Expression of *SOX18* in Mycosis Fungoides

Alina JANKOWSKA-KONSUR[1], Christopher KOBIERZYCKI[2], Adam REICH[1], Aleksandra PIOTROWSKA[2], Agnieszka GOMULKIEWICZ[2], Mateusz OLBROMSKI[2], Marzenna PODHORSKA-OKOLOW[2], Piotr DZIEGIEL[2,3] and Jacek C. SZEPIETOWSKI[1]
Departments of [1]Dermatology, Venereology and Allergology and [2]Histology and Embryology, University of Medicine, and [3]Department of Physiotherapy, University School of Physical Education, Wroclaw, Poland

***SOX18* is a transcription factor involved in the development of hair follicle, blood and lymphatic vessels, as well as regenerative processes. In addition, accumulated data indicate the role of SOX18 in tumourigenesis. So far, no studies on the role of *SOX18* expression in mycosis fungoides (MF), the most common primary cutaneous T-cell lymphoma, have been performed. Therefore, we evaluated *SOX18* expression in MF at the mRNA and protein level. *SOX18* expression was observed predominantly on the blood and lymphatic vessels, in the intratumoural and peritumoural microenvironment of MF. The intratumoural, but not peritumoural, expression of *SOX18* correlated positively with the advancement of the disease, cutaneous involvement and extracutaneous metastases at the protein level ($p < 0.001$, $p < 0.001$, $p = 0.004$, respectively). Significantly lower *SOX18* mRNA expression was correlated with lymph node involvement ($p = 0.01$). In conclusion, we hypothesize that *SOX18*, as a marker of neovascularization, may be involved in the progression of MF.**

Key words: mycosis fungoides; SOX18; angiogenesis; lymphangiogenesis; proliferation markers; Ki-67.

Accepted May 16, 2016; Epub ahead of print May 27, 2016

Acta Derm Venereol 2017; 97: 17–23.

Corr: Jacek C. Szepietowski, Department of Dermatology, Venereology and Allergology, University of Medicine, Chalubinskiego 1, PL-50-368 Wroclaw, Poland. E-mail: jacek.szepietowski@umed.wroc.pl

Mycosis fungoides (MF) is the most common subtype of primary cutaneous T-cell lymphomas (CTCL), defined by skin-only involvement without extracutaneous spread at the time of presentation. MF is characterized by a long-term disease course, slow progression and favourable prognosis, in which it resembles indolent nodal non-Hodgkin's lymphomas. Clinically, in the early stages, MF manifests with itchy patches and plaques that strongly assimilate benign dermatoses, i.e. eczema, lichen planus or psoriasis vulgaris, and therefore represents a diagnostic challenge for dermatologists and pathologists (1, 2). The skin lesions tend to withdraw and relapse, and the disease can proceed in this way for many years. In some cases, however, MF may rapidly progress, by skin tumour development and systemic dissemination, which significantly worsens the prognosis and survival. Despite intensive research over past decades, mechanisms impacting MF development and spread, as well as factors affecting the disease course have not been fully disclosed. Male sex, advanced age, advanced clinical staging, increased serum lactate dehydrogenase level and eosinophilia have been identified as unfavourable prognostic factors; however, novel diagnostic and prognostic tools in the MF evaluation are needed (3–6). Recent studies have also focused on the role of neovascularization in MF growth and dissemination (7).

SRY-related HMG-box 18 (*SOX18*) belongs to the *SOX* family of genes encoding transcription factors, structurally characterized by a high-mobility group (HMG) domain, which specifically binds to the 5'-(A/T)(A/T)CAA(A/T)G-3' DNA sequence motif (8, 9). Based on their amino acid homology, members of the SOX family have been divided into 10 groups (A–J). Along with *SOX7* and *SOX17*, *SOX18* belongs to the group F, and plays an important role in the vascular development and lymphatic fate specification (10). Mutation of *SOX18* has been associated with aberrant blood and lymphatic system development, resulting in hypotrichosis-lymphoedema-telangiectasia syndrome, the rare developmental condition, affecting hair follicles, blood as well as lymphatic vasculature (11). In addition, a *SOX18 de novo* mutation has been associated with congenital disorders, including aortic dilation, hypotrichosis and telangiectasia (12). Since pathological vascularization is likely to repeat genetic programmes and signalling pathways during embryonic vessel development, it is not surprising that the markers of physiological angiogenesis and lymphangiogenesis are also found in the processes of tumourigenesis. Recently, *in vitro* and *in vivo* models, as well as clinical studies have indicated the role of *SOX18* in tumour growth and spread (13–15). Duong et al. (14) in the study on a mouse model of melanoma have observed that *SOX18* disruption impairs neolymphangiogenesis during tumour growth and the partial loss of *SOX18* reduces cancer cell metastasis. Similarly, in another study, allograft melanoma tumours in the mice heterozygous for the dominant-negative *SOX18* mutation (Sox18RaOp) or null for *SOX18* showed reduced growth and microvessel density (16). Increased *SOX18* expression has been demonstrated in endothelial cells of blood and lymphatic vessels indicating its role in vascularization of the neoplastic tissue (17). On the other hand, recent *in vitro* studies have documented *SOX18* expression in breast, gastric and pancreatic cancer as well as

in melanoma cell lines (13). *SOX18* overexpression has been reported in gastric cancer tissues compared with the normal control (18). In addition, recent papers have also implied the association of *SOX18* expression and aggressiveness of the tumour in terms of malignancy grade and distant metastases. In the study focusing on *SOX18* expression in tissue samples and cell lines of invasive breast cancer, Pula et al. (15) have noted increased *SOX18* expression in cancer cells that correlated with higher malignancy grades. In another study by Wang et al. (19) *SOX18* knockdown cell lines derived from hepatocellular carcinoma (HCC) showed inhibition of the proliferation as well significantly impaired migration and invasion.

