Associations of keratin 8 levels in aqueous humor with treatment outcomes after intravitreal ranibizumab for neovascular age-related macular degeneration

Joo Youn Shin

Department of Medicine

The Graduate School, Yonsei University

Associations of keratin 8 levels in aqueous humor with treatment outcomes after intravitreal ranibizumab for neovascular age-related macular degeneration

Directed by Professor Suk Ho Byeon

The Doctoral Dissertation
submitted to the Department of Medicine,
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the degree
of Doctor of Philosophy in Medical Science

Joo Youn Shin

December 2021

This certifies that the Doctoral Dissertation of Joo Youn Shin is approved.

Thesis Supervisor : Suk Ho Byeon

Thesis Committee Member#1 : Hyung Keun Lee

Thesis Committee Member#2 : Hyewon Chung

Thesis Committee Member#3: Changsoo Kim

Thesis Committee Member#4: Jong Eun Lee

The Graduate School
Yonsei University

December 2021

ACKNOWLEDGEMENTS

First of all, I appreciate my supervisor, Prof. Suk Ho Byeon, for his great advice and guidance, which have been invaluable in the course of the degree study.

I also appreciate the contribution of the members of the thesis committee members, Prof. Hyung Keun Lee, Prof. Hyewon Chung, Prof. Changsoo Kim, and Prof. Jong Eun Lee, who have offered expert and personal support. I also thank all members of the Department of Ophthalmology, Yonsei University School of Medicine, for their support.

Finally, I would like to thank my parents for their support and, specially, my husband, Young-wook, who always believes in me and supports me. My kids, Seojin and Juwon, I love you with all my heart.

<TABLE OF CONTENTS>

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Associations of keratin 8 levels in aqueous humor with treatment outcomes after intravitreal ranibizumab for neovascular age-related macular degeneration

Joo Youn Shin

Department of Medicine
The Graduate School, Yonsei University

(Directed by Professor Suk Ho Byeon)

Anti-vascular endothelial growth factor agents have recently shown remarkable improvement in neovascular age-related macular degeneration (nAMD) treatment. However, some patients still show poor responses to treatment; hence, novel prognostic markers and therapeutic targets are needed. This study investigated changes in the levels of keratin 8 (KRT8), a marker protein of retinal pigment epithelium (RPE), in the aqueous humor (AH) of patients with nAMD treated with intravitreal ranibizumab (IVR) injections, and their associations with clinical parameters, including treatment outcomes after IVR injection.

This study included 58 patients with nAMD treated with three monthly IVR injections and 46 control subjects. Samples of AH were collected at baseline and 2 months after initial treatment in nAMD group, and were obtained immediately before cataract surgery in control group. The levels of KRT8 in the AH were quantitatively assessed using a sandwich enzyme-linked immunosorbent assay kit.

Treatment outcomes, both visual and anatomical, were evaluated 3 months after initial injection by measurement of best corrected visual acuity and optical coherence tomography (OCT). Poor anatomical outcome (a poor responder) was defined as the presence of persistent fluid on OCT at month 3.

Baseline KRT8 levels were significantly higher in nAMD-treated eyes than control eyes. In AMD group, a significant decrease in KRT8 levels was observed between baseline and 2 months after IVR injections. When patients were classified according to treatment responses, responders demonstrated a significant decrease in KRT8 levels between baseline and month 2, whereas poor responders showed no significant change. In addition, higher KRT8 levels at month 2 were significantly associated with persistent fluid on OCT at month 3. These findings suggest that monitoring aqueous KRT8 may aid early determination of the therapeutic effects of IVR in nAMD patients and reflect the health condition of RPE during course of the disease.

Key words : age-related macular degeneration, anti-vascular endothelial growth factor, aqueous humor, keratin 8, retinal pigment epithelium

Associations of keratin 8 levels in aqueous humor with treatment outcomes after intravitreal ranibizumab for neovascular age-related macular degeneration

Joo Youn Shin

Department of Medicine
The Graduate School, Yonsei University

(Directed by Professor Suk Ho Byeon)

I. INTRODUCTION

Age-related macular degeneration (AMD) is the leading cause of blindness among elderly individuals in developed countries.[1,2] AMD is classified into dry and neovascular (wet) types, and neovascular AMD (nAMD) accounts for 90% of AMD-related vision loss. Neovascular AMD is characterized by pathologic choroidal neovascularization (CNV) that breaks through Bruch's membrane into sub-retinal pigment epithelium (RPE) space and/or the subretinal space, leading to retinal edema, hemorrhage, exudation, and fibrous scarring, which can cause serious visual impairment.[3] Although anti-vascular endothelial growth factor (VEGF) agents have recently shown dramatic improvement in nAMD treatment, the response to these drugs varies among individuals, and some patients still have poor or no response to treatment.[4]

The reasons for poor responsiveness to anti-VEGF agents are complex and varied.[5] However, since RPE cells are heavily implicated in the pathogenesis of AMD[6], the functional status of RPE in individual AMD patients may be one of the major contributors in AMD development and progression as well as to treatment outcomes. Various studies have attempted

to correlate morphologic changes in the retina, RPE, and choroid upon optical coherence tomography (OCT) with the clinical aspects and prognosis of AMD.[7,8] However, even with OCT, the morphological study of RPE is difficult, and molecular and proteomic studies of RPE *in vivo* have been limited.

Proteomic research analyses the nature of peptides or proteins in various biological samples of multifactorial diseases. It may help to access the biology of cells and tissues involved in diseases and, thus, find new biomarkers and target-based therapy. Recent investigations have demonstrated particular proteomic signatures in nAMD patients. These studies collected and profiled the aqueous humor (AH) of nAMD patients and controls, identified several differentially expressed proteins in nAMD AH, and selected potential biomarker candidates besides VEGF.[9-13] A Previous study identified RPE-secreted proteins in the AH of nAMD patients and showed that, among them, the expression of epithelial marker protein keratin 8 (KRT8) increased ~2-fold in nAMD patients compared with that in control subjects, and it varied after anti-VEGF treatments.[9]

KRT8, which is predominantly expressed in epithelium, is known to support the mechanical integrity of cells, modulate response to stress stimuli, and contribute to cell resistance to apoptosis.[14-16] KRT8 expression levels differ in various tumors: upregulated in the head and neck[17] and in oral cavity carcinoma[18], but downregulated in breast[19] and colorectal carcinomas.[20] Several studies have suggested that KRT8 levels are associated with the prognosis[18,21] and drug resistance of these tumors.[22,23] In the retina, KRT8 is a well-known epithelial marker of RPE[24,25]; it has been reported to be a major cytokeratin in RPE cells isolated from the human eyeball, and its level of expression increases in proliferating RPE cells with good maintenance of cuboidal morphology.[25] These findings suggest that RPE proliferation, as manifested by increased KRT8 expression in eyes under pathologic conditions,

might be a proper RPE wound healing response. Additionally, a previous study demonstrated increased KRT8 expression in oxidatively stressed RPE cells, along with autophagy, to protect RPE cells from cell death.[26]

However, the clinical implications of upregulated KRT8 levels in nAMD patients and their changes during treatments were not elucidated in the previous study.[9] Thus, the aim of this prospective study was to investigate the associations of visual and anatomical treatment outcomes with changes in the levels of KRT8 in the AH of treatment-naïve nAMD patients treated with intravitreal ranibizumab (IVR).

