Clinical and Immunological Characterization of Paraneoplastic Retinopathy

Yukiko Makiyama,1 Takanobu Kikuchi,2 Atsushi Otani,1 Akio Oishi,1 Congrong Guo,1 Satoko Nakagawa,1 Ken Ogino,1 Hiroshi Kojima,1 Masafumi Kurimoto,1 and Nagahisa Yoshimura1

1Department of Ophthalmology, Kyoto University Graduate School of Medicine, Kyoto, Japan
2Department of Instrumental Analysis, Research Center for Human and Environmental Science, Shinshu University, Nagano, Japan

Correspondence: Atsushi Otani, Department of Ophthalmology, Kyoto University Graduate School of Medicine, 54 Kawaharacho, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan; otan@kuhp.kyoto-u.ac.jp.
Submitted: February 14, 2013
Accepted: July 7, 2013

PURPOSE. To report the clinical and immunological characterization of paraneoplastic retinopathy (PR) and to investigate the association between spectral-domain optical coherence tomography (SDOCT) findings and the targets of autoantibodies in PR.

METHODS. We retrospectively enrolled eight patients (age range, 57–85 years; four men and four women) suspected of having PR. All patients underwent comprehensive ophthalmic examinations, including best-corrected visual acuity (BCVA) measurement, slitlamp examinations, kinetic visual field testing with Goldmann perimetry, electroretinography (ERG), fundus photography, fluorescein angiography, fundus autofluorescence (FAF), SDOCT, and serum sample tests (Western blot analysis and immunohistochemistry [IHC]).

RESULTS. Three patients had a history of malignant tumors, and four patients were newly diagnosed as having neoplastic tumors (small cell lung carcinoma [SCLC], thymoma, pancreatic neuroendocrine neoplasm, and colon cancer). Another de novo malignancy (SCLC) was detected in a patient with a history of malignancy (bladder cancer and liposarcoma). The BCVA in these patients ranged from hand motion to 1.5. Goldmann perimetry revealed island, ring-shaped, concentric, or central scotoma. All patients showed nonrecordable or reduced amplitude results on ERG. Fluorescein leakage was detected in five patients. Hyperautofluorescence and/or hypoautofluorescence on FAF was detected in six patients. The serum sample tests identified anti-retinal antibodies in all patients. Patients whose serum contained anti-photorceptor or anti-retinal pigment epithelium antibody on IHC showed damage of the outer retina on SDOCT.

CONCLUSIONS. In this case series, PR was associated with a variety of neoplasms and autoantibodies. Spectral-domain OCT can be used to characterize morphologic changes, and the changes were associated with the targets of autoantibodies.

Keywords: paraneoplastic retinopathy, spectral-domain optical coherence tomography, Western blot analysis, immunohistochemistry, autoantibody, anti-retinal antibody
Paraneoplastic retinopathy (PR) is a rare and potentially serious vision-threatening syndrome characterized by the development of retinal dysfunction associated with an underlying neoplasm. This condition is often characterized by neuro-ophthalmologic symptoms, such as decreased visual acuity, metamorphopsia, and scotomas, which may not be fully explained by other retinal or optic nerve diseases.

**Subjects and Clinical Examinations**

The study sample comprised eight patients (four men and four women) who were seen at Kyoto University Hospital (Kyoto, Japan) from June 2008 to August 2011 and who were suspected of having PR. The study was conducted in accordance with the tenets of the Declaration of Helsinki. The study design was approved by the ethics committee of Kyoto University. Two of eight patients were presented in a prior case report without description of Western blot analysis.

**Methods**

**Subjects and Clinical Examinations**

The study sample comprised eight patients (four men and four women) who were seen at Kyoto University Hospital (Kyoto, Japan) from June 2008 to August 2011 and who were suspected of having PR. The study was conducted in accordance with the tenets of the Declaration of Helsinki. The study design was approved by the ethics committee of Kyoto University. Two of eight patients were presented in a prior case report without description of Western blot analysis.