The aim of our study was to evaluate the diagnostic and prognostic value of *SOX18* in the most common CTCL, mycosis fungoides. Utilizing immunohistochemical (IHC) and molecular methods, we assessed the expression of *SOX18* in relation to clinicopathological data, mostly the progression of the disease. In addition, based on the observations linking *SOX18* expression with aggressive behaviour of the malignancy, we also correlated its expression with the proliferation markers Ki-67 and mini-chromosome maintenance proteins 3 and 7 (MCM-3, MCM-7). To the best of our knowledge, no studies analysing the role of *SOX18* expression in CTCL, including MF, have been published until now.

METHODS

Patients

The study was conducted on archival paraffin-embedded samples collected during diagnostic procedures from 80 patients with MF (29 women, 51 men, mean age: 59.2 ± 14.2 years, median: 62.5 years, range: 19–91 years), treated between the years 1994 and 2015 in the Department of Dermatology, Venereology and Allergology (Wroclaw Medical University, Poland). The diagnosis of the disease was established or re-evaluated based on clinical, histopathological and IHC examinations, according to the World Health Organization (WHO) classification (2008) (20). The staging was assessed according to TNMB (Tumour, Nodes, Metastases, Blood) system (ISCL/EORTC revision) (Tables SI and SII[1]) (21). Forty-seven patients were classified as the early stage (IA–IIA) and 33 were in the advanced stage (IIB–IVB). Healthy skin and chronic benign dermatitis paraffin-embedded blocks (15 lichen planus, 3 eczema disseminatum) served as controls. We used chronic dermatitis samples in order to elucidate the differences between benign and malignant skin conditions. From 26 patients (13 women, 13 men; 16 in the early and 10 in the advanced stage, mean age 55.3 ± 15.7 years) and 7 controls skin biopsies were collected into RNAlater (Qiagen, Hilden, Germany) and stored at –20°C until real-time PCR method was performed. In addition, 19 MF skin biopsies obtained from 10 women and 9 men (mean age 58.0 ± 15.8 years, 11 in the early and 8 in the advanced stage) and 6 controls were also collected and stored in –80°C in order to perform Western blot analysis. The study was approved by the ethics committee of Wroclaw Medical University (approval no. KB 574/2011).

[1]https://doi.org/10.2340/00015555-2466

Immunohistochemistry

All IHC reactions were performed on 4-µm thick tumour paraffin sections using Dako Autostainer Link 48 (Dako, Glostrup, Denmark) using murine monoclonal antibodies directed against SOX18 – 1:25 (sc-166025, epitope: 161–300 aa, Santa Cruz Biotechnology Inc., Dallas, TX, USA) to ensure repeatable reaction conditions. In order to validate the IHC reactions, we carried out additional analysis using rabbit polyclonal antibody Sox-18 – 1:50 (sc-20100, epitope: 161-300 aa, Santa Cruz Biotechnology Inc.) and we obtained convergent results.

Deparaffinization and antigen retrieval were performed using EnVision FLEX Target Retrieval Solution (pH 9.0, 97°C, 20 min; Dako) in PT Link (Dako). The sections were then washed in EnVision FLEX Wash Buffer (Tris-buffered saline (TBS)/0.05% Tween-20) and endogenous peroxidase was blocked using EnVision FLEX Peroxidase-Blocking Reagent (5 min at room temperature; RT, Dako) followed by a washing step with EnVision FLEX Wash Buffer. Primary antibodies were applied for 20 min at RT and then washed in EnVision FLEX Wash Buffer. Following this EnVision FLEX/HRP – secondary antibodies were applied (20 min at RT; Dako). Diaminobenzidine (DAB, Dako) was utilized as the peroxidase substrate and the sections were incubated for 10 min at RT. Finally, the sections were counterstained with EnVision FLEX Hematoxylin (7 min at RT, Dako), dehydrated in graded ethanol concentrations (70%, 96%, 99.8%) and xylene and mounted in the SUB-X Mounting Medium (Dako). Primary antibody was diluted in EnVision FLEX Antibody Diluent (Dako). Negative controls were performed by omitting the incubation with primary antibody, whereas as positive controls served healthy skin samples with hair follicle and endothelial SOX18 expression (**Fig. 1A, B**, respectively). The IHC sections were evaluated using a BX-41 light microscope (Olympus, Tokyo, Japan) by 2 pathologists who were blinded to the patients' clinical data. Nuclear SOX18 expression assessed in intratumoural and peritumoural area separately was predominantly observed in endothelial cells. Initially, whole slide was scanned at low power (×40 and ×100 magnification) to identify the hot-spots (areas of potentially highest vascular density) as it is used in the microvessel density (MVD) count. Subsequently, these areas were examined under ×200 magnification using a Chalkley Point Array graticule (Pyser Sgi., Edenbridge, UK). The Chalkley count was regarded as the number of grid points that hit stained microvessels. A mean score was determined for 3 intratumoural and peritumoural hot-spots.

