RESEARCH ARTICLE

Expression of Granulysin and FOXP3 in Cutaneous T Cell Lymphoma and Sézary Syndrome

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Abstract

Background: Multiple complex pathways are operable in the evolution of cutaneous T cell lymphomas (CTCLs). These pathways involve interaction between neoplastic T cells and cells of the immune system (especially dendritic cells and the non-malignant T cells). Granulysin is a proinflammatory antimicrobial peptide which has an immune alarmin function, activating dendritic cells, as well as an active role in tumor immunology and prognosis. FOXP3+ regulatory T cells Tregs are an important player in the immune system. Much controversy is found in the literature about the role of Tregs in CTCL. Aim: The present study aimed to investigate the expression of granulysin and FOXP3 in mycosis fungoides (MF), its precursor lesion large plaque parapsoriasis and its leukemic form Sézary syndrome (SS). Materials and Methods: Immunohistochemical expression of granulysin and FOXP3 were assessed in lesional skin biopsies taken from 58 patients (4 large plaque parapsoriasis, 48 MF and 6 SS). Results: Granulysin positivity was cytoplasmic and higher in MF than in parapsoriasis en plaque and higher in the more advanced stages of MF ($p<0.001$). All groups showed significant differences between each other except between MF tumor stage and SS. FOXP3 positivity was nuclear and higher in early stage MF (plaque and patch stages) than in tumor stages and SS ($p<0.001$). However the FOXP3 count was lower in parapsoriasis en plaque than in other stages of MF. All the groups showed significant differences between each other except between parapsoriasis and SS and between patch and plaque stages of MF. Conclusions: The present study supports a role for granulysin in MF progression and proposes a novel hypothesis about the effect of FOXP3 +veTregs in the suppression of the activity of the neoplastic cells in MF.

Keywords: Mycosis fungoides - sézary syndrome - cutaneous T cell lymphoma - granulysin - FOXP3

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Introduction

Primary cutaneous T-cell lymphomas (CTCLs) represent a heterogeneous group of lymphoproliferative disorders characterized by monoclonal proliferation of skin-homing CD4+ve T lymphocytes. (Willemze et al., 2005) The major variant is Mycosis Fungoides (MF), which is confined to the skin and has a slowly progressing course over years to decades. (Quaglino et al., 2012) The less common Sézary syndrome (SS) carries a worse prognosis and is characterized by erythroderma, generalized lymphadenopathy, and leukemic blood involvement with Sézary cells (Wilcox, 2014).

Granulysin is a cytotoxic molecule expressed, together with granzymes and perforin, in granules of cytotoxic T lymphocytes (CTLs) and natural killer cells (NK). It was originally discovered in 1998 as an antimicrobial peptide, with subsequently described roles in innate immunity (cytolytic and pro-inflammatory), dendritic cell chemotaxis and activation (immune alarmin function) and functions in tumor immunology (inducing tumor cell lysis by apoptosis). (Wang, 2014)

Early investigations showed that impaired expression of granulysin by NK cells correlates with progression of cancer and lower levels of granulysin expression denotes an immunosuppressive state in cancer bearing patients (Kishi et al., 2002). Subsequent studies confirmed this observation (Nagasawa et al., 2005; Saigusa et al., 2007). However in NK cell tumors, serum granulysin was found to be proportionately increased with cancer cell load in NK cell leukemia and was significantly reduced after treatment. (Sekiguchi et al., 2012) Based on these findings, some authorities suggested that granulysin level may be used as a marker for tumor prognosis. (Okada and Morishita, 2012) To the best of our knowledge, granulysin expression has not yet been investigated in CTCL.

Regulatory T cells (Tregs), characterized by a CD4+ CD25+ phenotype, represent 5% to 10% of peripheral T cells. They are defined as a T-cell population that can influence other lymphoid cells with suppression of the immune response. Tregs play an essential role in immune regulation by facilitating peripheral immune self-tolerance and thus precluding autoimmunity. (Zou, 2006) Tregs express the transcriptional repressor Forkhead box P3

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(FOXP3) which is essential for their development and considered the most specific marker for them. (Zhan et al., 2012).