**Western Blot Analysis**

Serum samples were obtained from each patient and analyzed by Western blot as previously reported. Briefly, retina, brain, kidney, liver, and spleen tissue samples from C57BL/6 mice were homogenized. Extracted proteins were used to detect organ-specific antibodies. Proteins from each organ were separated by 8% or 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes. The membrane was probed with the patient’s serum and then rinsed and incubated with alkaline phosphatase–conjugated goat anti-human IgG (Jackson Immunoresearch, West Grove, PA). The associated signal was detected with a 5-bromo-4-chloro-3-indolylphosphatase–nitro blue tetrazolium chloride detection system.

**Characterization of Paraneoplastic Retinopathy**

The diagnosis of PR was supported by fundus examination findings or the pathophysiology of other retinal or optic nerve diseases. The attenuation of retinal function was considered to support a diagnosis of PR. In such cases, we performed Goldmann perimetry, ERG, fluorescein angiography (FA), fundus autofluorescence (FAF), and SDOCT. Additional endoscopy or bronchoscopy examinations were performed as needed. Informed consent was obtained from the participants after the objectives and the nature of the procedures had been fully explained; blood samples were collected thereafter. We diagnosed patients as having PR when they showed the following: (1) reduced amplitude ERG, (2) the presence of anti-retinal autoantibodies, (3) a previous or newly detected neoplasm, and (4) acute or subacute disease progression.

**Methods**

In the context of the study, we performed additional diagnostic procedures to support the diagnosis and understand the molecular basis of the pathologic changes. Western blot analysis was employed to detect organ-specific antibodies. Proteins from each organ were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes. The membrane was probed with the patient’s serum and incubated with alkaline phosphatase–conjugated goat anti-human IgG. The associated signal was detected using a 5-bromo-4-chloro-3-indolylphosphatase–nitro blue tetrazolium chloride detection system.

**TABLE.** Clinical Characteristics, Treatment of Neoplasm and PR, and Results of Western Blot Analysis in Eight Cases

<table>
<thead>
<tr>
<th>Case n/Sex/ Age at Diagnosis, y</th>
<th>Initial BCVA</th>
<th>Tumor</th>
<th>Western Blot Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chief Complaint</td>
<td>FA Leakage</td>
<td>History</td>
</tr>
<tr>
<td>1/M/85</td>
<td>Defect of VF</td>
<td>No</td>
<td>Bladder cancer and liposarcoma in mediastinum</td>
</tr>
<tr>
<td>2/F/64</td>
<td>Defect of VF, metamorphopsia</td>
<td>Yes</td>
<td>No specific finding</td>
</tr>
<tr>
<td>3/M/57</td>
<td>Decrease in VA, contraction of VF</td>
<td>Yes</td>
<td>Thymoma</td>
</tr>
<tr>
<td>4/F/81</td>
<td>Decrease in VA, photopsia</td>
<td>Yes</td>
<td>No specific finding</td>
</tr>
<tr>
<td>5/F/78</td>
<td>Decrease in VA, photopsia</td>
<td>Yes</td>
<td>No specific finding</td>
</tr>
<tr>
<td>6/M/59</td>
<td>Decrease in VA, night blindness</td>
<td>No</td>
<td>Renal cancer</td>
</tr>
<tr>
<td>7/M/61</td>
<td>Decrease in VA</td>
<td>Yes</td>
<td>Lymphoma (40 y previously), renal cancer</td>
</tr>
<tr>
<td>8/F/68</td>
<td>Decrease in VA, photopsia</td>
<td>No</td>
<td>No specific finding</td>
</tr>
</tbody>
</table>