Polymerase chain reaction

Total RNA was isolated from studied tissue samples with RNeasy Mini Kit (Qiagen) according to the manufacturer's protocol. To eliminate genomic DNA contamination, on-column DNase digestion was performed using RNase-Free DNase Set (Qiagen). Quantity and purity of RNA samples were assessed by measuring the absorbance at 260 and 280 nm with NanoDrop1000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). First-strand cDNA was synthesized using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Carlsbad, CA, USA). The *SOX18* mRNA expression was determined by quantitative real-time PCR with 7900HT Fast Real-Time PCR System and TaqMan Gene Expression Master Mix (Applied Biosystems). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as reference gene. For the reactions, the following sets of primers and TaqMan probes were used: SOX18 Hs00746079_s1 and GAPDH Hs99999905_m1 (Applied Biosystems). All the reactions were performed in triplicate under the following conditions: activation of polymerase at 50°C for 2 min, initial denaturation at 94°C for 10 min and 40 cycles of denaturation at 94°C for 15 s. followed by annealing and elongation at 60°C for 1 min. The relative SOX18mRNA expression was calculated with the ΔΔCt method.

Western blot

Frozen samples were thawed in CelLytic MT Cell Lysis Solution (Sigma Aldrich, Munich, Germany) with the addition of protease inhibitors, Benzonase – 50 U/ul (Merck; Millipore, Bedford, MA, USA) and 0.2 mM phenylmethylsulfonyl fluoride (PMSF). Protein concentrations of whole cell lysates were determined by bicinchoninic acid (BCA) assay (Pierce, Rockford, IL, USA). Equal amounts of total protein (30 µg) were mixed with sample buffer and dithiothreitol (DTT) and resolved by SDS-PAGE. After the completion of the electrophoresis, the samples were transferred to polyvinylidene difluoride (PVDF) membranes (Immobilon; Millipore, Bedford, MA, USA) and incubated in a 4% BSA solution in Tris-buffered saline (TBS) with the addition of 0.1% Tween-20. Later on, the membranes were incubated with mouse anti-human SOX18 antibody – 1:100 (sc-166025; Santa Cruz Biotechnology Inc.) for a night at 4ºC. At the end the membranes were treated with the peroxidase-conjugated donkey anti-mouse secondary antibody – 1:3,000 (715-035-150; Jacksons Immunoresearch, Suffolk, UK) for 1 h, rinsed, and incubated with the Immun-Star-HRP Chemiluminescent Substrate (Bio-Rad, Hercules, CA, USA). Protein quantifications were based on the total protein normalization.

Statistical analysis

All data were analysed with Statistica12.0 software (Statsoft, Krakow, Poland). Patients and controls were compared using χ^2 test, unpaired Student's *t*-test, Mann-Whitney *U* test and analysis of variance with Scheffé *post hoc* test. Relationships between quantitative data were verified with Spearman rank correlation test. Kaplan–Meier curves overall survival were calculated from the date of the start of therapy until the latest follow-up. The differences between the curves were assessed by log-rank test. *p*-values <0.05 were considered as statistically significant.

RESULTS

Expression of SOX18 *in regard to patients' clinical and pathological data*

The expression of *SOX18* was observed in 77 out of 80 MF samples and 11 out of 18 controls ($p<0.001$). The studied marker was expressed predominantly in the nuclei of endothelial cells of blood and lymphatic vessels. The expression was noted in the peritumoural area as well as in the intratumoural microenvironment (Fig. 1C–D). In some cases nuclear expression of SOX18 was found in neoplastic cells, but reactivity was weak, therefore, it was not taken into further consideration. Among the tested samples, the intratumoural expression of *SOX18* was significantly higher in MF compared with chronic benign dermatoses (means ± standard deviation (SD): 5.9 ± 3.0 and 4.2 ± 2.0, respectively, $p=0.03$). To determine the correlation between *SOX18* expression and the advancement of the disease, its expression was analysed with respect to the MF staging. *SOX18* intratumoural expression was significantly higher in advanced stages compared with early ones ($p<0.001$) (**Table I**). We also found increased expression of intratumoural *SOX18* in more infiltrated skin lesions (tumours, T3) compared with the less infiltrated ones (patches, plaques (T1, T2) ($p<0.001$) and erythroderma (T4) ($p=0.03$) (Table I). Regarding metastases, the increased expression of intratumoural *SOX18* was linked with the disease dissemination to the lymph nodes ($p=0.04$). Analysing the peritumoural *SOX18* expression, no significant differences were observed between MF and benign dermatoses, as well as between various MF groups divided based on its advancement.

