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Low titre autoantibodies against recoverin in sera of patients with small cell lung cancer but without a loss of vision

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Abstract

To date, many authors have described the presence of autoantibodies against various neuronal proteins, paraneoplastic antigens (PNA), in a serum of patients with different kinds of malignant tumors located outside the nervous system. These autoantibodies may cross-react with the corresponding PNA or their epitops present in neurons and thus initiate the development of a variety of neurological disorders, paraneoplastic syndromes (PNS), even though the primary tumor and its metastases have not invaded the nervous system. Cancer-associated retinopathy (CAR) is a rare ocular PNS induced by autoantibodies against several retinal antigens, one of which is a photoreceptor calcium-binding protein, recoverin. Only several CAR patients with a few kinds of cancer (endothelial carcinoma, breast cancer, epithelial ovarian carcinoma) have so far been found to contain autoantibodies against recoverin in their sera. As for lung cancer, the majority of CAR cases mediated by anti-recoverin autoantibodies have been revealed in patients with the most malignant lung cancer, small cell lung carcinoma (SCLC), and only one similar case has been described for a patient with non-small lung carcinoma. The common feature of all these anti-recoverin-positive patients, irrespective of the type of cancer, is the presence of both the CAR syndrome and high titres (as a rule, more than 1:1000) of the underlying autoantibodies in their serum. In this study, we have used recombinant myristoylated recoverin to screen serum samples of 50 patients with SCLC by Western blot and revealed 5 individuals

Abbreviations: CAR, cancer-associated retinopathy; PNS, paraneoplastic neurological syndromes; RAbs, anti-recoverin autoantibodies; SCLC, small cell lung carcinoma.

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with low titres of anti-recoverin antibodies, who have no manifestation of a loss of vision. To our knowledge, this is the first report on the presence of low titre autoantibodies against recoverin in a serum of patients with cancer, but without visual dysfunction.

Keywords: Small cell lung carcinoma; Paraneoplastic neurological syndrome; Autoimmune; Cancer-associated retinopathy; Paraneoplastic autoantigen; Recoverin

1. Introduction

Paraneoplastic neurological syndromes (PNS) are rare neurological disorders that are related to cancer, even though the primary tumor and its metastases have not invaded the nervous system. PNS are thought to be autoimmune diseases that develop when malignant tumors express proteins, paraneoplastic antigens (PNA), which are normally present only in neurons. PNA expression outside the nervous system triggers the host's immune response toward the corresponding PNA or their epitops present in neurons, resulting in the development of PNS [1]. One of the PNS is cancer-associated retinopathy (CAR), a paraneoplastic ocular disorder characterized by progressive retinal degeneration and as a result by a loss of vision [2].

The first indication that the retinal protein with an apparent molecular weight 23-kDa could be 'a CAR antigen' was obtained by Thirkill [2]. More recently [3] the CAR antigen was shown to be homologous to a retinal Ca^{2+}-binding protein, p26 or recoverin, almost simultaneously discovered by our and other groups [4–6]. Four potential Ca^{2+}-binding sites of the EF-hand type are present in a recoverin molecule of which only two (the 2nd and the 3rd ones) are capable of binding calcium ions [5,7]. The immunopathogenic region of recoverin (residues 64–70) is contained within the 2nd EF-hand structure [8].

Only a small number of CAR patients with a few kinds of cancer (endothelial carcinoma [9], breast cancer [10], epithelial ovarian carcinoma [11]) have so far been found to contain anti-recoverin autoantibodies (RAbs) in their serum. As for lung cancers, the majority of RAbs-positive CAR cases have been revealed in patients with the most malignant lung tumor of neuroendocrine origin, small cell lung carcinoma (SCLC) [2,3,8,12,13]; only one similar case has been described for a patient with non-small lung carcinoma [14], the tumor of bronchial epithelial origin.

It should be stressed that the common feature of all anti-recoverin-positive patients described earlier, irrespective of the type of cancer, is the presence of both the CAR syndrome and high titres (as a rule, more than 1:1000) of the underlying autoantibodies in their serum [2,3,9–14]. In this study, we have used recombinant myristoylated recoverin to screen serum samples of 50 patients with SCLC by Western blot and have revealed five patients with low titres — from 1:20 to 1:160 — of anti-recoverin antibodies, who have no manifestation of a loss of vision. None of sera of the other 45 patients with SCLC and of 30 healthy individuals contain RAbs.

