Clinical and Immunological Characterization of Paraneoplastic Retinopathy

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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, including fluorescein angiography (FAF), 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 perimeter 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 hypouautofluorescence 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

Autoimmune retinopathy (AIR) is a progressive retinal degeneration caused by autoimmune processes. These processes are considered to be mediated by autoantibodies directed to retinal proteins. AIR cases with underlying malignant or benign tumors are termed paraneoplastic retinopathies (PRs). This classification includes cancer-associated retinopathy (CAR), melanoma-associated retinopathy,1 and lymphoma-associated retinopathy.2 One hypothesized mechanism for the development of PR is the production of autoantibodies against common proteins expressed in both the neoplasm and the retina.3,4 Paraneoplastic retinopathy (PR) was first described in 1976 by Sawyer et al.5 Subsequent case reports classified characteristic clinical features, including a sudden and progressive loss of vision associated with photopsia, ring scotoma or concentric visual field defects, attenuated retinal arterioles, and abnormal electroretinography (ERG) findings.5 The presence of circulating serum autoantibodies specific to retinal antigens is necessary for a diagnosis of PR; however, this laboratory test is not available in most ophthalmological settings.5 Western blot analysis can be used to detect anti-retinal antibodies.7 However, the lack of standardization across studies makes it difficult to compare the results obtained by various authors.8–10 Recent progress in imaging technology has allowed us to detect detailed retinal changes and has provided insight into the pathologic mechanisms underlying retinal diseases. Optical coherence tomography (OCT), which allows for the noninvasive visualization of normal retina and of pathologic conditions, is now widely accepted as a standard tool in clinical practice.11 The currently available spectral-domain OCT (SDOCT) machines achieve resolution up to 5 μm; indeed, recent SDOCT devices allow for layer by layer evaluation of the retina.12,13 Previous studies14,15 showed that various imaging modalities can be used to strengthen a diagnosis of CAR or PR. However, there is limited information about the SDOCT findings associated with PRs. One of the objectives of this study was to identify any clinical characteristic features, including SDOCT findings, seen in patients with PR. We also investigated the association between clinical characteristics and serum sample test results (e.g., Western blot analysis and immunohistochem-
Characterization of Paraneoplastic Retinopathy

Patients were referred to our clinic for suspected PR or 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. 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. Informed consent was obtained from the participants. 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.

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.

<table>
<thead>
<tr>
<th>Case n/Sex/ Age at Diagnosis, y</th>
<th>Chief Complaint</th>
<th>Initial BCVA</th>
<th>FA Leakage</th>
<th>ERG</th>
<th>Tumor</th>
<th>History</th>
<th>New Discovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/M/85</td>
<td>Defect of VF</td>
<td>OD OS</td>
<td>VF</td>
<td></td>
<td>Subnormal in cone, NR in remainder</td>
<td>Bladder cancer and liposarcoma in mediastinum</td>
<td>SCLC</td>
</tr>
<tr>
<td>2/F/64</td>
<td>Defect of VF, metamorphopsia</td>
<td>0.4 0.1</td>
<td>Ring scotoma (R), central scotoma (L)</td>
<td>Yes</td>
<td>Subnormal</td>
<td>No specific finding</td>
<td>SCLC</td>
</tr>
<tr>
<td>3/M/57</td>
<td>Decrease in VA, contraction of VF</td>
<td>0.2 0.01</td>
<td>Concentric contraction (R), central scotoma (L)</td>
<td>Yes</td>
<td>Subnormal</td>
<td>Thymoma</td>
<td>No specific finding</td>
</tr>
<tr>
<td>4/F/81</td>
<td>Decrease in VA, photopsia</td>
<td>0.1 0.08</td>
<td>Central scotoma (both sides)</td>
<td>Yes</td>
<td>Subnormal</td>
<td>No specific finding</td>
<td>Thymoma</td>
</tr>
<tr>
<td>5/F/78</td>
<td>Decrease in VA, photopsia</td>
<td>0.2 0.08</td>
<td>Ring scotoma (R), island scotoma (L)</td>
<td>Yes</td>
<td>Subnormal</td>
<td>No specific finding</td>
<td>Pancreatic neuroendocrine neoplasm</td>
</tr>
<tr>
<td>6/M/59</td>
<td>Decrease in VA, night blindness</td>
<td>0.8 1.2</td>
<td>Island scotoma (R), not particular (L)</td>
<td>No</td>
<td>Subnormal (R), normal (L)</td>
<td>Renal cancer</td>
<td>Brain metastasis</td>
</tr>
<tr>
<td>7/M/61</td>
<td>Decrease in VA</td>
<td>0.3 0.2</td>
<td>Ring scotoma and concentric contraction (both sides)</td>
<td>Yes</td>
<td>NR</td>
<td>Lymphoma (40-y previously), renal cancer</td>
<td>No specific finding</td>
</tr>
<tr>
<td>8/F/68</td>
<td>Decrease in VA, photopsia</td>
<td>0.3 0.03</td>
<td>Central and ring scotoma (both sides)</td>
<td>No</td>
<td>Subnormal</td>
<td>No specific finding</td>
<td>Colon cancer</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, CA). In some cases, the retinal slices were coincubated with rhodamine-conjugated peanut antibody (Invitrogen, Carlsbad, CA). In some cases, the retinal sections were coincubated with rhodamine-conjugated peanut antibody (Invitrogen, Carlsbad, CA). 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 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 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, CNGA1, CNGB1, MERTK, PED6A, PED6B, PNR, RDH12, RGR, RBP1, SAG, TULP1, CRB, RPE65, USH2A, USH3A, LRAT, PROM1, and 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. Electroretinography revealed abnormalities in all cases, but the degree of impairment ranged from nonrecordable to slight changes in the cone response in one eye.

