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Antirecoverin autoantibodies in the patient with non-small cell lung cancer but without cancer-associated retinopathy

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Summary The goal of the present study was to analyze serum and tumor tissue of a patient with non-small cell lung cancer (NSCLC) for the presence of autoantibodies against recoverin (anti-Rc) and recoverin expression, correspondingly. Using immunoblotting with recombinant recoverin as an antigen, we have detected anti-Rc in serum of the patient. At the same time, the patient did not manifest any signs of cancer-associated retinopathy (CAR). Polyclonal (monospecific) antibodies against recoverin used for immunohistochemical analysis of the patient's tumor revealed recoverin expression in the tumor sections. To our knowledge, this is the first case of the presence of serum anti-Rc in NSCLC patients in the absence of paraneoplastic retina degeneration.

1. Introduction

Paraneoplastic antigens are neuronal proteins which can also be expressed in transformed cells outside the nervous system. The aberrant expression of these antigens is believed to be the cause of a number of paraneoplastic neurological syndromes (for a review, see refs. [1–3]). One of paraneoplastic antigens, Ca^{2+}-binding protein recoverin, is normally specific for retina [4,5], but it can also be detected in tissues different from retina providing their malignant transformation [6–11]. A number of patients with small cell lung carcinoma (SCLC) were found to have serum autoantibodies against recoverin (anti-Rc) [6,7,12–16]. In contrast to SCLC, only one anti-Rc-positive patient with another kind of lung cancer, non-small cell lung carcinoma (NSCLC), was known so far [11]. For a time, serum anti-Rc were only detected in patients when they had both lung cancers and cancer-associated retinopathy (CAR) [6–15]. However,

Abbreviations: anti-Rc, autoantibodies against recoverin; CAR, cancer-associated retinopathy; NSCLC, non-small cell lung carcinoma; SCLC, small cell lung carcinoma.

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we have recently revealed several patients with SCLC who had serum anti-Rc, but had no manifestation of the CAR-syndrome [16]. In this work, we used recombinant recoverin to reveal anti-Rc in serum of a patient with NSCLC. In addition, we analyzed paraffin tumor sections of the patient for recoverin expression using antibodies against recoverin.

2. The patient and methods

A 69-year-old male with a history of chronic obstructive pulmonary disease had been smoking 20 cigarettes per day for 59 years. He gave up smoking in 1997 when he underwent lobectomy of the right lower lobe of the lung due to squamous cell carcinoma (pT1N0M0). During the next 2 years, the patient felt satisfactorily and continued to work. In June 1999, fibreoptic bronchoscopy detected a tumor at the right lower lobe stump of 2 cm in diameter. Histological analysis of the tumor confirmed the relapse of a poor differentiated squamous cell carcinoma. Chest computer tomography revealed metastases in the right ipsilateral mediastinal lymph nodes. As the patient refused to undergo any anti-cancer treatment, he got only a supportive care before the next hospitalization in September 2000. Between hospitalizations in 1999 and 2000, the state of the patient's health was gradually deteriorating: he lost 7 kg (from 67 to 60 kg), there appeared a pain in the back. Computer tomography and fibreoptic bronchoscopy detected the development of the middle lobe atelectasis and metastases in the ipsilateral mediastinal lymph nodes, spine radiography and bone scintigraphy revealed vertebral body metastases (Th6-Th8), i.e. the objective examination confirmed the progressive disease (cT2N2M1). In addition to supportive care, the patient received prednisolone (25 mg per os one time a day for 10 days) for treatment of COPD exacerbation. He died of progressive lung cancer in January 2001.

2.1. Immunoblotting of serum samples

Serum samples were prepared from whole blood of the NSCLC patient and stored at $-70\,^{\circ}C$. Western blot analysis of the samples was performed as described in [16] with homogeneous recombinant myristoylated recoverin as an antigen [17]. Rabbit polyclonal (monospecific) anti-recoverin antibodies were prepared as described in [4,5] and used as a positive control; normal human sera served as a negative control.