GCL, ganglion cell layer; HM, hand motion; INL, inner nuclear layer; NR, nonrecordable; OPL, outer plexiform layer; VA, visual acuity; VF, visual field; WB, Western blot.
cells, the outer segments of s-cone cells, and Müller cells, antibodies were used to label the outer segments of cone CA), or S-100 protein (Dako, Glostrup, Denmark); these clonal antibodies against blue opsin (Chemicon, Temecula, rabbit poly- slices were coincubated with rhodamine-conjugated peanut antibody (Invitrogen, Carlsbad, CA). In some cases, the retinal incubated with the AlexaFluor 488–conjugated anti-human IgG 0.05% NP-40 in PBS for 1 hour at room temperature. The slides blocked with 4% normal goat serum, 0.1% Triton X-100, and were then incubated with a 1:50 dilution of the patient’s serum 8 hours in a freshly prepared solution of 0.5% zinc chloride, sections (4 μm thick) were prepared, deparaffinized, and then embedded in paraffin. Sections (4 μm thick) were prepared, deparaffinized, and blocked with 4% normal goat serum, 0.1% Triton X-100, and 0.05% NP-40 in PBS for 1 hour at room temperature. The slides were then incubated with a 1:50 dilution of the patient’s serum overnight at 4°C. Next, the specimens were rinsed in PBS and incubated with the AlexaFluor 488–conjugated anti-human IgG antibody (Invitrogen, Carlsbad, CA). In some cases, the retinal slices were coincubated with rhodamine-conjugated peanut agglutinin (Sigma-Aldrich Corp., St. Louis, MO), rabbit polyclonal antibodies against blue opsin (Chemicon, Temecula, CA), or S-100 protein (Dako, Glostrup, Denmark); these antibodies were used to label the outer segments of cone cells, the outer segments of s-cone cells, and Müller cells, respectively. In these cases, AlexaFluor 594–conjugated anti-rabbit IgG was used as a secondary antibody. The slides were finally mounted in a mounting medium containing diamidino-2-phenylindole (Vector Laboratories, Burlingame, CA). The contribution of secondary antibodies to the observed immune reaction was verified by staining without the primary antibody. The sections were examined using a confocal scanning microscope (LSM 5 Exciter; Carl Zeiss Microimaging, Jena, Germany).

**Immunohistochemistry**

C57BL/6 mouse eyes were fixed by immersion at 4°C for 12 hours in a freshly prepared solution of 0.5% zinc chloride, 17.16 mM zinc trifluoroacetate, and 0.05% calcium acetate in 0.1 M Tris- HCl (pH 6.5) and then embedded in paraffin. Sections (4 μm thick) were prepared, deparaffinized, and blocked with 4% normal goat serum, 0.1% Triton X-100, and 0.05% NP-40 in PBS for 1 hour at room temperature. The slides were then incubated with a 1:50 dilution of the patient’s serum overnight at 4°C. Next, the specimens were rinsed in PBS and incubated with the AlexaFluor 488–conjugated anti-human IgG antibody (Invitrogen, Carlsbad, CA). In some cases, the retinal slices were coincubated with rhodamine-conjugated peanut agglutinin (Sigma-Aldrich Corp., St. Louis, MO), rabbit polyclonal antibodies against blue opsin (Chemicon, Temecula, CA), or S-100 protein (Dako, Glostrup, Denmark); these antibodies were used to label the outer segments of cone cells, the outer segments of s-cone cells, and Müller cells, respectively. In these cases, AlexaFluor 594–conjugated anti-rabbit IgG was used as a secondary antibody. The slides were finally mounted in a mounting medium containing diamidino-2-phenylindole (Vector Laboratories, Burlingame, CA). The contribution of secondary antibodies to the observed immune reaction was verified by staining without the primary antibody. The sections were examined using a confocal scanning microscope (LSM 5 Exciter; Carl Zeiss Microimaging, Jena, Germany).

**Genetic Analysis**

Genomic DNA was prepared from peripheral blood using a DNA extraction kit (QuickGene-610L; Fujifilm, Tokyo, Japan) according to the manufacturer’s protocol, and the A260/A280 optical density was evaluated. The DNA from all patients except case 3 was submitted to genetic analysis to exclude the presence of the following genes linked to retinal degeneration:

- CERKL
- CNGAI
- CNGBI
- MERTK
- PED6A
- PED6B
- PNR
- RDH12
- RGR
- RBP1
- SAG
- TULP1
- CRB
- RPE65
- USH2A
- USH3A
- LRAT
- PROML1
- PBP3

The tests were performed using a microarray (Asper Ophthalmics, Tartu, Estonia).