To our knowledge, this is the first report on the presence of low titre autoantibodies against recoverin in a serum of patients with cancer, but without any evidence of visual dysfunction.

2. Materials and methods

Serum samples were prepared from whole blood of 50 patients with SCLC and of 30 healthy individuals and stored at -70°C. Normal pooled human sera were used as a negative control.

Recombinant myristoylated recoverin was obtained according to [15] and was used in Western blot analysis (see below). Rabbit polyclonal (monospecific) anti-recoverin antibodies were prepared as described in [4,5] and were used as a positive control in Western blot analysis.

Western blot analysis was performed with rocking at room temperature after SDS-PAGE [16] of recombinant myristoylated recoverin (2 μg per track) in 12% gel. The gel slabs were electrotransferred to Hybond-C Extra nitrocellulose mem-

branes (Amersham) in Tris–glycine–methanol buffer, pH 8.3. Then nonspecific sites were saturated by incubation for 1.5 h with 10% solution of delipidated powdered milk and membranes were incubated for 12 h with: (i) a serum of SCLC patients; (ii) a serum of healthy individuals as a negative control (initial dilution was 1:20 in both cases); and (iii) rabbit's polyclonal (monospecific) anti-recoverin antibodies (1 μg/ml) as a positive control. All incubations were performed in buffer B (20 mM Tris–HCl, pH 7.4, 500 mM NaCl, 0.05% TWEEN-20, 1 mM $CaCl_2$). Blots were rinsed three times (each for 10 min) with buffer B, incubated for 1.5 h with sheep anti-human IgG peroxidase conjugate (Amersham) at dilution of 1:500 with buffer B, rinsed again with 50 mM Tris–HCl, pH 7.6 and finally incubated with 10 mM 3,3′-diaminobenzidine as a substrate in 0.01% hydrogen peroxide.

3. Results

In the process of raising antibodies against recoverin, we measured a level of the antibodies in the serum of two rabbits after recoverin injection and found that the maximal levels were different in each animal. Investigation of an eye bottom and light microscopy of retina slices detected retinal degeneration in the rabbit with a high titre of the antibodies, whereas the eye characteristics of the rabbit with the low titres did not differ from those of the control animals (data not shown). The finding that anti-recoverin antibodies circulated in the blood stream of the experimental animal without causing retina degeneration stimulated us to screen serum samples of patients with SCLC irrespective of the presence of the CAR syndrome in them.

We have screened 50 patients with diagnosis SCLC and revealed five patients with RAbs in their sera before treatment (Fig. 1 and Table 1); in none of the cases a loss of vision has been manifested. When 'exogeneous' recoverin was used as a competitor for RAbs binding to recoverin on the blot, the intensity of the reaction was supressed in an exogeneous recoverin-dependent manner (not shown). None of serum samples of the other 45 patients with SCLC and of 30 healthy individuals investigated as a negative control contain RAbs.

Determination of the RAbs titres in the RAbs-positive patients mentioned gave values in the range between 1:20 and 1:160. It is interesting to note that chemotherapy caused disappearance of RAbs from the blood stream of 3 patients investigated. In one case (patient coded 'SCLC-4'), when the analysis was repeated 3 months after the last course of chemotherapy, RAbs appeared again in the patient's serum (see Table 1).

The example of the RAbs titre determination in patient SCLC-4 is shown in Fig. 2. One can see that the intensity of staining of the recoverin bands decreases with the increase of the serum dilution. The band can be detected at the dilution between 1:10 and 1:80, but it fully disappears at further dilution; the titre of RAbs in this case is estimated at 1:40.