Tregs play an important role in immune evasion by cancer cells, by suppression of cancer cell specific CTLs, thus helping cancer cells to escape immune surveillance. In keeping with this, several studies have showed that increased numbers of Tregs are associated with poor prognosis and decreased survival in several types of carcinoma. (Du et al., 2012; Zhang et al., 2012) However, an opposite role is played by these in cells in many hematological malignancies, including CTCL, where higher counts of FOXP3+ve Tregs were associated with improved survival and lower counts were associated with higher stage and more tumor burden. (Kelley and Parker, 2010) Moreover, down regulation of FOXP3 gene expression was linked to the progression of MF. (Johnson et al., 2014) However, the issue was complicated by many observations in CTCL, where large cell transformation in MF was associated with FOXP3 expression in the malignant large cells (Hallermann et al., 2007), CTCL cells could be induced to express FOXP3 in vitro (Berger et al., 2005; Kasprzycka et al., 2008), and the neoplastic cells in few reported CTCL cases were expressing FOXP3 (Gjerdrum et al., 2007; Clark, 2009; Heid et al., 2009; Marzano et al., 2009).

The present study aims to investigate the expression of granulysin and FOXP3 in MF and its precursor lesion large plaque parapsoriasis and its leukemic form SS.

Materials and Methods

The study was conducted on 58 patients (4 large plaque parapsoriasis, 48 MF and 6 SS), which were recruited and clinically diagnosed in Dermatology and Venereology Department, Tanta University Hospital and confirmed pathologically in Pathology Department, Faculty of Medicine, Tanta University. 4 mm punch biopsies were taken from the lesional skin of all patients. Biopsies were fixed in 10% neutral buffered formalin solution, processed and embedded in paraffin wax. The blocks were sectioned and stained routinely by Hematoxylin and eosin. Blocks showing less than 5 high power fields (x400) with lymphomononuclear infiltrate (per section) were excluded.

All selected blocks were immunohistochemically stained for FOXP3 and granulysin. For immunostaining, 4 μm sections were deparaffinized in xylene, and then rehydrated in descending concentrations of ethanol. Blockage of endogenous peroxidases (by incubation in 0.3% H_2O_2 for 30 min) was followed by microwave treatment (15 min in 10 mM sodium citrate buffer pH 6.0) for antigen retrieval. Slides were then incubated with the primary antibodies FOXP3 (Mouse Anti-FOXP3 Monoclonal Antibody, Cat. #A00744, GenScript, USA, dilution 1:300 overnight at 4°C) and granulysin (Mouse Anti-granulysin IgG2b Monoclonal Antibody, Biotec, Monosan, Netherlands, dilution: 1:80 for 30 minutes at 25°C). Specific binding was detected using the LSAB 2 kit (Dako, Carpentaria, CA, U.S.A.) according to the manufacturer's instructions. Sections were washed by phosphate buffered saline after each step. Phosphate buffered saline was used instead of the primary antibody as negative control.

Slides were examined blindly (without knowledge of the clinical data). Counting the positive cells was done by the use of LEICA image analysis system (LEICA DFC290 HD, Leica Microsystems Ltd., Heerbrugg, Switzerland). The fields that contained the infiltrate were determined by low power magnification (x100). Five high power fields (x400 fields) were counted in each case. Cells with nuclear positivity for FOXP3 and those with cytoplasmic positivity for granulysin were counted. The counts were statistically analyzed using SPSS version 16 (SPSS Inc., Chicago, Illinois, USA).

Results

The present study included 58 cases of CTCL formed of 54 cases of CTCL (48 MF and 6 SS) and 4 cases of its precursor large plaque parapsoriasis. Different stages of MF were included namely; patch stage (n=10), plaque stage (n=30), tumor stage (n=8) and SS (n=6). The study included 35 male patients and 23 females. The age ranged from 31-71 years with mean 48.9±9.29 years.