To support the results obtained by IHC reaction we also studied *SOX18* expression in frozen samples of MF and control group (19 and 6 subjects, respectively) using the Western blot technique. The analysis revealed slightly higher expression of *SOX18*

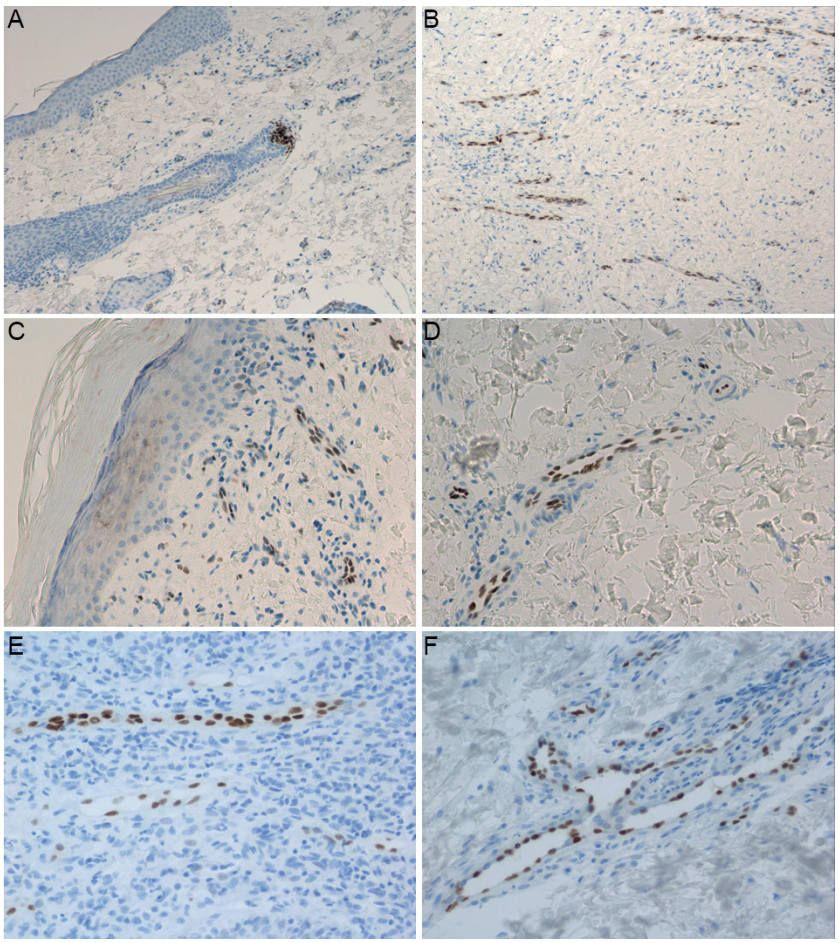

Fig. 1. Immunohistochemical expression of SOX18 in healthy skin samples. (A) In hair papilla and (B) in endothelial cells (×100). Nuclear expression of *SOX18* in endothelial cells in vessels of (C) dermis and (D) subcutaneous layer (×200), whereas in mycosis fungoides in (E) intratumoural and (F) peritumoural area (×400).

Table I. Expression intensities of intratumoural and peritumoural SOX18 in mycosis fungoides

	Intratumoural SOX18			Peritumoural SOX18		
	Mean ± SD	Median (range)	*p*-value	Mean ± SD	Median (range)	*p*-value
Stage						
T1	4.8 ± 2.1	5.3 (0–9.3)	**< 0.001** (T3 vs. T1 or T2: $p < 0.001$, T3 vs. T4: $p = 0.03$)	2.5 ± 2.8	2.3 (0–10.7)	0.97
T2	4.9 ± 2.5	5.0 (0–8.7)		2.5 ± 3.0	1.7 (0–8.7)	
T3	9.0 ± 3.8	8.0 (4.0–16.0)		2.7 ± 2.3	2.3 (0–8.0)	
T4	6.2 ± 1.8	6.3 (3.3–10.0)		3.0 ± 4.0	2.0 (0–14.0)	
Stadium						
a	4.9 ± 2.2	5.3 (0–8.7)	0.69	2.8 ± 2.9	2.3 (0–10.7)	0.49
b	5.2 ± 2.5	5.2 (0–9.3)		2.1 ± 2.6	0 (0–7.0)	
Lymph nodes						
N0	5.4 ± 2.9	5.3 (0–14.0)	**0.04**	2.7 ± 3.0	2.3 (0–10.7)	0.71
N1–3	6.7 ± 3.0	6.3 (3.3–16.0)		2.5 ± 3.1	2.3 (0–14.0)	
Metastases						
B0	5.9 ± 3.0	5.7 (0–16.0)	0.83	2.7 ± 3.1	2.3 (0–14.0)	0.42
B1	5.7 ± 1.8	5.7 (3.3–8.0)		1.6 ± 1.5	2.3 (0–3.3)	
Stage						
Early	4.9 ± 2.3	5.3 (0–9.3)	**< 0.001**	2.6 ± 2.9	2.3 (10.7)	0.45
Advanced	7.4 ± 3.2	6.7 (3.3–16.0)		2.7 ± 3.2	2.3 (0–14)	
Status						
Alive	5.5 ± 2.9	5.3 (0–16.0)	0.31	2.5 ± 2.9	2.2 (0–14.0)	0.94
Dead	6.2 ± 2.7	6.0 (0–14.0)		2.5 ± 2.7	2.0 (0–10.7)	

Analysis of variance with *post hoc* test Scheffe and Student's *t*-test. Significant values are shown in bold.

in the MF group (0.07 ± 0.06 vs. 0.06 ± 0.04, $p = 0.79$), albeit not reaching statistical significance.