4. Case report

In all five RAbs-positive cases revealed, the diagnosis of the extensive stage of SCLC was established by physical examination, plain chest radiography, bronchoscopy with brushed biopsy with the subsequent histological and cytology analyses. The visual acuity of all the patients remained immutable in comparison with that before the disease and during the whole observation period. However, only one patient coded as

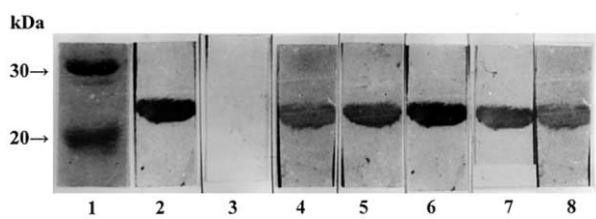

Fig. 1. Immunoblotting of sera (dilution 1:20) of RAbs-positive patients. 1, blot of standard proteins stained with Panceau S. 2,3, control samples: blots of recombinant myristoylated recoverin stained with (2) rabbit polyclonal (monospecific) anti-recoverin antibodies (1 μg/ml) or with (3) a normal human serum (1:20). 4–8, blots of recombinant myristoylated recoverin stained with a serum of patients SCLC-1 (4), SCLC-2 (5), SCLC-3 (6), SCLC-4 (7) and SCLC-5 (8).

Table 1
RAbs titres in five RAbs-positive patients with SCLC[a]

Patient	Initial analysis (before treatment)		Subsequent analyses (after treatment)	
	Date	Titre	Date	Titre
SCLC-1	15.01.1999	1:160		No data[d]
SCLC-2	27.05.1999	1:160		No data[d]
SCLC-3	12.06.2000	1:80	27.07.2000	0[b,d]
SCLC-4	16.06.2000	1:40	05.09.2000	0[b]
			29.11.2000	1:40[c]
SCLC-5	22.09.2000	1:20	06.12.2000	0[b]

[a] Extensive stages of SCLC.
[b] Several days after chemotherapy.
[c] A total of 2.5 months after chemotherapy.
[d] The patient died.

SCLC-4 was available for ophtalmological investigation; three patients (SCLC-1, 2 and 3) died and one patient (SCLC-5) was not available for ophtalmological investigation due to bronchial asthma. That is why the description of patient SCLC-4 is the only one presented in Section 4.

Patient SCLC-4, a 58-year-old man was admitted to the Pulmonology Research Institute, Moscow in June 2000. He had been smoking 40 cigarettes per day for 48 years. First symptoms of the disease appeared 3 months before the diagnosis of SCLC. He complained of a cough with mucopurulent sputum, fever about 37°C, exertional dyspnea, a loss of voice and progressive weakness. At the moment of the 1st blood sampling for the RAbs analysis, the diagnosis was the extensive stage of SCLC. The tumor obturated the left upper lobe bronchus involving the left main bronchus and did not reach the carina to 3 cm. There were methastases into ipsilateral hilar lymphatic nodes, ipsilateral and contralateral mediastinal nodes, ipsilateral supraclavicular lymphatic nodes and pancreas. The patient had no loss of vision before the disease and during the whole observation period. Ophtalmological investigation was carried out about 5 months after the diagnosis at the moment of the last serum sampling when the RAbs titre was equal to 1:40 (see Table 1). The patient's visual acuity was determined as OD 0.5 and OS 1.0. Ophtalmoscopy revealed the retina and optic nerve to be normal in appearance. Reaction of pupils to light was normal. The patient had no narrowing of the visual field (Fig. 3). Thus, the manifestation of retina degeneration was not found.

5. Discussion

The symptoms of PNS can be manifested long before the clinical diagnosis of the underlying tumor, thus enabling the clinician to predict the future development of a particular cancer [17]. It is postulated that the reason for the PNS development is an expression of PNA in the tumor localized outside the nervous system that triggers an immune response toward the corresponding PNA present in neurons [1]. PNS are shown to be

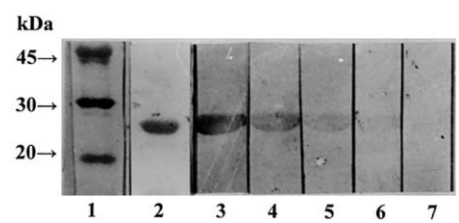

Fig. 2. Immunoblotting of a serum (dilutions from 1:10 to 1:160) of RAbs-positive patient SCLC-4. 1, blot of standard proteins stained with Panceau S. 2, a control sample: blot of recombinant myristoylated recoverin stained with rabbit polyclonal (monospecific) anti-recoverin antibodies (1 μg/ml). 3-7, blots of recombinant myristoylated recoverin stained with a serum of patient SCLC-4 at dilutions of 1:10 (3), 1:20 (4), 1:40 (5), 1:80 (6) and 1:160 (7).

