Increased Expression of PLS3 Correlates with Better Outcome in Sezary Syndrome

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TO THE EDITOR

Patients with Sézary syndrome (SS), a rare erythrodermic and leukemic form of cutaneous T-cell lymphoma, have a poor prognosis with a 5-year overall survival (OS) of 20–42% and a median OS between 2.5 and 5.0 years (Agar et al., 2010; Bernengo et al., 1998; Diamandidou et al., 1999; Kim et al., 2003; Kubica et al., 2012; Talpur et al., 2012).

Prognostic factors associated with a worse survival reported in SS include advanced age (Agar et al., 2010; Diamandidou et al., 1999; Foulc et al., 2003; Kim et al., 2003; Kubica et al., 2012; Talpur et al., 2012), short duration of skin lesions before diagnosis of SS (Foulc et al., 2003), previous history of mycosis fungoides (Bernengo et al., 1998; Kubica et al., 2012), elevated serum lactate dehydrogenase levels (Agar et al., 2010; Bernengo et al., 1998; Diamandidou et al., 1999; Foulc et al., 2003; Kubica et al., 2012; Talpur et al., 2012), degree of nodal involvement (Diamandidou et al., 1999; Kim et al., 2003), and factors reflecting blood tumor burden, such as increased leukocyte counts (Bernengo et al., 1998; Talpur et al., 2012; Vidulich et al., 2009) or high Sézary cell counts (Bernengo et al., 1998).

However, the results of these studies are not consistent, which may be due to different diagnostic criteria for SS, such as inclusion of patients without a T-cell clone in the peripheral blood, and analysis of mixed populations of patients with SS and erythrodermic mycosis fungoides.

Recently, we investigated the diagnostic significance of a large number of immunophenotypic and molecular biomarkers for SS in a group of patients with SS (Boonk et al., 2016) that fulfilled the diagnostic criteria of the World Health Organization–European Organization for Research and Treatment of Cancer classification (Willemze et al., 2005). None of these patients had a history of mycosis fungoides. Molecular biomarkers diagnostic for SS were copy number alterations in *MYC* (gain) and/or *MNT* (loss); increased expression of *DNM3*, *TWIST1*, *EPHA4,* and *PLS3;* and decreased expression of *STAT4*.

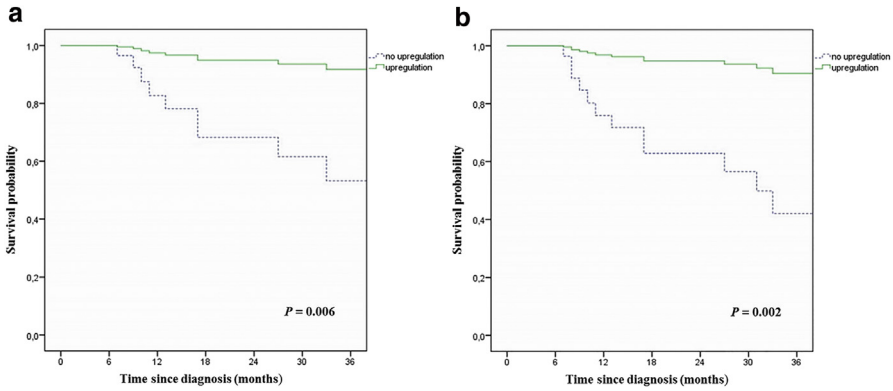

Figure 1. Survival curves. (**a**) Disease-specific survival and (**b**) overall survival curve according to the groups with and without up-regulation of *PLS3*.

We investigated the prognostic significance of these molecular biomarkers and previously reported clinical prognostic markers using the same cohort of SS patients. Between September 2009 and October 2013, 64 SS patients from six European Organization for Research and Treatment of Cancer centers, including Helsinki, Finland; London, UK; Leiden, The Netherlands; Mannheim, Germany; Turin, Italy; and Paris, France were included and followed up until January 30, 2015. At the inclusion of the study, clinical variables (sex, age at diagnosis, duration of skin lesions before diagnosis if SS, lymph node involvement, leukocyte count, absolute CD4 count, and Sézary cell count) were recorded, and peripheral blood samples were collected for copy number variation and gene expression quantitative PCR analysis, as described previously (Boonk et al., 2016). Lymph node involvement was defined by presence of enlarged lymph nodes of 1.5 cm or larger in the longest transverse diameter on computed tomography scan or histologically confirmed lymph node involvement.