II. MATERIALS AND METHODS

1. Study design and participants

The present prospective study was performed at Severance Hospital and Gangnam Severance Hospital of Yonsei University and Isan Paik Hospital of Inje University, between April 2016 and April 2018 (ClinicalTrial.gov trial number NCT02707575). This study was performed in accordance with the tenets of the Declaration of Helsinki and approved by the ethics committee of each institution (Severance Hospital: 2018-0061-002, Gangnam Severance Hospital: 2015-0611-015, and Ilsan Paik Hospital: 2016-04-010). All study participants provided written informed consent.

Each enrolled nAMD patient was required to be least 50 years of age with newly diagnosed (treatment-naïve) nAMD, with a recent onset of disease confirmed by history and clinical findings. Excluded eyes exhibited one of the following features: myopia with a refractive error > ±3.0 dioptres or evidence of pathologic myopia (preoperative refractive data were used to assess pseudophakic eyes); any history of vitrectomy, anti-VEGF therapy, laser treatment, or photodynamic therapy; a history of cataract surgery within 3 months prior to presentation; evidence of end-stage AMD such as subfoveal fibrosis or atrophy; eyes with large submacular hemorrhage (SMH) over 1

disc-diameter; evidence of other retinal diseases, including central serous chorioretinopathy, diabetic retinopathy, hypertensive retinopathy, and other neovascular maculopathies; glaucoma; poor imaging data caused by media opacity; or unstable fixation. Patients with uncontrolled systemic diseases, use of immunosuppressive drugs, or malignant tumours in any location, were also excluded. The control group consisted of patients who underwent cataract surgery during the same period. Through preoperative evaluation, eyes with ophthalmic diseases other than cataracts, or eyes that met the exclusion criteria were excluded from the control group.

2. Baseline evaluation, treatment, and aqueous humor sampling

At baseline, each patient in the nAMD group underwent a comprehensive ophthalmological examination assessing best-corrected visual acuity (BCVA) and intraocular pressure (IOP), autorefractometry/keratometry (ARK), slit lamp biomicroscopy, indirect ophthalmoscopy, colour fundus photography (FP), fluorescein angiography (FA), indocyanine green angiography (ICGA) (Optos® P200Tx, *Optos* PLC, Dunfermline, UK), and OCT (Swept Source OCT DRI OCT Triton, Topcon, Tokyo, Japan). After baseline evaluation, three consecutive monthly injections of 0.5 mg IVR (Lucentis; Novartis, Basel, Switzerland) were administered to nAMD patients. At every visit for injection, and one month after the third injection (month 3), ophthalmic examination, including BCVA, IOP, slit lamp biomicroscopy, FP, and OCT, were performed to monitor treatment outcome.

AH samples were taken at baseline and 2 months after the initial treatments (month 2). Preoperatively, each eye was anesthetized topically with 0.5% proparacaine hydrochloride. Patients received standard disinfection with povidone–iodine scrub of the eyelids and surrounding skin and povidone–iodine eye drops to the conjunctival sac. After inserting a sterile lid speculum, a 30-gauge needle was inserted bevel up through the peripheral

cornea and 0.1 ml of AH was collected. Consecutively, a dose of 0.5 mg IVR was administered through the pars plana. Antibiotic eyedrops (0.5% moxifloxacin hydrochloride) were given postoperatively for 3 days.

The control subjects also underwent a comprehensive ophthalmological examination, including BCVA, IOP, ARK, slit lamp biomicroscopy, indirect ophthalmoscopy, FP, and OCT preoperatively. AH samples of the control group were obtained immediately before cataract surgery.

3. Measurement of KRT8 levels in AH

Immediately after collection, AH samples were transferred to sterile plastic tubes (safe-lock microcentrifuge tubes, 1.5 ml) and immediately frozen and stored at -80 ℃ until analysis.

The levels of KRT8 in the AH were quantitatively assessed using a sandwich enzyme-linked immunosorbent assay (ELISA) kit (Cloud-Clone Corporation, Houston, TX, USA). The assays were performed according to the manufacturers' protocols. Samples were added to 96-well microplates and the plates were incubated for 2.5 h at room temperature (RT), followed by gentle shaking for 2 h at 37 ℃. Biotinylated antibodies were incubated for 1 h at RT with gentle shaking at 37 °C. Horseradish peroxidase-streptavidin solution was incubated for 45 min at RT, followed by gentle shaking for 30 min at 37 ℃. Tetramethylbenzidine dihydrochloride substrates were added to each well for 30 min in the dark. The enzyme-substrate reaction was terminated by the addition of a sulphuric acid solution, and the colour change was measured at a wavelength of 450 nm. The concentration of KRT8 in the samples was then determined by comparing the optical density of the samples to the standard curve.

4. Imaging and data analysis

Neovascular AMD was diagnosed based on the results of FP, FA, ICGA, and OCT, with evidence of hyperfluorescence and late leakage associated with detachment of pigmented epithelium, serous retinal detachment, subretinal exudation, and SMH. CNV types were subdivided into four categories as follows: a) Polypoidal choroidal vasculopathy (PCV) was diagnosed based on a finding of ICGA with the presence of a branched vascular network and on evidence of terminal polypoidal lesions in the sub-pigment epithelial layer with orange-red protrusions corresponding to the polypoidal lesions revealed by ICGA, or both. b) Type 1 CNV was characterized by new vessels located beneath the RPE. c) Type 2 CNV was defined as new vessels penetrating the RPE layer and localized in the subretinal space as observed upon OCT. d) Type 3 CNV, retinal angiomatous proliferation, was defined as the intraretinal proliferation of new vessels, which may originate from both retinal and choroidal circulation. The presence of retinal-choroidal anastomosis was identified by ICGA and/or intraretinal hemorrhages on FP and/or intraretinal fluid (IRF) upon OCT. The size of the CNV area was calculated by defining the boundaries of the CNV on FA. The central macular thickness (CMT) was automatically calculated on OCT as the average retinal thickness within a circle with a 1000 μm diameter centered on the fovea (the center circle of the Early Treatment Diabetic Retinopathy Study grid). All images were reviewed prior to measurement, and re-segmentation or re-centration of the fovea was undertaken if there were significant errors. The choroidal thickness was measured under the foveal center vertically from the outer border of the hyper-reflective line of the RPE to the inner border of the sclera. Morphologic features upon FP or OCT were also evaluated. These parameters included the presence of IRF, subretinal fluid (SRF), SMH, drusen, or hard exudate. This study defined the presence of drusen as one or more large (>125 μm) druse or extensive (20 soft or 65 hard without any soft) intermediate-sized drusen (63–124 μm), assessed within 2 disc-diameters of the center of the macula.

