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T-plastin (PLS3) gene expression differentiates SS from MF and inflammatory skin diseases and can serve as a biomarker to monitor disease progression

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Abstract

Background—Cutaneous T-cell lymphoma (CTCL) is a group of lymphoproliferative disorders that includes mycosis fungoides (MF) and Sezary syndrome (SS). T-Plastin (PLS3) is an actin-bundling protein that has been found to be highly expressed in Sezary cells but not in normal PBMCs. Here, we describe the value of using PLS3 as a sensitive molecular marker for differentiating stages of MF from SS, and for monitoring development of SS from MF.

Objectives—To determine the relationship between PLS3 expression level and SS, and disease progression and response to treatment in patients with MF/SS.

Methods—Total RNA from PBMCs from normal volunteers, MF/SS, and psoriasis patients were measured by quantitative PCR for PLS3 gene expression.

Results—In PBMCs from MF/SS and psoriasis patients, PLS3 expression was increased markedly in SS (greater than 400-fold) compared to that seen in early and late stage MF patients without SS cells or in patients with psoriasis. In a patient whose disease progressed to SS from MF, the PLS3 level in PBMCs showed an increased with disease progression. With treatment, the level of PLS3 decreased with chemotherapy and bone marrow transplantation.

Conclusions—PLS3 expression is a valuable and sensitive molecular biomarker to differentiate MF from SS. The measure of this gene may have value in conjunction with gene rearrangement studies to monitor disease severity or progression from MF to SS. Furthermore, PLS3 is not expressed in other inflammatory skin diseases, and may be valuable to distinguish SS from other cutaneous diseases associated with generalized erythroderma.

Introduction

The most common forms of cutaneous T-cell lymphoma (CTCL) are mycosis fungoides(MF) and Sezary syndrome(SS), malignancy of CD4 T cells. MF is skin restricted in the early stage, but can progress to systemic involvement. SS is characterized by erythroderma, lymphadenopathy and atypical T cells with cerebriform nuclei in the blood(Harris et al, 1994). MF/SS has numerous molecular abnormalities, include chromosomal changes and loss of surface markers CD7 and CD26 (Vonderheid, 2006). Lacking are consistent markers that increases in expression that can aid in diagnosis and monitoring of clinical progression.

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Though clonality is valuable for confirmation of malignancy, gene rearrangement may yield negative results (Jones et al, 2003). Furthermore clonality can be observed in reactive situations. In addition, substantially increased expression of cytokines is detected in early MF/CTCL, followed by decreased cytokines in late stages of CTCL (Chong et al, 2008). Nevertheless, there remains a need for specific biomarker that is simple to measure for improved diagnosis of MF/SS.

T-plastin (PLS3) was identified as a gene that is differentially expressed in T cells from Sezary patients (Zhou 2003). PLS3 is an actin-bundling protein found to be expressed highly in patients with Sezary disease and is not expressed in normal PBMCs (Lin et al, 1988, Kari 2003, Capriotti et al 2008). In this report, we analyzed the expression of PLS3 in different stages of MF/SS and erythrodermic skin diseases and describe the specificity of PLS3 in SS. We show the value of measuring PLS3 gene expression levels during disease progression, and demonstrate its decrease in response to treatment, illustrating the value of PLS3 as a sensitive molecular marker. Because increased expression of PLS3 was not observed in patients with psoriasis, PLS3 may be a useful marker in differentiating Sezary syndrome from other erythrodermic skin diseases.

Materials and Methods

All volunteers consented to Henry Ford Hospital IRB protocol for research studies. The PBMCs were isolated and were stimulated with PMA (25 ng/ml) and A23187 (0.1 ug/ml) as described (Chong et al, 2008).

Total RNA was isolated from PBMCs with TRIzol (Invitrogen). cDNA was synthesized from total RNA with Superscript II (Invitrogen), and PLS3 level was determined by quantitative PCR (in duplicates) using an ABI 7000 (40 cycles at 95°C × 15S, 60°C × 1min). Duplicates samples that deviated by more than one-half Ct were repeated. Primers for PLS3 (5-TGGGAACCAAACCCTGACTTT-3 and 5-GATCTTCCAGGACATTGAGGGTAT-3) and β 2-microglobulin (5-TCTACTTTGAGTGCTGTCTCCATGT-3 and 5-AAGTTGCCAGCCCTCCTAGAG-3) have similar amplification efficiencies. β 2-microglobulin expression remained stable upon stimulation. The relative gene expression was calculated using the 2-ddCT method (Livak et al, 2001). The mean expression of PLS3 in each sample was normalized relative to β 2-microglobulin. The mean level of PLS3 expression from normal samples at T=0 represents the level by which other samples were normalized. Sezary count was determined by the Henry Ford Cytopathology laboratory.