Aberrant gene expression in the SS samples was compared with samples from patients with erythrodermic inflammatory dermatoses and healthy control samples. Receiver operating characteristic curve analysis was used to determine fixed cutoff thresholds for each individual gene expression quantitative PCR assay with a specificity of 100% and an accuracy above 0.80. A one-tailed Mann-Whitney test was applied to test for significant differential expression between the SS and erythrodermic inflammatory dermatoses samples. *P*-values below 0.05 were regarded as statistically significant. The results of aberrant expression of the *DNM3*, *TWIST1*, *EPHA4*, *PLS3,* and *STAT4* genes were included in the statistical analysis. A more detailed Methods section including these thresholds is included in the Supplementary Materials and Supplementary Table S1 online.

Abbreviations: DSS, disease-specific survival; OS, overall survival; SS, Sézary syndrome

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Table 1. Results of univariate and multivariate analysis for variables at Sézary syndrome diagnosis[1]

	Survival in Months	Univariate Analysis DSS		Multivariate Analysis DSS		Univariate Analysis OS		Multivariate Analysis OS	
Variables	**Median (Range)**	**HR (95% CI)**	***P*-Value**	**HR (95% CI)**	***P*-Value**	**HR (95% CI)**	***P*-Value**	**HR (95% CI)**	***P*-Value**
Gain in copy number of *MYC*	—	—	0.843	—	—	—	0.366	—	—
Yes, n = 21	72 (1–129)	0.90 (0.32–2.54)	—	—	—	0.66 (0.26–1.63)	—	—	—
No, n = 31	49 (7–86)	1	—	—	—	1	—	—	—
Loss in copy number of *MNT*	—	—	0.355	—	—	—	0.388	—	—
Yes, n = 35	68 (1–129)	0.61 (0.22–1.74)	—	—	—	0.68 (0.28–1.65)	—	—	—
No, n = 17	49 (7–80)	1	—	—	—	1	—	—	—
Up-regulation of *DNM3*	—	—	0.337	—	—	—	0.008	—	0.658
Yes, n = 36	71 (9–129)	0.56 (0.17–1.83)	—	—	—	0.29 (0.12–0.72)	—	0.74 (0.20–2.75)	—
No, n = 13	33 (1–72)	1	—	—	—	1	—	1	—
Up-regulation of *TWIST1*	—	—	0.197	—	—	—	0.043	—	0.342
Yes, n = 32	71 (1–129)	0.48 (0.16–1.46)	—	—	—	0.39 (0.16–0.97)	—	0.57 (0.18–1.81)	—
No, n = 17	31 (7–80)	1	—	—	—	1		1	
Up-regulation of *EPHA4*	—	—	0.312	—	—	—	0.311	—	—
Yes, n = 32	49 (7–129)	1.95 (0.53–7.12)	—	—	—	1.70 (0.61–4.74)	—	—	—
No, n = 17	68 (1–80)	1	—	—		1	—	—	—
Up-regulation of *PLS3*	—	—	0.027	—	0.006	—	0.001	—	0.002
Yes, n = 32	71 (9–129)	0.29 (0.10–0.87)	—	0.14 (0.03–0.56)	—	0.19 (0.07–0.49)	—	0.12 (0.03–0.46)	
No, n = 17	33 (1–72)	1	—	1	—	1	—	1	—
Down-regulation of *STAT4*	—	—	0.356	—	—	—	0.250	—	—
Yes, n = 44	68 (1–129)	#	—	—	—	3.28 (0.43–24.77)	—	—	—
No, n = 5	Not reached	1	—	—	—	1	—	—	—
Sex	—	—	0.665	—	—	—	0.830	—	—
Female, n = 25	49 (1–129)	1.21 (0.51–2.85)	—	—	—	0.92 (0.43–1.98)	—	—	—
Male, n = 39	68 (1–115)	1	—	—	—	1	—	—	—
Age at SS diagnosis in years, n = 64	—	1.01 (0.96–1.05)	0.827	—	—	1.00 (0.97–1.04)	0.850	—	—
Duration of skin lesions, n = 60	—	0.98 (0.96–1.00)	0.075	0.99 (0.97–1.02)	0.447	0.99 (0.97–1.00)	0.152	—	—
Lymph node involvement	—	—	0.356	—	—	—	0.420	—	—
Yes, n = 18	49 (1–74)	1.82 (0.51–6.53)	—	—	—	1.55 (0.53–4.52)	—	—	—
No, n = 23	Not reached	1	—	—	—	1	—	—	—
Leukocyte count, n = 61	—	1.04 (1.01–1.06)	0.007	1.06 (1.02–1.10)	0.005	1.03 (1.01–1.06)	0.011	1.05 (1.01–1.08)	0.005
Absolute CD4 count, n = 59	—	1	0.146	—	—	1	0.265	—	—
Sézary cell count, n = 47	—	1	0.123	—	—	1	0.204	—	—