Real-time PCR analysis of *SOX18* mRNA expression was performed in 26 MF samples and 7 control cases. Significantly lower mean *SOX18* mRNA levels were observed in MF group than in chronic benign dermatoses (RQ 0.6 ± 0.3 and 1.4 ± 0.9, respectively, $p < 0.001$). In addition, higher *SOX18* mRNA expression was associated with less advanced lymph node involvement (stage N0: 0.68 ± 0.37 vs. N1: 0.48 ± 0.17 vs. N2: 0.18 ± 0.03, Scheffé *post hoc* test: N2 vs. N0: $p < 0.001$, N2 vs. N1: $p = 0.01$), but with more infiltrative nature of MF (stage b vs. stage a: 0.6 ± 0.2 vs. 1.0 ± 0.4, $p = 0.03$).

Correlation of SOX18 *expression with proliferation markers: Ki-67, MCM-3 and MCM-7*

In the present study we found a statistically significant correlation between intratumoural and peritumoural *SOX18* expression (r = 0.37; $p < 0.001$) (**Fig. 2A**). We also focused on the expression of the proliferation markers: Ki-67, MCM-3 and MCM-7 in relation to *SOX18* and we noted a statistically significant correlation between intratumoural *SOX18* and Ki-67, MCM-3 and MCM-7 expressions (Fig. 2B–D). In contrast, statistical analysis did not reveal any significant correlation between peri-

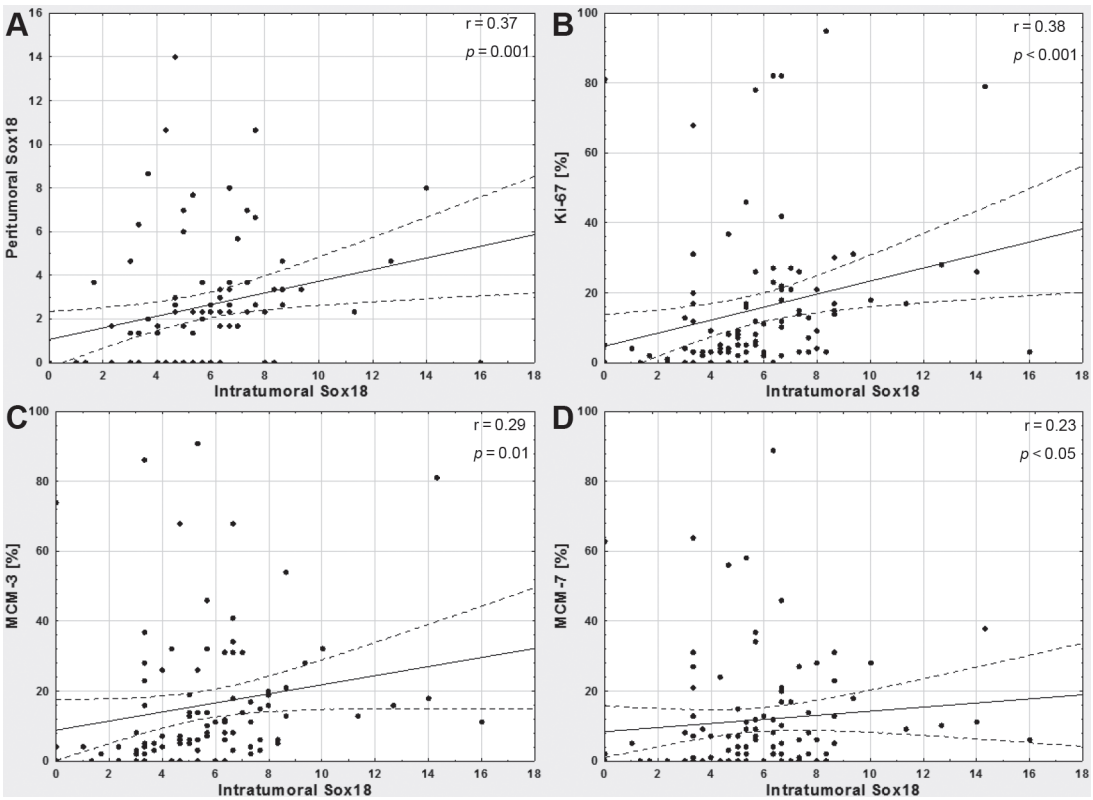

Fig. 2. Spearman-rank correlation test revealed moderate, albeit significant, positive associations between intratumoural expression of *SOX18* and peritumoural SOX18 (A), Ki-67 (B), MCM-3 (C) and MCM-7 (D) in patients with mycosis fungoides.

tumoural *SOX18* expression and studied proliferation markers. Detailed IHC analysis of the expression of proliferation markers Ki-67, MCM-3 and MCM-7 in CTCL was published previously (22).

The detailed analysis of patients' survival in regard with advancement of the disease and the epidemiological data were shown in our previous report (22). In the present study we focused on the patients' survival in regard to the *SOX18* expression and we did not find any significant relationship between patient's survival and intratumoural, as well as peritumoural *SOX* expression. In addition, no other associations between *SOX18* immunoreactivity and sex, age and disease duration were noted (data not shown).

DISCUSSION

Accumulated data indicate the role of *SOX18* in tumourigenesis. Recent studies have revealed the increased *SOX18* expression in tumour cells as well as in endothelial cells of blood and lymphatic vessels in intratumoural and peritumoural areas, which may indicate a double role of *SOX18* in the growth and spread of neoplasms (15, 19, 23).

