Review: Clinical and Pathological Aspects of Cancer-Associated Retinopathy (CAR)

Akiko Maeda, Tadao Maeda, Kei-ichi Ogawa, Ikuyo Maruyama, Takashi Nakagawa and Hiroshi Ohguro*

Department of Ophthalmology, Sapporo Medical University School of Medicine South 1, West 16, Chuo-ku, Sapporo, Hokkaido, 060-8543 Japan

Corresponding author: Hiroshi Ohguro

ABSTRACT

Cancer-associated retinopathy (CAR) is an ocular manifestation of paraneoplastic syndrome and is further classified into two distinct diseases, CAR and MAR (melanoma-associated retinopathy). CAR is associated with epithelial cancers, mostly lung small cell carcinoma, and is characterized by retinitis pigmentosa-like retinal degeneration, such as ring scotoma, night blindness, and lowering of a and b-waves in electroretinogram (ERG). Usually CAR can be found before an underlying primary cancer is diagnosed. In contrast, MAR is associated with cutaneous malignant melanoma and is characterized by the relatively sudden onset of photophobia, nyctalopia, and lowering of b-wave in ERG. Histopathological study has revealed that photoreceptor and bipolar degeneration are primarily involved in CAR and MAR, respectively. Both CAR and MAR are believed to result from an autoimmune basis. In CAR, a calcium binding protein called recoverin, heat shock cognate protein 70 (hsc 70), and other proteins are identified as retinal autoantigens, while the retinal antigens in MAR have not yet been identified.

In this text, we review up-to-date information about the clinical and pathological aspects of these diseases.

Key words: CAR, MAR, Recoverin, hsc 70, Autoimmunity

INTRODUCTION

Several lines of evidence have revealed that the central nervous system could be a target of the remote effect of a malignancy, and a variety of paraneoplastic syndromes, such as the Lambert-Eaton myasthenic syndrome,

*To whom requests for reprints should be addressed

paraneoplastic cerebellar degeneration and paraneoplastic sensory neuropathy have been reported (summarized in Table 1) (1). The pathogenesis of these

Table 1 Paraneoplastic Syndromes

Paraneoplastic Syndrome	Associated Malignancy	Associated Antibodies
CAR	SCLC, less commonly cervix	Anti-recoverin, Anti-HSC70
MAR	Cutaneous malignant melanoma	Anti-retinal bipollar cell
Cortical cerebellar degeneration	SCLC, breast, gynecologic, Hodgkin's	Anti-Yo (anti-Purkinje cell)
Encephalomyelitis/pure sensory neuropathy	SCLC, occasionally Hodgkin's	Anti-Hu (ANNA-1)
Limbic encephalitis		
Myelitis		
Brainstem encephalitis		
Cerebellar dysfunction		
Pure sensory neuropathy		
Progressive sensorimotor neuropathy	SCLC, breast, and other tumors	
Guillain-Barre	Hodgkin's disease	
Relapsing-remitting neuropathy	Lung. breast, lymphoma, myeloma	
Subacute motor neuropathy	Hodgkin's and non-Hodgkin's lymphoma	
Opsoclonus-myoclonus	Neuroblastoma in children Lung and occasionally breast in adults	Anti-Ri (ANNA-2)
Lambert-Eaton syndrome	SCLC, breast, gastrointestinal, others	Antibodies to voltage-gated calcium channels
Inflammatory myopathy	Breast, lung, ovarian, gastric	

SCLC: small cell carcinoma of the lung

disorders appears to be an immune reaction toward antigens shared by the cancer and the nervous system, but neither removal of the autoantibody nor treatment of the cancer is effective. In the field of ophthalmology, paraneoplastic retinopathy is known to be associated with patients with malignamcies. So far two types of the diseases have been described, cancer-associated retinopathy (CAR), and melanoma-associated retinopathy (MAR). CAR is characterized by sudden and progressive visual loss, ring scotoma, photophobia, impairment of dark adaptation, and abnormalities of the a and b-waves of the electroretinogram (ERG) like retinitis pigmentosa. Among the underlying primary cancers, small cell carcinoma has been reported most frequently. In most cases, CAR is diagnosed before an underlying primary cancer is discovered.