Abbreviations: CI, confidence interval; DSS, disease-specific survival; HR, hazard ratio; OS, overall survival; SS, Sézary syndrome.

[1]Parameters significant at the 0.1 level were included in multivariate analysis.

means no statistics can be computed.

Survival was calculated with the Kaplan-Meier method from the date of diagnosis until the patient's death or date of last follow-up. The median follow-up time after diagnosis was 45 months (range = 1–129 months). Twenty-seven patients died during follow-up, including 21 SS-related deaths. The disease-specific survival (DSS) after 1, 2, 3, and 5 years was 89%, 82%, 76%, and 59%, respectively, and OS was 86%, 79%, 72%, and 49%, respectively.

Univariate analysis of parameters with possible prognostic significance for DSS and OS was performed using Cox proportional hazards regression analysis, and parameters that were significant at the 0.1 level were included in a multivariate analysis model. *P*-values below 0.05 were regarded as statistically significant.

Both in univariate and multivariate analyses, up-regulation of *PLS3* was associated with a significantly better outcome for DSS and OS (multivariate $P = 0.006$ and $P = 0.002$,

respectively). Patients with up-regulation of *PLS3* had a median survival of 71 months (range = 9−129 months), compared with only 33 months (range = 1−72 months) in SS patients with normal expression of *PLS3* (Figure 1). Up-regulation of *DNM3* and *TWIST1* were associated with a better OS in univariate analysis ($P = 0.008$ and $P = 0.043$, respectively) but not in multivariate analysis ($P = 0.658$ and $P = 0.342$, respectively). Gain of *MYC*, loss of *MNT*, up-regulation of *EPHA4*, and down-regulation of *STAT4* showed no association with DSS and OS (Table 1).

Of the clinical parameters, both univariate and multivariate analyses showed that leukocyte count was a significant prognostic factor for DSS and OS (multivariate $P = 0.005$ and $P = 0.005$, respectively), whereas sex, age, duration of skin lesions before diagnosis, lymph node involvement, absolute CD4 count, and Sézary cell count were not (Table 1).

PLS3 (T-plastin) is an actin-binding protein that is expressed in all normal cells of solid tissues that have a replicative role, but it is normally not expressed in T cells (Lin et al., 1999). Expression of *PLS3* has been described as a specific marker of Sézary cells (Jones et al., 2012; Nebozhyn et al., 2006). Studies investigating the mechanism underlying dysregulation of *PLS3* expression in SS cells found no evidence for *PLS3* mutations within coding or promoter regions but showed significant hypomethylation of 5′-cytosine-phosphate-guanine-3′ dinucleotides 95−99 within the *PLS3* 5′-cytosine-phosphate-guanine-3′ island, which was restricted to the $PLS3^+$ cells (Jones et al., 2012). Reanalysis of recently published DNA methylation profiles (van Doorn et al., 2016) from nine patients with SS and four healthy control subjects included in this study confirmed this correlation between DNA methylation and *PLS3* expression (data not shown). A recent study found that constitutive *PLS3* expression was associated with apoptotic resistance to etoposide and suggested a role for cell survival in SS (Begue et al., 2012). How T-plastin expression is linked to a better outcome in patients with SS is not known and should be the subject of further study.

Although for a disease as rare as SS the number of included patients is relatively high, a limitation of this study is a small sample size, which yielded wide confidence intervals, and these observations should be confirmed in an independent study.

In conclusion, we show that up-regulation of *PLS3* is associated with a favorable disease outcome in patients with SS and that increased leukocyte count is a significant adverse prognostic factor for survival.

In all participating centers the study was approved by their local institutional ethical review boards and written informed patient consent was obtained (Boonk et al., 2016).