Granulysin immunohistochemistry

Granulysin positivity was cytoplasmic (figures 1-A to 1-D) and was detected in the reactive mononuclear cells. The mean counts for Granulysin in the different stages were represented in Table (1). Granulysin positivity was higher in MF than in the precursor lesion (parapsoriasis en plaque) and higher in the more advanced stages of MF ($p<0.001$). In post-hoc test, all groups showed significant differences between each other except between tumor stage and SS ($p=0.08$). No significant difference was found between male and female cases as regards granulysin counts ($p=0.85$).

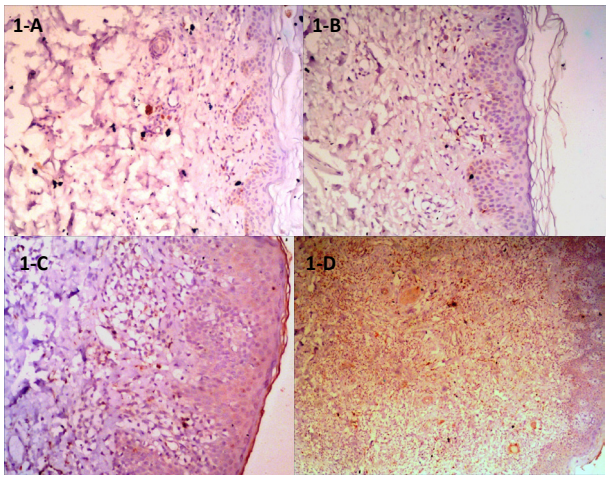


Figure 1. Granulysin Immunohistochemical Expression (cytoplasmic stain). (Figure 1-A) in parapsoriasis en plaque, few positive cells were observed (original magnification x 200); (Figure 1-B) in Mycosis Fungoides (MF), patch stage, more granulysin positive cells are observed compared to Figure 1-A of parapsoriasis en plaque (original magnification x 200); (Figure 1-C) in MF, plaque stage, higher number of granulysin positive cells than in patch stage (original magnification x 200) and (Figure 1-D) highest count of granulysin +ve cells was observed in MF tumor stage (original magnification x 100)

Table 1. Mean Granulysin Counts in Studied Cases

Diagnosis:	Granulysin mean counts	Kruskal Wallis test	P value	Post Hoc
Parapsoriasis (n=4)	2.25±0.95			P1<0.001*
				P2<0.001*
		48.18	<0.001*	P3<0.001*
				P4<0.001*
				P5<0.001*
				P6<0.001*
Patch stage (n=10)	7.70±1.49			P7= 0.001*
Plaque stage (n=30)	16.7±2.92			P8<0.001*
Tumor stage (n=8)	27.9±2.53			P9=0.004*
Sézary syndrome (n=6)	39.5±7.09			P10=0.08
Gender:				
Male (n=35):	18.02±10.8	0.18	0.85	
Female (n=23):	18.1±10.1			

*: significant; P1 between parapsoriasis and patch stage; P2 between parapsoriasis and plaque stage; P3 between parapsoriasis and tumor stage; P4 between parapsoriasis and Sézary syndrome; P5 between patch stage and plaque stage; P6 between patch stage and tumor stage; P7 between patch stage and Sézary syndrome; P8 between plaque stage and tumor stage; P9 between plaque stage and Sézary syndrome and P10 between tumor stage and Sézary syndrome