Treatment outcomes, both visual and anatomical, were evaluated 3 months after initial injection (month 3) by measurement of BCVA and OCT. The visual outcome was the BCVA at month 3 using the Snellen visual acuity chart, which was converted to logarithm of the minimum angle of resolution (logMAR) units. Poor anatomical outcome was defined as the presence of persistent fluid (IRF or SRF) upon OCT at month 3. The treatment outcome after 6 months was evaluated by reviewing BCVA and OCT retrospectively after the prospective study was completed. All measurements and diagnosis were conducted by two retinal specialists (J.Y.S. and J.L), and averaged values were used for evaluation.

5. Statistical analysis

To compare the baseline characteristics and KRT8 levels between the nAMD and control groups, the independent *t*-test was used for continuous variables and the Chi-squared test for categorical variables. To evaluate the relationship between baseline KRT8 levels and baseline characteristics, Pearson correlation was used for continuous variables, while the independent *t*-test was used for comparisons between baseline KRT8 levels and categorical variables. To compare KRT8 levels before and after IVR, the paired *t*-test was used, and the independent *t*-test was used for comparisons of KRT8 levels between responders and poor responders. To investigate the association between KRT8 level and visual outcome, linear regression analysis was performed, and logistic regression analysis was used for associations between KRT8 levels and anatomical outcome. In multivariate regression analysis, treatment outcome was the dependent variable, and clinically significant parameters significantly associated with treatment outcome in the univariate analysis were used as independent variables. Statistical analyses were performed using SPSS for Windows (version 21.0; IBM Corp., Armonk, NY, USA). A *p*-value of 0.05 was considered statistically significant.

III. RESULTS

1. Demographics and baseline characteristics

This prospective case-control study evaluated 58 eyes of 58 patients with treatment-naïve nAMD and 46 eyes of control patients. The characteristics of nAMD patients and those in the control group are summarized in Table 1. There was no significant difference in age ($p = 0.19$), sex ($p = 0.23$), or presence of systemic hypertension (HTN) ($p = 0.80$) or diabetes mellitus (DM) ($p = 0.30$) between the nAMD and control groups. Baseline visual acuity was better in the control group than in the nAMD group ($p < 0.001$).

Table 1. Baseline characteristics and baseline KRT8 levels in the nAMD and control groups

	nAMD	Control	p-value
n	58	46	NA
Age (years)	75.7 ± 9.5	73.5 ± 7.6	0.19
Sex (male; n, %)	39 (73.6)	25 (54.3)	0.23
HTN (n, %)	11 (18.9)	7 (15.2)	0.80
DM (n, %)	6 (10.3)	2 (4.3)	0.30
Visual acuity (logMAR)	0.65 ± 0.41	0.11 ± 0.16	<0.001*
(Snellen equivalents)	20/89	20/26	
Baseline KRT8 (ng/ml)	8.48 ± 1.21	4.99 ± 0.82	<0.001*

KRT8, keratin 8; nAMD, neovascular age-related macular degeneration; HTN, systemic hypertension; DM, diabetes mellitus; logMAR, logarithm of the minimum angle of resolution; NA, not applicable.
*$p < 0.05$

In the nAMD group, baseline visual acuity was 0.65 ± 0.41 logMAR, and the size of the CNV area was 8.75 ± 9.46 mm^2. Eighteen eyes (31%) were phakic and 40 eyes (69%) were pseudophakic. In terms of CNV type, 29 eyes (50%) had PCV, 9 (15.5%) had type 1 CNV, 9 had type 2 CNV, and 11 (19.0%) had type 3 CNV. In terms of morphologic features at baseline, 46 eyes (79.3%) had SRF, 24 (41.4%) had IRF, 17 (29.3%) had SMH, 17 (29.3%) had drusen, and 9 (15.5%) had hard exudate.

2. Baseline KRT8 levels in the nAMD and control groups

At baseline, the mean KRT8 levels in AH were 8.48 ± 1.21 ng/ml in the nAMD group, which was significantly higher than that in the control group (4.99 ± 0.82 ng/ml, $p < 0.001$) (Table 1).

In the control group, the baseline KRT8 levels were not correlated with age ($p = 0.83$), and there was no difference in KRT8 levels in terms of sex ($p = 0.22$), presence of HTN ($p = 0.27$) or DM ($p = 0.54$). In the nAMD group, the baseline KRT8 levels were not correlated with age ($p = 0.10$), and there was no difference in KRT8 levels in terms of sex ($p = 0.74$), the presence of HTN ($p = 0.14$) or DM ($p = 0.54$), or lens status ($p = 0.59$). In addition, the baseline KRT8 levels showed no correlation with CMT ($p = 0.89$), choroidal thickness ($p = 0.73$), or CNV size ($p = 0.92$). There was no significant difference in baseline KRT8 levels in terms of the type of CNV ($p = 0.25$) or other morphologic characteristics upon OCT, including SRF ($p = 0.33$), IRF ($p = 0.80$), SMH ($p = 0.20$), drusen ($p = 0.50$), and exudate ($p = 0.49$) (Table 2).

Table 2. Relationship between baseline KRT8 levels and baseline characteristics of the nAMD and control groups

Variables		nAMD	p-value	Control	p-value
Age (year)	r	0.223	0.10	-0.033	0.83
Sex	Male	8.52 ± 1.27	0.74	4.83 ± 0.53	0.22
	Female	8.40 ± 1.10		5.14 ± 1.07	
HTN	Yes	7.99 ± 0.89	0.14	5.61 ± 1.61	0.27
	No	8.59 ± 1.26		4.86 ± 0.56	
DM	Yes	8.19 ± 0.97	0.54	5.33 ± 0.06	0.54
	No	8.51 ± 1.24		4.96 ± 0.84	
Lens status	Phakic	8.61 ± 1.47	0.59	NA	NA
	Pseudophakic	8.42 ± 1.09			
CMT (μm)	r	0.019	0.89	-0.007	0.96
CT (μm)	r	-0.047	0.73	-0.008	0.60
CNV size (mm^2)	r	-0.012	0.92	NA	NA
CNV type					
PCV		8.56 ± 1.15	0.25	NA	NA
Type 1		8.92 ± 1.95			
Type 2		7.80 ± 0.64			
Type 3		8.46 ± 0.85			
Morphologic characteristics upon OCT					
SRF	Yes	8.56 ± 1.30	0.33	NA	NA
	No	8.17 ± 0.72			
IRF	Yes	8.43 ± 0.82	0.80	NA	NA
	No	8.51 ± 1.44			
SMH	Yes	8.16 ± 0.99	0.20	NA	NA
	No	8.61 ± 1.28			
Drusen	Yes	8.31 ± 0.78	0.50	NA	NA
	No	8.55 ± 1.35			
Exudate	Yes	8.22 ± 0.98	0.49	NA	NA
	No	8.53 ± 1.25			

KRT8, keratin 8; nAMD, neovascular age-related macular degeneration; r, correlation coefficient; HTN, systemic hypertension; DM, diabetes mellitus; CMT, central macular thickness; CT, choroidal thickness; CNV, choroidal neovascularization; PCV, polypoidal choroidal vasculopathy; OCT, optical coherence tomography; SRF, subretinal fluid; IRF, intraretinal fluid; SMH, submacular hemorrhage; NA, not applicable.