In our study, for the first time in the literature, we have documented the expression of *SOX18* on both mRNA and protein level, and its correlation with the progression of MF. In other recent studies focusing on the *SOX18* expression in lung, breast and ovarian tumours, the expression of the analysed transcription factor was observed in both cancer and endothelial cells of blood and lymphatic vessels (15, 23, 24). On the contrary, in MF, the significant expression of *SOX18* was found almost exclusively in the blood and lymphatic endothelium of intratumoural and peritumoural areas. Therefore, we assumed that the role of this factor in the development and spread of MF might be associated with neovascularization of the malignancy. Vascularization of the tumour is essential for its growth and dissemination. It is also one of the key markers of disease outcome. Numerous studies published over the past decades showed a close relationship between the vascularization of the tumour, its histological grade and clinical progression and poor prognosis in non-Hodgkin's lymphomas (NHL) (25–28). In contrast, relatively little attention was paid to primary CTCLs in the aspect of angio- and lymphangiogenesis. In our study, comparing malignant and non-malignant skin samples, we observed a higher *SOX18* reaction intensity in the vessels of MF than in vessels from lichen planus and disseminated eczema samples, representing benign inflammatory dermatoses. The *SOX18* overexpression in MF tissues suggests the role of this factor in tumour growth and angiogenesis rather than in the inflammatory processes. Similar results were obtained by Vacca et al. (29) who evaluated angiogenesis, measured by the MVD assessment in the skin biopsies obtained from MF patients and noticed significantly higher MVD in the skin lesions compared with the unchanged clinically patients' skin samples. We also noted the correlation between *SOX18* reaction intensity and advancement of the disease. The endothelial expression of *SOX18* was significantly higher in advanced stages compared with the early ones, which may reflect the increase in vascularity associated with disease progression. These results are consistent with our previous observations concerning angiogenesis in MF, which demonstrated increased angiogenesis measured by CD34 expression in advanced vs. early stages (30). Similarly, in Sézary syndrome (SzS), an aggressive leukaemic counterpart of MF, blood and lymphatic vessel density measured by CD31 and podoplanin expression, respectively, increased in parallel with progression of the disease (7). Even though MF represents a different type of malignant disease compared to solid tumours, MF data are also supported by the study of Pula et al. (24) who reported the correlation between higher *SOX18* expression and advanced disease stage in ovarian cancer.

In our study we documented the differential *SOX18* expression regarding skin and lymph node involvement in MF patients. More infiltrated cutaneous lesions were characterized by a higher intratumoural *SOX18* expression, whereas no correlation between peritumoural *SOX18* expression and tumour staging was found. Similarly, in the studies of Mazur et al. (31) and Jankowska-Konsur et al. (30), progression of the skin lesions correlated with higher MVD in MF. We also observed that the higher intratumoural, but not peritumoral *SOX18* reaction intensity was also associated with the dissemination of malignant cells to the lymph nodes. Our results are in concordance with other studies focusing on the impact of *SOX18* on cancer development and spread, e.g. the experiments on melanoma allografts in mice models have documented the metastatic potential of *SOX18* tumoural expression (14). In addition, the putative impact of *SOX18* on cancer dissemination was also demonstrated by Wang et al. (19) who have observed the impaired migration and invasion of *SOX18* knock-down hepatocellular cancer cells in the transwell migration assay. Interestingly, although our results strongly suggest the role of *SOX18* in the disease progression, its expression did not affect patient survival. On the contrary, in solid tumours (breast, ovarian and non-small cell lung cancer) higher *SOX18* expression correlated with poor prognosis (15, 23, 24). Insignificant correlations between survival and intratumoural *SOX18* expression might be associated with small sample size. However, these differences may also be partially explained by the different nature of MF, and further research determining the impact of *SOX18* expression on the clinical outcome in MF is needed.

In the present study, we obtained divergent results concerning the levels of *SOX18* mRNA and protein expression levels in MF and chronic benign dermatoses. Significantly lower *SOX18* mRNA expression was

observed in MF compared with the control group. Conversely, the *SOX18* protein expression level was higher in MF group. Recent *in vitro* and *in vivo* studies on the lung cancer have documented similar discrepancy of *SOX18* mRNA and protein level (23, 32, 33). According to the authors the reason for this divergence could be the hypermethylation, frequently observed in this type of malignancy. Accumulated data over the last decade indicates that gene expression may be regulated at the transcriptional and posttranscriptional level by a number of epigenetic mechanisms and epigenetic-related microRNA (miRNA) activity. In tumours these phenomena may be of particular importance, especially in the context of genetic instability, characteristic feature of malignancies (34, 35).

Uncontrolled proliferation is one of the hallmarks of a malignancy, indicating a tumour's aggressiveness potential. Over 2 decades proliferation rate has been routinely evaluated using Ki-67 antigen, a non-histone nuclear protein; however, new proliferation markers, i.e. MCM, have been implemented in cancer assessment recently. Accumulated data have pointed to the correlation between proliferation rate and aggressiveness of CTCL, disease advancement and poor clinical outcome (30, 32, 37, 38). Therefore, in the current study, we checked the association between the expression of *SOX18* and the proliferation markers Ki-67, MCM-3 and MCM-7 as indicators of the malignancy aggressiveness. The significant correlations between all analysed markers of proliferation and *SOX18* found in our study suggest that both, high proliferation rate and increased vascularization of the tumour, are determinants of the aggressive course of the malignant process. Our results are consistent with the study of Jethon et al. (23), who found a positive correlation between *SOX18* and Ki-67 expression in non-small lung cancer.

