Correlation of SATB1 expression with clinical course of cutaneous T-cell lymphomas

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Cutaneous T-cell lymphomas (CTCLs) are slowly progressive diseases with a poor prognosis. There are no specific prognostic factors in development of cutaneous lymphomas. SATB1 protein controls expression of many genes, including the cellular cycle and apoptosis. The subject of our study was the expression of SATB1 protein in the skin sample in patients with mycosis fungoides and Sezary syndrome and its correlation with clinical course. Immunohistochemical reaction with SATB1 antibody was observed in 29 cases of mycosis fungoides of different stages (15 patients) and two cases of Sezary syndrome. SATB1 expression was observed in 22 cases of mycosis fungoides, 7 of which were in the patch stage, 11 were in the plaque stage and 4 were in the tumor stage. SATB1 expression was not found in 2 cases of the patch stage, 4 cases of the plaque stage and one case of the tumor stage. Negative reaction was confirmed in both cases of the Sezary syndrome. There were no changes in SATB1 expression during progression of the disease. A group of patients with the positive reaction of the SATB1 is characterized by a noticeably longer time to progression between the stages. The SATB1 expression seems to be a potential prognosis factor confirming the inner heterogeneous features of CTCLs.

Key words: cutaneous T-cell lymphoma, CTCL, SATB1, prognostic marker, mycosis fungoides.

Introduction

Cutaneous T-cell lymphoma (CTCL) is a diverse clinical group of diseases in which symptoms are seen primarily in the skin i.e. lymphocytic infiltration. During the past decade, a more than a two-fold increase in the CTCL incidence has been noticed, and at now it comes to 6.4 per million people a year. CTCL are 75-80% of primary cutaneous lymphomas and the remaining 20-25% are B-cell lymphomas. Although mainly older patients are diagnosed with this type of lymphomas, CTCL can also occur in children and youths [1]. The most common form of CTCL is mycosis fungoides and its natural course can be characterized by three stages: patch, plaque and tumor. The disease has a mild clinical course with a slow, long progression. Patients in the advanced tumor stage with involvement of lymph node and internal organs always have a poor prognosis. The rate of progression usually takes many years but in fact it is unpredictable. In

the last years, a group of CD30-positive lymphoproliferative diseases was recognized by WHO-EORTC classification in which the primary cutaneous anaplastic large cell lymphoma and lymphomatoid papulosis (which also have a mild course and are the second most common type of lymphomas) were classified together [2]. The most aggressive disease is the Sezary syndrome which is characterized by three symptoms: erythroderma, lymphadenopathy and the presence of Sezary cells in the blood. This form of disease has a fast clinical course with an unfavorable patient prognosis and it affects one patient in ten to twenty cases of CTCL. The remaining forms of cutaneous lymphoma occur very rarely and are presented in the WHO 2008 classification [3]. Early diagnosis is of major clinical significance as it makes the possible treatment proper, but also it might inhibit further progression of the disease. Mycosis fungoides is a chronic progressive disease and there is no effective way to cure it. The therapeutic treatment comes down to the choice of clinical methods, which can temporarily stop the progression of the illness with as few side effects as possible. Here, the PUVA therapy has a significant meaning. In early stages it is used as a monotherapy and in later stages it is combined with interferon or retinoids. Immunomodulatory treatment is used to reduce side effects i.e. interferon α, bexarotene, denileukin diftitox and methotrexate [4, 5]. It is thought that in most cases the main mechanism of the effect of the drugs is to trigger apoptosis of cancer cells, induction of growth and modulation of immune response to the cancer cells [4, 6].

Current studies of primary cutaneous lymphoma are concentrated on optimization of diagnostic methods and further identification of specific markers and improvement of the 2008 WHO classification [7, 8]. There are no many reports on the specific markers that can be valuable in prognosis of cutaneous lymphoma which can predict the clinical course and choice of adequate treatment. Special AT-rich sequence-binding protein 1 (SATB1) seems to be an especially attractive potential marker. It is a protein of a nuclear matrix. On one hand, it is considered an indicator of unfavorable prognosis in the breast cancer, bladder cancer and laryngeal cancer, on the other hand it is studied as a key element of the growth of thymocytes in connection with Wnt/β-catenin signaling pathway. The subject of present study was the expression of SATB1 protein in the skin sample in patients with mycosis fungoides and Sezary syndrome and its correlation with the clinical course.