**RESULTS**

**Clinical Findings**

The clinical findings are summarized in the Table. Initial BCVA ranged from hand motion to 1.5. All patients complained of reduced visual acuity or visual field defects. Goldmann perimetry revealed island, ring-shaped, concentric, or central scotoma. The ERG results are shown in Figure 1. Electrotoretinography revealed abnormalities in all cases, but the degree of impairment ranged from nonrecordable to slight changes in the cone response in one eye. Vascularitis as confirmed by fluorescein leakage from the vasculature was observed in five cases (cases 2, 3, 4, 5, and 7). Two patients (cases 3 and 4) also had vitreous opacities. Fluorescein angiography revealed a petaloid leakage in case 4.

Three patients had a history of malignant tumors (bladder cancer, liposarcoma, thymoma, renal cancer, and lymphoma), and four patients were newly diagnosed as having neoplastic tumors (small cell lung carcinoma [SCLC], thymoma, pancreatic neuroendocrine neoplasm, and colon cancer). Another de novo malignancy (SCLC) was detected in a patient with a history of malignancy (bladder cancer and liposarcoma).

**FAF Findings**

In most cases, abnormality of FAF was detected. Abnormal hyperautofluorescence and/or hypoautofluorescence was not ed at presentation in all patients except cases 5 and 8 (Fig. 2). Although cases 5 and 8 displayed no detectable abnormality at

---

**Table.**

<table>
<thead>
<tr>
<th>Treatment of Neoplasm</th>
<th>Treatment of PR</th>
<th>Serum Sample Test Results, Western Blot Analysis kDa*/IHC</th>
<th>Course</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemotherapy</td>
<td>Oral prednisolone (corticosteroid 25 mg/d)</td>
<td>36, 39, 43, 64, 74, 217/Müller cell</td>
<td>Died within 5 mo</td>
</tr>
<tr>
<td>Chemotherapy, radiation therapy</td>
<td>Oral prednisolone (corticosteroid 30 mg/d)</td>
<td>23, 33, 37, 38, 60, 97, 215/OS of photoreceptor (s-cone)</td>
<td>VA stable for 2 y</td>
</tr>
<tr>
<td>Surgery</td>
<td>Steroid therapy (pulse+oral), plasmapheresis, intravenous immunoglobulin</td>
<td>29, 43, 100/OPL, IS of photoreceptor</td>
<td>Died within 1 y</td>
</tr>
<tr>
<td>Nontreatment</td>
<td>Ophthalmic solution (betamethasone sodium phosphate)</td>
<td>17, 21, 34, 41/GCL and IS of photoreceptor</td>
<td>Dropout</td>
</tr>
<tr>
<td>Surgery</td>
<td>Steroid therapy (pulse+oral)</td>
<td>46, 48, 60, 63, 98, 189, 222/GCL, OPL, OS of photoreceptor</td>
<td>VA declined to 0.03 OU in 9 mo</td>
</tr>
<tr>
<td>Surgery for renal, nontreatment for brain</td>
<td>Nontreatment</td>
<td>34, 71, 94, 102/GCL, INL, OPL</td>
<td>VA stable for 2 y</td>
</tr>
<tr>
<td>Radiation therapy for lymphoma, surgery for renal</td>
<td>Nontreatment</td>
<td>26, 46, 75, 108, 121/GCL, INL, outside of RPE</td>
<td>VA declined to 0.07 OD and 0.05 OS in 10 mo</td>
</tr>
<tr>
<td>Surgery</td>
<td>Nontreatment</td>
<td>25, 50, 90, 174/RPE</td>
<td>VA declined to HM OU in 3 y</td>
</tr>
</tbody>
</table>

* Specific antibodies against retina or brain are marked in bold.
the initial examination, case 8 developed abnormal hyper-autofluorescent and hypoautofluorescent lesions within 3 years. Abnormal FAF was eventually confirmed in seven of eight patients.

