ORIGINAL INVESTIGATIONS

Multifunctional protein nucleobindin 1 as a marker of vascular damage in systemic lupus erythematosus

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The search for new biomarkers for the early diagnosis of systemic lupus erythematosus (SLE) is a crucial task.

Objective: *a comparative study of concentrations of conservative protein nucleobindin 1 (NUCB1) in the blood serum of patients with SLE and healthy donors and assessment of correlation of NUCB1 level with clinical and serological manifestations of the disease.*

Material and methods. *The study included 21 patients with SLE who fulfilled SLICC criteria and 23 healthy donors. SLEDAI-2K index was used to assess SLE activity. Organ damage was assessed using SLICC damage index. Standard laboratory markers of SLE were analyzed in all patients. Concentration of NUCB1 in blood serum was determined using the enzyme immunoassay method.*

Results and discussion. *The group of SLE patients included 20 women and 1 man (median age 33 [27; 40] years, disease duration 5 [3; 10] years), mainly with high disease activity (median SLEDAI-2K 8.5 [6.0; 14.0]). Kidney involvement was found in 52% of cases (nephritis), involvement of joints – in 67% (arthritis), vessels – in 33%, skin – in 67%, pericarditis – in 29%, hematological abnormalities – in 71%, antinuclear factor – in 76% and antibodies against double-stranded DNA – in 71%.*

An increase in the mean NUCB1 level to 3881 ng/ml was found in the blood serum of SLE patients compared to the control group (2766 ng/ml; $p=0.048$). Correlations of NUCB1 levels with vascular damage ($r=0.653$; $p<0.05$) and pericarditis ($r=-0.490$; $p<0.05$) were found.

Conclusion. *Elevated NUCB1 levels in the blood serum of SLE patients may indicate involvement of this protein in autoimmune and apoptotic processes. The observed correlation of NUCB1 levels with vascular affection and pericarditis is the basis for further studies on the involvement of this protein in the development of various diseases, including SLE.*

Keywords: *systemic lupus erythematosus; NUCB1; endothelial cell apoptosis; vascular damage; pericarditis*

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Introduction

Systemic lupus erythematosus (SLE) is a systemic autoimmune rheumatic disease of unknown etiology, characterized by the overproduction of organ-specific autoantibodies to various components of the cell nucleus and by the development of immune-mediated inflammatory damage to internal organs [1]. The etiology of SLE is still unclear, and treatment is aimed at reducing disease activity. At present, significant progress has been achieved in increasing the survival of patients with SLE [2], however, there is a need for more effective early diagnosis of SLE and an active search for new SLE biomarkers is underway [3; 4].

Nucleobindin 1 (NUCB1) is a conserved Ca2+-binding eukaryotic protein involved in many processes in the body, such as stress response, immune response, and calcium homeostasis [5, 6]. NUCB1 forms a functional dimer and consists of several domains: a signal peptide, a DNA-binding domain, a Ca-binding domain, and a leucine zipper [7]. NUCB1 was first discovered as a B-cell growth and differentiation factor from KLM1-7 cells (spleen cells from MRL/l mice, mice that develop SLE-like disease).

NUCB1 has been shown to be able to interact with DNA isolated from this cell culture [8]. Systematic injections of NUCB1 into SLE-prone mice resulted in development of SLE-like disease (increased production of anti-ssDNA and anti-dsDNA autoantibodies) [9].

A mutant form of NUCB1 lacking the putative sites of interaction with DNA (the DNA-binding domain or "leucine zipper") does not stimulate the appearance of antibodies to DNA [10]. It has been shown that the level of NUCB1 in the blood serum of MRL/lpr mice, used as a model for studying SLE, is significantly higher than in the blood serum of normal mice (MRL/n) [11]. These data suggest that the DNA-binding activity of NUCB1 plays a role in inducing autoimmune reactions. In addition, it is known that the protein is a transcription factor and is involved in the epithelial-mesenchymal transition [12]. We have recently shown the RNA-binding and RNA-melting activities of NUCB1 [13]. Since NUCB1 has a high affinity for microRNAs (microRNAs are small non-coding RNA molecules 18–25 nucleotides long, with an average of 22, that participate in transcriptional and post-transcriptional regulation of gene

expression by RNA interference) that are associated with the development of SLE, it can be assumed that this protein is involved in the network of regulatory processes leading to the development of this severe autoimmune disease.

