the survival of RGCs. An excessive reduction in VEGF levels due to bevacizumab treatment may result in unintended damage to RGCs. Hence, balancing the therapeutic benefits of VEGF modulation with its potential consequences for RGC health is a key consideration in the management of retinal conditions.

Intravitreal injections of VEGF inhibitors, notably bevacizumab, have been commonly used in clinical settings owing to their cost-effectiveness. However, our study, along with previous studies, raised concerns about the potential risks associated with repeated VEGF-inhibitor injections, particularly their interference with the neuroprotective actions of VEGF. While some studies have suggested the safety of bevacizumab treatment for RGCs, future studies should explore the potential side effects, including serious eye conditions

such as glaucoma, of multiple bevacizumab injections.

In light of these considerations, alternative treatments for ischemic retinal conditions, such as AMD, retinal vein occlusion, and proliferative diabetic retinopathy, must be developed. In the present study, the effects of various VEGF inhibitors were evaluated to identify potential alternatives that might offer better safety profiles compared with bevacizumab. Our findings suggest that sorafenib, a multi-kinase inhibitor, has the potential to replace bevacizumab. Indeed, sorafenib demonstrated effective VEGF-inhibitor activity in vascular endothelial cells while causing less damage to RGCs. These promising results suggest that sorafenib could serve as a safe and viable alternative to bevacizumab for the treatment of ischemic retinal conditions. Further research and clinical studies are warranted to validate these findings and determine the full scope of the efficacy and safety of sorafenib in the treatment of various retinal diseases. Such investigations will guide clinicians in making informed treatment decisions and providing better options for patients seeking optimal care for their eye conditions.

Sorafenib is a kinase inhibitor approved for the treatment of various conditions, including advanced renal cell carcinoma, hepatocellular carcinoma, certain types of acute myeloid leukemia, and advanced thyroid carcinoma that does not respond to radioactive iodine treatment.[49,50] This drug exerts its effects by targeting several protein kinases, including VEGF receptor, platelet-derived growth factor (PDGF) receptor, and rapidly accelerated fibrosarcoma (RAF) kinases.[49,50] Initially identified as an RAF kinase inhibitor, sorafenib's action extends to the inhibition of multiple receptor tyrosine kinases involved in angiogenesis, the process of new blood vessel formation.[49,50] Its anti-proliferative and antiangiogenic properties are derived from its ability to block the RAF/mitogen-activated protein (MAP)/extracellular signal-regulated kinase (ERK) kinase cascade and its impact on receptor tyrosine kinases, including VEGF receptor 2 (VEGFR2), VEGFR3, PDGF receptor, FLT3, Ret, and c-Kit.[49,50] Additionally, sorafenib interacts with hypoxia-inducible factors 1 and 2, influencing the expression of growth factors such as VEGF and PDGF.[49,50]

In the context of ocular health, a prior study examined the potential of sorafenib to

counteract the overexpression of VEGF, PDGF, and PlGF in human retinal pigment epithelial cells subjected to light-induced stress.[51] The authors presented the promising viability of sorafenib as an antiangiogenic treatment for AMD.[51] Moreover, various *in vitro* studies have explored the effects of sorafenib. Sorafenib administration to primary human optic nerve head astrocytes and primary human retinal pigment epithelial cells under white light exposure can significantly reduce the light-induced overexpression of VEGF.[52,53] In the rat oxygen-induced retinopathy model, sorafenib could inhibit retinal neovascularization in a dose-dependent manner.[54] These findings strongly suggest sorafenib as a potentially effective therapeutic approach for patients with retinal diseases, specifically AMD, aligning closely with the results of the current study. These findings offer hope for advancements in treatment options and improved outcomes in the management of this complex and challenging retinal condition.

Nonetheless, further in-depth research and rigorous clinical trials are imperative to thoroughly validate the effectiveness and safety of sorafenib for this specific application. Additional investigations are critical to translating these promising preliminary findings into established treatments that can offer substantial benefits to individuals with retinal diseases, such as AMD.

We studied several candidate signaling molecules using RNA sequencing to better understand the signaling pathways downstream of VEGF and conducted a comprehensive functional analysis using the DAVID bioinformatics package to investigate the biological significance of the identified genes. We also visualized the intricate web of gene interactions and relationships using Cytoscape software, a tool that facilitates the organization of genes into functionally grouped networks, offering a clear and structured representation of how these genes collaborate and contribute to specific biological processes. Our investigation yielded intriguing insights into the PI3-Akt, Ras, MAPK, and JAK-STAT signaling pathways. These pathways are key components of the intricate network of signaling cascades activated by VEGF, shedding light on their critical involvement in RGC survival. Notably, our analysis unveiled the significant involvement

of the PI3-Akt, Ras, and MAPK signaling pathways in the context of our study. However, genes associated with the JAK-STAT pathway showed no significant involvement in our investigation. Interestingly, several genes were shared among the PI3-Akt, Ras, and MAPK pathways, whereas the JAK-STAT pathway exhibited a distinct genetic profile. This observation suggests that the JAK-STAT pathway may exhibit a comparatively independent response mechanism. Future investigations should delve deeper into this phenomenon to understand its implications and mechanisms.