2.2. Immunohistochemical staining

Tissue samples were fixed in 4% formaldegyde and embedded in paraffin. For an immunohistochemical study, the deparaffinized tissue sections were incubated with polyclonal (monospecific) antibodies against recombinant recoverin [4,5] (in a dilution of 1:100) as the first antibody. Immunoperoxidase staining was applied to the specimens by the streptavidin–biotin–peroxidase-complex. Bovine retina sections were used as a positive control of the reaction. The tumor cells were positively stained with monoclonal antibodies to chromogranin (Dako) in a dilution of 1:200 and to pancytokeratin (Immunotech) in a dilution of 1:100, suggesting neuroendocrine characteristics (data not shown).

3. Results

The first analysis of serum of the patient made in June 1999 detected anti-Rc with a titre of 1:10 (Fig. 1A). The second measurement in September 2000 showed an increase of the titre up to 1:20 (Fig. 1B). Thus, an amount of anti-Rc in the patient's serum tends to increase with the tumor progression. Normal human sera used as a negative control contain anti-Rc (see Fig. 1). Exogeneous recoverin as a competitor for anti-Rc binding to recoverin on the blot decreased the intensity of the reaction in an exogeneous recoverin-dependent manner (not shown) what proves the specificity of the reaction.

It is of interest to note that prednisolone administrated for the treatment of COPD exacerbation in the patient caused a disappearance of anti-Rc from the patient's blood stream 10 days after the treatment (see Fig. 1B), what was rather due to immunosupression by steroid therapy.

It should be stressed that the patient had no loss of vision before the disease and during the whole period of the observation. Detailed ophtalmological investigation of him performed in September 2000 showed that the patient possessed normal visual acuity, his retina and optic nerve were without any changes, the fundus picture remained intact and the visual field was unchanged (Fig. 2). Hence, one may conclude that the patient had no manifestation of the CAR-syndrome despite the presence of anti-Rc in his serum. To our knowledge, this is the first report on the anti-Rc-positive case in a NSCLC patient in the absence of the CAR-syndrome.

To present day expression of recoverin has been demonstrated immunohistohemically for one case

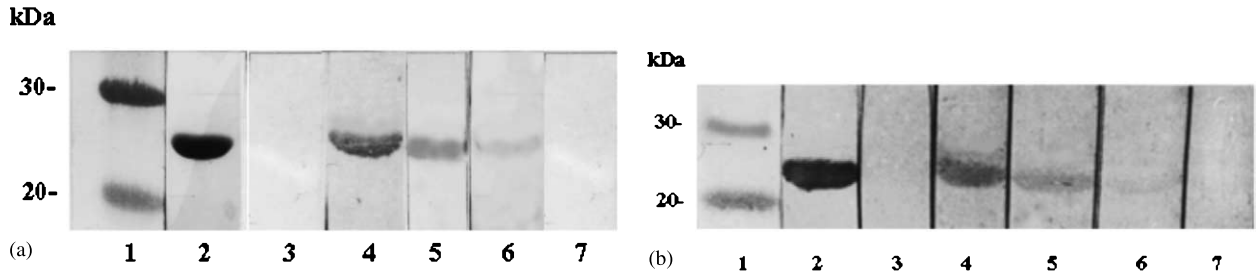

Fig. 1 Immunoblotting of serum of the NSCLC patient. (A) The first analysis in June 1999. 1, blot of standard proteins stained with Panceau S. 2, 3, controls samples: blots of recombinant recoverin stained with (2) rabbit polyclonal (monospecific) anti-recoverin antibodies (1 μg/ml) or with (3) a normal human serum (1:20). 4–7, blots of recombinant recoverin stained with the NSCLC serum at dilutions of 1:5 (4), 1:10 (5), 1:20 (6), and 1:40 (7). (B) The second analysis in September 2000. Tracks 1, 2 and 3 are the same as in A. 4–7, blots of recombinant myristoylated recoverin stained with the NSCLC serum at dilutions of 1:10 (4), 1:20 (5, 7), and 1:40 (6). Tracks 5 and 7 correspond to serum samples before and after prednisolone treatment.