This report for the first time documents the expression pattern of *SOX18* in MF, as well as its impact on the clinical progression of the disease. We found that intratumoural *SOX18* expression in the endothelial cells of the tumour vessels correlates with advanced stage, cutaneous involvement and metastatic status. Interestingly, although our results clearly indicate the role of *SOX18* in the disease progression, its expression did not affect patient survival. In our opinion, *SOX18* could be considered as a new marker of the disease advancement, as well as the subject of research on new therapeutic strategies; however, further studies are needed.

ACKNOWLEDGEMENTS

This work was financially supported by the National Science Center (Decision No. DEC-2011/01/B/NZ4/01052).

REFERENCES

1. Arps DP, Chen S, Fullen DR, Hristov AC. Selected inflammatory imitators of mycosis fungoides: histologic features and utility of ancillary studies. Arch Pathol Lab Med 2014; 138: 1319–1327.
2. Maryniak RK, Jankowska-Konsur A. The principles of clinicopathological and immunohistochemical diagnosis of primary cutaneous lymphomas. Pol J Pathol 2011; 62: s1–s23.
3. Zackheim HS, Amin S, Kashani-Sabet M, McMillan A. Prognosis in cutaneous T-cell lymphoma by skin stage: long-term survival in 489 patients. J Am Acad Dermatol 1999; 40: 418–425.
4. Kim YH, Liu HL, Mraz-Gernhard S, Varghese A, Hoppe RT. Long-term outcome of 525 patients with mycosis fungoides and Sezary syndrome: clinical prognostic factors and risk for disease progression. Arch Dermatol 2003; 139: 857–866.
5. Sausville EA, Worsham GF, Matthews MJ, Makuch RW, Fischmann AB, Schechter GP, et al. Histologic assessment of lymph nodes in mycosis fungoides/Sezary syndrome (cutaneous T-cell lymphoma): clinical correlations and prognostic import of a new classification system. Hum Pathol 1985; 16: 1098–1109.
6. Agar NS, Wedgeworth E, Crichton S, Mitchell TJ, Cox M, Ferreira S, et al. Survival outcomes and prognostic factors in mycosis fungoides/Sézary syndrome: validation of the revised International Society for Cutaneous Lymphomas/European Organization for Research and Treatment of Cancer staging proposal. J Clin Oncol 2010; 28: 4730–4739.
7. Karpova MB, Fujii K, Jenni D, Dummer R, Urosevic-Maiwald M. Evaluation of lymphangiogenic markers in Sézary syndrome. Leuk Lymphoma 2011; 52: 491–501.
8. Wegner M. From head to toes: the multiple facets of Sox proteins. Nucleic Acids Res 1999; 27: 1409–1420.
9. Harley VR, Lovell-Badge R, Goodfellow PN. Definition of a consensus DNA binding site for SRY. Nucleic Acids Res 1994; 22:1500–1501.
10. Bowles J, Schepers G, Koopman P. Phylogeny of the SOX family of developmental transcription factors based on sequence and structural indicators Dev Biol 2000; 227: 239–255.
11. Irrthum A, Devriendt K, Chitayat D, Matthijs G, Glade C, Steijlen PM, et al. Mutations in the transcription factor gene SOX18 underlie recessive and dominant forms of hypotrichosis–lymphedema–telangiectasia. Am J Hum Genet 2003; 72: 1470–1478.
12. Wünnemann F, Kokta V, Leclerc S, Thibeault M, McCuaig C, Hatami A, et al. Aortic dilatation associated with a de novo mutation in the sox18 gene: expanding the clinical spectrum of hypotrichosis-lymphedema-telangiectasia syndrome. Can J Cardiol 2016; 32: 135.e1–7.
13. Saitoh T, Katoh M. Expression of human SOX18 in normal tissues and tumors. Int J Mol Med 2002; 10: 339–344.
14. Duong T, Proulx ST, Luciani P, Leroux JC, Detmar M, Koopman P, Francois M. Genetic ablation of SOX18 function suppresses tumor lymphangiogenesis and metastasis of melanoma in mice. Cancer Res 2012; 72: 3105–3114.
15. Pula B, Olbromski M, Wojnar A, Gomulkiewicz A, Witkiewicz W, Ugorski M, et al. Impact of SOX18 expression in cancer cells and vessels on the outcome of invasive ductal brest carcinoma. Cell Oncol 2013; 36: 469–483.
16. Young N, Hahn CN, Poh A, Dong C, Wilhelm D, Olsson J, et al. Effect of disrupted SOX18 transcription factor function on tumor growth, vascularization, and endothelial development. J Nat Cancer Inst 2006; 98: 1060–1067.
17. Luo M, Guo XT, Yang W, Liu LQ, Li LW, Xin XY. Inhibition of tumor angiogenesis by cell-permeable dominant negative SOX18 mutants. Med Hypotheses 2008; 70: 880–882.
18. Eom BW, Jo MJ, Kook MC, Ryu KW, Choi IJ, Nam BH, et al. The lymphangiogenic factor SOX 18: a key indicator to stage gastric tumor progression. Int J Cancer 2012; 131: 41–48.
19. Wang G, Wei Z, Jia H, Zhao W, Yang G, Zhao H. Knockdown of SOX18 inhibits the proliferation, migration and invasion of hepatocellular carcinoma cells. Oncol Rep 2015. doi: 10.3892/or.2015.4112.