3. Changes in KRT8 levels between baseline and 2 months after the initial intravitreal ranibizumab injections in the nAMD group

A significant decrease in KRT8 levels was observed between baseline and month 2 ($p = 0.017$) (Fig. 1).

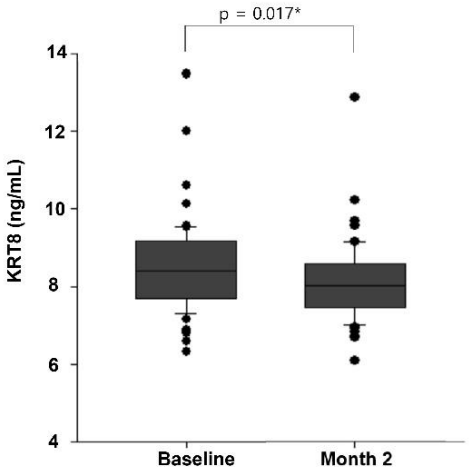

Figure 1. Changes in keratin 8 (KRT8) levels in eyes treated with intravitreal ranibizumab for neovascular age-related macular degeneration. KRT8 levels were significantly decreased between baseline and month 2. Box indicates median and inter-quartile range.

To investigate the relationship between the changes in KRT8 levels and treatment response, the KRT8 levels over time after IVR were compared by classifying the nAMD group into responders (dry) and poor responders (persistent fluid). In comparing KRT8 levels between responders and poor responders, the difference in baseline KRT8 levels failed to reach statistical significance between the two groups ($p = 0.053$). However, poor responders showed significantly higher KRT8 levels than responders at month 2 ($p < 0.001$). In addition, responders showed a significant decrease in KRT8 levels between baseline and month 2 ($p = 0.002$), whereas poor responders showed

no significant change in KRT8 levels (p = 0.73) (Figs. 2 and 3). Changes in KRT8 levels between baseline and month 2 showed a significant difference between responders and poor responders (-0.56 and -0.10, respectively; p = 0.045).

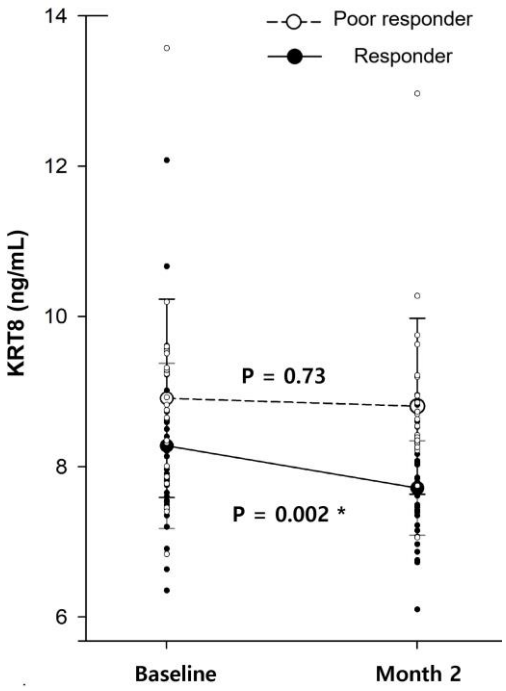

Figure 2. Changes in keratin 8 (KRT8) levels in responders and poor responders to intravitreal ranibizumab (IVR) treatment for neovascular age-related macular degeneration. Responders to IVR showed a significant decrease in KRT8 levels between baseline and month 2, whereas poor responders showed no significant change in KRT8 levels.

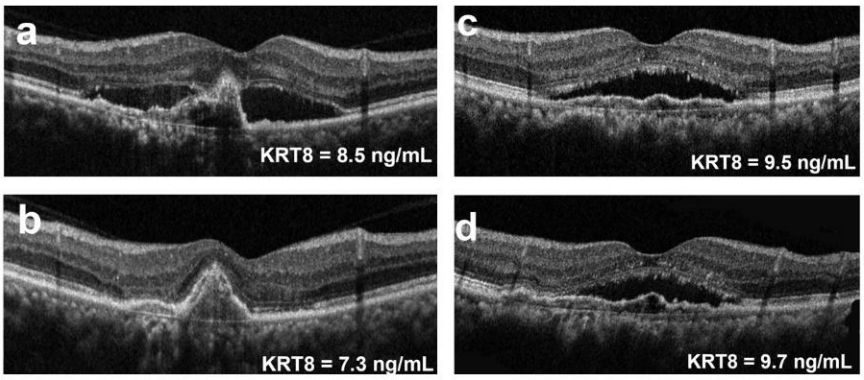

Figure 3. Treatment outcomes and keratin 8 (KRT8) levels after intravitreal ranibizumab injection for neovascular age-related macular degeneration. In a responder, optical coherence tomography (OCT) showed subretinal fluid (SRF) before intravitreal ranibizumab (IVR) (a), whereas SRF was resolved after IVR (b). Keratin 8 (KRT8) level decreased from 8.5 ng/mL to 7.3 ng/mL after treatment. In a poor responder, baseline OCT showed SRF (c), and KRT8 level was 9.5 ng/mL. Persistent fluid was observed on OCT after the treatment (d), and KRT8 level was 9.7 ng/mL, which was slightly higher than the baseline measurement.

4. KRT8 levels and treatment outcome after intravitreal ranibizumab injection

After three monthly injections of IVR (month 3), visual acuity improved from 0.65 ± 0.41 logMAR to 0.47 ± 0.38 ($p = 0.002$). Upon OCT, 33 eyes (56.9%) were dry, while 25 (43.1%) showed persistent fluid at month 3. CMT was significantly improved from 455.9 ± 252.3 μm to 263.9 ± 110.1 μm ($p < 0.001$).

The relationship between treatment outcome at month 3 and KRT8 levels is shown in Table 3. Visual outcome was not associated with KRT8 levels at baseline ($p = 0.63$), but worse visual outcome was associated with higher KRT levels at month 2 ($p = 0.045$). Association of anatomical outcome with KRT8

levels failed to reach statistical significance at baseline (p = 0.07), but a significant association was observed between poor anatomical outcome (persistent fluid on OCT) and higher KRT levels at month 2 (p = 0.001).