There are very few publications devoted to the study of NUCB1 levels in various diseases. They are mainly devoted to the study of changes in the level of antibodies to this protein in various tumor formations compared to their level in unchanged cells and tissues [5, 14 - 16]. Studies of changes in NUCB1 levels in human autoimmune diseases have not been conducted.

The aim of the study is a comparative analysis of the concentration of NUCB1 in the blood serum of patients with SLE and healthy donors and an assessment of the correlation of NUCB1 levels with clinical and serological manifestations of the disease.

Materials and methods

The study included 21 patients with SLE according to the 2012 SLICC criteria [17] (20 women and 1 man), median age 33 [27; 40] years, disease duration 5 [3; 10] years, with high activity (median SLEDAI-2K – 8.5 [6.0; 14.0] points). The patients were observed in the clinic of the Federal State Budgetary Scientific Institution "V.A. Nasonova Research Institute of Rheumatology" (V.A. Nasonova Research Institute of Rheumatology) and signed informed consent to participate in the study.

For all patients, disease activity was assessed using the SLEDAI-2K index [18], and irreversible organ damage was assessed using the SLICC/ACR (American College of Rheumatology) damage index (DI) [19]. Before inclusion in the study and during observation, concomitant therapy was assessed, standard laboratory tests were performed, including a complete blood count and urine test, determination of immunological markers of SLE: antibodies to double-stranded DNA (anti-dsDNA), antinuclear factor on Hep2 cells (ANF-Hep2), C3 and C4 components of the complement, antibodies to Sm (anti-Sm) – to U1, U2, U4 ribonucleoproteins.

NUCB1 concentration was assessed by western blotting technique using polyclonal antibodies specific to human NUCB1 that were purified and loaded onto Protein A Sepharose resin. The resulting resin was incubated with blood serum. The sample bound to the resin (antibodies) was eluted and analyzed using electrophoresis under denaturing conditions (PAAG with the addition of sodium dodecyl sulfate). The proteins were then transferred to a PVDF (PolyVinylidene DiFluoride) membrane and the presence of NUCB1 was determined using specific NUCB1 antibodies and secondary antibodies conjugated with peroxidase.

The absence of SLE activity was defined as a SLEDAI-2K score = 0, low activity as 1–5, moderate activity as 6–10, high activity as 11–19, and very high activity as >20 [18].

Inclusion criteria: male and female patients aged 18 to 65 years, with a confirmed diagnosis of SLE.

Exclusion criteria: severe concomitant pathology (infections, malignant neoplasms) or pregnancy, participation in other clinical trials. The study was approved by the local Ethics Committee of V.A. Nasonova Research Institute of Rheumatology and was conducted on the basis of a cooperation agreement between the Federal State Budgetary Scientific Institution "Protein Institute" of the Russian Academy of Sciences and V.A. Nasonova Research Institute of Rheumatology and Rheumatology dated 26.01.2018.

The characteristics of the patients are presented in the table.

The control group consisted of 23 healthy donors, matched for age and gender (median age – 32 [26; 39] years, 96.0% women; $p>0.05$ in both cases), without rheumatic, oncological or infectious diseases.

Statistical processing of the data was carried out on a personal computer using parametric and nonparametric statistical methods of the application programs Statistica 8.0 (StatSoft Inc., USA). Variables are presented as median and interquartile range (Me [25th; 75th percentile]). The significance of differences between two unrelated groups was assessed using the Mann–Whitney test. The relationship between the features was determined using Spearman's rank correlation criterion (r). Differences were considered statistically significant at $p<0.05$ [20].