Results

PLS3 mRNA expression was analyzed in total RNA from PBMCs from normal volunteers (n= 14), MF/SS patients (n=35), and psoriasis/pityriasis rubra pilaris (PRP) patients (n=6/ n=1) as described in Table 1 using quantitative PCR (Fig 1). Psoriasis represents an immune skin disease control where T cells are involved and patients can develop erythroderma. In normal and psoriasis PBMCs, the level of PLS3 expression was low and expressed in a similar range. The level of PLS3 expression in PBMCs from patients with early stage (I and II) and late stage MF without SS cells was not significantly different compared to normal controls. In PBMCs from SS patients, the level of PLS3 was higher than that in normal controls or patients with psoriasis/(PRP). The differences were not lost by activation. In the unstimulated state, T=0hr, PLS3 in SS was 408-fold greater than normal (p=0.056); at T=2hr, PLS3 was 1269-fold over normal (p=0.038); and at T=6hr, PLS3 was 914-fold greater than normal (P=0.023).

To test the value of monitoring PLS3 in disease progression, PBMCs from a 59 year-old female patient who presented initially with stage 1b MF and subsequently developed SS was

analyzed. During a 22 month period, PBMCs were isolated from 11 different time points and PLS3 expression measured (Fig 2). During this period, hematologic studies revealed the presence of SS cells in the blood. Examination of PLS3 expression in PBMCs showed a pattern that parallels the Sezary count. At 9 months after initial monitoring of PLS3 and with progressive disease, the patient was treated with systemic chemotherapy (CHOP, ONTAK and Fludarabine) with temporary clinical response. The PLS3 expression in PBMCs showed a 40% decrease from peak expression, and a corresponding decrease in the Sezary count. Subsequently, the patient received a bone marrow transplant, after which the PLS3 expression gradually decreased to undetectable levels. The PLS3 expression of the PBMC sample from the BMT donor was undetectable.

Discussion

PLS3 is expressed in a variety of tissues, however it is undetectable in normal PBMCs. Our findings showed that PLS3 expression is increased in the PBMCs in SS compared to earlier stages of MF and psoriasis. This suggests that this gene is a sensitive marker to distinguish SS from other stages of MF and inflammatory skin diseases. We also show for the first time that PLS3 expression can be correlated to disease extent in a patient who developed SS. In SS PBMCs, PLS3 expression was greater than 400-fold compared to normal, MF without SS cells, and psoriasis/PRP. Normal PBMCs from inflammatory skin disease, such as psoriasis do not express PLS3. Furthermore, patients with erythroderma, such as pityriasis rubra pilaris, did not express PLS3.

A previous study found that PLS3 levels did not correlate with tumor burden; the authors questioned whether that was due to lower levels of expression in some tumor cells or to expression only in a distinct subclass (Nebozhyn et al 2006). Our findings in longitudinal follow-up of a patient show that PLS3 does correlate with disease severity as reflected by the level of Sezary cells in the peripheral blood. The function of PLS3 in SS is unclear, as is the mechanism responsible for the expression of the gene. However, the mechanism for activating this gene may be associated with the transformation process.

In summary, PLS3 is a potentially valuable marker to differentiate MF from SS that can be measured for following transformation or progression. The level of PLS3 in PBMCs from SS patients is significantly higher than in patients without circulating atypical cells, psoriasis/PRP or in normal controls. PLS3 shows a strong relationship to the level of SS cells and its expression may be a potential marker of disease severity, progression and treatment response. Furthermore PLS3 may be a valuable diagnostic marker to differentiate SS from diseases associated with erythroderma, such as psoriasis or PRP.

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Figure 1.

PLS3 gene expression level in PBMCs. PLS3 was analyzed in early MF - Stage I (n = 11), Stage II (n=10) and late MF with positive Sezary cells (n = 8) and late MF with negative Sezary cells (n = 6) patients compared with normal controls (n = 14) and psoriasis/PRP patients (n = 6/1). PBMCs were isolated and total RNA prepared as described in the Materials and Methods. Quantitative RT-PCR was performed in duplicates, normalized to β 2M level of gene expression and expressed relative to the level of PLS3 in unstimulated normal PBMCs. (Points=mean; bars, SE).

Figure 2.

Temporal change in PLS3 gene expression and relationship to Sezary count. The level of PLS3 gene expression was measured by qRT-PCR normalized to normal in a patient with MF who progressed to Sezary Syndrome. PBMCs were isolated as described, and quantitative RT-PCR was performed on unstimulated PBMCs collected on the indicated dates. Relative level of PLS3 gene expression at T=2 was normalized to β 2M level of gene expression and plotted to the unstimulated level of gene expression for normal PBMCs (bar graph). Analysis of PBMCs from marrow donor are included for comparison. On the right axis, percent of Sezary cells are plotted for each specimen time point (line graph). Red dashed line indicates initiation of chemotherapy. Green line indicates BMT.

Table 1

Patients	Stage/TNMB	N	Avg age	Male/Female	Avg Sezary count	Sezary count range	Erythrodermic patients
Stage 1							
MF/CTCL	I/T1	11	58	6/5	0	0	0
Stage 2							
MF/CTCL	II/T2, N0–N1	10	71	8/2	0	0	0
Late MF/CTCL	III–IV/T3–T4, N0–N3,						
–SS	B2	6	56	2/4	0.6	0–2	0
Late MF/CTCL	III–IV/T3–T4, N0–3,						
+SS	B0–B1	8	74	3/6	29.5	12–63	8