Table 3. Association between KRT8 levels and treatment outcome after intravitreal ranibizumab injection

	Visual outcome			Anatomical outcome		
	B ± SE	95% CI	p-value	OR	95% CI	p-value
KRT8 at baseline	0.02 ± 0.04	-0.07–0.11	0.63	1.61	0.96–2.69	0.07
KRT8 at Month 2	0.10 ± 0.05	0.003–0.20	0.045*	7.97	2.48–25.66	0.001*

KRT8, keratin 8; Visual outcome, logMAR visual acuity at month 3; Anatomical outcome, persistent fluid on OCT at month 3; B, unstandardized beta coefficient; SE, standard error; CI, confidence interval.
*p < 0.05.

5. Association between KRT8 levels at month 2 and treatment outcome at month 3 after intravitreal ranibizumab injection

In the univariate analysis, a worse visual outcome was associated with higher KRT8 level at month 2 (p = 0.045) and the presence of IRF (p = 0.002). However, these associations were not significant in multivariate analysis (Table 4).

Poor anatomical outcome was associated with higher KRT8 levels at month 2 (p = 0.001) and larger CNV size (p = 0.04) in the univariate analysis. In multivariate logistic regression analysis, poor anatomical outcome was still found to be associated with higher KRT levels at month 2 (OR 8.32, 95% CI 2.02 – 34.2, p = 0.003) (Table 5).

Table 4. Association between KRT8 levels at month 2 and visual outcome at month 3

	Univariate			Multivariate		
	B ± SE	95% CI	p	B ± SE	95% CI	p
KRT8 (ng/ml)	0.10 ± 0.05	0.003–0.20	0.045*	0.06 ± 0.05	-0.04–0.17	0.23
Baseline characteristics for adjustments						
Age (year)	0.01 ± 0.01	-0.004–0.02	0.18	0.001 ± 0.01	-0.011–0.012	0.88
Sex (male)	0.11 ± 0.11	-0.11–0.32	0.32	0.08 ± 0.12	-0.16–0.33	0.49
HTN	0.02 ± 0.13	-0.24–0.28	0.87			
DM	-0.19 ± 0.16	-0.53–0.14	0.25			
ChT (μm)	0.00 ± 0.001	-0.001–0.002	0.52			
CNV size (mm^2)	0.01 ± 0.01	-0.002–0.02	0.10	0.01 ± 0.01	-0.01–0.03	0.44
CNV type						
PCV		Ref			Ref	
Type 1	0.26 ± 0.14	-0.03–0.54	0.08	0.17 ± 0.15	-0.14–0.47	0.27
Type 2	-0.02 ± 0.14	-0.31–0.26	0.87	-0.13 ± 0.16	-0.46–0.19	0.43
Type 3	0.26 ± 0.13	-0.01–0.52	0.06	-0.01 ± 0.17	-0.34–0.33	0.97
Morphologic characteristics of baseline OCT						
SRF	0.04 ± 0.13	-0.21–0.29	0.75			
IRF	0.31 ± 0.09	0.12–0.50	0.002*	0.23 ± 0.12	0.001–0.47	0.051
SMH	0.14 ± 0.11	-0.09–0.36	0.22			
Drusen	0.12 ± 0.11	-0.10–0.34	0.28			
Exudate	0.14 ± 0.11	-0.09–0.32	0.23			

B, unstandardized beta coefficient; SE, standard error; CI, confidence interval; KRT8, keratin 8 level at month 2; HTN, systemic hypertension; DM, diabetes mellitus; ChT, subfoveal choroidal thickness; CNV, choroidal neovascularization; PCV, polypoidal choroidal vasculopathy; Ref, reference; OCT, optical coherence tomography; SRF, subretinal fluid; IRF, intraretinal fluid; SMH, submacular hemorrhage.
*$p < 0.05$

Table 5. Association between KRT8 levels at month 2 and anatomical outcome at month 3

	Univariate			Multivariate		
	OR	95% CI	p	OR	95% CI	p
KRT8 (ng/ml)	7.97	2.48–25.7	0.001*	8.32	2.02–34.2	0.003*
Baseline characteristics for adjustments						
Age (year)	0.99	0.94–1.05	0.74	0.96	0.87–1.05	0.32
Sex (male)	0.42	0.13–1.38	0.15	0.62	0.07–5.33	0.66
HTN	0.51	0.12–2.15	0.36			
DM	3.47	0.58–20.8	0.17			
ChT (μm)	1.00	0.99–1.01	0.26			
CNV size (mm^2)	1.14	1.01–1.30	0.04*	1.16	0.95–1.41	0.15
CNV type						
PCV	Ref			Ref		
Type 1	0.49	0.11–2.22	0.35	0.35	0.05–2.73	0.32
Type 2	4.89	0.54–44.6	0.16	1.74	0.09–32.58	0.71
Type 3	0.51	0.13–2.07	0.67	0.99	0.06–17.44	0.99
Morphologic characteristics of baseline OCT						
SRF	2.31	0.55–9.65	0.25			
IRF	2.84	0.95–8.44	0.06	2.11	0.34–13.20	0.42
SMH	0.53	0.16–1.79	0.31			
Drusen	0.77	0.24–2.48	0.66			
Exudate	1.26	0.30–5.30	0.75			

OR, odds ratio; CI, confidence interval; KRT8, keratin 8 level at month 2; HTN, systemic hypertension; DM, diabetes mellitus; ChT, subfoveal choroidal thickness; CNV, choroidal neovascularization; PCV, polypoidal choroidal vasculopathy; Ref, reference; OCT, optical coherence tomography; SRF, subretinal fluid; IRF, intraretinal fluid; SMH, submacular hemorrhage.
*$p < 0.05$

6. Association between KRT8 levels at month 2 and treatment outcome at month 6 after intravitreal ranibizumab injection

After the prospective study was completed, treatment outcomes were retrospectively reviewed in 51 patients who were followed up at month 6. In the univariate analysis, visual outcome at month 6 was not associated with the KRT8 level at month 2 ($p = 0.87$), but anatomical outcome at month 6 was associated with the KRT level at month 2 ($p = 0.039$). In multivariate analysis, poor anatomical outcome at month 6 was still found to be associated with higher KRT levels at month 2 (OR 2.63, 95% CI 1.18 – 5.88, $p = 0.019$) (Table 6).