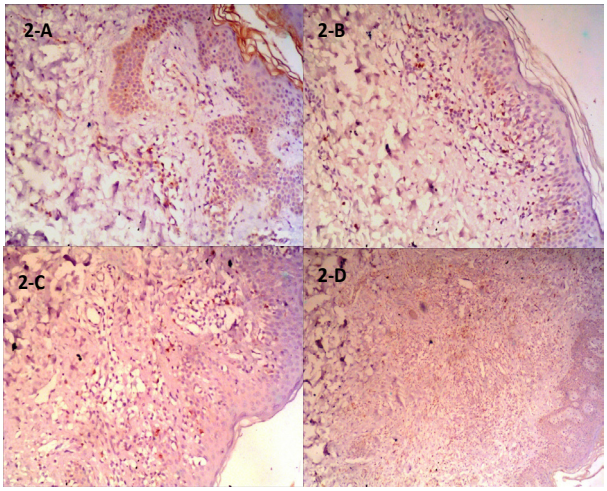
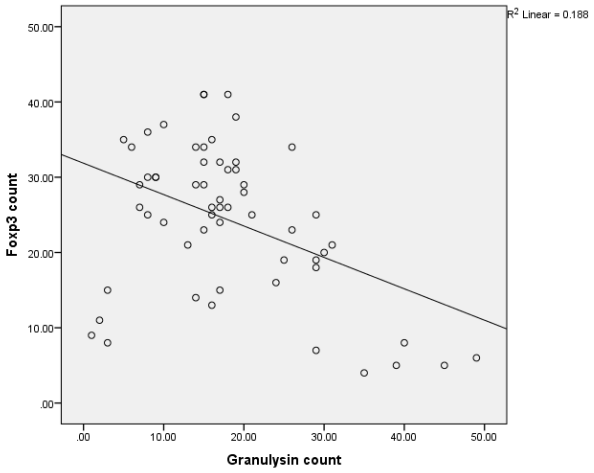


Figure 2. FOXP3 Immunohistochemical Expression (nuclear stain); (Figure 2-A) in parapsoriasis en plaque, few positive cells were observed (original magnification x 200); (Figure 2-B) in Mycosis Fungoides (MF), patch stage, nearly equal number of cells are observed compared to Figure 2-A of parapsoriasis en plaque (original magnification x 200); (Figure 2-C) in MF, plaque stage, lower number of granulysin positive cells than in patch stage (original magnification x 200) and (Figure 2-D) the lowest count of FOXP3 +ve cells in MF tumor stage (original magnification x 100)



Figureure 3. Correlation between FOXP3 and Granulysin Counts in the Studied Cases

Table 2. Mean FOXP3 Counts in Studied Cases

Diagnosis	FOXP3 mean counts	Kruskal Wallis test	P value	Post Hoc
Parapsoriasis (n=4)	10.8±3.8			P1<0.001*
				P2<0.001*
				P3=0.02*
		32.9	<0.001*	P4=0.35
				P5=1
				P6<0.001*
Patch stage (n=10)	29.9±4.2			P7<0.001*
Plaque stage (n=30)	29.1±7.4			P8<0.001*
Tumor stage (n=8)	20.1±2.85			P9<0.001*
Sézary syndrome (n=6)	5.83±1.47			P10<0.001*
Gender				
Male (n=35)	24.5±10.9	0.35	0.72	
Female (n=23)	24.1±8.7			

*: significant; P1 between parapsoriasis and patch stage; P2 between parapsoriasis and plaque stage; P3 between parapsoriasis and tumor stage; P4 between parapsoriasis and Sézary syndrome; P5 between patch stage and plaque stage; P6 between patch stage and tumor stage; P7 between patch stage and Sézary syndrome; P8 between plaque stage and tumor stage; P9 between plaque stage and Sézary syndrome and P10 between tumor stage and Sézary syndrome

FOXP3 immunohistochemistry:

FOXP3 positivity was nuclear and restricted to the reactive T cell population [no neoplastic cell positivity could be detected in all included cases] (figures 2-A to 2-D). The mean FOXP3 counts in the studied cases were summarized in Table (2). The mean FOXP3 count was higher in early stage MF [plaque and patch stages] than in the late tumor stage and Sézary syndrome (p<0.001). However, FOXP3 count was lower in the precursor lesion (parapsoriasis en plaque) than in other stages of MF. In post-hoc test: all the groups showed significant differences between each other except between parapsoriasis en plaque and SS (p=0.35) and between patch and plaque stages of MF (p=1). No significant difference was found between male and female gender as regard FOXP3 mean counts (p=0.72).