Cancer-associated retinopathy

1. Clinical aspects of CAR

Sawyer et al. first reported three patients with malignancies (65 year-old male: lung adenocarcinoma, 76 year-old female: lung small cell carcinoma, and 62 year-old female: endometrial stromal sarcoma) involving lost visual acuity by idiopathic retinal degeneration (2). Histopathological examination using electron microscopy demonstrated destruction of photoreceptor cells and granule cells in the retina of these patients (3). In this report, they emphasized that these malignancies were clinically detected after the visual symptomes appeared. Keltner et al. reported similar clinical cases with serum autoantibodies reacted with retinal cells by immunocytochemical study. Therefore, they suggest that serum autoantibodies to retinal antigens may contribute to the retinal degeneration (4). Grunwald et al. and Korngruth et al. reported additional cases and found that several retinal proteins including 23 kDa protein, 65 kDa, 145 kDa and 205 kDa were detected as autoantigens by western blot analysis (5-6). Until now, over 50 CAR cases have been reported. Jacobson et al. proposed the criteria of diagnosis of CAR as photosensitivity, ring scotomatous visual field loss, attenuated retinal arteriole caliber, and presence of serum autoantibodies toward retinal antigens (7).

In terms of Japanese cases of paraneoplastic syndromes related to the field of ophthalmology, Ohhira et al. first reported a case of paraneoplastic optic neuropathy with thyroid cancer in 1990 (8). In 1991, Nagai et al. reported a case of paraneoplastic opsoclonus with small cell carcinoma of the lung (9). The following are some case reports of CAR in Japan: Ohhira et al. (1990), Nagai et al. (1991), Suzuki et al. (1996), Ohkawa et al. (1996) and our group (8-12).

2. Retinal autoantigens recognized by sera from CAR patients

Polans et al. and Thirkill et al. independently identified a retinal calcium binding regulatory protein called recoverin, which is a retinal autoantigen recognized by serum autoantibodies obtained from CAR patients (13-14). Recoverin is known to be functionally involved in the adaptation to dark and light by regulating the activity of rhodopsin kinase and PrP2A (15). Interestingly, Polans et al. and Yamaji et al. found that recoverin has been identified as being expressed in cancer cells of CAR patients and its cell lines (16-17). Therefore, autoimmune reaction is triggered by recoverin aberrantly expressed in tumor cells which then in turn mediate retinal degeneration. In addition, it was also reported that other retinal antigens including 65 kDa protein, enolase (46 kDa protein), and neurofilament (58-62 kDa, 145 kDa and 205 kDa proteins) were also recognized by CAR patient's sera (Tabel 2) (5-7, 10-14, 16-23). Although the relationship among these antigens during

the pathogenesis of CAR is still unknown, these observations suggest that several retinal antigens may be involved in the pathogenesis of CAR.

Most recently, we analyzed sera from four Japanese patients with CAR associated with different types of cancers (2 patients; lung small cell cancer, 1 patient; lung adenocarcinoma, 1 patient; gastric cancer) and found that the sera (1:500 dilution) from all four commonly reacted with both 23 kDa and 65 kDa retinal soluble proteins (24). However such immunoreactivities were not detected in any control subjects including 20 cancerous patients at the same serum dilution in analysis by western blot (Fig 1). The 23 kDa protein has