Table 6. Association between KRT8 levels at month 2 and anatomical outcome at month 6

	Univariate			Multivariate		
	OR	95% CI	p	OR	95% CI	p
KRT8 (ng/ml)	2.13	1.04–4.39	0.039*	2.63	1.18–5.88	0.019*
Baseline characteristics for adjustments						
Age (year)	0.96	0.90–1.02	0.19	0.95	0.87–1.03	0.20
Sex (male)	0.35	0.09–1.28	0.11	1.01	0.16–6.27	0.99
HTN	0.15	0.02–1.32	0.09			
DM	3.63	0.60–3.63	0.16			
ChT (μm)	1.00	1.00–1.02	0.06			
CNV size (mm^2)	1.03	0.97–1.09	0.37	1.07	0.97–1.17	0.18
CNV type						
PCV	Ref			Ref		
Type 1	0.75	0.14–4.10	0.74	0.55	0.06–4.73	0.58
Type 2	8.00	0.86–74.22	0.07	0.79	0.04–13.96	0.87
Type 3	2.67	0.57–12.56	0.22	0	NA	>0.99
Morphologic characteristics of baseline OCT						
SRF	$>10^3$	NA	>0.99			
IRF	0.99	0.32–3.08	0.99	1.30	0.22–7.65	0.77
SMH	0.61	0.17–2.12	0.43			
Drusen	0.21	0.05–0.88	0.03*			
Exudate	0.92	0.19–4.35	0.91			

OR, odds ratio; CI, confidence interval; KRT8, keratin 8 level at month 2; HTN, systemic hypertension; DM, diabetes mellitus; ChT, subfoveal choroidal thickness; CNV, choroidal neovascularization; PCV, polypoidal choroidal vasculopathy; Ref, reference; OCT, optical coherence tomography; SRF, subretinal fluid; IRF, intraretinal fluid; SMH, submacular hemorrhage.
*$p < 0.05$

IV. DISCUSSION

In the present study, 58 treatment-naïve nAMD patients were enrolled, and the AH levels of KRT8 before and after IVR treatments were examined. Significantly increased KRT8 levels in nAMD eyes compared with those in the controls were observed before treatments. After IVR, responders showed a significant decrease in KRT8 levels, whereas poor responders showed no change. Higher KRT8 levels were associated with the presence of persistent fluid upon OCT after IVR, suggesting a potential role for KRT8 as a prognostic indicator for patients with nAMD.

KRT8, a well-known epithelial marker protein, has been known to support the mechanical integrity of cells, modulate stress response, and contribute to cell resistance to apoptosis.[14-16, 27] KRT8 has been identified as an RPE marker [24,25], and our previous study found that KRT8 levels are elevated about 2-fold in nAMD patients compared with those in controls.[9] In this study, KRT8 levels were 1.7-fold higher than in controls, which is in line with the previous study. The involvement of KRT8 in nAMD has been supported by keratin expression in the CNV membrane (CNVM).[28] Only RPE cells are immunoreactive for keratin within the retina; in surgically excised nAMD-related CNVMs, many RPE cells are strongly positive for this marker [28], indicating that increased AH KRT8 is likely derived from RPE cells in these patients.[9]

Oxidative and/or mechanical stress can trigger cytoskeleton activation.[29,30] A previous study reported that oxidative stress in human RPE cells induces upregulation of KRT8 and autophagy, resulting in the protection of RPE cells from apoptotic cell death under oxidative stress.[26] CNV in nAMD shares much with the process of a wound-healing response [28], and proliferation of RPE is speculated to serve as a reparative process to cover and regenerate damaged tissue and seal off leaking vascular channels.[31] Therefore, elevated KRT8 levels in the AH are likely to be related to a good reparative mechanism in these treatment-naïve nAMD patients.

After two consecutive IVR treatments, KRT8 levels decreased. However, although the average levels of KRT8 at month 2 were lower than those before the treatments, when patient groups were divided into responders and poor responders, only responders demonstrated a significant decrease in KRT8 levels after IVR, whereas poor responders showed persistent elevated KRT8 levels. Furthermore, higher KRT8 levels at month 2 were associated with the presence of persistent fluid upon OCT at month 3 after adjusting for other variables. Elevated KRT8 levels in nAMD eyes early in the disease

course are possibly a reparative or protective mechanism; however, prolonged elevation of KRT8 levels might be detrimental, as it could be related to epithelial-mesenchymal transition (EMT).[26] In EMT, polarized epithelial cells convert to motile mesenchymal cells, and transdifferentiated RPE cells are the principal nonvascular stromal cells in vascular and fibrotic nAMD-related CNVMs.[28] EMT ultimately results in the loss of RPE characteristics [24], which is concomitant with a rearrangement of the cytoskeleton.[32] Our previous study showed that under prolonged oxidative stress, high KRT8 levels induce EMT via its phosphorylation, resulting in loss of RPE cell junction integrity and degeneration of the RPE.[26] Similar results have been reported in pancreatic and gastric cancer cells.[33] Although there has been no study investigating how EMT causes resistance to treatment in nAMD patients, several studies have shown that EMT is associated with resistance to anti-VEGF treatment in various tumors, including pancreatic cancers [34], genitourinary cancers [35], and brain tumors. [36] In gastric cancer, KRT8 overexpression leads to EMT and enhances the proliferation and migration of cancer cells, and patients with high KRT8 levels tend to have unfavorable outcomes.[37] Moreover, EMT in RPE contributes to retinal fibrosis in nAMD eyes [38], and fibrosis often develops in poor responders to anti-VEGF treatment.[39] Based on these findings, we speculate that in responders, KRT8 expression is elevated as a reactive RPE change with the development of CNV and then decreases when the wound healing process proceeds and CNV regresses with anti-VEGF treatments. An unsuccessful treatment response could result in progression of tissue injury, inflammation, and prolonged loss of RPE cell-to-cell contact, which are responsible for initiating EMT and fibrosis. These processes might contribute to the persistence of KRT8 upregulation in poor responders. It remains to be determined whether upregulated KRT8 expression reflects the consequences or the causes of poor treatment response to IVR; in other words, prolonged KRT upregulation in poor responders might induce EMT, leading

to resistance to anti-VEGF treatments.

Although anti-VEGF agents have shown remarkable results in nAMD treatment, some patients have poor or no response to anti-VEGF agents or experience a loss of efficacy of anti-VEGF after repeated administration. Several proteins or pathways, other than VEGF, could cause variability in behavior of the disease and response to anti-VEGF treatment, and thus could be therapeutic targets for nAMD patients, particularly those who have poor response to treatment. For example, our previous study suggested that the upregulation of KRT8 and downregulation of phosphorylated KRT8 may promote cell survival while suppressing EMT [26]; thus, KRT8 could be a novel therapeutic target for the treatment of nAMD, which is supported by our present findings.

The limitations of this study include its small sample size and short follow-up period. With a short follow-up period, it is difficult to elucidate the association of KRT8 with recurrence or long-term treatment response. Although there was no statistically significant difference in age and sex between the nAMD and the control group, the matched case-control studies with larger sample size using sample size calculation design are needed. Although statistically significant, the relatively low beta coefficients and wide confidence intervals for the associations between KRT8 levels and treatment outcomes suggest that more research is needed on nAMD pathophysiology and confounding factors before using KRT8 levels in clinical practice. In addition, since the levels of both VEGF and KRT8 in the AH could not be obtained due to technical limitations, whether the change in KRT8 levels in the AH is an independent marker of treatment or it is associated with the change in VEGF levels, could not be determined. Despite these limitations, our results suggest that KRT8 could be a possible prognostic biomarker in nAMD patients.