Correlation between FOXP3 and Granulysin counts:

A significant negative correlation between FOXP3

and granulysin counts were observed (r=-0.43, p=0.001), figure 3.

Discussion

Although the prognosis of CTCL is generally good, reliable markers are needed to identify the subset of patients at risk for a more aggressive course, including leukemic transformation. (Escher et al., 2006; Zhou et al., 2014) Multiple complex pathways are operable in the evolution of MF through the different stages, ending in leukemic or large cell transformation. These pathways involves the interaction between the neoplastic T cells and cells of the immune system (dendritic cells, cytotoxic T cells and Tregs), with the intermediary action of many cytokines. (Jawed et al., 2014)

To the best of our knowledge, this is the first study to explore granulysin expression in CTCL. Granulysin expression was found to increase with progression of MF from the pre-lymphomatous phase (parapsoriasis en plaque) to the progressive phases of MF. This is well-explainable under the light of the immune alarmin function of Granulysin that activates antigen presenting DCs, which have a central role in the initiation and progression of CTCL (Edelson, 2001). It has been reported that cross talk between malignant T cells and CTL, with the intermediary role of bacterial superantigens, shares substantially in CTCL progression. (Willerslev-Olsen et al., 2013) Granulysin is expressed in, and released on activation of, the CTL, and hence an intermediary role of granulysin, and its alarmin-like action on DC, in CTCL progression is strongly suggested. So, Granulysin may be the junction, in a vicious circle, between CTL and DC in CTCL for perpetuation and progression of the proliferation of the malignant T cells. Hence, granulysin may be a potentially useful marker for aggressive behavior as well as a therapeutic target in CTCL, a fact that should be further validated by correlation with the patient survival.

The role of CD4+ve CD25+ve FOXP3+ve Tregs in MF has been a matter of long controversy. Initially, the work of Berger et al. (2005) suggested that CTCL cells have a regulatory phenotype. Thereafter, FOXP3 expression by the neoplastic cells in CTCL was demonstrated in a series of 5 patients with large-cell transformation of MF. (Hallermann et al., 2007) However, this was soon self-criticized by the lack of specificity of the utilized antibody. (Banham et al., 2008)

Several other groups have provided evidence that FOXP3 expression is rare in the neoplastic population in CTCL and that FOXP3 expression is found mainly in the non-neoplastic Tregs (Klemke et al., 2006; Tiemessen et al., 2006; Gjerdrum et al., 2007; Capriotti et al., 2008; Solomon and Magro, 2008; Wada et al., 2010) In our study, there was no detectable FOXP3 positivity in the neoplastic T cells in any of the studied cases. This observation was in agreement with the results of Klemke et al. (2006) and Wada et al. (2010). Rare examples of CTCL with convincing evidences of neoplastic cell expression of FOXP3 have represented SS (Capriotti et al., 2008; Heid et al., 2009; Wada et al., 2013), aggressive epidermotropic CD8-positive cytotoxic T-cell lymphoma (Gjerdrum et al., 2007) or an extraordinary aggressive epidermotropic CD4+ medium-/large sized primary cutaneous T cell lymphoma (Marzano et al., 2009) rather than classic MF.

In the present study, higher counts of FOXP3 + Tregs were observed in the earlier stages of MF (plaque/patch stages) than in the late tumor stage and SS. This is in keeping with the results of the most of the above mentioned studies of FOXP3 in CTCL (Klemke et al., 2006; Tiemessen et al., 2006; Gjerdrum et al., 2007; Capriotti et al., 2008; Solomon and Magro, 2008; Wada et al., 2010; Alcantara-Hernandez et al., 2014). Moreover, Gjerdrum et al. (2007) found that increased number of Treg was associated with a better prognosis in CTCL (including MF and its leukemic variant SS). Another study showed that very low Treg counts were detected in SS, in both the cutaneous lesion and peripheral blood and even claimed that low counts of FOXP3+ Tregs distinguishes MF from SS. (Klemke et al., 2006) A molecular support for these findings was provided by the work of Johnson et al. (2014), who found a decrease in the FOXP3 mRNA in skin samples with progression of MF stages.