CONFLICT OF INTEREST

MF received travel grants from TEVA.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at http://dx.doi.org/10.1016/j.jid.2016.10.025.

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The Involvement of Serum Amyloid A in Psoriatic Inflammation

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TO THE EDITOR

Psoriasis vulgaris is a common inflammatory skin disorder that is characterized by abnormal epidermal proliferation and a cellular infiltrate including neutrophils and T cells (Nestle et al., 2009). The number of T helper (Th) type 1 and Th17 cells are particularly increased in psoriatic patients, and they are thought to play critical roles in the pathogenesis (Lowes et al., 2013; Nestle et al., 2009). In addition, psoriatic keratinocytes are morphologically abnormal and may influence infiltrating T-cell function by producing cytokines and chemokines (Lowes et al., 2013; Nestle et al., 2009).

Serum amyloid A (SAA) is a highly conserved, acute-phase protein that is synthesized predominantly by the liver and is an apolipoprotein that is a component of high-density lipoprotein particles in the blood (Eklund et al., 2012). SAA isoforms are precursors of the amyloid A protein, a major component of the fibrous deposit in secondary amyloidosis associated with chronic inflammatory disorders such as rheumatoid arthritis (Perfetto et al., 2010). A persistent high concentration of serum SAA in the sera is considered to be a prerequisite for the development of secondary amyloidosis (Gillmore et al., 2001). In addition, SAA is known to activate immune cells and induce cytokines and chemokines (Eklund et al., 2012). Furthermore, SAA is involved in the pathogenesis of atherosclerosis, because it is produced and deposited in atherosclerotic lesions (Meek et al., 1994; O'Brien et al., 2005).

Some reports have already shown the association between psoriasis and SAA. Dogan et al. reported that SAA is a more specific marker of psoriasis rather than C-reactive protein (Dogan and Atakan, 2010). Yu et al. showed that SAA expression is up-regulated in psoriatic keratinocytes and that a Th17 cytokine, IL-17A, induces SAA expression in the cells (Yu et al., 2015). In addition, they also reported that SAA induces IL-1β secretion via nucleotide binding and oligomerization domain-like receptor family pyrin domain-containing 3 (NLRP3) in the cells. These findings suggest that keratinocyte-derived SAA is involved in psoriatic inflammation, although the mechanism has not yet been fully elucidated.

We sought other molecular mechanisms that might explain the SAA increase in psoriasis patients, and we hypothesized that some cytokines, other than IL-17A, that are expressed in psoriatic lesions may also induce SAA expression in keratinocytes. We stimulated normal human epidermal keratinocytes (NHEKs) with panels of cytokines, and the analyses using real-time PCR and ELISA showed that the Th1 cytokines IFN-γ and tumor necrosis factor (TNF)-α, in addition to IL-17A, significantly induced SAA expression in NHEKs; in contrast, the Th2 cytokines, IL-4 and IL-13, did not (Figure 1a–b). The induction by TNF-α and IL-17A was NF-κB dependent, whereas that by IFN-γ was signal transducer and activator of transcription-1 dependent (see Supplementary Figure S1a–b and S2a–b online). Furthermore, IFN-γ, TNF-α, and IL-17A synergistically enhanced SAA expression in NHEKs (Figure 1c). In contrast, the addition of IL-4 or IL-13 under the TNF-α stimulation significantly suppressed the TNF-α induced SAA expression in NHEKs (Figure 1d).

Skin and serum samples were collected with written informed consent from patients with atopic dermatitis or psoriasis and from normal healthy volunteers at Okayama University Hospital. This study was approved by the Ethics Committee of Okayama University. In addition to the in vitro data, immunohistochemical analysis showed that SAA expression was increased in the epidermal keratinocytes and dermal infiltrating cells in psoriatic lesions when compared with normal skin, as previously reported (Figure 1e and f). ELISA analysis showed that psoriasis patients ($n = 25$) also had significantly higher levels of SAA compared with normal healthy volunteers ($n = 17$) and atopic dermatitis patients ($n = 14$) (Figure 1g). Furthermore, the serum SAA protein levels of psoriasis patients significantly correlated with the Psoriasis Area and Severity Index scores (Figure 1h) ($n = 31$ (the 25 subjects shown in Figure 1g plus 6 more with SAA

Abbreviations: NHEK, normal human epidermal keratinocyte; SAA, serum amyloid A; Th, T helper; TNF, tumor necrosis factor

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