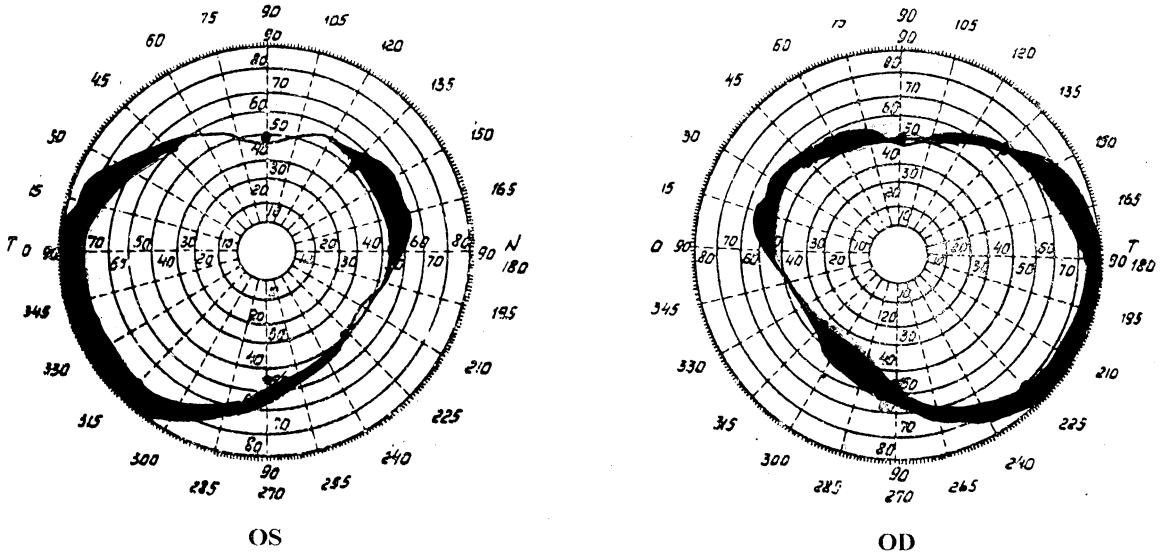

Fig. 3. Goldmann visual fields of RAbs-positive patient SCLC-4.

associated with different tumors, one of which is the most malignant lung tumor, SCLC. Although a number of PNS is developed in patients with SCLC, their frequency is quite rare: the total amount of various PNS associated with SCLC is estimated at 4% [18]. One of the most extensively studied groups of PNA is a family of Hu antigens, autoantibodies against which underlie a multifocal neurological disease, paraneoplastic sensory neuropathy [19]. The frequency of PNS in anti-Hu-positive patients with SCLC having high titres of the autoantibodies and corresponding PNS, is equal only to 1% [20]. However, SCLC patients with low titres of anti-Hu autoantibodies and without PNS have been found, and the frequency of these cases lying in the range of 15–20% [21], is far above that of the high titre anti-Hu cases. The presence of anti-PNA antibodies in the absence of PNS syndromes has also been demonstrated for Yo and Ri antigens, but here autoantibodies were detected only in 2–4% of the patients [22].In regard to recoverin, to our knowledge, this study is the first one to describe SCLC patients (as well as those with other cancers) when low titres of RAbs have been revealed in the patients' serum without any evidence of visual dysfunction in them. So far we have found five patients of this type among 50 SCLC patients investigated, whereas sera of the other 45 patients with SCLC and of 30 healthy individuals have no RAbs. Thus, the frequency of the RAbs low titre cases can be preliminary estimated as 10%. It should be stressed that this number corresponds to the measurements made before the treatment because chemotherapy causes disappearance of RAbs from the blood stream (see Table 1) presumably due to repression of the patients' immune activity.

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