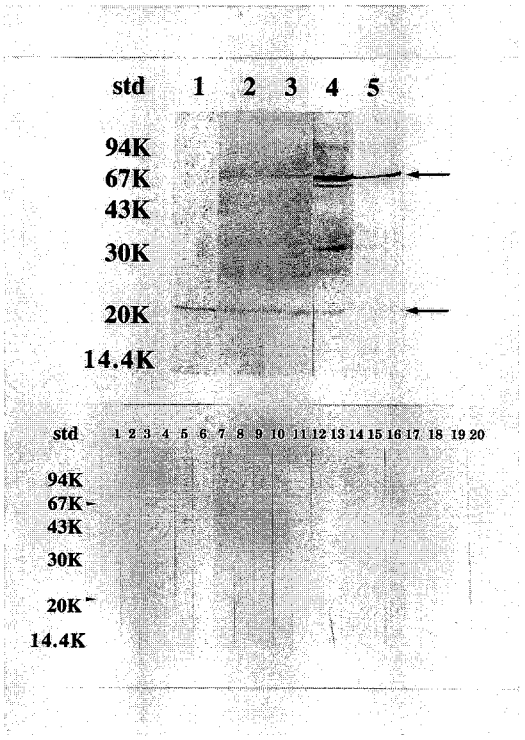
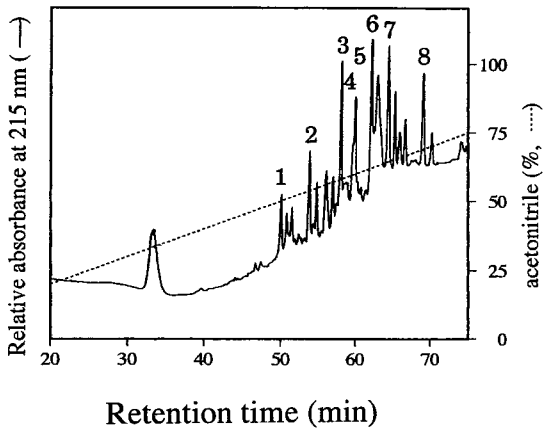


Figure 1 Western blot analysis of sera from four patients with cancer-associate reinopathy and from 20 non-CAR patients with malignancies.

Upper panel; Anti-recoverin serum (lane 1, 1:3000 dilution) and sera from CAR patients (#1-4; lanes 2-5, 1:500 dilution) were tested with bovine retinal soluble extract. The protocol of immunoblotting is described in Materials and Methods section. 23 kDa and 65 kDa proteins were commonly proved by patients' sera (indicated by arrows).

Lower panel; Sera from non-CAR patients were tested with bovine retinal soluble extract. The type of cancer, age, and sex, respectively, in each lane are as follows; small-cell lung carcinoma, lanes 1, 66 M; 2, 60 M; 3, 68 M; 4, 58 F; 5, 70F; 6, 68 F; 7, 62 M; 8, 65 M; 9, 69F; and 10, 63 F; lung adenocarcinoma lanes 11, 70 M; 12, 78 M; 13, 62 M; 14, 65 M; and 15, 60 M; gastric cancer, lanes 16, 65 F; 17, 60 F; 18, 68 M; 19, 70 F; and 20, 69 F. The positions of the 23 kDa and 65 kDa proteins are indicated by arrows.

identical molecular masses to that of recoverin because the immunoreactivity against recoverin using anti-recoverin antibody migrated to the identical position of the 23 kDa band (Fig.1 Upper panel; lanes 1 and lanes 2-5). The in-gel digestion of partially purified 65 kDa retinal antigen from fresh bovine retinas with endoproteinase Lys C and the peptide sequence analysis identified heat shock cognate protein 70 (hsc 70) (fig 2).



1 MSKGPAVGID LGTTYSCVGV FQHGKVEIIA NDQGNRTTPS YVAFTDTERL IGDAAKNQVA
P8
61 MNPTNTVFDA KRLIGRRFDD AVVQSDMKHW PFMVVNDAGR PKVQVEYKGE TKSFYPEEVS
121 SMVLTKMKEI AEAYLGKTVT NAVVTVPAYF NDSQRQATKD AGTIAGLNVL RIINEPTAAA
P6
181 IAYGLDKKVG AERNVLIFDL GGGTFDVSIL TIEDGIFEVK STAGDTHLGG EDFDNRMVNH
P2
241 FIAEFKRKHK KDISENKRAV RRLRTACERA KRTLSSSTQA SIEIDSLYEG IDFYTSITRA
301 RFEELNADLF RGTLDPVEKA LRDAKLDKSQ IHDIVLVGGS TRIPKIQKLL QDFFNGKELN
P4
361 KSINPDEAVA YGAAVQAAIL SGDKSENVQD LLLLDVTPLS LGIETAGGVM TVLIKRNTTI
P5
421 PTKQTQTFTT YSDNQPGVLI QVYEGERAMT KDNNLLGKFE LTGIPPAPRG VPQIEVTFDI
481 DANGILNVSA VDKSTGKENK ITITNDKGRL SKEDIERMVQ EAEKYKAEDE KQRDKVSSKN
P6
541 SLKSYAFNMK ATVEDEKLQG KINDEDKQKI LDKCNEIINW LDKNQTAEKE EFEHQQKELE
601 KVCNPIITKL YQSAGGMPGG MPGGMPGGFP GGGAPPSGGA SSGPTIEEVD
P3