It was proven that increasing Tregs counts in solid malignancies is associated with poorer prognosis; probably due to the suppressive effect of Tregs on CD8 +ve CTLs, responsible for the tumor immunity and killing of tumor cells. (Du et al., 2012; Zhang et al., 2012) The reverse was observed in many hematological malignancies, especially T cell lymphomas, including CTCL (Kelley and Parker, 2010). This paradox in the prognostic significance of Tregs in CTCL compared to solid (non-hematologic) malignancies may be explained by the fact that Tregs not only suppress the anti-tumor immune response, but also have the potential to suppress tumor growth in malignant T cells as well.

However, Fried and Cerroni (2012) denied the progressive decrease of Tregs with progression of the MF lesion in sequential biopsies of 14 MF patients. They claimed that FOXP3 expression can be acquired or lost during the course of the disease, comparable to other phenotypic markers. Nevertheless, the low number of cases in thier study may weaken their conclusion.

No significant difference was found between patch and plaque stage of MF in our study. It should be noted that both stages are early stages of MF, with nearly similar prognosis (Jawed et al., 2014), even prompting some authors to consider both stages as one entity during investigation for FOXP3 counts (Gjerdrum et al., 2007).

In our study, the FOXP3 counts in the prelymphomatous large plaque parapsoriasis were significantly lower than the early stages of MF and very near to that of SS. This may apparently contrast with many studies showing higher counts in the non-neoplastic dermatoses in comparison to CTCL. (Fujimura et al., 2008; Solomon and Magro, 2008; Wada et al., 2010; Hanafusa et al., 2013) However, most of these studies did not include the prelymphomatous lesions in the non-neoplastic entities and also all used the percentage of the FOXP3+ cells rather than absolute counts (as was used in our study). One of these studies, examining FOXP3 counts in prelymphomatous lesions, found even lower counts in large plaque parapsoriasis than in the early stages of MF, agreeing with our results. The

same study showed that reactive lymphomatoid conditions have higher Tregs counts than the prelymphomatous T cell dyscrasias. (Solomon and Magro, 2008)

In the present study, the expression of granulysin, which is known to localize in the granular compartment of CTL, was inversely correlated to FOXP3 expression. This makes sense in the light of the negative effect of Tregs on CTLs, where upregulation of FOXP3 cells leads to down regulation of CTLs. This may be another mechanism by which FOXP3 +ve cells may affect progression of MF in addition to direct suppression of the neoplastic T cells, where Tregs may down regulate CTLs in the lesion, with loss of the alrmin effect of granulysin on DCs. This may lead to loss of the trophic effect of DC on the malignant T cells. When Tregs go down, this inhibitory effect is lost with the release of CTL activity, causing activation of DC and progression of malignancy. However, further mechanistic studies may be needed to substantiate this hypothesis.

In conclusion, the present study supports a role of granulysin in MF progression, with potential diagnostic and therapeutic implications, and proposes a novel hypothesis about the effect of FOXP3 +ve Tregs in the suppression of the activity of the neoplastic cells in MF.

References

Alcantara-Hernandez M, Torres-Zarate C, Perez-Montesinos G, et al (2014). Overexpression of hypoxia-inducible factor 1 alpha impacts FoxP3 levels in mycosis fungoides--cutaneous T-cell lymphoma: clinical implications. *Int J Cancer*, **134**, 2136-45.

Banham AH, Brown PJ, Lyne L, et al (2008). Is FOXP3 expressed in cutaneous T-cell lymphomas? *Eur J Haematol*, **80**, 90-1.

