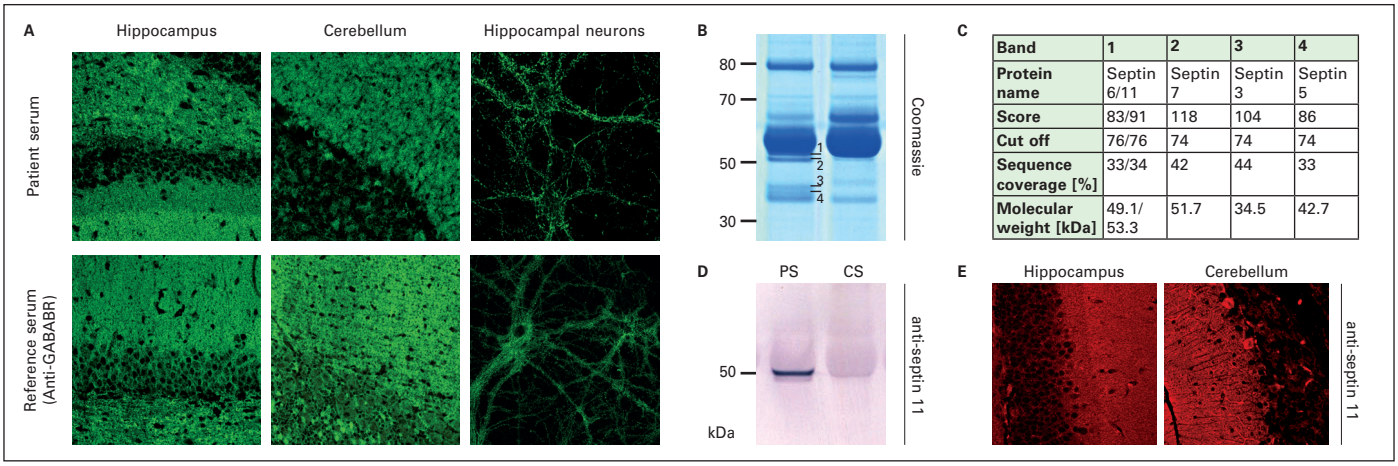
Identification of septin complex as an autoantibody target in paraneoplastic cerebellar ataxia

R. Bahtz[1], I.-M. Bloecker[1], N. Begemann[1], Y. Denno[1], S. Tiede[1], B. Teegen[1], W. Stoecker[1], C. Probst[1], K. Ruprecht[2], and L. Komorowski[1]

[1]Institute for Experimental Immunology, affiliated to EUROIMMUN AG, Luebeck, Germany
[2]Department of Neurology, Charité, Berlin, Germany



Band	1	2	3	4
Protein name	Septin 6/11	Septin 7	Septin 3	Septin 5
Score	83/91	118	104	86
Cut off	76/76	74	74	74
Sequence coverage [%]	33/34	42	44	33
Molecular weight [kDa]	49.1/ 53.3	51.7	34.5	42.7

A. Indirect immunofluorescence assay (IFA) on rat neuronal tissue sections and primary neurons with patient and anti-GABABR reference serum. B. Immunoprecipitates of patient (PS) or control serum (CS) with rat cerebellum lysate (SDS-PAGE, coomassie). C. Results of mass spectrometry analysis. D. Western blot analysis of immunoprecipitates generated as in B with anti-septin 11 rabbit antibody. E. IFA on rat neuronal paraffin sections with anti-septin 11 rabbit antibody.

Introduction

Neuronal autoimmune disorders are often accompanied by tumours outside the brain. In patients who exhibit **paraneoplastic neurological syndromes (PNS)**, autoantibodies against neuronal antigens can frequently be found. We identified a novel neuronal antigen-complex recognized by the serum of a 64-year-old man with malignant melanoma associated with cerebellar ataxia.

Methods

The localisation of the antigen was studied by **indirect immunofluorescence assay (IFA)** combined with confocal microscopy on rat brain sections and primary hippocampal neurons. Immunoprecipitation experiments with rat brain lysate followed by mass spectrometry (MS) were used to identify the antigen, and Western blot analysis was used for confirmation. Specificity of the individual experiments was controlled using sera from healthy controls.

Results

The patient serum revealed high-titer (1:3,200) IgG antibodies against the molecular layer of rat cerebellar and hippocampal tissue sections. On hippocampus, the outer molecular layer was recognized predominantly. This pattern differed markedly from anti-GABAB receptor reactivity, present in the serum as well, but at low titer (1:100). In the immunoprecipitate, five members of the septin family (septin 3, 5, 6, 7, and 11) were identified by MS and the presence of the main constituent, septin 11, was confirmed by Western blot analysis with a polyclonal rabbit antibody. Immunolabeling paraffin sections of rat brain with this antibody revealed the same staining pattern as with the patient serum. Recombinant septins 7 and 11 expressed in HEK293 did not bind patient or control IgG in IFA. Neither immunoprecipitated nor recombinant septins reacted with patient or control IgG in Western blot.

Conclusion

Septins are a large family of GTPases which have been associated with neurodegeneration and tumorigenesis, and septins 3, 5, 6, 7, and 11 have previously been reported to form a complex in hippocampal neurons. Our data indicate that autoantibodies against this complex, rather than against one of the individual septins, represent novel biomarkers in paraneoplastic cerebellar ataxia.

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EUROIMMUN AG · D-23560 Luebeck (Germany) · Seekamp 31 · Tel +49 451 58550 · Fax 5855591 · E-mail euroimmun@euroimmun.de