V. CONCLUSION

In summary, this study reveals that monitoring aqueous levels of KRT8 during IVR treatments shows an association between decreasing KRT8 levels and better treatment responses to anti-VEGF. The increase in KRT8 levels before treatment may suggest that RPE cells proliferate to envelop CNV and thus regress; KRT8 levels seem to decrease once they have proliferated to some degree. Although long-term data are needed to show that the levels of KRT8 in nAMD patients return to those in controls after regression of CNV, monitoring aqueous KRT8 levels may be a practical approach to predicting therapeutic effects during the course of early treatment. In addition, it may also help in determining the treatment strategy of anti-VEGFs, including treatment intervals, as an aid to imaging biomarkers. In addition, identification of poor responders to anti-VEGF treatments will help clinicians decide whether to switch to other agents available in the near future, which would optimize customized treatment for nAMD.

REFERENCE

1. Bressler NM. Age-related macular degeneration is the leading cause of blindness. JAMA. 2004;291:1900–1.
2. Pascolini D, Mariotti SP, Pokharel GP, Pararajasegaram R, Erya'ale D, Négrel AD et al. 2002 Global update of available data on visual impairment: a compilation of population-based prevalence studies. Ophthalmic Epidemiol. 2004;11:67-115.
3. Mitchell P, Liew G, Gopinath B, Wong TY. Age-related macular degeneration. The Lancet 2018;392:1147-59.
4. Rosenfeld PJ. Brown DM, Heier JS, Boyer DS, Kaiser PK, Chung CY et al. Ranibizumab for neovascular age-related macular degeneration. N. Engl. J. Med. 2006;355:1419–31.
5. Yang S, Zhao J, Sun X. Resistance to anti-VEGF therapy in neovascular age-related macular degeneration: a comprehensive review. Drug Des. Devel. Ther. 2016;10: 1857–67.
6. Handa JT, Rickman CB, Dick AD, Gorin MB, Miller JW, Toth CA et al. A systems biology approach towards understanding and treating non-neovascular age-related macular degeneration. Nat. Commun. 2019;10: 3347.
7. Lai TT, Hsieh YT, Yang CM, Ho TC, Yang CH. Biomarkers of optical coherence tomography in evaluating the treatment outcomes of neovascular age-related macular degeneration: a real-world study. Sci. Rep. 2019;9: 529.
8. Simader C. Ritter M, Bolz M, Deák GG, Mayr-Sponer U, Golbaz I et al. Morphologic parameters relevant for visual outcome during anti-angiogenic therapy of neovascular age-related macular degeneration. Ophthalmology 2014;121: 1237–45.
9. Kang GY, Bang JY, Choi AJ, Yoon J, Lee WC, Choi S et al. Exosomal Proteins in the Aqueous Humor as Novel Biomarkers in Patients with

Neovascular Age-related Macular Degeneration. J. Proteome Res. 2014;13: 581–95.

10. Kim TW, Kang JW, Ahn J, Lee EK, Cho KC, Han BNR et al. Proteomic analysis of the aqueous humor in age-related macular degeneration (AMD) patients. J. Proteome Res. 2012;11: 4034–43.
11. Lee H, Choi AJ, Kang GY, Park HS, Kim HC, Lim HJ et al. Increased 26S proteasome non-ATPase regulatory subunit 1 in the aqueous humor of patients with age-related macular degeneration. BMB Rep. 2014;47: 292–7.
12. Yao J, Liu X, Yang Q, Zhuang M, Wang F, Chen X et al. Proteomic analysis of the aqueous humor in patients with wet age-related macular degeneration. Proteomics Clin. Appl. 2013;7: 550–60.
13. Kersten E, Paun CC, Schellevis RL, Hoyng CB, Delcourt C, Lengyel I et al. Systemic and ocular fluid compounds as potential biomarkers in age-related macular degeneration. Surv. Ophthalmol. 2018;63: 9–39.
14. Zatloukal K. Stumptner C, Lehner M, Denk H, Baribault H, Eshkind LG, Franke WW. Cytokeratin 8 Protects from Hepatotoxicity, and Its Ratio to Cytokeratin 18 Determines the Ability of Hepatocytes to Form Mallory Bodies. Am. J. Pathol. 2000;156: 1263–74.
15. Ku NO, Omary MB. A disease- and phosphorylation-related nonmechanical function for keratin 8. J. Cell Biol. 2006;174: 115–25.
16. Caulin C, Ware CF, Magin, TM, Oshima RG. Keratin-Dependent, Epithelial Resistance to Tumor Necrosis Factor-Induced Apoptosis. J. Cell Biol. 2000;149: 17–22.
17. Gires O, Mack B, Rauch J, Matthias C. CK8 correlates with malignancy in leukoplakia and carcinomas of the head and neck. Biochem. Biophys. Res. Commun. 2006;343: 252–9.
18. Makino T, Yamasaki M, Takeno A, Shirakawa M, Miyata H, Takiguchi S et al. Cytokeratins 18 and 8 are poor prognostic markers in patients with

squamous cell carcinoma of the oesophagus. Br. J. Cancer 2009;101: 1298–306.

19. Woelfle U, Sauter G, Santjer S, Brakenhoff R, Pantel K. Down-Regulated Expression of Cytokeratin 18 Promotes Progression of Human Breast Cancer. Clin. Cancer Res. 2004;10: 2670–74.
20. Knösel T, Emde V, Schlüns K, Schlag PM, Dietel M, Petersen I. Cytokeratin profiles identify diagnostic signatures in colorectal cancer using multiplex analysis of tissue microarrays. Anal. Cell. Pathol. 2006;28: 167–75.
21. Fillies T, Werkmeister R, Packeisen J, Brandt B, Morin P, Weingart D et al. Cytokeratin 8/18 expression indicates a poor prognosis in squamous cell carcinomas of the oral cavity. BMC Cancer 2006;6: 10.
22. Fortier AM, Asselin E, Cadrin M. Keratin 8 and 18 loss in epithelial cancer cells increases collective cell migration and cisplatin sensitivity through claudin1 up-regulation. J. Biol. Chem. 2013;288: 11555–71.
23. Wang Y, He QY, Tsao SW, Cheung YH, Wong A, Chiu JF. Cytokeratin 8 silencing in human nasopharyngeal carcinoma cells leads to cisplatin sensitization. Cancer Lett. 2008;265: 188–96.
24. Zhao C, Yasumura D, Li X, Matthes M, Lloyd M, Nielsen G et al. mTOR-mediated dedifferentiation of the retinal pigment epithelium initiates photoreceptor degeneration in mice. J. Clin. Invest. 2011;121: 369–83.
25. Hunt RC, Davis AA. Altered expression of keratin and vimentin in human retinal pigment epithelial cells in vivo and in vitro. J. Cell. Physiol. 1990;145: 187–99.
26. Baek A, Yoon S, Kim J, Baek YM, Park H, Lim D et al. Autophagy and KRT8/keratin 8 protect degeneration of retinal pigment epithelium under oxidative stress. Autophagy 2017;13: 248–63.
27. Tao GZ, Looi KS, Toivola DM, Strnad P, Zhou Q, Liao J et al. Keratins

modulate the shape and function of hepatocyte mitochondria: a mechanism for protection from apoptosis. J. Cell Sci. 2009;122: 3851–5.