Vasculitis 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.

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Fluorescein angiography revealed a petaloid leakage in case 4.

FAF Findings

In most cases, abnormality of FAF was detected. Abnormal hyperautofluorescence and/or hypofluorescence was noted at presentation in all patients except cases 5 and 8 (Fig. 2). Although cases 5 and 8 displayed no detectable abnormality at

<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 3 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, RHOD, RGR, RLBP1, SAG, TULP1, CRB, RPE65, USH2A, USH3A, LRAT, PMOM1, 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
other systemic neoplasms, including bladder cancer, thymoma, liposarcoma, and renal cancer, have been associated with PR. Neuroendocrine carcinoma of the fallopian tube has also been reported as a cause of PR. To our knowledge, pancreatic neuroendocrine neoplasm has not been reported as a cause of PR.

In PR, autoantibodies against retinal antigens are considered to cause retinal dysfunction and retinal cell death. Anti-recoverin antibody, which recognizes a 25 kDa retinal protein found in rod and cone photoreceptors and in tumor cells, is the most studied autoantibody. Anti-recoverin autoantibodies bind to photoreceptor cells and trigger apoptosis. In the present study, anti-recoverin antibody was detected in only one patient (case 2), and the other patients had unidentified antibodies. To date, the protein targets of autoantibodies reported to cause PR include retinal alpha-enolase (46 kDa), arrestin (48 kDa), tubby-like protein 1 (78 kDa), heat shock cognate protein 70 (65 kDa), transducin beta (35 kDa), anti-carbonic anhydrase II (30 kDa), photoreceptor cell-specific nuclear receptor (46.5 kDa), interphotoreceptor retinoid-binding protein (145 kDa), and transient receptor potential cation channel, subfamily M, member 1 (TRPM1) (~180 or ~200 kDa). A number of retinal antigens that were not identified have been characterized by Western blot analysis. 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, SDOCT 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 “on response” bipolar 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 SDOCT might be associated with the presence of anti-photoreceptor or anti-RPE antibodies. Although this was an observational study and further investigations are needed, SDOCT 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,\textsuperscript{15} 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.\textsuperscript{15} 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.\textsuperscript{4} In fact, neoplasms were detected after the presentation of retinopathy in five of eight patients in the present series. Thus, it is important to diagnose PR in a timely fashion. The characteristics of PR in our study were similar to those of previously reported cases,\textsuperscript{15} including rapid, progressive, painless vision loss associated with photosensitivity that continued over a period ranging from weeks to months. In addition, patients with PR sometimes show asymmetric involvement in the right and left eyes, as shown in case 6. This is atypical among patients with inherited retinal degeneration. Although it is still a challenge to diagnose PR in many cases because of the variety of clinical appearances and the technical difficulty in detecting autoantibodies in serum,\textsuperscript{6} clinicians should be careful when faced with a patient manifesting rapid, atypical, or asymmetric retinal degeneration.

In addition to the difficulty in achieving an accurate PR diagnosis and despite the common use of steroids to treat PR, no definitive therapeutic protocol has been established.\textsuperscript{7} The prognosis is not good in many cases. In fact, five of 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).\textsuperscript{42} 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.\textsuperscript{1} 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.\textsuperscript{40} 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\textsuperscript{6} 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\textsuperscript{5,18,20,31,55,56,40,44,47,48} 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.

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