Figure 2 Identification of 65 kDa autoantigen recognized by CAR patients.
Upper panel; HPLC separation of proteolytic peptides of 65 kDa protein after in-gel digestion by endoproteinase Lys C. The immunoreactive 65 kDa protein to CAR patient's serum in 2D gels was treated with endoproteinase Lys C. Digested peptides were separated from each other by reverse-phase HPLC as described in Materials and Methods. Major peaks designated as 1 through 8 were subjected to Edman sequencing analysis.
Lower panel; Amino Acid sequence of 65 kDa proteolytic peptides and their homology with heat shock cognate protein 70. The amino acid sequences of eight proteolytic peptides from 65 kDa protein are indicated as bold letters with underlines in the bovine heat shock cognate 70 protein sequence (35). The peptide designations (P1-P8) as bold correspond with those in upper panel.

3. Possible roles of recoverin and hsc 70 in the pathogenesis of CAR

Among the retinal autoantigens reported so far, many authors agree that recoverin is a target antigen of autoimmune response in CAR for the following reasons. 1) recoverin is a retinal specific protein. 2) immunoreactivity toward recoverin is unique in CAR patients. 3) recoverin is expressed in cancer cells and its cell lines from CAR patients. In addition, our data strongly suggested that hsc 70 is also involved in the molecular pathology of CAR with recoverin. In fact, among the reports presenting the data of western blot analysis, both 23 kDa (recoverin) and 65 kDa protein were most frequently identified as immunoreactive bands, suggesting that the 65 kDa antigen is most likely to be hsc 70.

In terms of the relationship between recoverin and hsc 70, we do not know definitely, but can speculate that an autoimmune response toward recoverin might be essentially required for photoreceptor degeneration, and response toward hsc 70 might somehow assist in this process. To test our hypothesis, western blots were performed using different serum dilutions (100, 200, 500, 1000, 2000, 4000 and 8000 times) and the maximum dilutions to detect either recoverin or hsc 70 were plotted (in figure 3) in order to

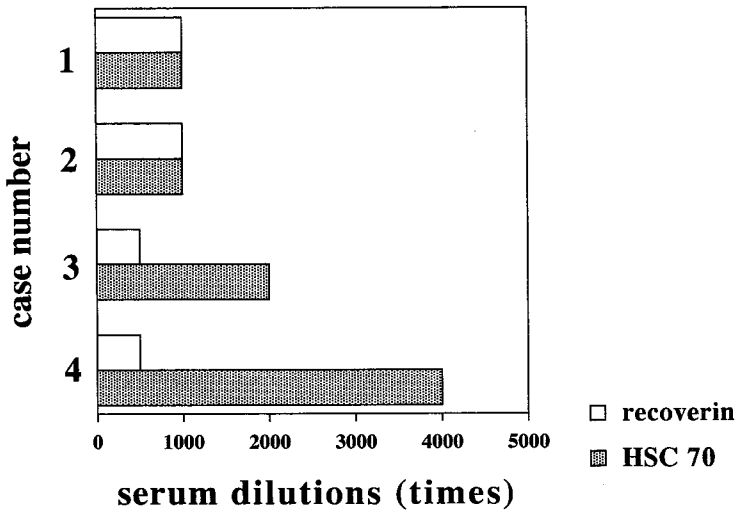


Figure 3 Maximum serum dilutions to detect either recoverin or hsc 70 in western blot analysis.