Results

The concentration of NUCB1 in patients with SLE was higher than in the control group ($p=0.048$): the median level of NUCB1 was 3881 [2182; 6218] and 2766 [1074; 3973] ng/ml, respectively. The 25th percentile value in the SLE group (2182 ng/ml) was slightly lower than the median NUCB1 level in the control group (2766 ng/ml). The median NUCB1 level in the SLE group (3881 ng/ml) was almost identical to the 75th percentile in the control group (3973 ng/ml; Fig. 1).

The upper limit of the normal serum concentration of NUCB1 was taken as the value corresponding to the 95th percentile of NUCB1 concentration in the control group – 4879 ng/ml. Increased NUCB1 levels (>4879 ng/ml) were detected in 38% of SLE patients and 4.3% of healthy donors.

In patients with SLE, a positive correlation of NUCB1 concentration with vascular damage ($r=0.653$; $p<0.05$) and a negative correlation with pericarditis ($r=-0.490$; $p<0.05$) were found.

Taking into account the identified relationship between the NUCB1 level and vascular damage and pericarditis, a comparison was made of the NUCB1 concentration in patients who had and did not have these disorders. In the group of SLE patients, the NUCB1 level was higher in the presence of vascular lesions (median – 6218 [4225; 13048] ng/ml; n=7) than in the absence of vascular pathology (2188 [1597; 3881] ng/ml; n=14), $p=0.020$ (Fig. 2).

In patients with SLE in the presence of pericarditis (n=6), the NUCB1 level was lower than in its absence (n=15), and its median was 2182 [1703; 2188] and 4795 [3048; 8994] ng/ml, respectively ($p=0.049$; Fig. 3).

Discussion

Based on the results of the analysis of previously obtained data on the interaction of NUCB1 with DNA [10], as well as the ability of NUCB1 to induce the appearance of autoantibodies to DNA [8, 9], we believed that an increased level of NUCB1 in the blood serum of patients with SLE would be associated with disease activity or the level of anti-dsDNA. However, no correlation between NUCB1 concentration and these parameters was found.

Comparing our results with literature data on the processes in which NUCB1 is involved, we suggested that increased NUCB1 level in patients with SLE is associated with the participation of this protein in apoptosis.

According to the Human Protein Atlas database, the level of NUCB1 is low in tissues with reduced proliferative capacity (muscles, eyes) and, accordingly, apoptosis. The epithelium of the skin, mucous membranes of the respiratory tract, gastrointestinal tract, genitourinary system, and hematopoietic tissue are characterized by maximum regenerative capacity. Thus, NUCB1 is

detected mainly in the liver, kidneys, digestive and respiratory systems (Human Protein Atlas). It is worth noting that the Human Protein Atlas database contains information on the distribution of protein in the tissues of healthy people, but not in the tissues of patients with autoimmune diseases.

Increased NUCB1 levels were associated mainly with vascular endothelial damage (Raynaud's syndrome, vasculitis, interstitial lung disease), and decreased levels were associated with pericarditis in our patients with SLE.

The vascular endothelium is the most metabolically active tissue, having a high capacity for apoptosis and cell proliferation under non-physiological conditions [21–23]. According to RNA-Seq (Human Protein Atlas) data, the level of NUCB1 messenger RNA (mRNA) in vascular and lung tissues is quite high (in vascular cells, the NUCB1 level is 1,143,849 nTNP/10 thousand cells, in lung cells it is 1,974,975 nTNP/10 thousand cells). The pericardium is a tissue with a weak capacity for the combined processes of proliferation and apoptosis. In cardiac tissues, including the pericardium, a very low level of NUCB1 mRNA is detected compared to vascular and lung cells - 200.918 nTNP/10 thousand cells (Human Protein Atlas).

We suggest that it is the involvement of NUCB1 in the apoptosis process that explains the positive correlation of the level of this protein with vascular damage and its negative correlation with pericarditis.