To the best of our knowledge, studies exploring the complex interplay between VEGF-inhibitor therapies and RGC survival are limited. Therefore, our findings offer novel insights into the molecular mechanisms underlying RGC survival and may guide the development of efficacious treatments for retinal diseases, potentially improving the outcomes of individuals with these conditions.

This study has a few limitations that merit consideration. First, our *in vitro* model focused exclusively on RGCs, whereas *in vivo*, RGCs exist in a complex milieu alongside various other cell types, including astrocytes, Müller cells, and glial cells. Thus, this controlled environment may not accurately replicate the *in vivo* interactions that affect RGC survival. Second, the conditions in neonatal rat RGCs may not completely align with those observed in adult human RGCs. Third, numerous factors beyond VEGF may contribute to cell survival. Although our study examined the correlations with VEGF, a comprehensive analysis of all potential contributing factors was not performed. Additionally, our study was conducted over a relatively short incubation period (48 h) because of the various constraints of the *in vitro* primary RNA culture system. This limited duration may not fully capture the long-term effects and complexities associated with RGC survival and treatment responses. Lastly, rat RGCs may differ from human RGCs, limiting the direct extrapolation of our findings to clinical contexts. Despite these inherent limitations, our study introduces novel clinical perspectives, suggesting that sorafenib holds promise as a safe treatment option for patients.

However, further experimental and clinical investigations are required to validate and

substantiate our *in vitro* findings in real-world clinical settings.

V. CONCLUSION

Our study indicated that sorafenib is a potentially more effective and safer treatment option than bevacizumab for various retinal diseases, uncovered new genes, and provided insights into the complex roles of multiple signaling pathways in this context. These findings will help facilitate the development of safe therapeutic approaches for managing retinal diseases associated with glaucoma. This study marks a significant advancement in the literature by improving the management and treatment outcomes for complex ocular conditions.

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ABSTRACT (IN KOREAN)

망막 질환 치료에서의 베바시주맙과 소라페닙의 망막신경절세포 생존에 대한 비교 분석

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목적: 항-혈관내피성장인자 제제인 베바시주맙이 망막신경절세포의 생존에 미치는 영향을 조사하고, 망막신경절세포 생존율을 증가시키기 위한 향상된 치료 전략을 탐색하고자 하였다.

방법: 신생아 쥐로부터 망막신경절세포를 분리하고, 베바시주맙을 기준으로 다양한 항-혈관내피성장인자 치료법들이 망막신경절세포의 생존율에 미치는 영향을 평가하였다. 항-혈관내피성장인자의 효과는 인간 탯줄 혈관내피세포를 사용하여 평가하였다. 혈관내피성장인자와 관련된 망막신경절세포의 생존 경로들을 확인하였으며, 항-혈관내피성장인자 치료에 영향을 받는 유전자를 식별하기 위해 RNA 시퀀싱을 수행하였다.

결과: 소라페닙과 베바시주맙 모두 탯줄 혈관내피세포에 강력한 항-혈관내피성장인자 효과를 나타내었다. 특히, 2mg/mL 베바시주맙은 탯줄 혈관내피세포에서 0.5μM 소라페닙과 유사한 항-혈관내피성장인자 효과를 나타내었다. 그러나 0.5μM 소라페닙은 2mg/mL 베바시주맙에 비해 더 높은

망막신경절세포 생존율을 보였다. Western blot 분석에서 베바시주맙 치료로 인한 Akt 수준 감소가 소라페닙에 비해 더 두드러졌음을 나타났으며, RNA 시퀀싱 결과 PI3K/AKT 경로, Ras 신호 경로 및 MAPK 신호 경로가 망막신경절세포 생존과 관련 있다는 것이 확인 되었다. 반면, JAK/STAT 경로는 주요 역할을 하는 것으로 보이지 않았다.

결론: 본 연구 결과는 소라페닙이 다양한 망막 질환에 대하여 베바시주맙에 비해 망막신경절세포에 더 안전한 치료 옵션을 제공할 수 있음을 시사하였다. 더불어, 망막신경절세포의 생존에 중요한 역할을 하는 신호 전달 경로를 밝혔고, 이와 관련된 유전자들을 제시하였다. 이러한 결과는 향후 망막신경절세포 손상에 대한 치료법과 보다 안전한 망막 질환 치료 방식을 개발하는 데 도움이 될 수 있을 것으로 기대된다.

핵심되는 말 : 혈관내피성장인자, 베바시주맙, 소라페닙, 망막신경절세포, 녹내장