OCT Findings
Spectral-domain OCT images revealed evidence of retinal degeneration in all cases, especially in the outer retina (Fig. 2, right). The detected changes included obscuration, interruption, or disappearance of the inner segment/outer segment (IS/OS) junction of the photoreceptors. The external limiting membrane was also affected in all patients except case 6. Thinning of the foveal or parafoveal outer nuclear layer was noted in all patients except cases 1 and 5. Case 4 showed cystoid macular edema.

Western Blot Analysis
The results of Western blot analysis are shown in Figure 3 and in the Table. Western blot analysis detected anti-retinal antibodies in all serum samples. Several bands specific to retinal proteins were observed. Anti-recoverin antibody was present in case 2. The molecular weights and the number of bands detected varied among patients.

Comparison Between IHC and SDOCT
High-magnification IHC and SC-OCT images are shown in Figure 4. Abnormalities of the outer retina were more easily confirmed in magnified SDOCT images (Fig. 4, top). Serum samples from the patients reacted to retinal tissue, as shown in the bottom panels of Figure 4.

In cases 2, 3, 4, and 5, SDOCT showed disruption of outer retinal structures (Fig. 4). Immunohistochemistry staining was detected in the inner or outer segment in these patients (Fig. 4B–E). In cases 7 and 8, IHC staining was detected in the retinal pigment epithelium (RPE) layer (Fig. 4G, H), and SDOCT showed obscuration of the IS/OS line. Case 1, which showed strong staining in the inner retinal layers on IHC, had a relatively preserved outer retina on SDOCT (Fig. 4). In case 6, IHC staining was most prominent in the ganglion cell layer (Fig. 4F); SDOCT showed thinning of the outer nuclear layer and interruption of the IS/OS (Fig. 4). In addition, thinning of the retinal nerve fiber layer was noted in this case (Fig. 2R).

Genetic Analysis
To rule out genetic retinal degeneration, mutation screening was carried out using a microarray from Asper Ophthalmics. Candidate gene mutations included CERKL, CNGA1, CNGB1, MERTK, PED6A, PED6B, PNR, RDH12, RGR, RLBP1, SAG, TULP1, CRB, PF665, USH2A, USH3A, LRAT, PROM1, and PBP3. No mutations were detected in seven patients by genetic analysis. Mutation screening was not performed in case 3 because informed consent for genetic analysis was not obtained.

DISCUSSION
In the present study, we characterize eight patients with PR induced by various tumors. The underlying neoplasms that induced PR were bladder cancer, liposarcoma, thymoma, lymphoma, and renal cancer. In five patients, diagnoses of SCLC, thymoma, pancreatic neuroendocrine neoplasm, and
Characterization of Paraneoplastic Retinopathy

In the present study, anti-recoverin antibody was detected in only one patient, and Western blot analysis detected various bands. We were unable to determine antigens for some molecular weight proteins. However, in case 1 for example, a band of molecular weight 36 kDa was detected on Western blot analysis, which was close to the molecular weight of 35 kDa reported by Peek et al. as an antigen against Müller cells; in fact, Müller cells were stained in this case. In case 5, Western blot analysis detected a band of molecular weight 46 kDa. Although the protein was not identified, anti-alpha-enolase antibodies (46 kDa) have been reported as a cause of cone dysfunction.

Central visual field defect and almost nonrecordable cone ERG in case 5 are consistent with this study. Although it is unclear whether all the detected bands have pathologic significance, the existence of these various autoantibodies suggests that the pathology, symptoms, and clinical signs of PR are multifaceted.