Clinical characteristics of patients included in the study (n=21)

Variables	Value
Gender, male/ female, n	20/1
Age, years, Me [25th; 75th percentile]	33 [27; 40]
Disease duration, years, Me [25th; 75th percentile]	5 [3; 10]
SLE activity, n (%):	
I	3 (14)
II	11 (53)
III	7 (33)
SLEDAI-2K, score, Me [25th; 75th percentile]	8.5 [6.0; 14.0]
SLE manifestations, n (%):	
nephritis	11 (52)
arthritis	14 (67)
pericarditis	6 (29)
skin damage	14 (67)
vascular damage	7 (33)
hematological disorders	15 (71)
Immunological disorders, n (%):	
positive ANA	16 (76)
positive anti-dsDNA	15 (71)
positive anti-Sm	5 (24)
hypocomplementemia	15 (71)
Medications at the time of inclusion in the study, n (%):	
GC	19 (90)
Hydroxychloroquine	13 (62)
Cytotoxic medications	9 (43)
Azathioprine – cytotoxic medication	1
Cyclophosphamide – cytotoxic medication	1
Mycophenolate mofetil - cytotoxic medication	7 (33)
Rituximab	12 (57)

Notes: GC – glucocorticoids.

NUCB1 involvement in the processes of apoptosis, proliferation, migration, and epithelial-mesenchymal transition has been shown in different model cell lines [8, 10, 12, 24]. It is also known that during endoplasmic reticulum stress caused by the accumulation of misfolded or damaged proteins, NUCB1 synthesis is activated. As a result, NUCB1 can interact with the transmembrane protein ATF6 [25] and negatively affect the misfolded protein response pathway. Signaling pathways can switch to apoptosis, which, in turn, is an important factor in the pathogenesis of SLE. Apoptosis is normally an "immunologically silent" process, since it is not accompanied by disruption of the integrity of the cell membrane and the release of intracellular antigens into the surrounding tissues. It is known that dysregulation of apoptotic processes is one of the key links in the pathogenesis of SLE [26]. Defects in apoptosis (both suppression and inappropriate enhancement) cause the formation of immune complexes and the production of inflammatory mediators.

A detailed study of the role of NUCB1 in the apoptotic process has not been conducted.

However, presumably regulation can be carried out in many ways. Firstly, through NUCB1 interaction with cyclooxygenases 1 and 2 located in the lumens of the endoplasmic reticulum [27]. As a result, mutual regulation of these proteins is possible and they influence not only inflammatory processes, but also apoptosis [28]. Secondly, through the interaction of NUCB1 with microRNAs that regulate the synthesis of mRNA proteins involved in apoptosis and the development of autoimmune diseases [29, 30]. We have recently shown that NUCB1 interacts with high affinity with SLE-associated miRNAs in vitro and has RNA chaperone activity, which may facilitate the interaction of miRNAs with target mRNAs [13]. Thirdly, during the participation of NUCB1 in the process of apoptosis. It is known that in apoptotic cells, the phospholipid phosphatidylserine is reoriented and is localized on the surface of the cell membrane (eat-me signal) starting from the early stage of apoptosis and up to complete degradation of the cell. Eat-me factors are recognized by phagocytes, as a result of which the process of absorption of the cell by phagocytes is launched. Recently, NUCB1 has been shown to bind to phosphatidylserine on the surface of cancer cells [24]. We suggest that NUCB1, by interacting with phosphatidylserine, can either screen this "black mark" on the membrane of apoptotic cells (similar to the structurally similar protein annexin 5 [31–33]) and prevent apoptosis, or, conversely, promote cell recognition by phagocytes and enhance apoptosis.

If we assume the participation of NUCB1 in the development of autoimmune and oncological diseases is associated with cell apoptosis, we can explain the different levels of expression of this protein in different tissues. Further detailed analysis of the role of NUCB1 in the process of cell apoptosis in autoimmune diseases is required. The obtained data may lead to the development of a new diagnostic parameter for SLE, which, in combination with existing methods, will improve the accuracy of diagnosis.