Material and methods

The studied group consisted of patients with cutaneous lymphoma with different stages of growth: 9 cases of mycosis fungoides in the patch stage, 15 cases in the plaque stage, 5 cases in the tumor stage and two cases of Sezary syndrome. Written informed consent was obtained from each patient before the tissue sample acquisition, and approval for the study was granted by the institution's Ethical Committee (No. 215/2008). Samples were fixed in 10% buffered formalin and embedded in paraffin block. All histopathological results were standardized according to the WHO classification (2008) using an immunohistochemical diagnostic panel of antibodies: CD3, CD4, CD7, CD8, CD20, CD30, CD45RO and the studies conducted confirmed monoclonal growth of the neoplasm using the PCR method. The classical immunohistochemical reaction was carried out with the use of polyclonal antibodies against the SATB1 protein (Santa Cruz) and EnVision+ Dual Link System-HRP visualization system (DAKO) on 5 μm paraffin sections, placed on the SuperFrost/Plus microscopic slides.

Results

Two different patterns of immunohistochemical reaction were observed: strong nuclear reaction with marked or lack of cytoplasmic reaction and a weak cytoplasmic reaction without a nuclear reaction. The first patterns was considered to be a positive reaction (Fig. 1A-B), and lack or weak cytoplasmic reaction were considered as a negative reaction (Fig. 1C-D).

Here, we observed a positive reaction in 22 cases of mycosis fungoides, 7 of which were in the patch stage, 11 were in the plaque stage and 4 were in the tumor stage, according to the criteria pointed above. Negative reaction was confirmed in 2 cases of the patch stage, 4 cases of the plaque stage and 1 case of the tumor stage. Both cases of the Sezary syndrome did not demonstrate the SATB1 expression. Simultaneously, a constant reaction pattern in a given patient with mycosis fungoides was observed, independently of the disease progression (Table 1). Average time of the disease in SATB1+ patient group was 14 years (3.5-23 years) in comparison to patients with negative expression of SATB1, in which the average follow-up of the disease was 6 years (3.5-10 years) (Fig. 2). During their observation, 3 mycosis fungoides patients died; 2 of them, who had positive staining of SATB1, died after 23 and 15 years after the diagnosis of the disease and 1 who did not demonstrate SATB1, died after 7 years of disease. The patients with the Sezary syndrome died 18 and 30 months after the diagnosis. All of these patients died from complications in the course of disease progression. The group of patients with SATB1 expression was characterized by a distinctly longer time to progression between the stages. The average time to progression from the patch stage to the plaque stage in SATB1 positive patients lasted 16 years, whereas 5 years in SATB1 negative patients. The average time to progression from the plaque stage to the tumor stage lasted 6 and 2.5 years