Berger CL, Tigelaar R, Cohen J, et al (2005). Cutaneous T-cell lymphoma: malignant proliferation of T-regulatory cells. *Blood*, **105**, 1640-7.

Capriotti E, Vonderheid EC, Thoburn CJ, et al (2008). Expression of T-plastin, FoxP3 and other tumor-associated markers by leukemic T-cells of cutaneous T-cell lymphoma. *Leuk Lymphoma*, **49**, 1190-201.

Clark RA (2009). Regulation gone wrong: a subset of Sezary patients have malignant regulatory T cells. *J Invest Dermatol*, **129**, 2747-50.

Du Y, Chen X, Huang ZM, et al (2012). Increased frequency of Foxp3+ regulatory T cells in mice with hepatocellular carcinoma. *Asian Pac J Cancer Prev*, **13**, 3815-9.

Edelson RL (2001). Cutaneous T cell lymphoma: the helping hand of dendritic cells. *Ann N Y Acad Sci*, **941**, 1-11.

Escher N, Spies-Weisshart B, Kaatz M, et al (2006). Identification of HNP3 as a tumour marker in CD4+ and CD4- lymphocytes of patients with cutaneous T-cell lymphoma. *Eur J Cancer*, **42**, 249-55.

Fried I, Cerroni L (2012). FOXP3 in sequential biopsies of progressive mycosis fungoides. *Am J Dermatopathol*, **34**, 263-5.

Fujimura T, Okuyama R, Ito Y, et al (2008). Profiles of Foxp3+ regulatory T cells in eczematous dermatitis, psoriasis vulgaris and mycosis fungoides. *Br J Dermatol*, **158**, 1256-63.

Gjerdrum LM, Woetmann A, Odum N, et al (2007). FOXP3+ regulatory T cells in cutaneous T-cell lymphomas: association with disease stage and survival. *Leukemia*, **21**, 2512-8.

Hallermann C, Niermann C, Schulze HJ (2007). Regulatory T-cell phenotype in association with large cell transformation of mycosis fungoides. *Eur J Haematol*, **78**, 260-3.

Hanafusa T, Matsui S, Murota H, et al (2013). Increased frequency of skin-infiltrating FoxP3+ regulatory T cells as a diagnostic indicator of severe atopic dermatitis from cutaneous T cell lymphoma. *Clin Exp Immunol*, **172**, 507-12.

Heid JB, Schmidt A, Oberle N, et al (2009). FOXP3+CD25- tumor cells with regulatory function in Sezary syndrome. *J Invest Dermatol*, **129**, 2875-85.

Jawed SI, Myskowski PL, Horwitz S, et al (2014). Primary cutaneous T-cell lymphoma (mycosis fungoides and Sezary syndrome): part I. Diagnosis: clinical and histopathologic features and new molecular and biologic markers. *J Am Acad Dermatol*, **70**, 1-16.

Johnson VE, Vonderheid EC, Hess AD, et al (2014). Genetic markers associated with progression in early mycosis fungoides. *J Eur Acad Dermatol Venereol*, **28**, 1431-5.

Kasprzycka M, Zhang Q, Witkiewicz A, et al (2008). Gamma c-signaling cytokines induce a regulatory T cell phenotype in malignant CD4+ T lymphocytes. *J Immunol*, **181**, 2506-12.

Kelley TW, Parker CJ (2010). CD4 (+)CD25 (+)Foxp3 (+) regulatory T cells and hematologic malignancies. *Front Biosci (Schol Ed)*, **2**, 980-92.

Kishi A, Takamori Y, Ogawa K, et al (2002). Differential expression of granulysin and perforin by NK cells in cancer patients and correlation of impaired granulysin expression with progression of cancer. *Cancer Immunol Immunother*, **50**, 604-14.