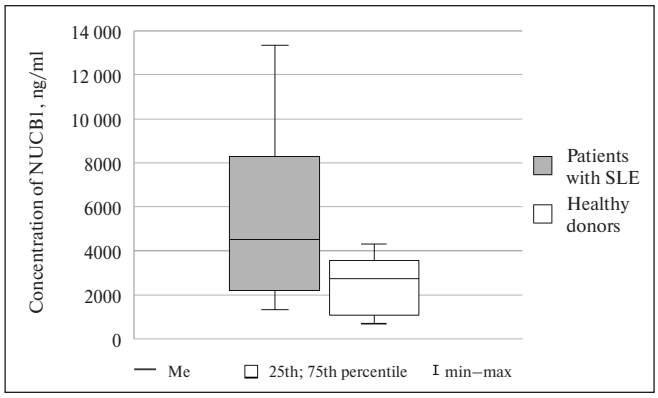

Fig. 1. *NUCB1 concentration (ng/ml) in the blood serum of patients with SLE (n=21) and healthy donors (n=23). In the blood serum of healthy donors, the concentration of NUCB1 is 2766 [1074; 3973] ng/ml, and in the blood serum of patients with SLE 3881 [2182; 6218] ng/ml (p<0.05)*

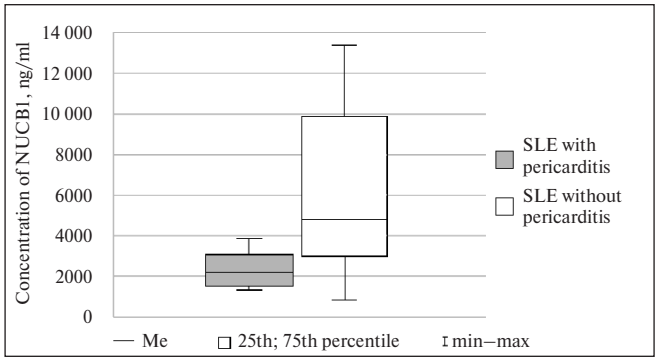

Fig. 3. *NUCB1 concentration (ng/ml) in the blood serum of patients with SLE with pericarditis (n=6) and without pericarditis (n=15). The NUCB1 concentration (ng/ml) in the blood serum of patients with SLE with pericarditis is 2182 [1703;2188] ng/ml, and in the blood serum of patients with SLE without pericarditis 4795 [3048; 8994] ng/ml (p<0.05)*

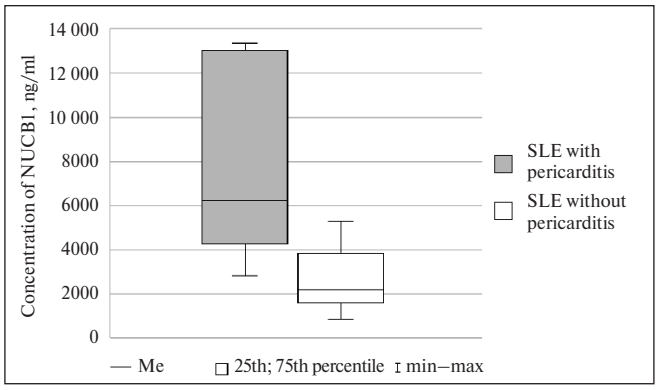

Fig. 2. *NUCB1 concentration (ng/ml) in the blood serum of patients with SLE with vascular damage (n=7) and without vascular damage (n=14). The concentration of NUCB1 (ng/ml) in the blood serum of patients with SLE with vascular damage is 6218 [4225; 13048] ng/ml, and in the blood serum of patients with SLE without vascular damage 2188 [1597; 3881] ng/ml (p<0.05)*

Conclusion

Increased serum NUCB1 levels in patients with SLE compared to healthy donors may indicate previously unknown involvement of this protein in autoimmune processes. We explain the positive correlation of NUCB1 levels with symptoms of vascular damage in patients with SLE by its active participation in the process of apoptosis of endothelial tissue cells.

The pilot study provides a basis for further full-scale investigation of the correlation between NUCB1 levels in vascular and serous membrane lesions in patients with SLE and assessment of the possibility of using this protein as a biomarker for early diagnosis of SLE.

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Author Contributions

There are no conflicts of interest. The authors are solely responsible for submitting the final version of the manuscript for publication. All the authors have participated in developing the concept of the article and in writing the manuscript. The final version of the manuscript has been approved by all the authors.

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