In the present study, SD-OCT images were compared with the results of IHC or Western blot analysis. The patients with presumed anti-photoreceptor antibodies (cases 2, 3, 4, and 5) and anti-RPE antibodies (cases 7 and 8) exhibited severe damage to the outer retina and poor visual acuity. However, the patients without presumed anti-photoreceptor or anti-RPE antibodies (cases 1 and 6) exhibited relatively preserved IS/OS lines and had good visual acuity. This result is consistent with the study by Kondo et al. In their study, a patient with anti-TRPM1 antibody, which is expressed in Müller cells, manifested inner nuclear layer and outer plexiform layer signals but not photoreceptor degeneration on IHC and almost normal retinal structure in OCT images. The severe degeneration of photoreceptors depicted with SD-OCT might be associated with the presence of anti-photoreceptor or anti-RPE antibodies. Although this was an observational study and further investigations are needed, SD-OCT may facilitate the management of PR cases.

FAF was also useful to detect abnormalities in patients with PR, as shown in previous studies. In fact, abnormal FAF was noted in seven of eight patients herein. A varied...
In the present study, one patient showed normal FAF. A patient having RP with normal FAF was also noted in the previous study, and the authors suggested the following explanations: (1) the patient was identified very early in the disease process, (2) autoantibodies alter normal retinal function but did not cause cell loss, or (3) vision loss was due to inner retinal dysfunction rather than photoreceptor disruption or loss. In the present study, the first explanation above was not likely because FAF abnormality was not observed even after 12 months. The second and third explanations were not applicable either because SDOCT and ERG showed outer retinal abnormality. Further studies are required to understand the relationships among retinal dysfunction, the pathologic process, and the finding of FAF in patients with PR.

It was reported that retinopathy sometimes precedes the detection of malignancy. In fact, neoplasms were detected in eight patients described herein eventually displayed visual acuity worse than 0.1 in both eyes. Further investigations are warranted to establish effective treatment for this disease.

Some cases with cancer-associated retinopathy have shown specific ERG patterns. For example, negative ERG results were reported in patients with melanoma-associated retinopathy or with anti-TRPM1 antibody (~180 or ~200 kDa). In the present study, scotopic and photopic ERG results were equally affected in seven cases, none of which exhibited a negative pattern. Adamus et al. reported that anti-enolase antibody (46 kDa) predominantly affected the photopic response, while anti-transducin antibody (40 kDa) predominantly affected the scotopic response, and anti-recoverin antibody reduced both responses. Anti–46 kDa protein antibody was seen in cases 5 and 7, and scotopic and photopic ERG results were equally affected. Although case 6 exhibited a reduced cone response in the right eye, he did not have anti–46 kDa protein antibody. We found no specific correlation between laboratory data and ERG findings in this series of patients.

There are several limitations to our study. The first is its retrospective design and the small number of patients. The second limitation is that we were unable to identify the anti-retinal antibody. Considering that Shimazaki et al. reported the presence of anti-retinal antibodies in normal serum, the antibodies detected in the present study might not be the cause of a patient’s PR. Another limitation with regard to the anti-retinal antibody is that Western blot analysis and IHC were performed using mice retina. Although the human retina would be most suitable for the detection of autoantibodies, human samples were not available. Considering that a previous study indicated that the retina from other species can be a substitute for research as long as there is an appropriate control, we believe that the effect of using mice retina instead of human retina should be minimal. In fact, most previous studies have used bovine, pig, monkey, rat, and mouse retina, as well as human retina. A third limitation is the clinical diagnosis of PR. Although we screened for major genetic mutations and the presentation is not typical, we could...
not completely rule out genetic retinal degeneration in our cases.

In conclusion, we reported the clinical findings and experimental serum sample test results in eight patients with PR. Although there was variety in the morphologic changes depicted with SDOCT and in the targets of autoantibodies, the SDOCT and IHC findings showed some correlation. Spectral-domain OCT may be a potential tool for further investigation of the pathophysiology associated with PR.

Acknowledgments

Disclosure: Y. Makiyama, None; T. Kikuchi, None; A. Otani, None; A. Oishi, None; C. Guo, None; S. Nakagawa, None; K. Ogino, None; H. Kojima, None; M. Kurimoto, None; N. Yoshimura, None

References

36. Ogihoro H, Ogawa K, Maeda T, Maeda A, Maruyama I. Cancer-associated retinopathy induced by both anti-recoverin and...


