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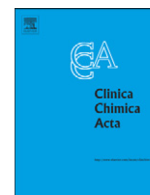

Betaine homocysteine methyl transferase 1, a novel auto-antigen associated with anti-Golgi immune reactivity

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ABSTRACT

Anti-Golgi antibodies are rare autoantibodies that have been described in systemic autoimmune diseases. Not all Golgi auto-antigens are known. The objective of this study was to identify a novel auto-antigen associated with anti-Golgi immune reactivity.
Sera from a patient with Golgi immune reactivity and from a control individual were used for Western blotting after 2-dimensional gel separation of a rat Golgi-enriched extract.
Betaine homocysteine S-methyltransferase 1 (BHMT1) was identified as an auto-antigen by MALDI-TOF/TOF mass spectrometry. Using human recombinant BHMT1, a strong positive blotting signal was obtained with serum from the patient but not from a control. Pre-absorption of the serum sample with reactivity to BHMT1 with recombinant human BHMT1 resulted in decreased reactivity on Western blotting and in disappearance of the Golgi-like pattern on indirect immunofluorescence. Using immunocytochemistry, we confirmed the subcellular localization of BHMT1 to the Golgi apparatus. Antibodies to BHMT1 were found in four of 80 samples with a Golgi-pattern on indirect immunofluorescence. The antibodies were not associated with a specific clinical condition.
We identified BHMT1 as a novel auto-antigen associated with anti-Golgi immune reactivity.
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1. Introduction

Anti-Golgi antibodies are rare autoantibodies that give a characteristic speckled staining flanking one side of the nucleus on anti-nuclear antibody testing. The antibodies have been described in systemic autoimmune diseases such as Sjögren's syndrome and systemic lupus erythematosus. The finding of Golgi autoantibodies is, however, not specific for the diagnosis of systemic autoimmune disease [1]. Several Golgi autoantigens have been identified (for review, see [2]). The Golgi autoantigens (Giantin, Golgin-245, Golgin-160, Golgin-95/GM130, Golgin-97) are generally high molecular weight proteins that are rich in coiled-coil domains in the central region [2]. In a study in which 80 human anti-Golgi autoimmune sera were evaluated, Giantin (370 kDa) was reported to be the predominant target of human anti-Golgi complex antibodies [3]. The antibodies were found in 50% of anti-Golgi autoimmune sera [3]. In 31% of anti-Golgi autoimmune sera, however, the Golgi antigens were uncharacterized.

The objective of this study was to identify novel auto-antigens associated with anti-Golgi immune reactivity.

2. Materials and methods

2.1. Materials

Recombinant his-tagged betaine homocysteine S-methyltransferase 1 (BHMT1) and mouse anti-BHMT1 antibodies were obtained from Genway, San Diego, CA, USA.

2.2. Antinuclear and anti-cytoplasmic antibody testing

Anti-nuclear and anti-cytoplasmic antibodies were detected by indirect immunofluorescence using HEp-2000® cells (Immunoconcepts, Sacramento, CA) with an Axioplan 2 fluorescence microscope

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(Carl Zeiss MicroImaging, Oberkochen, Germany) and Cytovision 3.6 software (Genetix, New Milton, UK).

2.3. Preparation of Golgi membrane vesicles

Golgi vesicles were prepared as previously described [4].

2.4. Gel electrophoresis and western blotting

For the separation of proteins in 2 dimensions, immobilized pH-gradient strips (pH range 3–10, GE Healthcare) were rehydrated overnight with approximately 300 μg of protein extract. Loaded proteins were first separated by isoelectric focusing and then by SDS-PAGE (12.5%) on a Multiphor II Electrophoresis System (GE Healthcare, Chalfont St. Giles, United Kingdom) according to the supplier's instructions. The proteins were either subjected to Western blotting or stained by Coomassie Brilliant blue. Western blotting was performed as previously described [5].

2.5. Protein identification by MALDI-TOF/TOF

Proteins were identified as by MALDI-TOF/TOF (Applied Biosystems 4800 Life Technologies Corporation, Proteomics Analyzer, Carlsbad CA, USA) as previously described [5].

2.6. Immunocytochemical staining

The subcellular localization of the novel auto-antigen was confirmed in HEp-2000® cells. The Golgi apparatus was stained using a primary mouse anti-Golgin 97 (Life Technologies Corporation). The novel auto-antigen was stained using diluted patient serum. Bound primary antibodies were detected using goat anti-mouse Alexa-568 and goat anti-human Alexa-488 (Life Technologies Corporation). Nuclei were stained with TOPRO-3 (Life Technologies Corporation). HEp-2000® cells were examined and colocalization was confirmed using a LSM510 confocal laser scanning microscopy (Carl Zeiss MicroImaging). Obtained images after optimal contrast and brightness setting with relevant scale bars were exported in JPEG format without compression or any additional modification.

3. Results and discussion

Golgi-immune reactivity (titer 1:640) was identified by immmunofluorescence in a 54-year-old woman with a thyroid goiter and arterial hypertension. Antinuclear antibodies were requested because she suffered from painful wrists and fingers, as well as swollen hands. Connective tissue disease and rheumatic arthritis were excluded on the basis of blood and radiographic analyses. She was successfully treated with Voltaren. The arterial hypertension was treated with Aldomet (α-methyldopa), a drug that has been suggested to induce anti-nuclear antibodies [6].