28. Lopez PF, Sippy BD, Lambert HM, Thach 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: 855–68.
29. Girouard MP, Pool M, Alchini R, Rambaldi I, Fournier AE. RhoA Proteolysis Regulates the Actin Cytoskeleton in Response to Oxidative Stress. PloS One 2016;11: e0168641.
30. Wu S, Lu Q, Wang N, Zhang J, Liu Q, Gao M et al. Cyclic stretch induced-retinal pigment epithelial cell apoptosis and cytokine changes. BMC Ophthalmol. 2017;17: 208.
31. Hu DN, Gentile RC, McCormick SA, Yang P-Y, Muldoon TO, Lin H-Y et al. Role of RPE Cells in Pathogenesis of Proliferative Vitreoretinopathy and Age-Related Macular Degeneration: Cell Culture Study of Surgical Excised Pre- and Sub-Retinal Membranes. J Clin Ophthalmol Eye Disord. 2017;1: 1002.
32. Grisanti S, Guidry C. Transdifferentiation of retinal pigment epithelial cells from epithelial to mesenchymal phenotype. Invest. Ophthalmol. Vis. Sci. 1995;36: 391–405.
33. Busch T, Armacki M, Eiseler T, Joodi G, Temme C, Jansen J et al. Keratin 8 phosphorylation regulates keratin reorganization and migration of epithelial tumor cells. J. Cell Sci. 2012;125: 2148–59.
34. Carbone C, Moccia T, Zhu C, Paradiso G, Budillon A, Chiao PJ et al. Anti-VEGF Treatment Resistant Pancreatic Cancers Secrete Proinflammatory Factors that Contribute to Malignant Progression by Inducing an EMT cell phenotype. Clin. Cancer Res. 2011;17: 5822–32.
35. Hammers H, Fu C, Gerber S, Van Den Berg S, Steenwinkel Fm Keizman

WM et al. Epithelial-mesenchymal transition: A mechanism of resistance to VEGF pathway inhibition in genitourinary cancers. J. Clin. Oncol. 2012;30: 390.

36. Piao Y, Liang J, Holmes L, Henry V, Sulman E, Groot JF. Acquired Resistance to Anti-VEGF Therapy in Glioblastoma Is Associated with a Mesenchymal Transition. Clin. Cancer Res. 2013;19: 4392–403.
37. Fang J, Wang H, Liu Y, Ding F, Ni Y, Shao S. High KRT8 expression promotes tumor progression and metastasis of gastric cancer. Cancer Sci. 2017;108: 178–86.
38. Ishikawa K, Kannan R, Hinton DR. Molecular mechanisms of subretinal fibrosis in age-related macular degeneration. Exp. Eye Res. 2016;142: 19–25.
39. Dikmetas O, Kadayıfcılar S, Eldem B. The effect of CFH polymorphisms on the response to the treatment of age-related macular degeneration (AMD) with intravitreal ranibizumab. Mol. Vis. 2013;19: 2571–8.

ABSTRACT(IN KOREAN)

삼출성 나이관련 황반변성 환자의 방수 내 케라틴8과 라니비주맙 치료 후 반응과의 연관성

<지도교수 변석호>

연세대학교 대학원 의학과

신주연

항 혈관내피세포성장인자 억제항체의 사용은 최근 신생혈관성 나이관련 황반변성의 치료에 현저한 개선을 보여주었다. 하지만 일부 환자는 여전히 치료에 대한 반응이 좋지 않으므로 이에 대한 새로운 예후 표지자와 치료 표적이 필요하다. 본 연구는 항 혈관내피세포성장인자의 하나인 라니비주맙의 유리체 내 주입술을 시행받은 신생혈관성 나이관련 황반변성 환자의 방수 내에 존재하는 망막색소상피의 마커 단백질인 케라틴 8 의 수치와 치료 결과 및 임상 변수와의 연관성을 알아보고자 하였다.

본 연구는 신생혈관성 나이관련 황반변성 환자군 58 명과 대조군 46 명을 대상으로 하였다. 환자군은 신생혈관성 나이관련 황반변성으로 새로 진단받고 3 개월간 매달 유리체 내 라니비주맙 주입술을 시행받은 환자를 모집하였다. 방수 채취는 환자군은 치료 전과 치료시작 2 개월 후 시행하였고, 대조군은 백내장 수술 직전에 채취하였다. 방수 내 케라틴 8

수치는 효소결합 면역흡착검사를 이용하여 정량적으로 측정하였다. 치료 결과는 시력과 빛간섭 단층촬영에서의 해부학적 결과로 구분하여 치료 시작 3 개월째에 판정하였으며, 치료반응이 적은 경우는 치료 시작 3 개월에 촬영한 빛간섭 단층촬영에서 망막하액이나 망막내액이 관찰되는 경우로 정의하였다.

케라틴 8 수치는 대조군에 비해 치료 전 환자군에서 유의하게 높았다. 환자군의 케라틴 8 수치는 치료 전에 비해 치료시작 2 개월 째 유의하게 감소했다. 치료반응에 따라 환자군을 반응군과 비반응군으로 분류하였을 때, 반응군은 치료 전에 비해 치료시작 2 개월 째 케라틴 8 이 유의하게 감소했던 반면에, 비반응군에서는 유의한 변화를 보이지 않았다. 또한 치료시작 2 개월 째 케라틴 8 수치가 높을수록 치료시작 3 개월째와 6 개월째 치료반응이 적을 가능성이 높았다.

본 연구 결과는 유리체 내 라니비주맙 주입술을 시행받은 신생혈관성 나이관련 황반변성 환자에서 방수 내 케라틴 8 이 치료결과와 연관성이 있음을 보여주었으며, 방수 내 케라틴 8 의 수치를 모니터링하는 것이 망막색소상피의 상태를 반영하여 라니비주맙 치료효과를 조기에 판정하는데 도움을 줄 가능성이 있음을 제시하였다.

핵심되는 말 : 나이관련 황반변성, 항 혈관내피세포성장인자 억제항체, 방수, 케라틴 8, 망막색소상피