Western blots were performed using different serum dilutions (100, 200, 500, 1000, 2000, 4000 and 8000 times) and the maximum dilutions to detect the recoverin (open columns) or hsc 70 (shaded columns) are plotted.

estimate the relationship of autoimmune responses between recoverin and hsc 70 among our patients. The immunoreactivities against recoverin and hsc 70 were almost identical in cases 1 and 2, while the immunoreactivities were much stronger in hsc 70 than recoverin in cases 3 and 4. If our speculation is true, we could reasonably understand that retinal degeneration can be caused by autoimmune response to recoverin, even if its titers are relatively low, in the presence of high titers of anti-hsc 70 antibody, as shown in our cases 3 and 4. Alternatively, if the titers of anti-recoverin antibody are high enough, anti-hsc 70 antibody may not be necessary for retinal degeneration, as shown in some previous CAR cases in which only 23 kDa antigen was detected (Table 2).

Table 2 Western blot analysis in the previous reports of CAR

Author, year	ref. No.	pt. No.	type of cancer	western blot (kDa)
Korngruth, 1982	3	1	small-cell, lung	20/65
Grunwald, 1985	7	2	1) small-cell, lung	23/65
			2) small-cell, lung	145/205
Thirkill, 1987	9	4	1) cervical cancer	23
			2) non small-cell, lung	23/48
			3) small-cell, lung	23/48
			4) small-cell, lung	23
Crofts, 1988	10	1	endometrial cancer	50
Thirkill, 1989	11	1	small-cell, lung	23/65'[a)]
Jacobson, 1990	1	2	1) small-cell, lung	1) 23/48
			2) adeno, lung	2) 23
Keltner, 1992	12	1	small-cell, lung	23/65
Thirkill, 1993	13	10	various	many bands[b)]
Adamus, 1993	18	1	small-cell, lung	23/65
Polans, 1991, 1995	1620	3	1) small-cell, lung	23/65
			2) small-cell, lung	23/65
			3) ovarian cancer	23/65
Suzuki, 1996	14	1	small cell, lung	62
Ohkawa, 1996	15	1	endometrial cancer	34
Adamus, 1996	22	8	various	46
Ohguro, 1998	24	4	1) small-cell, lung	23/65
			2) adeno, lung	23/65
			3) stomach cancer	23/65
			4) small-cell, lung	23/65

a) tumor cell 65 kDa was recognized by patient's serum

b) western blots revealed many immunoreactive bands, but their molecular masses could not be estimated because molecular standards were not shown.

combination of 23 kDa and 65 kDa is shown with a underline.

Hsc 70 is a member of the heat shock protein 70 family of proteins which were originally identified as being synthesized following a variety of cellular stresses, and which are present in normal unstressed cells, in which they play important roles as chaperons: 1) to assist in the correct folding and intracellular transport of proteins, 2) maintaining proteins in an active form until required and 3) in protein degradation (fig 4) (25-29). In addition to