In an attempt to identify the target antigen of the antibodies, we applied an immunoproteomics approach. Sera from the patient and from a control individual were used for Western blotting after 1- and 2-dimensional gel separation of a rat Golgi-enriched extract. Proteins to which there was differential (control versus patient) reactivity were excised and identified by MALDI-TOF/TOF mass spectrometry. Betaine homocysteine S-methyltransferase 1 (BHMT1) (EC 2.1.1.5) (O09171_RAT), Swiss-Prot database; molecular weight 44,976 Da and pI of 8.02, reacted with serum from the patient but not with serum from the control (Fig. 1). We confirmed the presence of anti-BHMT1 antibodies in the patient's serum by 1-dimensional SDS-PAGE and Western blotting using human recombinant his-tagged BHMT1. BHMT1 from rat has 92% identity at the amino acid sequence level with human BHMT1 (Q93088, Swiss-Prot database; molecular weight 44,998 Da and pI of 6.58) (Clustal W analysis). A strong positive blotting signal was obtained with serum from the patient but not from a control (Fig. 1). Pre-absorption of the serum sample with reactivity to BHMT1 with recombinant human BHMT1 resulted in decreased reactivity on Western blotting (Fig. 2, panel A) and in disappearance of the Golgi-like pattern on indirect immunofluorescence analysis (Fig. 2, panel B). Incubating HEp-2000® cells with a monoclonal mouse anti-BHMT1 antibody (Genway) resulted in a speckled cytoplasmic pattern, with peri-nuclear accentuation (Fig. 2, panel C). Commercial mouse anti-BHMT1 also reacted with purified Golgi vesicles (data not shown). The subcellular location of the novel autoantigen to the Golgi apparatus was supported by showing co-localization with golgin97 (Fig. 3).

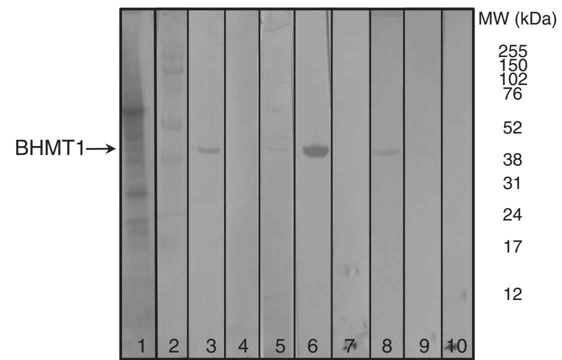

Fig. 1. Gel electrophoresis and reactivity to BHMT1 on Western blot analysis. Lane 1 shows one-dimensional gel electrophoresis of rat Golgi extract (Coomassie Brilliant Blue Staining). Lane 2 shows the molecular weight marker (Coommassie Brilliant Blue Staining). Lanes 3–4 represent Western blot analysis of rat Golgi extract after incubation (i) with serum from a patient treated with α-methyldopa and a Golgi-like anti-nuclear antibody pattern (lane 3) or with (ii) serum from a healthy subject (lane 4). Lanes 5–8 show Western blots of recombinant human BHMT1 (MW 45 kDa) after incubation (i) with serum from a patient treated with α-methyldopa and a Golgi-like anti-nuclear antibody pattern (lane 5), (ii) with serum from a healthy subject (lane 6), and (iii) with serum from two other patients with a Golgi-like anti-nuclear antibody pattern, with titer 1:80 and 1:320, respectively (lanes 7–8).

We screened 79 additional patients with a Golgi-like pattern for the presence of antibodies to BHMT1 by Western blotting (25 from University Hospitals Leuven, Belgium, 47 from University Hospital Zurich, Switzerland, and 7 from ULB Hôpital Erasme, Brussels, Belgium). Antibodies to BHMT1 were found in three of these samples. The clinical conditions of the patients in which the antibodies were found were (i) corneal ulcer (after pterygium excision), (ii) fibromyalgia, and (iii) chronic HBV infection, cirrhosis and hepatocellular carcinoma. Given the heterogeneity of clinical findings, no correlation between anti-BHMT1 antibodies and a specific clinical condition could be observed. This is in accordance with our recent observation that anti-Golgi antibodies were not clinically informative diagnostic markers of systemic rheumatic diseases [1].

Betaine homocysteine S-methyltransferase1 (BHMT1) is a Zn^{2+}-metalloenzyme which converts betaine and homocysteine to dimethylglycine and methionine, respectively, using betaine as methyl donor (for review, see [7]). BHMT1 is characterized by the presence of a putative coiled-coil domain at the C-terminus (predicted by the Coils Server of EMBnet (http://www.ch.embnet.org/)) and has some structural similarities to classical Golgi complex autoantigens (Giantin, Golgins) [8]. The enzyme is abundantly expressed in liver hepatocytes and kidney [9]. BHMT1 is generally regarded as a cytosolic enzyme. However, BHMT1 (degradation fragment) has been reported to be particularly enriched in the delimiting membrane of the autophagosome [10, 11], perhaps indicating that methylation/demethylation of membrane components could play a role in autophagy [11]. The membrane sources of autophagosomes are not completely understood, but it is possible that the membrane structures are derived

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