20. Rakfkiaer E, Cerroni L, Sander CA, Smoller BR, Willemze R. Mycosis fungoides. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, et al, editors. In: WHO classification of tumours of hematopoietic and lymphoid tissues. Lyon, International Agency for Research on Cancer (IARC); 2008: p. 269–317.
21. Olsen E, Vonderheid E, Pimpinelli N, Willemze R, Kim Y, Knobler R, et al. Revisions to the staging and classification of mycosis fungoides and Sezary syndrome: a proposal of the International Society for Cutaneous Lymphomas (ISCL) and the cutaneous lymphoma task force of the European Organization of Research and Treatment of Cancer (EORTC). Blood 2007; 110: 1713–1722.
22. Jankowska-Konsur A, Kobierzycki Ch, Reich A, Grzegrzolka J, Maj J, Dziegiel P. Expression of MCM-3 and MCM-7 in primaty T-cell cutaneous lymphomas. Anticancer Res 2015; 35: 6017–6026.
23. Jethon A, Pula B, Olbromski M, Werynska B, Muszczynska-Bernhard B, Witkiewicz W, et al. Prognostic significance of SOX18 expression in non-small cell lung cancer. Int J Oncol 2015; 46: 123–132.
24. Pula B, Kobierzycki C, Solinski D, Olbromski M, Nowak-Markwitz E, Spaczynski M, et al. SOX18 expression predicts response to platinum-based chemotherapy in ovarian cancer. Anticancer Res 2014; 34: 4029–4037.
25. Fukushima N, Satoh T, Sano M, Tokunaga O. Angiogenesis and mast cells in non-Hodgkin's lymphoma: a strong correlation in angioimmunoblastic T-cell lymphoma. Leuk Lymphoma 2001; 42: 709–720.
26. Ma SP, Lin M, Liu HN, Yu JX. Lymphangiogenesis in non-Hodgkin's lymphoma and its correlation with cyclooxygenase-2 and vascular endothelial growth factor-C. Oncol Lett 2012; 4: 695–700.
27. Paydas S, Seydaoglu G, Ergin M, Erdogan S, Yavuz S. The prognostic significance of VEGF-C and VEGF-A in non-Hodgkin lymphomas. Leuk Lymphoma 2009; 50: 366–373.
28. Vacca A, Ribatti D, Ruco L, Giacchetta F, Nico B, Quondamatteo F, et al. Angiogenesis extent and macrophage density increase simultaneously with pathological progression in B-cell non-Hodgkin's lymphomas. Br J Cancer 1999; 79: 965–970.
29. Vacca A, Moretti S, Ribatti D, Pellegrino A, Pimpinelli N, Bianchi B, et al. Progression of mycosis fungoides is associated with changes in angiogenesis and expression of the matrix metalloproteinases 2 and 9. Eur J Cancer 1997; 33: 1685–1692.
30. Jankowska-Konsur A, Maj J, Woźniak Z, Baran E. Angiogenesis assessment in patients with Mycosis Fungoides. Post Dermatol Alergol 2009; 26: 186–199.
31. Mazur G, Woźniak Z, Wróbel T, Maj J, Kuliczkowski K. Increased angiogenesis in cutaneous T-cell lymphomas. Pathol Oncol Res 2004; 10: 34–36.
32. Dammann R, Strunnikova M, Schagdarsurengin U, Rastetter M, Papritz M, Hattenhorst UE, et al. CpG island methylation and expression of tumour-associated genes in lung carcinoma. Eur J Cancer 2005 41: 1223–1236.
33. Azhikina T, Kozlova A, Skvortsov T, Sverdlov E. Heterogeneity and degree of TIMP4, GATA4, SOX18, and EGFL7 gene promoter methylation in non-small cell lung cancer and surrounding tissues. Cancer Genet 2011; 204: 492–500.
34. Heyn H, Vidal E, Ferreira HJ, Vizoso M, Sayols S, Gomez A, et al. Epigenomic analysis detects aberrant super-enhancer DNA methylation in human cancer. Genome Biol 2016; 26: 11.
35. Lee ST, Muench MO, Fomin ME, Xiao J, Zhou M, de Smith A, et al. Epigenetic remodeling in B-cell acute lymphoblastic leukemia occurs in two tracks and employs embryonic stem cell-like signatures. Nucleic Acids Res 2015; 43: 2590–2602.
36. Tobisawa S, Honma M, Ishida-Yamamoto A, Saijo Y, Iizuka H. Prognostic factors in 105 Japanese cases of mycosis fungoides and Sézary syndrome: clusterin expression as a novel prognostic factor. J Dermatol Sci 2013; 71: 160–166.
37. Edinger JT, Clark BZ, Pucevich BE, Geskin LJ, Swerdlow SH. CD30 expression and proliferative fraction in nontransformed mycosis fungoides. Am J Surg Pathol 2009; 33: 1860–1868.
38. Gambichler T, Bischoff S, Bechara FG, Altmeyer P, Kreuter A. Expression of proliferation markers and cell cycle regulators in T cell lymphoproliferative skin disorders. J Dermatol Sci 2008; 49: 125–132.