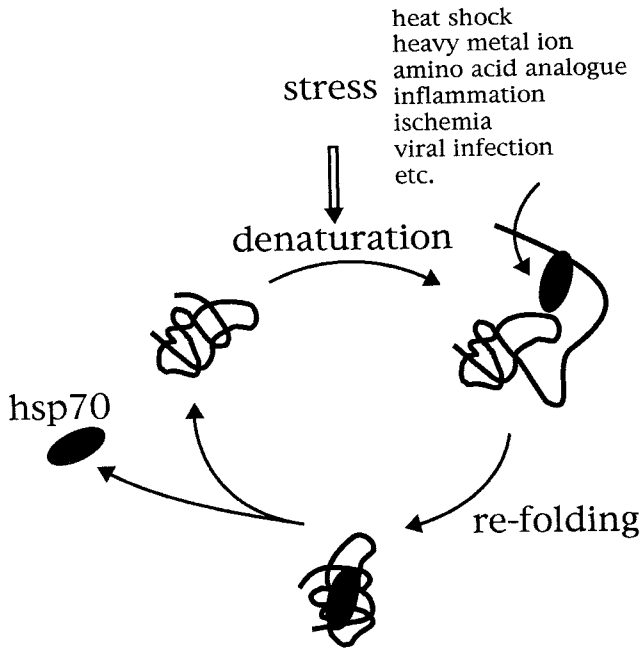


Figure 4 The possible roles of heat shock protein as molecular chaperons

this, elevated levels of hsps in the peripheral blood mononuclear cells (30) or serum autoantibodies against hsps have been identified in patients with several autoimmune diseases, such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA) and viral diseases (31-33). With regard to the autoantibody production, a molecular mimicry mechanism has been suggested as an underlying factor since hsps in eukaryote share homology with certain immunodominant proteins of infectious microorganisms. Although it is still unknown how such autoimmune reactions affect the onset and course of diseases, the above observations suggest that autoimmune response against hsps might play pivotal roles in the pathophysiology of autoimmune diseases, including CAR. These observation suggest that the presence of autoantibody against 65 kDa protein in some none CAR subjects does not conflict with our speculation that hsc 70 is related with CAR.

4. Clinical and pathological aspects of MAR

Melanoma-associated retinopathy (MAR) described in patients with cutaneous malignant melanoma is characterized by a progressive retinal degeneration with night blindness, a sensation of shimmering light, evoked dark-adapted thresholds, and the finding of a preserved a-wave with absent or diminished b-wave in the ERG. Clinical aspects of CAR and MAR are summarized in Table 3. Milam et al. first demonstrated that sera from MAR

Table 3 Clinical and pathological aspects in CAR and MAR

	CAR	MAR
Legion	Photoreceptor	Retinal bipolar cell
ERG Abnormality	a and b-wave	b-wave
Associated Malignancy	Lung , cervix, stomach e.t.c.	Cutaneous malignant melanoma
Associated Antibodies	Anti-recoverin, Anti-HSC 70	Anti-retinal bipolar cell

ERG: electroretinogram

patients specifically reacted with retinal bipolar cells detected by immunochemical studies (34). Therefore both the clinical electrophysiological and immunohistochemical findings suggest that retinal degeneration in MAR is caused primarily by dysfunction of retinal second neuron cells (retinal bipolar cells) resulting from an autoimmune basis (fig 5). However, unlike CAR,

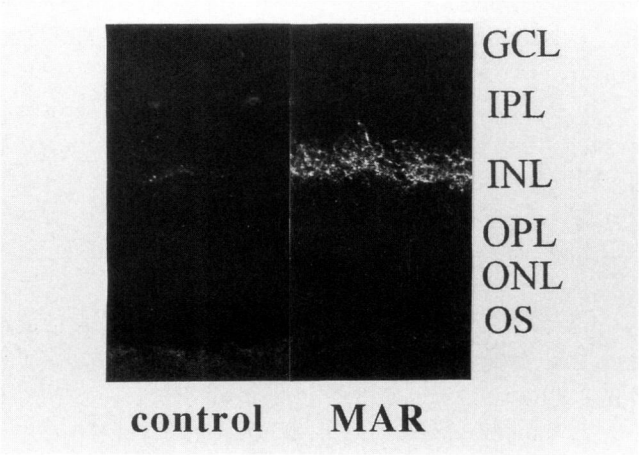


Figure 5 Detection of the presence of MAR antigen using immnocytochemistry.
Cyostat sections of unfixed rat retina processed for indirect immunofluorescence. Sections processed with MAR patient's IgG (1:150) preincubated with ROS membranes (rignt). GCL, layer of the ganglion cells; IPL, inner plexiform layer; INL, inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer; OS, outer segment.

the specific retinal antigen molecules in MAR have not been identified yet, because routine biochemical approaches to detect the presence of the MAR antigen, such as western blotting and ELISA were not successful.

CONCLUSION

There are two types of retinopathy, cancer-associated retinopathy (CAR), and melanoma-associated retinopathy (MAR), associated with malignancies resulting from an autoimmune basis. CAR is associated with lung small cell carcinoma and other epithelial cancers, and is characterized by retinitis pigmentosa-like retinal degeneration. Sera from CAR patients reacted with retinal calcium binding protein called recoverin and heat shock cognate protein 70 (hsc 70). On the other hand, MAR is associated with cutaneous malignant melanoma and sera of the patients contain autoantibodies against a melanoma antigen which cross react with bipolar cells of the retina.

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