of SCLC only [18]. Whereas no immunohistochemical data have been obtained on expression of the protein in NSCLC tumors. In the present study, we used polyclonal (monospecific) antibodies against recoverin for immunohistochemical detection of recoverin in the primary tumor of the NSCLC patient. Fig. 3 shows the sections of the tumor stained with the antibodies. One can see in Fig. 3A that some tumor cells give recoverin-positive reaction (brown color). At the same time the staining is absent when the antibodies are omitted (Fig. 3B) or preadsorbed with recoverin (Fig. 3C). Thus, we can make a conclusion on specificity of the recoverin-positive reaction revealed.

4. Discussion

Paraneoplastic neurological syndromes associated with malignant tumors usually occur months or even years before the cancer has been detected using traditional diagnostic techniques [1] what allows to consider the underlying autoantibodies as a promising tool for an early diagnostics of cancer. Our present study demonstrates that anti-Rc can be detected in a NSCLC patient who has no CAR-syndrome. While paraneoplastic neurological syndromes are very rare [1], frequencies of the autoantibodies vary over a wide range: from 2 to 4% for anti-Yo and anti-Ri [19] to about 20% for anti-Hu [20]. A frequency of anti-Rc in sera of patients with SCLC was preliminary estimated as 10% [16], but no statistical data have yet been obtained on the serum anti-Rc frequency in patients with NSCLC. That is why consequent steps of our research are aimed at accumulation of statistical data to estimate the anti-Rc frequencies in sera of patients with NSCLC irrespective of the presence of ften recoverin is expressed in tumors of patients with lung cancers.

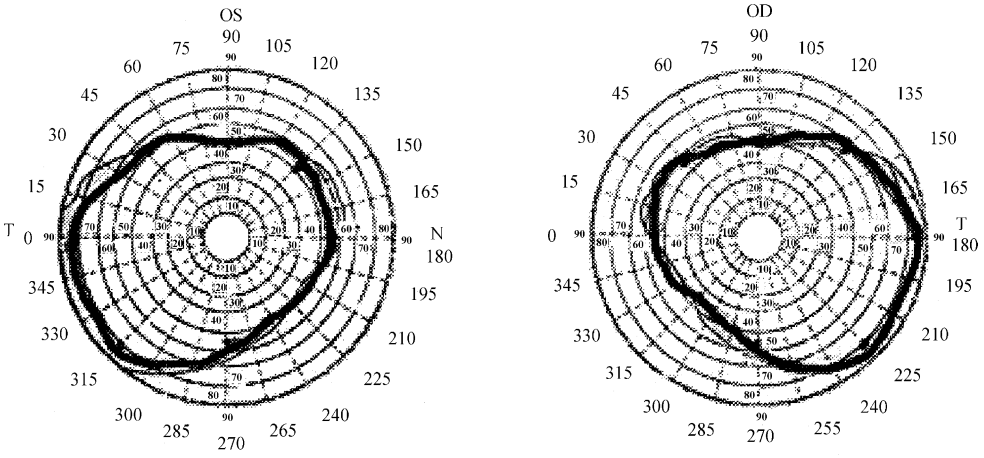

Fig. 2 Goldmann visual fields of the NSCLC patient.