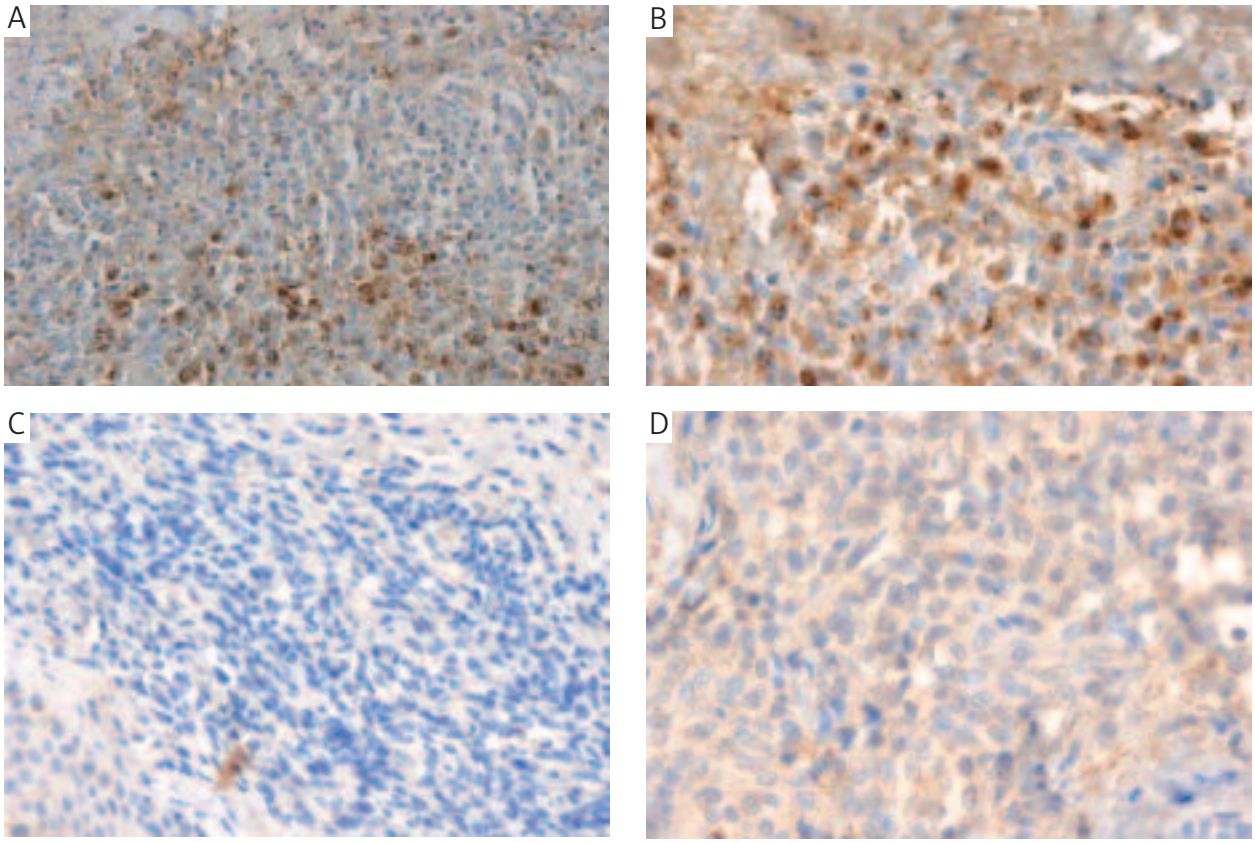

Fig. 1. SATB1 expression in neoplasmatic lymphocyte infiltration of the skin: strong nuclear reaction with a marked cytoplasmic reaction was considered as positive reaction (A, B – SATB1+) and weak or lack of cytoplasmic reaction without a nuclear reaction (C, D – SATB1–). A, C – magnification 100×, B, D – magnification 200×

for SATB1 positive and SATB1 negative patients, respectively (Fig. 3).

Discussion

SATB1 is a nuclear matrix protein, which creates a three-dimensional structure (cage-like structure) inside the nucleus, folds and remodels chromatin, but also attracting even the most distant parts of the genome. The new organization of the chromatin enables common and coordinated regulation of expression simultaneously in many genes. Another mechanism of action of this protein is recruitment of enzymes modifying histones in different places of chromatin, and as a consequence enabling the regulation of chosen gen transcription [9, 10]. Current researches show that SATB1 protein is expressed in cells, which have to change their function, e.g. in differentiating progenitor cells. The typical example is the maturation of thymocytes into T-lymphocytes, well described in the literature [11].

Recent papers indicate that SATB1 is an independent indicator of bad prognosis in some tumors. A high level of the expression this protein has been marked in samples of the tumor taken from the patients with breast cancer distinctly correlated with the metastasis (no matter what the condition of the lymph nodes was at the moment of the marking) and a time left. The assessment of the expression profiles of genes that have

Table I. SATB1 expression in mycosis fungoides patients

Patient No.	Patch stage	Plaque stage	Tumor stage
1	SATB1+	SATB1+	SATB1+
2	SATB1+	SATB1+	SATB1+
3	SATB1+	SATB1+	SATB1+
4	SATB1+	SATB1+	–
5	SATB1+	SATB1+	–
6	SATB1+	SATB1+	–
7	SATB1+	SATB1+	–
8	–	SATB1+	SATB1+
9	–	SATB1+	–
10	–	SATB1+	–
11	–	SATB1+	–
12	SATB1 –	SATB1 –	SATB1 –
13	SATB1 –	SATB1 –	–
14	–	SATB1 –	–
15	–	SATB1 –	–