Klemke CD, Fritzsching B, Franz B, et al (2006). Paucity of FOXP3+ cells in skin and peripheral blood distinguishes Sezary syndrome from other cutaneous T-cell lymphomas. *Leukemia*, **20**, 1123-9.

Marzano AV, Vezzoli P, Fanoni D, et al (2009). Primary cutaneous T-cell lymphoma expressing FOXP3: a case report supporting the existence of malignancies of regulatory T cells. *J Am Acad Dermatol*, **61**, 348-55.

Nagasawa M, Kawamoto H, Tsuji Y, et al (2005). Transient increase of serum granulysin in a stage IVs neuroblastoma patient during spontaneous regression: case report. *Int J Hematol*, **82**, 456-7.

Okada S, Morishita T (2012). The Role of Granulysin in Cancer Immunology. *ISRN Immunology*, **2012**, 5.

Quaglino P, Pimpinelli N, Berti E, et al (2012). Mycosis fungoides: disease evolution of the "lion queen" revisited. *G Ital Dermatol Venereol*, **147**, 523-31.

Saigusa S, Ichikura T, Tsujimoto H, et al (2007). Serum granulysin level as a novel prognostic marker in patients with gastric carcinoma. *J Gastroenterol Hepatol*, **22**, 1322-7.

Sekiguchi N, Asano N, Ito T, et al (2012). Elevated serum granulysin and its clinical relevance in mature NK-cell neoplasms. *Int J Hematol*, **96**, 461-8.

Solomon GJ, Magro CM (2008). Foxp3 expression in cutaneous T-cell lymphocytic infiltrates. *J Cutan Pathol*, **35**, 1032-9.

Tiemessen MM, Mitchell TJ, Hendry L, et al (2006). Lack of suppressive CD4+CD25+FOXP3+ T cells in advanced stages of primary cutaneous T-cell lymphoma. *J Invest Dermatol*, **126**, 2217-23.

Wada DA, Pittelkow MR, Comfere NI, et al (2013). CD4(+) CD25(+)FOXP3(+) malignant T cells in Sezary syndrome are not necessarily functional regulatory T cells. *J Am Acad Dermatol*, **69**, 485-9.

Wada DA, Wilcox RA, Weenig RH, et al (2010). Paucity of intraepidermal FoxP3-positive T cells in cutaneous T-cell lymphoma in contrast with spongiotic and lichenoid dermatitis. *J Cutan Pathol*, **37**, 535-41.

Wang G (2014). Human antimicrobial peptides and proteins.

Pharmaceuticals (Basel), **7**, 545-94.
Wilcox RA (2014). Cutaneous T-cell lymphoma: 2014 update on diagnosis, risk-stratification, and management. *Am J Hematol*, **89**, 837-51.
Willemze R, Jaffe ES, Burg G, et al (2005). WHO-EORTC classification for cutaneous lymphomas. *Blood*, **105**, 3768-85.
Willerslev-Olsen A, Krejsgaard T, Lindahl LM, et al (2013). Bacterial toxins fuel disease progression in cutaneous T-cell lymphoma. *Toxins (Basel)*, **5**, 1402-21.
Zhan HL, Gao X, Zhou XF, et al (2012). Presence of tumour-infiltrating FOXP3+ lymphocytes correlates with immature tumour angiogenesis in renal cell carcinomas. *Asian Pac J Cancer Prev*, **13**, 867-72.
Zhang GQ, Han F, Fang XZ, et al (2012). CD4+, IL17 and Foxp3 expression in different pTNM stages of operable non-small cell lung cancer and effects on disease prognosis. *Asian Pac J Cancer Prev*, **13**, 3955-60.
Zhou QY, Wang YL, Li X, et al (2014). Metabolomics investigation of cutaneous T cell lymphoma based on UHPLC-QTOF/MS. *Asian Pac J Cancer Prev*, **15**, 5417-21.
Zou W (2006). Regulatory T cells, tumour immunity and immunotherapy. *Nat Rev Immunol*, **6**, 295-307.