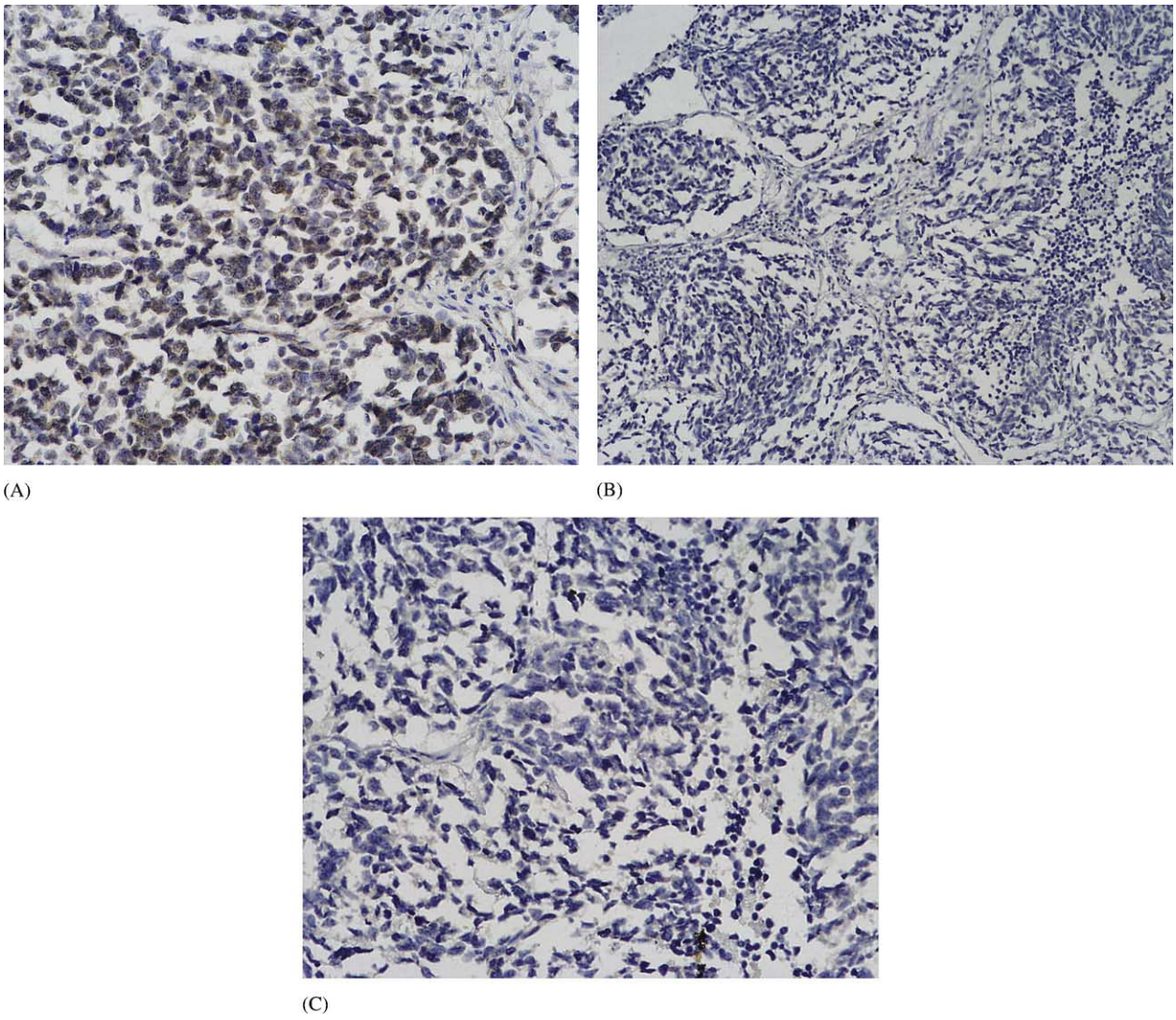

Fig. 3 Immunohistochemical analysis of recoverin expression in the tumor of the NSCLC patient. (A) Rabbit polyclonal (monospecific) antibodies against recombinant recoverin (5 μg/ml) and anti-rabbit IgG peroxidase conjugate were used to visualize recoverin-positive cells in paraffin tumor sections. (B) The same as ''A'' but without anti-recoverin antibodies. (C) The same as ''A'' but anti-recoverin antibodies were preadsorbed with recoverin. Magnification: × 400 (A, C) or × 200 (B).

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