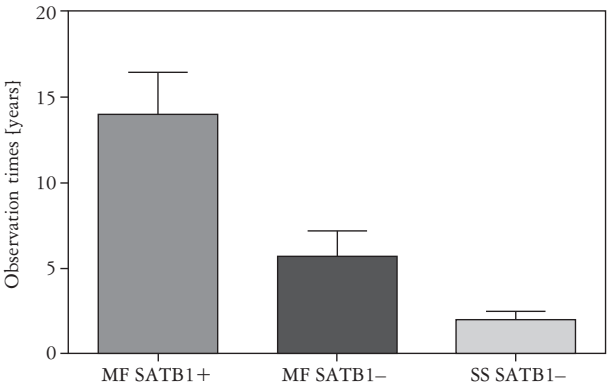

Fig. 2. Average disease duration from the onset of symptoms

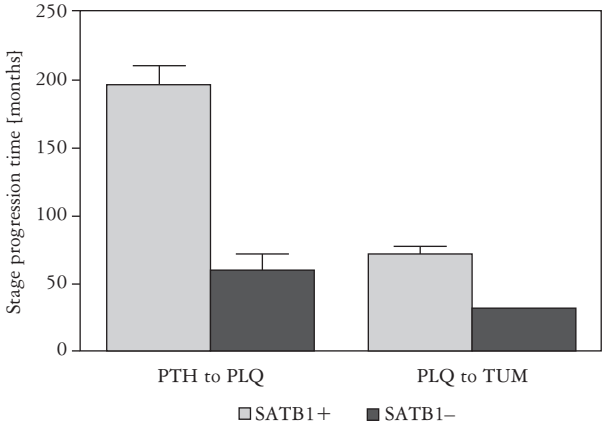

Fig. 3. Average time of mycosis fungoides stage-to-stage progression

been simultaneously taken on the breast cancer cell lines using the shRNA method to down-regulate the expression of SATB1 has shown changes in expression of over 1000 genes. Many of these genes were associated with metastasis and an aggressive course of disease with a simultaneous stop of tumour suppressor genes. These observations coincided with the changes in morphology of the cells *in vitro* and with *in vitro* marked in mice with metastasis potential of implanted tumour cells which correlated with the controlled level of SATB1 expression [12]. These observations were confirmed by other authors who pointed out to the correlation of SATB1 expression at the level of mRNA with classical unfavorable prognosis factors (grade, stage, NPI) [13]. Similarly, the correlation between the level of expression of SATB1 mRNA and metastasis and unfavorable prognosis was demonstrated for non-small-cell lung carcinoma [14], squamous laryngeal cancer [15], and bladder cancer [16]. Different results were currently presented by Irons *et al.* in studies on the breast cancer cell lines as well as the mice model analogous to the Kohwi-Shigematsu. The influence of silencing and overexpression of SATB1 protein in accordance with previous reports on a change of morphology of cultured cells, forming of tumors and metastasis in case of xenograft in mice was not observed. Moreover, using the tissue matrix to determine the expression of mRNA for SATB1 did not lead to confirmation of the correlation between its high level and unfavorable course of the disease [17].

Many basic researches on the mechanism of how SATB1 works were made on the JURKAT cell line, which comes from immortalized T lymphocytes, including the assessment of the SATB1 participation in the process of apoptosis and these concerning thymocyte differentiation [18, 19]. The JURKAT cells are often used as a cellular model of CTCL [19]. In this aspect the SATB1 expression and correlation with clinical data seems to be particularly interesting, especially with reference to the above mentioned mechanisms of therapeutic methods used in treatment of primary cutaneous lymphoma, and towards the new literature data concerning the correlation between SATB1 protein and Wnt/β-catenin pathway in development of T lymphocytes [21].

In the presented research we observed a correlation between nuclear expression of SATB1 and a better clinical course of disease, reflected by slow progression of the disease or lack of progression to a more advanced stage. No changes in SATB1 expression together with the progress of the disease were noted. Simultaneously, a negative SATB1 reaction was observed in both cases of Sezary syndrome, an aggressive cutaneous lymphoma with the shortest lifetime.

Up to now, there have been no reports in the literature describing the SATB1 expression in mycosis fungoides. Recently, Wang *et al.* have demonstrated the lack of SATB1 expression in the cells isolated from blood of nine patients with the Sezary syndrome [22]. The results of their studies showed that the restoration of SATB1 expression in the cellular module trigger a spontaneous death of cells and a strong sensitization of Hut78 cells (an established tumour cell line of Sezary syndrome) for activation-induced cell death (AICD). These observations combine the lack of SATB1 expression with the high resistance of Hut78 cell line for apoptosis (especially AICD), which, as proposed by other authors, can be responsible for the CTCL development and progression [23, 24]. Reports cited above are also reflected in our studies – worse prognosis of lymphoma was in the case of lack of SATB1 expression and it can be connected with the proposed mechanism of immunity to apoptosis.

The connection of SATB1 expression with the apoptosis immunity can also explain a different meaning of SATB1 protein in cutaneous lymphoma, which is different from meaning in cancers and cutaneous melanoma where the high expression of this protein correlated with an unfavorable prognosis [25]. In that aspect, cutaneous lymphomas are a group of diseases of absolutely different biology than the diseases previously studied. The SATB1 expression seems to be not only a potential prognostic marker but also a stratification factor confirming the inner diversity of CTCL. The lack of factors that can

determine the rate of CTCL progression makes the choice of adequate treatment difficult. It also makes hard to create unambiguous guidelines and precise classification of that diverse group of tumors.

The present research is an initial report conducted on a relatively small group of patients. The results provide the basis for an analysis based on a larger group of patients with the use of additional methods which will make the unambiguous evaluation of SATB1 expression possible.

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